TWNK in Parkinson's Disease: A Movement Disorder and Mitochondrial Disease Center Perspective Study

Marco Percetti, MD,[1,2] Giulia Franco, MD,[1,3] Edoardo Monfrini, MD,[1,3] Leonardo Caporali, PhD,[4] Raffaella Minardi, PhD,[4] Chiara La Morgia, MD, PhD,[4] Maria Lucia Valentino, MD,[4,5] Rocco Liguori, MD,[4,5] Ilaria Palmieri, PhD,[6] Donatella Ottaviani, MD,[7] Maria Vizziello, MD,[1,3] Dario Ronchi, PhD,[1] Federica Di Berardino, MD,[8] Antoniangela Cocco, MD,[9,10] Bertil Macao, PhD,[11] Maria Falkenberg, PhD,[11] Giacomo Pietro Comi, MD,[1,3] Alberto Albanese, MD,[10] Bruno Giometto, MD,[7] Enza Maria Valente, MD, PhD,[6,12] Valerio Carelli, MD, PhD,[4,5] and Alessio Di Fonzo, MD, PhD[1,3*]

[1]*Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy* [2]*Neurology Unit, San Gerardo Hospital, ASST Monza, Monza, Italy* [3]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan, Italy* [4]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy* [5]*Unit of Neurology, Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, Bologna, Italy* [6]*Neurogenetics Research Center, IRCCS Mondino Foundation, Pavia, Italy* [7]*Neurology Unit, Rovereto Hospital, Azienda Provinciale per i Servizi Sanitari (APSS) di Trento, Trento, Italy* [8]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Audiology Unit, Milan, Italy* [9]*University of Milan, Milan, Italy* [10]*Department of Neurology, Istituto di Ricovero e Cura a Carattere Scientifico Humanitas, Research Hospital, Milan, Italy* [11]*Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden* [12]*Department of Molecular Medicine, University of Pavia, Pavia, Italy*

ABSTRACT: Background: Parkinsonian features have been described in patients harboring variants in nuclear genes encoding for proteins involved in mitochondrial DNA maintenance, such as *TWNK*.
Objectives: The aim was to screen for *TWNK* variants in an Italian cohort of Parkinson's disease (PD) patients and to assess the occurrence of parkinsonism in patients presenting with *TWNK*-related autosomal dominant progressive external ophthalmoplegia (*TWNK*-adPEO).
Methods: Genomic DNA of 263 consecutively collected PD patients who underwent diagnostic genetic testing was analyzed with a targeted custom gene panel including *TWNK*, as well as genes causative of monogenic PD. Genetic and clinical data of 18 *TWNK*-adPEO patients with parkinsonism were retrospectively analyzed.
Results: Six of 263 PD patients (2%), presenting either with isolated PD (n = 4) or in combination with bilateral ptosis (n = 2), carried *TWNK* likely pathogenic variants. Among 18 *TWNK*-adPEO patients, 5 (28%) had parkinsonism.
Conclusions: We show candidate *TWNK* variants occurring in PD without PEO. This finding will require further confirmatory studies. © 2022 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson Movement Disorder Society.

Key Words: *TWNK*; twinkle; Parkinson's disease; parkinsonism; mitochondrial DNA

*__Correspondence to:__ Dr. Alessio Di Fonzo, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit Via Francesco Sforza, 35, 20122, Milan (MI), Italy; E-mail: alessio.difonzo@policlinico.mi.it

Alessio Di Fonzo, Valerio Carelli, and Enza Maria Valente share senior authorship.

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Pathogenesis of Parkinson's disease (PD) has long been associated with mitochondrial dysfunction.[1] Dopaminergic neurons of the substantia nigra pars compacta seem to be particularly vulnerable to mitochondrial damage.[2] Although sequencing of mitochondrial DNA (mtDNA) failed to reveal pathogenic mutations associated with PD, population-specific common variants defining mtDNA haplogroups have been implicated as possible risk factors.[3] In addition, age-related accumulation of somatic mtDNA deletions in the substantia nigra has been reported to occur more significantly in PD patients than in age-matched controls.[4,5] Moreover, the regulation of mtDNA copy number seems to be affected in PD, leading to a relative mtDNA depletion.[6,7]

