DNAJC6 Mutations Disrupt Dopamine Homeostasis in Juvenile Parkinsonism-Dystonia

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ABSTRACT: Background: Juvenile forms of parkinsonism are rare conditions with onset of bradykinesia, tremor and rigidity before the age of 21 years. These atypical presentations commonly have a genetic aetiology, highlighting important insights into underlying pathophysiology. Genetic defects may affect key proteins of the...
endocytic pathway and clathrin-mediated endocytosis (CME), as in DNAJC6-related juvenile parkinsonism.

Objective: To report on a new patient cohort with juvenile-onset DNAJC6 parkinsonism-dystonia and determine the functional consequences on auxilin and dopamine homeostasis.

Methods: Twenty-five children with juvenile parkinsonism were identified from a research cohort of patients with undiagnosed pediatric movement disorders. Molecular genetic investigations included autozygosity mapping studies and whole-exome sequencing. Patient fibroblasts and CSF were analyzed for auxilin, cyclin G–associated kinase and synaptic proteins.

Results: We identified 6 patients harboring previously unreported, homozygous nonsense DNAJC6 mutations. All presented with neurodevelopmental delay in infancy, progressive parkinsonism, and neurological regression in childhood. $^{123}$I-FP-CIT SPECT (DaTScan) was performed in 3 patients and demonstrated reduced or absent tracer uptake in the basal ganglia. CSF neurotransmitter analysis revealed an isolated reduction of homovanillic acid. Auxilin levels were significantly reduced in both patient fibroblasts and CSF. Cyclin G–associated kinase levels in CSF were significantly increased, whereas a number of presynaptic dopaminergic proteins were reduced.

Conclusions: DNAJC6 is an emerging cause of recessive juvenile parkinsonism-dystonia. DNAJC6 encodes the cochaperone protein auxilin, involved in CME of synaptic vesicles. The observed dopamine dysregulation in patients is likely to be multifactorial, secondary to auxilin deficiency and/or neurodegeneration. Increased patient CSF cyclin G–associated kinase, in tandem with reduced auxilin levels, suggests a possible compensatory role of cyclin G–associated kinase, as observed in the auxilin knockout mouse. DNAJC6 parkinsonism-dystonia should be considered as a differential diagnosis for pediatric neurotransmitter disorders associated with low homovanillic acid levels. Future research in elucidating disease pathogenesis will aid the development of better treatments for this pharmacoresistant disorder. © 2020 The Authors. Movement Disorders published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.

Key Words: auxilin; DNAJC6; dopamine; dystonia; parkinsonism

Classical Parkinson’s disease (PD) is an age-related neurodegenerative disorder, mainly affecting adults aged >50 years. Patients typically present with resting tremor, bradykinesia, rigidity, and postural instability. To date, a number of early-onset genetic forms of PD (Parkin, PINK1, and DJ-1)$^{13}$ and complex parkinsonism syndromes (ATP13A2, PLA2G6, FBXO7, SLC6A3, SLC39A14, and PANK2) have been described. Importantly, the study of such monogenic forms of disease have provided significant insight into the pathogenic mechanisms underlying sporadic PD.$^{10}$ More recently, two genes, namely SYNJ1$^{11}$ and DNAJC6,$^{12-14}$ encoding proteins involved in postendocytic recycling of synaptic vesicles, have been identified in early-onset parkinsonism.

In this study, we report on 6 children from three families, presenting with juvenile parkinsonism-dystonia associated with novel, biallelic DNAJC6 mutations. We delineate their clinical phenotype, neuroimaging features (including $^{123}$I-FP-CIT single-photon emission computed tomography [SPECT; DaTScan]) and pattern of cerebrospinal fluid (CSF) neurotransmitter metabolites. Furthermore, we utilized patient fibroblasts and CSF to investigate secondary effects on auxilin, cyclin G–associated kinase (GAK), and dopaminergic proteins.

