DNAJC12 and dopa-responsive non-progressive Parkinsonism

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ABSTRACT

Biallelic *DNAJC12* mutations were described in children with hyperphenylalaninemia, neurodevelopmental delay, and dystonia.

Using whole-exome sequencing we identified homozygous null variants in *DNAJC12* in two kindreds with early-onset parkinsonism: a nonsense mutation (c.187A>T;p.K63*, Proband-A); and a splicing variant (c.79-2A>G;p.V27Wfs*14, Proband-B). Both probands had mild, non-progressive, motor symptoms, sustained benefit from small dose of levodopa and substantial worsening of symptoms after levodopa discontinuation. Neuropathology (Proband-A) revealed no alpha-synuclein pathology. RNA analysis showed a significant reduction of *DNAJC12* transcripts in both patients. Our results suggest *DNAJC12* mutations can present with dopa-responsive non-progressive parkinsonism in adulthood, broadening the clinical spectrum of *DNAJC12* deficiency.

INTRODUCTION

Biallelic mutations in *DNAJC12* were recently described in four families with hyperphenylalaninemia, dystonia, and intellectual disability.[1] DNAJC12 function is still unclear; it was suggested[1] to interact with aromatic amino-acid hydroxylases, including phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), tryptophan hydroxylase-1 and -2 (TPH1, TPH2). These enzymes are central to the synthesis of dopamine and serotonin[2] and share the obligatory cofactor tetrahydrobiopterin (BH_4)(Fig 1).[3]

Mutations in enzymes involved in the biosynthesis of monoamine neurotransmitters, including those responsible for synthesis and salvage of BH_4, present with a wide spectrum of clinical manifestations, such as motor and autonomic dysfunction, cognitive impairment, and sleep disturbances (Fig 1).[4,5] Symptoms usually start during infancy, although a later onset may occur.[4] Motor symptoms, largely due to dopamine deficiency, comprise motor delay, dystonia, parkinsonism, and hypotonia.[4]

Here, we identified two novel homozygous *DNAJC12* null mutations in two families with early-onset dopa-responsive non-progressive parkinsonism not associated with overt dystonic features.

METHODS

The study was performed according to the Helsinki Declaration and approved by site-specific Ethics Committees. Written informed consent was obtained from all subjects. Parkinson's disease (PD) was diagnosed by Movement Disorders expert Neurologists, and the Hoehn and Yahr stage (H&Y) used to assess PD symptoms/progression.

Whole-exome sequencing

WES was performed using the Ion AmpliSeq™ Exome Kit and the Ion Proton™ System (Thermo Fisher Scientific, Carlsbad, USA). Reads were aligned against the human reference genome hg19. Variant

annotation was performed with ANNOVAR (http://annovar.openbioinformatics.org/en/latest/). Variant confirmation, segregation analysis and *DNAJC12* screening were performed by Sanger sequencing.

RNA analysis

RNA from frozen brain tissue was isolated using the RNeasy Mini kit (Qiagen, Hilden, Germany) and reverse-transcribed with Superscript VILO™ cDNA synthesis kit (Thermo Fisher Scientific). Whole-blood RNA was extracted using the PAXgene Blood miRNA Kit (Qiagen) and reverse-transcribed with the ImProm-II™ Reverse Transcription System (Promega, Madison, USA). For splicing assays, *DNAJC12* exons 1-3 were RT-PCR amplified and the obtained products analyzed by Sanger sequencing. *DNAJC12* transcripts were assessed by real-time RT-PCR using: i) a TaqMan assay on a 7900HT instrument (Thermo Fisher Scientific)(Proband-A); ii) the SYBR Premix ExTaqII (TAKARA, Mountain View, USA) on a LightCycler480 (Roche, Basel, Switzerland)(Proband-B).

