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Rare diseases: from basic science to clinical practice and public health

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Rare diseases: from basic science to clinical practice and public health

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Editorial: Rare diseases: from basic science to clinical practice and public health

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Editorial on the Research Topic
Rare diseases: from basic science to clinical practice and public health

Rare diseases (RDs), characterized by their occurrence in a small proportion of the population, have garnered significant attention in recent years as a burgeoning public health concern (1). According to the Global Genes organization, more than 10,000 distinct rare and genetic diseases affect approximately 400 million individuals worldwide. Unfortunately, half of those diagnosed with RDs are children, and many of these conditions are either fatal or cause severe disabilities. Since RDs are heterogeneous, complex, and individually rare, diagnosing and assessing them poses substantial challenges, such as delayed diagnosis, inadequate support systems, persistent disability, and lack of effective treatment, often at substantial financial cost (1). It is estimated that up to 95% of rare diseases lack approved treatments by the United States Food and Drug Administration (FDA), with patients typically waiting an average of 4.8 years for an accurate diagnosis (2).

Accurate diagnosis and prompt treatment represent recurring hurdles for healthcare professionals working with RD patients. Advances in genetic testing and molecular biology have elucidated the pathogenesis of rare diseases and facilitated earlier diagnosis, leading to improved patient outcomes (3). Genetic testing expedites diagnosis and mitigates healthcare expenditures by circumventing unnecessary investigations, procedures, hospitalizations, and medication regimens, ultimately leading to the best possible clinical outcomes for patients (1). Innovations such as genetic modification and traditional drugs have provided breakthroughs against genetically determined diseases (4) while emerging research areas such as oligonucleotide therapy, stem cell therapy, and gene therapy have revolutionized treatment paradigms for myriad complex diseases (3).

The available options remain limited given the significant scientific expertise and investment capital required to develop treatments for rare diseases (3). Nevertheless, recent years have witnessed an outburst in mergers and acquisitions, driven by novel incentives and mitigation methods (5). Accelerated data analysis, integration, and aggregation of data facilitate the dissemination of information, the development of treatments, and new international collaborative research endeavors, thereby

streamlining efforts for researchers, clinicians, patients, and their families (5). Standardization and streamlining of multi-site trial review and contracting processes are critical to improving patient access to new therapies, leveraging extensive datasets to foster novel medical insights, and refining trial designs (4). Thus, initiatives such as the Rare Diseases Clinical Research Network (RDCRN) or Rare Disease Europe (EURORDIS) encourage collaboration among diverse stakeholders, providing training opportunities for new investigators in rare disease research (3). Despite advances in technology, the 10-year survival rate post-diagnosis is still approximately 80%, with a significantly higher risk of death for men (6).

While developed countries have made significant advancements in addressing rare diseases, inequalities persist in less affluent regions, where access to healthcare improvements is limited, exacerbated by deficient public health infrastructure. Social customs and cultural practices rooted in history and tradition can hinder progress, particularly in regions with deep traditions (7). Varying definitions of rare diseases according to disease prevalence in different countries complicate intervention strategies.

Addressing rare diseases extends beyond pharmacotherapy to encompass holistic patient care, necessitating tailored interventions, symptom management, and environmental modifications to accommodate patient needs (7). The mental health toll exacted by RDs on affected children and their families is profound, resulting in psychological distress, diminished quality of life, heightened caregiver burden, and social upheaval (8, 9). The prolonged diagnostic journey, often culminating in unmet therapeutic needs, fosters prolonged uncertainty, fueling anxiety and emotional distress in patients and caregivers (8, 10). Even when patients are knowledgeable about their disease, knowing that the scientific understanding of the majority of rare diseases is lacking can increase anxiety about the future, create instability, and lead to various consequences even for family members (10). Additionally, caring for a sick child is highly time-consuming (due to hospital visits, specialized treatments, and diagnostic procedures since children require multidisciplinary care coordination across multiple sectors and a high frequency of inpatient stays), compounded by financial strain and disruptions to family dynamics that increase emotional distress (9). Siblings may feel overshadowed or neglected, giving up their free and leisure time. Moreover, there is a real medical financial hardship. All of this leads to higher emotional distress. Children afflicted with RDs encounter formidable barriers, such as compromised self-sufficiency and a lack of social support or understanding from others. They may also feel socially rejected and experience negative emotions from traumatic situations (e.g., separation from their parents during long hospital stays) (8, 9). Unfortunately, many families are unaware of psychosocial care services or do not have the time to seek them out, which makes psychological well-being even worse (8).

There is still a paucity of studies on the burden experienced by families of children with RDs. At present, the majority of family members report experiencing caregiver burden, which typically appears to increase in relation to the severity of the disease (9). Because RDs often start in childhood, the burden of the disease and its associated challenges most often fall on family members. Studies report that family members of individuals with RDs feel socially isolated and lonely (it is difficult for them to find other families facing the same disease and to share their caregiving burden); they also struggle to find enough time for themselves, which negatively impacts their work or occupation (9). It is worth mentioning that having a child with a rare disease places an emotional burden on family members and caregivers; there is a correlation between more debilitating forms of the disease and a greater impact on the family and their social life (9).

Children with RDs and their parents face multifaceted challenges, as existing healthcare models do not adequately address their medical and psychosocial needs. Communication barriers, compounded by speech and language disorders, impede daily care and social integration (10). Therefore, it is crucial to have good access to home healthcare services. However, due to funding problems and a need for more human resources, these families need more access to these services even in North America and Europe (10). Children with RDs and their families may encounter barriers to engagement in community activities due to a lack of accessible transportation and other adaptations to meet mobility or other needs. This creates significant barriers to attending medical appointments, engaging in educational and therapeutic activities, and playing, all of which require further adaptation to lead a full life.

In light of these challenges, several recommendations are warranted:

1. Active engagement of patients, families, and communities in the research process.
2. Adoption of a patient-centered model by healthcare companies leveraging that leverages digital and analytical technologies to personalize interventions.
3. To reduce the neglect and marginalization of the RD population, it is important to increase public awareness and competence in health and social care policies.
4. Comprehensive psychosocial assessments by healthcare providers to ensure appropriate support for patients and their family members.
5. Centralization of appropriate care, ensuring continuity, mitigating communication barriers, and enhancing access to specialist expertise, taking a broader view of the child's health and well-being than just one aspect of care by interacting with different medical recommendations from multiple specialists.

These measures collectively aim to optimize outcomes and improve the quality of life for individuals afflicted with RDs and their families.

Author contributions

UO: Conceptualization, Writing – original draft, Writing – review & editing. OY: Writing – original draft, Writing – review & editing. RU: Writing – original draft, Writing – review & editing. LJ: Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Psychobehavioral factors and family functioning in mucopolysaccharidosis: preliminary studies

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Introduction: Mucopolysaccharidoses (MPS) constitute a group of progressive and multisystemic inherited metabolic diseases that profoundly affect both the mental health of patients and the wellbeing of their families. This study aims to evaluate the impact of MPS on family functioning and related factors.

Methods and results: Twenty-five patients with MPS, including types I ($n = 4$), II ($n = 11$), IIIB ($n = 2$), IVA ($n = 3$), and VI ($n = 5$), and their families participated in this study. The mean patient age was 13 years [standard deviation (SD): 7.7 years]. Behavioral and emotional problems were noted in 9.1% of all patients. While the type of MPS did not directly influence mental problems, the presence of neuronal involvement did ($p = 0.006$). Patients with MPS III exhibited difficulties primarily in emotional areas, conduct, hyperactivity, and peer problems. Importantly, both patients with MPS II and those with MPS III experienced a significant impact on communication [mean scores for communication domain: MPS II, 35.6 (SD: 24.3); MPS III, 35.0 (SD: 22.6)]; poorer communication was directly linked to worse adaptive behavior ($p = 0.012$), and worse adaptive behavior was associated with lower quality of life ($p = 0.001$). Quality of life and caregiver burden among family members did not significantly differ across MPS types; however, higher caregiver burden was negatively associated with quality of life ($p = 0.002$). Concerning family functioning, the most impacted domains included independence, intellectual/cultural orientation, activity/recreation, and expressiveness. Domain scores did not vary based on MPS type, treatment, or neurological involvement. Quality-of-life scores were positively associated with the cultural/intellectual domain score.

Conclusion: The impacts of quality of life and family extend beyond clinical characteristics and MPS type, strongly influenced by patient cognition and communication, as well as type of family functioning, especially those with greater cultural/intellectual skills of their family members. A multidisciplinary approach addressing the broader needs of individuals with MPS becomes essential. Techniques aimed at improving communication, including prompt interventions such as speech therapy and augmentative and alternative communication strategies, can contribute to overall family functioning improvement.

KEYWORDS

mucopolysaccharidoses, family functioning, inherited metabolic diseases, cognitive function, psychobehavioral effects

1 Introduction

Mucopolysaccharidoses (MPS) are a group of rare inherited metabolic diseases (IMDs) caused by a lysosomal enzyme deficiency that affects the catabolism of glycosaminoglycans (GAGs). This deficiency causes accumulation of intracellular substances, leading to a complex cascade of events that lead to dysfunction of several cellular processes and pathways; these include an abnormal composition of membranes (impacting vesicle fusion and trafficking), impairment of autophagy, impairment of mitochondrial function, oxidative stress, and dysregulation of signaling pathways (1, 2). Depending on the deficient enzyme, MPS can be classified into the following 14 types: I, II, IIIA, IIIB, IIIC, IIID, IIIE (involving arylsulfatase G deficiency, encoded by the *ARSG* gene), IVA, IVB, VI, VII, IX, X, and the MPS-plus syndrome (MPSPS). MPSPS is caused by pathogenic or likely pathogenic variants in the *VPS33A* gene; although this gene codes for a lysosomal hydrolase, pathogenic or likely pathogenic variants in it result in a massive accumulation of GAGs (3–8). All MPS types are chronic, progressive, and multisystem diseases (4, 9).

MPS has an extremely variable prognosis, which is influenced by the MPS type, genetic variant, residual activity of the deficient enzyme, efficiency of GAG metabolism, age at onset, speed of disease progression, age at treatment initiation (enzyme replacement therapy or hematopoietic cell transplantation), socioeconomic status, and several other factors (10–12).

IMDs adversely affect the psychosocial wellbeing of parents (13). Furthermore, the severity and clinical manifestations of IMDs, including cognitive and motor impairment, are associated with the quality of life of caregivers (14). This could be attributed to the increased need for support among patients to perform activities of daily living. These added responsibilities can directly affect the health and wellbeing of the family, which disrupts work performance and social life (14).

Parents of patients diagnosed with MPS face various challenges arising from the multisystemic nature of the disease, which encompasses orthopedic, vision, and hearing issues; speech disorders; and cardiac problems (15). For patients, these issues extend beyond the physical aspect; even in milder cases, they may contribute to psychological problems and hinder appropriate societal adaptation. Some patients, despite having the capacity to work, may remain at home; conversely, some patients may face obstacles due to psychological challenges while attending school, making it difficult for them to form friendships (16).

Patients sometimes express fears of being scrutinized, harbor guilt concerning their parents, and grapple with anxieties about the future (including aspects such as forming friendships, getting married, bearing economic responsibilities, and having employment) (16). Even in attenuated forms of the condition, the psychological challenges faced by these patients and their family members can be profound; this is because owing to a better understanding of their own situation, these patients may experience a unique set of psychological complexities as compared to patients with the severe phenotype who have intellectual disabilities (16). Conversely, individuals with severe neurological impairment tend to grow increasingly reliant on care and often present with behavioral issues, such as hyperactivity, mouthing, unusual body movements, and inattention, which can be particularly pronounced in those with MPS III who are aged 2–9 years (17). The behaviors and psychological characteristics of these patients undergo significant changes, and the parents/caregivers experience extreme stress that directly affects their daily functioning. In light of this, the provision of psychological care to both patients and their family members or caregivers is indispensable (18).

Furthermore, since most MPS types involve autosomal recessive pattern of inheritance, parents may have two or three children with the disorder prior to the diagnosis of their first child (19). However, the psychosocial burden of MPS on parents in developing countries remain unclear.

Accordingly, this study aimed to evaluate the psychobehavioral effects of MPS on family functioning and related factors.

2 Materials and methods

2.1 Study design and population

This cross-sectional, observational, descriptive study was conducted in the Pequeno Príncipe Children's Hospital and approved by our Ethics Committee (protocol number 47925921.5.0000.0097). All methods were performed in accordance with the guidelines and regulations of the Brazilian National Commission of Health (Commission of Ethics in Human Research-CEP/CONEP). The parents provided consent to the use of all data and images and for publication of this report.

We included participants with increased urinary glycosaminoglycans and laboratory-confirmed reduction in enzymatic activity; specifically, the enzymatic deficiency was defined as a reduction in enzymatic activity of <10% of the normal laboratory reference value.

Participants were further subgrouped according to MPS type, central nervous system involvement, and treatment performed [no treatment, enzyme replacement therapy (ERT), or hematopoietic cell transplantation].

2.2 Cognitive function

Estimated full-scale IQ was assessed using the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) and Wechsler Abbreviated Scale of Intelligence (WASI); participants were administered the test that had been validated for their age.

The WPPSI-R was administered to children aged between 3 years 6 months and 5 years 11 months. The children received a four-subtest short version of the test comprising two subtests that assess perceptual–motor abilities. Raw scores obtained using the four subtests were converted into scaled scores (20).

The WASI was administered to children aged >6 years. It comprised four subtests (two verbal and two performance scales), which included the Vocabulary, Similarities, Block Design, and Matrix Reasoning subtests. Raw scores obtained using the four subtests were converted into scaled scores (21).

2.3 Children's behavioral and emotional mental health

The Child Behavior Checklist (CBCL) was used to assess behavioral and emotional problems in children and Adult Self Report (ASR) was

used for patients over 18 years of age during the previous 6 months (22, 23). It comprised 120 items, which were scored on a three-point scale: 0 (not true), 1 (somewhat or sometimes true), and 2 (very true or often true). It has excellent reliability and has been validated in the Brazilian population (24). The raw score was converted into T-scores by the Assessment Data Manager software and quantified within the following dimensions: Anxiety/Depression, Withdrawal, Somatic Complaints, Social Problems, Thinking Problems, Attention Problems, Rule-Breaking Behavior, Aggressive Behavior, Depressive Problems, Anxiety Problems, Somatic Problems, Attention Deficit/Hyperactivity Disorder, Oppositional Defiant Disorder, and Conduct Disorder. Additionally, the instrument can provide a Total Problem Score as well the Internalizing and Externalizing Problems scores (23). T-scores of ≤59, 60–64, and ≥65 indicate non-clinical symptoms, a risk for problem behaviors, and clinical symptoms, respectively (22, 23).

The Strength and Difficulties Questionnaire (SDQ) was used to assess problems related to mental health. The questionnaire comprises 25 items, including 10 items on abilities, 14 items on difficulties, and 1 neutral item. The instrument is divided into five subscales for assessing emotional symptoms (fears, excessive worries, sadness, and hopelessness), conduct problems (irritability, aggression, and antisocial behaviors such as lying), hyperactivity (restlessness, distraction, and inattention), problems with peer relationships (difficulties in relationships with other people, whether children or adults), and prosocial behavior (knowing how to cooperate, help, share). For each item, the individual could choose false (0 points), more or less true (1 point), and true (2 points). The score of each subscale ranges from 0 to 10, with a lower score indicating a better mental health status (25).

2.4 Adaptive behavior

Vineland Adaptive Behavior Scales (Vineland) was used to assess adaptative behavior. It involves a semi-structured interview using items scored as 0 (never performed), 1 (sometimes or partly performed), or 2 (behavior is usually or habitually performed). Normality was considered when score was 86 or higher (26, 27).

2.5 Family functioning

The Family Environment Scale (FES) is a self-reported 90-item scale for assessing family functioning across 10 different domains (28). We used the questionnaire version validated for Portuguese (29). It comprises five subscales, including Cohesion (commitment and family support); Expressiveness (direct communication of feelings), Conflict (express anger and conflict); Independence, Achievement Orientation, Intellectual Cultural Orientation, Active Recreational Orientation, Moral-Religious Emphasis, and Organization (maintenance of the family structure and organization); and Control (trust in rules and procedures to manage family life). The presence of problems is indicated by high scores on the Conflict and Control scales or low scores on the other scales (28). Table 1 presents the results grouped according to the type of family functioning.

2.6 Caregiver burden

Caregiver burden was used to assess the version of the Zarit Burden Interview that has been translated and adapted to Portuguese (30, 31). The ZBI comprises 22 items rated on a 5-point Likert scale that ranges from 0 (never) to 4 (nearly always), with the total score ranging from 0 to 88. This tool allows assessment of objective and subjective burden among informal caregivers with respect to health, social life, personal life, finances, emotions, and relationship types.

2.7 Quality of life

We used the family impact module of the Pediatric Quality of Life Inventory™ to assess the impact of the disease and treatment on family functioning as well as the child's adaptation to chronic diseases (32).

2.8 Coping techniques

The self-administered COPE Brief was used to investigate how individuals responded to stressful situations (33). It comprises 14 subscales for assessing coping techniques (self-distraction, active coping, denial, substance use, use of emotional support, use of instrumental support, behavioral disengagement, venting, positive reframing, planning, humor, acceptance, religion, and self-censorship).

2.9 Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 22.0 (IBM Corp, Armonk, NY,

TABLE 1 Classification according to the typology of the family environment (28).

