**Early-onset severe spinocerebellar ataxia 42 with neurodevelopmental deficits
(SCA42ND): case report, pharmacological trial and literature review**

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ABSTRACT

Early-onset severe spinocerebellar ataxia 42 with neurodevelopmental deficits (SCA42ND, MIM#604065) is an ultrarare autosomal dominant syndrome related to *de novo* *CACNA1G* gain-of-function mutations. All patients with SCA42ND show cerebellar atrophy and/or hypoplasia on neuroimaging and share common features such as dysmorphic features, global developmental delay, and axial hypotonia, all manifesting within the first year of life. To date, only ten patients with SCA42ND have been reported with functionally confirmed gain-of-function variants, bearing either of two recurrent mutations. We describe a girl with congenital ataxia, without epilepsy, and a *de novo* p.Ala961Thr mutation in *CACNA1G*. We review the published subjects with the aim of better characterizing the dysmorphic features that may be crucial for clinical recognition of SCA42ND. Cerebellar atrophy, together with digital anomalies, particularly broad thumbs and/or halluces, should lead to clinical suspicion of this disease. We describe the first pharmacological attempt to treat a patient with SCA42ND using zonisamide, an antiepileptic drug with T-type channel blocker activity, in an off-label indication using an itemized study protocol. No efficacy was observed at the dose tested. However, she showed a maintained neurodevelopmental improvement.

Keywords: Early-onset severe spinocerebellar ataxia 42 with neurodevelopmental deficits (SCA42ND); *CACNA1G*; T-type calcium channel blockers; zonisamide; broad hallux.

INTRODUCTION

Early-onset severe spinocerebellar ataxia 42 with neurodevelopmental deficits (SCA42ND, MIM#604065) is an ultrarare autosomal dominant syndrome first described in 2018 by Chemin et al. related to *de novo* *CACNA1G* gain-of-function mutations [Chemin et al., 2018]. This syndrome is allelic to spinocerebellar ataxia 42 (SCA42, MIM#616795), an autosomal dominant adult-onset neurodegenerative disorder characterized by slowly progressive ataxia accompanied by other cerebellar signs [Morino et al., 2015]. All patients with SCA42ND have cerebellar atrophy and/or hypoplasia on magnetic resonance imaging (MRI) and share common features such as global developmental delay and axial hypotonia, manifesting within the first year of life [Chemin et al., 2018; Barresi et al., 2020; Kunii et al., 2020]. They also exhibit dysmorphic facial features and digital anomalies. Two recurrent *CACNA1G* mutations, p.Ala961Thr and p.Met1531Val, have been described in patients with SCA42ND. Moreover, Kunii et al. described a patient with an unreported heterozygous variant in *CACNA1G* (p.Ile1273Phe) whose pathogenicity remained undetermined after electrophysiological evaluation [Kunii et al., 2020]. The description of the gain-of-function effect caused by the two recurrent mutations in the T-type $\text{Ca}_v3.1$ channel raised the hypothesis that T-type channel blockers might have a therapeutic potential [Chemin et al., 2018], but such treatments have not been tested to date. However, some common antiepileptic drugs are known to act on T-type calcium channels [Powell et al., 2014]. Here we describe a girl with a *de novo* p.Ala961Thr mutation in *CACNA1G* and review the previously published subjects, in an attempt to better characterize the clinical picture to identify the key features for clinical recognition of SCA42ND. We also report the lack of efficacy of off-label administration zonisamide (ZNS) in the context of an itemized study protocol.

