resulted in no significant change. The patient was referred to our department for surgical treatment for choreiform movements. She was totally dependent for her daily-life activities. Dysarthria, dysphagia, ocular pursuit abnormality, and generalized tonic–clonic seizures (once every 3 months) were confirmed. The motor assessment of the Unified Huntington's Disease Rating Scale (UHDRS; range, 0–60, with higher scores indicating higher severity) was 45 (Video SS1). The patient rejected having implanted DBS systems. There was significant atrophy in the globus pallidus (Fig. 1A). Therefore, we performed a left pallidothalamic tractotomy (Fig. 1B–D). The detailed operative procedure has been described in a previous study.[2] Unexpectedly, significant improvements were observed in bilateral choreiform movements immediately after the surgery (Video SS2). The improvements in choreiform movements gradually increased with time. Reduced volume in voice and worsened dysarthria developed after the surgery, which resulted in full recovery at the 3-month postoperative evaluation. The patient could stand and walk independently at the 6-month evaluation (Video SS3). The motor assessment of UHDRS was 26 (42.2% improvement).

The limited single case report and small cohort studies have indicated that deep brain stimulation of the globus pallidus internus could achieve significant improvement in choreiform movements.[3-5] In ablative procedures for HD, as far as we know, 5 patients have been reported in a total of 2 studies.[6,7] Spiegel and Wycis reported the effects of dorsomedial thalamotomy and pallidotomy in 4 cases of HD.[6] They reported that pallidotomy was more effective in improving choreiform movements and long-lasting effects than dorsomedial thalamotomy. Cubo et al reported a single case of a Westphal variant of HD with generalized dystonia and parkinsonism without chorea, treated by bilateral pallidotomy.[7] Ablation of the pallidothalamic tract implies neuromodulation of the pallidal efferents to the thalamus, which is expected to have efficacy similar to that of pallidotomy. ●

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

LRRK2 Loss-of-Function Variants in Patients with Rare Diseases: No Evidence for a Phenotypic Impact

Certain missense variants in the *LRRK2* gene, which encodes leucine-rich repeat kinase 2 (LRRK2), are a common cause of Parkinson's disease (PD).[1] As these variants increase the protein's kinase activity, treatment strategies aim at reducing LRRK2 function and/or abundance. Safety concerns for this approach are based on considerable *LRRK2* expression in numerous tissues and organs.[2] Indeed, LRRK2 knockout/inhibition in mammalian model organisms has been reported to affect the lungs, the kidneys, and the liver.[3] Findings from two recent studies on *LRRK2* loss-of-function (LoF) variants in humans have been interpreted as partially alleviating pertinent concerns: Blauwendraat and colleagues showed absence of enrichment of these variants in PD patients,[4] while Whiffin and colleagues revealed that their presence is not associated with reduced life expectancy, abnormal standard laboratory parameters, or common adverse phenotypes in a large cohort of healthy individuals.[5] However, the probably most relevant human subjects, that is, those presenting with (rare) disease phenotypes, have not yet been systematically analyzed. The present study describes the spectrum and frequency of *LRRK2* LoF variants in CentoMD®, a data repository for patients with rare disorders,[6] and analyzes the dataset for potential disease associations.

