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Research Paper

Long-Term Disease Course of Pontocerebellar Hypoplasia Type 10

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ABSTRACT

Background: Pontocerebellar hypoplasia type 10 (PCH10) due to CLP1 gene mutations is characterized by structural brain anomalies, progressive microcephaly, severe intellectual and physical disabilities, and spasticity. In this follow-up study, evolution of phenotypic and neurological characteristics of patients with PCH10 is discussed.
Methods: Phenotype, growth parameters, motor functions, developmental tests, spasticity assessments, functional independence assessments, electroencephalography (EEG), and brain magnetic resonance imaging (MRI) of 10 patients with PCH10 were monitored on separate examinations. Alterations were recorded.
Results: Patients were followed-up for an average of 2.83 years. The tone of the upper extremities was significantly higher than that of the lower extremities, according to Modified Ashworth Scale (MAS) values. Sixty percent of patients could sit unsupported; 20% achieved supported sitting initially but lost the ability during follow-up. Absence of grabbing or sitting was observed in 20% of patients. During follow-up, one person achieved supported sitting and one person achieved head holding. Only one patient was able to speak a few words. Cerebellar atrophy (two of 10), pons hypoplasia (four of 10), cortical atrophy (seven of 10), enlarged ventricles (10 of 10), thinning of the corpus callosum (10 of 10), hypomyelination (six of 10), and increased white matter signal intensity (six of 10) were the observed MRI findings.
Conclusions: Progressive cerebral and cerebellar atrophy was demonstrated radiologically for the first time in a PCH10 cohort. It is of crucial importance to identify these patients promptly with the help of dysmorphic findings and spasticity being pronounced in the upper extremities. Furthermore, we note that phenotypic and neurological examination findings tend to change slightly over time.

Introduction

Pontocerebellar hypoplasia type 10 (PCH10) (OMIM# 615803) is caused by biallelic *CLP1* gene mutations and is characterized by structural brain anomalies, progressive microcephaly, severe intellectual and physical disabilities, and spasticity.[1,2] The *CLP1* gene, located on chromosome 11q12.1, was first discovered by Weitzer and Martinez in 2007.[3] This gene encodes multifunctional kinase proteins, such as the tRNA splicing endonuclease (TSEN) complex and pre-mRNA cleavage complex II.[1] The gene plays a role in tRNA biogenesis and maturation,[3] and during their formation, tRNAs undergo post-transcriptional modifications as they pass from the pre-tRNA form to the fully functional state. Mutations in genes encoding all four TSEN subunits (TSEN54, TSEN2, TSEN34, TSEN15) and CLP1 are known to cause neurodegenerative disorders with PCH. Hanada et al. suggested that mutations in the CLP1 gene impair the CLP1-TSEN complex integrity and reduce pre-tRNA cleavage. Thus, an intact CLP1-TSEN complex is essential for efficient tRNA splicing.[4] A study also found that the neuronal death

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associated with the CLP1 R140H mutation may be related to the role of CLP1 in mRNA 3′-end processing.[5] Last, a study by Sekulovski et al. sought their roles in disease mechanisms and found that TSEN acts independently of CLP1 in catalyzing intron excision.[6]

Only a few cases of PCH10 have been reported in the literature. All published cases involve families living in the eastern region of Turkey, with one exception being a family from Sudan. Interestingly, all patients with PCH10 share the same homozygous missense c.419G > A; p.(Arg140His) mutation in the *CLP1* gene.[7]

This article presents changes in the phenotypic and neurological characteristics observed in patients with PCH10 diagnosed and followed up at our outpatient clinic.

Materials and Methods

Patients

This study was conducted by the Department of Pediatric Neurology at Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, and the Department of Medical Genetics at Istanbul University, Istanbul Faculty of Medicine. Patients were diagnosed using whole exome sequencing or direct sequencing of the *CLP1* gene after clinical suspicion. All patients carried the same previously reported R140H mutation. Written informed consent was obtained from the parents of all children for the publication of clinical features, laboratory results, radiological images, and photographs.

Following their initial examination, patients were referred for necessary supportive treatments, such as physical therapy and nutrition. During follow-up, prenatal histories, growth parameters (head circumference, height, weight), dysmorphic findings, Denver Developmental Screening Test-II (DDST-II), and neurological examination results were recorded. The same pediatric neurologist measured the Modified Ashworth Scale (MAS), Functional Independence Measure for Children (WeeFIM), and Gross Motor Function Measure-88 (GMFM-88) during the first and last examinations. Additionally, electroencephalography (EEG) was performed.

To assess height, weight, and occipitofrontal circumference, growth charts developed by Neyzi et al. were used,[8] with all three parameters measured by the same physician.

Magnetic resonance imaging analysis

All patients underwent a 1.5-Tesla brain magnetic resonance imaging (MRI) and were evaluated by the same neuroradiologist.

Scales

Modified Ashworth Scale (MAS)

MAS is the most widely used clinical scale for evaluating spasticity. The MAS assesses muscle tone by passively moving the joint through its possible normal range of motion and recording resistance to passive motion. This scale classifies muscle tone from 0 (normal) to 4 (severe spasticity).[9]

Functional Independence Measure for Children (WeeFIM)

WeeFIM determines the level of pediatric functional independence in activities of daily living and is used for children between ages six months and 12 years. WeeFIM consists of six domains containing 18 items such as self-care (eating, grooming, bathing, dressing upper and lower extremities, toilet), sphincter control (bladder-bowel), transfer aids (chair/bed/wheelchair transfer, toilet transfer, tub/shower transfer), locomotion (walking/wheelchair, climbing stairs), communication (expression, comprehension), and social cognition (social interaction, problem solving, memory). This scale consists of a seven-level rating system where the seventh level means total independence and 1 is complete dependency, with a maximum score of 126.[10]

Gross Motor Function Measure (GMFM-88)

