

SHORT REPORT

Variants in *CPLX1* in two families with autosomal-recessive severe infantile myoclonic epilepsy and ID

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For a large number of individuals with intellectual disability (ID), the molecular basis of the disorder is still unknown. However, whole-exome sequencing (WES) is providing more and more insights into the genetic landscape of ID. In the present study, we performed trio-based WES in 311 patients with unsolved ID and additional clinical features, and identified homozygous *CPLX1* variants in three patients with ID from two unrelated families. All displayed marked developmental delay and migrating myoclonic epilepsy, and one showed a cerebellar cleft in addition. The encoded protein, complexin 1, is crucially involved in neuronal synaptic regulation, and homozygous *Cplx1* knockout mice have the earliest known onset of ataxia seen in a mouse model. Recently, a homozygous truncating variant in *CPLX1* was suggested to be causative for migrating epilepsy and structural brain abnormalities. ID was not reported although it cannot be completely ruled out. However, the currently limited knowledge on *CPLX1* suggests that loss of complexin 1 function may lead to a complex but variable clinical phenotype, and our findings encourage further investigations of *CPLX1* in patients with ID, developmental delay and myoclonic epilepsy to unravel the phenotypic spectrum of carriers of *CPLX1* variants.

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INTRODUCTION

CPLX1 encodes complexin 1, a neuronal protein, which belongs to the highly conserved complexin protein family. Presumably, *CPLX1* regulates vesicle fusogenicity by interfering with the C-terminal stability of the SNARE complex (soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor), which is an essential effector of the synaptic vesicle fusion process and the neurotransmitter release cascade.¹ Complexin functions at presynaptic nerve terminals to inhibit spontaneous SNARE-mediated synaptic vesicle exocytosis, while enhancing stimulated neurotransmitter release.² Variation of *CPLX1* is thought to be responsible for loss of its inhibitory function and to enhance spontaneous neurotransmitter release.² Studies with knockout mice indicate that *Cplx1* is implicated in neurological and psychiatric disorders but without ID/developmental delay.^{3–5} Recently, Karaca *et al.* identified in a comprehensive WES study in a large set of consanguineous families with neurodegenerative disorders and brain malformations one homozygous nonsense variant, p.(Glu108Ter) in *CPLX1* in two sisters with malignant migrating epilepsy and cortical atrophy. ID was not reported.⁶

The individuals described here were detected in a whole-exome sequencing (WES) cohort of 311 individuals with unexplained ID (manuscript in preparation). Inclusion criteria to this cohort were developmental delay (DD)/ID with an IQ <70 either as non-syndromic ID (isolated, without additional features, present in 88 out of 311 individuals) or as syndromic ID with additional features (for example craniofacial dysmorphism, organ malformation, aberrant

body measurements, etc.) which could not be attributed to a clinically recognizable syndrome by experienced clinical geneticists (in 223 out of 311 individuals). In addition, clinically relevant chromosomal aberrations had to be excluded previously by chromosomal microarray analysis, and fragile-X testing had to be normal. The aim of this study was the analysis of these individuals by WES to provide insight into the genetic basis and to find new candidate genes for ID.

MATERIALS AND METHODS

In this study, we examined 311 patients with unexplained ID. The individuals were clinically assessed by experienced clinical geneticists. Ethics approval was obtained from the respective ethics committees (Ethics vote 5360/13 for the Technische Universität München and 08-3663 for the Universität Duisburg-Essen), and all participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki Principles. Trio-based WES was performed as described in Kuechler *et al.*⁷ and Sanger sequencing was performed to verify the variants and to perform segregation analysis (Supplementary Material).

Exons are numbered like in the Ensembl 87 entry ENST00000304062.10 (http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000168993;r=4:784957-826198;t=ENST00000304062). Variants were deposited at www.LOVD.nl/CPLX1 (patients ID 95415, 95416, 95418).

RESULTS

Patient 1 and patient 2 are sisters, the third and fifth children of healthy consanguineous parents originating from Lebanon. The parents are first-degree cousins.