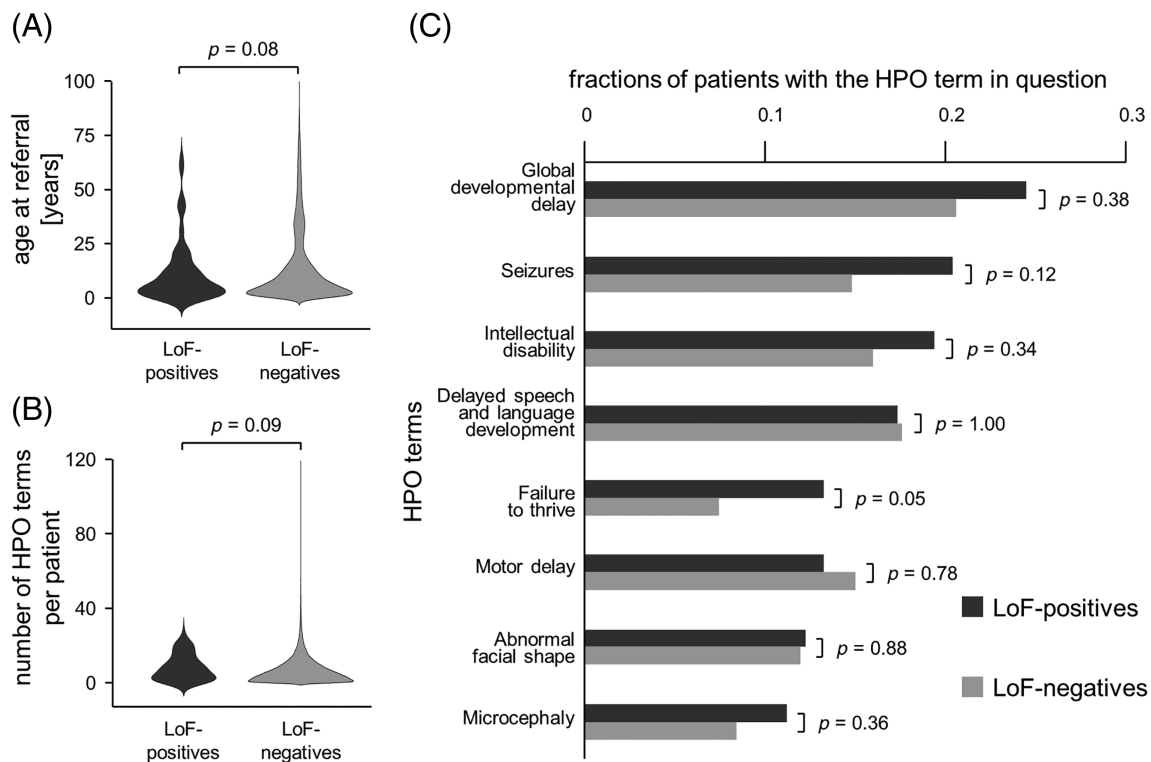--

FIG. 1. Comparison of *LRRK2* LoF-positive and *LRRK2* LoF-negative patients. (**A**) Age at referral. (**B**) Number of Human Phenotype Ontology (HPO) terms per patient. (**C**) Fractions of patients with HPO terms that are present in at least 10% of *LRRK2* LoF-positive patients. *P* values are two-tailed and are not corrected for multiple testing; they are based on Student's t-test in (**A**) and (**B**), and on Fisher's exact test in (**C**).

LRRK2 sequencing data from over 70,000 individuals (45,331 patients; 25,043 healthy family members) from diagnostic exome or genome sequencing were interrogated. In 154 cases, good quality[7] *LRRK2* LoF variants were present. There were a total of 52 distinct variants (22 nonsense, 20 frameshift, 10 splice-site) (Fig. S1). The fraction of patients was 0.636 among variant-positives and 0.644 among variant-negatives, demonstrating absence of an association of *LRRK2* LoF variants with disease. Focusing on only patients, there was no significant difference between variant-positives and variant-negatives with respect to age at referral and number of Human Phenotype Ontology (HPO) terms per patient (Fig. 1A,B). Furthermore, none of the HPO terms that were most frequently observed in *LRRK2* LoF-positives was significantly enriched in this cohort (Fig. 1C). Thus, rare disease patients with *LRRK2* LoF variants are not affected earlier or more severely than other patients, and do not have specific phenotypes.

Of note, four of the *LRKK2* LoF variant-positives were homozygous (Table S1; Fig. S2), of whom three were patients who did not show any obvious phenotypic overlap. The first patient had received a negative genetic report, while a homozygous variant of uncertain significance was reported in the second and a homozygous pathogenic variant in the third. Importantly, the fourth *LRRK2* LoF homozygous individual was a 32-year-old healthy father of a patient. Even complete absence of LRRK2 may thus have no overt early phenotypic impact in humans, but further studies are necessary to determine whether the above individuals are indeed "human *LRRK2* knockouts."

