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Pediatric toe-walking cohort with heterozygous SBF1 variants: A phenotypic description

David Pomarino [a,*], Amel Sidi Athmane [a], Bastian Fregien [b], Alexander Nazarkin [c], Kevin M. Rostásy [d]

[a] Pomarino Praxis für Ganganomalien, Hamburg, Germany
[b] ORTHOMAX, orthopädische üBAG GbR Langenhagen, Germany
[c] Praxis für Ganganomalien, Hamburg, Germany
[d] Fakultät für Gesundheit (Department für Humanmedizin), Universität Witten, Herdecke, Witten, Germany

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ABSTRACT

Purpose: Persistent toe walking is frequently labeled idiopathic; however, targeted genetic testing in selected cohorts can identify variants in genes implicated in neuromuscular disease. SBF1 is a known cause of autosomal recessive Charcot–Marie–Tooth disease type 4B3 (CMT4B3), whereas the clinical relevance of heterozygous SBF1 variants—particularly variants of uncertain significance (VUS)—remains unclear. We aimed to describe, in an exploratory manner, the clinical features of children with persistent toe walking in whom heterozygous SBF1 variants were identified, and to contextualize these observations using published CMT4B3 families and Human Phenotype Ontology (HPO) feature frequencies.

Methods: We retrospectively analyzed children referred to a specialized toe-walking clinic who underwent a standardized blinded clinical assessment and targeted 49-gene next-generation sequencing. Individuals with alternative sequencing approaches or known non-genetic causes of toe walking were excluded. Heterozygous SBF1 variants were summarized using HGVS nomenclature, ACMG classification, population allele frequency, and report date. Phenotypic frequencies were compared with published SBF1-related CMT4B3 families and with HPO-reported feature frequencies for CMT4B3.

Results: The cohort comprised 86 children (mean age 9.5 years), all with persistent toe walking. Common findings included skeletal features (e.g., pes cavus and lumbar hyperlordosis), whereas muscle weakness and deep tendon reflex abnormalities were less frequent than reported in recessive CMT4B3 families. Genetic testing identified a spectrum of **heterozygous SBF1 variants**, predominantly classified as **VUS**.

Conclusions: In this referral-based cohort, heterozygous SBF1 variants were observed in children with persistent toe walking and accompanying mild neuromotor/musculoskeletal features that partially overlap with reported CMT4B3 phenotypes; however, these findings are descriptive and do not establish causality or enrichment. Longitudinal follow-up, segregation/phase determination, and electrophysiological studies are needed to clarify clinical significance, potential biallelic configurations in some individuals, and possible gene-dosage or modifier effects.

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Introduction

Toe walking (TW) refers to a gait pattern characterized by predominant forefoot loading during walking. In this pattern, the normal heel-to-toe rolling motion of the foot is absent [1]. It represents a common developmental gait pattern in early childhood. However, when this behavior persists beyond the age of 2–3 years, further evaluation is recommended, as persistent toe walking may be associated with neurological or neuromuscular conditions such as cerebral palsy, muscular dystrophy, or autism spectrum disorder [2].

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* Correspondence to: Pomarino. Praxis für Ganganomalien, Rothenbaumchaussee 114, Hamburg 20149, Germany.
E-mail addresses: forschung@ptz-pomarino.de (D. Pomarino), info@ptz-pomarino.de (A. Sidi Athmane), info@orthomax.de (B. Fregien), buchhaltung@ptz-pomarino.de (A. Nazarkin), kevin.rostasy@uni-wh.de (K.M. Rostásy).

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Historically, children without identifiable orthopedic or neurological causes were often described as having idiopathic or habitual toe walking [3]. More recent studies, however, suggest a potential genetic contribution to this phenotype. Familial aggregation and findings from targeted next-generation sequencing (NGS), using panels that include genes implicated in hereditary neuropathies and myopathies associated with gait abnormalities, have increasingly identified rare variants in children previously classified as idiopathic toe walkers, with confirmatory Multiplex Ligation-dependent Probe Amplification (MLPA) performed when indicated [4].