Materials and Methods

Subject Recruitment:

A cohort of 232 children with undiagnosed movement disorders were recruited for research between 2012 and 2016 at UCL. Great Ormond Street–Institute of Child Health (London, UK). A subgroup of 25 patients were identified with juvenile parkinsonism, defined as onset of bradykinesia before 21 years of age, and at least one of the following signs: resting tremor, rigidity, and postural instability. All patients had detailed clinical assessment, undertaken by a movement disorder specialist. Review of (1) the clinical history, (2) features on neuroimaging, and (3) video recordings of the movement disorder at different time points was undertaken. Written informed consent was obtained from participating families, and the study was approved by the local ethics committees (Reference 13/LO/0168).

Diagnostic CSF Neurotransmitter Analysis:

In order to rule out a primary neurotransmitter disorder, where possible, patients had a routine diagnostic lumbar puncture for CSF neurotransmitter analysis. Using standardized protocols,$^{15}$ CSF samples were collected, snap frozen in liquid nitrogen, and stored at −80°C. Analysis was undertaken using high-pressure liquid chromatography (HPLC) with electrochemical detection and reversed-phase column.$^{15}$ Seven anonymized control pediatric CSF samples (with normal CSF neurotransmitter profiles) were obtained from the Neurometabolic Laboratory (National Hospital for Neurology and Neurosurgery, London, UK). All samples were processed and stored in accordance with the UK Royal College of Pathologists guidelines.

Molecular Genetic Investigation:

From the subgroup of 25 patients with juvenile parkinsonism (16 singletons and 9 familial cases from 3 kindreds), we prioritized two consanguineous families
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(Family A, 3 affected children; Family B, 2 affected children) for initial analysis. These families were investigated using an autozygosity mapping approach, given that the affected children were phenotypically similar and both families originated from the same region in Pakistan. Single-nucleotide polymorphism (SNP) genotyping was performed as previously described. In addition, whole-exome sequencing (WES) was performed for 2 children (A:III-1 and B:IV-2) by UCL Genomics (average WES coverage as previously reported), with an average DNAJC6 coverage of 30×, with minimum coverage 10× for 82% of the gene. WES data were probed for putative disease-causing DNAJC6 mutations in the remaining 20 cases (16 sporadic patients, 4 familial cases from a single kindred). This was undertaken through UCL Genomics (8 patients) and Wellcome Trust Sanger Institute (12 patients) within the Wellcome Trust UK10K Rare Diseases project, as previously reported. For patients where DNAJC6 mutations were identified, whole-exome data were also probed for other genes associated with early-onset dystonia-parkinsonism (Table 1).

**Direct Sanger Sequencing**

Sanger sequencing was used to confirm variants identified on WES and to establish familial segregation. A genomic DNAJC6 sequence (Ensembl transcript: ENST00000371069; NCBI reference sequence: NM_001256864) was utilized to design primers, using Primer3 software (http://bioinfo.ut.ee/primer3/). Primers and polymerase chain reaction (PCR) amplification conditions are available on request. PCR products were cleaned up with MicroCLEAN (Web Scientific) and directly sequenced using Big Dye Terminator Cycle Sequencing System (Applied Biosystems Inc., Foster City, CA). Sequencing reactions were run on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems Inc.) and analyzed with Chromas (http://www.technelysium.com.au/chromas.html).

**Fibroblast and CSF Immunoblotting:**

Methods to assess protein expression in patient fibroblasts were as previously reported. In brief, primary fibroblast lines were cultured from skin biopsies taken from Patients A-III:1 and B-IV:4 (c.766C>T; p.R256*) and 2 age-matched healthy donor controls. Antibodies for auxilin, GAK, and dopaminergic proteins as described previously. CSF protein was probed with the following antibodies: auxilin, GAK, tyrosine hydroxylase (MilliporeSigma, Burlington, MA), dopamine receptor 2 (MilliporeSigma), dopamine transporter (MilliporeSigma), vesicular monoamine transporter 2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and transferrin (Santa Cruz Biotechnology, Inc.) as the loading control. Relative protein levels were quantified using ImageJ software (National Institutes of Health, Bethesda, MD) and normalized to the loading control and the mean percentage of optical densitometry of three replicates analyzed with standard error of the mean.

