

Research Article

PINK1-Interacting Proteins: Proteomic Analysis of Overexpressed PINK1

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Recent publications suggest that the Parkinson's disease- (PD-) related PINK1/Parkin pathway promotes elimination of dysfunctional mitochondria by autophagy. We used tandem affinity purification (TAP), SDS-PAGE, and mass spectrometry as a first step towards identification of possible substrates for PINK1. The cellular abundance of selected identified interactors was investigated by Western blotting. Furthermore, one candidate gene was sequenced in 46 patients with atypical PD. In addition to two known binding partners (HSP90, CDC37), 12 proteins were identified using the TAP assay; four of which are mitochondrially localized (GRP75, HSP60, LRPPRC, and TUFM). Western blot analysis showed no differences in cellular abundance of these proteins comparing *PINK1* mutant and control fibroblasts. When sequencing *LRPPRC*, four exonic synonymous changes and 20 polymorphisms in noncoding regions were detected. Our study provides a list of putative PINK1 binding partners, confirming previously described interactions, but also introducing novel mitochondrial proteins as potential components of the PINK1/Parkin mitophagy pathway.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder with three cardinal manifestations: tremor, rigidity, and bradykinesia. In about 25% of all PD patients, at least one additional affected family member can be found, likely pointing to a direct, genetic cause of the disease. To date, eight genes have been confirmed to be associated with PD [1].

Besides the mutational analysis of PD-linked genes, the search for potential interactions between the protein products of these genes has recently gained increasing importance. Since the identification of the second monogenetic PD gene product Parkin, a common pathway leading to dopaminergic neurodegeneration has been proposed. To date, several studies reported such connections.

First, Shimura et al. hypothesized that Parkin plays a role in the coregulation of Alpha-synuclein (SNCA). The group identified a protein complex in normal human brain that

included the E3 ubiquitin ligase Parkin, UBC7 as its associated E2 ubiquitin-conjugating enzyme, and a novel form of SNCA as its substrate [2]. Later, an early-onset PD patient with a heterozygous missense mutation in both the *DJ-1* and the *PTEN-induced putative kinase 1* (*PINK1*) gene has been described. Additionally, overexpression of *DJ-1* and *PINK1* in SHSY-5Y cells revealed that the wildtype as well as the mutant forms of both proteins interact, and DJ-1 stabilized PINK1 [3]. Furthermore, *Drosophila pink1* and *parkin* loss-of-function mutants showed a similar mitochondrial phenotype. Since the *pink1*-related abnormalities could be rescued by *parkin* overexpression but not vice versa, it was suggested that pink1 acts upstream of parkin in a common pathway [4–6]. Recent studies provided evidence that the PINK1/Parkin pathway promotes mitochondrial fission as an initial step of mitophagy by ubiquitination of Mitofusins [7–9].

Still, only the minority of hereditary forms of PD can be explained by a mutation in one of the nine PD-associated

genes [10]. Therefore, this research focuses not only on proteins known to be involved in PD but also on novel interactors.

In the present study, we employed tandem affinity purification (TAP) to isolate proteins that are directly associated with PINK1. Using this approach, we aimed at a better characterization of the PINK1/Parkin mitophagy pathway.

2. Material and Methods

2.1. Patients. All patients underwent a standardized neurological examination performed by a movement disorders specialist. For sequencing of one of the candidate genes, *Leucine-rich PPR motif-containing (LRPPRC)*, 46 patients with atypical Parkinsonism including features of dementia, depression, or rapid disease progression were included in the present study. After obtaining informed consent, we collected blood from all patients for DNA extraction according to a published protocol [11].

Furthermore, fibroblasts from three PD patients with a homozygous p.Q456X PINK1 mutation and from two age-matched mutation-negative healthy controls were included in the study. Clinical features of these mutation carriers are described elsewhere [12, 13].

