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Short communication

Neurotransmitter trafficking defect in a patient with clathrin (*CLTC*) variation presenting with intellectual disability and early-onset parkinsonism

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ABSTRACT

Introduction: Clathrins play a key role in endocytosis, recycling, and trafficking as well as the generation of presynaptic vesicles. We report a new clinical condition associated with a *de novo* variant in the *CLTC* gene, which encodes the clathrin heavy polypeptide.

Case report: This 30-year-old woman presented with a developmental disorder during childhood that progressed to mild cognitive decline in late childhood and relapsing-remitting hypokinetic-rigid syndrome with severe achalasia, weight loss, and mood disorder in adulthood. ^{123}I-Ioflupane SPECT was normal. Blood phenylalanine was slightly increased and *PAH* sequencing revealed compound heterozygosity for two variants, p.[Asp151Glu]:[Thr380Met]. CSF examination unexpectedly detected a remarkable reduction of homovanillic, 5-hydroxyindolacetic, and 5-methylthetrahydrofolic acids, which could not be ascribed to any alteration of tetrahydrobiopterin and related biogenic amine pathways.

Methods: Trio-based exome sequencing was performed.

Result: A *de novo* missense variant (c.2669C > T/p.Pro890Leu) was detected in *CLTC*. Treatment with biogenic amine precursors was ineffective, while the inhibitor of MAO-A selegiline resulted in persistent clinical improvement.

Conclusions: We suggest *CLTC* defect as a new disorder of biogenic amine trafficking, resulting in neurodevelopmental derangement and movement disorder. Neurotransmitter depletion in CSF may be a biomarker of this disease, and selegiline a possible treatment option.

1. Introduction

Recent studies have revealed a link between proteins that participate in synaptic vesicle recycling and trafficking and early-onset parkinsonism [1]. Clathrins play a key role in endocytosis, intracellular recycling, and trafficking as well as the generation of presynaptic vesicles [2]. Clathrin heavy chain polypeptide gene (*CLTC*) (OMIM 118955) is highly expressed in the brain, as well as in various human tissues related to growth and development (Unigene: Hs.491351). Variants in *CLTC* (OMIM 118955) have recently been associated with variable clinical conditions involving epilepsy and neurodevelopmental disorders as recurrent features [3,4].

We report on the clinical phenotype of a patient with a *de novo CLTC* variant presenting with developmental disorders that progressed to parkinsonism plus in adulthood. An incidental finding was a mild hyperphenylalaninemia.

2. Patient and methods

This 30-year-old woman, the first offspring of nonconsanguineous

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Table 1
Clinical and molecular features in subject with *CLTC* variants.

Patient and sex	Age (years/ months)	Genotype	Clinical Phenotype	Neurological features	Facial dysmorphisms	Other	References
1F	30y/0m	c.2669C > T (p.Pro890Leu)	Moderate ID Mood disorder	Rigid-hypokinetic syndrome, clumsiness, attention instability	NO	Achalasia	Present case
2F	3y/6m	c.2737_2738dup (p.Asp913Glufs*59)	GDD	Hydrocephalus, hypotonia, hyporeflexia, nystagmus	Low-set ears, depressed nasal bridge, anteverted nares	Hypothyroidism, Ganglioneuroblastoma	[3]
3F	1 y/2m	c.977_980del (p.Ser326Cysfs*8)	GDD, ADHD, Social skills	Hypotonia	Epicanthic folds	NR	[4]
4M	20y/5m	c.1660_1668del (p.Met554_Tyr556del)	Borderline IQ, learning disabilities	Progressive paraparesis with lower-limb spasticity, ataxia, myoclonus	NR	NR	[4]
5M	3y/2m	c.2669C > T (p.Pro890Leu)	GDD	Mild ataxia, myoclonyc jerk	NR	NR	[4]
6M	4y/7m	c.2669C > T (p.Pro890Leu)	GDD	Mild hypotonia, oral and motor apraxia	NR	NR	[4]
7M	16y/0m	c.3140T > C (p.Leu1047Pro)	Profound ID	DEE, microcephaly, Hypotonia, choreo-athetotic movements myoclonic jerks	Oval-shaped face, upslanted palpebral fissures, long nose, tented upper lip, wide mouth, big central incisors, microretrognathia	Severe gastroinstestinal reflux, progressive kyphoscoliosis	[4]
8M	4y/0m	c.3322T > C (p.Trp1108Arg)	Severe ID	Microcephaly, seizures, bilateral convergent squint, visual impairment spasticity, dystonia, myoclonus, quadriplegic cerebral palsy	NR	Gastrooesophageal reflux and was gastrostomy fed	[4]
9M	10y/7m	c.3595C > T (p.Gln1199*)	GDD, mild ID	NR	Congenital ptosis zygomatic hypoplasia and peri-orbital puffiness and discoloration	NR	[4]
10F	7y/5m	c.3621_3623del (p.Asp1207del)	GDD, severe ID	DEE, severe hypotonia, ataxia, oral and motor apraxia acquired microcephaly, seizures	Upslanting palpebral fissures, long filtrum with thin upper lip, prominent ears, scattered and slow growing hair	Severe gastroesophageal reflux	[4]
11F	23y/0m	c.4575dup (p.Glu1526Argfs*18)	GDD, moderate ID, obsessive compulsive symptoms	DEE, seizures, neonatal hypotonia,	Abnormally long teeth	Scoliosis, flat feet	[4]
12M	12y/10m	c.4605+2T > C	GDD, moderate ID, anxiety disorder	Hypotonia, sensorineural hearing loss	NR	Neonatal feeding difficulties	[4]
13F	6y/8m	c.4663C > T (p.Gln1555*)	GDD, severe ID	NR	Tall forehead, broad nasal tip, high arched palate, shallow philtrum and wide mouth	Bilateral 5th finger clinodactyly, mild dilatation of the right renal pelvis	[4]
14M	11y/0m	c.4667G > A (p.Trp1556*)	Moderate ID, behavioral problems	Neonatal hypotonia	NR	NR	[4]
15F	5y/1m	17q23.1q23.2 del	Developmental delay	NA	Hypertelorism, flattened nasal bridge and midface	Atrial septal defect; left sided esotropia; long, thin fingers and toes; musculoskeletal disease	[12]
16?	NR	c.2669 C > T (p.Pro890Leu)	NR	Abnormalities of nervous system	NR	Abnormalities of the head, neck	[13]
17?	NR	c.5022_5023insA (p.Met1675Asnfs*4)	Typical development	NR	NR	NR	[14]
18?	NR	17q21.3–24 dup	NR	Hydrocephalus	NR	NR	[15]