GMFM-88 was developed to evaluate the gross motor functions of children between ages 15 months and 13 years. This scale is a questionnaire divided into five main categories: “Lying & Rolling,” “Sitting,” “Crawling & Kneeling,” “Standing,” and “Walking, Running & Jumping.” The values given to the scale are divided into four sections: “does not initiate (0),” “initiates (1),” “partially completes (2),” and “completes (3).” The obtained values are interpreted by converting them to the percentile system in each group, and a higher total score indicates better motor functions.[11,12]

Statistics

Statistical data analysis was performed using the “Statistical Package for Social Sciences” (SPSS) Version 16.0 (SPSS Inc, Chicago, IL, USA). The conformity of the variables to the normal distribution was evaluated using the Shapiro-Wilk test, Q-Q plots, and histograms. As a result of the analysis, normally distributed variables were presented with means and ranges, and non-normally distributed variables were presented with medians (25th to 75th percentiles). The Mann-Whitney U test was employed to compare two independent groups that did not exhibit normal distribution, whereas the Wilcoxon signed-rank test was used to compare two dependent groups that did not demonstrate normal distribution. For all analyses, $P < 0.05$ (two-sided) values were considered statistically significant.

Ethical consideration

Written informed consent was obtained from all families before they participated in the study, and ethics committee approval was obtained from the Istanbul University-Cerrahpasa Faculty of Medicine. The families have given consent for publishing photographs to be published in this article.

Clinical evaluation

Patient 1

The patient was delivered by emergency Caesarean section at 38 gestational weeks. The parents were second cousins. She was hospitalized in the intensive care unit for eight days due to difficulty in breathing, suspected to be caused by hypoxic-ischemic encephalopathy (HIE). She did not receive treatment for hypothermia. Myoclonic seizures were noticed at four months, and a burst suppression pattern was observed in the EEG. Antiepileptic treatment (valproic acid, levetiracetam, clonazepam) was initiated.

First examination (2.5 years)

She was an active and happy baby who made eye contact and had eye tracking. Physical examination revealed axial hypotonia, increased tone in the upper extremities compared with the lower extremities, increased deep tendon reflexes (DTR) in all extremities, and clonus. Dystonic contractions lasting one to three minutes were observed on her extremities and trunk during the examination, and these contractions were triggered by external factors such as pain and touch. She could sit unsupported for five to 10 seconds and had rotations in supine and prone positions. However, she did not produce any meaningful words.

Second examination (5.5 years)

The baby remains happy with eye contact and tracking abilities. She has voluntary smiles, reacts when angry, and cries. Although she lost her ability to sit, she could still hold her head. The difference in tone between her upper and lower extremities disappeared, but spasticity intensified. DTR were increased, and clonus and dystonia persisted. Her seizures are under control with a single drug (clonazepam). She is now able to sit and roll. Her nutrition continues with routine table foods that she can consume orally. Although she makes meaningless sounds, she does not produce any words.

Patient 2

First examination (6.1 years)

He was an active, happy baby with eye contact and tracking abilities. He could sit without support for about 10 seconds and roll from prone to supine positions. He did not produce any meaningful words. Physical examination revealed axial hypotonia, increased tone in the upper extremities compared with the lower extremities, increased DTR in all extremities, and ankle contractures, but no clonus. There was prominent lymphedema on the dorsum of his foot. A sinus with serous discharge was observed at the end of the sternocleidomastoid muscle, ending in the subcutaneous tissue in the anterior part of his neck.

Second examination (9.1 years)

The patient has eye contact and tracking abilities. He has voluntary laughing and crying reactions. He can briefly sit with support for five to 10 seconds and only roll 90° in supine and prone positions. During the physical examination, it was noticed that the difference in tone between his upper and lower extremities disappeared, the spasticity intensified, and DTR decreased, but no clonus was observed. Although he makes meaningless sounds, he does not produce any meaningful words. The lymphedema has regressed, but the skin remains thin and edematous.

Patient 3

First examination (1.5 years)

The child was active and happy, with eye contact and tracking abilities. He recognized his parents but did not produce any meaningful words. Although he could sit without support, he had head titubation and could only roll from supine to prone, or vice versa. While sitting, he tried to reach for objects. On physical examination, the patient had axial hypotonicity and increased tone in the upper extremities but a normal tone in the lower extremities. His DTR were normoactive, with no clonus, but the Moro reflex persisted. Rest and action tremors were observed in his distal extremities. The patient is most prominently displaying titubation and tremors, presenting cerebellar symptoms.

Second examination (4.5 years)

The patient has eye contact and tracking abilities. Although he makes sounds, he does not produce any meaningful words. He can sit with support and turn in any direction but does not crawl. It was observed that the tone in his lower extremities had increased, leading to a decreased difference in tone between his lower and upper extremities. Titubation was reduced, and his DTR were increased, but there were no signs of clonus. In the second examination, the Moro reflex and tremors were found to have disappeared.

Patient 4

First examination (8.1 years)

The patient was agitated and cachectic, with no eye contact, and unable to lift his head. He did not produce any meaningful words. Upon neurological examination, he was observed to have axial hypotonicity, peripheral hypertonicity, and intense spasticity in all extremities. He had multiple contractures in his shoulder, elbow, knee, and ankle. DTR were absent in his lower extremities but increased in his upper extremities, and there was no sign of clonus. The patient was admitted to the hospital at 9 years and 1 month with a complaint of fever and was diagnosed with H1N1 influenza. Once he was fever free and successfully treated, the patient was discharged on the third day. However, seizure frequency increased after discharge. Unfortunately, on the tenth day, he was found dead in his bed; this condition is assumed to have occurred due to sudden unexpected death in epilepsy or postinfectious causes. Autopsy was not performed.

Patients 5 and 6

Patient 4 has siblings from a twin pregnancy that was followed up for 40 weeks. Patients were diagnosed at age four months through gene analysis.

Patient 5

First examination (1.3 years)

The baby was active and happy, with eye contact and tracking abilities. He could use one meaningful word and sit briefly without support for 10 to 15 seconds, roll in supine and prone positions, and reach for objects. Neurological evaluation revealed axial hypotonicity, normoactive DTR, and no sign of clonus.