2.2. Tissue Culture. HEK cells and fibroblasts were cultured in Dulbecco's modified Eagle's medium (PAA Laboratories) and supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. All cells were maintained at 37°C in a saturated humidity atmosphere containing 5% CO₂. Passage numbers <10 were used for all experiments. To inhibit the mitochondrial membrane potential, fibroblasts were treated with the potassium ionophore valinomycin (1 μM, Sigma).

2.3. Protein Isolation by TAP and Mass Spectrometry (MS). The InterPlay Mammalian TAP System was employed according to the manufacturer's (Stratagene) protocol. In brief, human control RNA was extracted using the RNeasy Mini Kit (Qiagen) and *PINK1* cDNA was synthesized using Superscript II Reverse Transcriptase in combination with oligo dT primers (Invitrogen). Full-length *PINK1* cDNA was cloned into the pCTAP expression vector, which encodes two tandem affinity tags (a streptavidin binding peptide and a calmodulin binding peptide) after its multiple cloning site. Next, 10⁸ HEK cells were transiently transfected with the pCTAP-*PINK1* vector by means of the Ca²⁺PO₄ method [14]. After 24 h, cells were harvested and resuspended in lysis buffer supplemented with protease inhibitors (Sigma). Cells underwent three rounds of freeze-thawing, cell debris was pelleted by centrifugation at 16,000×g for 10 min, and the supernatant collected. Next, 2 mM EDTA, 10 mM β-mercaptoethanol, and the InterPlay streptavidin resin were added to the cell lysate and incubated at 4°C while rotating for 2 h. The resin was collected by centrifugation at 1,500×g for 5 min, washed twice, and incubated in biotin-containing buffer for 30 min at 4°C to elute the bound protein complexes. To further purify the protein complexes, calmodulin

resin and a calcium-containing buffer were added to the supernatant. After 2 h of incubation at 4°C, the resin was collected (centrifugation at 1,500×g for 5 min) and washed twice. From the calmodulin resin, protein complexes were eluted by adding EDTA-containing buffer and incubation for 30 min at 4°C on a rotator. The resulting eluate was concentrated by means of trichloroacetic acid precipitation and subsequently resuspended in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl pH7.6, 150 mM NaCl, 1% DOC, and 1% NP-40) with protease and phosphatase inhibitors (Roche Diagnostics). Next, purified proteins were resolved by one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized on the gel by silver staining as published [15]. In a control experiment, the pull-down approach was performed with beads only. Bands which appeared on the gel from the PINK1 overexpression experiment but not the control experiment were excised and sent for MS analysis to the Taplin Biological Mass Spectrometry Facility, Harvard Medical School, Boston, USA. According to the quality requirements of the facility, a protein can only be considered as interactor if two or more peptides match the respective protein in the Swiss-Prot database.

2.4. Knock-down Approach. For *PINK1* and *LRPPRC* knock-down, Hs_PINK1_4_HP (Qiagen) and *LRPPRC* UTR (complementary to the segment of the 3'UTR region of *LRPPRC* [16]) siRNAs were used with a final concentration of 50 nM. Scrambled siRNA (Silencer negative control 1 siRNA [Ambion]) with no known mammalian homology served as negative control (final concentration 50 nM). For transfection, the Nucleofector Device (Lonza) was used.

2.5. Mitochondrial Preparation. Mitochondria were isolated from fibroblasts as previously described [17]. In brief, cells were harvested and homogenized in buffer containing 250 mM sucrose, 10 mM Tris, and 1 mM EDTA, pH7.4. After that, nuclei and unbroken cells were removed by centrifugation at 1,500×g for 20 min. The supernatant containing intact mitochondria was transferred into a new tube and centrifuged at 12,000×g for 10 min. The resulting supernatant (cytosolic fraction) was transferred into another new tube and the mitochondria-enriched pellet (mitochondrial fraction) was dissolved in RIPA buffer containing protease and phosphatase inhibitors (Roche Diagnostics). Cytoplasmic fractions were concentrated by using Centricon YM-10 devices (Millipore) according to the manufacturer's instructions.