Charcot–Marie–Tooth (CMT) disease, also known as hereditary motor and sensory neuropathy, represents a clinically and genetically heterogeneous group of inherited peripheral neuropathies and is the most common inherited neuromuscular disorder. It is characterized by progressive distal muscle weakness, sensory loss, foot deformities such as pes cavus, and reduced or absent deep tendon reflexes. Most individuals develop symptoms during the first or second decades of life, typically beginning in the lower extremities and gradually involving the upper limbs.

Advances in next-generation sequencing (NGS) have expanded the genetic classification of CMT beyond the traditional demyelinating (CMT1) and axonal (CMT2) forms, uncovering numerous rare sub types caused by mutations in distinct genes involved in myelin structure and axonal maintenance [5]. One such gene of emerging interest is SBF1 (SET-Binding Factor 1), which encodes a catalytically inactive pseudophosphatase belonging to the myotubularin-related (MTMR) protein family. The SBF1 protein functions as a scaffold within the SBF1–SBF2–MTMR2 complex, contributing to phosphoinositide metabolism, endosomal trafficking, and Schwann cell myelin maintenance.

Pathogenic SBF1 variants cause Charcot–Marie–Tooth disease type 4B3 (CMT4B3), a rare autosomal recessive demyelinating neuropathy characterized by early-onset distal muscle weakness, areflexia, progressive sensory loss, and structural foot deformities [6,7].

To date, only a handful of families (approximately seven worldwide) have been reported with genetically confirmed SBF1-related CMT4B3, most harboring homozygous or compound heterozygous mutations. These patients typically exhibit a severe early-onset neuropathic phenotype, often accompanied by developmental abnormalities and cranial nerve involvement, including visual or facial weakness, as well as progressive distal muscle weakness [8,9].

In this exploratory, hypothesis-generating study, we build upon previously reported families with confirmed SBF1-related neuropathies to compare their clinical profiles with those of children presenting with persistent toe walking in whom heterozygous SBF1 variants of uncertain significance (VUS) were identified. We additionally descriptively contrast the clinical features observed in our cohort with those listed for Charcot–Marie–Tooth disease type 4B3 (CMT4B3) (OMIM:615284) in the Human Phenotype Ontology (HPO) database [10]. Through this dual comparative framework, we aim to (1) describe the range of clinical features observed in children with heterozygous SBF1 variants, (2) explore their possible relevance to pediatric gait abnormalities in an exploratory manner, and (3) provide groundwork for future investigations into gene-dosage effects and incomplete penetrance in peripheral neuropathies.

Methods

Children included in this study were drawn from a larger clinical population of approximately 1500 patients referred to our specialized toe-walking institute for evaluation of persistent toe walking. Eligibility for genetic testing was based exclusively on the clinical diagnosis of idiopathic persistent toe walking established through the structured diagnostic work-up before and/or after referral to our clinic, and this represented the first genetic evaluation for all included children. Genetic testing was performed without prior knowledge of SBF1 or any other specific gene status. Referrals occurred through two main pathways.

In the first pathway, children presented to our clinic for initial evaluation of persistent toe walking and were subsequently referred, as part of routine diagnostic work-up, to pediatric neurologists and/or pediatric orthopedists to exclude common neurological, neuromuscular, and orthopedic causes of toe walking.

In the second pathway, children had already undergone comprehensive evaluation in a Social Pediatrics Center or equivalent specialized setting, where alternative neurodevelopmental, neuromuscular, and myopathic diagnoses had been clinically excluded and were then referred to our institute for further assessment of persistent toe walking.

In both referral pathways, children were included only if the etiology of the gait abnormality remained unclear after this structured diagnostic evaluation. At our institute, each child underwent a standardized examination described in detail in the section Clinical Examination and Assessment Procedures. Persistent toe walking was documented when observed during clinical evaluation and reported by caregivers as the child's predominant walking pattern.

Genetic evaluation was subsequently performed using the same targeted 49-gene next-generation sequencing (NGS) panel applied in our previous work, as part of routine diagnostic evaluation for persistent toe walking.

For the present analysis, we performed a post hoc subset analysis including those children who were found to carry at least one heterozygous SBF1 variant on panel sequencing.

