Global Medical Genetics 13 (2026) 100081

Contents lists available at ScienceDirect

Global Medical Genetics

journal homepage: www.keaipublishing.com/en/journals/gmg/

A comparative phenotypic analysis of a heterogeneous PMP22 cohort presenting with persistent toe-walking versus classic PMP22-related Neuropathies

David Pomarino[a,*], Kevin M. Rostásy[b], Bastian Fregien[c], Jan Oliver Schönfeldt[d], Alexander Nazarkin[e]

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

ARTICLE INFO

Keywords:
PMP22 variants
Persistent toe-walking
Hereditary neuropathies
Phenotypic analysis

ABSTRACT

Background: The Peripheral Myelin Protein 22 (PMP22) gene plays a central role in peripheral nerve myelination, and dosage alterations (deletion, duplication, or point mutation) are established causes of hereditary neuropathies such as Charcot–Marie–Tooth disease type 1 A (CMT1A), CMT1E, and Hereditary Neuropathy with Liability to Pressure Palsies (HNPP). However, its potential contribution to atypical developmental motor phenotypes such as persistent toe-walking (PTW) has not been systematically explored.

Objective: To characterize the phenotypic spectrum of pediatric PMP22 variant carriers presenting with PTW and to compare their clinical features with those of established PMP22-related neuropathies.

Methods: This retrospective study analyzed 22 children with PMP22 variants (pathogenic, likely pathogenic, or of uncertain significance) identified through a targeted 49-gene next-generation sequencing panel. Detailed phenotypic data were collected across five clinical domains—genetic, developmental, gait and musculoskeletal, neurological, and associated comorbidities—and compared to standardized phenotype frequencies for CMT1A, CMT1E, and HNPP derived from Orphanet and the Human Phenotype Ontology (HPO) databases.

Results: Persistent tip-toe gait was universal, accompanied by pes cavus, lumbar hyperlordosis, tremor, and hyporeflexia. Speech and language difficulties were reported in 45 % of cases, and a family history of toe-walking in 40 %. Additional muscle symptoms and neurological findings were reported, developmental disorders were also reported.

Conclusions: Children carrying PMP22 variants with PTW exhibit a distinct phenotype differing from classic demyelinating neuropathies. The findings suggest that a subset of idiopathic toe-walking cases may represent a developmental manifestation within the PMP22-related disease spectrum, highlighting the value of genetic testing in reevaluating gait disorders of uncertain etiology.

* Correspondence to: Pomarino. Praxis für Ganganomalien, Rothenbaumchaussee 114, 20149 Hamburg, Germany.
E-mail address: forschung@ptz-pomarino.de (D. Pomarino).

https://doi.org/10.1016/j.gmg.2025.100081
Received 10 November 2025; Received in revised form 25 November 2025; Accepted 26 November 2025
Available online 11 December 2025

Introduction

The peripheral myelin protein 22 (*PMP22*) gene plays an essential role in the formation and maintenance of myelin in the peripheral nervous system [1]. PMP22 expression is dosage-sensitive, and alterations in gene copy number or sequence—through duplication, deletion, or pathogenic variants—give rise to a well-established spectrum of hereditary neuropathies [2 – 6].

Deletions of the PMP22 locus result in Hereditary Neuropathy with Liability to Pressure Palsies (HNPP), a condition characterized by recurrent, pressure-induced focal neuropathies and transient sensory or motor deficits, leading to temporary episodes of numbness and weakness [7,8]. Conversely, duplication of PMP22 causes Charcot–Marie–Tooth disease type 1 A (CMT1A), the most common inherited peripheral neuropathy. CMT1A is associated with demyelination and impaired peripheral nerve conduction, typically manifesting as distal muscle weakness, length-dependent sensory loss, and reduced or absent reflexes [3,4].

Certain PMP22 sequence variants are associated with other neuropathy subtypes, such as CMT1E, which may include additional features such as sensorineural hearing impairment [9,10]. Collectively, PMP22-related disorders illustrate how defined genetic alterations can produce characteristic patterns of peripheral nerve dysfunction.

In contrast, the etiology of persistent toe walking (PTW) remains poorly understood. PTW is typically considered a diagnosis of exclusion when no neurological, orthopedic, or developmental cause is identified, yet the phenotype may encompass more than an isolated gait abnormality [11,12].

Anatomically, toe walking is characterized by the absence of a normal heel strike during the initial stance phase of the gait cycle, with the forefoot making contact with the ground instead of the heel. This gait pattern is a consequence of sustained plantar flexion at the ankle joint. The primary structures involved are the calf muscles -the gastrocnemius and the soleus- and their common tendon, the Achilles tendon, which attaches to the heel bone, the calcaneus. Contraction of the gastrocnemius-soleus complex pulls the calcaneus upward via the Achilles tendon, creating plantar flexion. In cases of persistent toe walking, this muscle-tendon unit may be congenitally shortened or may develop adaptive shortening over time, simply put when calf muscles are tight or short, they pull on the Achilles tendon, which then pulls the heel up. This makes it hard for the child to put their heels down to walk flat-footed [13].

Initial genetic and clinical findings from this cohort were previously reported (Journal of Global Medical Genetics, to be published; manuscript ID GMG-D-25–00063). Building on that dataset, the present study provides a comparative analysis between PTW individuals carrying PMP22 variants and the established phenotypes of PMP22-related neuropathies.