2.6. Protein Extraction. Proteins were extracted using RIPA buffer containing 0.1% SDS. Cells or mitochondria-enriched pellets were dissolved in the appropriate amount of buffer and incubated on ice for 30 min. After that, the lysates were centrifuged at 16,000×g for 20 min at 4°C. The supernatant was transferred into a new tube for further processing.

2.7. Western Blot Analysis. SDS-PAGE was performed using NuPAGE 4%–12% Bis-Tris gels (Invitrogen). After electrophoresis, proteins were transferred to the nitrocellulose

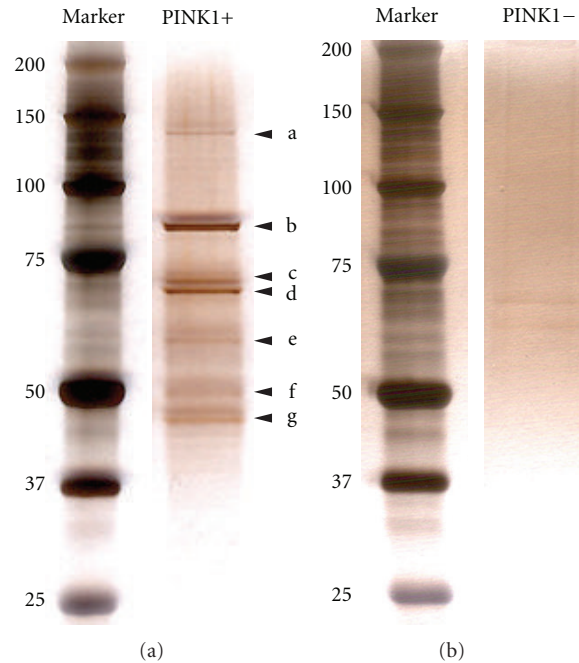


FIGURE 1: SDS-PAGE result after TAP with overexpressed PINK1. (a) A TAP approach was performed with HEK cells overexpressing *PINK1* (PINK1+). Purified proteins were resolved by SDS-PAGE and visualized on the gel by silver staining. Protein bands which were excised from the gel are marked by an arrow head (a–g). (b) In a control experiment, the pull-down approach was performed with beads only (PINK1–). A molecular weight marker was used to estimate the size of the detected protein bands.

membrane (Protran) and probed with antibodies raised against β -actin (Sigma), Mortalin (GRP75, Abcam), Heat shock 60 kDa protein (HSP60, Cell Signalling), Mitochondrially encoded cytochrome c oxidase I (MT-CO1, MitoSciences), Voltage-dependent anion channel 1 (VDAC1, Abcam) and Elongation factor TU (TUFM, Abcam). An antibody against LRPPRC was kindly provided by Professor S. Piñol-Roma, Brookdale Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, New York, USA [18].

2.8. Mutational Screening. All 38 coding *LRPPRC* exons and flanking intronic regions were sequenced on an ABI 3100 Genetic Analyzer. Primers and PCR conditions are summarized in Supplementary Table 1 available online at doi:10.4061/2011/153979.

3. Results and Discussion

3.1. New *PINK1* Interactors. Using a TAP approach with overexpressed PINK1 (PINK1+), or empty beads (PINK1–) we identified interactors of the target protein. The resulting TAP eluates were resolved by SDS-PAGE. Silver staining was used to visualize protein bands on the PAGE gels. Seven bands were excised from the PINK1+ gel (Figure 1(a), a–g) which were not detectable on the PINK1– gel (Figure 1(b)) and analyzed by MS (Table 1).

A total of 14 proteins were identified which met the requirement of having two or more peptides matched to it by the database-searching program. Out of those, seven

are predominantly found in the cytoplasm (Heat shock 90 kDa proteins alpha and beta, Heat shock 70 kDa proteins 1, 2, 8, and 1-like, and Hsp90 cochaperone Cdc37), two are components of microtubuli (Tubulin alpha-1C chain and Tubulin alpha-3C/D chain), one is associated with the endoplasmic reticulum (78 kDa Glucose-regulated protein) and four are mitochondrially localized (GRP75, HSP60, LRPPRC, and TUFM) (for details on subcellular localization see: <http://expasy.org/sprot/>). Of note, also PINK1 itself was identified by MS in most of the analyzed bands on the PAGE gel (Figure 1(a), b–g).