Second examination (4.3 years)

The child is happy and socially active, with eye contact and tracking abilities. He has three meaningful words (“mother,” “father,” and “sister”). Although he can sit with support for five to 10 seconds, roll in all directions, and reach and grasp objects, he does not crawl. Upon neurological examination, a difference in tone between his upper and lower extremities was observed, but his DTR were normoactive and there was no clonus.

Patient 6

First examination (1.3 years)

He was an active and happy baby who made good eye contact and could track objects with his eyes. However, he had not yet developed any meaningful words. He could sit independently for around 10 to 15 seconds and roll over both onto his back and stomach. He could also reach for and grasp objects.

During his neurological examination, he showed hypotonicity in his core muscles but had increased muscle tone in his upper extremities. However, his muscle tone was normal in his lower extremities. His DTR were within the normal range, with no sign of clonus.

Second examination (4.3 years)

This child is active and generally happy, with good eye contact and tracking skills. Although open to social communication, he has not yet developed any meaningful words. He can sit with support for up to 15 seconds and roll in any direction, but he has not yet begun to crawl. He can reach for objects, grasp them, transfer them hand to hand, and put them into a container. During the examination, a difference in muscle tone was noted between the patient's

upper and lower extremities. However, his DTR were within the normal range, with no signs of clonus.

Patient 7

First examination (2.5 years)

This child is active and has good eye contact and tracking skills but does not yet use meaningful words. He has been diagnosed with laryngomalacia. He can sit without support for 10 seconds, roll in any direction, and reach for objects. The patient exhibited axial hypotonicity and increased DTR in all extremities during the examination. However, there were no signs of clonus.

Second examination (5.5 years)

This child appears agitated and is visibly undernourished. He does not make eye contact, track objects, or smile. The only response observed was to painful stimuli. He continues to suffer from laryngomalacia and has lost the ability to lift his head or sit up.

During the examination, the patient's DTR were found to be decreased. He also had multiple contractures in all extremities and had suffered recurrent bone fractures in the lower extremities. Despite the absence of any identifiable positive trigger, this patient has shown the fastest progression of symptoms among all patients.

Patient 8

The younger brother of Patient 7 was born in a subsequent pregnancy. However, as Patient 7 had already received a genetic diagnosis, prenatal genetic testing was performed due to the known presence of the mutation in the family. Patient was diagnosed at age three months through gene analysis.

First examination (1.2 years)

This baby was active but had limited eye contact and tracking skills and had not yet developed meaningful words. He could sit with support, roll 90° from supine to prone positions, and grasp objects but could not reach for them.

During the examination, the patient exhibited axial hypotonicity, with increased muscle tone in his upper extremities but normal muscle tone in his lower extremities. His DTR were increased, but there were no signs of clonus.

Second examination (four years)

This restless child did not make eye contact or track objects and displayed occasional, aimless smiles. Although he could lift his head, he had lost the ability to sit or roll. During the examination, increased muscle tone was noted in the patient's upper extremities, and his DTR were also increased compared with the previous examination.

Patient 9

First examination (1.5 years)

He was an active and happy child who made eye contact and tracked objects but had no meaningful words. He could not lift his head, sit, roll, or reach for objects, although he could grasp them with his hands. He had axial hypotonicity, increased tone in his upper extremities, normal tone in his lower extremities, and no clonus.

Second examination (four years)

He was agitated and restless and did not make eye contact or track objects or smile responsively. He had been followed up in the intensive care unit for two weeks due to a generalized tonic-clonic seizure and status epilepticus three months before the last examination. Since the seizure, he has experienced a significant loss of motor and cognitive abilities and suffered from dystonic contractions. He could not lift his head or sit and had significant spasticity in all extremities. The difference in tone between his upper and lower extremities was found to have disappeared. His DTR were increased, but clonus was absent.

Patient 10

Patient 9's brother was also affected. Targeted genetic testing was performed prenatally due to the known mutation in the family. He is the first patient with the CLP1 mutation who was diagnosed prenatally.

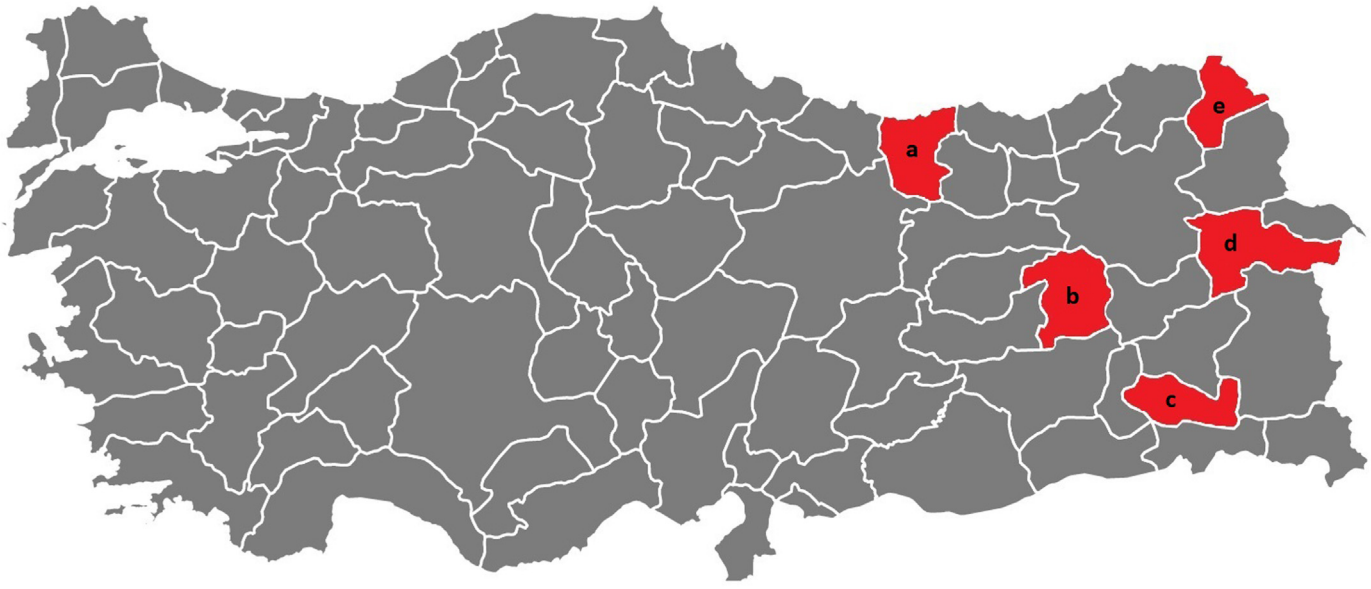

FIGURE 1. Regional origins of the affected patients are marked in the map of Turkey. Black Sea region (a: Patient 2). Eastern region (b, Patient 1; c, Patients 7 and 8; d, Patients 4, 5, 6, 9, and 10; e, Patient 3). The color version of this figure is available in the online edition.

