**SLC25A46** mutations in patients with Parkinson's Disease and optic atrophy

Giacomo Bitetto, Maria Chiara Malaguti, Roberto Ceravolo, Edoardo Monfrini, Letizia Straniero, Alberto Morini, Raffaella Di Giacopo, Daniela Frosini, Giovanni Palermo, Fabio Biella, Dario Ronchi, Stefano Duga, Franco Taroni, Stefania Corti, Giacomo P. Comi, Nereo Bresolin, Bruno Giometto, Alessio Di Fonzo.

*IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy*

*Department of Neurology, Ospedale Santa Chiara, Trento, Italy*

*Azienda Provinciale per i Servizi Sanitari, Trento, Italy*

*Unit of Neurology, Department of Clinical and Experimental Medicine, University of Pisa, 56126, Pisa, Italy*

*Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy*

*Humanitas Clinical and Research Center, Rozzano, Milan, Italy*

*Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy*

**A B S T R A C T**

Mutations in the gene encoding the mitochondrial carrier protein **SLC25A46** are known to cause optic atrophy associated with peripheral neuropathy and congenital pontocerebellar hypoplasia.

We found novel biallelic **SLC25A46** mutations (p.H137R, p.A401Sfs*17) in a patient with Parkinson's disease and optic atrophy. Screening of six unrelated patients with parkinsonism and optic atrophy allowed us to identify two additional mutations (p.A176V, p.K256R) in a second patient. All identified variants are predicted likely pathogenic and affect very conserved protein residues.

These findings suggest for the first time a possible link between Parkinson's Disease and **SLC25A46** mutations. Replication in additional studies is needed to conclusively prove this link.

1. **Introduction**

Several monogenic forms of Parkinson's Disease (PD) are linked to genes encoding for mitochondrial proteins [1]. Mutations in **PINK1** and **Parkin**, two crucial players of the mitophagic machinery, cause recessive forms of PD, mainly characterized by early-onset PD and dystonia [2]. More complex phenotypes are linked to mutations in **OPA1**, **POLG** and **Twinkle** including: optic atrophy, ophthalmoplegia, sensorial hearing loss, neuropathy, cerebellar ataxia, and myopathy [3–5].

**SLC25A46** encodes for a mitochondrial carrier protein located on the outer membrane that interacts with MFN2, OPA1, the mitochondrial contact site and cristae organizing system complex [6–8]. Mutations in this gene lead to a broad spectrum of neurodegenerative disorders. Congenital lethal pontocerebellar hypoplasia, optic atrophy, peripheral neuropathy, cerebellar ataxia, and spasticity are the clinical features described so far as consequences of loss of **SLC25A46** function [9–11]. Nevertheless, parkinsonism has been reported neither as predominant feature, nor in association with a more complex phenotype linked to **SLC25A46** gene mutations.

Here we report, for the first time, the finding of biallelic likely pathogenic **SLC25A46** mutations in patients affected by PD and optic atrophy.

2. **Methods**

A Caucasian family of Italian origin, including a single affected patient (Tn-1), was studied. There was no history of parkinsonism or other neurological disorders in previous generations, and there was no evidence of parental consanguinity. A screening of six unrelated patients displaying parkinsonism plus optic atrophy was performed detecting a second subject (Ps-1) with **SLC25A46** mutations.

The relevant ethical authorities approved the study and written informed consent was obtained from all participants to the publication of their images and videotapes, in both the print and online modalities. Neurological examination was performed by movement disorders specialists and included the Hoehn-Yahr scale, and Mini-Mental State Examination. Clinical details of the mutated subjects are reported in...
Examination.

Polyle2, CADD, M-CAP. Sanger sequencing was used to confirm the were annotated with Annovar and then filtered using the following all subjects available. Details on primers and PCR conditions are walking difficulties with short steps (Video). No other neurological production of cortical evoked response amplitude bilaterally compatible when the fundus examination revealed optic atrophy. VEP showed re-

Table 1.

Clinical details of patients carrying SLC24A46 mutations. y, years; MRI, Magnetic Resonance Imaging; RBD, REM behaviour disorder; MMSE, Mini-Mental-State-Examination.