CASE REPORT

The patient, a 25-month-old girl, was born after uneventful pregnancy and delivery of healthy, non-consanguineous Spanish parents. She was born at 41 weeks and 4 days of gestation with a weight of 3200 g (-0.3 standard deviation (SD)), a length of 50 cm

(0.0 SD), and an occipitofrontal circumference (OFC) of 35 cm (0.2 SD). The neonatal course was uneventful. At 4 months of age she was referred to early intervention services and pediatric ophthalmology due to axial hypotonia and strabismus. She achieved head control at 4 months of age. She was referred to our hospital at the age of 18 months, with normal height and weight but an acquired microcephaly (OFC 44 cm; -2.5 SD). She showed dysmorphic features including sparse, thin hair, high frontal hairline, broad forehead, deep-set eyes, upslanted palpebral fissures, retrognathia, and wide mouth (see all Human Phenotype Ontology [HPO] terms and iconographic details in Figure 1). Limb anomalies were also observed, including broad hallux, diffuse clubbing of toes, ulnar deviation of the second finger, prominent interdigital folds, fifth-finger clinodactyly, and thigh lipodystrophy (without inverted nipples); she presents a supernumerary nipple. Neurological examination revealed esotropia, axial hypotonia, normal strength and deep tendon reflexes, and no pyramidal signs. At that time, she had social smile, babbled, held hands together in the midline, and was unable to sit unsupported.

MRI revealed widening of the interfolia spaces predominantly in the superior vermis, denoting incipient cerebellar atrophy (Figure 1). General laboratory tests yielded normal results. Standard metabolic work-up for children with cerebellar atrophy (including isoelectric focusing of serum transferrin) showed normal results. A video-electroencephalogram and an array CGH were also normal. Trio-based whole exome sequencing (WES) identified a likely pathogenic *de novo* heterozygous missense mutation in the *CACNA1G* gene (NM_001256324, c.2881G>A, p.Ala961Thr), previously reported and classified as gain-of-function [Chemin et al., 2018].

Considering the unexplored potential benefit of T-type calcium channel blockers on SCA42ND [Matar et al., 2009; Chemin et al., 2018], ZNS was administered as an off-label treatment in the context of a 6-month study protocol accepted by the Local Pharmacy Commission. Still, although ZNS has been the last approved drug molecule with T-type channel activity for clinical use [Weiss and Zamponi, 2019], it also targets other channels and proteins to produce neuroprotection [Kopecky et al., 2014]. Baseline

evaluation included physical exam, cognitive assessment (Bayley Scales of Infant and Toddler Development III), language assessment (Communication and Symbolic Behavior Scales Developmental Profile (CSBS DP) Infant-Toddler Checklist), and motor assessment (Gross Motor Function Measure-88, GMFM-88). For the treatment of epilepsy, ZNS is usually initiated at 1-2 mg/kg/day and then increased until seizure control is reached (usual childhood maintenance dose is 4-8 mg/kg/day) [Panayiotopoulos, 2010]; given that our patient had no epilepsy, in the absence of adverse effects, our target dose was 2 mg/kg/day to be reached after slow titration at the 6th week. We selected that dose because low doses of 25 mg per day have been reported to be effective in the only previous report of an adult patient with SCA42 and severe tremor [Hara et al., 2019]. At the 14th week, ZNS would be continued or discontinued, depending on the clinical response, and a final evaluation after withdrawal would also be informative. Follow-up evaluation included: vital signs, cognitive, social skills and language, and gross and fine motor at 5, 7, 14 and 24 weeks (unexpectedly the final evaluation had to be postponed until the 30th week due to COVID-19 isolation recommendations). Results are detailed in Table 1.

Written informed consent for genetic testing, publication, and off-label use of ZNS was obtained from the parents of the patient. Treatment with ZNS with slow titration was initiated at 17 months and 24 days of age at an initial dose of 0.2 mg/kg/day (once daily). Due to the absence of adverse effects, the ZNS dose was increased up to 2 mg/kg/day (twice daily) at the 6th week of the study protocol. At the 14th week, no significant improvement was observed in the main outcomes; physical therapists reported a mild improvement in axial tone, gaze control, cephalic control, and object manipulation with both hands, but GMFM-88 total score only increased 4.5% above the change expected due to spontaneous development (see Table 1). Consequently, treatment with ZNS was withdrawn at that point. At the 30th week, without ZNS treatment, an overall improvement was observed in the main outcomes.

