



Clinical, electrophysiological, and neuroimaging characterization of childhood-onset non-5q spinal muscular atrophies: a retrospective pediatric case series

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Received: 16 January 2026 / Accepted: 28 May 2026

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Introduction

Spinal muscular atrophy (SMA) is a genetically inherited motor neuropathy characterized by degeneration of the lower motor neurons. The most common form of SMA is 5q-SMA, which is caused by mutations in the *SMN1* gene. However, with recent advancements in molecular genetic technologies, it has become possible to identify rarer subtypes known as “non-5q SMA,” which are not associated with *SMN1* mutations. These subtypes are genetically highly heterogeneous and present with a wide range of phenotypes depending on the underlying genetic mutation [1–5].

Among the genetic mutations evaluated in this study, genes such as *ASCC1*, *BICD2*, *VRK1*, *UBA1*, *TRPV4*, and *HEXB* are associated with lower motor neuron disorders

manifesting with early-onset hypotonia, respiratory distress, arthrogryposis, developmental delay, and progressive muscle weakness. In children harboring these genetic variants, neuropathic findings on electromyography (EMG), marked muscle atrophy, and in some cases central nervous system (CNS) involvement detectable on neuroimaging studies may also be present [3–6].

Current data in the literature regarding these genes are mostly derived from individual case reports. Due to the substantial phenotypic variability observed among patients, standardizing clinical approaches and diagnostic algorithms remains challenging. This creates a significant gap in clinical practice, particularly in terms of early diagnosis, genetic counseling, and therapeutic guidance [1–4].

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Recent studies have increasingly emphasized the complexity of genotype–phenotype correlations in non-5q spinal muscular atrophy and related hereditary motor neuron disorders. Advances in next-generation sequencing have expanded the spectrum of genes associated with SMA-like phenotypes, revealing substantial clinical and genetic heterogeneity and highlighting the challenges in establishing consistent genotype–phenotype relationships [7–10]. Theuriot et al. demonstrated that whole-exome sequencing significantly improves diagnostic yield in patients with SMA-like phenotypes who are negative for *SMN1* deletions, while Fernández-Eulate et al. reported a broad phenotypic overlap across different genetic subtypes [8, 9]. Similarly, Zambon et al. highlighted that early-onset hereditary neuronopathies represent a genetically diverse group requiring integrated clinical, electrophysiological, and genetic evaluation [10].

Despite these advances, most available data are derived from individual case reports or small case series, which limits the ability to generalize findings and to develop standardized diagnostic frameworks [1–4]. In this context, there remains a need for well-characterized cohorts integrating clinical, electrophysiological, neuroimaging, and genetic data to better define genotype–phenotype relationships in non-5q SMA.

The present study aims to address this gap by providing a detailed clinical, electrophysiological, neuroimaging, and genetic characterization of a pediatric cohort with genetically confirmed non-5q SMA. By integrating multimodal data across multiple genotypes within the same cohort, this study provides a more comprehensive evaluation of genotype-associated clinical and radiological patterns and contributes novel insights into the clinical spectrum of non-5q SMA, with potential implications for improving diagnostic awareness and early referral in children presenting with SMA-like phenotypes.

The aim of this study is to evaluate and compare the clinical, neurophysiological, and imaging findings of 11 pediatric patients with mutations in six different genes associated with non-5q SMA (*ASCC1*, *BICD2*, *VRK1*, *UBA1*, *TRPV4*, and *HEXB*) in the context of the current literature. The goal is to better characterize the clinical spectrum specific to these rare genetic SMA subtypes and to contribute to improved diagnostic awareness and earlier referral processes.

Study design and participants

In this study, “non-5q SMA-like” phenotypes were defined as pediatric-onset disorders primarily affecting lower motor neurons without *SMN1* deletions and characterized by predominant motor weakness and neurogenic EMG findings mimicking spinal muscular atrophy. Cases with overlapping

neuropathic or skeletal manifestations were included only when the primary clinical and electrophysiological phenotype was motor neuron predominant. Sensory involvement was not considered an exclusion criterion if lower motor neuron involvement remained the dominant feature.