Typology	Conditions
Independence orientation	Independence ≥69 and independence ≥ achievement/assertiveness
Achievement orientation/assertiveness	Achievement/assertiveness ≥60 and achievement/assertiveness ≥ intellectual/cultural AND moral / religiosity
Intellectual/cultural orientation	Intellectual/cultural ≥60
Moral and religious orientation	A—Moral/religious structure moral/religiosity ≥60 and moral/religiosity ≥ intellectual/cultural
	B—Moral/religious dysfunction moral/religiosity ≥60 and moral/religiosity ≥ intellectual/cultural and organization ≤50
Support guidance	Cohesion OR expressiveness OR both ≥60 and cohesion and expressiveness ≥ conflict
Conflict orientation	Conflict ≥60
Disorganization orientation	Organization ≤50

United States). Descriptive analyses were used to obtain summary measures depending on the nature of variables. Further, inferential analysis was performed using study-relevant statistical tests (chi-squared and Fisher's exact test). Continuous dependent variables were compared with categorical independent variables across all groups using the Kruskal–Wallis test. If the *p*-values obtained were significant, pairwise comparisons were performed to determine the differences between the groups. Statistical significance was set at $p < 0.05$.

3 Results

Among 56 patients diagnosed with MPS in our hospital (Curitiba, Paraná, Brazil), since 2005, 18 died before the commencement of neuropsychological assessments, eight refused to participate in the study, and five could not be contacted. Accordingly, 25 patients with MPS and their families were included, including four, 11, two, three, and five patients with MPS I, MPS II, MPS IIIB, MPS IVA, and MPS VI, respectively. The mean age was 13 years [standard deviation (SD): 7.7 years] (Table 2). ERT was performed in 50, 81.8, 100, and 100% of patients with MPS I, MPS II, MPS IVA, and MPS VI, respectively. Additionally, 50 and 18.2% of patients with MPS I and MPS II, respectively, underwent hematopoietic cell transplantation. None of the patients with MPS III received any specific treatment.

Table 2 summarizes the scores for the cognitive functioning and the strengths and difficulties subscales, as well as the total score of difficulties. Patients with MPS III exhibited difficulties predominantly

TABLE 2 Characteristics of the population studied according to the type of MPS[a], psychometric characteristics, and family burden.

Variables		MPS I (*n* = 4)	MPS II (*n* = 11)	MPS III (*n* = 2)	MPS IVa (*n* = 3)	MPS VI (*n* = 5)	Total (*n* = 25)	*P*
Age (Years) Mean (SD)		10.7 (5.7)	13.1 (5.4)	9.5 (7.8)	19.0 (12.1)	14.0 (9.0)	13.3 (7.3)	0.802
Intellectual Quotient Mean (SD)		81.75 (33.7)	51.0 (17.4)	50 (14.1)	70.7 (12.9)	80.8 (36.4)	63.5 (26.1)	0.203
Capabilities and difficulties Mean (SD)	Total difficulties	10.0 (2.8)	11.5 (4.4)	25.0 (7.1)	5.7 (6.0)	8.3 (9.0)	11.2 (6.3)	0.081
	Emotional problems	2.0 (1.4)	2.3 (1.8)	6.5 (0.7)	1.7 (2.9)	2.3 (1.5)	2.5 (2.1)	0.227
	Conduct problems	1.5 (2.1)	1.7 (1.9)	3.0 (4.2)	0.3 (0.6)	1.3 (1.3)	1.5 (1.9)	0.823
	Hyperactivity	3.0 (4.2)	4.8 (3.1)	10.0 (0.0)	0.7 (1.2)	4.0 (0.8)	4.4 (3.3)	0.060
	Peer problems	3.5 (4.9)	2.7 (1.8)	5.5 (2.1)	3.0 (3.0)	0.75 (0.96)	2.7 (2.4)	0.201
	Prosocial	7.5 (3.5)	5.4 (3.4)	7.0 (0.0)	5.3 (5.0)	8.5 (1.9)	6.3 (3.3)	0.504
	Impact	4.0	3.2 (2.8)	0	2.5 (3.5)	0.25 (0.5)	2.2 (2.6)	0.168
Adaptive behavior Mean (SD)	Communication	89.5 (9.2)	35.6 (24.3)	36.0 (22.6)	94.7 (16.5)	72.8 (33.4)	58.0 (33.9)	0.037*
	Daily living skills	81.0 (14.1)	38.3 (27.1)	42.0 (31.1)	78.3 (20.0)	76.0 (44.5)	57.4 (34.4)	0.143
	Socialization	84.5 (2.1)	49.7 (26.0)	53.0 (15.6)	86.3 (26.1)	78.6 (36.3)	65.4 (29.8)	0.250
	Motor skills	82.5 (0.7)	42.1 (24.1)	45.5 (21.9)	82.7 (24.7)	76.0 (37.1)	60.1 (30.8)	0.387
Problems score Mean (SD)	Total	56.0 (5.7)	56.9 (6.9)	63.5 (17.7)	47.7 (7.6)	54.8 (6.7)	55.8 (8.2)	0.269
	Externalizing	55.0 (1.4)	49.5 (9.3)	56.0 (18.4)	43.3 (0.6)	51.5 (7.1)	50.1 (8.8)	0.215
	Internalizing	63.0 (9.9)	55.4 (5.2)	65.5 (10.6)	50.7 (9.1)	56.0 (8.5)	56.5 (7.6)	0.483
Quality of life Mean (SD)		65.4 (26.0)	69.4 (15.5)	47.1 (2.7)	67.7 (31.0)	77.4 (21.6)	68.6 (19.5)	0.353
Family burden Mean (SD)		27.5 (4.9)	20.7 (1.3)	24.5 (6.5)	14.0 (8.0)	24.4 (14.2)	21.6 (8.5)	0.289
Family environment Mean (SD)	Cohesion	56.3 (10.3)	57.3 (7.6)	48.0 (24.0)	54.0 (14.2)	53.2 (13.2)	55.0 (10.8)	0.983
	Expressiveness	46.7 (6.5)	51.1 (7.7)	43.5 (4.9)	50.7 (9.7)	48.2 (2.7)	49.2 (6.7)	0.605
	Conflict	49.3 (10.5)	48.0 (9.7)	52.0 (11.3)	40.3 (6.4)	45.2 (13.9)	46.9 (10.2)	0.582
	Independence	42.3 (9.2)	43.4 (7.4)	57.0 (5.7)	42.3 (4.6)	48.2 (12.1)	45.3 (8.8)	0.380
	Achievement	49.0 (3.5)	48.8 (8.5)	50.0 (8.6)	53.3 (12.5)	53.2 (11.5)	50.5 (8.6)	0.801
	Intellectual-cultural	48.7 (8.6)	45.6 (9.1)	41.5 (7.8)	50.3 (8.6)	47.8 (13.2)	46.7 (9.4)	0.799
	Active-recreational	48.0 (5.0)	50.3 (11.0)	53.0 (0.0)	46.7 (15.0)	51.2 (10.8)	50.0 (9.8)	0.805
	Moral-religious	59.3 (2.9)	55.0 (9.9)	56.0 (7.1)	59.3 (7.6)	56.0 (6.1)	56.4 (7.7)	0.908
	Organization	65.0 (3.5)	59.5 (4.7)	63.5 (7.8)	60.0 (10.8)	64.2 (2.7)	61.7 (5.5)	0.367
	Control	62.7 (6.4)	58.3 (10.0)	54.0 (7.1)	61.0 (3.5)	60.6 (8.7)	59.3 (8.2)	0.775

[a]MPS, mucopolysaccharidoses; SD, standard deviation. *Asterisks indicate statistical significance.

in emotional areas, conduct, hyperactivity, and peer problems. The total score of difficulties was associated with the presence of neuronopathy ($p = 0.001$); however, no relationships were identified for the subscales.

The mean scores for the communication domain in adaptive behavior were below the cut-offs in patients with MPS II (35.6; SD: 24.3) and MPS III (35.0; SD: 22.6); significant differences were noted in the scores between patients with MPS II and those with MPS IV ($p = 0.018$). The total difficulty score was directly associated with impairment in the communication domain ($p = 0.002$). Both patients with high and very high scores in the conduct problems domain showed communicative impairment.

Additionally, 9.1, 4.5, and 9.1% of all patients exhibited behavioral or emotional problems, externalizing symptoms, and internalizing problems, respectively. These problems were more frequent ($p = 0.017$) in patients with neuronopathy (60.4, SD: 7.8) than in those without neurological involvement (51.9, SD: 6.4); however, they did not significantly differ according to age ($p = 0.078$) or time from diagnosis ($p = 0.351$).

The parents' quality-of-life scores did not differ significantly across the different MPS types ($p = 0.353$) (Table 2). Regarding the impact on family functioning, the quality-of-life scores were positively associated with the cultural/intellectual domain score ($p = 0.007$); however, they were not associated with the other domains of family functioning or coping techniques used. Regarding adaptive behavior, the mean quality-of-life score was 63.14 (SD: 17.7) for parents of patients with low adaptive behavior and 94.3 (SD:6.9) for parents of patients with normal adaptive behavior ($p = 0.001$). There was no difference ($p = 0.881$) between the quality of life reported by family members according to the type of MPS (Table 2). In addition, self-reported quality-of-life scores were obtained from only eight participants. The scores did not significantly differ among patients with MPS I (72), MPS II (72.5, SD: 12.0), MPS VI (74.2; SD: 3.3), and MPS IVA (83.5, SD: 20.5).

Regarding family functioning and its domains, the adverse effects of MPS were observed in the domains of independence, intellectual/cultural, activity/recreation, and expressiveness (Table 2). However, no differences existed in any family functioning domains according to the MPS type, treatment type, or neurological involvement.

Families with an intellectual/cultural orientation had a better ($p = 0.017$) Problem-Focused Coping techniques (mean: 2.7; SD: 0.19) than families without an intellectual/cultural orientation (1.9, SD: 0.56).

For all patients, the mean caregiver stress score was 21.6; SD: 8.5. Moreover, 44, 40, and 4% of the family members reported no/mild, moderate, and severe burdens, respectively. Caregiver stress did not significantly differ according to the type of MPS ($p = 0.289$), treatment type ($p = 0.489$), or neurological involvement ($p = 0.203$). Caregiver burden was negatively associated with quality of life ($p = 0.010$).

The most reported coping techniques were Problem-Focused Coping techniques (average: 2.02; SD: 0.6), followed by Emotion-Focused Coping (1.68; SD: 0.43) and Avoidant Coping (0.26; SD: 0.26). The reported coping techniques did not significantly differ according to the type of MPS ($p = 0.679$, $p = 0.209$, and $p = 0.534$, respectively), type of treatment ($p = 0.669$, $p = 0.991$, and $p = 0.762$, respectively), or neurological involvement ($p = 0.722$, $p = 0.107$, and $p = 0.418$, respectively).

The main symptoms of the patient's mental health, the family's quality of life and functioning, and the coping techniques identified, as well as the relationships among them, are summarized in Figure 1.

4 Discussion

The neurocognitive impact of MPS widely varies from minor attention and executive function difficulties to severe intellectual disability (34). Similarly, we observed a wide range of cognitive impacts in patients with MPS II, ranging from mild effects to drastically low IQ scores, which indicates brain involvement and functional impairments despite them being considered as "non-neuronopathic" (34). Contrastingly, patients with MPS IV and MPS VI did not show progression of neurocognitive abnormalities, with most of them showing normal cognitive function (34). This is consistent with previous reports of relatively preserved cognitive functioning in these patients compared with those with other MPS types. Taken together, our findings emphasize the heterogeneity in the cognitive impacts of MPS and the need to perform individualized assessments and interventions.

Notably, we observed difficulties in cognitive functioning and adaptive behavior across several domains. Patients with MPS II and MPS III exhibited relatively lower scores in the communication domain than in the adaptive behavior domain. Indeed, speech, language, and communication impairments have been reported in patients with MPS, especially MPS II and III (34, 35). These impairments manifest as delayed language and speech development, limited vocabulary, speech absence, and overall impaired communication skills. Moreover, these communication deficits adversely affect their activities of daily life, especially expression of needs and desires (36, 37). Additionally, the total difficulty score was associated with adaptive performance in communication. The impact of these communication difficulties extends beyond the linguistic domain; instead, it limits social interactions, educational opportunities, and participation in various activities. Specifically, patients may experience frustration, social isolation, and difficulties in forming meaningful relationships (38). Over time, adaptative behavior may allow these children to cope with interpersonal issues even with persistent or worsening physical problems (36, 39). Therefore, adequate adaptive capacities can improve the psychosocial quality-of-life, which is consistent with the previous report by Shapiro et al. (39). Interventions targeting speech, language, and communication skills are crucial in supporting individuals with MPS to enhance their quality of life and promote their overall wellbeing (40). Specifically, prompt interventions, including speech therapy as well as augmentative and alternative communication strategies, can significantly improve communication outcomes and overall functioning (41). Additionally, multidisciplinary approaches that address the broader needs of individuals with MPS, including educational support and social skills training, can further enhance their communication abilities and optimize their participation in various aspects of life.

Our findings indicated a relationship between behavior disturbance and cognition in patients with MPS; specifically, IQ scores were negatively associated with a risk of behavioral issues. Consistent with previous reports, we found that scores for adaptive behaviors were lower in patients with MPS II and MPS III than those in general

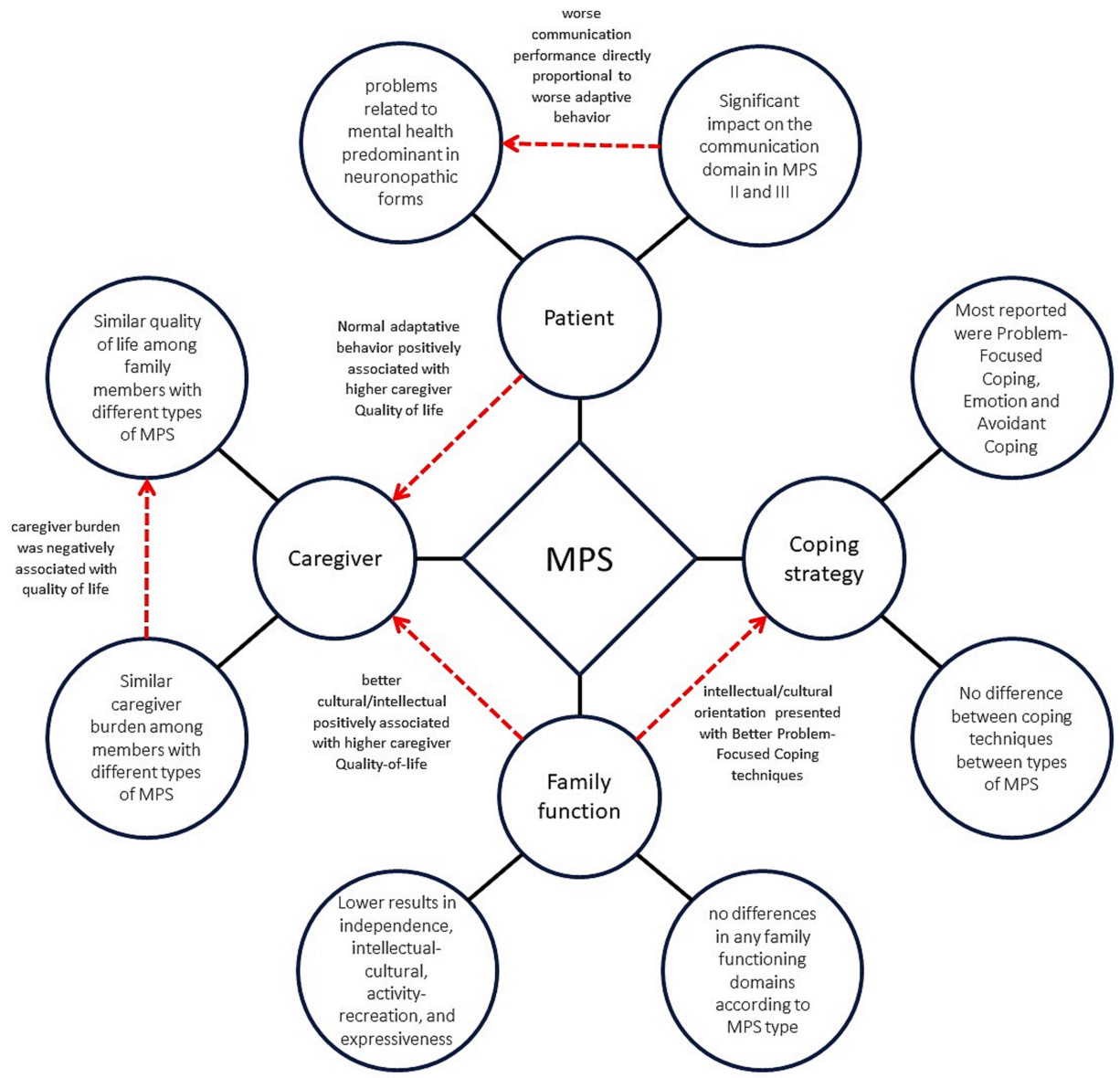

FIGURE 1
Psycho-behavioral factors and family functioning in mucopolysaccharidosis. The chart depicts the key elements of the mental health of individuals with MPS, the quality of life and stress experienced by their family members, and the family functioning and coping techniques that were identified in this study. It also depicts the correlations among these variables. Black lines represent the main factors identified, while red lines indicate the relationships among the different factors.

population, irrespective of treatment (36, 42). There has been insufficient research on behavioral, attentional, and executive function abnormalities in patients with MPS IV and MPS VI, which negatively affect the quality of life (34). However, our findings demonstrated the presence of emotional and peer problems in the MPS IVA group, albeit to a lesser extent than those in the MPS III group. Various behavioral problems have been reported in patients with MPS IVA, including anxiety/depression, attention difficulties, and somatic complaints (43). These findings demonstrated the need to address both cognitive and behavioral aspects when managing patients with MPS.