Pathogenic variants in nuclear genes encoding for proteins primarily involved in mtDNA maintenance, such as *POLG*, *TWNK*, *MPV17*, *OPA1*, *DGUOK*, or *SLC25A46*,[8-13] have been described in patients with mitochondrial syndromes featuring parkinsonian signs as part of their complex phenotypic manifestation.[14] Among these genes, *TWNK* encodes for the mitochondrial twinkle helicase, which is essential for mtDNA replication.[15] Pathogenic variants in *TWNK* have been associated with different phenotypes, ranging from autosomal dominant progressive external ophthalmoplegia[16] (adPEO) to rare autosomal recessive syndromes, such as mtDNA depletion syndrome, Perrault syndrome, infantile-onset spinocerebellar ataxia, mitochondrial recessive ataxia syndrome, and sensory ataxia neuropathy dysarthria and ophthalmoplegia.[17-21] Thus far, 10 *TWNK*-adPEO patients also presenting with parkinsonism have been reported in the literature.[9,22-27]

We here explore the frequency of *TWNK* variants in an Italian cohort of PD patients and describe the associated clinical and neuroradiological phenotypes (*TWNK*-PD). In addition, we reassess clinical and genetic data from a cohort of adPEO-carrying *TWNK* pathogenic variants and manifesting parkinsonism (*TWNK*-adPEO-P) and compare the clinical and genetic findings of *TWNK*-PD and *TWNK*-adPEO-P. Finally, we evaluate the defects of mtDNA maintenance in either blood or muscle biopsies (when available) associated with detected *TWNK* variants.

Patients and Methods

We included in the study 263 patients with a diagnosis of PD made by a movement disorder specialist and referred for genetic testing for diagnosis at the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) or at IRCCS Mondino Foundation (Pavia, Italy). Data were consecutively collected from 2017 to 2021. Patients with adPEO were evaluated at the Mitochondrial Diseases Center of the IRCCS Institute of Neurological Sciences (Bologna, Italy). Of 302 adPEO, data from 18 carrying *TWNK* variants were retrospectively analyzed. The Ethics Committees of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Mondino Foundation, and Institute of Neurological Sciences (Comitato Etico Interaziendale Bologna-Imola, CE-BI 13036) approved the study, and all patients provided written informed consent to study participation.

Genetic Analysis

PD patients underwent either next-generation sequencing of a targeted panel of 19 genes associated to PD and parkinsonism (Table S1), which included *POLG*, *TWNK*, *OPA1*, and *SLC25A46* (Haloplex Technology, Agilent, Santa Clara, CA, United States), or whole exome sequencing (WES) with subsequent analysis of the same virtual gene panel (Twist Core Exome Kit, Twist Biosciences, San Francisco, CA, United States). Exon rearrangements were assessed through Multiplex Ligation Probe Amplification (MS-MLPA) using Salsa MLPA Probemix P051-D2 or P052 Parkinson (MRC-Holland, Amsterdam, Netherlands) following manufacturer's instructions. All *TWNK*-adPEO-P patients had been diagnosed through WES and subsequent analysis of the same virtual gene panel used for the PD cohort. Further information is available in Appendix S1.

Results

Genetic Results

Monoallelic variants of *TWNK* were detected in 6 of 263 (2%) patients with PD (162 men, 62%; 85 with positive family history for PD, 32%; mean age at onset 51.35 ± 13.03 years) (Table 1; Fig. 1; Table S3 in Appendix S1). The *TWNK* variants identified in PD were searched in in-house exomes of Italian non-PD subjects (n = 2529) and in two databases, Network for Italian Genomes (n = 1492) and NIG—Exomes from Italy (n = 1686). None of the variants were present in any databases, except for the p.G540R that was found in the heterozygous state in a pediatric patient with neurodevelopmental syndrome (one of 5707, 0.0001752). None of the *TWNK*-PD and *TWNK*-adPEO-P patients carried additional pathogenic variants or genomic rearrangements in other autosomal dominant or autosomal recessive PD-related genes. The variant identified in patient 4 was detected also in the mother who had also developed PD. To obtain support for the functional relevance of the *TWNK* variants identified in PD patients, we performed molecular modeling. Our in silico analysis predicted that all the identified variants could have negative effects on twinkle activity (see Appendix S1, Fig. S3).