This retrospective descriptive file review study was conducted with the approval of the Karadeniz Technical University Ethics Committee. A total of 11 pediatric patients were included who were followed in the pediatric neurology department between 2015 and 2025 and were found to have mutations associated with non-5q SMA (*ASCC1*, *BICD2*, *VRK1*, *UBA1*, *TRPV4*, and *HEXB*) based on genetic analysis. All patients were selected from those who were negative for homozygous deletions in the *SMN1* gene but exhibited clinical features resembling SMA.

In the present study, pediatric patients with genetically confirmed variants associated with non-5q SMA were included if they were identified during the evaluation of children presenting with SMA-like phenotypes and negative *SMN1* testing.

Data collection

This retrospective observational case series included pediatric patients evaluated for suspected non-5q spinal muscular atrophy between March 11, 2015, and May 30, 2025. Patients were identified through collaborative follow-up among several tertiary pediatric neurology centers in Türkiye. Most cases were evaluated and longitudinally followed at the Pediatric Neurology Department of Karadeniz Technical University, a regional referral center for pediatric neuromuscular disorders.

During the 10-year study period, genetic evaluation was performed in pediatric patients referred with suspected spinal muscular atrophy (SMA). A total of 198 patients with SMA-like phenotypes underwent *SMN1* deletion analysis using multiplex ligation-dependent probe amplification (MLPA). Among these, 46 patients were diagnosed with 5q-SMA. The remaining 152 patients with negative *SMN1* results were further evaluated using next-generation sequencing approaches, including targeted gene panels and/or whole-exome sequencing (WES), to investigate non-5q SMA and other genetic etiologies.

The study adhered to the principles of the Declaration of Helsinki, revised in 2013. Data retrieved from patient records included age, sex, age at symptom onset, initial symptoms, family history, consanguinity, socioeconomic background, muscle strength distribution, electrophysiological findings (EMG and nerve conduction studies), laboratory values (e.g., creatine kinase), brain and spinal MRI findings, muscle biopsy results, and genetic analysis reports.

Motor function and muscle strength were evaluated using standard clinical assessment tools commonly applied in neuromuscular disorders. Muscle strength was graded using the Medical Research Council (MRC) scale. When available from clinical documentation, standardized motor function scales such as the Hamersmith Functional Motor Scale Expanded (HFMSE) and the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) were reviewed retrospectively to provide additional information on functional motor performance. Because this study was retrospective and included patients of different ages and phenotypic severity, these assessments were not uniformly available for all individuals.

Genetic analysis

Genetic testing was performed using targeted neuromuscular gene panels and/or whole-exome sequencing, depending on the period of evaluation. Earlier cases were reanalyzed using updated variant databases. Variant interpretation followed the guidelines of the American College of Medical Genetics and Genomics (ACMG). Classification incorporated multiple lines of evidence, including population allele frequency databases (e.g., gnomAD), in silico pathogenicity prediction tools, previously published literature, and segregation analysis when available. Variants of uncertain significance were reported descriptively but were not used to establish definitive genotype–phenotype associations. For suspicious or novel variants, Sanger sequencing confirmation was performed, and parental carrier status was evaluated when necessary.

Statistical analysis

Data were analyzed using SPSS for Windows version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as medians and ranges, and categorical variables as frequencies (n).

Results

Among the 152 patients with negative *SMN1* testing, 11 patients were identified as having variants associated with non-5q SMA. This corresponds to a diagnostic yield of 7.2% within the *SMN1*-negative subgroup. When considering the entire cohort of patients evaluated for SMA-like phenotypes, non-5q SMA accounted for 5.6% of cases.

A total of 11 patients diagnosed with non-5q spinal muscular atrophy (SMA) were included in the study, in whom six

different pathogenic gene variants were identified (*BICD2*, *TRPV4*, *ASCC1*, *VRK1*, *UBAI*, and *HEXB*). The detailed clinical, electrophysiological, neuroimaging, and genetic characteristics of these patients are summarized in Table 1. The most frequently detected variants were in the *BICD2* and *TRPV4* genes (3/11 each), followed by *ASCC1* variants (2/11). All patients had an infantile-onset disease course, with symptoms manifesting either at birth or before 8 years of age.