A Brazilian study reported a mean quality of life score of 48.1 in 11 mothers of children with MPS (14). In our study, except for MPS III, the other MPS groups had values higher than the aforementioned one, even in the presence of cognitive impairment. Contrastingly, they were lower than that reported by an Irish study (mean of 93.8) on patients with MPS, predominantly those with mild forms of the disease. The better quality of life observed in the Irish study could be attributed to a high level of social support (44). These inconsistencies in the reported impact of MPS on the quality of life may be attributed to several factors, including variability in the disease manifestations, treatment availability, and social support systems across different regions and healthcare systems. These factors can significantly affect the perception of quality of life by both individuals with MPS and their families. Furthermore, these inconsistencies can be attributed to the small

sample sizes and potential cultural differences in the studied populations.

In our study, scores related to family functioning were lower in patients with MPS than in the healthy Brazilian population, especially in the domains of independence, intellectual/cultural, activity/recreation, and expressiveness (29). Children with MPS greatly rely on family members for assistance and support, which places a significant burden on the family and affect their overall functioning (36, 45, 46).

Furthermore, the responsibility to care give to children with MPS can adversely affect the parents' working lives. Specifically, parents may be unemployed or forced to reduce their working hours to provide care for their children with MPS (13). Balancing caregiving responsibilities with work obligations can be challenging and cause financial strain and changes in career trajectories. These findings highlight the substantial impact of MPS on the family unit and the need for comprehensive support systems.

Inconsistent with previous reports, we observed no correlation between the severity of MPS and impact on families, which could be attributed to coping characteristics, including recognizing positive aspects of the caring process, reevaluating life goals, and receiving support from other affected families (13, 14, 36, 47).

The progressive and complex nature of MPS places significant demands on families and caregivers. The clinical manifestations of MPS can limit activities of daily living; moreover, the chronic and progressive disease nature can result in functional disability and a decrease in quality of life (39, 45, 46). Various MPS forms are related to behavioral problems that require coping strategies as well as time and physical presence from caregivers, which directly contribute to social isolation among families (48). This may explain the relatively greater impact in the independence domain. Generally, the severity of MPS symptoms is negatively associated with family functioning (36).

The caregiver burden reported by family members of children with MPS was found to be lower than that reported by family members of children with other chronic diseases (49) or Down syndrome (50). However, it was higher than that reported by family members of healthy Brazilian children (50). The caregiving responsibilities limit opportunities for leisure activities and social engagement. Moreover, these caregivers often experience parental stress, grief, feelings of loss, guilt, marital strain, and conflicts in their roles. Additionally, the chronicity of the disease contributes to family stress and imposes psychosocial demands on caregivers (36).

The caregiver burden is negatively associated with the quality of life for the patient, which indicates that the patient's wellbeing influences the family dynamics. Notably, the wellbeing of the caregiver significantly influences the overall care provided to the child. Specifically, stress and burden levels among caregivers are negatively associated with their ability to provide optimal care and support to the child with MPS (51). Moreover, the caregiver burden is negatively associated with the perception of quality of life in pediatric patients (52). It is difficult to determine the causal relationship between caregiver stress and the child's quality of life since these domains are interconnected and influenced by various factors. Accordingly, to elucidate this relationship, it is important to consider the multifaceted nature of MPS and its impact on the entire family unit.

In conclusion, our preliminary findings indicated that the impact of MPS on family functioning extends beyond physical aspects and encompasses social and emotional dimensions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Pequeno Principe Hospital Ethics and Ethics Committee (protocol number 47925921.5.0000.0097). All methods were performed in accordance with the guidelines and regulations of the Brazilian National Commission of Health (Commission of Ethics in Human Research-CEP/CONEP). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

DV: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. TB: Formal analysis, Investigation, Software, Writing – review & editing. VF: Investigation, Writing – review & editing. MS: Investigation, Resources, Writing – review & editing. MC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Taliglucerase alfa in the longterm treatment of children and adolescents with type 1 Gaucher disease: the Albanian experience

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Introduction: Enzyme replacement therapy is already recognized as the gold standard of care for patients with Gaucher disease. Taliglucerase alfa is one of the three alternatives recommended for treatment of Gaucher disease in children and adults.
Aim: This study aims to evaluate the long-term efficacy and safety of Taliglucerase alfa in children and adolescents with Type 1 Gaucher disease.
Patients and methods: Over a six-year period, we monitored the efficacy of continuous treatment in 10 patients by assessing various parameters, including hemoglobin concentration, platelet count, liver and spleen volume, bone mineral density, glucosylsphingosine level, chitotriosidase activity, and growth parameters. Safety was evaluated by immunogenicity and adverse event monitoring.
Results: The mean age of patients was 13.4 ± 3.6 years and the treatment duration was 60.24 ± 13.4 months. From baseline to end line the parameters change as follows: hemoglobin concentration improved from 12.7 (±1.3) to 14.6 (±1.5) and platelet count from 180 (±74) to 198 (±79). The spleen volume, was reduced by 46% ($p = 0{,}007$). The chitotriosidase activity decreased from 4,019.7 (±3,542.0) nmoles/ml/hr to 2,039.5 (±1,372.2) nmoles/ml/hr (46% reduction). Glucoylsphingosine level dropped from 119.2 (±70.4) ng/ml to 86.2 (±38.1) ng/ml, indicating a reduction of 28%. Bone mineral density Z-score, improved from −1.47 (±1.76) to −0.46 (±0.99) (69.7% reduction). Out of the 1,301 total administrations, our patients reported only 37 (2.8%) infusion-related adverse events which were mild and transitory.
Conclusion: Taliglucerase alfa exhibits good efficacy and a safe profile in the treatment of children and adolescents with Type 1 Gaucher disease.

KEYWORDS

Gaucher disease, children, adolescent, Taliglucerase alfa, enzyme replacement therapy, efficacy, safety

Abbreviations
ERT, enzyme replacement therapy; GD, gaucher disease; TGa, taliglucerase alfa; HGB, hemoglobin; PLT, platelet; BMD, bone mineral density; Lyso-GB1, glucosylsphingosine; AEs, adverse events; MRI, resonance imaging; CT, computed tomography; DXA, dual x-ray absorptiometry; DBS, dried blood spot; ADA, anti-taliglucerase alfa antibodies (ADA).

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1 Introduction

Gaucher disease (GD), OMIM #230800, ORPHA355) is an autosomal recessive genetic disorder affecting approximately 1 in 40,000 to 1 in 100,000 of the global population (1–3). It is a lysosomal storage disorder caused by pathogenic biallelic variants in the *GBA1* gene, which encodes the lysosomal enzyme acid beta-glucocerebrosidase. The *GBA1* pathogenic variants lead to a deficiency in the glucocerebrosidase enzyme, resulting in the accumulation of glucosylceramide in the lysosomes of macrophages in various organs, notably in the reticuloendothelial system, responsible for most clinical manifestations (4–7). The two main phenotypes of this illness are the non-neuronopathic form (GD type 1), the most frequent, accounting for about 85%–90% of cases, and the neuronopathic phenotype (GD types 2 and 3). However, a continuum of phenotypes can be observed, ranging from asymptomatic or mild to lethal perinatal forms (4, 8, 9). The significant phenotypic heterogeneity is partly explained by the numerous (>860) pathogenic variants found in the *GBA1* gene to date, as well as by several genetic, epigenetic, and environmental factors (3, 9).

Enzyme replacement therapy (ERT) is already recognized as the gold standard of care for GD patients (10–12). Three ERT drugs are available for the treatment of GD patients: Imiglucerase (Sanofi/Genzyme); Velaglucerase alfa (Takeda-Shire); and Taliglucerase alfa (Pfizer-Protalix). Taliglucerase alfa (TGa) is produced in carrot cells and is the first recombinant therapeutic protein produced in a plant cell expression system to be approved for use in humans by the American Food and Drug Administration (10, 11, 13). TGa differs from native glucocerebrosidase by 2 amino acid residues at the C-terminus and up to 7 amino acid residues at the N-terminus (5). Imiglucerase differs from native glucocerebrosidase at amino acid residue 495, where it has a histidine instead of an arginine in the C-terminus. Velaglucerase alfa has an identical secondary structure to native glucocerebrosidase (5). Taliglucerase alfa does not require additional steps to create glycan structures necessary for cellular uptake by Gaucher cells (14–16). As it is plant-derived, it can cause more adverse reactions than mammalian-derived enzymes (16). TGa appeared to have similar safety and efficacy profiles compared to imiglucerase and velaglucerase alfa (5).

Taliglucerase alfa is recommended for treating adults and children with GD1, and in some countries, including ours, it is also used to treat the hematologic and visceral symptoms of type 3 Gaucher disease (10–13). Our research study aims to evaluate the long-term results on safety and safety of TGa in children and adolescents with GD1.

2 Material and methods

This prospective study was conducted at the Gaucher Unit of the "Mother Teresa" University Hospital in Tirana, Albania. The period of data collection was January 2016–December 2021. The study obtained approval from the national ethical committee. All patients or their parents, in the case of minors, before inclusion in the study, provided signed informed consent forms.

2.1 Patients

A total of 10 children and adolescents treated with Type 1 GD, treated with TGa for at least 2 years, were enrolled and monitored for a period of 6 years. Upper age limit for adolescents is defined according to the American Academy of Pediatrics, which categorizes adolescence as 11–21 years of age, further divided into early adolescence (ages 11–14), middle adolescence (ages 15–17), and late adolescence (ages 18–21) (17). At the baseline, we collected epidemiologic data for all patients, including age, gender, height, weight, race, ethnicity, and genotype.

2.2 Efficacy assessment

To evaluate efficacy, the following parameters were monitored: Hemoglobin concentration (HGB) and platelets count (PLT) were evaluated once a year. Spleen and liver volumes were evaluated once a year by Magnetic Resonance Imaging (MRI) and Computed Tomography (CT scan) in some cases (due to patient refusal of MRI or its unavailability). Volumes were expressed in multiples of the normal volume (MN). The normal liver volume was calculated as 25 ml/kg/weight, whereas the normal spleen volume was 2 ml/kg/weight. Bone mineral density (BMD) was evaluated by Dual x-ray Absorptiometry (DXA) performed for the vertebral spine once a year. Chitotriosidase activity was evaluated every six months using DBS cards sent to the Child Institute of Health in Athens, Greece. The reference value is <150 nmoles/ml/hr. We have not done genetic testing for eventual mutations of the CHIT gene, since our patients had high levels of chitotriosidase activity at the time of diagnosis. Glucosylsphingosine (Lyso-GB1) was assessed every three months using Dried Blood Spot (DBS) cards sent to Centogene Laboratory in Rostock, Germany. The normal reference value for Lyso-GB1 is <6.8 ng/mol. Growth was assessed by measuring and recording weight and height every 12 months, while puberty development was evaluated annually using the Tanner Scale. Assessments stopped once the patient reached stage 5 of the Tanner scale.

2.3 Safety assessment

Taliglucerase alfa safety was evaluated by immunogenicity, which was determined by looking for anti-taliglucerase alfa antibodies (ADA), and monitoring of adverse event (AE). Blood samples for ADA were collected every six months and subsequently sent to Covance Laboratory in Indianapolis, USA.

2.4 Statistical analyses

Categorical variables were represented as frequency and percentages, and descriptive continuous variables were represented as means and standard deviations. The student's *T*-test was performed to test changes from baseline. Statistical analysis was conducted using IBM SPSS Statistics 26.0 software.

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3 Results

3.1 Epidemiologic data

As depicted in Table 1, there are 10 children and adolescents enrolled in this study, comprising 7 boys and 3 girls, all of Caucasian race and Albanian ethnicity. The mean age of patients at the initiation of treatment with TGa was 13.4 ± 3.6 years, ranging from 6.9 to 16.5 years old. Eight of them had previously undergone treatment with Imiglucerase, while two were treatment-naïve patients.

TABLE 1 Epidemiologic data.

Age	
Mean ± SD	13.4 ± 3.6 years
Range	6.9–16.5 years
Gender	
Male	3 (30%)
Female	7 (70%)
Race	White (100%)
Ethnicity	Albanian (100%)
Genotype	
p.Asn409Ser/p.Asp448His:p.His294Gln	7 (70%)
p.Asn409Ser /p.Ser146Leu	1 (10%)
p.Asn409Ser /p.Ser146Leu	1 (10%)
*p.Asn409Ser/p.Arg86**	1 (10%)
Patients treatment status	
Switched	8 (80%)
Naïve	2 (20%)
Treatment duration (months)	
Mean ± SD	60.24 ± 13.4
Range	41.2–72
Infusions per month/patient	
Mean	1.84
Dosage (mean)	
Baseline	55.23 UI/kg weight
Endline	47.20 UI/kg weight

The starting dose for switched patients was identical to the last dose of imiglucerase, whereas for treatment-naïve patients, according to our National Gaucher Disease protocol, the starting dose was 60 UI/kg weight. The mean dose of TGa at baseline was 55 UI/kg/weight, and by the end, it had decreased to 47 UI/kg/weight, representing a 15% reduction. The average duration of treatment was 60.24 ± 13.4 months, ranging from 41.2 to 72 months.

Only one patient stopped receiving TGa treatment, due to personal reasons unrelated to health or medical concerns. The total number of drug infusions administered was 1,301, averaging 1.84 drug infusions per month per patient, as opposed to the normal rate of 2.1 per month (26 infusions/year). This discrepancy was attributed to temporary constraints in the drug supply as well as difficulties accessing the Gaucher Unit due to COVID-19 pandemic-related limitations.

Among the patients, seven of them exhibited the genotype *p.Asn409Ser/p.Asp448His:p.His294Gln*, while three others had the genotype *p.Asn409Ser/other* (Table 1).

3.2 Blood parameters

The mean average of HGB concentration remained within the normal range, with a significant increase graphic (Figure 1), changing from 12.7 (±1.3) at baseline to 14.6 (±1.5) at the endpoint ($p = 0.02$), corresponding to a 13% rise. The mean platelet count, despite individual variations in some patients (three patients had a PLT level between 120,000 and 150,000 at some time point), has practically remained stable, within normal range, increasing from 1,180 (±74) at baseline to 198 (±79) at the endpoint ($p = 0.72$), corresponding to a 6% change (Figure 2).

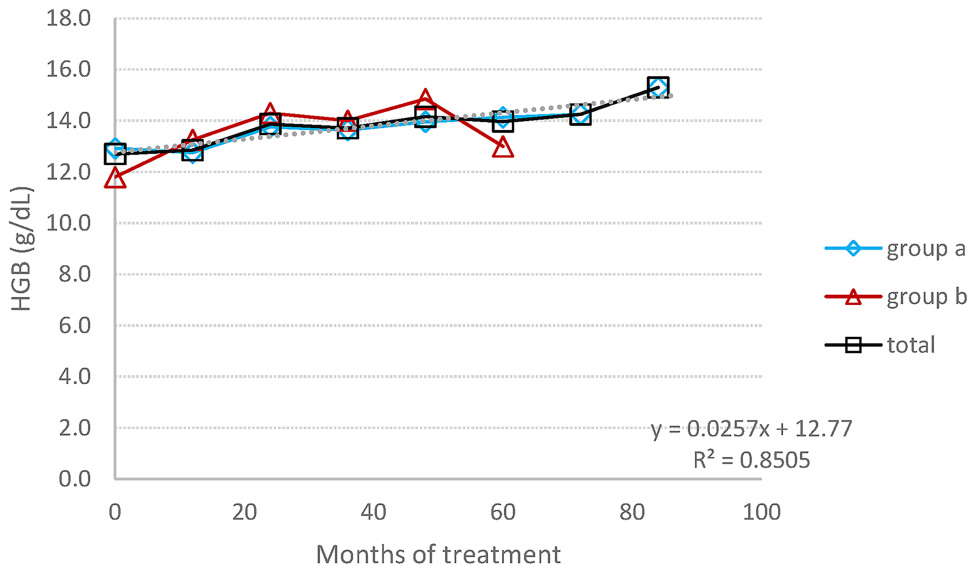

FIGURE 1
HGB concentration: the average change in the concentration of HGB during the entire follow-up period.

3.3 Spleen and liver volumes

Spleen volumes exhibited a noteworthy decrease of approximately 45%, dropping from −1.47 (±1.76) MN to −0.46 (±0.99) MN, indicating a 69.7% reduction (Figure 3). In treatment-naïve patients, the reduction of the spleen volume was more pronounced than in switched patients, decreasing from 8 MN to 3.4 MN, corresponding to a 58% reduction.

The liver volume was essentially normal at baseline, with two patients having liver volumes of 1.2 and 1.5 MN. By the end of the study, all patients' liver volumes had returned to normal, and there were no signs or symptoms of liver cirrhosis.