Legend: ? = not reported; GDD = global developmental delay; ID = intellectual disability; NR = not reported; DEE = developmental and epileptic encephalopathy.

Italian parents, was born after a normal pregnancy and caesarean section delivery due to breech presentation. Anthropometric measurements were within normal ranges from birth to adulthood. No dysmorphic features were detected throughout the follow-up. During early childhood, psychomotor delay and impairment of social skills were observed. At the age of 4 years, motor incoordination and proximal limb rigidity became evident. In the following years she experienced cognitive development stagnation, and at the age of 11, mild intellectual disability, drooling, and slight gait ataxia were reported on clinical examination. Brain MRI, EEG recording, and visual and auditory evoked potentials were normal. An extensive metabolic work-up at the age of 13 detected a mild increase in blood phenylalanine (Phe, 180–240 μmol/L in several repeated evaluations; normal 60–120 μmol/L), low urinary neopterin (0.15 mmol/mol creat.; normal 0.2–1.7 mmol/mol creat.), and low levels of homovanillic acid (HVA, 56 nmol/L; normal 133–551 nmol/L), 5-hydroxyindolacetic acid (5HIAA, 31 nmol/L; normal 74–163 nmol/L), and 5-methylthetrahydrofolic acid (5MTHF, 32.5 nmol/L; normal 41–117 nmol/L) with normal levels of biopterin and high Phe (145 μmol/L; normal < 20 μmol/L) in cerebrospinal fluid (CSF).

Molecular examination of genes involved in tetrahydrobiopterin (BH4) and related biogenic amine pathways (*GCH1, FMRT, PTS, SPR, DHPR, TH, DDC*) detected no alterations. *PAH* sequencing revealed compound heterozygosity for two missense variants, p.[Asp151Glu]:[Thr380Met]. Lacking any alternative diagnostic clues, the condition was regarded as an atypical presentation of a very mild *PAH* deficit [5]. At the age of 13, a BH4 loading test was found to normalize blood Phe and was added to the therapy, resulting in transient stabilization of the clinical condition. In the following years, the girl experienced further cognitive decline and a relapsing-remitting disorder characterized by hypo- and bradykinesia, dysphagia, hyporexia with weight loss, and mood disorder. CSF examination at the age of 23 years confirmed the pattern of neurotransmitter alterations previously detected (HVA, 41 nmol/L; normal 115–488 nmol/L; 5HIAA, 30 nmol/L; normal 66–141 nmol/L), showed low neopterin and biopterin (1.4 nmol/L; normal 9–20 nmol/L; and 1.9 nmol/L; normal 10–30 nmol/L, respectively) with normal 5-methylthetrahydrofolic acid.

L-dopa/carbidopa (10/1 mg/kg/day), 5-OH-tryptophan (4 mg/kg/day), pyridoxine (300 mg/day), and folinic acid (30 mg/week) therapy failed to improve her neurological condition, which remained characterized by frequent fluctuations between periods of (A) extreme bradypsychism, bradykinesia, anorexia with weight loss, and depression, and (B) periods of relative wellbeing with hypomanic behaviour. At the age of 30, brain MRI and ^{123}I-Ioflupane SPECT were normal. Unified Parkinson Disease Rating Scale (UPDRS) part III score was 12. Neuropsychological assessment showed a moderate intellectual disability (Wechsler Adult Intelligence Scale −IV edition: Full Scale IQ = 43), impaired sustained attention and semantic verbal fluency with relatively good adaptive skills (Vineland Adaptive Behaviour Scales II edition: Communication Domain = 69; Daily Living Skills Domain = 73; Socialization Domain = 68; Composite Score = 67). During a subsequent relapse, she experienced anorexia, postprandial vomiting and remarkable weight loss; an oesophageal function examination disclosed achalasia type I (100% absence of peristalsis) without alterations of intrinsic muscle structure at biopsy, suggesting the diagnosis of parkinsonism. A treatment with selegiline (7.5 mg/day) was then initiated, with improvement of rigid-hypokinetic syndrome, mood disorder, and oesophageal motility.

