PTPA variants and impaired PP2A activity in early-onset parkinsonism with intellectual disability

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Supplementary Appendix 1

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Supplementary Appendix 2

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Supplementary Appendix 3 - Clinical case-reports - Family 1

In the South African family (Family 1), the affected 33-year-old male sibling (Fig. 1A; **F1-II-1**) developed right-sided rest tremor of the hand when he was 11 years old and a diagnosis of levodopa-responsive parkinsonism was made at the age of 15. He was unable to write, needed assistance with feeding himself and had dystonia of his right-ankle affecting gait. Treatment with levodopa was effective from the onset.

His mother had placenta praevia leading to a complicated birth at 37 weeks pregnancy. He was oxygen-dependent for the first week after birth but did not need respiratory support. No jaundice was recorded. Early childhood development was slow with delayed motor and communication milestones. He never attended a mainstream school but learned to write and now communicates well. In the early part of primary school, he was able to participate in field athletics and tug-of-war.

Levodopa remained effective but he required increased dosages to remain functional. Levodopa induced motor complications three years after initiation of the treatment and led to the addition of amantadine, entacapone and pramipexole. Pramipexole was stopped due to increased impulsivity with pathological gambling and hypersexuality. Non-motor symptoms included constipation and sialorrhea.

Subthalamic nucleus deep brain stimulation (STN-DBS) was performed at the age of 22 years old with sustained effect on motor score. At the time of surgery, his levodopa equivalent daily dose was 2000mg/day with severe motor fluctuations and peak dose axial dyskinesia. The levodopa challenge showed an improvement of 75% after an increased dose of levodopa. He had mild gait problems mostly caused by right ankle dystonia without retropulsion or freezing-of-gait. With follow up 11 years after surgery, he had occasional freezing-of-gait and falls due to retropulsion, tremor in the right hand and dystonia of the right ankle. Best treatment- and stimulation-on UPDRS-3 was 22 and his levodopa equivalent daily dose 1050mg/day. Any increases in levodopa caused dyskinesia.

He is independent in all basic activities of daily living. Although no formal testing was done to compare with, there does not seem to be any decline in cognitive and language function.

His sister (Fig. 1A; **F1-II-2**), a 24-year-old female, started with features of parkinsonism at the age of 11 years old. She was initially seen when she was 9 years old with no parkinsonism. First symptoms included gait hypokinesia and impairment of hand dexterity that responded well to levodopa with the early development of motor complications after two years. Subthalamic STN-DBS was done when she was 15 years old.

No pregnancy-related complications were recorded. Normal motor and communication milestones were observed but early identification of a learning disability led to special schooling, where she was active in netball and field athletics.

Depression and anxiety were recognized from the onset and treatment with fluoxetine and psychological support was started at an early age.

Treatment with levodopa remained effective, although with persistent nausea. Increased dosages were needed to maintain a functional on-state. After two years motor fluctuations led to the addition of entacapone and pramipexole. By the age of 15 years old, DBS was considered. At this stage she had a LEDD of 1448mg/day and a recorded improvement of 72% with the levodopa challenge. Axial symptoms included off-treatment freezing-of-gait and right ankle dystonia.

At last follow up best-on treatment UPDRS-3 was 14; at a LEDD of 950mg/day with minor increases in levodopa dose causing dyskinesia. She had some axial impairment with freezing-of-gait and mild retropulsion.

Non-motor features included mild anxiety, depression, and restless legs syndrome. There was no subjective decline in cognitive or language ability and she remains independent in all basic activities of daily living.

Clinical examination in both siblings was negative for ataxia, pyramidal signs (hyperreflexia, extensor plantar responses), or autonomic nervous system involvement – with normal bladder control, erectile function (II-1) and orthostatic blood pressure response. Neither of the two siblings had anosmia. Difficulty in independent turning was the only sleep-related complaint and recent onset restless legs syndrome in individual II-2. There were no complaints related to dream enactment behavior. MRI brain was normal in both siblings and both had normal metabolic screening tests, including 24-hour urine copper and ceruloplasmin.