Children with established orthopedic, neuromuscular, or neurodevelopmental diagnoses known to cause toe walking (e.g., cerebral palsy, autism spectrum disorder, muscular dystrophy, tethered cord syndrome) were excluded. Severe structural deformities unrelated to neuropathic etiologies (e.g., congenital talipes equinovarus) were also considered exclusion criteria.

Exclusion of alternative neurological and neurodevelopmental diagnoses was based on prior documented assessments by referring pediatricians, pediatric neurologists, pediatric orthopedists, or Social Pediatrics Centers. Neuroimaging studies (e.g., brain MRI) and formal neuropsychological or developmental assessments were only performed when indicated.

Clinical examination and assessment procedures

All children underwent a standardized clinical evaluation blinded to the genetic testing results. The assessment followed a structured protocol consisting of detailed history taking, musculoskeletal evaluation, gait analysis, and neurological examination. The procedures are described below.

Patient history

A structured medical history was obtained from parents or caregivers. Information collected included:

- Age, sex, age at independent walking, and age at onset of toe walking
- Pattern and progression of the gait abnormality
- Family history of toe walking or known hereditary neuropathies
- Presence of muscular symptoms (e.g., muscular pain, muscle cramps, weakness)
- Developmental history (speech or language delay, social or behavioral difficulties)
- Visual impairment
- Bladder and bowel control-assessed through caregiver-reported frequency of daytime and nighttime voiding and bowel movements. Based on developmental benchmarks, most children typically acquire daytime and nighttime bowel control by age 4–5 years [11].

Physical examination

Postural and structural assessment

A full morphological inspection was performed to document structural abnormalities. Evaluated features included:

- Lumbar hyperlordosis
- Pes cavus
- Foot drop
- Thoracic deformities: pectus excavatum or pectus carinatum
- Digital anomalies: clinodactyly or brachydactyly

Balance and motor function

Balance was tested using a standard single-leg stance:

- Single-leg stance test:

Inability to maintain stance for ≥ 5 s without support was considered abnormal.

Motor function was assessed using two functional tasks performed in standing position:

- **Heel Walking Test:** inability to maintain heel walking indicated weakness of ankle dorsiflexors.
- **Foot Drop Test (active toe-lift):** difficulty lifting the forefoot against gravity was considered positive for dorsiflexor weakness or impaired motor control.

Ankle range of motion (ROM)

Ankle ROM was measured using a goniometer following the neutral (0°) method [12].

- **Dorsiflexion:** restricted when < 20°
- **Plantarflexion:** restricted when < 50°

Measurements were taken with knees extended in supine position to assess gastrocnemius vs. soleus contributions.

Neurological examination

Neurological assessment included:

- **Deep tendon reflexes (patellar):** The patellar reflex was tested with the patient seated and legs relaxed. The patellar tendon was tapped briskly with a reflex hammer, and a normal response was defined as a brief knee extension [13]. Diminished or absent reflexes were documented as hyporeflexia or areflexia.
- Tremor evaluation:

Position: arms extended forward, palms down

Provocation: repeated opening and closing of hands

Tremor was considered present when rhythmic oscillatory movements appeared during or after the maneuver.

Genetic testing

Genetic analysis for all included children was carried out using a targeted next-generation sequencing (NGS) approach aimed at detecting hereditary neuropathies and myopathies that may present with gait abnormalities. Only individuals tested with this specific NGS workflow were eligible; children who had undergone alternative sequencing methods, such as whole-exome sequencing, were excluded to maintain methodological uniformity.

All samples were processed with the same validated 49-gene NGS panel (Thermo Fisher Scientific), which includes genes previously associated with neuromuscular and neuropathic conditions. The gene list is provided in Supplementary Table 1. The panel design was informed by Human Phenotype Ontology (HPO) terms related to neuro- and myopathies. Saliva samples were collected by the treating pediatric specialists as part of routine diagnostic work-up. DNA isolation followed standard clinical procedures, and sequencing was performed on the Ion Torrent platform (Thermo Fisher Scientific, Waltham, MA, USA). Target regions comprised all coding exons with flanking intronic boundaries (± 10 bp).