The genetic characteristics and variant classifications of the patients are presented in Table 2. Overall, a male predominance was observed, particularly among patients harboring *BICD2*, *TRPV4*, and *ASCC1* variants. Variants in *UBAI* were detected exclusively in male patients, consistent with X-linked inheritance patterns. Consanguinity was reported in approximately 70% of cases and was especially frequent among patients with *ASCC1* and *TRPV4* variants. A positive family history was documented in patients carrying variants in *ASCC1*, *UBAI*, and *HEXB*.

Pathogenic, likely pathogenic, and variants of uncertain significance were identified across multiple genes associated with motor neuron disease. *BICD2* variants were detected in three patients as heterozygous missense changes occurring de novo. Two of these variants were previously reported and classified as pathogenic, while one novel variant was classified as likely pathogenic. Heterozygous missense variants in *TRPV4* were identified in three patients, two of which were classified as pathogenic or likely pathogenic and one as a variant of uncertain significance.

Autosomal recessive inheritance was observed in patients with *ASCC1*, *VRK1*, and *HEXB* variants, all of whom carried homozygous missense variants with heterozygous parents. Among these, variants in *VRK1* and *HEXB* were previously reported, whereas the *ASCC1* variant was novel. The two patients carrying the *ASCC1* variant were unrelated individuals from different families. In addition, segregation analysis was available for one of the patients carrying the *ASCC1* variant (c.836 C>T, p.Thr279Ile). Family screening demonstrated that both parents and the unaffected sibling were heterozygous carriers, while the affected individual was homozygous for the variant. This segregation pattern, together with the compatible clinical phenotype, provided additional supportive evidence for pathogenicity according to ACMG criteria, allowing reclassification of this variant to likely pathogenic. A hemizygous missense variant in *UBAI* was identified in one male patient and was maternally inherited.

Muscle involvement patterns varied according to genotype, as summarized in Table 1. Generalized muscle weakness was the predominant clinical presentation in patients with *BICD2* and *ASCC1* variants, whereas a scapuloperoneal distribution of weakness was characteristic of patients with *TRPV4* variants. Nearly all patients exhibited inability to walk or severe gait impairment.

Table 1 Clinical, electrophysiological, neuroimaging, and genetic characteristics of patients with non-5q SMA

	<i>BICD2</i>	<i>TRPV4</i>	<i>ASCC1</i>	<i>VRK1</i>	<i>UBA1</i>	<i>HEXB</i>
Demographic and clinical features						
n	3	3	2	1	1	1
Sex (M/F)	1/2	3/0	2/0	1/0	1/0	0/1
Current age (years)	9 years, 6 years, 12 years	13 years, 4 months, 16 years, 7 months, 14 years 2 months	At birth/At birth	9 years 6 months	4 years 9 months	15 years
Age at symptom onset (<18 years)	At birth, 2 years 4 months, 18 months	8 years 4 months, 10 years 7 months, 9 years 2 months	At birth/ At birth	3 years 6 months	1 years 6 months	8 years
Family history/Pattern of inheritance	-/AD	-/AD	+ /AR	-/AR	+ /X-linked recessive	+ /AR
Consanguinity	+ /+ /+	+ /- /-	+ /+	-	+	+
Generalized muscle weakness (Proximal SMA)	+ /- /+	- /- /-	+ /+	-	-	+
Scapuloperoneal	- /- /-	+++	-	-	-	-
Muscle weakness	+ /- /-	- /- /-	-	-	-	-
Facial weakness	+ /- /-	- /- /-	+	-	-	+
Axial weakness						
Deep Tendon Reflexes	Absent+ /+ /absent	n /n /n - /- /-	+ /+ - /-	Absent -	+	Brisk +
Spasticity	+ /- /-					
Walking difficulty	Immobility/ Normal/ +	- /- /+	Immobility/Immobility	+	+	+
Key clinical findings						
Arthrogyposis	+ /- /-	- /- /-	+ /+	-	-	-
Contractures	+ /- /-	- /- /-	- /-	-	-	+
Pes cavus/planus	+ /+ /+	- /- /-	+ /+	-	+	-
Skeletal dysplasia	- /- /-	+ /+ /+	- /-	-	-	-
Bone fractures	- /- /-	- /- /-	- /-	-	-	+
Myoclonic epilepsy						
Respiratory or heart insufficiency	Respiratory insufficiency	-	Respiratory insufficiency	-	-	Respiratory and heart insufficiency
Weakness distribution	Predominantly lower limb involvement	Scapuloperoneal pattern (distal > proximal)	Generalized hypotonia at birth	Progressive proximal and distal weakness	Mild distal weakness	Progressive generalized weakness
CK (0-200 U/L)	98/ 120/ 150	189/285/312	136	88	101	149
Motor function scales						
HFMSE (0-66)	40-55NA	35-50NA	15-35NA	20-40NA	35-55NA	15-35NA
CHOP-INTEND (0-64)	3-4/5	Dis-tal LL 3-4/5	1-3/5	2-4/5	3-4/5	2-3/5
MRC muscle strength						
Ambulation	Usually ambulant	Ambulatory with steppage gait	Often non-ambulant	Progressive loss of ambulation	Mild-moderate impairment	Progressive decline
Neuroimaging and Electrophysiology						