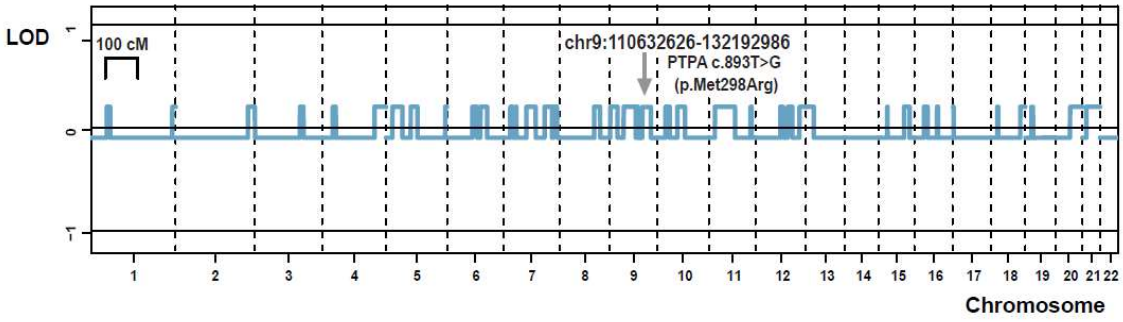
Supplementary Appendix 4

Screening for *PTPA* loss-of-function (LOF) variants

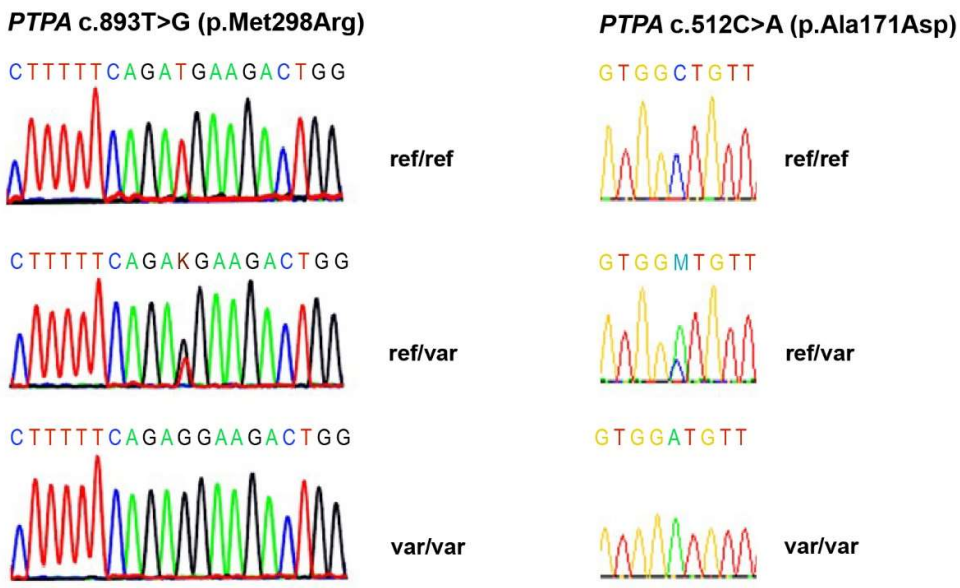
We searched for *PTPA* homozygous or heterozygous LOF variants in the French and Mediterranean PD Genetics Study group (FMPD cohort) as well as in the PD variant browser v0.2.1.[56] We included frameshift, stopgain, stoploss, startgain, startloss, splice donor (+1,+2bp from any intron exon boundary), and splice acceptor (-1,-2bp) variants. We also looked for CNVs in the FMPD cohort, using Dragen (Illumina).