Copy-number variation (CNV) analysis was incorporated into the workflow. When the NGS output suggested possible deletions or duplications, confirmatory testing was undertaken using Multiplex Ligation-dependent Probe Amplification (MLPA) with PMP22-specific probe sets (MRC-Holland). This enabled reliable detection of clinically relevant CNVs—most notably the classical PMP22 duplication observed in CMT1A.

Variant interpretation followed the ACMG classification framework, assigning findings to pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign categories. All interpretations were date-stamped to account for ongoing updates in population frequency data and database annotations. Only P, LP, and VUS variants were retained for analysis; benign findings can be provided on request.

In silico tools (e.g., Mutation Taster, PolyPhen-2, Mutation Assessor) and multiple variant databases (HGMD Professional, LOVD-IARC, dbSNP, ClinVar, and additional resources when relevant) supported variant evaluation. The testing approach does not fully exclude variants outside captured regions—such as deep intronic, regulatory, or highly homologous genomic segments—and detection of low-level mosaicism or single-exon CNVs may be limited.

According to validation data from the accredited diagnostic laboratory (Labor Dr. Heidrich & Colleagues, Hamburg, Germany), the overall analytical sensitivity for clinically relevant variants exceeded 96 %. Documentation of the laboratory's internal validation procedures can be provided upon request. Segregation testing was not routinely undertaken due to feasibility and cost constraints. The complete list of genes included in the 49-gene panel is provided in (Supplementary Table 1).

Statistical analysis

Descriptive statistical methods were applied to characterize demographic variables, clinical findings, and genetic results within the study cohort. All analyses were conducted using LibreOffice Calc (v.25.2.5.2). Categorical data—such as sex, variant classification, and recorded clinical features—were summarized as absolute counts and percentages. Percentages were calculated using only available data, with missing values excluded from denominators.

Where relevant, 95 % confidence intervals for proportions were estimated using Fisher's exact test. The study was not designed to support multi variable or adjusted statistical modeling; therefore, all comparisons are exploratory and descriptive in nature.

Results

Study population

The study population comprised 86 individuals between 3 and 20 years of age, with a mean age of 9.5 years. Males represented the majority of the cohort (60 patients, 69.8 %), while females accounted for 26 participants (30.2 %). To further characterize the pediatric cohort, age at evaluation was stratified into predefined groups: early childhood (3–5 years), middle childhood (6–10 years), early adolescence (11–15 years), and late adolescence (16–20 years) (Table 1).

Table 1
Age distribution of the study cohort.

Age group (years)	N (%)
3–5	10 (0.12 %)
6–10	46 (53.5 %)
11–15	26 (30.2 %)
16–20	5 (5.8 %)

Table 2
Clinical characteristics of the published CMT4B3 cases compared with our cohort.

Feature category		Our cohort—N(%)	Published CMT4B3 families—N = 7 (%)
Gait disturbances[a]		86 (100 %)	6 (86 %)
Skeletal anomalies	**Pes cavus**	75 (87.2 %)	4 (57.1 %)
	Spine[b]	42 (48.8 %)	4 (57.1 %)
	Hands[c]	63 (57.1 %)	3 (42.8 %)
Muscle weakness		18 (20.9 %)	7 (100 %)
Neurological findings	**DTR**[d]	5 (5.8 %)	7 (100 %)
	Visual impairments	8 (9.3 %)	1 (14.3 %)
Developmental features		**BBC**[e] **difficulties**—27 (31.4 %) **Social difficulties**—12 (13.9 %) **Speech difficulties**—47 (54.7 %)	**Intellectual impairment**—3 (42.8 %)

[a] In our cohort, this refers exclusively to persistent toe walking, whereas in previously published SBF1 family cases, gait abnormalities primarily manifested as unsteady gait;
[b] Our patients most commonly showed lumbar hyperlordosis. Reported CMT4B3 families exhibited lumbar hyperlordosis (n = 2), scoliosis (n = 1), and kyphoscoliosis (n = 1).
[c] Findings in our cohort included clinodactyly and brachydactyly, while published families showed syndactyly (n = 2), mild joint laxity, and a positive thumb sign.
[d] (DTR) Deep tendon reflexes: In our cohort, three children had reduced reflexes, while two had exaggerated reflexes. In contrast, previously reported families exhibited areflexia.
[e] (BBC) Bowel and bladder control: In our cohort, BBC difficulties denote lack of bladder or bowel continence beyond age 5, as per developmental norms.