To our knowledge, only the interaction between the HSP90/CDC37 chaperone system and PINK1 has been described so far [19, 20]. CDC37 is a molecular cochaperone that functions with HSP90 to promote folding of kinases [21]. With respect to PINK1, the chaperone system was found to influence the protein's subcellular distribution. The authors of this study proposed that the HSP90/CDC37/PINK1 complex is destined for a translocation that leads to PINK1 processing, whereas in the absence of HSP90 in the complex, PINK1 might be attached to mitochondria as full-length precursor [19].

Given that PINK1 was reported to be associated with mitochondria [17, 22], we focused primarily on the mitochondrial proteins GRP75, HSP60, LRPPRC, and TUFM in the ensuing experiments.

3.2. Cellular Abundance of GRP75, HSP60, LRPPRC, and TUFM. First, we determined the quality of the noncommercially available LRPPRC antibody by means of a knock-down

TABLE 1: Potential interactors of PINK1.

Swiss-Prot accession no.	Gene	Protein name	Subcellular localization	No. of unique peptides	Sequence coverage in %	Band on PAGE gel	Previous report of interaction
P42704	<i>LRPPRC</i>	Leucine-rich PPR motif-containing protein	Mitochondrion	2	1.8	a	none
P07900	<i>HSP90A</i>	Heat shock 90 kDa protein alpha	Cytoplasm	27	28.5	b	Weihofen et al. [19]
P08238	<i>HSP90B</i>	Heat shock 90 kDa protein beta	Cytoplasm	18	21.9	b	Weihofen et al. [19]
P38646	<i>GRP75</i>	75 kDa glucose-regulated protein/Mortalin	Mitochondrion, cytoplasm	6	10.8	c	none
P11021	<i>GRP78</i>	78 kDa glucose-regulated protein	Endoplasmic reticulum	15	28.7	c	none
P08107	<i>HSPA1</i>	Heat shock 70 kDa protein 1	Cytoplasm, organelles	10	17.3	c	none
P34931	<i>HSPA1L</i>	Heat shock 70 kDa protein 1-like	Cytoplasm, organelles	8	13.7	c	none
P54652	<i>HSPA2</i>	Heat shock 70 kDa protein 2	Cytoplasm, organelles	4	7.2	c	none
P11142	<i>HSPA8</i>	Heat shock 70 kDa protein 8	Cytoplasm	7	12.7	c	none
P10809	<i>HSP60</i>	Heat shock 60 kDa protein	Mitochondrion	7	16.2	d	none
Q9BQE3	<i>TUBA1C</i>	Tubulin alpha-1C chain	Microtubule	2	5.3	f	none
Q13748	<i>TUBA3C</i>	Tubulin alpha-3C/D chain	Microtubule	3	10.7	f	none
Q16543	<i>CDC37</i>	Hsp90 cochaperone Cdc37	Cytoplasm	10	22.2	g	Weihofen et al. [19]
P49411	<i>TUFM</i>	Elongation factor Tu	Mitochondrion	3	8.0	g	none