Table 1 (continued)

	<i>BICD2</i>	<i>TRPV4</i>	<i>ASCC1</i>	<i>VRK1</i>	<i>UBA1</i>	<i>HEXB</i>
MRI	-/ Normal	Periventricular lacunar-like lesions on T2-weighted MRI	Nonspecific cerebral microhemorrhages on SWI	Pontocerebellar hypoplasia with pontine and cerebellar atrophy	Normal	Bilateral thalamic T2 hyperintensity
Electrophysiological findings (NCS/EMG) Reduced CMAP amplitudes with neurogenic motor unit potentials	Reduced CMAP amplitudes with preserved motor conduction velocities; sensory studies normal; needle EMG showing chronic denervation features (reduced recruitment, fibrillation potentials) <i>when available</i>	Reduced CMAP amplitudes predominantly in distal muscles; motor conduction velocities preserved or mildly reduced; sensory studies largely normal; needle EMG consistent with chronic denervation <i>when available</i>	Markedly reduced CMAP amplitudes; limited electrophysiological data due to early-onset severe phenotype; available studies consistent with neurogenic involvement	Reduced CMAP amplitudes; preserved motor conduction velocities; sensory studies normal; needle EMG showing chronic denervation consistent with anterior horn cell involvement <i>when available</i>	Mildly reduced CMAP amplitudes; motor conduction velocities preserved; sensory studies normal; needle EMG demonstrating chronic neurogenic changes <i>when available</i>	Reduced CMAP amplitudes; preserved motor conduction velocities; sensory studies normal; needle EMG showing chronic denervation features <i>when available</i>
Sensory nerve conduction	Normal sensory nerve conduction	Normal sensory nerve conduction	Normal sensory nerve conduction	Normal sensory nerve conduction	Normal sensory nerve conduction	Normal sensory nerve conduction
Genetic analysis WES	c.2239C>T (p.Arg747Cys) c.2080C>T (p.Arg694Cys) c.1496T>C (p.Leu499Pro)	c.1781G>A (p.Arg594His) c.925C>T (p.His309Tyr) c.1847G>A (p.Arg616Gln)	c.836C>T (p.Thr279Ile)	c.706G>A (p.Val236Met)	c.449C>T (p.Pro150Leu)	c.1597C>T (p.Arg533Cys)

Abbreviations: *AD* autosomal dominant, *AR* autosomal recessive, *CK* creatine kinase, *CMAP* compound muscle action potential, *EMG* electromyography, *MUAP* motor unit action potential, *WES* whole-exome sequencing. The symbols “+” and “−” indicate presence or absence of the corresponding clinical feature, *NA* not available, *HFMSE* Hammersmith Functional Motor Scale Expanded, *CHOP-INTEND* Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders. HFMSE and CHOP-INTEND assessments were not systematically available for all patients due to the retrospective design and heterogeneous clinical follow-up

Functional severity and standardized motor assessments across genotypes are summarized in Table 1.

Additional clinical features included arthrogryposis in patients with *ASCC1* variants, joint contractures in four patients, pes cavus or pes planus in three patients, skeletal dysplasia in three patients, bone fractures in one patient, and myoclonic epilepsy in one patient.