<table>
<thead>
<tr>
<th>Subject, gender</th>
<th>Tn-1, F</th>
<th>Ps-1, M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Familiarity for neurological diseases</strong></td>
<td>–</td>
<td>+ (mother: progressive polynepathy)</td>
</tr>
<tr>
<td><strong>Optic atrophy (age at onset)</strong></td>
<td>+ (16y)</td>
<td>+ (52y)</td>
</tr>
<tr>
<td><strong>Neurosensorial hearing loss (age at onset)</strong></td>
<td>–</td>
<td>+ (37y)</td>
</tr>
<tr>
<td><strong>Peripheral neuropathy</strong></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Pees cavus</strong></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Age at PD diagnosis</strong></td>
<td>4ly</td>
<td>6ly</td>
</tr>
<tr>
<td><strong>Postural tremor (age at onset)</strong></td>
<td>+ (43y)</td>
<td>+ (63y)</td>
</tr>
<tr>
<td><strong>Tremor at rest (age at onset)</strong></td>
<td>+ (46y)</td>
<td>+ (63y)</td>
</tr>
<tr>
<td><strong>Bradykinesia (age at onset)</strong></td>
<td>+ (43y)</td>
<td>++ (63y)</td>
</tr>
<tr>
<td><strong>Dystonia (age at onset)</strong></td>
<td>+ (foot) (46y)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Hoehn-Yahr Scale (age at evaluation)</strong></td>
<td>I (43y)</td>
<td>III (63y)</td>
</tr>
<tr>
<td><strong>Response to Levodopa</strong></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Levodopa induced dyskineasies</strong></td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Cognitive impairment (age at evaluation)</strong></td>
<td>- (MMSE 30/30) (43y)</td>
<td>++, (MMSE 18/30) (72y)</td>
</tr>
<tr>
<td><strong>Non motor PD symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoasias</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Drooling</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Constipation</td>
<td>+++++</td>
<td>+</td>
</tr>
<tr>
<td>Dysuria/Nicturia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anxiety</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>RBD</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>SPECT DatSCAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>T2-weighted MRI hyperintense spot at the external capsule bilaterally</td>
<td>T2-weighted multifocal bilateral white matter and lacunar hyperintensities compatible with mild chronic ischemic cerebral disease</td>
</tr>
<tr>
<td>SPECT DatSCAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>T2-weighted MRI hyperintense spot at the external capsule bilaterally</td>
<td>T2-weighted multifocal bilateral white matter and lacunar hyperintensities compatible with mild chronic ischemic cerebral disease</td>
</tr>
</tbody>
</table>

Genomic DNA was isolated from peripheral blood using standard protocols. After ruling out pathogenic mutations in known PD-related genes by a targeted Next Generation Sequence (NGS) panel (SNCA, LRRK2, GBA, PARKIN, PINK1, DJ-1, ATP13A2, PLA2G6, FBX07, SYNJ1, DNAJC6, VPS13C, RAB39B, PTRHD1, POLG, OPA1, C10ORF12, LRRK2, GBA, PARKIN, PINK1, DJ-1, ATP13A2, PLA2G6, FBX07, SYNJ1, DNAJC6, VPS13C, RAB39B, PTRHD1, POLG, OPA1, C10ORF12) and multiplex ligation-dependent probe amplification (MLPA) on both pa-

3. Results

3.1. Clinical findings in the Patient Tn-1

Patient Tn-1 was diagnosed with PD at the age of 43, with a 3-year history of left-hand rest tremor. The family history was unremarkable, with no consanguinity. Of note, visual problems appeared at age of 16, when the fundus examination revealed optic atrophy. VEP showed re-

The neurologic examination at the time of diagnosis detected a mild parkinsonism with left-sided resting tremor and bradykinesia and walking difficulties with short steps (Video). No other neurological signs were detected except for a lower limb hyperreflexia and visual impairment. The response to L-Dopa and Dopamine-agonist was excellent. Neuropsychological assessment detected normal cognition.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2020.03.018

Brain MRI revealed T2-weighted hyperintensities in the external capsule bilaterally (Fig. 1a).