**Statistical Analysis**

Statistical analysis was performed using Prism software (version 8; GraphPad Software Inc., La Jolla, CA), with data tested for Gaussian distribution and compared by the Student t test.

**Data Availability Statement:**

All clinical and experimental data relevant to this study are contained within the article. For Families A, B, and C, there is no ethical approval in place for deposition of whole-exome sequencing genomic data into a public repository. Genomic data from UK10K are available at theEGA European Genomen Phenome Archive (https://www.ebi.ac.uk/ega/home), EGAS00001000128(UK10K RARE FIND). Details of statistical analysis can be shared upon request.

**Results**

**Patient Cohort**

A total of 232 children were referred with undiagnosed movement disorders for genetic research (Fig. 1A). Of these, 25 children (10.7%) had juvenile parkinsonism, 16 females and 9 males with a current median age of 14 years (range, 4–28). Fourteen of 25 had additional neurological features, including dystonia (14 patients), developmental delay/learning difficulties (14 patients), and seizures (4 patients; Fig. 1B).

**Molecular Genetic Investigations**

Families A and B (Fig. 1A) were prioritized for autozygosity mapping studies. SNP genotyping revealed a 4.33-Mb region of common homozygosity in both families on chromosome 1, between rs640407 (64,267,606 base pairs [bp]) and rs2566784 (68,602,735 bp; Fig. 2A,B). This region showed a common haplotype in all affected children whereas unaffected siblings had a different haplotype. It was therefore considered to be the likely disease locus (Fig. 2B).

WES performed in Patients A-III:1 and B-IV:2 revealed 23,369 and 23,549 variants, respectively. Given familial...
FIG. 1. Juvenile parkinsonism cohort: clinical features and molecular genetic investigation. (A) Flowchart demonstrating the pathway of molecular genetic investigations in a subcohort of 25 children with juvenile parkinsonism. (B) Clinical characteristics of 20 children from 17 families. Early infancy <3 months; infancy 3 to 12 months; toddler 12 to 24 months; childhood 2 to 13 years; adolescence 13 to 18 years. *Consanguineous family. M, male; F, female; N, normal; NP, not performed; NR, not reported. [Color figure can be viewed at wileyonlinelibrary.com]
**FIG. 2.** Molecular genetic investigations and DaTSCAN imaging. (A) Family A and B SNP array results showing homozygous regions detected. For each chromosome, the start and end point is specified using the Reference SNP Cluster ID (rs number) and physical position. (B) Homozygous SNPs are represented in light blue (AA) and dark blue (BB), heterozygous SNPs in red (AB), and “no calls” in white. (C) Sanger Sequencing confirms a homozygous *DNAJC6* mutation, c.766C > T (p.R256*), in all affected children of Family A (A-III:1, A-III:4, and A-III:5) and Family B (B-IV:2, B-IV:4). Parents are heterozygous carriers. (D) I-123-DaTSCAN™ with SPECT imaging in a control subject, Patient A-III:1 (19 years 3 months), Patient A-III:4 (11 years 4 months), and Patient B-IV:4 (17 years). In Patients A-III:1 and B-IV:4, DaTSCAN findings indicate virtually complete absence of tracer uptake in the basal ganglia, with very high background activity, suggesting loss of presynaptic dopaminergic terminals, whereas Patient A-III:4 showed significantly reduced, albeit still visible, uptake in the head of caudate (left better than right, white arrows). [Color figure can be viewed at wileyonlinelibrary.com]
consanguinity and the autozygosity mapping results, targeted analysis for recessive pathogenic variants within the putative disease locus on chromosome 1 was undertaken. A single homozygous nonsense variant c.766C>T (p.R256*) in DNAJC6 (Chr1, 65,248,219–65,415,869) was identified both in A-III:1 and B-IV:2 on WES, located within the common region of heterozygosity. No other pathogenic changes in previously reported genes causing juvenile parkinsonism-dystonia phenotypes were identified from the WES data. Direct Sanger sequencing confirmed the homozygous c.766C>T mutation in all 5 affected patients, and familial segregation studies revealed that all parents were obligate carriers in both kindreds, with unaffected siblings either wild type or heterozygous for the identified variant (Fig. 2C). WES/whole-genome sequencing data for the remainder of the parkinsonism-dystonia cohort (n = 20) was interrogated for DNAJC6 mutations. This led to the identification of a homozygous nonsense variant (c.2416C>T) in a sixth unrelated patient (Patient C), which was confirmed on Sanger sequencing.