Creatine kinase (CK) levels were generally within normal limits, except for mild to moderate elevation (189–312 U/L) observed in patients with *TRPV4* mutations. Electromyographic evaluations revealed a neurogenic pattern in all patients.

Brain MRI findings, summarized in Table 1 and illustrated in Fig. 1, revealed lacunar-like infarcts in the *TRPV4* group, cerebral microhemorrhages in patients with *ASCC1* variants, pontocerebellar hypoplasia in the patient with the *VRK1* mutation, and thalamic involvement in the patient with the *HEXB* mutation.

Electrophysiological evaluation included motor and sensory nerve conduction studies as well as needle electromyography. Motor nerve conduction studies demonstrated reduced compound muscle action potential (CMAP) amplitudes in affected nerves, while motor conduction velocities were within or near the normal range. Sensory nerve conduction studies were largely preserved, supporting a predominantly motor neuron process rather than a primary peripheral neuropathy. F-wave responses, when available, were either normal or mildly prolonged. Needle electromyography revealed reduced recruitment, fibrillation potentials, positive sharp waves, and chronic neurogenic motor unit action potentials in clinically affected muscles. These findings are consistent with previously reported electrophysiological patterns in hereditary motor neuron disorders and several non-5q SMA phenotypes.

Brain MRI findings, summarized in Table 1 and illustrated in Fig. 1, revealed lacunar-like infarcts in the

Table 2 Genetic features of the patients with non-5q spinal muscular atrophy (SMA)

Gene	RefSeq ID	Transcript change	Protein change	Zygosity	Inheritance	Variant type	ACMG classification	gnomAD frequency	Clinvar	Novelty
<i>BICD2</i>	NM_001003800.2	c.2080 C>T	p.Arg694Cys	Heterozygous parents	De novo (AD)	Mis-sense	Pathogenic (PS4, PM2, PM5, PP3, PM1, PP5)	Not reported	Pathogenic/Likely pathogenic	Previously reported
<i>BICD2</i>	NM_001003800.2	c.2239 C>T	p.Arg747Cys	Heterozygous parents	De novo (AD)	Mis-sense	Pathogenic (PS4, PP1, PM2, PP3, PM5, PP5)	Not reported	Pathogenic/Likely pathogenic	Previously reported
<i>BICD2</i>	NM_001003800.2	c.1496T>C	p.Leu499Pro	Heterozygous parents	De novo (AD)	Mis-sense	Likely Pathogenic (PP1, PP4, PM2, PM6)	Not reported	-	Novel
<i>TRPV4</i>	NM_021625.5	c.1781G>A	p.Arg594His	Heterozygous parents	Unknown (AD)	Mis-sense	Pathogenic (PS3, PS2, PM2, PM5, PM1, PP3, PP5)	Not reported	Pathogenic/Likely pathogenic	Previously reported
<i>TRPV4</i>	NM_021625.5	c.925 C>T	p.His309Tyr	Heterozygous parents	Unknown (AD)	Mis-sense	VUS (PM1, PM2)	Not reported	VUS	Previously reported
<i>TRPV4</i>	NM_021625.5	c.1847G>A	p.Arg616Gln	Heterozygous parents	Unknown (AD)	Mis-sense	Likely Pathogenic (PS4, PM2, PM1, PP3)	Not reported	Pathogenic	Previously reported
<i>ASCC1</i>	NM_001198800.3	c.836 C>T	p.Thr279Ile	Homozygous parents	Parents: Het. (AR)	Mis-sense	Likely Pathogenic (PM2, PP3, PP1, PP4)	Not reported	-	Novel
<i>VRK1</i>	NM_003384	c.706G>A	p.Val236Met	Homozygous parents	Parents: Het. (AR)	Mis-sense	Likely Pathogenic (PM3, PM2, PP3, PP2)	0.0009%	Pathogenic/Likely pathogenic	Previously reported
<i>UBA1</i>	NM_153280.3	c.449 C>T	p.Pro150Leu	Hemizygous parents	Maternal (X-linked)	Mis-sense	VUS (PM2, PP2)	Not reported	-	Novel
<i>HEXB</i>	NM_000521.4	c.1597 C>T	p.Arg533Cys	Homozygous parents	Parents: Het. (AR)	Mis-sense	Pathogenic (PM3, PM2, PM5, PM1, PP3, PP5)	0.0021%	Pathogenic/Likely pathogenic	Previously reported

TRPV4 group, cerebral microhemorrhages in patients with *ASCC1* variants, pontocerebellar hypoplasia in the patient with the *VRK1* mutation, and thalamic involvement in the patient with the *HEXB* mutation.