TABLE 1.
Demographic Characteristics and Developmental Parameters

Patients	1	2	3	4	5	6	7	8	9	10
Gender	F	M	M	M	M	M	M	M	M	M
Date of birth	01.12.2015	17.05.2012	05.07.2017	03.11.2010	30.05.2017	30.05.2017	28.4.2016	30.10.2017	4.9.2017	26.03.2019
City	Bingöl (b)	Giresun (a)	Ardahan (e)	Ağri (d)			Siirt (c)		Ağri (d)	
Consanguineous	Second-degree cousins	First-degree cousins	First-degree cousins	Second-degree cousins			First-degree cousins		First-degree cousins	
Mutation	c.419G>A	c.419G>A	c.419G>A	C419G>A			c.419G>A		c.419G>A	
Pregnancy duration	38 w	38 w	42 w	41 w	40 w	40 w	40 w	40 w	40 w	40 w
Polyhydramnios	-	-	+	-	-	-	-	-	-	-
Diagnosis age	2.4 years	3.7 years	1.4 years	6.9 years	4 months	4 months	10 months	3 months	10 months	Prenatal
Growth parameters										
Birth weight (kg)	3.7	3.4	3.9	3.4	2.8	2.9	3.3	3.25	4.1	4.6
	75-90p	25-50p	75-90p	25-50p	3-10p	10-25p	25-50p	25-50p	90-97p	>97p
Birth head circumference (cm)	35	36	35	36	34.5	34.8	35.2	34.9	36	36
	50-75p	75-90p	50-75p	75-90p	25-50p	25-50p	50-75p	50p	75-90p	75-90p
Length at birth (cm)	48	49	53	51	48	49	50	49	53	56
	25-50p	25-50p	90-97p	50-75p	10-25p	25-50p	50p	25-50p	90-97p	>97p
Examination age (years)	2.5	6.1	1.5	8.1	1.3	1.3	2.5	1.2	1.5	0.25
	5.5	9.1	4.5	-	-	4.3	5.5	4	4	2.5
Head circumference (cm)	44	46.5	41	46	43	42	43.1	41.9	45.5	40.3
	45	48	43	-	45.4	45.4	46.5	44.1	47	45.1
	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p
	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p
Length (cm)	89	110	95	101	71	94	70	81	69	77	94	111	78	72	83	63
	25-50p	25-50p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	<3p	50-75p	25-50p	25-50p	<3p	<3p	50-75p
Weight (kg)	14	15	16	20	8.5	12	17.2	9.4	12.8	9.2	12.5	13	12	13	8.5	9.1	6.8	13
	50-75p	3-10 p	<3p	<3p	<3p	<3p	<3p	<3p	3-10p	<3p	25-50p	<3p	75-90p	<3p	<3p	50-75p

Abbreviations:
F = Female
M = Male
p = Percentile
The alphabets a, b, c, d, and e denote cities on the Turkey map in Fig 1.

First examination (three months)

He was an active, breast-fed baby with a continuing sucking reflex. He had limited eye contact and only smiled reactively. Although he was able to lift his head, he could not roll. He had hypertonicity in all extremities, increased DTR, and clonus. The Moro reflex was present.

Second examination (2.5 years)

He was an active and happy baby who responded to sound and movement with a smile. When angry, he cried. The patient did not make eye contact or track objects. Although he babbled, he did not have any meaningful words. He could reach for objects and grasp them but could not lift his head or roll. His upper extremities were hypertonic, whereas his lower extremities were hypotonic. DTR were increased, and clonus was absent.

Results

This study evaluated 10 patients from six families. The mean follow-up period for our patients was 2.83 years (range: 2.25 to 3 years). The mean age at diagnosis was 1.6 years (range: prenatal to 6.9 years). Patient 10 was diagnosed in the prenatal period. The regional classification of patients is shown on the map (Fig 1). Only Patient 2 was from the Black Sea region, and all other patients were from the Eastern Anatolia Region. None of the patients were born with microcephaly in our study, but as they aged, the growth of their head circumference stopped (Table 1). At the first examination, all patients except one had microcephaly (Patient 10) (Table 1). Sixty percent of the patients could sit without support (Patients 1, 2, 3, 5, 6, and 7). Twenty percent of the patients achieved supported sitting (Patients 1 and 7) but lost this ability during follow-up. No grabbing or sitting was observed in 20% of all patients (Patients 4 and 9). During follow-up, 10% reached the supported sitting stage (Patient 8) and 10% (Patient 10) could only hold their head (Table 2). At the first examination, 60% of the patients (Patients 1, 2, 4, 7, 8, and 10) had increased DTR, whereas 40% had normal DTR. During follow-up examinations, DTR were decreased in two patients who initially had increased DTR (Patients 2 and 7), and in two patients who initially had normal reflexes, DTR were found to be increased (Patients 3 and 9) (Table 2). Severe intellectual disability was observed in all subgroups according to the DDST-II, except for one patient diagnosed prenatally during the initial examination (Table 2). In the language section of the Denver test, during follow-ups, it was observed that the scores increased for four patients (Patients 1, 5, 6, and 10), decreased for three patients (Patients 7, 8, and 9), and remained the same for two patients (Patients 2 and 3). Only one patient could speak, and the number of words increased to three during the follow-up (Patient 5). In the social development section, it was observed that the scores increased for five patients (Patients 1, 2, 3, 5, and 10), decreased for three patients (Patients 7, 8, and 9), and remained the same for one patient (Patient 6).