The aim of this analysis is to examine whether individuals with PTW who carry PMP22 variants exhibit features that overlap with known PMP22-related neuropathies (CMT1A, HNPP, CMT1E). While these conditions appear clinically distinct, a subset of our cohort presents with overlapping features—such as pes cavus, areflexia, and tremor—that are hallmarks of peripheral nerve dysfunction.

The objective of this retrospective study is to describe the clinical features of PTW individuals with PMP22 variants and to explore potential areas of phenotypic overlap with established PMP22-related neuropathies. The analysis is intended to be exploratory and hypothesis-generating, rather than diagnostic.

By characterizing this cohort, we aim to contribute to a more nuanced understanding of the clinical variability associated with PMP22 variants and their potential relationship to PTW.

Materials and methods

Study design and population

This retrospective observational study was conducted over a four-year period and examined the genetic and clinical characteristics of pediatric patients presenting with persistent toe walking (PTW). Children were referred by pediatricians, neuropediatricians, and orthopedic specialists for evaluation of gait abnormalities. A total of 1500 patients underwent genetic testing as part of routine clinical assessment.

Inclusion and exclusion criteria

Patients were eligible for inclusion if they met the following criteria:

1. Persistent toe walking observed during daily activities, as reported by parents or caregivers.
2. Completion of a 49-gene next-generation sequencing (NGS) panel specifically designed for neuromuscular and neuropathy-associated genes [14].
3. Provision of written parental consent for the use of anonymized clinical and genetic data for research purposes.
4. For the present comparative analysis, only patients in whom the NGS or MLPA testing identified a PMP22 variant (pathogenic, likely pathogenic, or VUS) were included.

Patients were excluded if they had:

- A known neurological, orthopedic, or developmental condition associated with toe walking (e.g., cerebral palsy, autism spectrum disorder, tethered cord syndrome, limb-length discrepancy).
- A history of significant perinatal or birth complications.

Data collection and analysis

Data were systematically collected through a standardized questionnaire and a detailed physical examination. The collection process and the five core clinical domains we focused on are illustrated in the workflow below (Fig. 1).

The specific data points captured within each clinical domain were:

Genetic profile

Developmental history. Perinatal history, age of bowel and bladder control, mode of delivery, and family history of toe walking or neuropathy.

Gait & musculoskeletal features. Age of toe-walking onset, proportion of daily time spent toe walking, progression over time, ankle ROM, and foot morphology (e.g., pes cavus). or other structural anomalies (e.g., clinodactyly, brachydactyly, pectus excavatum).

Neurological features

Deep tendon reflexes, balance difficulties, presence of tremor, distribution of musculoskeletal pain (calves, heels, back), and muscle-related symptoms.

Associated comorbidities

Speech/language difficulties, social developmental concerns, and visual impairments.

Clinical assessment

Because this study was retrospective, all clinical data were derived from existing records documented at the time of presentation. The clinical evaluation protocol routinely applied in the treating clinics is summarized in Table 1

Sensory testing: Formal assessments of sensory modalities were not systematically available in the retrospective dataset. In addition, nerve conduction studies were not performed because the clinical examinations were conducted independently of the genetic results; at the time of evaluation, neuropathy was not suspected and therefore NCV testing was not clinically indicated.

Genetic testing

All included patients underwent a targeted 49-gene NGS panel designed to identify pathogenic variants associated with neuromuscular disorders potentially linked to PTW. Whole-exome sequencing was excluded because it did not form part of the standardized diagnostic workflow during the study period.

Saliva samples were collected by the treating clinicians. Sequencing was performed on the Ion Torrent platform, targeting coding exons and ± 10 bp of flanking intronic regions.

Because PMP22 dosage alterations are a major cause of hereditary neuropathies, Multiplex Ligation-Dependent Probe Amplification (MLPA) was subsequently performed in all cases where NGS suggested a possible copy-number change, in order to confirm or exclude a pathogenic CNV.

Prior to testing, written informed consent for the use of anonymized data in research was obtained. The study received ethical approval from the ethical board of the Deutschen Verbandes für Physiotherapie an der Physio-Akademie in Wremen, Germany (project number 2025–02).

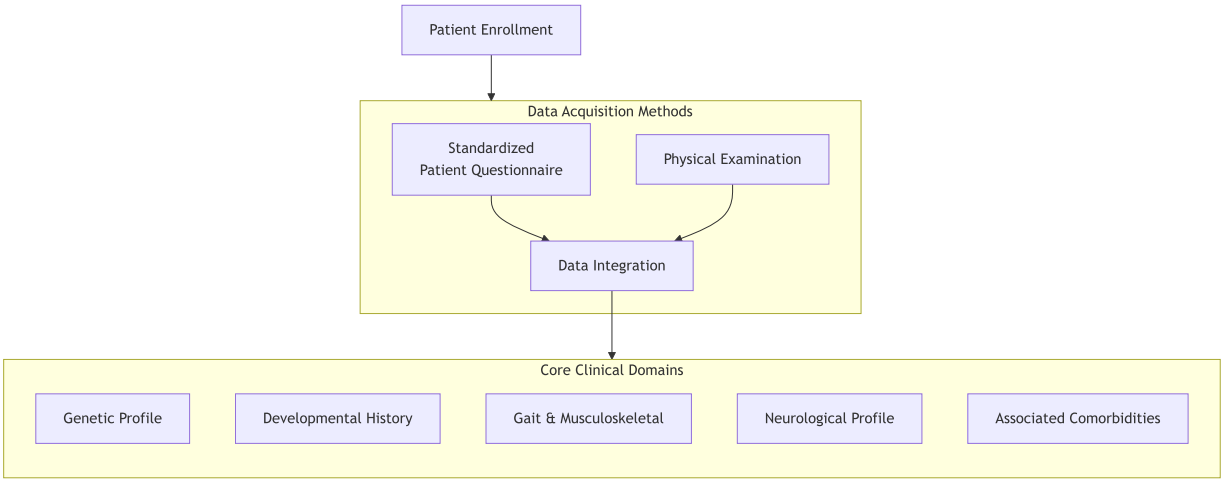

Fig. 1. Data collection and categorization workflow.