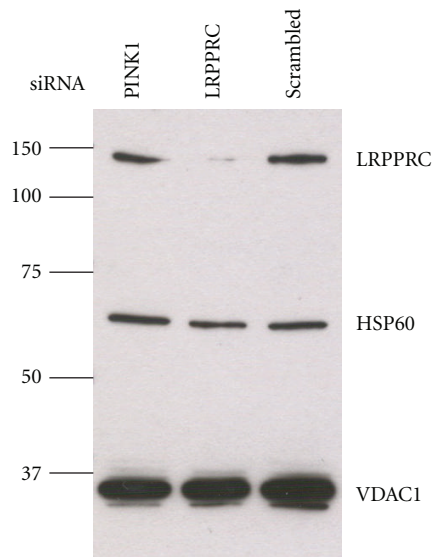


FIGURE 2: Specificity of an anti-LRPPRC antibody. Fibroblasts were incubated with *PINK1* siRNA, *LRPPRC* siRNA, or scrambled siRNA for 24 h. Whole cell lysates were analyzed by Western blotting with an antibody against LRPPRC. LRPPRC levels decreased only when *LRPPRC* siRNA was employed, confirming the specificity of the anti-LRPPRC antibody used in our study. The mitochondrial markers HSP60 and VDAC1 served as loading controls. HSP60: Heat shock 60 kDa protein; LRPPRC: Leucine-rich PPR motif-containing protein; VDAC1: Voltage-dependent anion channel 1.

approach. This experiment showed a drop in LRPPRC levels when siRNA against *LRPPRC* was employed but not when scrambled siRNA was used, confirming the specificity of the antibody. LRPPRC protein levels were also not affected by a *PINK1* knockdown (Figure 2).

Next, the abundance of GRP75, HSP60, LRPPRC, and TUFM was investigated in mitochondrial fractions from control and *PINK1*-mutant fibroblasts. This experiment revealed comparable levels of GRP75, HSP60, and LRPPRC in both groups (Figures 3(a) and 3(b)-left half). The abundance of TUFM was variable in all investigated samples showing no clear trend when comparing mutants and controls (Figure 3(a)).

Furthermore, we tested the quality of the GRP75 antibody by investigating GRP75 in the cytosolic fraction under valinomycin stress conditions. GRP75 is a mitochondrial matrix chaperone, synthesized as a 679-amino acid preprotein, which contains a 51-residue N-terminal mitochondrial targeting sequence (MTS). After the membrane potential-dependent import into mitochondria, it is cleaved into the mature protein which is ~5.5 kDa shorter than the preprotein [23–25]. When treating cells with the mitochondrial membrane potential inhibitor valinomycin, the full-length form of GRP75 (MTS-GRP75) was detected in the cytosolic fractions from mutants and controls. Consequently, we considered the anti-GRP75 antibody as specific. In both groups, the levels of MTS-GRP75 were comparable (Figure 3(b)-right half). The abundance of the processed form of GRP75 in the cytosolic fraction was likely due to contamination with

the mitochondrial fraction, although previous studies have shown that GRP75 can also be cytosolically localized [26].

Though our Western blot results are not supporting a direct link between *PINK1* and any of the detected mitochondrial proteins, it should be noted that GRP75, HSP60, and LRPPRC have been identified by proteomic analysis as potential interactors of Parkin earlier [27]. Furthermore, their molecular functions render them interesting targets in the context of PD.

GRP75 serves as a major mitochondrial molecular chaperone and plays a key role in the import and partitioning of nuclear-encoded proteins within the two mitochondrial membranes and the matrix [28–30]. Furthermore, GRP75 seems to function in the management of oxidative stress via the PD-associated protein DJ-1. Mutations in DJ-1 were found to weaken the protein's interaction with GRP75 [31, 32]. GRP75 has also been described as an antiapoptotic agent. By binding of the transcription regulator p53, GRP75 prevents the formation of the proapoptotic p53/Bcl-xL/Bcl-2 complex [33, 34]. Furthermore, putative mutations in *GRP75* were suggested to contribute to the risk of developing PD [35] and a decrease in GRP75 expression was detected in PD patient brains compared to controls [36].

HSP60 is a mitochondrial chaperone responsible for the transport of nuclear-encoded proteins via the mitochondrial membranes and their refolding in the matrix [37, 38] and has been linked to the pathogenesis of Alzheimer's disease. Apparently, HSP60 provides protection against intracellular β -amyloid stress through maintenance of mitochondrial respiratory complex IV activity [39]. Complex IV deficiency in turn, has been implicated in PD [40, 41] opening the possibility for a role of HSP60 in the pathogenesis of the disease.