DISCUSSION

Here we report the first Spanish patient with SCA42ND and review the previously published cases (**Supplementary Table 1**) in an attempt to better document the homogeneous clinical characteristics of this disease and to identify the dysmorphic features and, particularly, digital anomalies that may be helpful clues for the diagnosis. This is the eleventh individual with SCA42ND reported in the literature [Chemin et al., 2018; Barresi et al., 2020; Kunii et al., 2020]. For the first time, we describe the experience with off-label administration of ZNS in the context of an itemized study protocol.

All SCA42ND patients have been diagnosed in the context of trio-based WES analysis in cohorts of patients with cerebellar atrophy.

Overall, 3/11 patients are male and median age at initial signs/symptoms was 3 months of age (range 2 days to 6 months of age) (for more detail see **Supplementary Table 1**). All patients show severe-to-profound developmental delay/intellectual disability with impairments in motor, language and social domains. Epilepsy has been reported in all the patients harboring the p.Met1531Val mutation (3/3), most of them with early-onset epileptic encephalopathy. In contrast, only one of the 8 patients harboring the p.Ala961Thr mutation presents epilepsy. The most common neurological findings are: cerebellar ataxia (10/11), distal hypertonia (8/11), axial hypotonia (8/11), strabismus (7/11), dysmetria (3/11) and nystagmus (2/11). All patients show MRI abnormalities, with the most common being global cerebellar atrophy or vermis atrophy.

In contrast with the relative non-specificity of these neurological manifestations, some characteristic dysmorphic features warrant special consideration, so as to avoid unnecessary delay in diagnosis. The most common dysmorphic facial features in the nine patients with available information are: microcephaly (3/9), hair frontal hairline (9/9), sparse and/or thin hair (9/9), frontal bossing and/or broad forehead (7/9), deep-set eyes (5/9), upslanted palpebral fissures (5/9), short palpebral fissures (3/9), thin nasal pyramid (3/9), prominent columella (2/9), hypoplastic alae (4/9), wide mouth (4/9), downturned corners of mouth (4/9), prognathism (3/9), retrognathia or micrognathia (2/9) and large

earlobes (4/9). Regarding digital anomalies, 6/9 patients show 5th and/or 2nd finger clinodactyly, 4/9 broad thumbs and 4/9 broad halluces. We also observed thigh lipodystrophy, which, together with the cerebellar syndrome, raised the differential diagnosis with phosphomannomutase deficiency (PMM2-CDG) [Grünwald et al., 2002]. Overall, 8/11 patients with SCA42ND have the recurrent p.Ala961Thr mutation (including the present case) and 3/11 have the recurrent p.Met1531Val mutation, both located within the transmembrane segment S6 of *CACNA1G* [Chemin et al., 2018; Barresi et al., 2020] and leading to a gain-of-function effect. This raised the hypothesis that T-type channel blockers would have a therapeutic potential [Chemin et al., 2018]. Interestingly, the blockade action of ZNS on T-type channels has been reported to rely on its capacity to enhance channel inactivation [Kito et al., 1996], therefore specifically counteracting one of the gain-of-function effects induced by mutation p.Ala961Thr and justifying its therapeutic use. Given that our patient did not have epilepsy, we considered off-label administration of ZNS for its known calcium channel blocker effect [Powell et al., 2014]. We report an itemized study protocol to objectively measure the benefit of a repurposed treatment administered to a single patient. Given the difficulties in launching clinical trials in ultrarare diseases, this approach might be a way to produce preliminary data, always considering risks and benefits for the patient. In order to objectively measure the potential benefit of a treatment in the developing child, it is important to use age-adjusted scales to take into consideration the expected change due to spontaneous development.

We did not observe a significant improvement in the main outcomes with the administration of ZNS at the dose tested. Its low blockade efficiency on human Cav3.1 channels (compared to that over T-type Cav3.2) [Matar et al., 2009] may explain the insufficient efficacy of ZNS treatment in this study. Therefore, future studies might consider the administration of higher doses of ZNS or other drugs with known calcium channel blocker effect, such as ethosuximide and valproate [Powell et al., 2014]. These antiepileptic drugs should also be taken into consideration in patients with *CACNA1G*-related epilepsy, with an eye toward an etiopathogenic effect. In this sense, for this type

of ultra-rare disorders, the use of animal models is likely to be also required to fully test and demonstrate the effect of such therapies.