Epilepsy was present in half of the patients (Patients 1, 2, 4, 8, and 9). The earliest age of seizure onset was one month (Patient 8). The EEG revealed a disorganized pattern in two of five patients (Patients 8 and 9), burst suppression in one patient (Patient 1), generalized spike-wave in one patient (Patient 2), and hypsarrhythmia in one patient (Patient 4). Sixty percent of seizure-free patients had normal patterns (Patients 3, 7, and 10), whereas the others had a disorganized background (Patients 5 and 6) (Table 2). Seizures were controlled in 60% of the patients during follow-up. Burst suppression (Patient 1) and hypsarrhythmia pattern (Patient 4) continued as generalized spike-waves during follow-ups. In the patient with generalized spike-wave (Patient 2), the pattern changed to a disorganized background. Those with a

TABLE 2.
Neurological and Systemic Evaluation

Examination	1		2		3		4	5		6		7		8		9		10	
	First	Second	First	Second	First	Second	First	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
DDST-II (months)																			
Gross motor	6	4	7	6	7	7	2	7	6	8	9	8	2	5	3	3	3	2	2
Fine motor	9	7	5	6	10	13	3	6	9	8	11	6	3	5	5	4	2	2	6
Language	10	11	9	9	10	10	3	12	14	9	10	8	3	6	4	6	3	3	8
Social	10	11	10	11	12	14	3	12	15	11	11	8	3	6	3	6	2	3	7
Deep tendon reflex	↑	↑	↑	↓	N	↑	↑	N	N	N	N	↑	↓	↑	↑	N	↑	↑	↑
Seizures																			
Seizure onset	4 months		2 years		−		5 months	−		−		−		1 months		3.3 years		−	
EEG	BS, GSW		GSW		Normal		HA	Disorganized		Disorganized		Normal		Disorganized		Disorganized		Normal	
Seizure control	+		−		None		−	None		None		None		+		+		None	
Seizure type	MC		MC (eyes)		None		T/MC	None		None		None		T		T/C		None	
Startle reaction	+	+	+	+	−	−	+	−	−	−	−	−	−	+	+	−	−	−	−
Visual finding																			
Following & fixation	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−	+	−	+	−
Strabismus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
Nystagmus	+	−	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−	−	+
Skeletal abnormalities																			
Kyphoscoliosis	+	+	+	+	−	+	+	−	+	−	+	−	+	−	+	+	+	−	+
Hip abnormalities	+		−		−		−	−		−		−		−		+		−	
Skin																			
Hypertrichosis	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	+	+	+
Long lashes	−		+		+		+	+		+		+		+		−		+	
Eczema	−		−		−		−	−		−		−		+		−		−	
Lymphedema	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Loose skin	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cold extremities	−	−	+	+	−	+	+	−	−	−	−	−	+	−	+	−	+	−	−
GIT manifestations																			
Constipation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Feeding difficulties	−	−	+	+	−	−	+	−	−	−	−	−	+	−	+	−	+	−	−
Short frenulum	−	−	−	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−
Genitourinary system																			
Retractile testis			+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+

Abbreviations:
BS = Burst suppression
C = Clonic
DDST-II = Denver Developmental Screening Test-II
EEG = Electroencephalography
GIT = Gastrointestinal tract
GSW = Generalized spike wave
HA = Hypsarrhythmia
MC = Myoclonic
N = Normal
T = Tonic
↑ = Increased
↓ = Decreased
+ = Presence of the finding
− = Absence of the finding

FIGURE 2. In Patient 3, time-dependent evolution of dysmorphic features has been demonstrated. In the first examination, high-arched eyebrows, prominent eyes, down-slanting palpebrae, long palpebral fissures, bitemporal narrowing, synophrys, micrognathia, smooth philtrum, and a round face were observed. In the second examination, dysmorphic findings became more prominent over time. The color version of this figure is available in the online edition.

disorganized background (Patients 8 and 9) continued in the same manner.

Dystonia was observed in two patients (Patient 1 and 9). Patient 1 had a history of HIE. In Patient 9, dystonia may have developed as a sequel to status epilepticus and intensive care admission.

During follow-up examinations, following-fixation and strabismus progressed; on the contrary, nystagmus was found to be regressed. Clinodactyly (Patient 9) and short metatarsal (Patient 1) were nonspecific findings of the skeletal system. All patients developed kyphoscoliosis during follow-up. Skin findings observed on the patients were hypertrichosis, long eyelashes, eczematous lesions (Patient 8), lymphedema (Patient 2), loose skin (Patient 3), and cold extremities (50%) (Table 2). Dysphagia was present in 20% of patients during the initial examination and increased to 50% during follow-up.

Dysmorphic finding

Some dysmorphic findings, such as prominent eyes and a round face, appeared in early childhood and vanished as the patients aged. Conversely, dysmorphic features include long palpebral fissures, a broad nasal root, synophrys, bulbous nasal tip, low-hanging columella, tapered fingers, smooth philtrum, large ear pinna, and widely spaced teeth that appeared as the patient aged (Fig 2). Changes in dysmorphic findings from the first to the last examination are shown in Table 3.

Scales

The MAS evaluation on the first examination revealed increased tonus on the upper extremities in all 10 of 10 patients and increased tonus on the lower extremities in five of 10 patients. Two of the five patients had milder increased lower extremity tonus than upper extremity tonus, whereas the rest had equal upper/lower extremity tonus (Table 4).

According to the MAS values in the first and second examinations, the degree of spasticity on the upper extremities increased in five patients and remained the same in four patients. There was no observed tendency to decrease spasticity in any of the patients. The degree of spasticity in the lower extremities increased in eight patients, whereas it decreased in only one patient (Patient 10) (Table 4).