Discussion

Recent studies have emphasized the substantial genetic and clinical heterogeneity of non-5q spinal muscular atrophy and related hereditary motor neuron disorders. Advances in next-generation sequencing have expanded the spectrum of genes associated with SMA-like phenotypes and improved

diagnostic yield in *SMN1*-negative patients [7–10]. In particular, Theuriet et al. reported that whole-exome sequencing significantly increases the likelihood of identifying a genetic diagnosis in such patients, while Fernández-Eulate et al. demonstrated considerable phenotypic overlap across different genotypes [8, 9]. Similarly, Zambon et al. highlighted that early-onset hereditary neuronopathies represent a genetically diverse group requiring integrated clinical, electrophysiological, and genetic evaluation [10].

In our cohort, the diagnostic yield of next-generation sequencing among *SMN1*-negative patients was 7.2%, which is notably lower than the approximately 30–40% yield reported in previous studies [8–10]. This difference

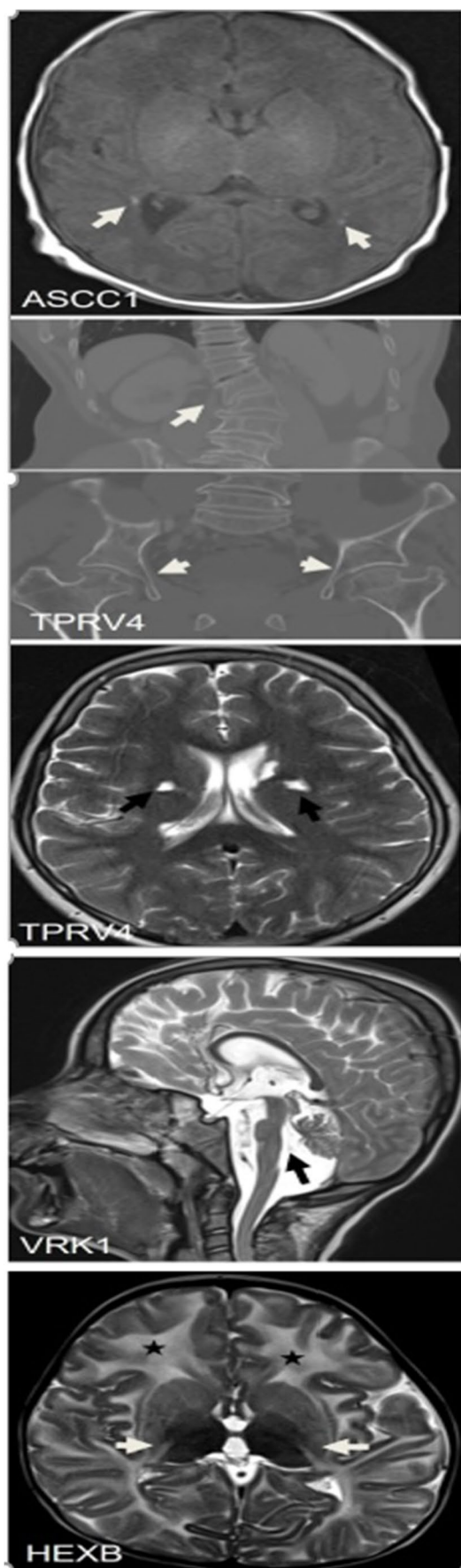


Fig. 1 Representative neuroimaging and skeletal findings in childhood-onset non-5q spinal muscular atrophy (SMA)

(A) *ASCCI*: Axial susceptibility-weighted imaging (SWI) demonstrating multiple punctate cerebral microhemorrhages within the supratentorial white matter (white arrows)

(B) *VRK1*: Sagittal T2-weighted brain MRI showing pontocerebellar hypoplasia with marked atrophy of the pons and cerebellar hemispheres (black arrow)

(C) *TRPV4*: Coronal radiographic image demonstrating skeletal abnormalities involving the long bones, characterized by metaphyseal irregularities and cortical thinning (white arrows)