Comparison with previously reported SBF1-related CMT4B3 families

To contextualize the clinical presentation of our cohort, we first compared their phenotypic features with those described in the seven previously published families carrying biallelic SBF1 variants and clinically diagnosed with Charcot–Marie–Tooth disease type 4B3 (CMT4B3) [9,21]. Reported features in those families included gait abnormalities, skeletal deformities, muscle weakness, hyporeflexia, neurological involvement, and developmental delay (Table 2).

Genetic findings

Genetic analysis in our cohort identified a spectrum of heterozygous SBF1 variants. Variant classification followed ACMG guidelines, and interpretations reflect the evidence available at the time of laboratory reporting. Because variant interpretation is dynamic and may evolve as new population data or functional evidence emerges, the corresponding report date and population allele frequencies are provided for each variant. Population frequencies were drawn primarily from gnomAD; when unavailable, alternative databases were referenced.

A summary of the detected SBF1 variants, including HGVS nomenclature, ACMG classification, allele frequency, and report date, is presented in (Supplemntary Table 2).

Comparison between VUS patients in our cohort and VUS families

To further refine the potential clinical significance of heterozygous SBF1 variants of uncertain significance (VUS), we compared the phenotypes of VUS carriers in our cohort with those reported in the few published CMT4B3 families harboring heterozygous or compound VUS variants. Although the published VUS families are rare (N = 3) and their phenotypes heterogenous, this comparison provides an additional layer of context for interpreting the milder presentations observed in our cohort. The analysis focuses on key domains commonly affected in SBF1-related neuropathies—gait abnormalities, skeletal deformities, muscle weakness, neurological signs, and developmental features—allowing evaluation of both shared and divergent characteristics between our VUS carriers and previously documented cases (Table 3).

Comparison with human phenotype ontology (HPO) frequencies for CMT4B3

To further characterize the clinical relevance of the SBF1 heterozygous variants in our cohort, we compared the prevalence of their observed symptoms with the approximate frequencies listed in the Human Phenotype Ontology (HPO) entry for CMT4B3 (Table 4).

Discussion

In this exploratory study, we investigated the clinical relevance of **heterozygous SBF1 variants** in a large pediatric cohort referred for persistent toe walking. While pathogenic or likely pathogenic *biallelic* SBF1 mutations are a well-established cause of **Charcot–Marie–Tooth disease type 4B3 (CMT4B3)**—a severe, early-onset demyelinating neuropathy—little is known about the phenotypic consequences of **heterozygous** variants, particularly those classified as variants of uncertain significance (VUS). Our findings show that heterozygous SBF1 variants were identified in children referred for persistent toe walking and were accompanied by mild, variably penetrant neuromuscular or musculoskeletal features that only partially overlap with the classical CMT4B3 phenotype. These observations are descriptive and do not demonstrate statistical enrichment or a causal relationship between heterozygous SBF1 variants and persistent toe walking. In the absence of a control cohort or population-based comparison sequenced using the same panel, we cannot assess whether the frequency of heterozygous SBF1 variants observed in this cohort exceeds what would be expected by chance.

Phenotypic comparison with published CMT4B3 families

The contrast between our cohort and previously described CMT4B3 families underscores the **distinct clinical profile** associated with heterozygous versus biallelic SBF1 variants. Patients with confirmed recessive CMT4B3 consistently exhibit **severe distal muscle weakness and universal areflexia**, frequently accompanied by structural deformities such as pes cavus, scoliosis, and digital anomalies. In comparison, children in our cohort displayed a **much milder phenotype**, with low frequencies of muscle weakness and hyporeflexia, and without evidence of progressive neuropathy. Moreover, none presented with the global developmental impairment or cranial nerve involvement reported in several CMT4B3 families.

The feature most consistently shared between the two groups was the presence of **skeletal abnormalities**, particularly **pes cavus** and **lumbar hyperlordosis**, although these were typically mild in our cohort. This partial phenotypic overlap indicates a descriptive similarity between the two groups but does not establish a causal role of heterozygous SBF1 variants in musculoskeletal or neuromotor development.