Neuropathology

Autopsy was performed 60h after death. Brain specimens were paraffin-embedded, cut into 5-μm-thick sections, deparaffinized, and rehydrated. Following epitope unmasking monoclonal antibodies to α-synuclein and p62 (BD Transduction Laboratories, Oxford, UK); β-amyloid (Signet, BioLegend, San Diego, USA); phospho-tau (Innogenetics, Ghent, Belgium); and phospho-TDP-43 (Cosmo Bio, Tokyo, Japan) were applied, incubated overnight (4°C), and detected using the Dako REAL EnVision System (Agilent Technologies, Santa Clara, USA). Alzheimer's disease neuropathological diagnosis was based on β-amyloid plaques distribution (according to Thal's phase), neurofibrillary tangle pathology distribution (according to Braak's staging), and neocortical neuritic plaque density (according to CERAD).[6]

Biochemical analyses

Phenylalanine, biogenic amine metabolites (5-hydroxyindoleacetic acid, 5-HIAA; homovanillic acid, HVA), pterins, DHPR activity, and amino acids were measured either in blood, dried blood, cerebrospinal fluid (CSF), or urine as described.[1]

RESULTS

We analyzed two kindreds, from Saskatchewan (Canada; family A) and from Italy (family B) with recessive early-onset parkinsonism (Fig 2). Both probands had mild, non-progressive motor symptoms and derived marked and sustained benefit from small dose of levodopa. These clinical features challenged the diagnosis of idiopathic PD, so that attempts were made to discontinue levodopa, invariably leading to worsening of symptoms in both probands.

Proband-A[7] had juvenile parkinsonism (age at onset, AAO=13y) that marginally progressed to H&Y 3 by age 31, and remained stable over the subsequent 43 years (Video 1). Psychometric testing at 32y revealed mild intellectual disability (IQ=68) that did not worsen (Table 1). ^{18}Fluoro(F)-Dopa PET imaging at 56y and 73y revealed a mild reduction in uptake that was non-progressive over time, and some degree of asymmetry (Fig 2; Table 1). The patient died at 74y.

Proband-B was diagnosed with early-onset tremulous parkinsonism (AAO=32y). She had marginal progression of motor and non-motor symptoms over 30 years (H&Y 1)(Video 2). She developed mild peak-dose levodopa-induced dyskinesias that disappeared by fractioning levodopa dose. Her scholastic performance was below average. Neuropsychological assessment (55y) revealed mild cognitive impairment and borderline intellectual ability (IQ=71)(Table 1). DaT-SPECT imaging at 52y was at the lower limit of normality in the right putamen. A second DaTSCAN at 57y was normal (Fig 2).

Proband-B's younger brother had mild non-progressive tremulous parkinsonism (AAO=51y). Early and prominent psychotic symptoms were noted, without overt cognitive dysfunction. Presynaptic nigrostriatal function appeared normal at DaTSCAN (54y; data not shown).

WES identified *DNAJC12* homozygous null variants in both subjects: a nonsense substitution (NM_021800.2:c.187A>T;p.K63*) in Proband-A and a splicing mutation (NM_021800.2:c.79-2A>G;p.V27Wfs*14) in Proband-B. Both variants are absent from public databases (GnomAD). No other

mutations were detected in genes previously linked/associated with PD. Variants were confirmed by Sanger sequencing; segregation analysis within families was consistent with an autosomal-recessive inheritance pattern (Fig 2). No other causal mutations were identified in 127 early-onset (<45y) PD in house exomes, nor in 87 Asian and 283 Caucasian early-onset patients screened by Sanger sequencing.

RT-PCR and sequence analysis on Proband-B's RNA revealed that the c.79-2A>G mutation causes exon-2 skipping, resulting in a frame-shift and a premature stop (p.V27Wfs*14). *DNAJC12* transcript levels were significantly decreased in Proband-A's cerebellum and Proband-B's whole blood (Fig 2).