Delineation of the Clinical Phenotype of DNAJC6 Patients

**Family A (3 Affected Patients)**

Patients A-III:1, A-III:4, and A-III:5 are 3 affected children born to first-cousin parents, currently 20, 12, and 10 years old (Table 1). Two other brothers (A-III:2 and A-III:3), aged 17 and 15 years, have mild learning difficulties without evidence of a movement disorder. The paternal grandfather was diagnosed with PD in his fifties.

All 3 children were born at term after an uneventful antenatal period. Microcephaly was evident at birth (head circumference: <0.4th centile), but nonprogressive over time. All siblings had early neurodevelopmental delay and moderate learning difficulties.

A-III:1 is the eldest daughter, aged 20 years. She presented at 10 years, with a 6-week history of feeding difficulties, vomiting, and weight loss. Over time, she developed fever, unsteady gait, facial asymmetry, left-sided tremor, and generalized seizures and was diagnosed with an encephalitis of uncertain etiology. She recovered from this acute illness, but subsequently had progressive bradykinesia, with tremor and rigidity, and loss of independent ambulation at 13 years, associated with cognitive decline. She is now wheelchair dependent, with generalised cogwheel-rigidity, severe bradykinesia, multiple limb contractures and emotional lability (Video 1). She also has severe gut dysmotility, with recurrent vomiting, and required a gastrostomy for deteriorating bulbar dysfunction. CSF neurotransmitter analysis (age 11 years), while on levodopa therapy, revealed an isolated low 5-hydroxyindoleacetic acid (5-HIAA; Fig. 3A). At 12 years 11 months, when off l-dopa, CSF HVA, and HVA:5-HIAA ratio were low. Brain MRI showed evidence of right-sided atrophy of the perisylvian region and right cerebellum by 19 years of age (Supporting Information Fig. S1). At 19 years, 123I-FP-CIT SPECT (DaTScan) showed absent uptake in the basal ganglia when compared to normal subjects (Fig. 2D). At this stage, while on l-dopa treatment, her CSF HVA levels normalized (Fig. 3A). Her condition is refractory to medical treatment, with no clinical response to trihexyphenidyl, benzhexol, procyclidine, clonazepam, rotigotine, and apomorphine. l-dopa has proven difficult to titrate because of marked drug sensitivity. She experiences an improvement in motor function and speech 30 minutes postdose, after which she returns to the off state. l-dopa dosages >150 mg/d have resulted in drug-related dyskinesias.

Her two brothers (A-III:4 and A-III:5) presented with fine motor difficulties at 8 years of age. They subsequently developed positional tremor, upper limb dystonic posturing, hypophonia, hypomimia, bradykinesia, cogwheel rigidity, and postural instability over 12 months (Videos 2 and 3). Like their sister, both have gastrointestinal complications with sialorrhea, recurrent vomiting, and feeding difficulties, necessitating gastrostomy insertion. A-III:4 is currently 12 years old and suffers from anxiety and perseveration. His CSF-HVA levels are at the lower limit of normal, with a low HVA:5-HIAA ratio <1.0 (Fig. 3A). MRI brain scan was normal. 123I-FP-CIT SPECT (DaTScan) at 11 years showed profound reduction in tracer uptake in the basal ganglia (Fig. 2D). Both boys responded to treatment with transdermal rotigotine and oral trihexyphenidyl, but with increasing doses, both experienced dyskinesias, necessitating dose reduction.