Table 1
Clinical Assessment Protocol.

Category	Assessment	Procedure	Positive Finding
1. Postural & Structural	Lumbar Hyperlordosis	Goniometric measurement (lumbosacral angle)	-
	Pes Cavus, Pectus excavatum, clinodactyly, and brachydactyly	Morphological inspection	Observation of abnormality
2. Balance & Motor	Balance **Disorder**	Single-Leg Stance	< 5 s or requires support
	Motor	Heel Walking Test	Inability to perform correctly
		Foot Drop Test: Toe lifting test (standing)	
3. Ankle ROM	Dorsiflexion	Goniometry (neutral/0° method) [15]	< 20° (Restricted)
	Plantarflexion		< 50° (Restricted)
4. Neurological	Tremor (Essential)	a) Arms extended, palms down	Visible rhythmic oscillations
		b) Hand opening-closing provocation	
	Dysarthria	Parent/Caregiver report	Reported speech difficulty

All detected variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines [16]. Variants categorized as pathogenic, likely pathogenic, or VUS were included for further analysis. Classification was supported by in silico prediction tools (MutationTaster, PolyPhen-2) and curated databases including HGMD, ClinVar, LOVD, and dbSNP.

The analysis had certain limitations. It could not reliably detect variants in non-coding or regulatory gene regions, variants in areas with high sequence homology or repeats, copy number variations affecting single exons or entire genes nor, genetic mosaicism with a low frequency component.

Statistical analysis

Descriptive statistics were performed using LibreOffice Calc to summarize demographic data (age, gender), genetic findings (PMP22 variant classifications), and clinical features (e.g., toe walking duration). Categorical variables, including variant types (Pathogenic, Likely Pathogenic, VUS) and symptom prevalence, were expressed as frequencies and percentages.

Comparator disease phenotypes

Phenotypic frequencies for CMT1A, CMT1E, and HNPP were obtained from Orphanet and the Human Phenotype Ontology (HPO) database [17]

Features were categorized using standard HPO frequency descriptors:

- Obligate: 100 %
- Very frequent: 80–99 %
- Frequent: 30–79 %
- Occasional: 5–29 %

These descriptors represent the aggregated phenotypic expression across the disease course and do not encode age of onset or progression.

Results

Study population

A total of 22 patients carrying PMP22 variants were included in the study. All presented with persistent toe walking and had undergone targeted NGS and, when indicated, MLPA testing. The cohort ranged in age from 17.5 months to 17 years (mean age 7.7 years) and comprised 14 girls (63.6 %) and 8 boys (36.4 %).

Prevalence of family history in the cohort

A family history of toe walking was reported in 9/22 patients (40.9 %). Paternal history accounted for 31.8 %, maternal history for 9.0 %, and sibling history for 13.5 % (brothers 9.0 %, sisters 4.5 %). Additional affected relatives were reported in 9.0 % of cases.

Clinical findings

All patients were unable to stand with their heels in contact with the ground. Ankle joint range-of-motion restriction was present in 21/22 patients (95.5 %), involving reduced dorsiflexion, plantarflexion, or both.

Musculoskeletal pain was frequently reported, affecting the calves, heels, feet, or back. Speech and language difficulties were documented in 10/22 patients (45.6 %).

Structural features were frequent in the cohort. Pes cavus was present in 90.9 % of patients, lumbar hyperlordosis in 90.9 %, and clinodactyly or brachydactyly in 86.4 %. Pectus excavatum was observed in 36.4 % of cases.

Muscular symptoms were also common: muscle spasm occurred in 50.0 % of patients, myalgia in 31.8 %, and muscle fatigue in 27.3 %.

Neurological signs included tremor in 68.2 % of patients, balance disorders in 45.5 %, hyporeflexia in 22.7 %, and visual impairments in 13.6 %.

Developmental features were reported in some cases, including voluntary bladder and bowel control not achieved by age five (27.3 %)and social interaction challenges (22.7 %).

Genetic testing

NGS and MLPA identified 23 PMP22 variants across the 22 patients. One patient carried both a sequence variant and a duplication. Variant classifications were based on ACMG guidelines using the evidence available at the time of analysis. It is important to note that the interpretation of genetic variants is dynamic. The ACMG classifications and population frequencies presented here reflect the evidence available at the time of analysis and reporting (Report Date). These interpretations are subject to change as new evidence emerges in the literature. Variant population frequencies were derived from the Genome Aggregation Database (gnomAD), except for patient 11, for whom data were obtained from the Exome Aggregation Consortium (ExAC).

A summary of the identified variants, their HGVS (coding DNA reference sequence by Human Genome Variation Society) nomenclature, and ACMG classification is provided in Table 2.