TABLE 3.
Dysmorphic Findings

Patients	1	2	3	4	5	6	7	8	9	10
High-arched eyebrows	+	+	+	+	+	+	+	+	+	+
Prominent eyes	+	+	+	+	−	−	(+−)	+	+	+
Down slanting palpebra	+	+	+	−	+	+	+	−	+	+
Long palpebral fissures	+	+	+	+	+	+	+	+	+	(−+)
Broad nasal root	+	+	(−+)	−	+	+	+	+	(−+)	(−+)
Bitemporal narrowing	+	+	+	+	−	+	+	+	+	+
Synophrys	−	−	+	+	−	−	+	+	−	(−+)
Bulbous nasal tip	−	−	−	−	−	−	+	+	(−+)	−
Low hanging columella	−	+	−	+	+	−	−	−	(−+)	(−+)
Widely spaced teeth	−	(−+)	−	−	+	+	−	(−+)	−	−
Short neck	−	+	−	−	−	−	−	−	−	−
Tapered fingers	+	+	+	+	+	+	+	+	+	(−+)
Micrognathia/ retrognathia	−	+	+	+	+	+	+	+	+	(−+)
Smooth philtrum	+	+	+	+	+	+	+	+	−	(−+)
Round face	−	(+−)	(+−)	−	−	−	(+−)	+	+	(+−)
Big ears	+	−	−	−	−	−	+	+	−	−
Big ear pinnae	+	+	+	+	+	+	−	+	−	(−+)

(+): Presence of the dysmorphic finding, (−): absence of the dysmorphic finding, (−+): absence in first examination presence in the last examination, (+−): presence in first examination, absence in the last examination.

TABLE 4.
Results of MAS, WeeFIM, and GMFM-88 Scales

Patients	1		2		3		4	5		6		7		8		9		10	
Examination	First	Second	First	Second	First	Second	First	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
MAS																			
Shoulder	2	3	2	2	1	1	3	2	2	2	2	1	3	2	3	2	3	2	3
Elbow	2	3	3	3	2	2	4	2	2	2	2	2	4	2	3	2	4	2	4
Hand	1	2	2	2	2	2	4	2	2	2	2	2	3	2	3	2	4	2	3
Hip	1	3	1	2	0	2	3	0	1	0	1	1	4	0	2	0	3	2	1
Knee	1	3	2	3	0	1	4	0	1	0	1	2	3	0	2	0	4	2	1
Foot & ankle	1	2	4	4	0	1	4	0	1	0	1	2	4	0	2	0	4	2	1
WeeFIM	23	21	19	19	23	24	18	21	22	20	20	20	18	19	18	18	18	*	18
GMFM-88	19.4	17.8	19.4	18.6	24.5	22.8	4.7	24.1	23.6	20.9	23.7	18.4	5.8	15.1	9.5	7.5	3.4	*	6.4

Abbreviations:
GMFM-88 = Gross Motor Function Measure
MAS = Modified Ashworth Scale
WeeFIM = Functional Independence Measure for Children
MAS: This scale classifies muscle tone from 0 (normal) to 4 (severe spasticity).
WeeFIM: In this scale, scoring ranges from 18 to 126. As the score increases, independence also increases.
GMFM-88: In this scale, each question is scored on a scale of 0 to 4: "does not initiate (0)," "initiates (1)," "partially completes (2)," and "completes (3)." As the score increases, motor functions improve.
* Not suitable for age testing.

There was a statistically significant difference between the MAS values in all calculated body parts in the first and second examinations. MAS values were not higher in the upper extremity compared with the lower extremity in four of nine patients in the first and second examinations. The *P* values were 0.034 and 0.030 in the upper and lower extremities, respectively (Table 5).

However, the WeeFIM and GMFM-88 tests did not reveal significant differences ($P = 0.334$, $P = 0.069$) (Table 5).

Radiological findings

MRI was conducted in all children, revealing the following findings: cerebellar atrophy in two of 10 patients, pons hypoplasia in four of 10 patients, cerebral cortex atrophy in seven of 10 patients, enlarged ventricles in all 10 patients, thinning of the corpus callosum in all 10 patients, hypomyelination in six of 10 patients, and an increase in white matter signal intensity in six of 10 patients (Table 6 and Fig 3). In patients with observed cerebral cortex atrophy, particularly forebrain involvement was noted. We observed pontocerebellar hypoplasia in Patients 2 and 8 only. Patients 1 and 6 had only pons hypoplasia, with a normal cerebellum.

Patients 1 and 2 had two magnetic resonance images taken at different time points. In Patient 2, the cerebellum was observed to have a normal volume in the first MRI (Fig 3-2a), but there was volume loss in the cerebellum in the follow-up MRI (Fig 3-2b). Additionally, although there was no cortical atrophy in the first MRI of Patient 1 (Fig 3-1a), it appeared in the second MRI (Fig 3-1b).

Discussion

Loss-of-function mutations in the *CLP1* gene cause loss of kinase and cleavage activity, as well as alterations to the function of the tRNA endonuclease complex; this leads to abnormal cellular accumulation of unspliced pre-tRNAs.[13]

Schaffer et al. demonstrated cerebellar and motor degeneration in *CLP1*-null zebrafish, suggesting that *CLP1* mutations may contribute to PCH10 in humans.[2] Hanada et al. found that homozygous *CLP1* mutant mice suffered from prenatal death and spinal motor neuron degeneration.[4] Our study describes the youngest patient reported in the literature thus far who was found to carry the *CLP1* R140H mutation prenatally and presented with abnormal neurological findings at age just three months. This finding further supports animal studies suggesting that PCH10 begins prenatally.