Persistent toe walking as a potential early neuromotor marker

While all of the children in our cohort presented with persistent toe walking, the previously published CMT4B3 cases

Table 3
Comparison of Clinical Features Between SBF1 VUS Carriers in Our Cohort and Published CMT4B3 VUS Families.

Feature category		Our cohort (VUS)—N = 87 (%)	Published cMT4B3 families (VUS)—N = 3 (%)
Gait disturbances[a]		87 (100 %)	3 (100 %)
Skeletal anomalies	**Pes cavus**	73 (87 %)	2 (66.7 %)
	Spine[b]	41 (49 %)	2 (66.7 %)
	Hands[c]	61 (72.6 %)	1 (33.3 %)
Muscle weakness		17 (20.2 %)	3 (100 %)
Neurological findings	**DTR**[d]	5 (6 %)	3 (100 %)
	Visual impairments	8 (9.5 %)	0
Developmental features		**BBC**[e] **difficulties**—26 (31 %)	**Intellectual impairment**—0
		Social difficulties—12 (14.2 %)	
		Speech difficulties—47 (56 %)	

[a] In our cohort, this refers exclusively to persistent toe walking, whereas in previously published SBF1 family cases, gait abnormalities primarily manifested as unsteady gait;
[b] Our patients most commonly showed lumbar hyperlordosis. Reported CMT4B3 families exhibited scoliosis (n = 1), and kyphoscoliosis (n = 1).
[c] Findings in our cohort included clinodactyly and brachydactyly, while one family showed syndactyly,
[d] (DTR) Deep tendon reflexes: In our cohort, three children had reduced reflexes, while two had exaggerated reflexes. In contrast, previously reported families exhibited areflexia.
[e] (BBC) Bowel and bladder control: In our cohort, BBC difficulties denote lack of bladder or bowel continence beyond age 5, as per developmental norms.

exhibited more generalized gait abnormalities, such as unsteady gait. Persistent toe walking may represent a **milder or earlier neurodevelopmental manifestation** associated with impaired distal motor control or subtle proprioceptive deficits—phenomena that are difficult to detect during early childhood.

Persistent toe walking may represent a nonspecific early functional finding that can precede or occur without progression to clinically overt neuropathy. This raises the possibility that heterozygous SBF1 variants may act as modifiers or coincidental findings rather than independent causes of neuromotor abnormalities.

Comparison with human phenotype ontology (HPO) data

Our comparison with HPO frequencies for CMT4B3 provides additional insight. While classical CMT4B3 shows high frequencies of **muscle weakness**, **areflexia**, and **progressive loss of ambulation**, these features were uncommon or absent in our cohort. Conversely, toe walking—a phenotype not described in HPO profiles—was universal in our cohort, suggesting that **current HPO entries may not capture the full phenotypic spectrum linked to SBF1 variation**, particularly for heterozygous states. However, the presence of heterozygous SBF1 variants in this cohort may be incidental, given the absence of a control group and the lack of demonstrated enrichment compared with background population frequencies.

Interestingly, several features considered “frequent” or “very frequent” in CMT4B3—such as pes cavus or spinal deformities—were also common in our cohort, albeit typically milder. This supports a possible **shared underlying biological vulnerability** affecting distal muscle balance or structural development.

Possible mechanisms: gene dosage, modifier effects, and sub clinical neuropathy

The emergence of symptoms in heterozygous carriers raises important biological questions. Three mechanistic explanations merit consideration:

Gene-dosage effects

Although CMT4B3 is classically recessive, reduced SBF1 function in heterozygous individuals may produce partial disruption of the MTMR2-SBF1-SBF2 complex, potentially affecting phosphoinositide signaling or Schwann cell biology in subtle ways.

Modifier or susceptibility effects

Heterozygous SBF1 variants may not be sufficient to cause neuropathy independently but could modify neuromotor development, especially when combined with environmental or biomechanical factors (e.g., ligamentous laxity, early gait abnormalities).

Sub clinical peripheral neuropathy

The low but notable frequencies of weakness and hyporeflexia suggest that some children may harbor mild peripheral nerve involvement below the threshold for clinical diagnosis.