Gene-burden association analysis

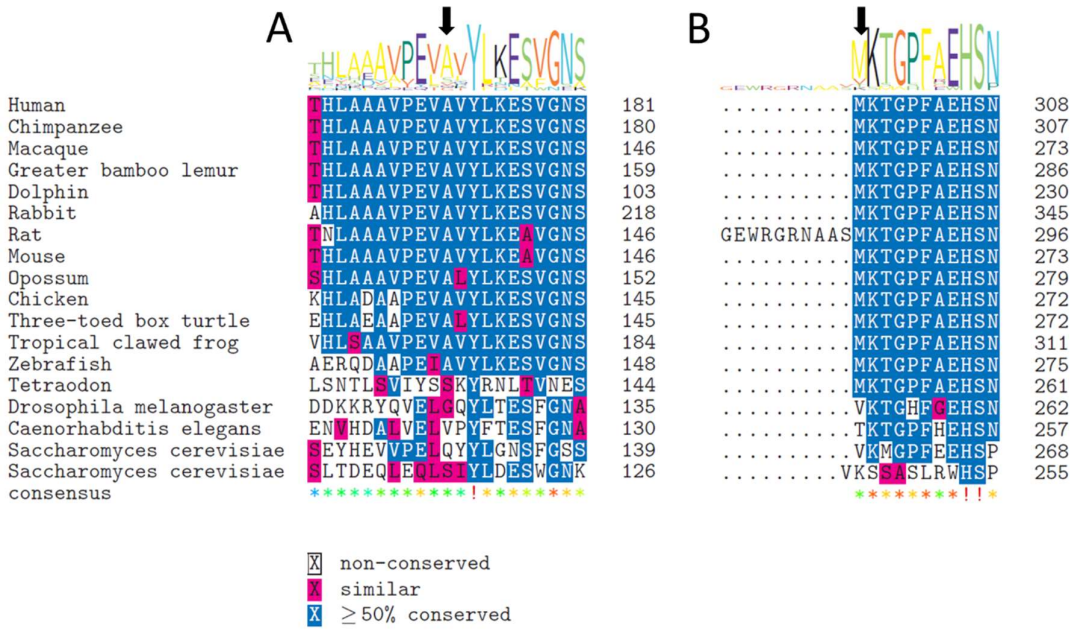
We looked for *PTPA* variants in the publicly available PD variant browser v0.2.1.[56] Due to the more uniform coverage and the limited variant missingness, we included data from the PD Genome Project and the UK Biobank cohort (excluding the IPDGC array datasets and IPDGC Exome Sequencing Project). Data from 2,859 PD patients and 42,334 controls were included in our analyses. We performed gene-burden association analysis by fixed-effect Mantel-Haenszel test, using the rma.mh function from the R v.4.2.0 package metafor v3.4-0.[111] We took along all variants regardless of zygosity, or impact on the coding sequence. We found no evidence of enrichment of *PTPA* variants in PD for variants regardless of minor allele frequency (MAF) (OR 0.964, CI95 0.869-1.070, p-value 0.509), variants with MAF <5% (OR 0.916, CI95 0.772-1.088, p-value 0.338), or variants with MAF <1% (OR 0.895, CI95 0.750- 1.068, p-value 0.236).



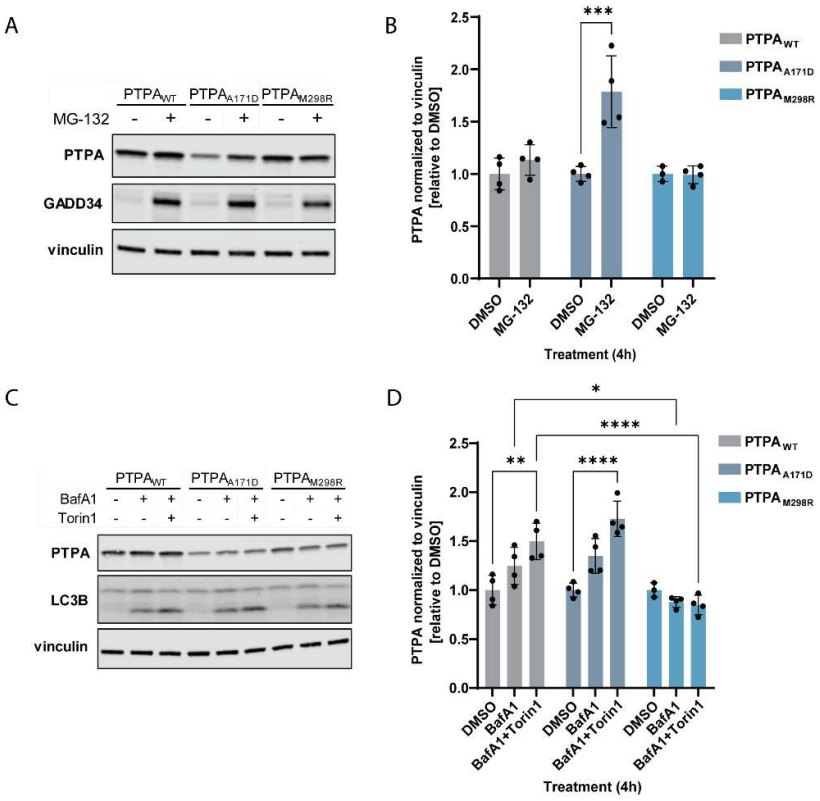
Supplementary Figure 1: Autosomal recessive linkage analysis plot in Family 1. The relative position of the *PTPA* variant is depicted in the corresponding 21,56 Mb region of interest. LOD: logarithm of odds.