LRPPRC has been linked to cytochrome C oxidase deficiency. Mutations in the gene lower *MT-CO1* and *MT-CO3* mRNA levels and, in turn, impair complex IV assembly [42, 43]. Recent functional studies further strengthen the link between LRPPRC and mitochondrial RNA metabolism [44, 45]. However, when we compared *MT-CO1* protein levels in fibroblasts from *PINK1* mutants and controls, no differences were observed (Figure 3(a)). Furthermore, LRPPRC was identified as a component of the PGC-1 α complex which itself is also linked to energy homeostasis in the cell [46]. Like in the case of HSP60, LRPPRC's impact on the respiratory chain offers a potential connection with PD.

TUFM is part of the translational apparatus of mitochondria. During protein biosynthesis, it mediates the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes [47]. Additional described functions of TUFM comprise recognition and translocation of cotranslationally damaged proteins to the proteasome [48], rearrangement of cytoskeletal components [49, 50], and regulation of cell survival [51]. Mutations in the *TUFM* gene cause combined oxidative phosphorylation deficiency type 4 due to decreased mitochondrial protein synthesis [52]. Interestingly, however, there is also a report connecting TUFM and PD, where TUFM was found to co-immunoprecipitate with Leucine-rich repeat kinase 2 which is encoded by the *PARK8* gene *LRRK2*. Coincubation with recombinant TUFM reduced the kinase