To conclude, we suggest that cerebellar atrophy together with digital anomalies such as broad thumbs and/or halluces should lead to clinical suspicion of this disease, for which targeted therapies are likely to appear in the coming years.

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Figure legend.

Figure 1. Clinical features and brain MRI of the proband at 19 months of age.

Human phenotype ontology (HPO) codes are included on the left side. **A.** Front view of the face. **B.** Lateral view of the face. **C.** Posterior view of the head. **D.** Front view of the thorax. **E.** Left thigh. **F.** Hands. **G.** Feet. **H and I.** Brain MRI T1-weighted sagittal image, and T2-weighted coronal image, respectively, show the widening of interfolia spaces (see arrows).

Table 1. Results of the cognitive, language, and motor assessment during the study protocol.

Timeline	Baseline assessment	5 weeks	7 weeks	14 weeks	30 weeks
ZNS dosage	No treatment	1.2 mg/kg/d	2 mg/Kg/d	2 mg/kg/d	No ZNS

ZNS titration	After assessment started dosage at 0.2 mg/kg/d; slow titration, reached 1.2 mg/kg/d at the 4 th week	Increased up to 2 mg/kg/d, reached at the 6 th week	Maintained	ZNS was stopped abruptly after assessment	
Age	17 m 24 d	19 m 10 d	19 m 24 d	21 m 15 d	25 m 5 d
Cognitive assessment: Bayley-III cognitive subtest					
Direct score	25	23	24	25	32
Composite score	55	55	55	55	55
Percentile	0.1	0.1	0.1	0.1	0.1
Equivalent age	5 m 10 d	5 m	5 m	5 m 10 d	8 m
Language assessment: CSBS DP Infant-Toddler Checklist (concern)					
Emotion and eye gaze	6	7	7	8	8
Communication	6	7	8	7	7
Gestures	1	2	2	2	4
Social composite	13 (concern)	16 (concern)	17 (concern)	17 (concern)	19 (NOT concern)
Sounds	4	5	7	7	7
Words	0	0	0	0	0
Speech composite	4 (concern)	5 (concern)	7 (concern)	7 (concern)	7 (concern)
Understanding	4	4	4	3	4
Object use	3	3	3	3	3
Symbolic composite	7 (concern)	7 (concern)	7 (concern)	6 (concern)	7 (concern)
Total	24 (concern)	28 (concern)	31 (concern)	30 (concern)	33 (concern)
Motor assessment: GMFM-88[†]					
Lying & rolling domain score	25	31	31	31	51
Lying & rolling domain percentile	49.0	60.7	60.7	60.7	49.0
Sitting domain score	7	13	13	13	21
Sitting domain percentile	11.6	21.6	21.6	21.6	35.0
Total score	12.00%	16.46%	16.46%	16.46%	27.00%

CSBS DP, Communication and Symbolic Behavior Scales - Developmental Profile; GMFM-88, Gross Motor Function Measure-88; d: days; m: months; ZNS: zonisamide.

[†]Gross Motor Function Measure (GMFM-88) consists of 88 items categorized into five domains: A. Lying and Rolling; B. Sitting; C. Crawling and Kneeling; D. Standing; E. Walking, Running and Jumping. Results in the last three domains are not shown as they were always 0.

Supplementary Table 1. Summary of clinical, EEG and brain MRI data. Adapted from Barresi et al. [Barresi et al., 2020].