Supplementary Figure 2: Representative electropherograms of the *PTPA* variant Sanger sequencing in Family 1 and Family 2. Ref/var: heterozygous genotype for the corresponding variant; var/var: homozygous variant genotype; ref/ref: wild-type (reference) genotype.



Supplementary Figure 3: PTPA orthologue alignment at position A. 171 and B. 298. Sequences of PTPA orthologues were obtained from ensembl (https://www.ensembl.org/index.html) and aligned using the R package msa using ClustalOmega method. The Alanine 171 and Methionine 298 are relatively conserved amino acid (indicated in blue).



Supplementary Figure 4: Comparison of PTPA levels upon inhibition of proteasome and autophagy-lysosome degradation pathways in cultured cells. (A) Representative Western blot of protein extracts from cultured cells transfected with plasmids expressing wild-type PTPA ($PTPA_{WT}$), p.Ala171Asp PTPA ($PTPA_{A171D}$) and p.Met298Arg PTPA ($PTPA_{M298R}$) treated for 4h with DMSO (vehicle control) or 20 µM MG-132. Blots were probed for expression of PTPA, vinculin, and GADD34, a marker for activation of the integrated stress response pathway, which is a consequence of proteasomal inhibition. **(B)** Quantification showing a significant increase in PTPA levels in p.Ala171Asp PTPA-expressing cells (n=4). **(C)** Representative Western blot of PTPA in transfected cells treated for 4h with DMSO or 200 nM Bafilomycin A1 (BafA1) alone or in combination with 200 nM Torin1. Blots were probed for expression of PTPA, vinculin, and LC3B, an autophagosome marker. **(D)** Quantification of PTPA upon treatments, showing a similar significant increase in PTPA wild-type and p.Ala171Asp levels upon treatment with BafA1 and Torin1, indicating sensitivity to autophagosomal degradation, in contrast to p.Met298Arg PTPA that showed no differences upon treatment. The bars indicate mean PTPA levels relative to DMSO-treated cells (n=4). Error bars represent ± standard deviation. Only significant changes $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****) are shown.