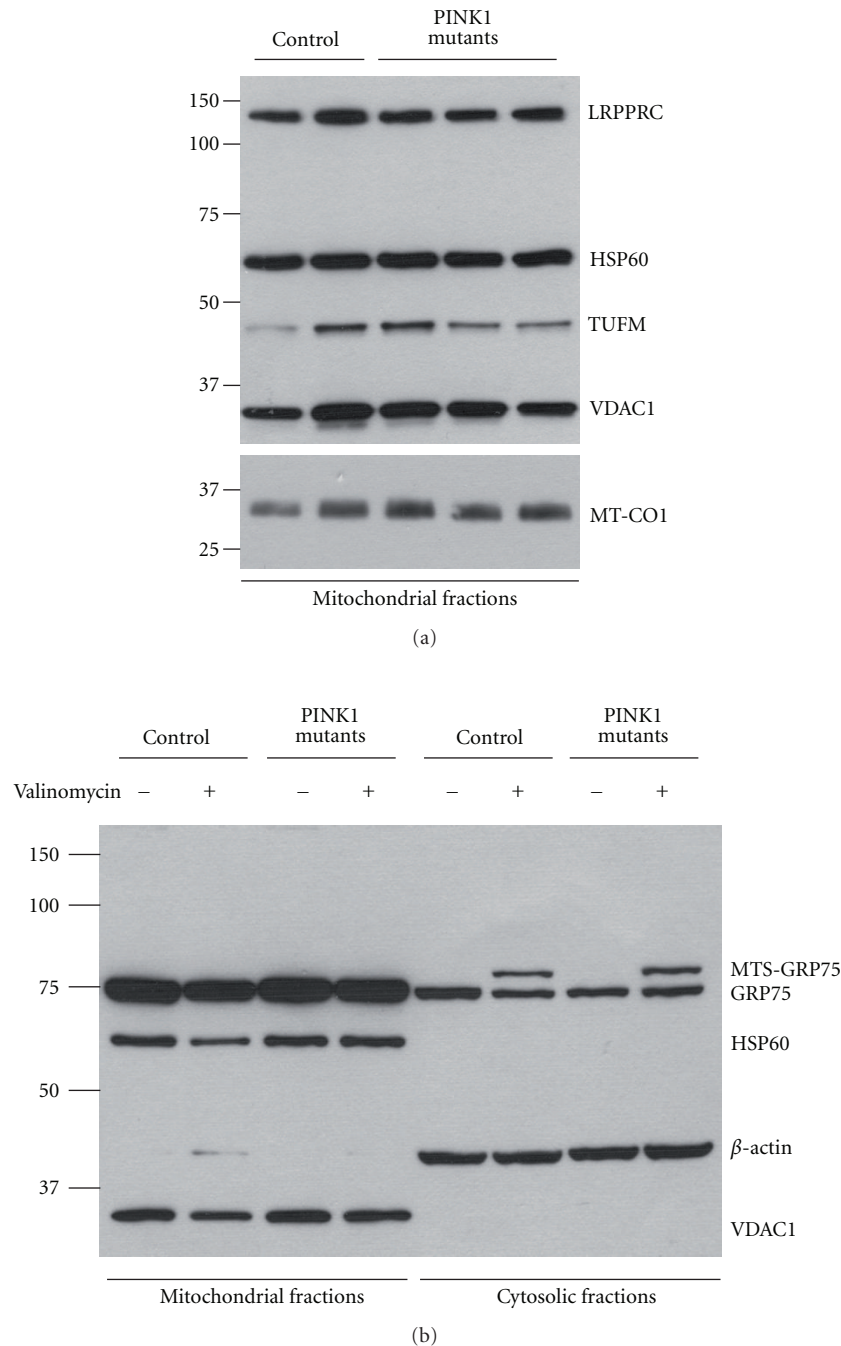


FIGURE 3: Cellular abundance of potential mitochondrial PINK1-interacting proteins. Mitochondrial and cytosolic fractions from fibroblasts were analyzed by Western blotting using antibodies against HSP60, LRPPRC, TUFM, MT-CO1, and GRP75. (a) The mitochondrial localization of LRPPRC and TUFM was confirmed, and no differences in their cellular abundance were detected when comparing *PINK1* mutants and controls. Furthermore, the level of LRPPRC-associated MT-CO1 was not altered in *PINK1* mutants. (b) In the mitochondrial fractions, the abundance of (processed) GRP75 was comparable in *PINK1* mutants and controls under basal and valinomycin stress conditions (1 μ M for 24 h). In the cytosol, an additional band representative of accumulation of nonprocessed MTS-GRP75 was detected when cells were treated with the mitochondrial membrane inhibitor valinomycin. Due to a possible contamination of the cytosolic fraction with mitochondria and/or partially cytosolic localization of GRP75, also the processed form of the protein is apparent in this fraction. The mitochondrial marker VDAC1 and the cytosolic marker β -actin served as loading controls. GRP75: 75 kDa glucose-regulated protein; HSP60: Heat shock 60 kDa protein; LRPPRC: Leucine-rich PPR motif-containing protein; MT-CO1: Mitochondrially encoded cytochrome c oxidase I; MTS-GRP75: GRP75 with mitochondrial targeting sequence; TUFM: Elongation factor Tu; VDAC1: Voltage-dependent anion channel 1.

TABLE 2: Allelic frequencies of sequence variations identified in *LRPPRC*.

Gene position	DNA variation	NCBI no.	AF PD	AF DB	Database*
5'UTR	c.-45G>A	rs11124961	7.6%	1.4%	pilot.1.CEU
Exon 2	c.246G>A (p.Q82Q)	rs6741066	66.7%	65.5%	HapMap-CEU
Intron 3	IVS3-132C>G	rs6721144	6.8%	13.3%	HapMap-CEU
Intron 6	IVS6-70T>C	rs17031786	14.4%	13.8%	HapMap-CEU
Exon 9	c.1068A>G (p.Q356Q)	rs4953042	16.3%	19.2%	HapMap-CEU
Intron 9	IVS9+30A>G	rs7593842	15.2%	12.7%	HapMap-CEU
Intron 13	IVS13+28T>C	rs62135104	9.5%	1.5%	pilot.1.CEU
Intron 15	IVS15+11C>G	rs58811869	7.8%	13.9%	pilot.1.CEU
Intron 17	IVS17-28T>G	rs72877186	15.2%	15.3%	pilot.1.CEU
Intron 20	IVS20-40A>C	rs7594526	42.4%	47.5%	HapMap-CEU
Intron 22	IVS22+27T>G	rs28394191	43.5%	40.3%	pilot.1.CEU
Exon 23	c.2481A>G (p.P827P)	rs115993634	1.1%	none	none
Intron 27	IVS27+26C>T	rs4952694	51.1%	53.0%	AoD_Caucasian
Intron 27	IVS27-38A>G	none	2.2%	none	none
Intron 28	IVS28+21C>A	rs7568481	43.5%	47.4%	HapMap-CEU
Intron 30	IVS30+97T>C	rs17424482	8.7%	3.7%	HapMap-CEU
Intron 32	IVS32-3C>T	rs35113761	6.5%	none	none
Intron 35	IVS35+14C>T	rs3795859	15.2%	15.0%	HapMap-CEU
Intron 35	IVS35+15C>T	rs76850904	8.7%	none	none
Intron 36	IVS36-42G>C	none	1.1%	none	none
Exon 37	c.4023T>C (p.Y1341Y)	none	1.1%	none	none
Intron 37	IVS37+37G>A	rs2955280	51.1%	53.4%	HapMap-CEU
3'UTR	*399G>A	none	2.3%	none	none
3'UTR	*556A>T	rs1136998	7.6%	8.3%	HapMap-CEU