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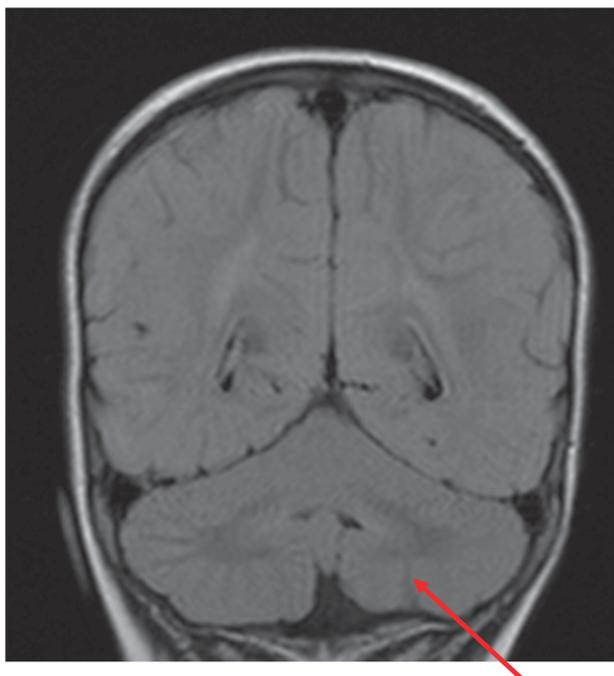


Figure 1 MRI of patient 1 at the age of 12 months. Axial Inversion recovery (left and middle) and coronal FLAIR (right) sequences depicting a small cleft of lobule VIII (arrows) of the left cerebellar hemisphere.

Patient 1 was born in 2005 spontaneously after an uneventful pregnancy at week 41 of gestation. Birth measurements were normal (length: 53 cm (+0.36 SD), weight: 3550 g (-0.07 SD), OFC: 37.5 cm (+1.77 SD)). The first clinical signs of the disease appeared when she was 6 weeks old. She first experienced epileptic spasms. Next, she developed striking seizure exacerbations and migrating myoclonic epilepsy. Seizures occurred more than 100 times daily. Beyond the progressive myoclonic seizures, the diagnosis of generalized epilepsy was established at the age of three years. Seizures were pharmacoresistant and did not respond to ketogenic diet. Electroencephalography (EEG) repeatedly showed generalized epileptiform activity and hyperexcitability. MRI of the brain performed at age 12, 24 and 36 months showed a small cleft of lobule VIII of the left cerebellar hemisphere with malorientation of the adjacent cerebellar folia (Figure 1). No other pathologic findings were noted, especially no cortical atrophy. She was unable to sit unsupported, to crawl or to walk. No head control was achieved. Speech was absent. Assessment at age 2⁵/₁₂ years showed massive hypotonia, normal measurements (height: 88 cm (-0.82 SD), weight: 11 kg (-1.32 SD), OFC: 47.5 cm (-1.26 SD)) and mild craniofacial dysmorphism (Figure 2). She passed away at 8 years of age most likely due to a severe seizure episode during sleep.

Patient 2 was born in 2013. Pregnancy and birth at week 40 were uneventful. Birth measurements were normal (length: 51 cm (-0.32 SD), weight: 3490 g (+0.04 SD), OFC: 35 cm (+0.08 SD)). First non-febrile related epileptic spasms occurred at age 2½ months. During course of disease seizure types were changing. At the age of 6 months, the girl presented with predominantly myoclonic epilepsy. These were progressive and occurred more than 150 times per day. Respiratory infections as well as febrile infections were triggers, leading to generalized seizures. Epilepsy did not respond to antiepileptic drugs. EEG showed generalized spikes and waves as well as hyperexcitability.

An MRI scan of the brain performed at age 2 months and ultrasonography of the brain at age 2 and 8 months did not detect any abnormalities, especially no cortical atrophy. At physical examination at around 33 months, she was unable to sit unsupported, to crawl, to walk and to speak. OFC (50 cm, +0.61 SD) and height (90 cm, -0.85 SD) were found to be normal, whereas her weight (9.4 kg) diminished relatively (-2.95 SD). Craniofacial dysmorphism comprising hypertelorism, bowed eyebrows, flat midface and thin upper lip were noted (Figure 2).

WES detected the homozygous nonsense variant chr4:g.786 591G>T (GRCh38/hg38); NM_006651.2:c.315C>A; NP_006642.1:p.(Cys105Ter) in Exon 4 of *CPLX1* in the affected sisters. The unaffected parents were found to be heterozygous for this variant.