Comparative clinical features

Supplementary Table 1 summarizes the frequency of key clinical findings in our cohort and compares them descriptively with reported phenotypic frequencies of PMP22-related neuropathies (CMT1A, CMT1E, HNPP). Frequencies for comparator disorders were extracted from Orphanet and HPO and are presented as percentage categories (e.g., 80–99 %, 30–79 %).

No inferential statistics were performed, as an internal comparison group (PTW patients without PMP22 variants) was not available. While other neuropathies involve PMP22 alongside other genes, these four conditions (CMT1A, HNPP, CMT1E) represent the clearest clinical entities for a focused comparison on PMP22's contribution to disease phenotype.

Frequency key (HPO conventions)

Obligate (100 %)

Very frequent (80–99 %)

Frequent (30–79 %)

Table 2
Summary of genetic findings in the study patients.

Patient	c. HGVS	ACMG Classification	Freq. (%)	Report Date
P1	c.*33 G > A	VUS	0.0273 %	08.10.2021
P2	c.*3 C > T	VUS	0.0088 %	14.02.2022
P3	c.103 G > A	VUS	0.00318 %	15.11.2022
P4	c.353 C > T	VUS	0.4020 %	07.06.2023
P5	c.353 C > T	VUS	0.4020 %	10.01.2023
P6	c.353 C > T	VUS	0.4020 %	10.01.2023
P7	c.353 C > T	VUS	0.4020 %	19.12.2022
P8	c.353 C > T	VUS	0.4020 %	15.11.2022
	Duplication	P	-	
P9	c.353 C > T	VUS	0.4020 %	13.07.2022
P10	c.353 C > T	VUS	0.4020 %	01.04.2022
P11	c.353 C > T	VUS	0,468 %	18.11.2020
P12	c.353 C > T	VUS	0,468 %	16.02.2021
P13	c.353 C > T	VUS	0.4020 %	11.02.2025
P14	Duplication	P	-	01.08.2022
P15	Duplication	P	-	13.06.2023
P16	Duplication	P	-	07.03.2023
P17	Duplication	P	-	08.04.2022
P18	Duplication	P	-	16.06.2021
P19	Duplication	P	-	17.03.2021
P20	Duplication	P	-	16.03.2021
P21	Duplication	P	-	28.03.2022
P22	Exon 2 Deletion	P	-	06.10.2020

c.HGVS, coding DNA reference sequence by Human Genome Variation Society; Freq, Frequency.

Occasional (5–29 %)
Rare (1–4 %)
NR = Not Reported

Supplementary Table 1: Comparative Phenotypic Analysis of our heterozygote PMP22 carriers cohort and PMP22- Linked Disorders

Text Supplementary Table 1: VBC, Voluntary Bladder and Bowel Control; MW, Muscle Weakness; CMT1A, Charcot-Marie-Tooth disease type 1A; CMT1E, Charcot-Marie-Tooth disease type 1E; HNPP, Hereditary Neuropathy with Liability to Pressure Palsies

Discussion

This study provides a descriptive comparative analysis of children with persistent toe walking who were found to carry PMP22 variants and contrasts their clinical profiles with the reported phenotypes of established PMP22-related neuropathies, including Charcot–Marie–Tooth disease type 1A (CMT1A), CMT1E, and Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) *[18,19,20]*. These disorders represent primary conditions in which pathogenic alterations in PMP22 are known to impair peripheral myelination and produce characteristic neurological deficits *[21]*.

To enable a standardized comparison, our analysis focused on clinical features that are consistently represented within Human Phenotype Ontology (HPO) and Orphanet datasets. While each of the comparator neuropathies includes additional manifestations not addressed here (e.g., hearing loss in CMT1E or pressure palsies in HNPP), limiting the comparison to common, well-defined terms allowed for clearer alignment with the phenotypic data available in our cohort [22,23].

It is important to emphasize that the comparison with CMT1A, CMT1E, and HNPP was not intended to determine the pathogenicity or clinical relevance of PMP22 variants in our cohort. A definitive assessment would require a comparison between toe-walking children with PMP22 variants and those without such variants within the same population, which was beyond the scope of this retrospective dataset. Instead, the comparison presented here is descriptive and contextual, intended only to contrast the observed clinical frequencies with those reported in established PMP22-related neuropathies. These exploratory observations should therefore be interpreted as hypothesis-generating rather than diagnostic or causal.

Future work within the same clinical cohort comparing PMP22-positive and PMP22-negative toe-walking children will be essential to determine whether the observed features are variant-associated or reflect the broader toe-walking population. We are currently engaged in this next phase of research, which includes extending the analysis to children carrying variants in other neuropathy-associated genes, allowing a more robust evaluation of gene-specific and gene-independent phenotypic patterns.

It is important to emphasize that PMP22 participates in complex regulatory networks, and the phenotypic consequence of individual variants may vary widely. For this analysis, we restricted comparison to disorders in which PMP22 is recognized as the principal monogenic contributor, as this provides the most appropriate clinical benchmark for interpreting our findings [20], [24].

A major limitation of this retrospective cohort is the absence of nerve conduction studies (NCS), which represent a key diagnostic tool in PMP22-related neuropathies. In our setting, clinical examinations were conducted independently of the genetic results, and neuropathy was not suspected at the time of evaluation; therefore, NCS were not clinically indicated. Despite this limitation, the available clinical and genetic information permitted a structured descriptive comparison across functional domains [20].