**Family B (2 Affected Patients)**

Patients B-IV:2 and B-IV:4 are 2 affected girls, born to first-cousin parents, and currently 28 and 19 years old. Both were born uneventfully following a normal pregnancy, presenting with early feeding difficulties, hypotonia, and delayed milestones by 6 months old. Both made slow developmental progress, achieving independent ambulation and spoken language by 3 years of age.

B-IV:2 presented at 9 years with generalized seizures that stabilized with lamotrigine therapy. From 13 years of age, motor and cognitive deterioration ensued, with onset of parkinsonism and loss of speech and ambulation. She experienced anxiety and recurrence of seizures. She has severe antecollis, hypomimia, tremor, generalised cogwheel rigidity, bradykinesia, and positive glabellar tap (Video 4). MRI was normal until 18 years, after which there was radiological evidence of mild generalized atrophy. Several medications were tried without clinical benefit, including l-dopa (maximum, 10 mg/kg/d), selegiline, rotigotine, and trihexyphenidyl. The younger sibling, B-IV:4, presented at 7 years with gait deterioration, bradykinesia, and cogwheel rigidity. She lost...
FIG. 3. CSF neurotransmitter analysis and patient fibroblast and CSF immunoblotting. (A) CSF neurotransmitter analysis. Age-related reference ranges indicated in brackets after each value. Red: abnormal result. Gray: borderline result. Symbol (#) indicates reference range:1Keith Hyland, Robert A.H. Surtees, et al. Pediatr Res 1993;34:10–14; 2Keith Hyland, Future Neurol 2006;1:593–603; 3Surtees R, Hyland K. Biochem Med Metab Biol 1990;44:192–199. (B) Scatterplot of CSF HVA and 5-HIAA levels (nmol/L) measured by high performance liquid chromatography (patient = red shapes, control = black triangles). Medication at time of CSF sampling: A-III-1: co-careldopa, melatonin, glycopyronium; A-III-4: none; B-IV-4: L-dopa, pyridoxine; Control 1: none; Control 2: none. Immunoblot of auxilin and GAK in patient fibroblasts (C) and CSF (D) compared to controls. (E) Immunoblot of patient CSF for TH, DAT, VMAT, and D2R protein levels measured compared to controls. Graphs show mean protein percent optical density (OD) normalized to loading control in patients (red) and controls (black). LP, lumbar puncture; y, years; m, months; 5-MTHF, 5-methyltetrahydrofolate; NP, not performed. [Color figure can be viewed at wileyonlinelibrary.com]
independent ambulation and speech by 10 years (Video 5). She has developed dystonic posturing, bulbar dys-
function (necessitating gastrostomy), and a disrupted sleep pattern. The MRI brain scan was initially normal,
but by 16 years showed subtle global cerebral atrophy (particularly in the posterior regions) as well as cerebellar
atrophy (Supporting Information Fig. S2). $^{123}$I-FP-CIT SPECT (DaTScan) at 17 years showed profound re-
duction in tracer uptake in the basal ganglia (Fig. 2D). CSF HVA and HVA:5-HIAA ratio were reduced at ages 4 and
14 years. CSF-HIAA levels were reduced at age 4 years (Fig. 3A). She showed an initial response to l-dopa, but
developed emotional lability at 5.5 mg/kg/d, leading to drug withdrawal. There was no clinical improvement
observed with trihexyphenidyl or chloral hydrate. She had a modest response to pramipexole, with improved
facial expression, reduced tremor, and increase in voluntary movements.