Patient 3 was born in 2005 as second son of healthy distantly consanguineous parents originating from the Turkmen population. Pregnancy with patient 3 was impaired by placental insufficiency, resulting in a preterm delivery at week 31 of gestation. Birth measurements were normal (length: 39 cm (-0.85 SD), weight: 1350 g (-0.85 SD), OFC: 28 cm (-0.79 SD)). Fetal outcome was poor. He displayed infant respiratory distress syndrome, hypotonia and feeding difficulties (no sucking reflex). Beginning from the first months postpartum, EEG revealed marked persistent generalized seizure activity despite the absence of overt clinical seizure episodes. The first seizure event was recognized in a non-febrile context at 2 years. The boy experienced first myoclonic seizures. During course of disease, seizures switched to alternately myoclonic, tonic and generalized seizures, which occurred >50 times a day. Treatment with distinct anticonvulsants showed no improvement of patient's clinical signs. He experienced cerebral movement disorder and cerebral palsy. An MRI scan of the brain at around 17 months of age was normal and no cortical atrophy was noted. All milestones of motor and speech development were delayed. He was able to sit at 36 months and to walk with support at age 6 years. Speech was limited to nearly absent. On physical examination at age 7 years, weight (18.5 kg, -2.03 SD) was in the normal range, whereas height (110–112 cm, -2.68 SD) and OFC (48.5 cm, -3.11 SD) were decreased. He presented with mild craniofacial dysmorphism (Figure 2).

WES identified homozygosity for the variant chr4:g.786,524G>T (GRCh38/hg38); NM_006651.2:c.382C>A; NP_006642.1:p.(Leu128Met) in exon 4 of *CLPX1* in the patient and heterozygosity in the unaffected parents.

This variant is known as rs371709824, and six heterozygous but no homozygous allele carriers among 33 197 individuals are listed in the ExAC database (<http://exac.broadinstitute.org>). Three *in silico* tools (SIFT v5.2.2, PolyPhen-2 and MutationTaster) predict this variant as possibly disease causing. The amino acid exchange affects a highly conserved residue of the C-terminal domain (CTD) of complexin.¹ The CTD is required to bind complexin to SNARE-mediated synaptic vesicles (SVs) by membrane interaction and is of particular importance for its inhibitory function.² Thus, we consider the p.(Leu128Met) as the causative variant in patient 3.

Experimental data for WORM COMPLEXIN indicates that two motifs in the C-terminal domain of CPXL1 are involved in vesicle binding: The first motif spanning residues 110–124 mediates a weak interaction, whereas the second motif comprising residues (128–143) mediates a tight interaction. Both motifs combine to localize and gate complexin function on SVs. (data from Snead *et al.*)² The Cys105* variant lacks both of these interaction motifs and the Leu128Met variant exhibits a variant of a conserved residue within the second motif responsible for tight binding.



Figure 2 Clinical photographs of the patients with homozygous *CPLX1* variants. (a–c) Patient 1 at the age of 2⁵/₁₂ years with hypertelorism, bowed eyebrows, flat midface, long philtrum with thin upper lip and full cheeks. Normal left foot. (d–g) Patient 2 at the age of 2⁹/₁₂ years with hypertelorism, bowed eyebrows, flat midface, thin upper lip. Underriding second toe. Normal hands. (h–k) Patient 3 at the age of seven years with marked eyebrows, large nose with bulbous tip, long philtrum with thin upper lip. Underriding third toe. Normal hands.

The clinical features of the patients with homozygous *CPLX1* variants are summarized in Table 1.

DISCUSSION

Recently, Karaca *et al.*⁶ reported two sisters with homozygous loss-of-function variants in *CPLX1* who displayed migrating epilepsy and cortical atrophy without reported ID. The present study is the first to identify homozygous *CPLX1* variants in patients with severe myoclonic epilepsy and in addition confirmed marked developmental delay and ID. No further variants were identified.

Although one might conclude that ID in our patients is completely dependent on myoclonic encephalopathy,⁸ we do not believe that this explanation is sufficient as (i) developmental delay/ID in our patients is remarkably severe and (ii) patient 3 showed ID and developmental delay before onset of epilepsy. This rather points to overlap in terms of the genetic etiology of migrating epilepsy and ID and suggests shared pathological pathways. This hypothesis is supported by studies of *Cplx1*-deficient mice, which showed marked abnormalities in complex behavior, deficits in sensory and motor skills and suffered from seizures.^{9–11} However, the potential functional connection remains unclear.