Further electrophysiological or imaging-based studies would be essential to test these hypotheses.

An important alternative explanation for the observed phenotypes is the absence of segregation and phase determination. Several individuals in our cohort harbor more than one SBF1 variant; without parental testing, it is not possible to determine whether these variants are located in cis or in trans. Consequently, some children may carry compound heterozygous SBF1 variants and represent atypical or milder presentations of autosomal recessive CMT4B3 rather than true heterozygous carriers. This possibility must be considered when interpreting genotype–phenotype associations in the present study. Moreover, the use of a targeted gene panel rather than whole-exome or whole-genome sequencing limits the ability to exclude alternative genetic diagnoses, and some phenotypes observed in this cohort may be attributable to variants in genes not captured by the panel.

Developmental findings and their implications

We observed increased rates of speech difficulties, social challenges, and bowel/bladder control difficulties compared with

Table 4
Comparison of HPO frequencies with our cohort.

Phenotypic category	Our cohort	HPO frequency for CMT4B3 (OMIM:615284)
Gait disturbances	Toe walking (always)	N/A Loss of ambulation (frequent)
Skeletal anomalies	Pes cavus (very frequent)	Pes planus (very frequent)
	Lumbar hyperlordosis (frequent)	Scoliosis (very frequent)
	Clinodactyly/Brachydactyly (frequent)	Syndactyly (occasional)
Muscle weakness	Occasional	Very frequent
DTR	Hyporeflexia (uncommon)	Areflexia (very frequent)

HPO frequency categories: uncommon (1–4 %), occasional (5–29 %), frequent (30–79 %), very frequent (80–99 %), always (100 %).

population expectations. While these findings are **not typical of CMT4B3**, they may indicate broader neurodevelopmental variability within the cohort. Whether these features reflect underlying genetic contributions, subtle neuromotor impairments, or unrelated developmental variation remains unclear. However, their presence supports the possibility that **SBF1 may have broader roles in neurodevelopment** beyond peripheral myelination.

Strengths and limitations

A key strength of this study is the **large, systematically evaluated cohort**, with standardized clinical and genetic assessments. The blinded clinical examination minimizes bias in phenotype recording.

However, several limitations must be acknowledged:

- Absence of electrophysiological data:
  Nerve conduction studies could help determine whether subtle neuropathy is present.
- VUS interpretation remains dynamic:
  Some variants may eventually be reclassified as benign or pathogenic as population databases expand.
- Referral bias:
  Since all participants were referred for persistent toe walking, the cohort may not represent the full spectrum of heterozygous SBF1 carriers in the general population.
- Absence of a control or enrichment analysis:
  Without a population-based or clinically comparable control cohort sequenced using the same panel, we cannot determine whether heterozygous SBF1 variants are enriched in children with persistent toe walking compared with background population frequencies; therefore, the observed association may be coincidental.
- Lack of segregation analysis and phase determination:
  Parental testing was not performed; therefore, the phase (cis vs. trans) of multiple SBF1 variants identified in some individuals could not be determined. As a result, a subset of patients may carry compound heterozygous SBF1 variants and could, in fact, meet genetic criteria for autosomal recessive CMT4B3 with an atypical or milder phenotype. This uncertainty limits genotype–phenotype interpretation and has implications for clinical counseling.
- Restricted scope of the targeted gene panel:

Genetic testing was limited to a predefined 49-gene panel focused on hereditary neuropathies and myopathies associated with gait abnormalities. Consequently, pathogenic variants in genes not included in the panel may have been missed. This represents an important diagnostic blind spot, particularly given the exploratory nature of the study.

Clinical and research implications

Our findings suggest that heterozygous SBF1 variants may be observed in association with a mild, variably penetrant neuromotor phenotype, in contrast to the severe neuropathy associated with biallelic mutations. This highlights a possible extension of the range of clinical features observed in individuals carrying heterozygous SBF1 variants, without establishing a causal disease spectrum, and underscores the importance of considering neuromuscular genetic testing in children with persistent toe walking and musculoskeletal anomalies.