Gross examination of Proband-A's brain was unremarkable except the substantia nigra (SN) appeared hypopigmented. There was no evidence of α-synuclein pathology in brainstem nuclei. The pigmented neurons in the locus coeruleus (LC) appeared well-preserved, whereas in the SN there was a moderate loss with marked depigmentation. Some tau-positive neurofibrillary tangles (NFTs) and neurites were noted in the LC, reticular formation, basis pontis, and hippocampus and moderate NFTs/neurites in the entorhinal cortex fulfilling Braak's NFT stage 2. Moderate diffuse amyloid plaques were seen in frontal/parietal cortices together with sparse neuritic plaques (CERAD A). Some β-amyloid deposits were found in the central grey of the midbrain and red nucleus, reaching Thal amyloid phase 4. Thus, the case would be assigned A3-B1-C1.[6] No changes were seen with phospho-TDP-43, whereas p62 showed diffuse staining in spinal-cord motor neurons and glial aggregation that was positive for β-amyloid.

Proband-B had elevated blood phenylalanine (449μM)(Table 1) comparable to previosly described patients.[1] CSF analysis showed very low concentrations of 5-HIAA and HVA; increased HVA/HIAA ratio, increased phenylalanine concentration (74μM) along with an elevation of several amino acids (Table 1).

Defects in BH_4 metabolism were excluded based on normal levels of urinary pterins and DHPR activity in dried-blood spot (Table 1).

DISCUSSION

Homozygous null mutations in *DNACJ12* were identified in two kindreds with early-onset parkinsonism, broadening the clinical spectrum of *DNAJC12* deficiency, which was reported[1] to clinically mimic deficits in BH_4 metabolism, leading to a progressive movement disorder with prominent dystonia and intellectual disability.[1] However, our data suggest *DNAJC12* mutations may also present with disease onset in adolescence/adulthood, with parkinsonism as the main symptom, mild intellectual disability, and no overt dystonic features.

Such variability parallels that observed in patients with *GCH1* mutations.[8] While the classical phenotype is childhood-onset dopa-responsive dystonia, *GCH1* carriers may present in adulthood with slowly-progressing parkinsonism, similar to idiopathic PD.[9] Levodopa-responsive parkinsonism has been occasionally reported in patients with hyperphenylalaninemia due to PAH mutations or to defects in BH_4 metabolism.[10–13] However, even if some of these patients[13–15] can fulfil clinical criteria for idiopathic PD, little or no evidence of dopaminergic deficit is apparent by presynaptic nigrostriatal imaging. These patients might hence represent cases of "scans without evidence of dopaminergic deficit".[16]

In Proband-A, the biochemical neurotransmitter imbalance was not associated with a degenerative process. The SN hypopigmentation is likely a consequence of a chronic deficiency in dopamine, the main precursor of neuromelanin in dopaminergic neurons.[17] The lack of histologically-evident neurodegeneration is consistent with neuropathological and neuroimaging data reported in patients with biogenic amine disorders,[13] and with the non-progressive course of the disease.

Hitherto, all neuronally-expressed DNAJ class-III (DNAJC) members (*DNAJC6*/auxilin, *DNAJC26*/GAK, *DNAJC5*/CSPα, *DNAJC13*/Rme-8, *DNAJC12*/HSP40) have been implicated in parkinsonism.[18] DNAJC12 has a critical role in chaperoning amino-acid hydrolase interactions required for catecholamine synthesis.[1] In line with this, TH was shown to interact with VMAT2 transporter, which is required to package dopamine at the synapse.[19] Thus, the coupling between synthesis and packaging of dopamine may be

tightly regulated by the chaperone properties of DNAJC12. DNAJC proteins appear to contribute to the maintenance of an equilibrium within the presynaptic compartment that ensures viability of the synapse, repopulation of synaptic vesicle machinery, and folding of intrinsically-disordered proteins, such as α-synuclein.[18]

In summary, we here report a different phenotype associated with mutations in *DNAJC12*. This finding adds to the recent paper by van Spronsen et al.[20] describing a more mild and heterogeneous clinical spectrum of DNAJC12-deficient hyperphenylalaninemia. Pleiotropy is emerging as an important contributor to genetic disorders. The utilization of the same protein in multiple biological processes well suits the multiple functions and interactors hypothesized for DNJAC12. From a clinical perspective, our results suggest that patients with mild non-progressive parkinsonian symptoms, response to low-dose levodopa and possibly intellectual disability should be screened for hyperphenylalaninemia. If positive, they should be tested for *DNAJC12* variants. Such patients may have great benefit from combined administration of BH_4, dopamine, and serotonin precursors, as previously suggested.[1]