Note: AF: allelic frequency, DB: database, PD: Parkinson's disease and *Only studies based on European populations included.

activity of LRRK2, whereas the GTPase activity remained unchanged [53].

3.3. *LRPPRC* Mutational Screen. Among the identified PINK1 interactors, *LRPPRC* is the only protein which is unequivocally linked to a neurodegenerative disorder. Mutations in *LRPPRC* are the cause of the French-Canadian type of Leigh syndrome (LSFC). LSFC patients suffer from progressive focal necrotizing lesions of the brainstem, basal ganglia, and cerebellum, accompanied by capillary proliferation. Besides metabolic acidosis, clinical features include generalized developmental delay, cerebellar signs, and a striking paucity of facial and limb movement, as well as hypomimia [46]. Given the presence of Parkinsonian signs in LSFC patients, we decided to sequence the 38 exons and flanking intronic regions of *LRPPRC* in 46 patients with atypical PD with early onset and/or rapid disease progression and dementia. This mutational screen revealed 24 substitutions; four of which have not yet been reported in any database (Table 2). Four synonymous variations were detected in the coding region (c.246G>A [p.Q82Q], c.1068A>G [p.Q356Q], c.2481A>G [p.P827P], c.4023T>C [p.Y1341Y]). Seventeen changes were found in introns, one in the 5'UTR and two in the 3'UTR. The frequencies of most substitutions in our sample were similar to those reported in the NCBI SNP

database (<http://www.ncbi.nlm.nih.gov/>) for studies based on populations of European origin, such as pilot.1.CEU, HapMap-CEU, and AoD_Caucasian. Interestingly, frequencies of SNPs c.-45G>A, IVS13+28T>C and IVS30+97T>C, were markedly higher than those reported in the databases. The significance of this finding needs to be investigated in a larger sample. The screening techniques used here allow, however, only for the identification of qualitative sequence changes. Therefore, although no single nucleotide changes of likely pathogenic relevance have been found in the *LRPPRC* gene, gene dosage variations cannot be excluded.

4. Conclusions

In the current study, TAP technology was employed for the first time to identify PINK1-associated proteins. These experiments resulted in a list of 14 putative PINK1 binding partners, confirming two reported interactions (HSP90 and CDC37), but also introducing four novel mitochondrially localized proteins (GRP75, HSP60, *LRPPRC*, or TUFM) as potential components of the PINK1/Parkin mitophagy pathway. Although preliminary results from protein expression and DNA sequencing analyses do not strengthen a link between the PINK1/Parkin pathway and any of these interactors, it cannot be excluded that their connection

with the pathway may be more complex. Additional protein function studies, for instance under mitochondrial stress conditions, will be needed to fully characterize this potential link. In addition, future perspectives include association studies with SNPs in all identified genes in a larger PD patient sample.

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