Clinical Features

Clinical features are summarized in Table S3, Appendix S1. Two *TWNK*-PD patients also manifested ptosis without clinical and video-oculographic limitations of gaze. Interestingly, the mother of patient 4 was affected by ptosis and developed PD at age 60 years (see Appendix S1). Of 18 *TWNK*-adPEO patients, 5 (28%) also presented with parkinsonism. A more exhaustive clinical description of all *TWNK*-PD and *TWNK*-adPEO-P cases reported here is available in Appendix S1. Mean age at onset of parkinsonism was

TABLE 1 *Genetic data of* TWNK *variants reported in this article*

Patient	Phenotype	Base change	AA change	Zygosity	dbSNP (rs number)	gnomAD* v2.1.1	Italian controls**	ACMG criteria***	Classification
***TWNK*-PD**									
Patient 1	EOPD	c.500 T > C	p.L167P	Het	–	–	–	PM1m, PM2s PP2su, PP3m	LP
Patient 2	EOPD	c.1112 G > A	p.R371Q	Het	rs 143309797	13/129′034 (0.0001007)	–	PM1s, PM2su, PP2su, PP3m	LP
Patient 3	EOPD	c.1381 G > A	p.E461K	Het	rs 776518524	4/113′770 (0.00003516)	–	PM1s, PM2su PP2su, PP3m	LP
Patient 4	PD with ptosis	c.1618 G > A	p.G540R	Het	rs 568256888	3/113′770 (0.00002637)	1/5707 (0.0001752)	PM1m, PM2s PP2su, PP3m PP1su	P
Patient 5	PD with ptosis	c.1966 A > C	p.K656Q	Het	–	–	–	PM2su, PP2su PP3su	VUS-LP
Patient 6	PD	c.2010 G > C	p.Q670H	Het	rs 778236767	–	–	PM2su, PP2su, PP3su	VUS-LP
***TWNK*-adPEO-P**									
Patient 7	adPEO-P	c.907 C > T	p.R303W	Het	rs 1159929268	1/113′466 (0.000008813)	–	PM1m, PM5m, PP5m, PM2su PP2su, PP3su	LP
Patient 8	adPEO-P								
Patient 9	adPEO-P	c.1001 G > A	p.R334Q	Het	rs 28937887	–	–	PM1m, PP5s PM5m, PM2su PP2su, PP3su, BP6su	P
Patient 10	adPEO-P								
Patient 11	adPEO-P	c.1609 T > C	p.Y537H	Het	rs 144001072	33/113′770 (0.0002941)	-	PM1m, PM2su PP2su, BP4su	VUS-LP

AA, amino acids; ACMG, American College of Medical Genetics; EOPD early-onset Parkinson's disease; Het, heterozygous (monoallelic); LP, likely pathogenic; P, pathogenic; VUS, variant of unknown significance; VUS-LP, VUS with likely pathogenic effect; adPEO, autosomal dominant progressive external ophthalmoplegia.

** In-house exomes of patients with neurological diseases (non-PD, n = 2529), Network for Italian Genomes (n = 1492), and NIG—Exomes from Italy (n = 1686).

*** ACMG criteria: m, moderate; s, strong; su, supporting.

*Minor allele frequency in European (non-Finnish) population. All variants were absent in European (Finnish) population.

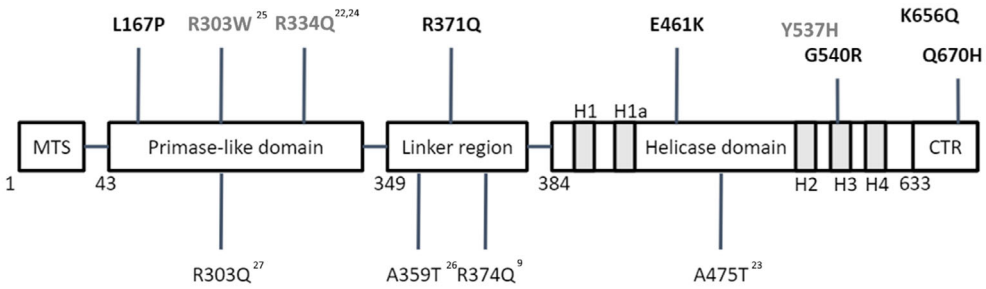

FIG. 1. Distribution of *TWNK* variants in patients with parkinsonism. *TWNK* variants here reported (bold) are in the upper part of the figure (black = *TWNK*-PD patients; gray = *TWNK*-adPEO-P). Variants related to previously reported *TWNK*-adPEO-P patients are in the lower part (see references 9 and 22–27). MTS, mitochondrial targeting sequence (1–42); primase-like domain (43–348); linker region (349–383); helicase domain (384–632), H1 (409–422), H1a (439–446), H2 (512–517), H3 (535–558), and H4 (569–587); CTR, C-terminal region (633–684). adPEO, autosomal dominant progressive external ophthalmoplegia. [Color figure can be viewed at wileyonlinelibrary.com]

