eFigure. Facial appearance of the family members.

A. The proband's mother (II-3). B. The proband (III-1). C. The proband's youngest sister (III-3). D. The proband's younger sister (III-2).

eTable 1. The assessment of cognitive function in female carriers in this family using the Japanese version of the Wechsler Adult Intelligence Scale, 3rd Edition (JWAIS-III).

		Proband		Proband's youngest sister	
Age at exam		51		45	
		raw score	scaled score	raw score	scaled score
Verbal Comprehension	Vocabulary	7	2	21	7
	Similarities	4	1	15	7
	Information	4	2	9	5
	Comprehension	3	1	9	3
Working Memory	Arithmetic	2	1	6	3
	Digit Span	2	1	7	3
	Letter-Number Sequencing	0	1	3	1

Perceptual Organization	Picture Completion	1	1	13	6
	Block Design	12	2	16	3
	Matrix Reasoning	3	1	18	11
	Picture Arrangement	2	2	10	7
Processing Speed	Digit Symbol	8	1	57	6
	Symbol Search	0	1	14	2
Verbal Comprehension Index (VCI)		50*		80	
Perceptual Reasoning Index (PRI)		50*		79	
Working Memory Index (WMI)		50*		50*	
Processing Speed Index (PSI)		50*		66	
Full Scale IQ		42		68	
Verbal IQ		47		65	
Performance IQ		47		76	

IQ: intelligence quotient. *: The minimum admissible value for this index is 50.

eTable 2. Genes known to be associated with or as risk factors for the conditions of parkinsonism and dementia.

DCTN1 PRKN FLNC ABCA7 MAPT NPC1 LRRK2 TGM6 ATXN2 STH APP GRN PSEN1 PSEN2 APOE CR1 BIN1 SORL1 CHMP2B PSENEN TREM2 SETX SIGMAR1 EWSR1 SPG11 GAK OGG1 GRIN2B MS4A7 TREML2 TREML4 MS4A1 VCP CHCHD10 CD33 TARDBP PRNP IL6 OPTN TBK1 THBS2 MKL2 DAAM2 PLEKHG5 CLECL1 CTNNA1 CD163L1 AKAP9 GALR3 MIEF1 INPP5D EPHA1 UNC5C NME8 PLCD1 UNC13C CD2AP CLU CHRNB2 CHRNA4 EPHA5 CDH2 EPHA6 CRMP1 PIN1 FUS C9orf72 PCDH11X NAMPT CPE CSF1R UBQLN2 SERPINI1 ADAM17 ERMP1 VLDLR SRCAP APH1A HFE NLGN1 MS4A6A CALHM1 TFCP2 CDKN1A CREB1 AGER TNF ABCG2 A2M AR IL6R CYP46A1 NOS1 MAPK8IP1 RTN3 MME CTSD CFH LPL CHAT ABCA1 GSTM3 SERPINA3 IL1B NCSTN BLMH PPP2R2B SLC19A1 BDNF ATP7B PICALM CST3 GSK3B ADRB2 TP53 CASP8 OTC MS4A6E TF IL1A TYROBP CTSF VPS35 MARK4 SLC30A3 MEOX2 FPR2 KLK1 SQSTM1 PLD3 GSTT1 XBP1 ABI3 LRP2 CAV1 BACE1 CYP19A1 CTNNA3 MPO ADAM10 PRKCA TTC3 TCOF1 MLKL AMBN MTHFR AXIN1 TNK1 TM2D3 TRIML2 TOMM40 TRAK2 MYH13 PGBD1 GALP ACAN LRP6 LRPAP1 TFAM IP6K3 NOTCH3 TSHZ3 CASP7 PLCG2 ESR2 COMT OLR1 ARMS2 ADAM9 ECE1 ARC DRD1 FGF1 VEGFA DRD3 CCR1 CETP HTR2C LRP1 DIO2 ESR1 CCL11 APOC1 MIR146A ADRA2B PNMT APBB3 LAMA1 DAPK1 PLAU NGF IDE HMGCR NGFR VDR NTRK1 NRG1 TLR2 PNP PCK1 GBA ZNF224 IL18 PGRN TDP hnRNPA1 hnRNPA2 hnRNPA2B1 UBQLN SNCA UCHL1 PINK1 ATP13A2 GIGYF2 HTRA PLA2G6 FBXO7 RAB29 EIF4G1 DNAJC6 SYNJ1 DNAJC13 CHCHD2 ABCB1 SCN9A MAOB SLC6A4 CYP2D6 NQO2 PITX3 HMOX2 XRCC1 FMR1 DRD4 CHRNB3 HNMT HTRA2 HSPA1A POLG ADH1C XRCC3 CPXM1 RAD51B ZFYVE26 RIC3 GLUD2 SLURP1 RUNDC3A COL12A1 EPPK1 ANKRD13A PAPD4 PMEL VPS53 SLC5A9 KCNV2 FBXL17 LCT SLC52A1 MKS1 PEPD PTEN MGA ACMSD VAPB PACRG RAB39B MTX1 HLA-DRA SLC6A3 NDUFV2 ERBB2 GCH1 COQ2 HMOX1 SLC18A2 VPS13C PODXL SLC39A14 CP SLC30A10 SPR ATP6AP2 TNK2 GSTP1 TYR SLC2A1 PRRT2 CACNA1A CLCN1 CLCN2 PIGT TERC PNKD CLEC7A IL10 ATG12 SNPD1 PTK2B TBP SIRT1 OVOS2 MS4A6ATV1 TMEM230 HSPA9 SLC41A1 SCARB2 EEF1D PLXNA4 LAMP2 NOD2 ATG7 FTH1 SNCAIP ARSBTV2 ATG5 GPATCH2L PTPRH UHRF1BP1L PARL