ARTICLE	[Chemin et al., 2018]				[Barresi et al., 2020]	
Patient	Subject 1	Subject 2	Subject 3	Subject 4	Subject 1	Subject 2
Nucleotide change (NM_018896.4)	c.2881G>A	c.4591A>G	c.2881G>A	c.2881G>A	c.2881G>A	c.2881G>A
Amino acid change	p.Ala961Thr	p.Met1531Val	p.Ala961Thr	p.Ala961Thr	p.Ala961Thr	p.Ala961Thr
Inheritance	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>
Age at last follow-up, years	11	13	3	8	11	2
Gender	Female	Female	Female	Female	Male	Female
Weight at birth, kg (SD)	2.4 (-2 SD)	2.8 (-1 SD)	2.9 (-1 SD)	2.9 (-1 SD)	2	4 (N/A)
Length at birth, cm (SD)	43 (-2 SD)	49 (N/A)	49.5 (N/A)	49.5 (N/A)	N/A	49 (N/A)
HC at birth, cm (SD)	32 (-2 SD)	N/A	35 (N/A)	N/A	N/A	35 (N/A)
Microcephaly (last exam)	+	+	-	-	-	-
Age at initial symptoms	3 months	8 days	7 days	4 months	< 1 month	6 months
DYSMORPHIC FEATURES						
Sparse and/or thin hair	+	+	+	+	+	+
High frontal hairline	+	+	+	+	+	+
Frontal bossing and/or broad forehead	N/A	N/A	+	+	+	+
Hypertelorism	N/A	+	N/A	N/A	Mild	Mild
Deep-set eyes	N/A	N/A	N/A	N/A	+	+
Palpebral fissures	Short, upslanted	Upslanted	N/A	Upslanted	Short	Upslanted
Ears	Anteverted	N/A	N/A	N/A	Large earlobe	Anterior creases, large earlobe
Thin nasal pyramid	N/A	N/A	N/A	N/A	+	+
Prominent columella	N/A	N/A	N/A	N/A	-	-
Hypoplastic alae	N/A	N/A	N/A	N/A	+	+
Wide mouth	N/A	N/A	N/A	N/A	+	+
Downturned mouth corners	N/A	N/A	N/A	N/A	+	+
Prognathism	N/A	+	N/A	+	-	+
Retrognathia or micrognathia	N/A	N/A	N/A	N/A	-	-
Clinodactyly	+	+	-	-	+	+
Broad thumbs	-	-	-	+	+	+
Broad halluces	-	-	-	+	-	+
Other (hands and feet)	Short	Short	Syndactyly	Fetal pads	-	Fetal pads
NEURODEVELOPMENT						
ID/DD	Severe ID	Profound ID	Severe DD	Profound ID	Severe ID	Severe DD
Motor delay	+	+	+	+	+	+
Language	Delayed	Delayed	Delayed	Delayed	+	+
Social	Delayed	Delayed	Delayed	Delayed	+	+
Seizures (onset)	-	+	+	-	-	-
NEUROLOGICAL FINDINGS						
Distal hypertonia	-	+	+	+	+	+
Axial hypotonia	+	+	+	+	-	+
Deep tendon reflexes	Normal	Normal	Normal	Normal	Normal	Normal
Nystagmus	-	-	-	-	+	-
Dysmetria	+	N/A	N/A	+	N/A	-
Cerebellar ataxia	+	+	+	+	+	-
Ophthalmological findings	Apraxia, strabismus	Apraxia, strabismus	Hyperopia	Apraxia, strabismus	Esotropia, Doll's eyes, pale papillae	Exotropia
INVESTIGATIONS						
EEG	Normal	Normal	Multifocal epileptic discharges	Slow background activity	Sharp waves with irregular morphology	Normal
MRI	Global cerebellar atrophy	Vermis atrophy	Global cerebellar atrophy	Cerebellar hypoplasia	Vermis atrophy Hyperintense dentate nuclei	Inferior vermis hypoplasia
Thyroid function tests	-	+	-	-	-	-
Cardiovascular	-	-	-	Prolonged QT	Atrial septal defect	-