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AUTHOR CONTRIBUTION

LS, IG, and RC contributed to acquisition and analysis of data and to drafting the text and preparing the figures. RA and GS contributed to data analysis and drafting the text. LP, VR, AY, VS, JS, AP, JF, KN, NH, AR, AHR, SG and NB contributed to acquisition and/or analysis of data; MJF and SD contributed to conception and design of the study.

POTENTIAL CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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FIGURE LEGENDS

Figure 1: Monoamine neurotransmitter metabolism and related clinical phenotypes.

Enzymes whose deficiencies can affect the pathway are boxed and shaded according to the associated spectrum of clinical manifestations. The possible interaction of DNAJC12 with different hydroxylases, as proposed by Anikster and colleagues,[3] is indicated by two-headed arrows.

Figure 2: *DNAJC12* mutations in families with early-onset recessive parkinsonism.

A. Pedigree of family A (left) and consanguineous family B (right). The affected subjects are represented by black symbols, the proband, analyzed by WES, is indicated by an arrow. The *DNAJC12* mutation genotype is reported, when available, under each family member. wt=wild type (c.187A, Family A; c.79-2C, Family B); Het=heterozygote (c.79-2A/c.79-2G, Family B); Homo=homozygote (c.187T, Family A; c.79-2G, Family B).

B. Imaging results. Imaging study of the presynaptic nigrostriatal function was performed by using ^{18}F-dopa PET for Proband-A (left), with the tissue input uptake rate constant K_{occ} as outcome variable and DaT-SPECT for Proband-B (right).^{18}Fluoro(F)-Dopa PET: Index case at 43 and 60y disease duration. Images represent tracer concentration (Bq/mL) averaged from 30-90 minutes after tracer injection. The patient was injected with the same amount of tracer and was of the same weight. Both images are scaled to the same maximum. DaT-SPECT: Index case at 20 and 25y disease duration. SPECT images were normalized and Z scores are displayed at the bottom of the scan. Below each scan, FP-CIT SPECT binding values are shown, as calculated using the Basal Ganglia Matching Tool V2 Semi-quantitative; caudate nucleus binding values are displayed on the left, putamen values on the right. The black line represents the population average values adjusted for age; the red line and the green line represent the 90% and the 97% inferior confidence limit.

C. Sanger sequencing electropherograms showing the nucleotide sequence surrounding the *DNAJC12* mutations (c.187A>T left; c.79-2A>G, right). The mutated nucleotide is indicated by an arrow and shaded in grey.

D. Quantification of total *DNAJC12* mRNA levels by real time RT-PCR. Left: Proband-A and two controls. All reactions were performed in triplicate on RNA extracted from cerebellum and data normalized using three housekeeping genes (*DNAJC12* Hs01113092_m1; *GAPDH*: Hs01105870_m1; *HPRT1*: Hs02800695_m1 and *SYP*: Hs00300531_m1 Thermo Fisher Scientific). Right: Proband-B and 24 controls. All reactions were performed in triplicate on RNA extracted from total blood. *HMBS* (Hydroxymethylbilane synthase) was used as internal reference. Results were analyzed with the software GeNorm (https://genorm.cmgg.be) and are presented as normalized rescaled values, and analyzed by unpaired t-test. *: $p<0.05$,**: $p<0.01$.

E.

Agarose-gel electrophoresis of the RT-PCR assay performed on the RNA extracted from total blood of the Proband B and of a control individual (Ctrl). PCR products showed amplification of a lower molecular weight fragment (199 bp) compared to the control (278 bp). Sequencing of the shorter PCR product confirmed complete skipping of exon 2. On the right, schematic representation of the amplicons obtained from the RT-PCR with the position of the primers used in the assay. Exons are represented by boxes, primers by arrows.