Previously reported cases of *CLP1* mutation have been isolated to the Eastern Anatolia Region of Turkey. In our series, only one patient (Patient 2) was from the Black Sea region (Fig 1-a) and their family was not genetically related to the Eastern Anatolia region. This fact suggests the possibility of a wider geographic region being affected by this specific mutation. Furthermore, during the preparation of this study, supporting evidence emerged from Sudan, where a geographically distant family with *CLP1* mutation was reported.[7]

The main clinical findings reported in patients have included hypotonia, global growth retardation, truncal ataxia, abnormal eye movement, dysarthria, intentional tremor, microcephaly, and seizures.[1] In our series, none of the patients were born with microcephaly, but their head circumferences ceased to grow as they aged (Table 1). Severe intellectual disability was observed in all

TABLE 5.
Median Values of MAS, WeeFIM, and GMFM-88

Examination	First	Second	*P*
WeeFIM*	20 (18.5-22)	19 (18-21.5)	0.334
GMFM-88*	19.4 (11.3-22.5)	17.8 (6.1-23.2)	0.069
MAS-Shoulder*	2 (1.75-2)	3 (2-3)	0.034
MAS-Elbow*	2 (2-2.25)	3 (2-4)	0.038
MAS-Hand*	2 (2-2)	2 (2-3)	0.034
MAS-Hip*	0.5 (0-1.25)	2 (1-3)	0.016
MAS-Knee*	0.5 (0-2)	2 (1-3)	0.020
MAS-Foot & ankle*	0.5 (0-2.5)	2 (1-4)	0.031
MAS-Upper extremity*	6 (5-6)	8 (6-10)	0.041
MAS-Lower extremity*	1.5 (0-6.25)	6 (3-10)	0.020
MAS-Upper & lower extremities†			0.034‡
			0.030§

Abbreviations:
GMFM-88 = Gross Motor Function Measure
MAS = Modified Ashworth Scale
WeeFIM = Functional Independence Measure for Children
* Wilcoxon signed-rank test.
† Mann-Whitney U test.
‡ First examination.
§ Second examination.

TABLE 6.
Radiological Outcomes

Patients	1		2		3	4	5	6	7	8	9	10
Age	4 Months	1.5 Years	4 Years	7 Years	2 Years	6 Months	18 Months	2 Years	1 Years	5 Months	6 Months	2.5 Years
Cerebellum atrophy	–	–	–	+	–	–	–	–	–	+	–	–
Pons hypoplasia	+	+	+	+	–	–	–	+	–	+	–	–
Cerebral cortex atrophy	–	+	+	+	–	+	+	+	+	+	–	+
Enlarged ventricles	–	+	+	+	+	+	+	+	+	+	+	+
White matter signal increase	–	+	+	+	+	–	+	+	–	–	–	+
Corpus callosum thinness	+	+	+	+	+	+	+	+	+	+	+	+
Myelination retardation	–	+	+	–	+	–	+	+	–	–	–	+

subgroups according to the DDST-II, except for one patient diagnosed prenatally (Table 2).

According to Karaca et al., none of the 11 subjects could move or sit unassisted. All had poor head control lasting between 10 and 15 seconds. The youngest patient, BAB5318, was six months old and had no head control.[1] In our cohort, 60% of patients could sit without support (Patients 1, 2, 3, 5, 6, and 7). However, despite receiving physical therapy during follow-up, 20% of these patients lost their ability to sit (Patients 1 and 7). One patient (10%) could sit with support (Patient 8). Patient 5 gained the ability to speak at least three words at the last examination and was the only patient in the cohort with speaking ability (Table 2).

All patients reported by Karaca et al. had hypertonia and increased DTR.[1] In our cohort, only 60% of patients had increased DTR, and DTR were decreased in two patients during follow-up examinations. Since mice with *CLP1* mutation develop progressive spinal motor neuron loss resembling amyotrophic lateral sclerosis, the decreasing DTR seem to support these findings (Table 2).

Epilepsy is a common finding in PCH10 and was present in 50% of our patients. The earliest age of seizure onset was one month. EEGs during the early period showed signs of epileptic encephalopathies, such as burst suppression and hypsarrhythmia. Seizures in three of five patients who received treatment were fully controlled (Table 2). When examining the evolution of EEGs over time, the EEG findings of patients with epileptic encephalopathy transformed into a generalized spike-wave pattern. The patient who exhibited generalized spike-wave changed to a disorganized background.

Dermatologic evaluations of our patients revealed several novel findings, including hypertrichosis (seven of 10), long eyelashes (eight of 10), loose skin (one of 10), lymphedema (one of 10), a sinus with active serous discharge located in the sternocleidomastoid region (one of 10), eczema (one of 10), and dry, cold skin on the distal parts of the extremities (five of 10). Loose skin, lymphedema, and eczema resolved during follow-up. Notably, one patient (Patient 9) did not initially exhibit hypertrichosis but developed it during later periods. Retractile testes, which had not previously been reported, were observed in 88% (eight of nine) of our male patients (Table 2).

MAS is the most commonly used scale in clinical practice to assess the severity of spasticity. In our study, we evaluated the degree of spasticity using the MAS and found statistically significant differences in all areas between the two examinations (Tables 4 and 5). The difference between the upper and lower limbs was particularly striking, with spasticity occurring earlier in the upper extremities. This early onset difference negatively affected our patients regarding functionality and mobilization. We used WeeFIM to assess functional independence in activities of daily living, and we found that functional results were lower compared with age groups. There was no significant difference in the statistical WeeFIM values between the two examinations. Among the patients whose gross motor functions were compared, we observed that the GMFM-88 values decreased