The observation that her apparently unaffected 24 year-old younger brother carried the same *PAH* molecular and biochemical status prompted to a trio-based exome sequencing analysis that identified a *de novo* missense variation, c.2669C > T (NM_004859.3, p.Pro890Leu) in *CLTC* (case 18052017 in Ref. [4]).

Written informed consent of patient and her parents was obtained. The study was approved by the local Ethics Committee.

3. Discussion

Table 1 summarizes the clinical characteristics of cases with heterozygous *de novo CLTC* variants reported thus far.

Facial dysmorphism, brain malformations, musculoskeletal defects, intellectual disability, and epileptic encephalopathies (EE) are the most common features [4]. The severity of neurological involvement is variable, ranging from learning disability to severe intellectual disability with or without EE. An interesting genotype-phenotype correlation can be found in *CLTC* defect: patients carrying variants in the first segment of clathrin light chain binding domain, exhibit refractory epilepsy; while patients with truncating variants on the protein C-terminus show hypotonia, developmental delay/intellectual disability [4]. Movement disorders have been reported in a few subjects as progressive paraparesis, ataxia with myoclonic jerks, spastic tetraplegia with dystonia and myoclonus (Table 1). The occurrence of the p.Pro890Leu variation in unrelated subjects suggests a missense clustering [4]. Two of these children presented with global developmental delay associated with mild ataxia (case 5 in Table 1) and mild hypotonia with oral and motor apraxia (case 6 in Table 1), respectively. Unfortunately, CSF examination has not been performed in any patient with the *CLTC* defect and the clinical follow-up of these two patients is limited in order to define the outcome of this condition.

A prolonged clinical follow-up characterizes the patient here reported. She presented with early-onset developmental delay, subsequent slow intellectual decline, and mood disorder and parkinsonism in adulthood. The finding of mildly elevated blood Phe was compatible with biallelic variants affecting *PAH*, while the remarkable depletion of neurotransmitters in CSF remained an unexplained biochemical feature. Reduced HVA and 5-HIAA has been reported in CSF of phenylketonuric patients, almost all with blood Phe above 600 μmol/L [6–8]. The levels of HVA and 5-HIAA are related to concurrent elevated concentrations of CSF and blood Phe and show remarkable interindividual variability [7]. In subjects with blood Phe lower than 360 μmol/L, biogenic amines in CSF are normal (VL and NB, personal observations). According to the present clinical experience, these levels of blood Phe do not entail any adverse effect on brain development and functioning and do not require treatment [9].

We propose that a defective CLTC protein, as such, may result in the depletion of biogenic amines in the brain by altering their synaptic turnover. A large number of proteins are involved in vesicle formation and recycling, docking, endocytosis and exocytosis, as well as in the mitochondrial ATP buffering machinery that supports all these processes [1]. The maintenance of synaptic transmission requires that vesicles are recycled after releasing neurotransmitters. The majority of synaptic vesicles appears to recycle through a pathway requiring clathrin, which is involved in the first steps of endocytic vesicle formation at the level of the synaptic terminal. Consistently, *in vitro* studies have provided evidence that a deficiency in clathrin function causes a progressive decline in synaptic transmission and results in massive bulk membrane retrieval [10]. Accordingly, treatment with dopamine and serotonin precursors in our patient failed to provide a beneficial effect, while selegiline resulted in improvement of neurological and psychiatric symptoms. This unusual response to treatment is consistent with the known effect of selegiline, which, inhibiting irreversibly monoamine oxidase A, enhances the synaptic effect of monoaminergic neurotransmitters.

Intellectual disability is the most common clinical feature of autosomal recessive defects of biogenic amine metabolism and transport, reflecting the crucial role of these neurotransmitters in the development and maintenance of higher mental functions [11].

In conclusion, we described a new clinical condition presenting with developmental disorders leading to intellectual disability and later-onset non-degenerative parkinsonism, with biogenic amine depletion as a probable pathogenic biochemical marker.

Contributors

FM, FN: substantial contributions to the acquisition, analysis, and interpretation of data for the work; drafting the work and revising it critically for important intellectual content. SB, MV, SP: acquisition, analysis and interpretation of data for the work; revising the work critically for important intellectual content. FFH, NB, AB, MT: interpretation of data for the work; revising the work critically for important intellectual content. VL: substantial contributions to the conception or design of the work, drafting the work and revising it critically for important intellectual content. All authors approved the final version of the manuscript for submission and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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