Table 1: Demographic, clinical, and biochemical characteristics of patients carrying *DNAJC12* variants.

Demographic and clinical characteristics				***Biochemical characteristics[a]***	
	Proband-A	***Proband-B***	***Proband-B's brother***	***Proband-B***	
Origin	Canada	Italy	Italy	***Blood***	
Family	A	B	B		
Gender	M	F	M	**Phenylalanine**	449 (37-115 µM)
AAO motor symptoms	13	32 (tremor)	51	**Prolactin** [b]	12.3 (4.8-23.3 ng/mL)
AAO non-motor symptoms	-	26 (anxiety/depression)	53	***Cerebrospinal fluid***	
Age at diagnosis	31	32	51	**HVA**	37 (115-455 nM)
At last assessment	73	59	58	**5-HIAA**	7 (51-204 nM)
Parkinsonism	++	++	+	**HVA/5-HIAA ratio**	5.3 (1.1-3.7)
Dystonic features	-	-	-	**7,8-dihydrobiopterin**	2 (<18 nM)
Levodopa response	+++	+++	++	**Tetrahydrobiopterin**	3 (18-53 nM)
Levodopa-induced dyskinesias	+	++	+	**Total neopterin**	19 (10-31 nM)
Intellectual disability	+ (IQ 68)	± (IQ 71)	+ (IQ n.a.)	**5- Methyl tetrahydrofolate**	40 (26-118 (nM)
Education	6 (scholastic performance below average)	8 (scholastic performance below average)	8	**3-O-Methyl-dopa**	35 (<50 nM)
Cognitive dysfunction	- Normal (MMSE 28/30, n.v.>24) one year before death	+ Mild global impairment (MMSE 22/30, n.v.>24) with mild frontal-lobe (FAB 12.1/18, n.v.>13.4) and visuo-spatial dysfunction	+	**L-Dopa**	6 (<15 nM)
Psychiatric features	None	Anxiety/depression	Psychosis (hallucinations, delusions)	**5-Hydroxy-tryptophan**	<2 (<10 nM)
Sleep Disorders	None	++[c]	++	**Phenylalanine**	74 (7-11 µM)
Fatigue	None	+++	+++	***Urine***	
Brain MRI	Normal	Normal	Normal		
Presynaptic nigrostriatal Imaging	F-Dopa PET. Mild non-progressive reduction	DAT SPECT normal (Putamen R 3.40, L 3.84;	DAT SPECT normal (Putamen R 4.06, L 3.95;	**Neopterin**	0.4 (0.2-1.7 mmol/molKrea)

	First scan: Putamen R 0.0065, L 0.0086; Put/Caud R 0.71, L 0.72, n.v.>0.7; Putamen asymmetry 32% L>R, n.v.<25% Second scan: Putamen R 0.0062, L 0.0081; Put/Caud R 0.77, L 0.74, n.v.>0.7; Putamen asymmetry 31% L>R, n.v.<25%	Put/Caud R 0.70, L 0.78, n.v.>0.7; Putamen asymmetry 12% L>R, n.v.<25%) [d]	Put/Caud R 0.80, L 0.77, n.v.>0.7; Putamen asymmetry 3% R>L, n.v.<25%)	**Biopterin**	0.9 (0.5-2.7 mmol/molKrea)
				%B = 100*B/(N+B)	69 (49-85)
				Dried blood	
				DHPR activity	1.4 (>1.1 mU/mg Hb)
Brain metabolism PET	n.a.	Normal	n.a.		

[a] Normal ranges are reported in brackets.

[b] Under chronic levodopa treatment.

[c] Sleep disturbances responded to 5-Hydroxy-Tryptophan (dose of 1mg/kg/day, slowly titrated from 25 mg to 75 mg/day).

[d] Data are presented only for the first scan.

Abbreviations: AAO, Age at onset; FAB, Frontal assessment Battery; MMSE, Mini Mental State Examination; n.a. data not available; n.v., normal values; PD; Parkinson's disease; PET, positron emission tomography; SPECT, single photon emission computed tomography.