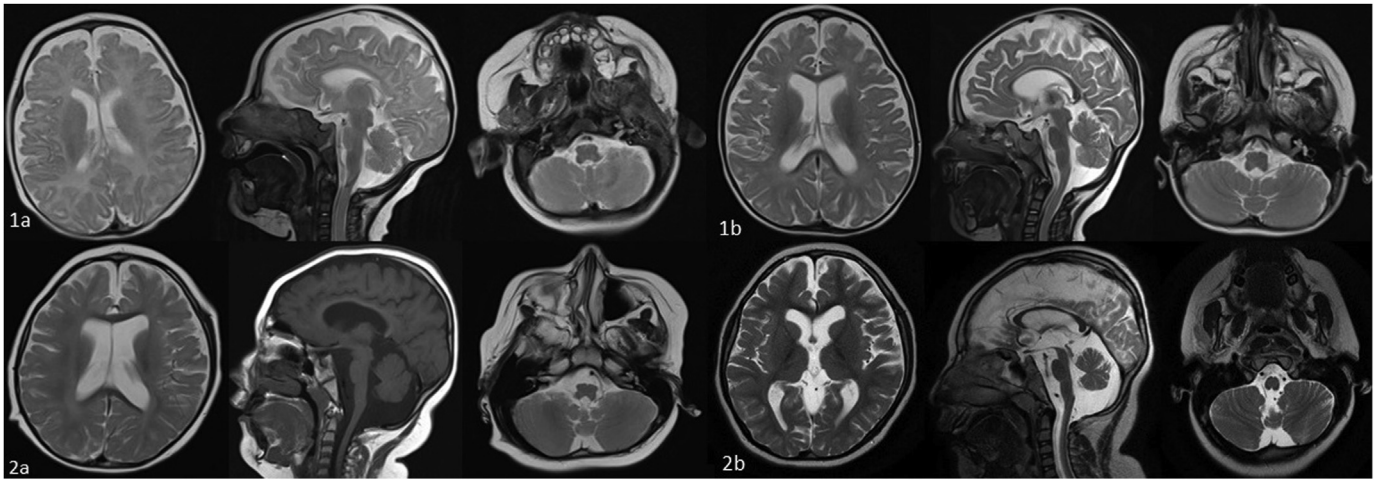

FIGURE 3. Consecutive magnetic resonance imaging (MRI) of Patients 1 (1a, 1b) and 2 (2a, 2b). In the first MRI of Patient 2, pons hypoplasia, cerebral cortex atrophy, enlarged ventricles, increased signal intensity in white matter, thinning of the corpus callosum, and delayed myelination were observed. In the second MRI, cerebellar atrophy was observed as a novel feature. The myelination was suitable for the patient's age (a: first MRI, b: second MRI).

except for one patient (Patient 6). It is well known that implementing an effective physical therapy and rehabilitation program in the early stages is beneficial in resolving spasticity and improving the functionality and gross motor functions. Therefore, we referred all our patients to physical therapy and rehabilitation support. However, the effectiveness of the supportive treatment may have been affected by various factors, such as the coronavirus disease 2019 outbreak, the treatment conditions in the cities they live in, their nutritional status (low muscle mass, swallowing problem), age at diagnosis, and the age of treatment initiation.

Dystonia was observed in two patients. Patient 1 had a history of HIE, which might have been the cause of dystonia. Similarly, in Patient 9, dystonia may have developed as a sequel to status epilepticus and intensive care admission. It is important to remember that dystonia can still be a secondary aspect of PCH10.

Patient 7 had the fastest progressive worsening among the patients we followed. This patient had the most intense contractures and the lowest GMFM-88 value. Interestingly, the two examinations showed no difference in tone between the upper and lower extremities. Patient 7 also had laryngomalacia present from birth that did not resolve.

Patient 3 had the most prominent cerebellar symptoms, despite a normal imaging of pons and cerebellum. Interestingly, the patient had persistent Moro reflex and jitteriness at 18 months in the first examination, but these symptoms disappeared in the second. These findings suggest that clinical severity may not be correlated with radiological findings. MRI typically shows cerebral (cortical) and cerebellar volume loss, brain stem hypoplasia, thinning of the corpus callosum, signal changes in the white matter, and enlarged ventricles. We interpreted the loss of cerebral and cerebellar volume as atrophy because in the MRI of Patient 2, the cerebellum was observed to have a normal volume in the first MRI (Fig 3-2a) but there was volume loss in the cerebellum in the follow-up MRI (Fig 3-2b). Additionally, although there was no cortical atrophy in the first MRI of Patient 1 (Fig 3-1a), cortical atrophy appeared in the second MRI (Fig 3-1b). These findings suggest that *CLP1* is a progressive disease.

Brain abnormalities reported in PCH10 appear to differ from those associated with other types of PCH. The dragonfly sign and involvement of the hindbrain and forebrain are typically seen in other PCH types but not in PCH10.[14] Interestingly, we did not observe the dragonfly sign in our patients. Karaca et al. suggested that cortical involvement in PCH10 is predominantly located in the frontal lobes.[1] However, Schaffer et al. reported no difference between forebrain and hindbrain involvement.[2] In one of the two patients reported by Wafik et al., posterior cortical involvement was dominant.[15] In all our patients with cortical atrophy, forebrain involvement was evident, as indicated by Karaca et al.[1]

Schaffer et al. reported pontocerebellar hypoplasia and thinning of the corpus callosum in five patients. In Patient 6, cerebellar hypoplasia and thinning of the corpus callosum were observed, but the pons appeared normal.[2] In contrast, we observed pontocerebellar hypoplasia in only two patients. Two patients had only pons hypoplasia, but their cerebellum was normal. All patients had a thin corpus callosum.

Conclusion

This study evaluated changes over time in the clinical findings of 10 patients with PCH10. The very early presentation at age three months suggests that progressive deterioration starts at an early age, even during the antenatal period. Early and late signs of the disease vary, and these should be considered during diagnosis. Our patient from the north coast of Turkey and later reported patients from Sudan showed that the R140H mutation is not limited to a specific region but rather is more widely spread. This study is the first to report tonus disparity between the upper and lower extremities. Increased tonus was observed earlier in the upper extremities, which could be a predictive sign for PCH10. Consecutive MRIs confirmed the progressive course of variable brain atrophy. It is important to note that not all patients with PCH10 develop pontocerebellar hypoplasia, so ongoing monitoring is necessary despite the name of the disease.

CRediT authorship contribution statement

Serhat Guler: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Ayca Dilruba Aslanger:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Turkan Uygur Sahin:** Data curation, Writing – review & editing. **Alpay Alkan:** Data curation, Investigation, Writing – review & editing. **Cengiz Yalcinkaya:** Data curation, Writing – review & editing. **Sema Saltik:** Data curation, Investigation, Writing – review & editing. **Gözde Yesil:** Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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