Family C

This 18-year-old girl is the third child of distantly related
Latin American parents, with 2 healthy siblings. She ini-
tially presented with neonatal feeding difficulties and
hypotonia. In infancy, she showed delay in attaining mile-
stones and developed seizures characterized by staring epi-
sodes with loss of tone. She walked independently from
2 years, but by 10 years of age her gait deteriorated, lead-
ing to frequent falls, postural instability, and losing the
ability to run. Over the next 4 years, she continued to dete-
riorate with worsening antecollis and bradykinesia (Video
6). She developed severe bulbar dysfunction with
sialorrhea, dysarthria, and, dysphagia, leading to consid-
erable weight loss. At 12 years, she developed generalized
tonic-clonic seizures and atypical absences, responsive to
lamotrigine and zonisamide therapy. MRI demonstrated
subtle generalized cerebellar atrophy and CSF HVA was
low (Fig. 3A). Her movement disorder responded to l-
dopa, with improved tremor, gait, and a reduction in dro-
oling. A maximum of 200 mg/d was tolerated, but further
increases led to intolerable drug-induced dyskinesias.
After 4 months of treatment, she developed aggressive
behavior and received treatment with quetiapine. By
16 years, she became increasingly sensitive to l-dopa, with
peak-dose agitation, restlessness, and dyskinesia. Lower-
ing the dose improved side effects, and continued to pro-
vide motor benefit, although the effects wore off 2 to 3
hours after administration. On-off phenomena were
commonly reported, and in the off state, she was often
akinetic and rigid. Introduction of trihexyphenidyl
improved rigidity, but not immobility.

Patient CSF and Fibroblast Analysis

CSF HPLC analysis of the DNAJC6 patient cohort
showed reduction in CSF-HVA levels ($P = 0.002$) com-
pared to controls (but not 5-HIAA levels) in 3 patients
(Fig. 3A,B). Patient fibroblasts showed reduced auxilin
($P = 0.009$) and a trend for increased GAK protein
($P = 0.11$; Fig. 3C). Patient CSF auxilin levels were even
more significantly reduced ($P = 0.0015$; Fig. 3D). Nota-
ably, CSF-GAK levels were significantly increased in
patients ($P = 0.0014$; Fig. 3D). CSF immunoblotting
studies showed that several key components of the
dopaminergic synapse were significantly reduced,
including tyrosine hydroxylase (TH; $P = 0.0001$), vesic-
ular monoamine transporter (VMAT; $P = 0.0002$),
dopamine transporter (DAT; $P = 0.0003$), and D2
receptor (D2R; $P = 0.002$; Fig. 3E).

Discussion

Juvenile parkinsonism attributed to DNAJC6 muta-
tions has only recently been reported. Here, we report
on a further 6 patients from three families, with two
previously unreported homozygous nonsense mutations
in DNAJC6. Moreover, our findings on $^{123}$I-FP-CIT
SPECT (DaTScan) imaging, CSF analysis, and immuno-
blotting suggest downstream dyshomeostasis of auxilin,
GAK, and dopaminergic proteins in DNAJC6-related
disease.

Our data confirms that all reported cases of juvenile-
onset DNAJC6-parkinsonism have core clinical charac-
teristics (Table 1), including (1) clinical presentation of
progressive parkinsonism toward of the first decade
(median, 10 years; range, 7–13), (2) significant neuro-
logical regression thereafter, and (3) loss of ambulation
in mid-adolescence. $^{12-14,21}$ In contrast to adult-onset
PD, childhood parkinsonian disorders rarely present with
a “pure” parkinsonian phenotype, as illustrated by the
classical primary pediatric monoamine neuro-
transmitter disorders. $^{20}$ Similarly, in early-onset
DNAJC6-related disease, parkinsonism is commonly
present in tandem with a multitude of other clinical fea-
tures, including dystonia, moderate learning difficulties,
epilepsy, and neuropsychiatric features $^{12-14,21}$
(Table 1). Furthermore, many of our patients had evi-
dence of bulbar dysfunction, gut dysmotility, and sleep
disturbance. The majority of our patients showed lim-
ited response to l-dopa and other standard therapies
for parkinsonism-dystonia. They experienced severe,
oncen intolerable, side effects with dopaminergic agents,
including on-off phenomenon and severe dyskinesia,
particularly at higher drug dosages.