ARTICLE	[Barresi et al., 2020]		[Kunii et al., 2020]*		Present study
Patient	Subject 3	Subject 4	Subject 1	Subject 3	Subject 1
Nucleotide change (NM_018896.4)	c.2881G>A	c.4591A>G	c.2881G>A	c.4591A>G	c.2881G>A
Amino acid change	p.Ala961Thr	p.Met1531Val	p.Ala961Thr	p.Met1531Val	p.Ala961Thr
Inheritance	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>
Age at last follow-up, years	3	1	11	8	2
Gender	Male	Male	Female	Female	Female
Weight at birth, kg (SD)	3 (N/A)	3 (N/A)	N/A	N/A	3.2 (-0.3 SD)
Length at birth, cm	N/A	50 (N/A)	N/A	N/A	50 (0.0 SD)
HC at birth, cm	N/A	N/A	N/A	N/A	35 (0.2 SD)
Microcephaly (last exam)	-	-	N/A	N/A	+
Age at initial symptoms	< 1 year	2 days	6 months	3 months	4 months
DYSMORPHIC FEATURES					
Sparse and/or thin hair	+	+	N/A	N/A	+
High frontal hairline	+	+	N/A	N/A	+
Frontal bossing and/or broad forehead	+	+	N/A	N/A	+
Hypertelorism	-	-	N/A	N/A	-
Deep-set eyes	+	+	N/A	N/A	+
Palpebral fissures	Short	Normal	N/A	N/A	Upslanted
Ears	Large earlobe	Anteverted	N/A	N/A	Large earlobe
Thin nasal pyramid	+	-	N/A	N/A	-
Prominent columella	+	+	N/A	N/A	-
Hypoplastic alae	+	+	N/A	N/A	-
Wide mouth	+	-	N/A	N/A	+
Downturned mouth corners	+	+	N/A	N/A	-
Prognathism	-	-	N/A	N/A	-
Retrognathia or micrognathia	+	-	N/A	N/A	+
Clinodactyly	-	+ (2 nd , 5 th)	N/A	N/A	+ (2 nd , 5 th)
Broad thumbs	+	-	N/A	N/A	-
Broad halluces	+	-	N/A	N/A	+
Other (hands and feet)		Syndactyly, koilonychia	N/A	N/A	Clubbing of toes
NEURODEVELOPMENT					
ID/DD	Severe DD	Severe DD	ID	ID	Severe DD
Motor delay	+ (unable to walk)	+	+ (wheelchair)	+ (bedridden)	+
Language	Absent	Absent	N/A	N/A	Delayed
Social	Delayed	Poor interaction	Delayed (behaviour issues)	N/A	Delayed
Seizures (onset)	-	+ (2 days)	-	+ (3 months)	-
NEUROLOGICAL FINDINGS					
Distal hypertonia	-	+	+	+	-
Axial hypotonia	+	+	N/A	N/A	+
Deep tendon reflexes	Normal	Normal	N/A	N/A	Normal
Nystagmus	-	+	N/A	N/A	-
Dysmetria	+	N/A	N/A	N/A	N/A
Cerebellar ataxia	Off balance	+	+	+	+
Opthalmological findings	Strabismus	Absent eye tracking, erratic eye movements	N/A	N/A	Esotropia
INVESTIGATIONS					
EEG	Normal	Multifocal spikes and waves discharges	N/A	Hypsarrhythmia (initial EEG)	Normal
MRI	Mild cerebellar parenchymal volume loss	Long vertical axis, mild atrophy of folia in the cerebellar cortex, plagiocephaly†	Normal cerebellum Cerebral atrophy	Progressive cerebellar atrophy Cerebral atrophy	Vermis atrophy
Thyroid function tests	N/A	-	N/A	N/A	-
Cardiovascular	-	-	N/A	N/A	-

N/A: not available; SD: Standar Deviation; DD: developmental delay; ID: intellectual disability; HC: head circumference.

* Patient 2 reported by Kunni et al. harboring an unreported heterozygous variant in *CACNA1G* (p.Ile1273Phe) is not included since its pathogenicity was undetermined after electrophysiological evaluation [Kunii et al., 2020].

† And hyperintense lesion in the mesial aspect of the left hemisphere.

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