significantly younger in *TWNK*-PD than in *TWNK*-adPEO-P patients (52.7 vs. 73.6 years, $P = 0.0018$). Two *TWNK*-adPEO-P patients were treated with levodopa (L-dopa) without a clear benefit. Other neurological features like postural tremor, head tremor, and apraxia appeared to be more frequent in *TWNK*-adPEO-P patients but without reaching statistical significance.

mtDNA Deletions and Copy Number

Quantitative analysis of mtDNA copy number between *TWNK*-PD patients and age- and sex-matched controls showed no differences (Appendix S1 in Fig. S1). In skeletal muscle biopsies, mtDNA amount and 7sDNA were comparable among groups, whereas quantifiable mtDNA deletions were detected only in a subgroup of *TWNK*-adPEO patients (Appendix S1, Fig. S2). COX negative fibers in muscle biopsies of patients 7 and 10 were 2.3% and 0.9%, respectively.

Discussion

Our study focused on the role of *TWNK* in patients from two cohorts: (1) a PD cohort of patients who consecutively underwent genetic testing, including *TWNK*; and (2) a retrospective adPEO cohort known to carry a heterozygous *TWNK* pathogenic variant and presenting parkinsonism. Interestingly, carriers of a *TWNK* variant were not rare among our cohort of PD patients (6 of 263, 2%). The screening in non-PD subjects indicates that these variants are not common in the Italian population. Most relevantly, *TWNK* variants were also found in PD patients lacking ptosis, a hallmark of twinkle -related myopathy, suggesting that the diagnostic screening of this gene should be considered also in PD patients without other signs suggestive of a mitochondrial disease.

Before this study, parkinsonism cases associated with *TWNK* variants without ptosis, PEO, or any other sign of myopathy have not been reported[9,22-27] (Appendix S1, Table S1). Our findings suggest that *TWNK* variants could be related to a clinical picture indistinguishable from idiopathic PD with good response to L-dopa and development of L-dopa complications (motor fluctuations and dyskinesias), only occasionally featuring ptosis.

Parkinsonism was relatively frequent in the *TWNK*-adPEO group (5 of 18, 28%). In line with previous reports, the phenotype of *TWNK*-adPEO-P was characterized by a complex association of neurological and nonneurological signs suggestive of an underlying mitochondrial syndrome (Appendix S1, Tables S3 and S4). Compared to *TWNK*-PD subjects, *TWNK*-adPEO-P patients showed a later onset of parkinsonian features (on average in the seventh decade of life) and, when assessed, a poorer response to L-dopa due to the development of adverse effects. In these patients, the cause of parkinsonian features was accompanied by the frequent co-occurrence of cardiovascular risk factors or magnetic resonance imaging evidence of vascular encephalopathy. In fact, I^{123} ioflupane scintigraphy (DaTSCAN) was repeatedly negative in a *TWNK*-adPEO-P patient, challenging the hypothesis of nigrostriatal degeneration as causative of parkinsonian features. These observations suggest that the atypical parkinsonian syndrome of *TWNK*-adPEO seems a distinct phenotype compared to that of *TWNK*-PD. A possible confounding factor is the different age of the two cohorts, having the PD group an earlier age of onset. *TWNK*-PD patients did not show any muscular involvement or complained of muscular symptoms; thus, electromyography (EMG) and muscle biopsy were not performed. Of note, mtDNA copy number abnormalities were not observed in this group (Appendix S1, Fig. S1). On the contrary, among *TWNK*-adPEO-P patients, 3 of 5 patients showed