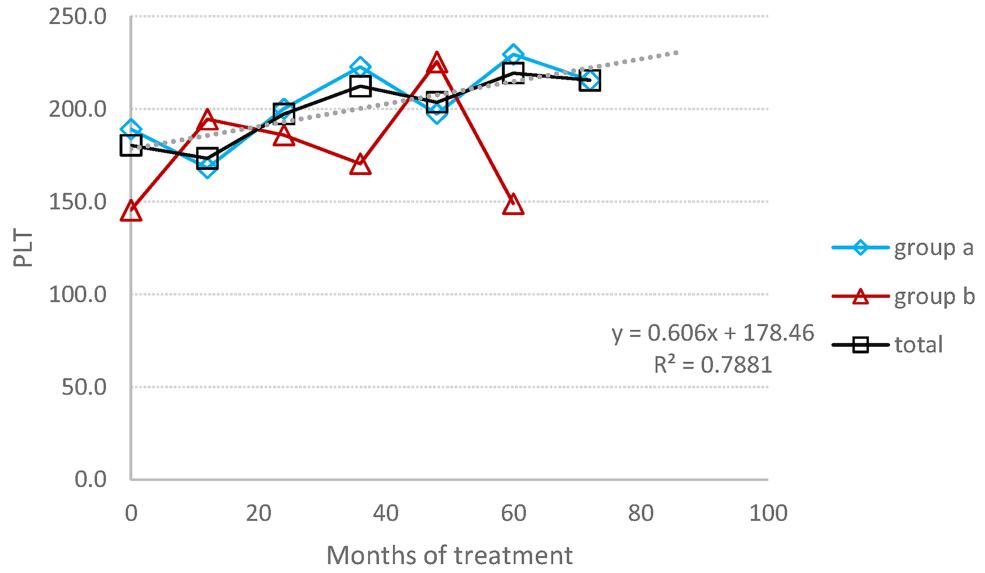

FIGURE 2
PLT count: the average change in the PLT number during the entire follow-up period. (A) Switched patients; (B) treatment-naïve patients and all 10 patients.

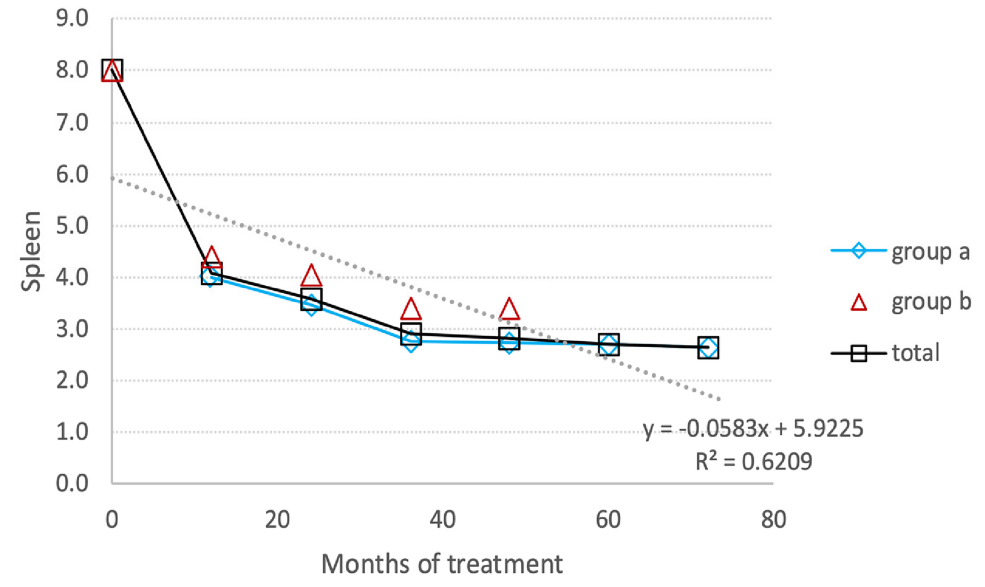

FIGURE 3
Spleen reduction: the average reduction in spleen volume during the entire follow-up period. (A) Switched patients; (B) treatment-naïve patients and total all patients.

3.4 Chitotriosidase activity

The chitotriosidase activity was measured every six months for the first 49.5 ± 2.77 months for switched patients and 19.5 ± 9.2 months for treatment-naïve patients. Afterward, chitotriosidase activity was not utilized for monitoring Gaucher patients. The mean change in chitotriosidase activity from baseline to endline was as follows: it decreased by 46% for all patients, from 4,019.7 (±3,542.0) nmoles/ml/hr to 2,039.5 (±1,372.2) nmoles/ml/hr. Switched patients experienced a decrease of 49%, going from 6,710.67 nmol/ml/hr to 4,089.78 nmol/ml/hr, while treatment-naïve patients had an 81% decline from 7,374 nmol/ml/hr to 1,420 nmol/ml/hr. The last measurement of Chitotriosidase activity shows an increase level (Figure 4). In fact, this represents the value for a single patient, which results to be higher than the average value of the entire group in the previous measurement. But, even in this patient we have a decrease in chitotriosidase activity from 10,725 nmol/ml/hr (baseline) to 3,330 nmol/ml/hr (last measurement).

Notably, in a 9-year-old girl, an increase in chitotriosidase activity was observed, rising from 792 nmol/ml/hr at baseline to 1,192 nmol/ml/hr and 2,427 nmol/ml/hr in two consecutive measurements, following an initial decrease in activity. At the same time that chitotrisosidase activity reached 2,426 nmoles/Ml/hr, the level of lyso-Gb1 dropped from 112 ng/ml to 82 ng/ml.

3.5 Glucosylsphingosine (lyso-Gb1)

The lyso-Gb1 level was monitored every three months, and Figure 5 presents the results for switched patients, treatment-naïve ones, and the entire group. At the endpoint, the group's total lyso-Gb1 levels decreased from 119.2 (±70.4) ng/ml at baseline to 86.2 (±38.1) ng/ml, indicating a 28% drop.

3.5.1 Switched patients

The most significant reduction, from a mean of 103.05 ng/ml to a mean of 60.95 ng/ml (40.8%), occurred after 24 months of monitoring lyso-Gb1. At this point, there was an involuntary treatment break lasting about three consecutive months, which was associated with a prominent increase in lyso-Gb1 levels, more than twice as high as the previous measurement. After the restart of TGa treatment, the reduction in lyso-Gb1 levels continued, albeit at a lower rate of 22.8%, resulting in a decrease from a mean of 131.27 ng/ml to 102.7 ng/ml (Figure 6). In this group, we found out that lyso-Gb1 levels were lower in five patients harboring the *p.Asn409Ser* mutation linked to the double allele mutation *p.Asp448His;His294Gln* than in three patients carrying the *p.Asn409Ser*/others genotype. The mean lyso-Gb1 levels were 69.88 ng/ml and 158.3 ng/ml at the baseline measurement and 67.74 ng/ml and 121.37 ng/ml at the final measurement, respectively, showing a roughly twofold decrease at both time points.

3.5.2 Naïve patients

Naïve patients exhibited a decline in lyso-Gb1 levels during the follow-up period, with a reduction from 159.7 ng/ml at baseline to 80 ng/ml at the last measurement, corresponding to a 50% decrease. The reduction rate trend was as follows: 42.1% at 6 months after TGa treatment; about 70% at the end of the first year, and 77.5%. at the end of the second year. Afterwards, the Lyso-GB1 reduction trend was affected by treatment break period, resulting in increase levels of this biomarker (Figure 5).

3.6 Bone mineral densitometry (BMD)

At baseline, three patients exhibited Z-score <−2.5, and three others showed a Z-score between −1 to −2.5. All patients, with the exception of one, demonstrated a significant improvement in BMD (Figure 7). The group's bone mineral density as defined by

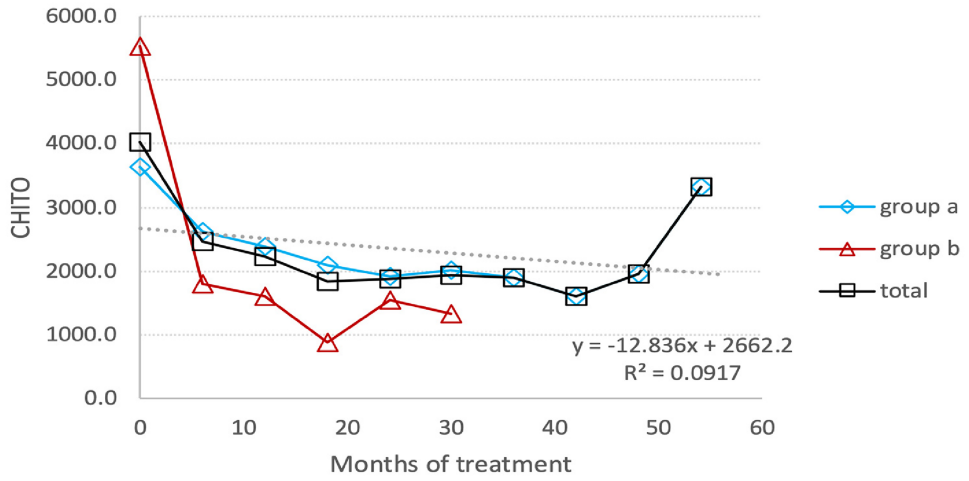

FIGURE 4
Chitotriosidase activity: the average reduction in the activity level of chitotriosidase under TGa treatment throughout the entire follow-up period. (A) Switched patients; (B) treatment-naïve patients and total all patients.

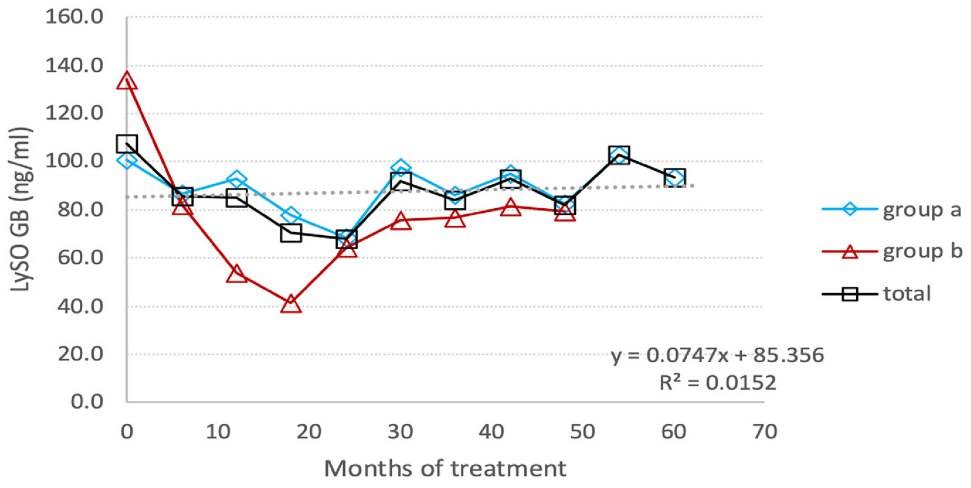

FIGURE 5
Lyso-GB1 level: the average change in the level of lyso-Gb1 under TGa treatment, during the entire follow-up period. (A) Switched patients; (B) treatment-naïve patients and total all patients.

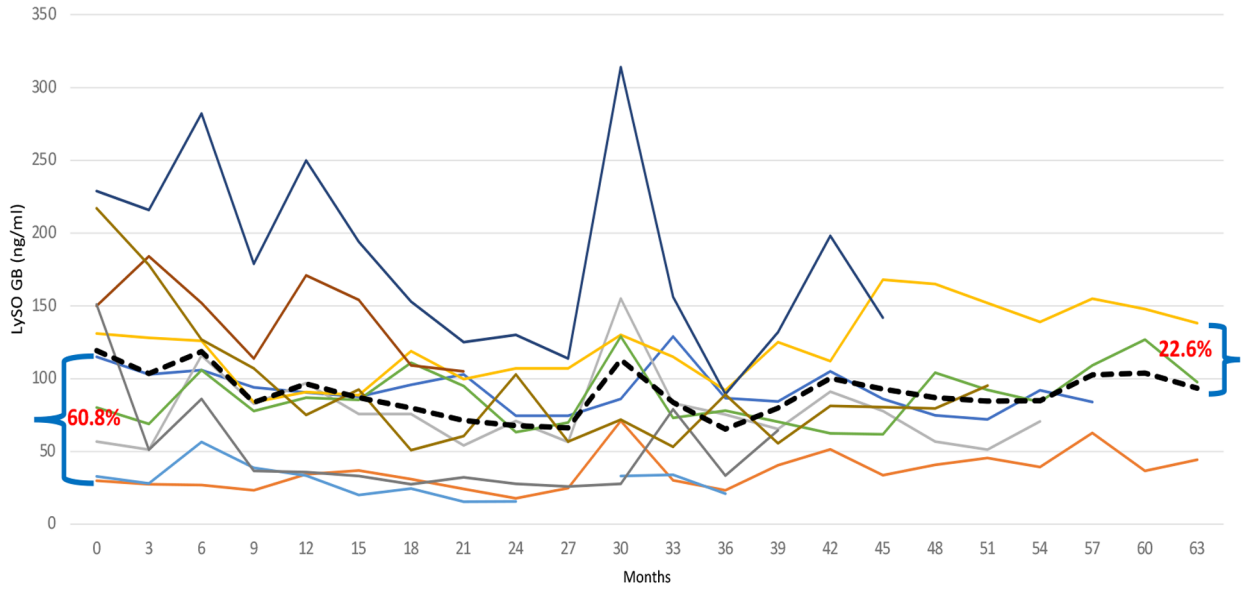

FIGURE 6
Individual Lyso-GB1 curves: the change in the level of Lyso-Gb1 depending on the treatment with TGa, during the entire follow-up period. The interrupted line indicates the mean values of Lyso-GB1.

the Z-score, increased by 69.7%; from −1.47 at baseline to −0.46 at the endpoint ($p = 0.161$).

Notably, the patient who did not show improvement in BMD exhibited consistently high and persistent Lyso-Gb1 levels >150 ng/mol, throughout the study, even though other parameters were normalized.

3.7 Growth

Out of the 10 patients, 7 had already reached puberty upon joining the study. The weight and height of these patients showed improvement, and they all fall within normal ranges according to WHO percentile charts. However, one patient exhibited a weight below the normal percentile, while the height remained within the normal range.

Only two girls began treatment at the ages of 6 and 7 years old. According to the Tanner Scale, both females experienced typical puberty development and achieved a normalized weight with TGa. One girl experienced her first menarche at 13 years old, and the other, at 11 years old, is at stage 3 of Tanner puberty. However, it's worth noting that the height of one of the girls was below 2 standard deviations.

4 Safety

4.1 Adverse events

Out of the 1,301 total administrations, our patients reported only 37 infusion-related adverse events, indicating a low incidence of 2,8%. These adverse events typically occurred during the initial infusions and were generally mild and temporary. The most frequently observed types of infusion-related AEs included urticaria (hives), cough, sneezing, pruritus, nausea, and lip edema reported by three of our patients (30%). The most common adverse events unrelated to drug infusion were rhinopharyngitis (36%), bone pain (15.7%), anxiety disorders (10.5.%), covid19 infection (10.5.%) (Table 2).

4.2 Immunogenicity

In terms of immunogenicity, blood samples collected during the first three years of treatment were tested. IgG anti-taliglucerase alfa antibodies (ADA) were identified in only one patient (10%), and at a low titer. At first, this patient showed a negative result in the initial year of treatment. However, after one year of TGa, he tested positive with a titer of 70. Subsequent measurements revealed titers of 74 and 59 in the following two years. Interestingly, this patient experienced three infusion-related adverse events: lipedema, urticaria, sneezing, and pruritus.

TABLE 2 Adverse events, infusions related and no infusion related.

AEs -infuions related	AEs number	%	AEs-no infusions related	AEs number	%
Urticaria	17	45.9	Rhinopharyngitis	7	36.8
Edema (lips)	5	13.5	Bone pain/arthralgia	3	15.7
Cough	4	10.8	Anxiety disorders	2	10.5
Sneeze	4	10.8	Covid 19	2	10.5
Pruritis	3	8.1	Others	5	26.3
Others	4	10.8			
Total AEs	37	100	Total	19	100

5 Discussion

Since its introduction in the 1990s, enzyme replacement therapy has become the standard of care for patients with Type 1 Gaucher Disease (GD) (3, 11, 12). Both children and adults, whether naïve or switched patients, have exhibited clinically and statistically significant improvements in the major clinical characteristics of GD1 under the treatment of TGa.

The results of our study indicate that all follow-up parameters, such as hemoglobin concentration, platelet count, liver and spleen volume, remained stable or improved, a consistent finding in other studies (10, 12).

Chitotriosidase activity was found to decline significantly over the study's course, showing the drug's efficacy. However, in a 9-year-old female patient, chitotriosidase activity changed from a decreasing profile in the first measurement from baselines to an increase of 50% over a two-time point course. This increase in chitotriosidase activity occurred without any episode of clinical deterioration or dose change. The coadministration of a corticosteroid is the most likely cause because the child was being treated for bronchial asthma, and corticosteroids have been shown by Van Duessen to affect chitotriosidase levels (18). Actually, the increase of chitotriocidase activity was accompanied by a decrease in the level of lyso-GB1, which further strengthens the idea of corticosteroids'influence in chitotriocidase activity.

