Heterozygous carriers of a Parkin or PINK1 mutation share a common functional endophenotype

ABSTRACT

Objective: To use a combined neurogenetic-neuroimaging approach to examine the functional consequences of preclinical dopaminergic nigrostriatal dysfunction in the human motor system. Specifically, we examined how a single heterozygous mutation in different genes associated with recessively inherited Parkinson disease alters the cortical control of sequential finger movements.

Methods: Nonmanifesting individuals carrying a single heterozygous Parkin (n = 13) or PINK1 (n = 9) mutation and 23 healthy controls without these mutations were studied with functional MRI (fMRI). During fMRI, participants performed simple sequences of three thumb-to-finger opposition movements with their right dominant hand. Since heterozygous Parkin and PINK1 mutations cause a latent dopaminergic nigrostriatal dysfunction, we predicted a compensatory recruitment of those rostral premotor areas that are normally implicated in the control of complex motor sequences. We expected this overactivity to be independent of the underlying genotype.

Results: Task performance was comparable for all groups. The performance of a simple motor sequence task consistently activated the rostral supplementary motor area and right rostral dorsal premotor cortex in mutation carriers but not in controls. Task-related activation of these premotor areas was similar in carriers of a Parkin or PINK1 mutation.

Conclusion: Mutations in different genes linked to recessively inherited Parkinson disease are associated with an additional recruitment of rostral supplementary motor area and rostral dorsal premotor cortex during a simple motor sequence task. These premotor areas were recruited independently of the underlying genotype. The observed activation most likely reflects a “generic” compensatory mechanism to maintain motor function in the context of a mild dopaminergic deficit. Neurology® 2009;72:1041–1047

GLOSSARY

BOLD = blood oxygen level–dependent; CMA = cingulate motor area; FDR = false discovery rate; fMRI = functional MRI; HRF = hemodynamic response function; IPS = intraparietal sulcus; M1HAND = primary motor hand area; PD = Parkinson disease; PMd = dorsal premotor cortex; SMA = supplementary motor area; SPM = statistical parametric mapping; SVC = small volume correction; TE = echo time; TMS = transcranial magnetic stimulation; TR = repetition time; VOI = volumes of interest.

Several genes have been identified that can lead to Parkinson disease (PD), including four recessively inherited forms caused by mutations in the Parkin (PARK2), DJ-1 (PARK7), PINK1 (PARK6), and ATP13A2 (PARK9) genes.1-3 These familial forms of PD show a substantial clinical overlap with sporadic PD. Nonmanifesting individuals who carry a single heterozygous mutation in the Parkin and PINK1 gene associated with recessively inherited PD...
have attracted particular interest. PET of dopaminergic neurotransmission showed that these individuals have a mild presynaptic dopaminergic dysfunction in the striatum. Therefore, nonmanifesting carriers of a single mutant allele provide a unique model to study the effect of a subclinical loss of dopamine-producing cells in the substantia nigra on the human motor system.

In a recent functional MRI (fMRI) study, we provided evidence for a compensatory redistribution of neuronal activity within the motor system in nonmanifesting carriers of a heterozygous mutation in the Parkin gene. With internally cued movements, mutation carriers displayed a stronger activation of the right rostral cingulate motor area and left dorsal premotor cortex (PMd) compared to externally cued movements. They also showed stronger functional coupling between the rostral cingulate motor area and posterior putamen in the context of internal movement selection. Because mutation and non–mutation carriers performed the task equally well, these activity changes were interpreted as adaptive redistribution of neuronal activity in rostral motor cortical areas which helps to maintain motor function in the context of a latent nigrostriatal dysfunction.

The present experiment extended our previous fMRI study in two directions. First, we used a different experimental task which required participants to quickly perform a brief “chunk” of three movements. In our previous fMRI study, the experimental task required the selection of single movements. The onset of each movement was externally paced at a low rate and consecutive movements were separated by periods of rest. By using a “real” motor sequence task, we examined how a heterozygous mutation in a gene linked to recessively inherited PD impacts on functional brain networks subserving sequential movements. We hypothesized that the regional expression of functional changes in motor cortical areas critically depends on the particular function probed by the experimental task. Therefore, the adaptive redistribution of cortical activity within preexisting motor networks was expected to be different for the motor sequence task as opposed to the previously used movement selection task. Specifically, we predicted that mutation carriers would show a compensatory recruitment of rostral premotor areas that are specialized for the control of complex motor sequences.