$^{123}$I-FP-CIT SPECT (DaTScan) was performed in 3
patients, demonstrating reduced tracer uptake in the basal
ganglia, suggestive of impaired presynaptic dopamine
uptake and striatonigral neurodegeneration. Postmortem
studies have confirmed striatal dopamine deficiency in
patients with parkinsonism. $^{22,23}$ Together, these observa-
tions suggest a neurodegenerative process in DNAJC6
patients. MRI brain imaging further corroborates this
hypothesis; the mild generalized cerebral and/or cerebellar atrophy in 4 patients suggests that DNAJC6-related disorders may also be associated with neuronal loss in other regions of the central nervous system.

All 6 cases fit the juvenile phenotype associated with this gene, though more recently, DNAJC6 mutations have been reported in early adult-onset PD. Although there are a number of overlapping features (progressive parkinsonism, psychiatric features), affected patients presented later (range, 21–42 years) and seizures and cognitive decline are not reported.

Homozygous and compound heterozygous mutations in DNAJC6 are predicted to result in loss of protein function. To date, splice-site variants, large multiexonic deletions, truncating mutations, and missense mutations have been reported. All 6 patients in our cohort had nonsense mutations, predicted to cause nonsense-mediated decay or premature protein truncation. Five of the 6 reported patients are from two consanguineous families originating from the same region in Pakistan, and all have the same nonsense mutation. SNP array confirmed a common haplotype at this disease locus for all affected children, suggesting a possible founder effect.

DNAJC6 encodes for auxilin, a neuronally expressed J-chaperone protein involved in the uncoating of clathrin-coated vesicles (Fig. 4). Auxilin modifies the three-dimensional conformation of heavy-chain clathrin triskelions, leading to clathrin coat distortion, instability, and subsequent disassembly. Neurotransmission involves rapid continuous recycling of synaptic vesicles through CME. Deficiency in auxilin ultimately results in impairment of synaptic vesicle recycling and impaired neurotransmission. Similarly, aberrant synaptic vesicular trafficking is also evident in other forms of early-onset parkinsonism, including LRRK2, VMAT2, and SNCA-related disease. Clathrin-mediated endocytosis is crucial for the regulation of developmental signaling pathways through internalization of receptors or ligands and is required for axon and dendrite outgrowth. Presence of developmental delay well before onset of parkinsonism in patients with DNAJC6 mutations further corroborates the notion that auxilin is likely to have a central role in neurodevelopment, given its role in CME. In Drosophila, auxilin is crucial for Notch signaling, a developmental pathway that regulates neural stem-cell proliferation, survival, renewal, and differentiation, as well as neuronal specification of dopaminergic neurons.

To investigate the downstream effects of DNAJC6 mutations, we studied auxilin and GAK protein levels in 2 patients using patient fibroblasts and CSF. Auxilin is a neuron-specific protein, enriched in presynaptic terminals, whereas GAK is an ubiquitously expressed.
protein.35,36 Auxilin and GAK are highly homologous proteins that both have the ability to bind clathrin and clathrin adaptor protein 2 in order to initiate clathrin uncoating of endocytosed vesicles.37 In the auxilin knockout mouse model, it is reported that upregulation of GAK can partially compensate for the loss of auxilin and decrease mortality.35 We therefore wished to determine whether a similar compensatory mechanism was evident in our patients. In our study, we observed that patient fibroblast auxilin protein levels were significantly reduced when compared to controls, as previously reported.14 We found that patient fibroblast GAK levels were slightly, but not significantly, increased, whereas patient CSF GAK protein levels were significantly increased. Our findings support upregulation of brain GAK levels in DNAJC6 patients, partially compensating for auxilin reduction, as evident in the auxilin knockout mouse model.35