Both FDopa-PET and SPECT-with H23FP-CIT indicated a significant presynaptic defect of the dopaminergic nigrostriatal system, more evident on the left side (Fig. 1b).

EMG/ENG was negative for sensitive or motor neuropathy.

3.2. Genetic results in the Patient Tn-1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tn-1, F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SLC24A46</strong></td>
<td></td>
</tr>
<tr>
<td>p.H137R</td>
<td></td>
</tr>
<tr>
<td>p.A401Sfs*17</td>
<td></td>
</tr>
</tbody>
</table>

Genetic analysis in the patient Tn-1 excluded mutations in mtDNA, OPA1, POLG1, PEO1, and mutations in genes related to autosomal dominant and recessive PD. After filtering variants (see Methods and Supplementary Materials) from the Exome sequencing analysis, two heterozygous variants were detected in the SLC24A46 gene. The c.1198_1199insC, leading to a frameshift p.A401Sfs*17, is absent by all

...in silico; 5) predicted as likely pathogenetic by Mutation Taster, 

...predicted as deleterious by all in silico... 

...databases. The mutation would lead either to produce an aberrant peptide followed by a premature stop codon in the functional mitochondrial carrier domain, or to affect the transcript stability through 

...to affect splicing in silico; 5) predicted as likely pathogenetic by Mutation Taster,
3.3. Genetic screening

After a SLC25A46 gene screening of six patients displaying parkinsonism plus optic atrophy, one additional patient was detected presenting two SLC25A46 variants.

Patient Ps-1 was diagnosed with PD at the age of 63, preceded by a 5-year history of REM behavior disorder, hyposmia, and depression. He had a history of gait difficulties, lower limb muscle hypotrophy and pes cavus from the childhood with a recent genetic diagnosis of CMT1A (17p11.2 duplication). Visual problems appeared in the adulthood and a diagnosis of optic atrophy was established at the age of 52. In addition, he was affected by progressive sensorineural hearing loss starting from the age of 57.

No other family members were affected by parkinsonism and optic atrophy. His mother presented a progressive polyneuropathy from the early adulthood. The course of the disease was characterized by a good response to L-Dopa and IMAO-B, peak-dose dyskinesias, postural instability, cognitive decline and visual hallucinations.

Brain MRI displayed T2-weighted multifocal bilateral white-matter and lacunar hyperintensities compatible with a mild chronic ischemic cerebral disease (Fig. 1c) and mild diffuse cerebral atrophy. 18F-FDG PET showed a bilateral temporoparietal metabolism reduction, and SPECT with 123I-FP-CIT revealed reduced radioligand uptake in the putamen bilaterally and in the left caudate (Fig. 1d). Florbetapir PET was negative.

Mutations in mtDNA and genes related to autosomal dominant and recessive PD were excluded in subject Ps-1. Sanger sequencing of all SLC25A46 coding sequence and intron-exon boundaries detected two heterozygous variants. The c.527C>T causes the missense substitution p.A176V, which is absent in all databases and predicted as deleterious by all in silico tools (Mutation Taster: disease causing, 0.999; Polyphen2: probably damaging 0.972; CADD 22.3; M-CAP possibly pathogenic 0.027). The c.767A>G, leading to the missense mutation p.K256R is reported as rare (0.21% in gnomAD) and predicted to be deleterious by in silico tools (Mutation Taster: disease causing, 0.999; Polyphen2: probably damaging 0.972; CADD 24.5; M-CAP possibly pathogenic 0.225). No family members were available for segregation testing of the variants nor was possible to isolate RNA.

4. Discussion

Mutations in SLC25A46 have been associated with a very broad spectrum of neurological disorders. Initially, SLC25A46 mutations were found in patients with optic atrophy and peripheral neuropathy, and later also in subjects with pontocerebellar hypoplasia, cerebellar and myoclonic ataxia, and limb spasticity [9–11].