Future research directions include:

- Longitudinal follow-up to assess progression or resolution of symptoms
- Segregation and functional studies to clarify variant pathogenicity
- Electrophysiological assessment to detect subclinical neuropathy
- Larger multi center studies to refine genotype–phenotype correlations

Conclusion

This study provides a descriptive account of children with heterozygous SBF1 variants presenting with subtle neuromotor or musculoskeletal abnormalities, without establishing a causal relationship. While the phenotype differs markedly from classical CMT4B3, partial overlap suggests that SBF1 may play a broader role in neuromotor development than previously recognized. These findings warrant further investigation into SBF1 gene dosage effects and underscore the value of detailed phenotyping in interpreting genetic variants of uncertain significance. Importantly, the lack of parental testing and phase determination precludes exclusion of atypical recessive cases in a subset of patients, underscoring the need for segregation analysis in future studies.

Author contribution

David Pomarino contributed to the conceptualization of the study, data curation, and formal analysis. Amel Sidi Athmane contributed to data curation and investigation and drafted the original manuscript. Bastian Fregien and Alexander Nazarkin contributed to the study methodology and validation. Kevin M. Rostásy contributed to formal analysis, supervision, validation, and manuscript review and editing. All authors reviewed and approved the final manuscript.

Ethical statement

This study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. All procedures were performed in compliance with relevant laws and institutional guidelines and were approved by the ethical board of the Deutschen Verbandes für Physiotherapie an der Physio-Akademie in Wremen, Germany (project number 2025–02).

Prior to participation, written informed consent was obtained from all subjects. The consent process included explanations of the study's purpose, procedures, and any potential implications of the results. All data collected were formally anonymized to protect participant confidentiality.

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Declaration of Competing Interest

The Authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

Generative AI Deepseek was used in the writing phase of this manuscript exclusively for linguistic polishing and enhancing clarity. All scientific reasoning, data analysis, and intellectual substance remain the sole contribution of the authors. The AI was not employed in the research process itself.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gmg.2026.100095.

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Glossary

ACMG (American College of Medical Genetics and Genomics): Professional organization that publishes standards and guidelines for the interpretation and classification of genetic variants (e.g., pathogenic, likely pathogenic, VUS).
Allele Frequency: The proportion of chromosomes in a population carrying a specific genetic variant, commonly derived from population databases.
Autosomal Recessive: A mode of inheritance in which disease manifests only when both alleles of a gene are affected.
CMT (Charcot-Marie-Tooth Disease): A group of inherited peripheral neuropathies characterized by distal muscle weakness, sensory loss, and skeletal deformities.
CMT4B3 (Charcot-Marie-Tooth Disease Type 4B3): A rare autosomal recessive demyelinating neuropathy caused by biallelic pathogenic variants in *SBF1*.
Deep Tendon Reflexes (DTRs): Involuntary muscle contractions elicited by tendon percussion, used to assess peripheral nerve and spinal cord function.
Electrophysiological Studies: Tests such as nerve conduction studies and electromyography used to evaluate peripheral nerve and muscle function.
Gene Dosage Effect: A phenomenon in which the number of functional gene copies influences phenotypic severity or expression.
HGVS (Human Genome Variation Society) Nomenclature: Standardized system for describing genetic variants at the DNA, RNA, and protein levels.
HPO (Human Phenotype Ontology): A standardized vocabulary used to describe and analyze human phenotypic abnormalities and their frequencies in genetic disorders.
Hyperlordosis (Lumbar Hyperlordosis): An exaggerated inward curvature of the lumbar spine, often associated with neuromuscular or postural abnormalities.
Idiopathic Persistent Toe Walking: Persistent toe walking without an identified neurological, orthopedic, or other cause.
Next-Generation Sequencing (NGS): High-throughput DNA sequencing technology enabling simultaneous analysis of multiple genes.
Pes cavus: A foot deformity characterized by a high medial arch, commonly associated with neuromuscular disorders.
Segregation Analysis: Genetic analysis examining whether a variant co-segregates with disease within a family.
SBF1 (SET Binding Factor 1): A gene encoding a pseudophosphatase involved in endosomal trafficking and myelination; biallelic pathogenic variants cause CMT4B3.
Variant of Uncertain Significance (VUS): A genetic variant for which current evidence is insufficient to classify it as benign or pathogenic.