(D) *TRPV4*: Axial T2-weighted brain MRI revealing periventricular lacunar-like lesions (black arrows)

(E) *HEXB*: Axial T2-weighted brain MRI demonstrating bilateral symmetric thalamic hyperintensity (white arrows)

may reflect the broad inclusion criteria and the marked clinical heterogeneity of SMA-like presentations in our cohort, as well as differences in sequencing strategies and variant interpretation. Our findings further indicate that a substantial proportion of patients with SMA-like phenotypes remain genetically unresolved despite advanced sequencing approaches, underscoring the need for more comprehensive genomic analyses and larger, well-characterized cohorts.

Consistent with previous reports, our cohort demonstrated heterogeneous clinical presentations associated with variants in multiple genes, including *BICD2*, *TRPV4*, *ASCCI*, *VRK1*, *UBA1*, and *HEXB*. Beyond confirming this heterogeneity, our findings provide additional insight into genotype-associated clinical variability within a single pediatric cohort. The integration of clinical, electrophysiological, and neuroimaging data allows a more comprehensive characterization of these conditions and supports the use of a multimodal diagnostic approach in children presenting with SMA-like phenotypes.

Three of our patients carrying *BICD2* variants demonstrated markedly different clinical presentations. *BICD2*-related spinal muscular atrophy with lower extremity predominance (SMALED) is commonly subdivided into SMALED type 2A and type 2B, reflecting differences in disease severity and distribution of muscle weakness [10–12]. In line with this classification, our cohort exhibited a broad phenotypic spectrum, ranging from a severe congenital presentation with neonatal-onset arthrogryposis and long-bone fractures, suggestive of a SMALED2A-like phenotype, to a milder, ambulatory course consistent with SMALED2B. These findings reinforce the considerable phenotypic variability associated with *BICD2* variants.

Although *BICD2*-associated SMALED is typically inherited in an autosomal dominant manner, intrafamilial variability has been described. In our cohort, the absence of a family history in the severely affected patient contrasted with the presence of a similarly affected relative in another case. While genetic testing in extended family members was not available, this pattern raises the possibility of reduced

or variable penetrance, a phenomenon previously reported in *BICD2*-related disorders [11]. In addition, the variants identified in our cohort (p.Arg747Cys and p.Arg694Cys) differ from commonly reported mutation hotspots such as p.Ser107Leu, further supporting the expanding mutational and phenotypic spectrum of *BICD2*-associated disease.

ASCC1-related spinal muscular atrophy with congenital bone fractures (SMABF2) is classically characterized by severe prenatal or neonatal hypotonia, respiratory insufficiency, arthrogryposis, and multiple long-bone fractures [13–20]. In contrast, our patients demonstrated a milder clinical course with preserved mobility and absence of overt skeletal fragility. However, skeletal involvement was still evident in the form of arthrogryposis and contractures, indicating that the phenotypic spectrum may be broader than previously recognized.

Notably, brain MRI revealed cerebral microhemorrhages in both cases, a finding not commonly emphasized in earlier reports of *ASCC1*-related disease. Given the limited number of observations and the lack of systematic comparison, this feature should be interpreted cautiously and may represent a nonspecific or secondary radiological finding rather than a disease-specific marker. Nevertheless, these observations further support the expanding clinical heterogeneity of SMABF2.

VRK1 encodes a serine/threonine kinase involved in cell-cycle regulation and neuronal proliferation, and biallelic variants have been associated with a spectrum of disorders including pontocerebellar hypoplasia and childhood-onset spinal muscular atrophy [3, 21–23]. Consistent with previous reports, our patient presented with progressive motor impairment and pontocerebellar hypoplasia on MRI, supporting the established neuroimaging phenotype associated with *VRK1* mutations [21–25].

UBA1 encodes a key enzyme initiating the ubiquitination cascade, and pathogenic variants have been associated with X-linked forms of spinal muscular atrophy and related motor neuron disorders [26, 27]. The clinical presentation observed in our cohort, including motor delay and distal weakness, is consistent with previously reported phenotypes [27, 28].