Diagnostic CSF neurotransmitter analysis revealed that levels of the stable dopamine metabolite (HVA) were either below the age-related reference ranges or close to the lower limit of normal in our patients, indicating impaired dopamine turnover. Indeed, CSF-HVA levels and HVA:5-HIAA ratios were comparable to those observed in TH deficiency, an inherited dopamine synthesis defect associated with central dopamine deficiency.20 In order to determine how DNAJC6 mutations may impact the dopaminergic system, we used patient CSF to analyze proteins involved in dopamine signaling and homeostasis. We observed that patient CSF had significantly reduced levels of VMAT, DAT, TH, and D2R when compared to controls.1231-FP-CIT SPECT (DaTScan) imaging additionally provides in vivo evidence of impaired DAT function in DNAJC6 patients. VMAT and DAT are both synaptic transporters recycled through clathrin-mediated endocytosis.38,39 The reduction in HVA associated with low VMAT/DAT protein levels may imply that the observed dopamine deficiency is associated with impaired clathrin-mediated neurotransmitter recycling. D2R is also postulated to be internalized through clathrin-mediated endocytosis.38,39 Neurons internalize receptors to adjust excitability and degrade, resensitize, and recycle desensitized receptors.38,39 DNAJC6 mutations thus may affect D2R protein levels and normal postsynaptic function. It is likely that presynaptic D2R autoreceptor function will also be affected, leading to aberrant TH regulation.40

Overall, our findings suggest that the mechanisms governing DNAJC6-associated parkinsonism are likely to be multifactorial. Another plausible explanation for the reduction in synaptic protein levels may be a result of neurodegeneration secondary to defective chaperone function. Auxilin and other J-chaperone proteins play a crucial role in regulating the folding and conformational change of proteins to maintain integrity in the neuron.41,42 Indeed, in the auxilin knockout mouse model, there is sequestration of clathrin cages in the cerebellum.35 With impaired auxilin function, a cumulative effect of sequestered misfolded proteins and accumulation of clathrin coat components in assembled coats and cages may lead to apoptotic cascades and neurodegeneration. There is growing interest in the role of such chaperone proteins in human disease and mutations in eight distinct J proteins (DNAJB2, DNAJB6, DNAJC5, DNAJC6, DNAJC12, DNAJC13, DNAJC19, and DNAJC29) have been described.33,44 Future research into such “chaperonopathies” may provide further insights into neurodegenerative disorders.

In conclusion, we report on a cohort of patients with previously unreported DNAJC6 mutations associated with early neurodevelopmental delay, juvenile parkinsonism, and neurological regression in the second decade of life. We further demonstrate disturbance of dopamine homeostasis in patient-derived CSF and report on a possible GAK-mediated compensatory mechanism for auxilin deficiency. Mutations in DNAJC6 are rare, but a likely under-recognized cause of parkinsonism-dystonia in infants and children. Elucidating the genetic diagnosis has important implications for families given that early diagnosis negates the need for extensive invasive investigations, facilitates treatment strategies, and aids genetic counseling for future pregnancies. The early clinical features and CSF neurotransmitter signature observed in our patients can mimic primary neurotransmitter disorders, and DNAJC6 mutations should thus be considered as a differential diagnosis. We observed reduced auxilin and increased GAK protein levels, suggesting a possible compensatory role for GAK in this condition. Study of CSF synaptic proteins suggest downstream effects on dopamine synthesis, recycling, homeostasis, and signaling that may result from a combination of primary auxilin deficiency and neurodegeneration. Abnormal synaptic vesicle dynamics are increasingly recognized as a disease mechanism in neurodegenerative parkinsonian disorders, and future research into elucidating the pathogenesis of such conditions will no doubt assist the development of novel targeted treatments.

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References