Complexins are small presynaptic proteins that bind to the SNARE complex where they modulate neurotransmitter release. *Cplx1* knock-out (*Cplx1*^{-/-}) mice developed a strong early onset ataxia in the absence of cerebellar degeneration.⁹ However, Kielar and colleagues

reported selective volume loss from the thalamus and cerebellum and atrophy of the molecular layer but no neuron loss in *Cplx1*^{-/-} mice.⁵

No such findings were noted in our patients or in the patients reported by Karaca *et al.* However, in contrast to the findings in the mouse model and our findings, Karaca *et al.*⁶ reported cortical atrophy in the homozygous *CPLX1* variant carriers. Although we cannot completely exclude that cortical atrophy was not detectable at time of investigation in our patients its presence is rather unlikely because patient 1 was followed-up by MRI brain scans during a period of three years and patient 2 did not show abnormalities pointing to specific brain damage at neuropsychiatric examination after a disease course of nearly three years. In patient 1, a small cleft of the left cerebellar hemisphere was noted. Cerebellar clefts are rare and thought to be of primary disruptive nature, most often caused by prenatal cerebellar hemorrhage.¹² In conclusion, the small number of reported individuals with presumably pathogenic *CPLX1* variants impedes a clear statement on whether the cortical atrophy in the patients of Karaca *et al.* and the cerebellar cleft in our patient 1 may either be coincidental findings or rare side features caused by the *CPLX1* deficiency.

We analyzed two independent screening cohorts, comprising 25, respectively 96 index cases with ID and epileptic encephalopathy, respectively spasms, and pedigrees compatible with autosomal-recessive inheritance (Supplementary Material). Among these cases, we did not detect *CPLX1* variants or any other pathogenic/likely

Table 1 Clinical features of the patients with homozygous *CPLX1* variants

	Patient 1	Patient 2	Patient 3
Gene with pathogenic variant	<i>CPLX1</i> c.315C>A;p.(Cys105Ter)	<i>CPLX1</i> c.315C>A;p.(Cys105Ter)	<i>CPLX1</i> c.382C>A;p.(Leu128Met)
Age at diagnosis (years)	Posthumously	2.5	10
Gender	Female	Female	Male
Consanguinity in parents	+	+	+
	First-degree cousins	First-degree cousins	Grandparents first-degree cousins
Age of mother at birth	24	32	27
Age of father at birth	27	35	27
ID	++	++	++
Sat/walked independently [mo]	n.r./n.r.	n.r./n.r.	36/n.r.
First words (months)	No speech	No speech	Speech nearly absent
Hypotonia	++	++	+
Seizures	++	++	++
Epileptic Spasms (weeks)	+ [6]	–	–
Myoclonic seizures (months)	+ [6–8]	+ [2–6]	+ [24]
Frequency of myoclonic seizures (per day)	100	> 150	> 50
Generalized seizures (months)	+ [36]	+ [12]	+ [24–36]
Frequent infections	+	+	–
Feeding problems	+	+	+
Birth (weeks)	41	40	31
Weight (g)	3350	3490	1350
Length (cm)	53	51	39
OFC (cm)	37.5	35	28
Age at examination (years)	2.5	2.9	7
Weight (kg)	11	9.4	18.5
Height (cm)	88	90	110–112
OFC (cm)	47.5	50	48.5
Cranofacial anomalies	+	+	+
	Hypertelorism, bowed eyebrows, flat midface, long philtrum, thin upper lip, full cheeks	Hypertelorism, bowed eyebrows, flat midface, thin upper lip	Marked eyebrows, large nose with bulbous tip, long philtrum, thin upper lip
Cleft palate	–	–	+
Internal abnormalities	–	–	–
Bilateral conductive hearing loss	–	–	+
Myopia	–	–	+
Strabismus	–	–	+
Hypothyreosis	–	+	–
Brain abnormalities	Unilateral cerebellar cleft	–	–

Reference cDNA sequence: NM_006651.2.

pathogenic variants or variants of unknown significance in other genes. Due to the rarity of the disorder and the widely dispersed, but very small, patient base no further screening cohort was at our disposal. Nevertheless, our results point to an involvement of *CPLX1* in the etiology of both, ID and migrating epilepsy and highlight the need of investigation of *CPLX1* in additional, enlarged cohorts of patients with either ID, epilepsy, brain structural abnormalities or combined abnormalities to define the clinical spectrum of *CPLX1* variant carriers.

A more precise elucidation of the disease pathophysiology would be a groundbreaking achievement for the understanding of complex neuronal processes and would shed further light on the biology of ID.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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