Second, we included nonmanifesting individuals carrying a single mutant allele in the Parkin or PINK1 gene. This enabled us to test whether the adaptive redistribution of neuronal activity in motor brain regions is specifically linked to mutations in a specific gene associated with recessively inherited PD. Given the closely related dysfunctional effects of mutations in both proteins in a drosophila model, our prediction was that the functional phenotype at a brain network level would be similar for both groups.

**METHODS**

**Participants.** We studied 13 subjects (mean age 38.9 ± 5.8 years, seven men) carrying a single heterozygous mutation in the Parkin gene, either a deletion of exon seven (n = 7) or a single base-pair deletion in exon nine (c.del1072T) (n = 6). Nine other subjects (mean age 41.9 ± 5.7 years, seven men) carried a heterozygous c.1366C>T nonsense mutation of the PINK1 gene. Three of the Parkin and five of the PINK1 mutation carriers had minor motor signs upon careful clinical examination, but were not aware of the motor signs and motor signs did not interfere with their daily activities. None of these subjects had a Unified Parkinson’s Disease Rating Scale score of more than 4 or met the international accepted diagnostic criteria of probable PD. Nine of the heterozygous carriers of a Parkin mutation had previously undergone 18F-DOPA PET showing a presynaptic dopaminergic deficit in the striatum.

We also studied two groups of healthy age-matched controls: 13 volunteers (mean age 38.7 ± 5.5 years, seven men) who served as controls for the nonmanifesting Parkin mutation carriers and 10 volunteers (mean age 40.0 ± 5.9 years, seven men) formed the control group for the nonmanifesting PINK1 mutation carriers. Controls were recruited from a departmental registry of volunteers and did not have mutations in Parkin or PINK1.

Participants had no history of a previous neuropsychiatric disease nor had they previously received dopaminergic or other antiparkinsonian drug treatment. All participants were consistent right-handers according to the Edinburgh handedness inventory. Written informed consent was obtained prior to the study. The experimental procedures had the approval of the local ethics committee.

**Experimental design.** The fMRI experiment consisted of 10 alternating blocks of REST and TASK. During the TASK periods, participants repeatedly performed sequential finger movements with their right dominant hand. Each sequence consisted of three thumb-to-finger opposition movements instructed by external visual cues. Participants produced three different motor sequences in pseudorandom order. The details of the experimental task are given in figure 1A. Before fMRI, participants were
Individuals sequentially tapped with the tip of their right thumb onto the tip of the indicated fingers. The position and order of the red dot specified the motor sequence that had to be performed within a given trial. When the instruction cue disappeared from the screen, participants were instructed to remain still and fixate the hand with their eyes. During the REST periods, the line drawing of the hand was continuously presented in the center of the visual field throughout the fMRI session. Participants performed 30 consecutive sequences per fMRI session. To assess performance during fMRI, we taped aluminum foil to the tips of the thumb and the fingers of the right hand. When the thumb and finger tips contacted each other, an electrical circuit was closed which was specific to a given finger. For each trial, we recorded the time during which the tip of the thumb had contact with the index, middle, ring, or little finger. This enabled us to calculate the time that elapsed between the first and last finger-to-thumb contact of the motor sequence, referred to as Tap1-Tap3 interval. To assess the stability of motor performance, we calculated the mean Tap1-Tap3 interval for each block of TASK and 10 blocks of REST. A two-dimensional drawing of the palm of the right hand was continuously presented for approximately 5 minutes.