TNR TNK2TV1 NPC2 APTX BSN PUS1 SHC2 ZNF231 COQ10D1 NACP MLASA1 CLN11 SHCB MSA1 SCK CXCL8 ICAM-1 EIF4EBP1 SLC1A4 CAV2 COX5B FGFR2 LAMA4 LTBP1 MAP1A PDGFC PSMB5 SPOCK TNFAIP TNFRSF21 HBB ABCA8 ACT RAB7L1 ADH7 UCHL-1 DM2 DBH USP24 AAOPD TH DDC ADORA1 DRD2 GLIS1 LINGO1 MCCC1 NR4A2 PDE8B PTRHD1 RIT2 PANK2 PARK7 PSAP NOS3 CYLD TIA1 CCNF

eTable 3. Female carriers with *SLC9A6* variations with atypical parkinsonism that developed in late life.

No.	Reference	*SLC9A6* variation	Onset age of parkinsonism	Type of parkinsonism	Intellectual disability	Psychiatric symptoms
1	Riess et al., 2013	c.1464_1465insT, p.Thr489TyrfsX23	55	Parkinson's disease	NR	Yes
2	Riess et al., 2013	c.1464_1465insT, p.Thr489TyrfsX23	70s	"Parkinsonism"	NR	NR
3	Sinajon et al., 2016	c.190G>T, p.E64X	60	CBD	Moderate	Yes
4	Pescosolido et al., 2019	NR	65	CBD	Severe	NR
5	Pescosolido et al., 2019	NR	Early 50s	Atypical parkinsonism	Mild	NR
6	Present Report	c.265T>C p.Trp89Arg	48	Atypical parkinsonism	Severe	No
7	Present Report	c.265T>C p.Trp89Arg	63	Atypical parkinsonism	Severe	No

NR, unknown or observation not recorded; CBD, corticobasal degeneration.

Supplementary Material

Methods

Whole-exome sequencing (WES)

The genomic DNA was isolated from peripheral blood leukocytes. Exome capture was performed with a SureSelect Human All Exon V6+UTR (89Mb) Kit (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing was carried out on a HiSeq2500 (Illumina, San Diego, CA, USA) using a HiSeq SBS Kit V4 (Illumina), which generated 100-bp reads. The average and minimum sequencing depths were

125× and 25×, respectively.

Plasmid construction

Expression plasmids encoding C-terminally flag epitope-tagged wild-type NHE6 were cloned by PCR amplification from human lymphocyte cDNA. Because the major isoform of NHE6 in the human lymphocyte cDNA had a short N terminus coding region, we amplified the upstream region of the gene corresponding to the N terminus variant from human genomic DNA and then connected them by In-fusion ligation system. Standard PCR reaction created the W89R variation. The amplified PCR fragments were ligated into the pCI-neo expression vector. We verified the complete nucleotide sequences of the expression plasmids.

Cycloheximide chase assay

The expression plasmids were transfected into HEK293T cells using polyethylenimine (PEI MAX, Polysciences). After 24 h of transfection, we add cycloheximide solution at a final concentration of 40 μg/ml and then chased. Cells were solubilized in TNET buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100), and then subjected to SDS-PAGE. After SDS-PAGE, proteins were transferred on to PVDF membrane and then labeled with anti-DDDDK-tag mouse monoclonal antibody (clone FLA-1, MBL) or anti-NHE6 rabbit polyclonal antibody (A304-448A, Bethyl laboratories). Blots were reprobed with a mouse monoclonal anti-β-actin antibody (#A3854) to control for loading. The proteins were visualized with HRP-labeled secondary antibodies using a chemiluminescence detection system (Immobilon Forte, Millipore). Digital images

were captured using ImageQuant LAS4000 (GE healthcare).

Immunofluorescence and confocal laser scanning microscopy

After 24 h of expression, cells were fixed in 4% paraformaldehyde and then permeabilized with 0.3% Triton X-100. Cells were subsequently labeled with anti-DDDDK-tag antibody and Alexa Fluor 568 labeled anti-mouse IgG antibody. Cells were counter-stained with anti-Rab antibodies against Rab5, Rab7, and Rab11 (Cell signaling technology), respectively, and then subsequently stained with Alex Fluor 488 labeled anti-rabbit IgG antibody. The images were captured as a single confocal plane using the FV1200 system (Olympus) using a ×100 oil immersion objective lens.

Image analysis and statistics

Image analysis of colocalization between NHE6 and Rabs in randomly selected 20 fields of images was performed using ImageJ software (http://rsbweb.nih.gov/ij/). To measure the intensity of fluorescence, the region of interest (ROI) was set according to the merge color, and the “measure” function was used to quantitate the averaged area and intensity in the ROI. The student’s t-test was performed using GraphPad Prism software.