TRPV4 encodes a calcium-permeable cation channel involved in mechanosensation and skeletal development. Mutations in *TRPV4* are associated with a spectrum of neuromuscular and skeletal disorders [29–33]. In our cohort, the observed scapulo-peroneal weakness and elevated creatine kinase levels are in line with previously described *TRPV4*-associated motor neuropathies, although genotype-specific conclusions remain limited by the small sample size.

HEXB encodes the β -subunit of β -hexosaminidase, and variants have been associated with neurodegenerative and neuromuscular phenotypes [34–39]. In our cohort, the presence of bilateral thalamic signal abnormalities on MRI, together with progressive motor impairment, is consistent

with previously reported findings, although interpretation remains limited by the small number of observations.

Our findings provide several important contributions to the current understanding of non-5q spinal muscular atrophy. First, by integrating clinical, electrophysiological, neuroimaging, and genetic data within a single pediatric cohort, this study offers a comprehensive characterization of genotype-associated variability across multiple rare genes. Second, the observed phenotypic diversity, including variability in disease severity, atypical neuroimaging findings, and patterns suggestive of variable penetrance, extends the currently recognized clinical spectrum of non-5q SMA.

From a clinical perspective, these findings underscore the importance of considering non-5q SMA in children presenting with SMA-like phenotypes despite negative *SMN1* testing. A multimodal diagnostic approach incorporating detailed clinical evaluation, electrophysiological studies, neuroimaging, and advanced genetic testing may facilitate earlier and more accurate diagnosis in this heterogeneous group of disorders.

This study has several limitations that should be acknowledged. First, the retrospective design may have resulted in incomplete or heterogeneous clinical documentation. Second, the relatively small sample size, particularly within genotype-specific subgroups, limits the ability to draw definitive genotype–phenotype correlations. Third, genetic testing in extended family members was not consistently available, which restricts the interpretation of inheritance patterns and variable penetrance.

Future studies including larger, prospectively collected cohorts and comprehensive family-based genetic analyses will be essential to better define genotype–phenotype relationships and to clarify the clinical and neuroimaging spectrum of non-5q spinal muscular atrophy.

Conclusion

In conclusion, this study provides a comprehensive clinical, electrophysiological, neuroimaging, and genetic characterization of pediatric patients with non-5q spinal muscular atrophy. Our findings highlight the marked genetic and phenotypic heterogeneity of this group and expand the recognized clinical spectrum across multiple rare genes. Importantly, the observed variability in disease severity, neuroimaging findings, and potential patterns of variable penetrance underscores the need for an integrated diagnostic approach. In children presenting with SMA-like phenotypes and negative *SMN1* testing, non-5q SMA should be systematically considered, and multimodal evaluation including genetic analysis is essential for accurate diagnosis.

Acknowledgements The authors are grateful to the personnel of Pediatric Neurology Policlinic and Electromyography Laboratories of University Medical Faculty for their assistance. Beril DİLBER, Elif Acar ARSLAN, Burcu Pariltan KÜÇÜKALIOĞLU, Hasan MERAL, Safiye Güneş SAGER, Ayberk Türkyılmaz, Tülay Kamaşak, and Ercan Demir equally contributed to the conception and design of the research; Beril DİLBER, Elif Acar ARSLAN, Burcu Pariltan KÜÇÜKALIOĞLU, Hasan MERAL, Safiye Güneş SAGER, Ayberk Türkyılmaz, Tülay Kamaşak, Ercan Demir contributed to conception and design of the research; Beril DİLBER, Elif Acar ARSLAN, Burcu Pariltan KÜÇÜKALIOĞLU, Hasan MERAL, Safiye Güneş SAGER, Emine TEKİN, Ayberk Türkyılmaz, Tülay Kamaşak, Ercan Demir contributed to interpretation of the data; and Beril DİLBER, Elif Acar ARSLAN, Burcu Pariltan KÜÇÜKALIOĞLU, Hasan MERAL, Safiye Güneş SAGER, Emine TEKİN, Ayberk Türkyılmaz, Tülay Kamaşak, Ercan Demir drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring integrity and accuracy of the work, and read and approved the final manuscript.

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Funding The authors have nothing to report.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Consent Informed consent was obtained from the participants to participate in the current study.

Competing interests The authors declare no competing interests.

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