clinical and/or EMG signs of myopathic involvement (Appendix S1, Table S3), and 2 patients had a muscle biopsy typical of mitochondrial myopathy (Appendix S1, Table S3). However, quantification of mtDNA deletions, as well as of mtDNA copy number or DNA 7S performed on muscle biopsy, failed to reveal differences with controls (Appendix S1, Fig. S2), probably due to the rare COX negative fibers in their muscle biopsy, confirming that myopathy in these patients is frequently very mild or even subclinical. Among *TWNK*-PD patients, 4 carried a pathogenic or likely pathogenic variant, whereas the remaining 2 carried variants formally classified as variants of unknown significance with likely pathogenic effect according to the American College of Medical Genetics guidelines (see Supplementary Methods in Appendix S1; Table 1). The co-segregation of the p.G540R variant with PD in the family of patient 4 further supports its likely pathogenic role. However, more evidence is needed to prove the role of these variants in the pathogenesis of PD, especially those identified in "pure" PD. Indeed, the lack of family history and functional demonstration of the impact on mtDNA raises caution in assigning a definitive association with PD. The distribution of the variants across the twinkle protein showed the involvement of all functional domains, without any clustering (Fig. 1). Although *TWNK* variants are more frequent in the N-terminal domain and linker region of the protein, we failed to demonstrate any evidence of clear genotype–phenotype correlations.

PD is a complex multifactorial disease, in which several predisposing genetic factors could interplay to promote the development of the disease. To our knowledge, the presence of other genetic contributors to the development of parkinsonian features in patients with mitochondrial syndromes, such as variants in PD-related genes, has not been ruled out to date. In our series of *TWNK*-PD and *TWNK*-adPEO-P patients, the co-occurrence of possibly causative variants in classical PD genes has been excluded, strengthening the likely pathogenic role of *TWNK* in contributing to PD. However, the development of parkinsonism in patients carrying variants in *TWNK*, as well as in other mtDNA maintenance genes, remains somehow enigmatic. Not all carriers eventually develop parkinsonism, denoting an incomplete penetrance of this trait. This incomplete knowledge has relevant implications on genetic counseling related to PD risk in families carrying *TWNK* variants.

In summary, we described the presence of *TWNK* variants in patients with PD or parkinsonism, with or without signs of myopathy. Our findings strengthen the relation between the *TWNK* gene and PD. Screening in larger PD populations, segregation analysis in additional familial cases, and functional studies are needed to confirm these interesting new observations. ■

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Data Availability Statement

Raw data were generated at IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy), IRCCS Mondino Foundation (Pavia, Italy), and IRCCS Institute of Neurological Sciences (Bologna, Italy). Derived data supporting the findings of this study are available from the corresponding author on request.

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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.

GRN Mutations Are Associated with Lewy Body Dementia

Paolo Reho, PhD,[1] Shunsuke Koga, MD, PhD,[2] Zalak Shah, PhD,[1] Ruth Chia, PhD,[3] the International LBD Genomics Consortium, The American Genome Center, Rosa Rademakers, PhD,[2,4] Clifton L. Dalgard, PhD,[5,6] Bradley F. Boeve, MD,[7] Thomas G. Beach, MD, PhD,[8] Dennis W. Dickson, MD,[2] Owen A. Ross, PhD,[2,9] and Sonja W. Scholz, MD, PhD[1,10*]

[1]*Neurodegenerative Diseases Research Unit, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, USA* [2]*Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA* [3]*Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, Maryland, USA* [4]*VIB Center for Molecular Neurology, Antwerp, Belgium* [5]*Department of Anatomy, Physiology & Genetics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA* [6]*The American Genome Center, Collaborative Health Initiative Research Program, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA* [7]*Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA* [8]*Banner Sun Health Research Institute, Sun City, Arizona, USA* [9]*Department of Clinical Genomics, Mayo Clinic, Jacksonville, Florida, USA* [10]*Department of Neurology, Johns Hopkins University Medical Center, Baltimore, Maryland, USA*

ABSTRACT: Background: Loss-of-function mutations in *GRN* are a cause of familial frontotemporal dementia, and common variants within the gene have been associated with an increased risk of developing Alzheimer's disease and Parkinson's disease. Although TDP-43-positive inclusions are characteristic of *GRN*-related neurodegeneration, Lewy body copathology has also been observed in many *GRN* mutation carriers.

Objective: The objective of this study was to assess a Lewy body dementia (LBD) case–control cohort for pathogenic variants in *GRN* and to test whether there is an enrichment of damaging mutations among patients with LBD.

***Correspondence to:** Dr. Sonja W. Scholz, Neurodegenerative Diseases Research Unit, National Institutes of Health, NINDS, Bethesda, MD 20892-3707, USA; E-mail: sonja.scholz@nih.gov

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