Supplementary Table 1: Primers for *PTPA* (NM_178001) PCR and Sanger sequencing

Oligo name	Oligo Sequence	Size
PTPA-exon1-Fwd	TGAGCACAACCCAAACTTGACG	502bp
PTPA-exon1-Rev	TGACTCTCGCCCTCCTGAGC	
PTPA-exon2-Fwd	TGTGTGTGTTGTAGGGGAGGATTC	275bp
PTPA-exon2-Rev	GCTTCTGAAAAGTCAGGCTCCAAG	
PTPA-exon3-Fwd	GGTTGGGAGTCAGGGTCAGG	383bp
PTPA-exon3-Rev	AGAGATGGGGTTTCACATGTTGG	
PTPA-exon4-Fwd	GCATTGCACACGATGATCTGG	468bp
PTPA-exon5-Rev	TCTGTGTTATCTTCTCATAGTGTTTTACATGG	
PTPA-exon5-Fwd	GAAAGCCTATCATGCTCTCCTGACC	399bp
PTPA-exon5-Rev	GCTATACAATGCAGGCCCTTCC	
PTPA-exon6-Fwd	AGTCGCCTGGGTAGTCTTCTGC	597bp
PTPA-exon6-Rev	GAGACCATTTTCACAAGAACAGAAAGC	
PTPA-exon7-Fwd	TCCATGTTGACCACCCTCCTC	297bp
PTPA-exon7-Rev	GCACCCCATTTCCCAGTTTG	
PTPA-exon8-Fwd	CTTCAGTGGTTATTTTGGGGTCTCC	331bp
PTPA-exon8-Rev	TGGGCAGGAAGAAGGGAAGG	
PTPA-exon9-Fwd	CCACGGGCAGGACTGAGG	386bp
PTPA-exon9-Rev	AGTCAGCAGCGGCTCTTTCG	
PTPA-exon10-Fwd	TGCATGGGTGTGACTTTGCTG	349bp
PTPA-exon10-Rev	GAGGCCCCAAGTGTCAGAGG	
PTPA-exon11-Fwd	GGAGTGGGTGTCTTTGGATAGAAGG	388bp
PTPA-exon11-Rev	CACCCCACCCCAGTAAACAGC	

Forward (Fwd) and reverse (Rev) primers.

Supplementary Table 2: Known PD/parkinsonism-causing or related genes screened in the French and Mediterranean Parkinson's Disease Genetics Study group (FMPD cohort)

ATP13A2
DNAJC6
FBXO7
GBA
GCH1
LRRK2
PANK2
PARK7
PINK1
PLA2G6
POLG
PRKN
PTRHD1
SNCA
SPG11
SYNJ1
TH
UCHL1
UQCRC1
VPS13C
VPS35

Supplementary Table 3: Known PD/parkinsonism-causing or related/candidate genes screened in the WES of the patient F1-II-2 (Family F1)

ARSA
ATP13A2
ATP6AP2
ATP6V1B2
CCN3
CHCHD2
CLTC
DCTN1
DNAJC13
DNAJC6
FBXO7
GBA
GCH1
LRP10
LRRK2
MECP2
NR4A2
NRXN2
NUS1
PANK2
PARK7
PINK1
PLA2G6
PLXNA4
PODXL
POLG
PPP2R5D
PRKN
PSAP
PTRHD1
RAB39B
RIC3
SIPA1L1
SLC9A6
SNCA
SPG11
SYNJ1
TBC1D24
TGM6
TH
TMEM230
UCHL1
UQCRC1
VPS13C
VPS35
WARS2
WASL
WDR45

No variants of interest were identified in the known PD/parkinsonism genes based on the following criteria: (i) variants with coding effect or variants with predicted splicing effect by at least one out of four tools (ADA,[37] RF,[37] SpliceAI,[38] SQUIRLS[39]) located within ±10 base-pairs from any intron-exon boundary, and (ii) with a minor allele frequency (MAF) <1% in public population databases and <1% in in-house reference datasets.

Supplementary Table 4: RT-qPCR primers for *PTPA*, *PP2A-C* and reference transcripts

Oligo name	Oligo Sequence
PTPA-Fwd	ACTCCAACCAGCTGTGGAAC
PTPA-Rev	AGTGCTGGATCACAGGGAAC
PP2A-C-Fwd	GCTGCAATCATGGAACTTGAC
PP2A-C-Rev	GACGAGTAACATGTGGCTCG
COPS5-Fwd	CCAGGAACCATTTGTAGCAG
COPS5-Rev	GTAGCCCTTTGGGTATGTCC
CLK2-Fwd	TCGTTAGCACCTTAGGAGAGG
CLK2-Rev	TGATCTTCAGGGCAACTCG

Forward (Fwd) and reverse (Rev) primers.