Familiarized with the task and practiced the respective finger sequences for approximately 5 minutes. By choosing a short sequence, we kept the task simple, favoring automatic performance without a high level of monitoring. The use of longer sequences would have increased the load on working memory, possibly forcing subjects to divide the sequence into separate chunks. We randomly presented three sequences rather than repeating the same sequence during a given block. This forced the participants to continuously switch between different motor representations of simple overlearned sequences.

Our decision to select sequential finger movements as experimental task was based on two considerations. First, sequential finger movements have been extensively studied in PD, providing evidence for compensatory overactivity in the PMd and intraparietal sulcus in PD during sequential movements. Second, healthy controls show a linear increase in activity with sequence complexity in the rostral part of the supplementary motor area (referred to as pre-SMA) and the rostrodorsal portion of the right PMd. Therefore, we hypothesized that the latent dopaminergic dysfunction in presymptomatic carriers of a Parkin or PINK1 mutation results in a compensatory recruitment of the pre-SMA and right PMd to maintain motor performance within a normal range.

Participants performed 30 consecutive sequences per fMRI session. To assess performance during fMRI, we taped aluminum foil to the tips of the thumb and the fingers of the right hand. When the thumb and finger tips contacted each other, an electrical circuit was closed which was specific to a given finger. For each trial, we recorded the time during which the tip of the thumb had contact with the index, middle, ring, or little finger. This enabled us to calculate the time that elapsed between the first and last finger-to-thumb contact of the motor sequence, referred to as Tap1-Tap3 interval. To assess the stability of motor performance, we calculated the mean Tap1-Tap3 interval for 10 consecutive trials during the fMRI session.

MRI data acquisition. Whole-brain MRI was performed on a 1.5 T Magnetom Symphony scanner (Siemens, Erlangen, Germany) equipped with a standard head coil. We used a T2*-weighted gradient-echo echoplanar sequence (repetition time [TR] = 3,000 msec, echo time [TE] = 40 msec, flip angle = 90°, matrix 64 × 64 voxels, field of view = 256 × 256 mm², 30 axial slices, slice thickness: 4 mm) to map task-related changes in the blood oxygen level–dependent (BOLD) signal. A total of 160 brain volumes were acquired per session. We also obtained a whole-brain structural MRI dataset using a three-dimensional T1-weighted FLASH sequence (TR = 15 msec, TE = 5 msec, 192 axial slices, voxel size = 1 × 1 × 1 mm³, axial field of view = 256 × 256 mm²).

Data analysis. Using the mean Tap1-Tap3 interval as dependent variable, we performed a two-factorial repeated-measures analysis of variance with the within-subject factor TIME (three levels: trial 1 to 10, trials 11–20, and trials 21–30) and between-groups factor GROUP (four levels: nonmanifesting PINK1 or Parkin mutation carriers and their respective control groups without mutation). The Greenhouse-Geisser method was used to correct for nonsphericity if appropriate. Depending on a significant F value, post hoc t tests were performed. Data are given as mean and onefold SD. A p value of <0.05 was considered significant.

The fMRI data were processed and analyzed using statistical parametric mapping (SPM) software (SPM2; Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.
Regional increases in task-related blood oxygen level–dependent (BOLD) signal changes in nonmanifesting carriers of a Parkin or PINK1 mutation

(A) Statistical parametric maps. Sagittal, coronal, and axial slices highlighting those voxels in the pre–supplementary motor area (SMA) and adjacent dorsal premotor cortex (PMd) that showed a relative increase in BOLD signal during the sequential finger movement task in mutation carriers relative to controls without a mutation. The statistical parametric maps are superimposed onto a T2-weighted structural MRI template provided by MRicro (http://www.sph.sc.edu/comd/rorden/mricro.html). The voxels of the activation maps are color-coded according to their $t$ values. For illustrative purposes, the maps are thresholded at an uncorrected $p$ value of $p < 0.01$. (B) Parameter estimates of task-related BOLD signal changes in the right and left pre-SMA and right dorsomedial PMd. The column plots give the mean $\beta$ values (as estimated by the general linear model) for the task-related change in BOLD signal during the sequential finger movement task for each of the four groups (red columns = mutation carriers; yellow columns = non-mutation carriers). The $\beta$ values are given in arbitrary units (AU) and refer to the voxel showing a peak difference between mutation carriers and noncarriers. Error bars equal the 95% confidence interval of the mean.