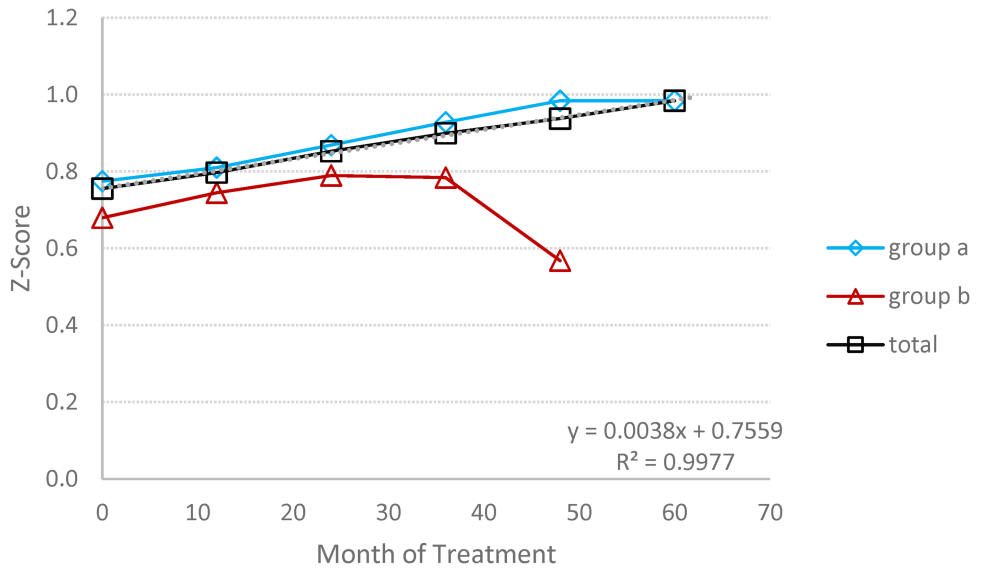

FIGURE 7
Bone mineral densitometry (BMD): the average changes in BMD expressed by Z score, during the follow-up period. (A) Switched patients; (B) treatment-naïve patients and total all patients.

Lyso-Gb1, the deacylated form of glucosylceramide, appears to be the most accurate biomarker for monitoring patients with Gaucher Disease. Its level correlates with the severity of the disease and significantly decreases under enzyme replacement therapy (7, 19–21). The reduction is higher in treatment-naïve patients than in switched ones. A reduction of 70% is observed in our two treatment-naïve patients during the first year of treatment, seemingly higher compared to other reports (19–21). Rather than the drug itself, the differences are probably related to the different characteristics of our patients, such as age, the severity of the disease, genotypes, and the limited number of patients. Switched patients in our study display a reduction of 41% after 22 months of taliglucerase alfa therapy. As previously mentioned, during that time, our patients had an involuntary break from treatment for about three months. Their lyso-Gb1 level significantly increased by more than twofold from the last measurement done before the break, regardless of the fact that neither their clinical symptoms nor their hematologic parameters had changed (22). This fact demonstrates that monitoring lyso-Gb1 enables the flagging of absent/insufficient treatment before clinical consequences arise (22).

The genotype *p.Asn409Ser/p.Asp448His; His294Gln* is the most frequent in the Albanian Gaucher population, accounting for more than 60% of GD patients, and it is generally associated with a mild to moderate genotype (23). Regarding the correlation between genotypes and lyso-Gb1 levels, we note that patients with the genotype *p.Asn409Ser/p.Asp448His;His294Gln* had lower levels of lyso-Gb1 at both baseline and the last measurement, compared to those with the genotype *p.Asn409Ser*/other.

Bone disease poses a significant challenge in GD1 treatment, with osteopenia and osteoporosis frequently observed in Gaucher patients across different ages and genders. Reduced bone mineral density (BMD) can be detected as early as 5 years of age, becoming more pronounced during adolescence (24–26). Various studies have demonstrated a positive effect of ERT therapy on the frequency of bone pain, bone crisis, and BMD (10, 26). Most clinical parameters, including BMD, have been reported to normalize or nearly normalize in children receiving ERT within 8 years (27). Our data align with this, as the mean Z-score for BMD at the endpoint results in a significant change of about threefold compared to the baseline.

However, despite this overall improvement, one of our patients reported occasional bone pain, experiencing a relatively severe episode of back pain lasting nearly a week. Our investigation found no evidence supporting other causes for this event.

Another patient exhibited persistence of BMD Z scores at approximately −2, along with a high lyso-Gb1 level of more than 150 ng/ml during the course of treatment. The persistent high level of lyso-Gb1 could potentially explain the inadequate treatment response in improving BMD, as Lyso-Gb1 may actively contribute to low mineral density by interfering with normal osteoblast function (7), as reported by Dekker. Additionally, the patient's genotype *p.Asn409Ser/p.Ser146Leu*, which is uncommon in our population, appears to be associated with a relatively severe phenotype.

Under the TGa treatment, all but one of our patients exhibited normalized growth and development parameters. A young girl measured at -2SD in height at the final assessment. It is plausible that the observed deviation in height for this girl could be attributed to concomitant pathology, specifically the solitary maxillary incisor syndrome. This condition might have influenced her growth pattern and contributed to the observed height measurement of less than -2SD. It is important to consider such additional factors when interpreting growth and development in patients, as comorbidities can play a significant role in shaping their overall health outcomes.

TGa has a good safety profile when treating children and adolescents (28, 29). Our study supports this through the very low incidence of infusion-related adverse events and the moderate severity and transient nature of these AEs. On the other hand, the development of anti-drug antibodies is commonly observed with recombinant therapeutic proteins. In GD patients, antitaliglucerase alfa antibodies have also been reported, but their presence has no impact on the effectiveness of ERT (28, 29). In our Gaucher unit, we also observed that anti-drug antibodies affected the efficacy of the drug in an adult, likely due to the patient's inherited immunological dysregulation, as the patient's family has a strong history of autoimmune/inflammatory disease (30).

Conclusion: Our long-term study demonstrates that TGa exhibits good efficacy and a safe profile in the treatment of children and adolescents with Gaucher disease.

Data availability statement

The raw data supporting the conclusions of this article will be made available by authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Albanian Medical Ethical Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

PC: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. ST: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Validation. VV: Methodology, Writing – review & editing. AG: Data curation, Methodology, Writing – review & editing. IA: Data curation, Software, Validation, Writing – review & editing. AT: Investigation, Writing – review & editing. ED: Investigation, Writing – review & editing. GH: Data curation, Writing – review & editing. MT: Investigation, Writing – review & editing. ET:

Investigation, Writing – review & editing. MT: Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Congenital absence of the gallbladder in a child: a case report

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Background: Congenital absence of the gallbladder (CAGB) is an exceedingly rare embryological anomaly of the biliary system, with a complex etiology involving the failure of gallbladder formation during embryogenesis. Clinical manifestations are diverse; most patients are asymptomatic, while some present with symptoms such as biliary colic. The complexity of its clinical presentation and radiological features renders diagnosis challenging.
Case presentation: Fetal ultrasound at 22 weeks of gestation revealed an absent gallbladder. At 9 years and 11 months of age, the child exhibited significant weight gain and abnormalities. Abdominal ultrasound and magnetic resonance images demonstrated fatty liver and gallbladder agenesis. Liver function tests indicated mild abnormalities, with aspartate aminotransferase at 67 IU/L and alanine aminotransferase at 44 IU/L. Following 6 months of hepatoprotective and lipid-lowering therapy, a satisfactory treatment response was achieved, with normalization of liver function and improvement in fatty liver.
Conclusions: CAGB may be associated with other congenital abnormalities, although isolated cases are uncommon. Clinically, it may manifest as nonspecific biliary, gastrointestinal, or urinary symptoms, mimicking various digestive disorders and leading to misdiagnosis. Genetic sequencing and in-depth embryological research may elucidate the etiology and enhance diagnostic accuracy.

KEYWORDS

congenital absence of the gallbladder, hepatic ultrasound, liver function abnormalities, fatty liver, case report

1 Introduction

Congenital absence of the gallbladder (CAGB) is an exceptionally rare congenital biliary malformation, with a reported global incidence of 0.01%–0.065% (1). Since Lemery's initial description in 1701, approximately 500 cases have been documented worldwide (2). While most individuals with CAGB remain asymptomatic, some may exhibit signs and symptoms reminiscent of gallbladder disease. The pathogenesis of CAGB remains enigmatic, but it is presently regarded as a congenital malformation (3), potentially attributable to the failure of gallbladder bud formation or a deficiency in vacuolization during embryonic development.

CAGB can be classified into three categories: multiple congenital anomalies, asymptomatic, and symptomatic (4). Although some individuals with CAGB may remain symptom-free throughout life without associated complications, abdominal ultrasonography can definitively diagnose asymptomatic cases (5). Additionally, some patients' symptoms may resemble those of other digestive disorders, primarily presenting as upper right abdominal pain, gastrointestinal symptoms, biliary colic, and occasionally

jaundice, with recurrent episodes (6, 7). These symptoms may be attributable to concomitant common bile duct stones and intrahepatic bile duct stones, or they may be caused by isolated CAGB, the reasons for which remain obscure, thus increasing the likelihood of misdiagnosis (8). Imaging studies may also have limitations, necessitating an enhanced understanding of this condition among surgeons and radiologists, who must synthesize clinical presentation and auxiliary examinations to make comprehensive judgments, thereby reducing misdiagnoses and unnecessary exploratory surgeries (9, 10).

We report a case of a male fetus diagnosed with CAGB during a prenatal checkup. The gallbladder was not visualized during the 22-week prenatal examination, and the fetus was born in March 2011, developing normally. In February 2022, at the age of 9 years and 11 months, the child underwent evaluation due to significant weight gain, and abdominal ultrasound and magnetic resonance images (MRI) revealed fatty liver and CAGB.

2 Case presentation

A male patient, born at full term with a birth weight of 2,750 g, was diagnosed with CAGB during a prenatal ultrasound at 22 weeks of gestation. The patient's growth and development were normal until the age of 9 years and 11 months when he presented with significant weight gain. At presentation (10 years old), the patient weighed 47 kg with a height of 142 cm, resulting in a BMI of 23.3 kg/m^2. His blood pressure and pulse rate were within normal limits, and his body temperature was 36.7°C. Liver function tests revealed mild abnormalities with aspartate aminotransferase (AST) at 67 IU/L and alanine aminotransferase (ALT) at 44 IU/L. These findings, along with the fatty liver observed on MRI, are likely associated with the patient's rapid weight gain rather than being a direct consequence of the congenital absence of the gallbladder. Importantly, the conjugated bilirubin level was within normal limits (0.2 mg/dl), which helped rule out biliary atresia, as elevated conjugated bilirubin is typically observed in that condition. Upper gastrointestinal endoscopy was not performed as the patient's symptoms and other diagnostic findings did not suggest acid peptic disease. Additionally, ultrasonography demonstrated an absent gallbladder (Figure 1A). At higher magnification (Figure 1B), the gallbladder absence was more apparent. This finding was consistent with the diagnosis of congenital absence of the gallbladder. In clinical practice, the absence of the gallbladder on ultrasound typically necessitates further diagnostic imaging or tests to confirm the diagnosis and exclude other conditions.

MRI of the abdomen revealed a fatty liver with an absence of the gallbladder (Figure 2). No other bile duct variations were observed in imaging studies. The images clearly illustrated various segments of the liver and surrounding abdominal anatomy. The grayscale property of the images facilitated a detailed examination of internal structures, with varying shades representing different tissues and densities. The liver and its vascular structures were distinctly visible (Figure 2A). A similar section with more pronounced vascular features was subsequently observed (Figure 2B). Moreover, the MRI revealed an area of altered tissue density, associated with fatty liver disease (Figure 2C), and provided additional views of the liver and surrounding tissues, further corroborating the diagnosis of fatty liver (Figures 2D,E). Variations in tissue density within the images may indicate fat deposition in the liver, while the absence of certain expected anatomical features could suggest underdevelopment or absence of the gallbladder. Following six months of hepatoprotective and lipid-lowering treatment, liver function normalized, and fatty liver improved. The patient was satisfied with the diagnostic and the proposed care. The episode of care is organized as a timeline in Table 1.

The patient and his family expressed satisfaction with the diagnostic process and the proposed care plan. They reported

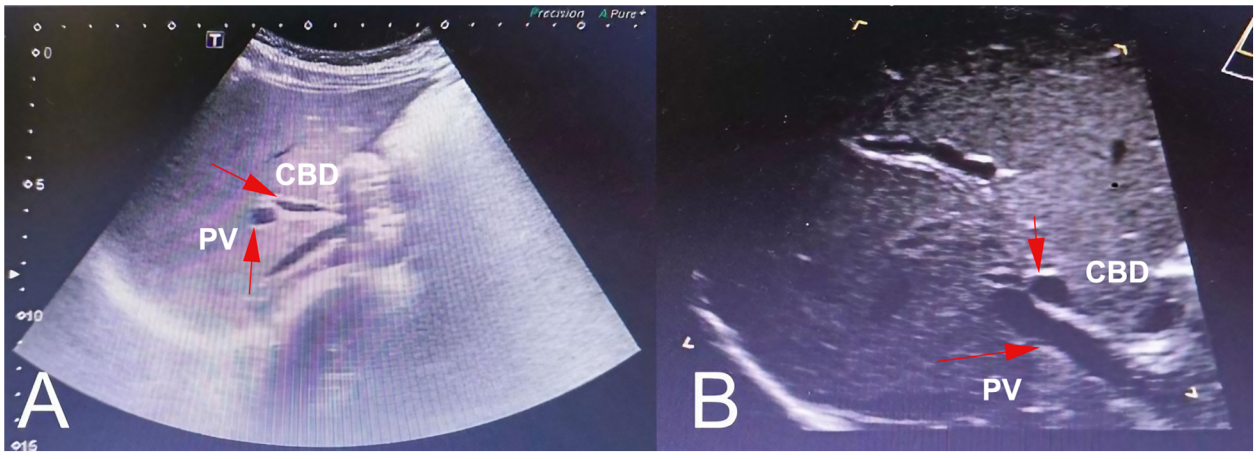

FIGURE 1
Ultrasound scans of a pediatric patient with CAGB. An ultrasonographic image (A) and after magnification (B) of the gallbladder showed a deficiency in the gallbladder. The common bile duct (CBD) and portal vein (PV) are indicated by arrows for reference. No gallbladder or gallbladder remnant was visible in the expected location.

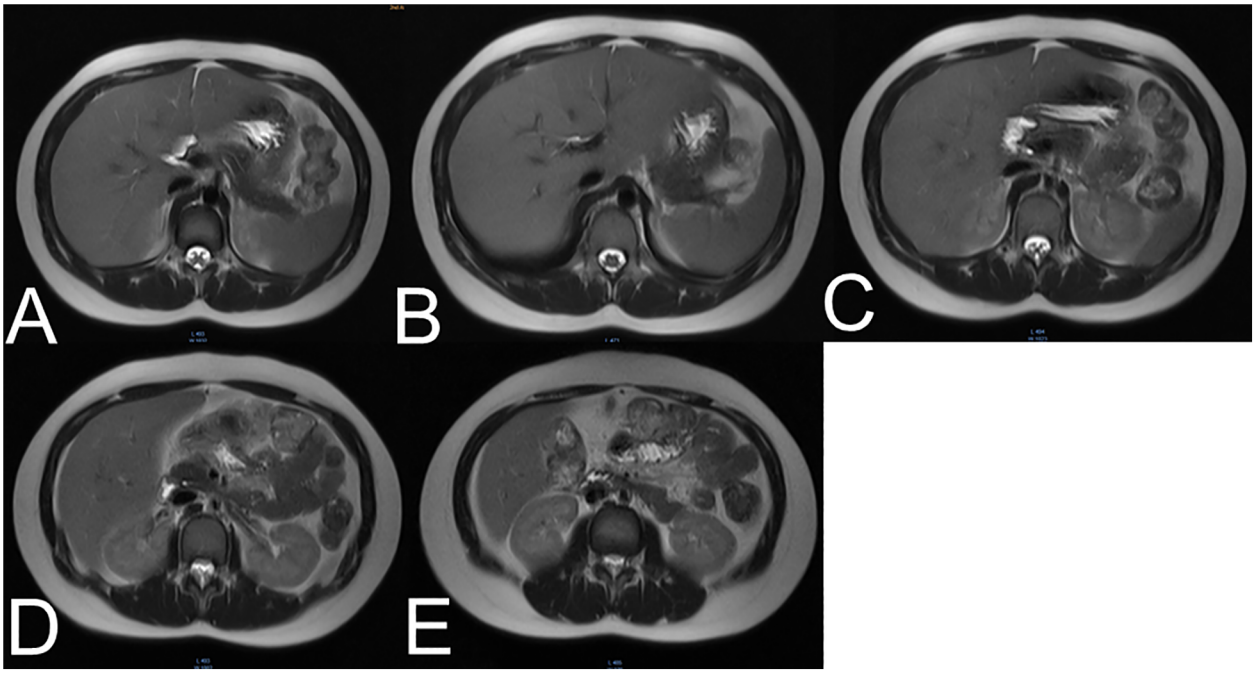

FIGURE 2
(A–E) Five different cross-sectional MRI scans of the abdomen. These findings usually illustrate various segments of the liver and surrounding anatomical structures, which indicate mild hepatic steatosis.

TABLE 1 A timeline with relevant data from the episode of care.

Time point	Event description
Gestational week 22	Gallbladder not observed during prenatal checkup.
March 2011	The male fetus was born, and normal growth and development were observed.
February 2022	At 9 years and 11 months of age, the patient presented with significant weight gain. Abdominal ultrasound and abdominal magnetic resonance imaging revealed: fatty liver, agenesis of the gallbladder, and mild liver function abnormalities.
6 months later	After 6 months of liver protection and lipid-lowering treatment, liver function returned to normal, and fatty liver improved.
One year later	At follow-up, the patient continued in good clinical condition.

feeling relieved to have a clear explanation for the prenatal findings and were committed to following the recommended lifestyle modifications and treatments. The patient's growth and development were monitored every six months from age 10 to 13, with all measurements within the normal range. At age 13, the patient's height was 164.5 cm and weight was 59 kg, indicating normal growth progression.