The analysis is structured by functional category to highlight key divergences as follows:

1. Gait Disturbances:

Our cohort is characterized primarily by a persistent, tip-toe gait. In contrast, secondary gait disturbances in PMP22-related neuropathies result from progressive neuromuscular deterioration:

- **CMT1A & CMT1E** primarily present with gait disturbance and gait imbalance. These arise from a combination of distal muscle weakness, sensory ataxia, and the development of secondary orthopedic deformities like pes cavus [25]. Steppage gait was also reported in CMTE1, representing a potential phenotypic overlap with the toe-walking observed in our cohort.
- **HNPP** patient records show no reported (NR) specific gait disturbance as a core feature, consistent with its episodic, focal nature.

2. Musculoskeletal Hallmarks:
Our cohort exhibited a high frequency of structural anomalies—including pes cavus, lumbar hyperlordosis, clinodactyly, brachydactyly, and pectus excavatum—features that are not uniformly characteristic of PMP22-related neuropathies.
In CMT1A and CMT1E, musculoskeletal deformities such as pes cavus and hammertoes arise as secondary consequences of chronic denervation and long-standing muscle imbalance, rather than as primary congenital findings.
HNPP, although capable of presenting with pes cavus, distal amyotrophy, and occasionally scoliosis, does so at substantially lower frequencies, and these features typically reflect the biomechanical impact of recurrent pressure palsies, not early developmental morphology.
Reports of scoliosis in HNPP often stem from postural asymmetry due to shoulder-girdle weakness rather than intrinsic axial skeletal variation.
Taken together, the high prevalence, early onset, and multisystem pattern of structural anomalies in our cohort are more consistent with developmental or congenital traits rather than the acquired, neuropathy-driven orthopedic changes seen in classic PMP22-associated disorders [26,27].

3. Muscular symptoms:
   The nature of muscular complaints shows notable similarities between the cohorts. Subjective symptoms, including muscle spasm, myalgia, and fatigue, were reported in our cohort. Similar subjective complaints, such as myalgia and spontaneous muscle pain, are also well-documented features of certain PMP22-related neuropathies.
   From a clinical perspective, muscle weakness represents a complex condition characterized by both subjective traits (e.g., perceived fatigue, debility) and objective measures (e.g., measurable loss of strength, atrophy). While objective weakness is a well-documented hallmark of progressive neuropathy, it's noteworthy that subjective weakness—often described as fatigue or lack of endurance—frequently coexists even in the presence of muscle tightness or spasm [28].
   The shared presentation of these muscular symptoms across cohorts suggests potential overlap in symptomatic experience. The interpretation of whether this similarity represents a common underlying pathophysiology or convergent symptomatic expression remains an open question.
4. Neurological signs:
   Tremor was present in our cohort, presenting a notable contrast to the PMP22-related neuropathies where it is not a defining feature. Current research suggests tremor is not a single disease entity but rather a family of disorders with multiple potential etiologies, including cerebellar degeneration, genetic inheritance, and other central nervous system pathologies [29].
   Deep tendon reflex abnormalities provide critical localizing information regarding neurological dysfunction. Hypoactive or absent reflexes typically indicate injury or disease involving the lower motor neurons (nerve roots or peripheral nerves), whereas hyperactive reflexes suggest a lesion involving upper motor neurons (brain, brainstem, or spinal cord) [30].
   In our cohort, hyporeflexia was observed, consistent with potential peripheral nervous system involvement. This finding shows overlap with the areflexia/hyporeflexia documented in CMT1E, CMT1A, and HNPP – all conditions primarily affecting peripheral nerves.
   Balance and postural disorders were present in our cohort and demonstrated overlap with those documented in PMP22-related neuropathies. While balance problems can arise from various etiologies including vestibular disorders, cardiovascular issues, and musculoskeletal problems, in the context of peripheral neuropathies they typically result from impaired proprioceptive feedback due to nerve damage [31–34].
   The similar prevalence of balance disorders across cohorts suggests possible convergent symptomatic expression despite potential differences in underlying mechanisms. In PMP22-related neuropathies, these symptoms likely reflect peripheral sensory impairment and disrupted neural signaling from damaged nerves. In our cohort, the presence of balance disorders alongside other neurological findings may indicate either peripheral nerve or musculoskeletal involvement.
   Visual impairments were another distinctive finding in our cohort, which contrasts with their absence in standard descriptions of PMP22-related neuropathies. This finding further supports the possibility of more widespread neurological involvement beyond peripheral nerve dysfunction.
5. Developmental aspects:
   Parent-reported social difficulties were noted occasionally in the cohort. The underlying nature of these challenges—whether intrinsic developmental traits or secondary consequences of living with a persistent gait abnormality— documenting these concerns is important, as persistent toe-walking may influence social participation, self-perception, and peer interactions, highlighting the need to consider potential psychosocial impact alongside physical findings.
   Regarding autonomic function, most children achieve daytime urinary continence by age 5. In our cohort, only two patients exhibited delays in achieving bowel and bladder control beyond the typical developmental window [35]. This low prevalence suggests that significant autonomic dysfunction is not a primary feature of our cohort's condition.
6. Genetic findings:

The central question of this study was to describe the phenotypic features of children with persistent toe walking who also carried PMP22 variants, without assuming pathogenicity.

In several patients, the PMP22 c.353 C > T (p.Thr118Met) variant was identified. Although some diagnostic laboratory reports between 2020 and 2025 classified this allele as ‘likely pathogenic (class 4)’ or a ‘variant with pathogenic potential,’ these classifications were accompanied by extensive cautionary statements noting the controversial and inconsistent interpretation of this variant in the literature.