RESULTS Behavior. All participants found the thumb-to-finger opposition tasks easy to perform. The maximum error rate was two sequential errors per session. Analysis of variance revealed no difference in mean Tap1-Tap3 interval among groups ($p > 0.5$). The mean Tap1-Tap3 interval was $1.44 \pm 0.18$ s among individuals carrying a Parkin mutation and $1.45 \pm 0.08$ s among controls without mutation. The mean Tap1-Tap3 interval was $1.40 \pm 0.11$ s in individuals with a PINK1 mutation and $1.36 \pm 0.08$s in the corresponding controls.

Functional MRI. Epoch related analysis identified a bilateral set of sensorimotor areas where the BOLD signal increased when participants performed the finger sequence task (figure 1B and tables e-1 through e-3 on the Neurology® Web site at www.neurology.org). Mutation carriers showed increased activation in right rostral PMd and the pre-SMA compared to controls (figure 2, table 1). The overactivity in these rostral premotor areas was independent of the geno-
Table 1 Differences in task-related blood oxygen level-dependent (BOLD) signal changes between mutation carriers of a Parkin or PINK1 mutation and healthy controls without mutation

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z score</th>
<th>p Value (SVC)</th>
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<tr>
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<tr>
<td>Rostral SMA</td>
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<td>8</td>
<td>62</td>
<td>4.31</td>
<td>0.018</td>
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<td>R</td>
<td>22</td>
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<td>60</td>
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Areas showing a relative increase in BOLD signal during the sequential finger movement in nonmanifesting carriers. Differences in BOLD signal are characterized by their regional maxima (Z score, p value, and x, y, z coordinates in MNI space). p Values are corrected for the number of voxels within the predefined spherical volumes of interest (for details, see Methods). SVC = small volume correction; SMA = supplementary motor area; PMd = dorsal premotor cortex.

Figure 3 Relative increases in task-related blood oxygen level-dependent (BOLD) signal changes in nonmanifesting carriers of a PINK1 mutation compared with nonmanifesting carriers of a Parkin mutation

Axial slices showing voxels in dorsal frontoparietal cortex with significant increase in BOLD signal during the sequential finger movement task in PINK1 mutation carriers relative to Parkin mutation carriers. The statistical parametric maps are superimposed onto T2-weighted structural MR images provided by MRicro (http://www.sph.sc.edu/comd/orden/micro.html). The voxels of the activation maps are color-coded according to their Z values. For illustrative purposes, the maps are thresholded at an uncorrected p value of p < 0.01.

DISCUSSION When nonmanifesting heterozygous carriers of a Parkin or PINK1 mutation perform a simple motor sequence task, they recruit the pre-SMA and right rostral PMd which are not utilized by healthy controls without mutation. This finding extends our recent morphometric MRI study showing an increase in gray matter volume in the basal ganglia in a comparable group of nonmanifesting carriers of a Parkin or PINK1 mutation. Together, the functional and structural MRI data suggest that mutations in the Parkin and PINK1 gene produce a very similar functional and structural endophenotype. This implies that single heterozygous mutations in these two genes have a similar impact on the human motor system.

Converging evidence from neuroimaging and transcranial magnetic stimulation (TMS) show that in healthy individuals, the pre-SMA mainly contributes to motor sequence control in nonroutine situations. Accordingly, functional neuroimaging demonstrated an activation of the pre-SMA and rostral right PMd with new or complex motor sequences but not with sequences that were highly overlearned or easy to perform. The activation of the pre-SMA during sequential movements was attributed to the formation of and switch between visuomotor associations rather than the control of the movements per se. In the presence of a mutant Parkin or PINK1 allele, the “extra-recruitment” of the pre-SMA and adjacent PMd most likely reflects an adaptive mechanism by which the motor system counteracts the preexisting
Areas showing relative differences in task-related BOLD signal changes between carriers of a single mutant PINK1 or Parkin allele.