3 Discussion

CAGB is an uncommon condition characterized by the failure of proper gallbladder development during fetal life (11). It is generally accepted that this anomaly is linked to the development of the hepatic diverticulum. During the fourth week of embryogenesis, the hepatic diverticulum arises from the endoderm at the junction of the foregut and yolk stalk, ultimately giving rise to the liver and gallbladder, with the cystic duct originating from its proximal portion (12). Patients with CAGB often experience vague symptoms such as pain in the upper right abdomen, nausea, or jaundice. Diagnosing CAGB can be challenging, as it may be mistaken for gallstones, occasionally leading to unnecessary surgeries (13).

The gallbladder is an organ that stores bile for digestion; its absence may affect digestive function (14). However, in isolated cases of gallbladder absence, no special treatment is generally required, and pregnancy can continue with regular postnatal observation (15). Non-visualization during prenatal screening, potentially due to technical issues or natural causes, necessitates a follow-up ultrasound to check for abnormalities. Gallbladder absence could indicate other anomalies and requires careful monitoring, as observed in a case at 22 weeks gestation. Furthermore, gallbladder absence may be associated with other anomalies, such as duodenal atresia or biliary atresia (16). In our case report, the gallbladder absence was noted during a 22-week gestational check-up, necessitating follow-up to ensure normal development. Moreover, if the child exhibits any abnormal symptoms during growth and development, medical treatment or other interventions may be warranted based on the specific situation. The concurrent occurrence of gallbladder agenesis and fatty liver in this young patient underscores the need for comprehensive evaluation and management of metabolic health, particularly in the context of rapid weight gain in pediatric patients. The concurrent occurrence of gallbladder agenesis and

fatty liver in a young patient underscores the need for early lifestyle and medical interventions to prevent advanced liver disease, particularly in the context of pediatric patients.

In summary, the preoperative diagnosis of CAGB is challenging. During the history-taking process, it is essential to inquire about the patient's family history of CAGB or other related systemic variations (17). Imaging studies follow, with surgical exploration being the last resort. Clinically, ultrasound is often used to examine gallbladder diseases, with a sensitivity of over 95% (18). In our case, the initial ultrasound examination indicated that the gallbladder was not visualized within the gallbladder fossa. According to the proposed diagnostic imaging protocol for biliary diseases, patients suspected of having cholecystitis with unclear ultrasound images or apparent atrophy should undergo upper abdominal MRI, CT, or magnetic resonance cholangiopancreatography (MRCP) examination, which may reduce the misdiagnosis rate of CAGB (19).

As patients with CAGB grow, they may experience various long-term effects on their digestive system and metabolism. The absence of a gallbladder can lead to biliary dyskinesia, which is believed to be caused by increased tonicity of the sphincter of Oddi. This condition is comparable to postcholecystectomy syndrome and may explain the biliary colic experienced by some patients with CAGB. Interestingly, ursodeoxycholic acid has shown promise in relieving symptoms of biliary dyskinesia and may potentially have a role in managing CAGB-related symptoms (20).

While many individuals with CAGB remain asymptomatic throughout their lives, suggesting that the body can adapt to the absence of a gallbladder, others may present with various symptoms. According to previous studies, right upper quadrant pain is present in 90% of symptomatic cases, nausea and vomiting in 60%, and jaundice in 35% (21, 22). These symptoms can mimic other biliary pathologies, leading to potential misdiagnosis.

It's important to note that CAGB has been associated with malformations in other systems, particularly cardiovascular, skeletal, and abdominal wall abnormalities. There is also an association with trisomy 18 and Klippel-Feil syndrome (23). This underscores the importance of comprehensive evaluation and long-term follow-up for patients diagnosed with CAGB.

Regarding the risk of malignancy, while there is limited research due to the rarity of the condition, some studies have noted that the common duct is frequently found to be dilated at exploration, sometimes in the presence of stones. This anatomical alteration might potentially impact the long-term health of the biliary system, although more research is needed to establish any definitive link to increased cancer risk.

In this case, fatty liver and gallbladder absence were identified upon reevaluation of the gallbladder after MRI. We believe that patients with congenital gallbladder absence require a thorough assessment of their condition. It is also necessary to broaden the diagnostic approach, combining various examination methods, such as genetic sequencing and in-depth embryological research, to continuously improve diagnostic accuracy and avoid misdiagnosis.

It is important to note that the mild elevations in liver enzymes (AST 67 IU/L, ALT 44 IU/L) and the fatty liver changes observed on MRI are more likely associated with the patient's rapid weight gain rather than being a direct consequence of congenital absence of the gallbladder. Childhood obesity is a known risk factor for non-alcoholic fatty liver disease, which can lead to elevated liver enzymes and hepatic steatosis. This case highlights the importance of considering multiple factors when evaluating liver function abnormalities in pediatric patients, even in the presence of rare congenital anomalies like gallbladder agenesis.

4 Conclusion

CAGB is a rare embryological anomaly of the biliary system, presenting challenges in diagnosis and treatment. CAGB may be associated with other congenital abnormalities, with isolated cases being uncommon. Clinically, it can manifest as nonspecific symptoms of the biliary, gastrointestinal, or urinary systems, often mimicking clinical presentations of many digestive diseases, leading to potential misdiagnosis. Genetic sequencing and in-depth embryological research may offer new perspectives for elucidating the etiology and improving diagnostic accuracy. This case highlights the importance of considering gallbladder agenesis in patients presenting with fatty liver, especially in the pediatric population.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Transition of patients with Gaucher disease type 1 from pediatric to adult care: results from two international surveys of patients and health care professionals

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Introduction: Gaucher disease (GD) is a rare, autosomal recessive lysosomal storage disorder caused by a deficiency in the enzyme glucocerebrosidase. The most common subtype in Europe and the USA, type 1 (GD1), is characterized by fatigue, cytopenia, splenomegaly, hepatomegaly, bone disease, and rarely pulmonary disease. Increased life expectancy brought about by improved treatments has led to new challenges for adolescents and their transition to adult care. Efficient healthcare transition to adult care is essential to manage the long-term age-related complications of the disease.
Methods: This international study consisted of two online surveys: one survey for patients with GD1 and one survey for healthcare professionals (HCPs) involved in treatment of patients with GD1. The aims of this international, multi-center project were to evaluate the current transition process in various countries and to understand the challenges that both HCPs and patients experience.
Results: A total of 45 patients and 26 HCPs took part in the survey, representing 26 countries. Our data showed that a third (11/33) of patients were aware of transition clinics and most stated that the clinic involved patients with metabolic diseases or with GD. Seven patients attended a transition clinic, where most patients (5/7) received an explanation of the transition process. Approximately half of HCPs (46%; 12/26) had a transition clinic coordinator in their healthcare center, and 10 of HCPs had a transition clinic for patients with metabolic diseases in their healthcare center. HCPs reported that transition clinics were comprised of multi-disciplinary teams, with most patients over the age of 18 years old managed by hematology specialists. The main challenges

of the transition process reported by HCPs included limited funding, lack of expertise and difficulty coordinating care amongst different specialties.
Discussion: Our study demonstrates the lack of a standardized process, the need to raise awareness of transition clinics amongst patients and the differences between the transition process in different countries. Both patients and HCPs expressed the need for a specialist individual responsible for transition, efficient coordination between pediatricians and adult specialists and for patient visits to the adult center prior to final transition of care.

KEYWORDS

healthcare transition, Gaucher, child and adolescent health, transition clinic, transfer of care

1 Introduction

Gaucher disease (GD) is a rare, autosomal recessive lysosomal storage disorder (LSD) caused by a deficiency of the enzyme glucocerebrosidase, which leads to the accumulation of its substrate, glucosylceramide, in macrophages, preventing their normal function (1). Enlarged macrophages containing undigested glucosylceramide are also known as Gaucher cells and are a pathological hallmark of GD (2). The buildup of Gaucher cells in the spleen, liver, bones, bone marrow, and other tissues cause a progressive loss of organ function, and account for the clinical symptoms associated with the disease (3). The incidence of GD is estimated at 1:40,000–1:60,000 live births and occurs in all ethnicities, although higher incidences are found in Ashkenazi Jews (1:800) (1, 4, 5). The incidence of type 1 GD (GD1) in Europe and North America was reported as between 0.45-22.9/100,000 live births (6).

GD historically has been classified into three main types based upon clinical signs and age of onset: type 1, type 2, and type 3 (7). Although the classification of GD subtype aids clinical management, GD comprises a wide phenotypic spectrum of disease, similar to other LSDs (7). GD1, the most common subtype in US and Europe, is characterized by fatigue, cytopenia, splenomegaly, hepatomegaly, bone disease, ophthalmic abnormalities and on occasion pulmonary disease, dental manifestations, lymphadenopathy and Gaucheroma (8–11). More recent studies have also demonstrated the presence of neurological conditions, including Parkinson's disease, indicating a broader spectrum of disease than previously thought (12, 13). Patients diagnosed with GD1 in childhood generally have more pronounced visceral and bone disease symptoms in comparison with those diagnosed in adulthood. In adulthood, bone disease is the most incapacitating manifestation of GD1, affecting one third of patients (8–11).

Diagnosis of GD is by biochemical testing and the age of GD1 diagnosis varies depending on the population under study. Results from a French Gaucher registry of over 500 patients reported a mean age at diagnosis of 17.4 years, compared to 27.2 years from the international ICGC Gaucher registry (NCT00358943) with over 1,600 patients (14, 15). Biochemical testing identifies deficient glucocerebrosidase activity in peripheral blood leukocytes or other nucleated cells, and genetic testing determines biallelic pathogenic variants in *GBA1*, the gene encoding glucocerebrosidase and is useful for risk prediction, stratification, and counseling (8). Biomarkers like glucosylsphingosine (lyso-Gb1) aid in diagnosis and monitoring disease progression and treatment response Current treatments for GD1 include enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) (16). Potential therapies in clinical trials include several gene therapies (NCT05487599; NCT05324943) and ambroxol, an oral chaperone therapy (NCT01463215), which is mainly studied for neuronopathic GD (type 2 and 3 GD).

Data from the ICGG Gaucher Registry demonstrated that patients diagnosed with GD1 treated with ERT showed improvement of both laboratory and clinical parameters, particularly if treatment was started early (17–21). Although advances in treatment of GD1 have improved quality of life, the disease can remain difficult to manage due to the heterogeneity of clinical presentation and some complex cases require a multi-disciplinary team of specialists to manage and coordinate care (22–26).

It is possible that the increased life expectancy brought about by improved treatments has led to new challenges for adolescents and their transition to adult care, including the requirement of additional specialists such as radiologists, gastroenterologists or dentists if patients develop further long-term manifestations and comorbidities of the disease (27–29). Smooth and coordinated transfer of care from pediatric specialists to adult specialists is essential to manage the long-term age-related complications of the disease and to monitor the compliance and response to available therapies (27). Healthcare transition is defined as the planned, purposeful process of preparing adolescents for adult-centered medical care and is recognized as an important aspect of care for patients with inherited metabolic diseases worldwide (22, 27). Successful transition enables the patient to remain engaged with the healthcare system while progressing towards self-directed management, which requires some readiness of the young adult to make their own healthcare decisions (23, 30). Not all centers have specific transition guidelines (31–33) and there appears to be considerable disparity in transition globally (34–37). A framework for a GD1 transition program is under development. However, well-structured guidelines on transition have been published by the UK's National Institute for Health and Care Excellence (NICE), and encompass a set of general guidelines for transition from children's to adult services (38). These guidelines include recommendations for planning transition and supporting

infrastructure, including the implementation of transition clinics. In the UK, transition clinics are prearranged and planned. Regular meetings prior to the transition clinic are an opportunity for both pediatric and adult teams to discuss any outstanding investigations, follow up on any social issues and plan which other specialists involved in a young person's care should be informed about the transfer of care to adult metabolic services.

The aims of this international and multi-center study were to evaluate the current transition process in various countries and to understand the challenges that both HCPs and patients experience. This study was designed by members of the International Working Group on Gaucher Disease (IWGGD), which promotes clinical and basic research into GD, and the International Gaucher Alliance (IGA), a patient led international organization.

2 Methods

This international study consisted of two online surveys: one survey for patients with GD1 and one survey for HCPs involved in treatment of patients with GD1, worldwide. The survey for patients with GD1 was open to individuals aged 16 years and over and to caregivers of an individual with GD1. The study was open to patients who were in the process of transitioning to adult services, had already transitioned to adult services or had remained under the same team's care throughout their life in their home countries without transitioning to adult care.

2.1 The surveys

Clinical experts who are members of the IWGGD (International Working Group for Gaucher Disease) prepared the surveys in collaboration with the IGA (International Gaucher Alliance) and two patient members of the IWGGD. The surveys were available in English only, were hosted on the Survey Monkey platform, and circulated electronically via email and social media. The local IWGGD representatives were able to translate the questions to those willing to participate. The patient survey included 15 questions aimed at gathering information on the transition process and the associated difficulties and needs from the perspective of patients and carers (see Supplementary Material for full list of questions). The HCP survey included 14 questions aimed at gathering information on the status of the transition process, its organization and the associated difficulties and needs from the perspective of HCPs (see Supplementary Material for full list of questions). The surveys included multiple choice questions, with the possibility to write additional text under the option "Other" when available. The survey was active for two months, from 5th October 2021 to 5th December 2021.

2.2 Recruitment

Patients were recruited via social media/newsletter advertisement by the IGA. The invitations contained a link to access the survey platform. In addition, 79 direct email invitations containing the link access were sent to potential participants from 57 IGA member countries. IGA members were asked to forward the invitation and link to HCPs in their country.

2.3 Ethical considerations

The study was registered as a service evaluation project with Research & Innovation at Salford Royal Organization (NCA) (registration number 23HIP04). The invitations contained details of the study and participants indicated their consent to take part by clicking the link to the survey. Participants completed the surveys anonymously and no personal data related to age, gender or ethnicity was collected.

2.4 Data analysis

Data was extracted from Survey Monkey and descriptive statistical analysis was performed using Microsoft Excel. Respondents were given the option to skip survey questions and not all respondents answered every question. The total number of respondents that answered each question are represented by the denominator.

3 Results

3.1 Responses

Forty-five responses to the patient survey were received. Of these, one patient only answered the first two questions (age and country) and was excluded from the analysis. A further six left the survey after Question 3 (Do you/your child remain under a specialist care?) and eight left after Question 5 (If "yes" to Q3, are you aware of transition clinic?). Most ($n = 28$) of the 30 remaining patients completed the survey to the end, with one stopping after question 11 and one after question 12.

Twenty-six responses to the HCP survey were received, including 20 complete responses. All six of the HCPs who did not complete left the survey after Question 4 (Do you have transition clinic for metabolic patients in your center?), including 3 who answered yes to this question.

3.2 Demographics

Of the 44 GD1 patients included in the analysis, most patients were from the Netherlands, the United Kingdom (UK) and the United States (US) (Figure 1; Table 1). Most patients were over the age of 18 years and were receiving specialist care at the time of the survey (Table 2). Of those patients receiving specialist care, most were cared for by metabolic medicine departments or centers (Table 2). Of the 26 who answered this question, half

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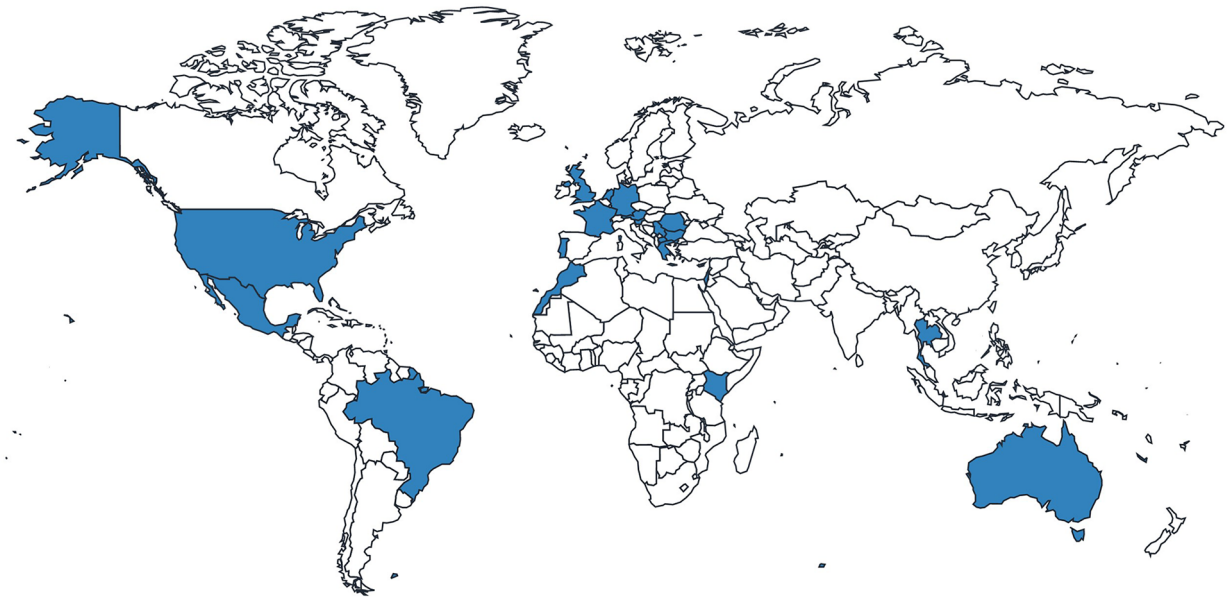

FIGURE 1
Distribution of responses from patients with Gaucher disease.