Across all reports, the laboratories emphasized the following:

1. the high allele frequency in population databases (~ 0.4–0.5 %) argues against a monogenic pathogenic effect;
2. published data describe a highly variable phenotype including asymptomatic carriers;
3. the variant has been classified in major databases predominantly as VUS or likely benign;
4. its clinical significance cannot be determined without segregation analysis, which was not available.

Given the inconsistent database classifications and lack of supportive clinical or electrophysiological data, we classified c.353 C > T as a variant of uncertain significance (VUS) for the purposes of this study, regardless of the initial classification provided in individual laboratory reports.

The aim of this work was not to determine causality, but to delineate whether a recognizable clinical pattern emerged among children with PMP22 variants who exhibit persistent toe walking.

Limitations & future directions

This exploratory study is limited by its retrospective design, absence of nerve conduction studies, and lack of systematic sensory testing. These limitations reflect real-world clinical practice, in which children with isolated toe walking are not routinely evaluated for neuropathy unless suggestive signs are present.

Future studies incorporating prospective neurological examinations, electrophysiological testing, segregation analysis, and broader genomic approaches would enable more definitive interpretation.

Deep phenotyping and molecular stratification may clarify whether persistent toe walking in some children arises from convergent developmental pathways or represents a phenotype that occasionally co-occurs with PMP22 variation independently of neuropathy.

Conclusion

This study provides a descriptive characterization of pediatric patients with persistent toe walking who were found to carry PMP22 variants. The clinical profiles observed in this cohort differed in several respects from the established phenotypes of PMP22-related neuropathies such as CMT1A, CMT1E, and HNPP, which are typically characterized by progressive sensorimotor impairment and electrophysiological evidence of demyelination. In contrast, the children in our cohort presented with a non-progressive gait anomaly, frequent musculoskeletal structural features, and selected neurological findings, without documented sensory loss or confirmed nerve conduction abnormalities.

Some features, including pes cavus, reduced reflexes, or nonspecific muscular complaints, were shared with known PMP22-associated neuropathies, although the underlying basis for these overlaps cannot be inferred from the available data. Given the retrospective design and absence of systematic neurophysiological testing, the presence or absence of peripheral neuropathy cannot be definitively established in this cohort.

The findings should therefore be interpreted as exploratory. They suggest that PMP22 variants—particularly those of uncertain significance or variable expressivity—may be present in a subset of children with persistent toe walking, but the clinical significance of this association remains unclear. Further studies incorporating control groups, standardized neurological examinations including sensory testing, and nerve conduction studies are required to determine whether these variants contribute directly to the observed phenotype or represent incidental findings.

In summary, this study highlights the importance of comprehensive clinical and genetic evaluation in children with persistent toe walking and underscores the need for future research to clarify the potential contribution of PMP22 variation to this presentation.

Ethical statement

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 and available if requested.

Study Registration: The study is registered in the German Clinical Trials Register at https://www.drks.de/DRKS00031141. Number: DRKS00031141.

Authors' contributions

David Pomarino conceptualized and designed the study, developed the methodology, collected and analyzed the primary data, and prepared the first draft of the manuscript. Kevin M. Rostásy provided critical revisions for intellectual content. Bastian Fregien participated in the orthopedic evaluation, contributed to the integration of genetic and clinical data, and reviewed the manuscript for accuracy. Jan Oliver Schönfeldt supported data curation, assisted in visualization and figure preparation, and contributed to the review and editing of the final manuscript. Alexander Nazarkin assisted with formal analysis, provided technical support for data processing, and contributed to the final review and editing of the paper. All authors read and approved the final version of the manuscript and agree to be accountable for the integrity of the work.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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.

Acknowledgment

We extend our heartfelt gratitude to the children and families who took part in this study; their willingness and cooperation made this research possible. We are equally thankful to the medical teams for their thorough clinical assessments, thoughtful project review, and invaluable feedback throughout the course of this work.

Our special thanks go to the dedicated administrative, technical, and support staff at Pomarino Praxis für Ganganomalien in Hamburg for their careful data collection and coordination efforts. We also acknowledge Labor Dr. Heidrich & Colleagues for their expert contribution to the genetic analyses.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gmg.2025.100081.