<table>
<thead>
<tr>
<th>Region</th>
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<th>y</th>
<th>z</th>
<th>Z score</th>
<th>p Value (SVC)</th>
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Areas showing relative differences in task-related BOLD signal changes between the two groups of nonmanifesting mutation carriers. Differences in BOLD signal are characterized by their regional maxima (Z score, p value, and x, y, z coordinates in MNI space). Z Values are corrected for the number of voxels within the predefined spherical volumes of interest (for details, see Methods).

SVC = small volume correction; PMd = dorsal premotor cortex; CMA = cingulate motor area; M1HAND = primary motor hand area; IPS = intraparietal sulcus.

latent nigrostriatal dysfunction.\(^5\)\(^6\)\(^8\) Mutation carriers and controls showed equal performance when performing the simple motor sequence task. We propose that the mechanism by which mutation carriers maintain a normal level of performance is to recruit additional premotor regions that are specialized for handling complex sequential movements. We argue that the subclinical nigrostriatal neurotransmission in nonmanifesting mutation carriers produced a dysfunction of the corticobasal ganglia-thalamocortical motor loops involved in the control of overlearned motor sequences. This latent dysfunction rendered the simple sequence task more demanding in terms of neuronal motor control and specifically called on the “support” of rostromedial premotor cortex to maintain task performance.\(^2\)\(^8\) Because the pre-SMA and rostromedial PMd are reciprocally connected with prefrontal areas\(^29\)\(^30\) and receive inputs from the “nonmotor” (associative) territories of the cerebellum and basal ganglia,\(^31\) the increased activation of the pre-SMA and rostral PMd might be driven by a compensatory increase in neuronal input from connected prefrontal or subcortical areas during the task.

The adaptive recruitment of cortical premotor areas was restricted to the pre-SMA and the dorsomedial part of right rostral PMd. In a previous fMRI study, we also found an increase in rostral motor areas in nonmanifesting carriers of a Parkin mutation, but the spatial pattern of increased activity was different from the one found in the present study. When participants selected a finger movement with their right hand based on internal cues, individuals with a mutant Parkin allele showed a stronger activation of the rostral cingulate motor area and the ventrolateral part of left rostral PMd but not in rostral SMA and right rostromedial PMd.\(^10\) These findings lend support to the notion that a latent nigrostriatal dopaminergic dysfunction gives rise to variable patterns of activity changes in rostral premotor regions which critically depend on the specific motor functions probed by the experimental task. The observation that the compensatory recruitment of cortical motor areas is task-specific underscores the capacity of human sensorimotor networks to flexibly adapt to a regional dysfunction.\(^32\)

We also identified some differences between carriers of mutations in the Parkin or PINK1 gene. In PINK1 mutation carriers, we observed an additional mainly left-hemispheric recruitment of frontoparietal areas, including distinct areas in left caudal PMd and intraparietal sulcus. It is unclear whether this reflects a true genotype-specific pattern of functional adaptation in the frontoparietal cortex. One possibility is that nonmanifesting PINK1 mutation carriers have a stronger functional impairment of the motor system, requiring recruitment of additional frontoparietal loops. Future studies could resolve this issue by correlating adaptive redistribution of cortical activity to the depth of the nigrostriatal dopaminergic deficit, for example using nuclear imaging.

An intriguing question is whether these increases in task-related activity persist, increase, or attenuate in mutation carriers who ultimately develop PD. A recent H\(^2\)\(^15\)O PET study provided some evidence that the extra-recruitment of frontoparietal areas still represents an effective mechanism of compensation in the early stage of sporadic PD.\(^33\) In that study, patients with PD achieved equal performance with healthy controls when learning short motor sequences. In patients, equal performance was associated with the additional recruitment of cortical areas that are normally specialized for learning more difficult sequences. Additional fMRI studies on high-risk populations as well as cross-sectional studies on drug-naïve patients with newly diagnosed sporadic or monogenic PD are needed to further address this important question.

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REFERENCES