($n = 13$) had been treated by a Gaucher disease team in childhood and adulthood.

Of the 26 HCPs included in the study, almost one-third were from the UK, followed by nearly equal representation of HCPs from other countries worldwide (Figure 2; Table 3). Most specialized in metabolic medicine (Table 4).

3.3 Transition process for patients

Of those patients remaining under specialist care, 33% (11/33) were aware of transition clinics (Table 5). Four patients were from the UK, five were from other European countries, one was from Israel and one was from Mexico (Supplementary Table S1). Of the patients that were not aware of transition clinics, 2/22 did not have a transition clinic available in their respective countries (Australia and Slovenia) and 2/22 patients were diagnosed too late to attend a transition clinic. Most patients who were aware of a transition clinic (8/9) stated that the transition clinic involved patients with GD or metabolic diseases in general.

One patient had been diagnosed as an adult, and while they were aware of transition clinics, they had no personal experience of attending one. Seven patients, from Greece, Israel, Mexico, North Macedonia, Romania, and the UK, provided details of their transition experience (Table 5). Three patients transitioned straight from adolescent care to adult care, two patients attended one transition clinic prior to the final transfer of care (Greece and Romania) and one patient attended four clinics (England). One patient did not say how many clinics they had attended. Patients were aged between 16 and 30 years when they started to

TABLE 1 Patient country of residence ($n = 44$).

Country	Patients, *n*	Country	Patients, *n*
Netherlands	$n = 8$	Bulgaria	$n = 1$
United Kingdom	$n = 8$	France	$n = 1$
United States	$n = 6$	Germany	$n = 1$
Mexico	$n = 3$	Israel	$n = 1$
Greece	$n = 2$	Kenya	$n = 1$
Romania	$n = 2$	Morocco	$n = 1$
Slovenia	$n = 2$	North Macedonia	$n = 1$
Australia	$n = 1$	Portugal	$n = 1$
Austria	$n = 1$	Serbia	$n = 1$
Brazil	$n = 1$	Thailand	$n = 1$

TABLE 2 Patient characteristics.

Category	*n* (%)
Age	
<18 years	1/44 (2)
≥18 years	42/44 (95)
Not answered	1/44 (2)
Specialist care received	
Yes	37/44 (84)
No	7/44 (16)
Specialist care[a]	
Metabolic medicine	13/33 (39)
Haematology	9/33 (27)
Clinical genetics	5/33 (15)
Internal medicine	4/33 (12)
Other[b]	1/33 (3)
Not answered	1/33 (3)

[a]Four patients receiving specialist care left the survey before this question.
[b]Other included: not sure ($n = 1$).

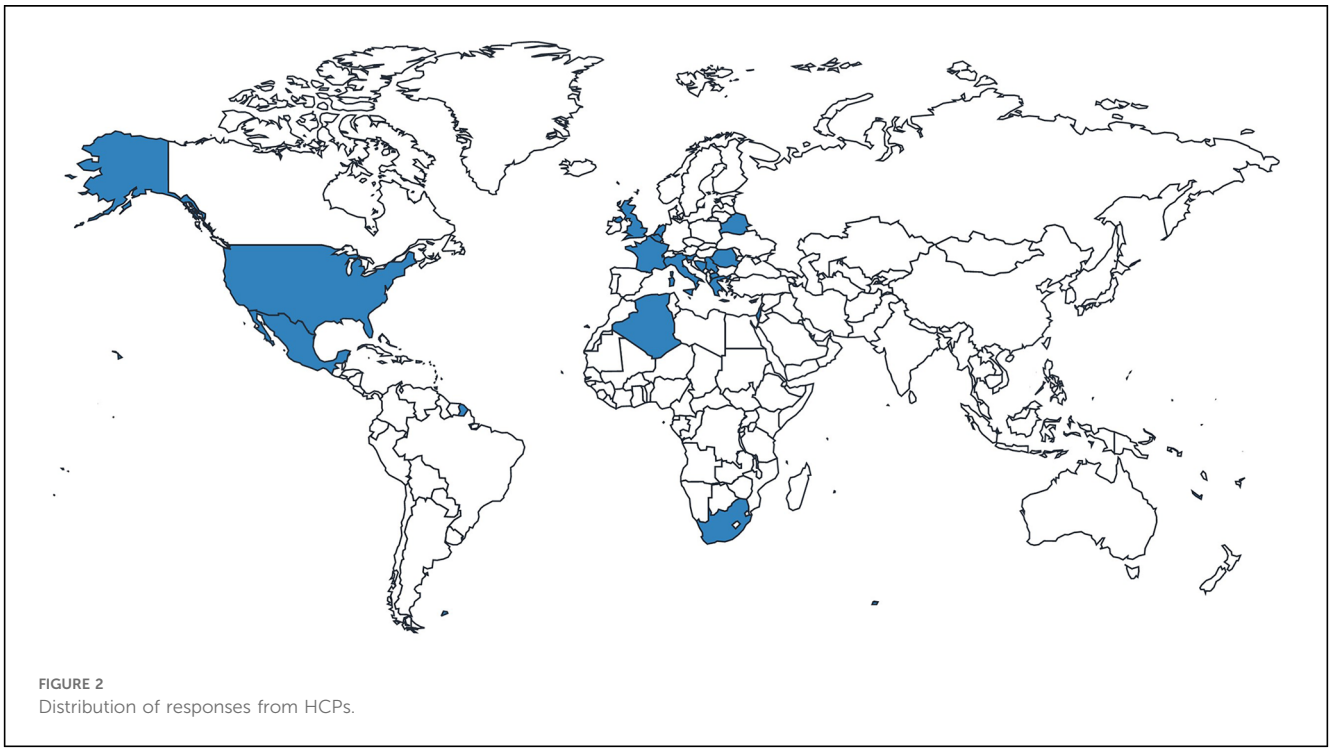

FIGURE 2
Distribution of responses from HCPs.

attend the transition clinic. Age of final transfer to adult care ranged between 18 and 31 years (Table 5).

Five patients (5/7) received an explanation of the transition process, but none were handed a leaflet about transition, and two received no explanation (Table 5). One patient was asked to complete transition documentation by completing the Ready Steady Go document (39).

3.3.1 Recommendations for improving the transition process

Patients were asked for their suggestions on how to improve the transition process (Table 6). Their recommendations included simplifying the referral process, improving the coordination of transition, including the transfer of patient documents, and making the process more patient friendly, with patients having more say in which specialists they see. Another patient suggested that doctors from the adult care team visit pediatric patients before transition to adult care to enable patients to become familiar with the adult care team and have the opportunity to ask questions about the adult service. One patient remained with the same GD1 specialist from childhood all through to adulthood.

3.4 HCP experience of the transition process

Almost half (12/26) of HCPs had a transition clinic coordinator in their healthcare center (Table 7). In some cases, the transition clinic coordinator was a clinical nurse specialist or a specialist in metabolic or LSDs. Of these HCPs, six were in the UK, with the remaining six of HCPs in other countries. (Belarus, Bosnia and Herzegovina, Mexico, Italy, North Macedonia and Romania).

3.4.1 Transition clinics for metabolic patients

Ten of 25 HCPs had a transition clinic for metabolic patients in their healthcare center (Table 7; Supplementary Table S1). Two HCPs working in the UK described monthly transition clinics that took place in the pediatric hospital, attended by both

TABLE 3 HCP country of work ($n = 26$).

Country	HCPs, *n*	Country	HCPs, *n*
United Kingdom	$n = 7$	Bosnia and Herzegovina	$n = 1$
France	$n = 2$	Greece	$n = 1$
Israel	$n = 2$	Italy	$n = 1$
North Macedonia	$n = 2$	Mexico	$n = 1$
Serbia	$n = 2$	Netherlands	$n = 1$
Algeria	$n = 1$	Romania	$n = 1$
Belarus	$n = 1$	Slovenia	$n = 1$
Belgium	$n = 1$	South Africa	$n = 1$

TABLE 4 HCP characteristics.

Category	*n* (%)
Specialist area	
Metabolic medicine	11/26 (42)
Haematology	5/26 (19)
Clinical genetics	3/26 (12)
Internal medicine	3/26 (12)
General Practitioner	0/26
Other[a]	4/26 (15)

[a]Other included: paediatric neurology ($n = 1$); gynaecology ($n = 1$); neurology ($n = 1$); paediatric haematology ($n = 1$).

TABLE 5 Patient experience of transition process.

Category	*n* (%)
Awareness of transition clinic	
Aware of transition clinic	11/33 (33)
Not aware of transition clinic	22/33 (67)
Age patient began attendance at transition clinic[a]	
15–18 years	2/7 (29)
19–22 years	3/7 (43)
23–27 years	0/7 (0)
28–31 years	2/7 (29)
Age of patient at final transfer	
15–18 years	1/7 (14)
19–22 years	2/7 (29)
23–27 years	1/7 (14)
28–31 years	3/7 (43)
Implementation of transition	
Patients received an explanation of the transition process	5/7 (71)
Patients received no explanation of the transition process	2/7 (29)

[a]Of the 11 patients who were aware of transition clinics, two left the survey before answering any further questions; one patient had not attended a transition clinic and had answered that the transition clinic did not involve metabolic/GD patients; one patient did not answer any of the questions regarding transition.

TABLE 6 Patient recommendations for improving the transition process.

"They need to get the adult doctors to visit the children when they are 16 so they can have a process to get use to going to another clinic, also visits in the adult clinic would help. I would also like to receive a document with all my history from the children clinic and discuss care with the adult doctor. Other problem is that we have no choice as we have to go to hematology, and we do have metabolic and endocrinology clinic and they said no to receiving Gaucher patients as they are all in hematology, which I think should change."
"We do not have transition clinics in Bulgaria. One day you're in pediatrics and after you turn 18 you're transitioned to the next clinic. The good thing is that both clinics are 5–10 min apart.
"I wish there would be more patient friendly transition process."
"We need a team of different specialists, not only a haematologist. Some of my paediatric doctors, like my cardiologist is still seeing me but because I specifically ask him to. Even though I have several bone problems I have never had an orthopaedist following my case."
"I would hope a process to be simpler and more natural. I think that transition process should be more automatic especially in cases where paediatric patients transition to adult [services] since that transition is inevitable."

pediatric and adult teams, and one HCP working in the Netherlands reported an individualized approach, where harmonization of protocols is standard practice. Physical transition takes place if needed, otherwise the transition is an administrative process. All HCPs (7/7), that had a transition clinic for metabolic patients in their healthcare center had transition protocols or guidelines (Table 7). In the UK, documentation included transition standard operating procedures and Growing Up and Gaining Independence (GUGI), a framework to encourage and support young people to become as independent as they can with their healthcare (40). In the Netherlands, protocols for treatment, diagnosis, follow-up and care pathways were harmonized, and treatment decisions were always made in a multidisciplinary team meeting.

Most HCPs reported the number of transition clinic appointments as a range, between one and four, although the

TABLE 7 HCP experience of transition process.

Category	*n* (%)
Transition clinics	
Presence of transition clinic coordinator	12/26 (46)
Presence of transition clinic for metabolic patients[a]	10/25 (40)
Metabolic transition clinic features[b]	
Presence of transition protocols and guidelines	7/7 (100)
Transition clinic team	
Paediatric team	6/7 (86)
Adult physician and nurse	6/7 (86)
Allied health professionals[c]	5/7 (71)
Subspecialities[d]	3/7 (43)
Common information discussed during transition	
Medical health issues	6/7 (86)
Wellbeing	6/7 (86)
Adult life-related issues[e]	6/7 (86)
Surgical procedures or pre-op assessment	5/7 (71)
Healthy living	5/7 (71)
Consideration of new therapies	4/7 (57)
Other[f]	3/7 (43)
Age patient began attendance at a transition clinic	
14 years	4/7 (57)
15 years	1/7 (14)
16 years	1/7 (14)
18 years	1/7 (14)
Centres with no metabolic transition clinic	
Management of adult patients with GD1	
Metabolic medicine	6/13 (46)
Haematology	7/13 (54)
Clinical genetics	3/13 (23)
Internal medicine	6/13 (46)
Other[g]	3/13 (23)
Main challenges to transition for patients with GD1	
Limited funding	3/13 (23)
Lack of expertise	2/13 (15)
No interest in metabolic medicine	4/13 (31)
Difficulty to coordinate care	5/13 (38)
Patient apprehension	1/13 (8)

[a]One HCP did not answer this question.
[b]Three HCPs with metabolic transition clinics left the survey before this question.
[c]Including physiotherapist, occupational health specialist, dietetics.
[d]Including orthopaedics, cardiology, neurology.
[e]Including pregnancy and mental health.
[f]Other included: dependent on individual ($n = 1$); dependent on consent and patient capacity ($n = 1$); long-term outcomes ($n = 1$).
[g]Other included: gynaecology ($n = 1$); endocrinology ($n = 1$); oncology ($n = 1$).

HCP from Bosnia and Herzegovina stated that patients attended six clinics before transition and one for a final report. One HCP explained that the number of clinics attended by patients was dependent on the individual needs of the patient.

HCPs reported that transition clinics were comprised of multi-disciplinary teams, including the pediatric team, adult physician and nurse, and allied health professionals, with additional subspecialities included on an individualized basis (Table 7).

The most common information discussed during transition included medical health issues, wellbeing, and adult life-related issues (Table 7). Patients were between 14 and 18 years old when they started to attend transition clinics (Table 7). Two HCPs

explained that age of attendance to transition clinic was dependent on the individual including the level of complex needs and severity of co-morbidities. The age of final transfer to adult care was generally between 16 and 18 years, apart from one center where the range was 16–21 years.

3.4.2 Centers without a metabolic transition clinic

In centers without a metabolic transition clinic, patients with GD over the age of 18 years were mostly managed by hematologists (7/13), internal medicine specialists (6/13) and metabolic specialists (6/13) (Table 7).

The main challenges to provision of a transition service for patients with GD in these centers included limited funding, lack of expertise and interest in adult metabolic medicine in many countries and difficulty coordinating care amongst different specialties. One HCP mentioned patient apprehension as the main challenge for transition (Table 7).

3.4.3 Recommendations for improving the transition process

Suggestions from HCPs included:

- Improved coordination and education of pediatric and adult teams on GD and other hereditary rare diseases with multisystem health problems.
- Standardization of care, guideline development and national approaches.
- Regular review of processes with patients and healthcare professionals to ensure transition is undertaken correctly.
- Visits to adult center for pediatric patients and the management of expectations for both adolescents and parents.
- Empowerment and support of young adults, with an individualized approach to adult care.
- A transition clinic coordinator who has experience within the pediatric service and close teamwork between pediatric and adult care teams.

4 Discussion

The results from our multi-center study give an international overview of the status of the transition process from the perspective of both patients with GD1 and HCPs involved in patient care. The majority of patients were cared for by metabolic specialists. The remainder were managed by hematology, genetics or internal medicine departments. This highlights the multi-disciplinary nature of GD, the coordination needed between specialists and the requirement for distinct types of care in the management of the disease. In a recent European study of transition in inherited metabolic diseases, while most centers had an adult metabolic team, only 31% were available to all metabolic conditions (27). This shortage of adult metabolic specialists often leads to fragmented care as patients require monitoring by different specialists, which exacerbates the impact of living with GD1, as patients must repeat information to different providers (27). Indeed, one of the greatest challenges that HCPs reported in this study, was that care of patients with GD1 was spread among different specialties, making it difficult to coordinate their care.

The management of GD is intrinsically challenging due to the phenotypic heterogeneity of the disease (22–26). Management of long-term age-related GD complications is, to a greater degree, complex and requires a smooth and coordinated transfer of care from pediatric specialists to adult specialists (27). Recommendations for the management of pediatric patients focus on the assessment of growth profile and routine neurological examination, including eye movements, while the follow-up of adult patients comprises monitoring of biochemical, hematological, and visceral parameters, in addition to a surveillance of bone disease and assessment of neuropathic pain (22–26). Patients with GD have a diminished health-related quality of life, with poorer outcomes reported in children. Special support should be given to adolescents, particularly during the transition of care to adulthood and a self-directed management. Transition clinics represent, thus, a foundational structure for both pediatric and adult teams to cooperate and effectively support the continuous management of GD tailored to the patients' developmental maturity and needs (23, 30).

4.1 The need for transition coordinators

In this study, only a third of patients were aware of transition clinics. Forty percent of HCPs had a transition clinic for metabolic diseases in general in their center. Patients with GD1 are reviewed in these clinics and none of the centers had a separate transition clinic for them. As patients and HCPs were not matched, these results may reflect the inter and intra-country differences in transition clinic provision, participation bias with HCPs with transition clinics being more likely to complete the survey or a lack of communication on the availability of transition clinics. While recommendations exist on the need for and importance of a named transition coordinator (41), our study suggests they are not available in many centers. In centers that provided metabolic transition clinics, only 70% had a transition clinic coordinator. In a recent European survey, only 31% of inherited metabolic disease centers had a designated transition coordinator, while a French study showed that only 48% of patients with LSD were appointed a transition coordinator (27, 35). A transition clinic coordinator is essential to ensure that adult teams are aware of the management required for rare disorders such as GD1 and are important to guarantee the continuity of care and improvement of outcomes (27).