Our observations in a large and clinically diverse diagnostic cohort represent conceptually novel support for the safety of currently pursued therapeutic strategies for patients with kinase-activating *LRRK2* mutations. Considering the accumulating evidence for an implication of increased LRRK2 activity also in the pathogenesis of other monogenic and idiopathic forms of PD,[1] our findings are of potentially even broader relevance. ●

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Supporting Data

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Increase of Prokineticin-2 in Serum of Patients with Parkinson's Disease

Prokineticin-2 (PK2) is a chemokine-like peptide abundantly expressed in the central nervous system (CNS), involved in multiple physiological and pathological conditions, which acts as an insult-inducible endangering mediator contributing to cellular defence.[1] Of relevance, PK2 is highly induced during early phases of dopaminergic degeneration in Parkinson's disease (PD), activating a neuroprotective response by the PGC-1α-mediated restoration of mitochondrial function.[2]

PK2 thus emerges as a crucial player in PD pathogenesis that may have relevance either as a biomarker of neuronal injury or a candidate target for disease-modifying treatments.

Consequently, our aim was to assess, for the first time, PK2 blood levels in PD patients, looking for correlations with the main clinical parameters and other neurodegeneration biomarkers.

Thirty-one PD patients and 14 age/sex-matched healthy volunteers (controls, CTLs) were enrolled (excluding those with relevant comorbidities),[3,4] and subjected to serum sampling for PK2 assay, as previously described (ELISA kit; LifeSpanBioSciences, Inc., Seattle, WA, USA).[1]

Clinical parameters for PD patients were assessed in the on state. For five of them, contemporary cerebrospinal fluid (CSF) biomarkers were also available[3] (Table 1). Ethical standards were respected in accordance with the Declaration of Helsinki, and informed consent was acquired.

Serum PK2 was significantly increased in PD patients compared to CTLs (one-way ANOVA on log10-transformed values [$F_{(1, 42)} = 10.55$, $P = 0.002$]) (Table 1). Receiver operating characteristic (ROC) analysis provided an AUC (area under the curve) of 0.75 ($P = 0.009$, 95% CI = 0.6–0.9). The cut-off value of 2.79 ng/ml differentiated PD patients from CTLs with sensitivity = 71% and specificity = 64%.

Pearson's analysis excluded correlations between serum PK2 and clinical parameters in PD patients, but indicated significant associations with CSF amyloid-β-42 (direct, R = 0.96, $P = 0.008$) and lactate (inverse, R = −0.89, $P = 0.04$) (n = 5). A regression model adjusted for age and sex confirmed the association only for amyloid-β-42 ([$F_{(3, 1)} = 320.9$, $R2 = 0.99$, $P = 0.04$; T = 24, $P < 0.026$]).

Our results thus indicate that the PK2 pathway is activated even in vivo in PD patients, with increased serum levels, which is in agreement with previous experimental data demonstrating a neuroprotective upregulation of PK2 in brain tissues from both animal models and human patients.[2]

Indeed, also in our small population, serum PK2 inversely correlated with CSF lactate, an index of oxidative stress and mitochondrial dysfunction,[3] suggesting a possible response to redox injury. Moreover, PK2 was independently associated with CSF amyloid-β-42, again implicating an eventual reaction to synaptopathy and amyloidopathy,[3] mirroring what happens in Alzheimer's disease, in which amyloid plaques and amyloid-β-42 peptide upregulate PK2 expression early during the neuropathological process, leading to increased PK2 serum levels in patients.[1]

Despite several limitations, including the sample size, the cross-sectional design, and the exiguity of neurodegeneration biomarkers, this pilot study draws attention to PK2 in PD, either as a biomarker or, especially, a therapeutic target. In fact, the PK2 pathway, which promotes mitochondrial biogenesis and counteracts oxidative stress,[5] is activated by metformin, a common antidiabetic agent with a promising neuroprotective effect.[6] Conversely, the PK2 pathway may intervene in the vascular system and neuronal resistance to ischemia,[5] which in turn are involved in PD pathogenesis.[7] Further studies are now needed to confirm and extend our preliminary observations.

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