Supplementary Table 5: Additional variants identified in the F1-II-2 WES under the autosomal recessive model that were excluded by co-segregation analysis (Sanger sequencing)

Chromosomal Position[a]	Ref allele[b]	Alt allele[c]	Gene	Transcript	cDNA	Protein	Exon	Zygosity	gnomAD v2.1.1[d]	CADD[e]	GERP[e]
11:62291388	C	T	AHNAK	NM_001620	c.10501G>A	p.Gly3501Arg	5	Heterozygous	2/251310	22.6	3.59
11:62295873	C	T	AHNAK	NM_001620	c.6016G>A	p.Asp2006Asn	5	Heterozygous	absent	18.41	2.71
11:62297070	C	G	AHNAK	NM_001620	c.4819G>C	p.Asp1607His	5	Heterozygous	1/251330	16.87	3.64
11:62382094	C	T	ROM1	NM_000327	c.839C>T	p.Ala280Val	3	Heterozygous	5/282846	12.17	-3.19
11:62382253	C	G	ROM1	NM_000327	c.998C>G	p.Ala333Gly	3	Heterozygous	absent	21.6	3.21

[a]Chromosomal position is given according to GRCh37/hg19.
[b]Ref allele = reference allele.
[c]Alt allele = alternative allele.
[d]gnomAD = Genome Aggregation Database[44]
[e]Predictions and scores across *in silico* algorithms were obtained via the Ensembl Variant Effect Predictor (VEP) (v105);[40] CADD = Combined Annotation Dependent Depletion,[54] CADD_phred_hg19; GERP = Genomic Evolutionary Rate Profiling.[110]

Supplementary Table 6: Predictions across *in silico* pathogenicity and conservation algorithms via the Ensembl Variant Effect Predictor (VEP) (v105)[40] for the two *PTPA* missense variants identified in this study

Chromosomal position[a]	9:131893865	9:131904725
cDNA[b]	c.512C>A	c.893T>G
Protein[b]	p.Ala171Asp	p.Met298Arg
CADD_phred_hg19	23.6	32
ClinPred	Damaging	Damaging
DANN	Damaging	Damaging
DEOGEN2	Tolerated	Tolerated
Eigen-phred	Pathogenic	Pathogenic
Eigen-PC-phred	Pathogenic	Pathogenic
FATHMM	Tolerated	Tolerated
fathmm-MKL_coding	Damaging	Damaging
fathmm-XF_coding	Damaging	Damaging
GenoCanyon	Damaging	Damaging
integrated_fitCons	Damaging	Damaging
LIST-S2	Damaging; tolerated	Damaging; tolerated
LRT	Deleterious	Deleterious
M-CAP	Tolerated	Damaging
MetaLR	Tolerated	Tolerated
MetaRNN	Damaging	Damaging
MetaSVM	Tolerated	Tolerated
MutationAssessor	Medium	Medium
MutationTaster	Disease causing	Disease causing
MutPred	Pathogenic	Pathogenic
MVP	Benign	Benign
Polyphen2_HDIV	Benign; probably damaging	Probably damaging; possibly damaging
Polyphen2_HVAR	Possibly damaging; benign	Probably damaging; possibly damaging
PrimateAI	Damaging	Damaging
PROVEAN	Neutral; damaging	Damaging
REVEL	Benign	Pathogenic
SIFT	Tolerated	Deleterious
SIFT4G	Tolerated	Deleterious
VEST4	Deleterious	Deleterious
GERP++_RS	4.94	5.39
phyloP100way_vertebrate	7.38	7.791
phastCons100way_vertebrate	1	1
SiPhy_29way_logOdds	17.5264	14.5924
ADA	No splicing effect	No splicing effect
RF	No splicing effect	No splicing effect
SpliceAI	No splicing effect	No splicing effect
SQUIRLS	Splicing effect	Splicing effect

[a]Chromosomal position is given according to GRCh37/hg19.
[b]Variants are annotated based on the *PTPA* transcript NM_178001.
Light blue for damaging/disease-causing/deleterious/pathogenic/likely pathogenic/conserved predictions; **Beige** for tolerated/benign/likely benign/nonconserved predictions.

Supplementary Videos 1 and 2

Video Legend: Video recordings showing the two affected South African patients without any Parkinson's disease treatment and with optimal pharmacological treatment and deep brain stimulation (DBS) switched on. Video 1 shows patient F1-II-1 and video 2 patient F1-II-2. In each video, segment 1 represents the worst off (no pharmacological treatment and DBS switched off) and segment 2 the best on state (optimal pharmacological treatment and DBS switched on).