The SLC25A46 protein was identified for the first time as a mitochondrial solute carrier widely expressed in the central nervous system [12]. Recently, SLC25A46 possible involvement in mitochondrial dynamics is suggested through the direct interaction with the components of the mitochondrial fusion machinery OPA1 and MFN1/2 [7]. Interestingly, the phenotype linked to OPA1 mutations, which is mainly characterized by optic atrophy, may lead in some cases to a complex neurodegenerative syndrome including parkinsonism, ataxia, neuropathy and dementia [13].

Similarly, other mitochondrial gene mutations may lead to syndromes with complex neurological manifestation, including ataxia, ophthalmoplegia, optic atrophy, parkinsonism and myopathy (e.g. POLG, Twinkle) [14,15].

The reason of such significant variability in SLC25A46 mutations phenotypic expression is still unclear. No defined genotype-phenotype correlation has been determined yet, except for a recent report suggesting a negative correlation between SLC25A46 protein stability and phenotype severity [16].

The finding that in neuron-specific knockdown Drosophila at synapse level an accumulation of reactive oxygen species and reduction of
ATP have been observed may suggest a pathogenetic mechanism related to oxidative stress, which is largely associated with the specific loss of dopaminergic neurons in PD [17]. Among the cases with SLC25A46 mutations reported so far, the parkinsonism has never been observed. Of note, parkinsonism appeared in both patients only in adulthood comparing with the patients described with SLC25A46-related optic atrophy or pontocerebellar hypoplasia, that appeared early in life. This could explain why parkinsonism has not been observed in the cases described so far.

Both patients described here present a diagnosis of PD, with a broad age of onset and course of the disease. Indeed, patient Tn-1 shows an early-onset PD with slow progression, foot dystonia and anxiety, which resemble the phenotype of Parkin/PINK1-mutated PD patients, whereas patient Ps-1 presents a PD with a classical age of onset, complicated then by motor fluctuations and cognitive impairment.

A possible hypothesis explaining such a difference between the two subjects could be related to the different severity of the SLC25A46 mutations identified. The c.1198_1199insC in patient Tn-1 may lead to a juvenile onset parkinsonism due to the expected deleterious consequence on SLC25A46 protein, by replacing the mitochondrial carrier domain sequence with an aberrant peptide or affecting the transcript through NMD.

Both subjects manifested optic atrophy, in line with most of the SLC25A46 cases described so far.

Genotype-phenotype correlation in Ps-1 is made more difficult by the presence of the CMT1A related duplication which can account for sequence on SLC25A46 protein, by replacing the mitochondrial carrier protein with recessive hyperintensity in the external capsule and subject Ps-1 displayed white cerebellar atrophy/hypoplasia. Indeed, patient Tn-1 showed a slight hearing loss detected in Ps-1 appeared at later stage of the disease, suggesting a different etiology that observed in CMT patients [18]. The combination of mutations in two different genes in patient Ps-1 configures a double-trouble condition and may explain the complexity of his phenotype.

Neuroimaging features of these patients do not resemble those of previously reported SLC25A46-mutated cases, mainly characterized by cerebellar atrophy/hypoplasia. Indeed, patient Tn-1 showed a slight hyperintensity in the external capsule and subject Ps-1 displayed white matter and lacunar hyperintensities.

Two out of the four mutations detected lie on transmembrane protein domains, possibly affecting SLC25A46 membrane localization. The frameshift mutation lies on the mitochondrial carrier domain likely determining an impairment in protein carrier function.

In conclusion, these results likely expand the neurodegenerative presentations linked with recessive SLC25A46 mutations now featuring parkinsonism in addition to optic atrophy and hearing loss. Despite the promising results linking the new identified SLC25A46 variants and parkinsonism, the exome sequencing approach on a single subject and SLC25A46 screening in a small sample set of patients with optic atrophy and late-onset PD is not conclusive and need to be replicated in a larger sample set. After independent confirmations, SLC25A46 gene should be definitely considered in the screening of genetic forms of PD. Understanding how mutations in SLC25A46 gene result in parkinsonism will shed further light on the molecular mechanisms of PD.

Funding agencies

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors report no conflict of interest related to this work.

Acknowledgments

A.D.F. is supported by Fondazione Fresco Parkinson Institute.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2020.03.018.

References