References

[1] G.J. Snipes, U. Suter, A.A. Welcher, E.M. Shooter, Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13), J. Cell Biol. 117 (1) (1992) 225–238, https://doi.org/10.1083/jcb.117.1.225.
[2] S. Pareek, U. Suter, G.J. Snipes, A.A. Welcher, E.M. Shooter, R.A. Murphy, Detection and processing of peripheral myelin protein PMP22 in cultured Schwann cells, J. Biol. Chem. 268 (14) (1993) 10372–10379.
[3] J.R. Lupski, R.M. de Oca-Luna, S. Slaugenhaupt, et al., DNA duplication associated with Charcot- Marie-Tooth disease type 1A, Cell 66 (2) (1991) 219–232, https://doi.org/10.1016/0092-8674(91)90613-4.
[4] P. Raeymaekers, V. Timmerman, E. Nelis, et al., Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a), HMSN Collab. Res. Group. Neuromuscul. Disord. 1 (2) (1991) 93–97, https://doi.org/10.1016/0960-8966(91)90055-w.
[5] U. Suter, A.A. Welcher, T. Ozcelik, et al., Trembler mouse carries a point mutation in a myelin gene, Nature 356 (6366) (1992) 241–244, https://doi.org/10.1038/356241a0.
[6] B.W. van Paassen, A.J. van der Kooi, K.Y. van Spaendonck-Zwarts, et al., PMP22 related neuropathies: charcot-marie-tooth disease type 1A and Hereditary Neuropathy with liability to Pressure Palsies, Orphanet J. Rare Dis. 9 (2014) 38, https://doi.org/10.1186/1750-1172-9-38.
[7] P.F. Chance, M.K. Alderson, K.A. Leppig, et al., DNA deletion associated with hereditary neuropathy with liability to pressure palsies, Cell 72 (1) (1993) 143–151, https://doi.org/10.1016/0092-8674(93)90058-x.
[8] E.C. Mariman, A.A. Gabreëls-Festen, S.E. van Beersum, et al., Prevalence of the 1.5-Mb 17p deletion in families with hereditary neuropathy with liability to pressure palsies, Ann. Neurol. 36 (4) (1994) 650–655, https://doi.org/10.1002/ana.410360415.
[9] B.G. Kousseff, T.A. Hadro, D.L. Treiber, T. Wollner, C. Morris, Charcot-Marie-Tooth disease with sensorineural hearing loss–an autosomal dominant trait, Birth Defects Orig. Artic. Ser. 18 (3B) (1982) 223–228.
[10] M.J. Kovach, J.P. Lin, S. Boyadjiev, K. Campbell, L. Mazzeo, K. Herman, L.A. Rimer, W. Frank, B. Llewellyn, E.W. Jabs, D. Gelber, V.E. Kimonis, A unique point mutation in the PMP22 gene is associated with Charcot-Marie-Tooth disease and deafness, Am. J. Hum. Genet 64 (6) (1999) 1580–1593, https://doi.org/10.1086/302420.
[11] J.E. Hall, R.B. Salter, S.K. Bhalla, Congenital short tendo calcaneus, J. Bone Jt. Surg. Br. 49 (4) (1967) 695–697 PMID: 6073187.
[12] O.M. Morozova, T.F. Chang, M.E. Brown, Toe Walking: When Do We Need to Worry? Curr. Probl. Pedia Adolesc. Health Care 47 (7) (2017) 156–160, https://doi.org/10.1016/j.cppeds.2017.06.004 Epub 2017 Jul 15. PMID: 28716514.
[13] Toe walking. American Academy of Orthopaedic Surgeons. ⟨https://orthoinfo.aaos.org/en/diseases–conditions/toe-walking⟩. Accessed Oct. 16, 2023.
[14] D. Pomarino, A. Emelina, J. Heidrich, et al., NGS-panel diagnosis developed for the differential diagnosis of idiopathic toe walking and its application for the investigation of possible genetic causes for the gait anomaly, Glob. Med. Genet 10 (2) (2023) 63–71, https://doi.org/10.1055/s-0043-57230.
[15] C.L. Brockett, G.J. Chapman, Biomechanics of the ankle, Orthop. Trauma 30 (3) (2016) 232 (-).
[16] S. Richards, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet Med. 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30.
[17] Human Phenotype Ontology. Accessed August 26, 2025. https://www.orpha.net/.
[18] R.H. Ziganshin, O.M. Ivanova, Y.A. Lomakin, et al., The pathogenesis of the demyelinating form of guillain-barre syndrome (GBS): proteo-peptidomic and immunological profiling of physiological fluids, Mol. Cell Proteom. 15 (7) (2016) 2366–2378.
[19] N. Hertzog, C. Jacob, Mechanisms and treatment strategies of demyelinating and dysmyelinating Charcot-Marie-Tooth disease, Neural Regen. Res 18 (9) (2023) 1931–1939, https://doi.org/10.4103/1673-5374.367834.
[20] P.F. Chance, Overview of hereditary neuropathy with liability to pressure palsies, Ann. New Y Acad. Sci. 883 (1999) 14–21.
[21] J. Li, B. Parker, C. Martyn, C. Natarajan, J. Guo, The PMP22 gene and its related diseases, Mol. Neurobiol. 47 (2) (2013) 673–698, https://doi.org/10.1007/s12035-012-8370-x.
[22] N.Y. Jung, H.M. Kwon, D.E. Nam, et al., Peripheral Myelin Protein 22 Gene Mutations in Charcot-Marie-Tooth Disease Type 1E Patients, Genes 13 (7) (2022) 1219, https://doi.org/10.3390/genes13071219.
[23] G. Galassi, A. Marchioni, Tackling respiratory failure in Guillain Barre' syndrome: burdens, management and prognosis, Can. J. Respir. Crit. Care Sleep. Med. 9 (1) (2025) 1–11, https://doi.org/10.1080/24745332.2024.2434480.
[24] Pashkova N. Lastname et al. 2019 (preprint) 1 PMP22 associates with MPZ via their transmembrane do- mains and disrupting this interaction causes a loss-of-func- tion phenotype similar to hereditary neuropathy associated with liability to pressure palsies (HNPP). A. Peterson T, P. Ptak C, eds. biorxiv. Published online December 24, 2023. doi:https://doi.org/10.1016/j.isci.2024.110989.
[25] J. Nonnekes, C. Hofstad, A. de Greef-Rotteveel, et al., Management of gait impairments in people with Charcot-Marie-Tooth disease: a treatment algorithm, J. Rehabil. Med. 53 (5) (2021), https://doi.org/10.2340/16501977-2831.
[26] K. Kikuchi, Clinical characteristics of gait disturbance in charcot-marie-tooth disease and future directions in physical therapy, Cureus 17 (6) (2025) e85581, https://doi.org/10.7759/cureus.85581.
[27] R.M. Pabón Meneses, G. Azcona Ganuza, J. Urriza Mena, A. Ibiricu Yanguas, L. Gila Useros, I. García de Gurtubay, Clinical and neurophysiological findings in patients with hereditary neuropathy with liability to pressure palsy and chromosome 17p11.2 deletion. Hallazgos clínico-neurofisiológicos en neuropatías hereditarias sensibles a la presión con deleción del cromosoma 17p11.2, Neurol. 37 (4) (2022) 243–249, https://doi.org/10.1016/j.nrl.2019.02.005.
[28] R. Bhimani, B. Punjani, C. Peden-McAlpine, Understanding clinical characteristics of muscle weakness, J. Neurosci. Nurs. 53 (2) (2021) 69–74, https://doi.org/10.1097/JNN.0000000000000574.
[29] E.D. Louis, Essential tremor' or 'the essential tremors': is this one disease or a family of diseases? Neuroepidemiology 42 (2) (2014) 81–89, https://doi.org/10.1159/000356351.
[30] F.Y. Rodriguez-Beato, O. De Jesus, Physiology, Deep Tendon Reflexes, StatPearls. Treasure Island (FL), StatPearls Publishing, 2023.
[31] J.M. Dougherty, M. Carney, M.H. Hohman, P.D. Emmady, Vestibular Dysfunction, StatPearls. Treasure Island (FL), StatPearls Publishing, 2023.

[32] A. Nordström, P. Nordström, Impaired balance predicts cardiovascular disease in 70-year-old individuals—an observational study from the healthy aging initiative, J. Am. Heart Assoc. 13 (19) (2024), https://doi.org/10.1161/JAHA.124.035073.
[33] Balance Disorders. National Institute on Deafness and Other Communication Disorders. Published March 6, 2018. Accessed September 9, 2025. doi: https://www.nidcd.nih.gov/health/balance-disorders#c.
[34] N. Alissa, A.G. Shipper, L. Zilliox, K.P. Westlake, A systematic review of the effect of physical rehabilitation on balance in people with diabetic peripheral neuropathy who are at risk of falling, Clin. Inter. Aging 19 (2024) 1325–1339, https://doi.org/10.2147/CIA.S459492.
[35] C. DEBORAH, 2025, Toilet Train. MSD Man. Consum. Version, ⟨https://www.msdmanuals.com/home/children-s-health-issues/health-supervision-of-well-children/toilet-training⟩.

Glossary

ACMG (American College of Medical Genetics and Genomics: Professional organization that provides internationally accepted guidelines for interpreting genetic variants based on pathogenicity evidence.
Achilles tendon: Strong fibrous cord connecting the calf muscles (gastrocnemius and soleus) to the heel bone (calcaneus), enabling plantar flexion of the foot.
Ankle dorsiflexion / plantarflexion: Movements of the foot at the ankle joint—dorsiflexion raises the foot upward, plantarflexion points it downward.
Brachydactyly: Shortening of the fingers or toes due to underdeveloped bones.
Charcot–Marie–Tooth disease (CMT): A group of inherited peripheral neuropathies characterized by progressive muscle weakness and sensory loss; subtypes include CMT1A, CMT1E, and others, depending on the affected gene.
Clinodactyly: Curvature or deviation of a finger or toe in the plane of the hand or foot.
Copy number variation (CNV): Genomic alteration in which sections of DNA are duplicated or deleted, changing gene dosage.
ExAC (Exome Aggregation Consortium): A large-scale database of human exome sequencing data used to estimate the population frequency of genetic variants.
Gastrocnemius–soleus complex: Pair of major calf muscles that function together to control ankle movement and posture during walking.
gnomAD (Genome Aggregation Database): Comprehensive population database integrating exome and genome sequencing data to provide allele frequency information for genetic variants.
Hereditary Neuropathy with Liability to Pressure Palsies (HNPP): A peripheral nerve disorder caused by deletion of the *PMP22* gene, leading to recurrent episodes of numbness or weakness after minor pressure or trauma.
Human Phenotype Ontology (HPO): A standardized vocabulary for describing human phenotypic abnormalities in genetic and clinical studies.
Idiopathic toe-walking (ITW): Persistent walking on the toes without a known neurological, orthopedic, or developmental cause, typically beyond age three.
Ion Torrent Platform: Next-generation sequencing (NGS) technology used for targeted genetic testing.
Lumbar hyperlordosis: Excessive inward curvature of the lumbar spine.
Myelination: Formation of the myelin sheath around peripheral nerve fibers, essential for rapid nerve impulse transmission.
Next-Generation Sequencing (NGS): High-throughput DNA sequencing method allowing simultaneous analysis of multiple genes.
Pectus excavatum: Congenital deformity where the chest wall appears sunken due to posterior displacement of the sternum.
Peripheral Myelin Protein 22 (PMP22): A transmembrane protein critical for peripheral nerve myelin stability; dosage alterations (deletion, duplication, mutation) cause various neuropathies.
Pes cavus: High-arched foot deformity often associated with neuromuscular disorders.
PolyPhen-2 / MutationTaster: In silico computational tools used to predict the functional impact of genetic variants.
Toe-walking: Gait pattern in which the forefoot contacts the ground first with little or no heel strike, often due to persistent plantar flexion at the ankle.
Variant of Uncertain Significance (VUS): A genetic alteration for which current evidence is insufficient to classify it as either pathogenic or benign.