4.2 Adolescent care

While patients first attended transition clinics between the ages of 16–30 years, HCPs reported that attendance usually started at 14–18 years. Young people have different needs to those of children and adults. Some symptoms of GD may also become more pronounced during adolescence. Therefore, starting the transition process in early adolescence allows the patient and parents time to adjust to the changes ahead and may avoid some

of the challenges encountered, such as reluctance to attend appointments with an unfamiliar adult team and attachment to pediatricians (30). Appropriate adolescent services prevent young adults with GD from transitioning to adult metabolic services prematurely and being treated as a child in other specialist clinics. Adolescent medicine needs better recognition, particularly in metabolic disorders.

4.3 Differences between centres

However, transition must also be flexible and personalized, taking into account the individual needs, cultural differences and circumstances of the patient (42, 43). This flexibility was commented on by the HCPs in this study, particularly in relation to the number of transition clinics offered. Our study showed variability in the number of transition clinics patients attended before final transfer. Patients with fewer health-related problems may only attend one transition clinic, while some other patients with more complex clinical presentation, will need input from other specialties and require more time to transition to adult care. Those patients are likely to remain under the care of several specialties and may attend their own transition clinics.

Although the patients in our study started attending their transition clinic between the ages of 16–30 years, they were 18–31 years old at the final transfer of care to adult services. HCPs reported that final transfer usually occurred at 16–18 years but could be as late as 21 years. The age ranges reported reflect both a personalized approach to individual patients and country differences in the care of adult patients with GD1. The ability of centers to offer flexibility based on the patients' developmental maturity often depends on the institution, cultural differences, or country regulations. The causes for the delayed transfer of care among patients with GD are not clear but are likely to be related to the availability and readiness of the adult team to take over the care rather than clinical reasons. For example, in Sweden and Italy, patients must transfer by the age of 18 years (44, 45). In the UK, final transfer must also take place before 18 years old as adult patients are not permitted to be hospitalized in pediatric hospitals due to legal restrictions. At 18 years of age, the primary receiver of information changes from the parent or caregiver to the young person (38). In some countries, transition can occur at a younger age. In Oman the transfer age is 13 years, although a recent study of transition readiness recommended that this be increased to 18 years (46).

In this and previous studies, a proportion of adult patients remain under the care of their pediatric team. In a French study of patients with LSD, including some patients with GD1, 24% of patients over the age of 21 were still cared for by pediatric departments (35). A European multi-center study found that 11% of patients remained under pediatric care throughout their lifetime (27). The reasons for patients remaining with pediatric services include a lack of transition organization, lack of disease knowledge in the adult center and refusal to transition by the family or patient (35). In addition, a successful transition process is dependent on standardized operating procedures and adequate financial resources and specific training. The European multi-center survey assessing the challenges associated with transition of patients with inherent metabolic disorders revealed that 90% of HCPs responders reported the absence of financial support for transition programs (27). Noteworthy, one patient in our study wished to remain with the same GD1 specialist from childhood through to adulthood. Patients and parents often get used to the pediatric team and find it difficult to adapt to the changes brought about by transition into adult care. Clear communication has been shown to increase patient and family satisfaction with the transition process (41, 47).

4.4 Transition documentation

Of the HCPs surveyed in our study, most stated that their transition clinics had transition protocols or guidelines, although there was a lack of standardization between centers. Transition clinics were comprised of a pediatrician, adult physician and a nurse, along with allied health professionals and subspecialities, reflecting the complexity and heterogenicity of the disease. We found that adult-health related issues such as pregnancy and mental health were amongst the most common issues discussed at transition clinics. Recommendations for patient management defined through a Delphi consensus in Spain include a multi-disciplinary care during pregnancy involving GD specialist, obstetrician, and anesthesiologist. Similarly, experts recommended the involvement of radiologists with experience in GD and orthopedic surgeons for an adequate monitoring of bone disease in patients with prosthetics (22–26). Our study indicates that most patients that attended transition clinics received an explanation of the transition process, although none were given a transition leaflet and only one completed transition support documentation. The absence of standard process and written information on transition may lead to a fear of adult care and an increase in patient and family anxiety (48). An understanding of the challenges related to the transition and long-term follow up is crucial to empower patients on their autonomy and participation in the decision-making process regarding choice of treatment or even frequency of ERT. There is growing evidence that transition programs improve patient outcomes and wellbeing (49). A structured transition program increases patient satisfaction, independence, and perceived health status. A systematic review of transitional care programs in patients with chronic illness or disability aged between 11 and 25 years has shown that the most frequently used strategies in successful programs were specific transition clinics and patient education (50). Initiatives such as the Ready Steady Go Program aim to give patients and their families the knowledge and skills needed to manage their condition into adulthood and have been shown to improve long-term outcomes (39, 51).

4.5 Improving the transition process

Patients and HCPs had similar views on how to improve transition through improved coordination and providing

opportunities to meet the adult team before transition. Patients wanted a more patient centric process that gave them more say in how their care was managed. HCPs emphasized the need for education, standards, and national approaches in combination with the flexibility to provide individualized care. One of the suggestions is a clinical review of a young adult after the transfer of care to the adult services jointly by pediatric and adult teams. It may empower patients and encourage them to engage with the management of their condition by the new team.

Our findings suggest that the transition process is not well developed in many countries, which may compromise patient care. The main challenges to provision of a transition service for patients with GD in these centers included limited funding, lack of expertise and interest in adult metabolic medicine in many countries and difficulty coordinating care amongst different specialties. One HCP also mentioned patient apprehension as the main challenge for transition.

In the UK, a set of general guidelines for transition from children's to adult services have been published by NICE (38). Within these guidelines, a set of quality standards have also been developed to measure progress and improve the quality-of-care providers are able to deliver. One of the overarching principles of the NICE guidelines was the encouragement of health and social care managers to work together and develop jointly agreed and shared transition protocols, information-sharing protocols and approaches to practice within the UK (38). The lack of harmonization of existing protocols, and inconsistency of outcomes and quality indicators between different countries remain a significant challenge during the transition process (43). The IWGGD have, therefore, developed transition and coordination of care guidelines to standardize care and support the transition process for patients with GD1 (52).

4.6 The multidisciplinary team

Transition of patients with GD1 should involve the relevant clinical specialties, psychologists and social workers in the multidisciplinary team and provide the education and information (e.g., leaflets or apps) necessary to support the patient's independence (Figure 3). Patients are encouraged to understand and manage their disease and acquire the skills and knowledge necessary for self-management, allowing them to act independently. They may attend transition clinics without their parents (52).

Patient organizations can play an important role in supporting the transition process by providing educational resources to help patients understand their condition, treatment, and transition itself, but also in being available for other support needs such as social issues and mental health. It has been recommended that patients are signposted to these services (41). Successful collaboration between clinicians and patient organizations, such as the IWGGD, can lead to the development of guidelines and practices that ensure patients are adequately supported during transition (52).

Well-developed transition guidelines may have potential application in other circumstances where patients need to move clinics, such as when relocating or moving country. There is also potential for new technologies such as Artificial Intelligence to facilitate transition by complementing the role of a transition

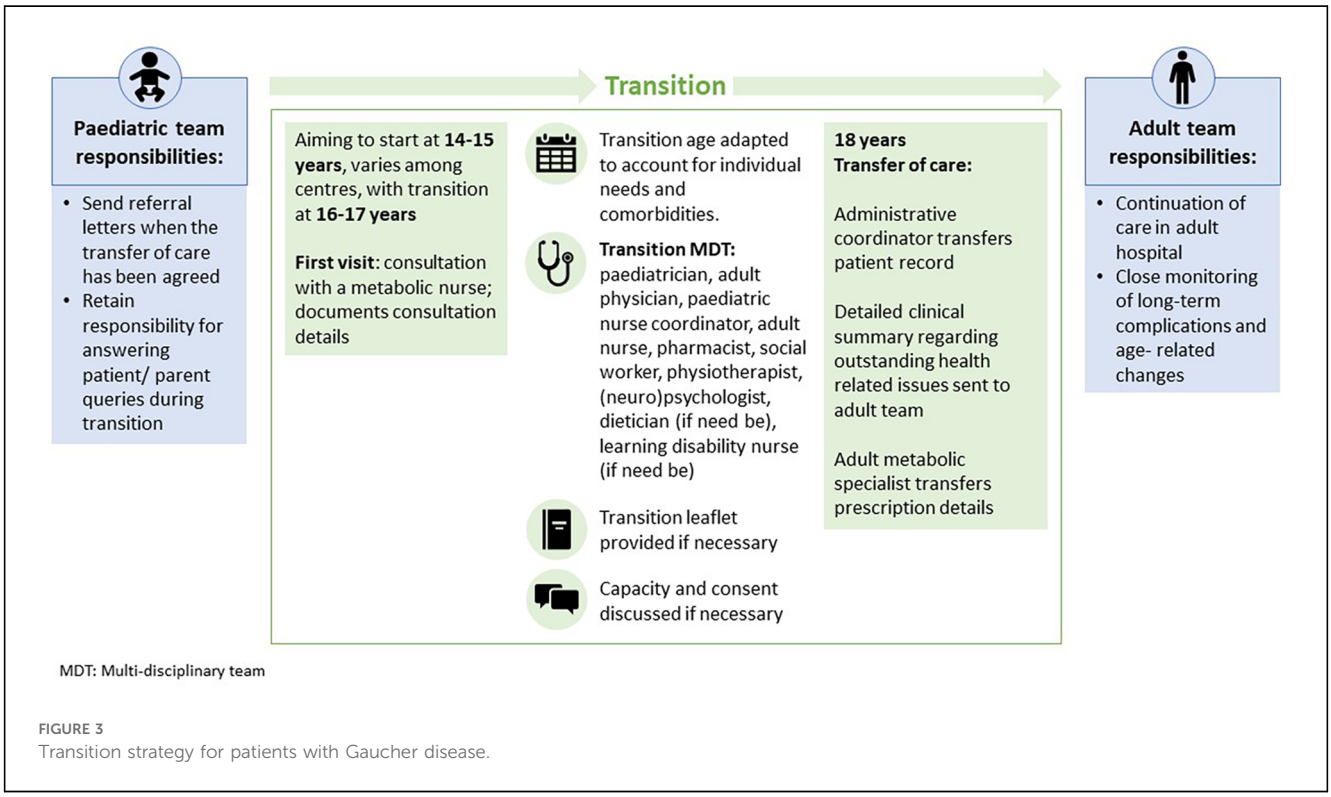

FIGURE 3
Transition strategy for patients with Gaucher disease.

coordinator; performing tasks such as scheduling, coordinating and reminding about appointments and the date/time of treatment (53).

4.7 Limitations of the study

To our knowledge, this is the first study to focus on the transition in GD1 specifically. Our study simultaneously reported the perspectives of patients and HCPs on the transition process worldwide, providing an overview of the current status of the transition process. One limitation of the study was the small number of HCPs surveyed per country. The results may not therefore provide a precise reflection of their clinical practice and we would need to survey a larger sample of HCPs to obtain a thorough picture of the status of international transition provision. To compound to this limitation, the survey was carried out in diverse regions with markedly different healthcare systems, which hinder the understanding and comparison of transition of care of patients diagnosed with GD. In addition, HCPs and patients were not matched so a direct comparison of their perspectives could not be made. Despite an equal opportunity to participate, the results of our survey might not shed a light on the transition of care across all surveyed countries. Half of the respondents (22/44) were mainly from 3 countries (Netherlands, United Kingdom, United States), while the remaining 22 respondents represented 17 surveyed countries, with 1 to 3 respondents each. Another limitation of our study is the reduced number of respondents from Israel, with only one patient and two HCPs participating in the survey. It would be relevant to have a higher participation from Israel to gain a better overview of the standard operating system in transition care in a country with a high prevalence of GD1. Finally, although our survey took place between October and December 2021, we did not assess whether any of the respondents went through the transition of care in the mist of the Covid-19 pandemic or how the disruption of global healthcare affected the transition process, particularly in GD1 centers that had to temporarily halted transition protocols involving multi-disciplinary teams.

5 Conclusions

In conclusion, the development of transitional care requires that pediatricians, adolescent medicine specialists and adult specialists work together with patients and GD association groups to manage the needs of patients with GD. An efficient transition process is essential to reduce patient fear and anxiety when moving to adult services. The results of the surveys demonstrate the variability in the transition process between countries, the lack of guidelines for a standardized process and the increasing clinical need for a harmonized transition program among pediatric and adult specialties. GD is one of the commonest rare diseases, with well understood pathophysiology and available therapies. Nevertheless, the implications of transitional care in the long-term follow-up of patients diagnosed with GD have not been previously analysed. As the complications in GD are treatable, it is important to understand the gaps in transition to empower these patients and encourage them to remain under the adult services' care. Further research, funding and education of adult physicians is required to improve patients' quality of life and indirectly their compliance to the therapies around the transfer of care.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving humans because this was a non-interventional online survey. The study was registered as a service evaluation project with Research & Innovation at Salford Royal Organization (NCA) (registration number 23HIP04). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Supervision, Visualization, Writing – review & editing. IŽ: Conceptualization, Methodology, Resources, Writing – review & editing. BK-W: Conceptualization, Methodology, Writing – review & editing. AJ: Data curation, Investigation, Software, Writing – review & editing. DC-G: Conceptualization, Methodology, Writing – review & editing. MA: Conceptualization, Methodology, Writing – review & editing. SR-V: Conceptualization, Methodology, Writing – review & editing. EL: Formal Analysis, Visualization, Writing – original draft. DH: Methodology, Writing – review & editing. UR: Conceptualization, Methodology, Writing – review & editing. TC-H: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1439236/full#supplementary-material

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The importance of ultrasound examination in care of juvenile idiopathic arthritis patients: 9 months follow-up study

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Introduction: Juvenile idiopathic arthritis (JIA) is a group of rare musculoskeletal disorders with chronic inflammation of joints, typically manifesting before the age of 16 years. The assessment of disease activity remains pivotal in JIA treatment decisions, particularly during clinical remission. While musculoskeletal ultrasound (MSUS) has shown promise in detecting subclinical synovitis, longitudinal data on MSUS features in JIA remains limited. The aim of this study was to evaluate the prevalence of subclinical synovitis observed in MSUS over a follow-up period in JIA patients. Additionally, it sought to assess the consistency and correlation between clinical findings, standardized composite clinical score (JADAS10), and MSUS-detected synovitis during 9 months follow-up.

Patients and methods: a prospective single-center study was conducted, enrolling all consecutive JIA patients (excluding systemic JIA) seen at the study center in one year period. At three-months intervals over a 9 months period (M0, M3, M6 and M9), patients underwent clinical examination, laboratory tests, and MSUS assessment. Data on demographic characteristics, disease profile, and treatment were collected. Patients were categorized into active disease (ACT) or remission (REM) groups based on Wallace criteria and JADAS10 scores using previously validated thresholds. The ultrasound assessments adhered to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) pediatric group, covering 40 joints, were performed by two ultrasonographers at every visit. Subclinical synovitis was defined as synovitis detected exclusively by MSUS. Spearman's correlation coefficients (r_s) were used to evaluate the association between MSUS, clinical data, and outcome measures, such as active joint count (ACJ), patient's/parent's global assessment of disease activity (PaGA), physician's global assessment of disease activity (PhGA) and JADAS10.

Abbreviations

ACJ, active joint count; ANA, antinuclear antibodies; AntiTNF, tumor necrosis factor inhibitors; cJADAS, clinical juvenile arthritis disease activity scale; ClinSUM, sum of all clinical signs; CRP, C-reactive protein; DA, disease activity; ESR, erythrocyte sedimentation rate; HLA B27, human leucocyte antigen B27; IQR, interquartile range; JADAS10, juvenile arthritis disease activity score 10; LOM, limited range of motion; MSUS, musculoskeletal ultrasound; MTX, methotrexate; M0, baseline visit; M3, 3months from inclusion into the study; M6, 6 months from inclusion into the study; M9, 9 months from inclusion into the study; MCP, metacarpophalangeal joints; MTP, metatarsophalangeal joints; N, number; NC, non correlated; NSAIDs, non-steroidal anti-inflammatory drugs; PaGA, patient/parent global disease activity; PhGA, physician's global disease activity; PIPs, proximal interphalangeal joints; REM, remission; RF, rheumatoid factor; SD, standard deviation; Sulfa, sulfasalazine.

Results: subclinical synovitis was evident in 5.2% of all joints and in 80.6% of the patients at baseline. During the follow-up period, signs of subclinical synovitis decreased to 3.8% of joints, however, the proportion of affected patients remained high (67.7%), with the majority in REM group. Despite the consistent strong correlation between PaGA and PhGA throughout the study ($r_s > 0.895$; $p < 0.001$), both measures displayed moderate ($r_s = 0.647$; $p < 0.001$) to weak ($r_s = 0.377$; $p = 0.04$) correlations with MSUS findings. Notably, PaGA remained significantly correlated with MSUS at the M9 visit ($r_s = 0.377$, $p = 0.04$), while PhGA showed no correlation ($p = 0.094$).

Conclusions: The study results indicate the persistence of subclinical inflammation detected by MSUS in a significant proportion of JIA patients, even during clinical remission. Moreover, the findings suggest that conventional measurements of JIA activity may be insufficient for assessing patients in clinical remission.

KEYWORDS

juvenile idiopathic arthritis, MSUS, ultrasound, imaging, children, pediatric

1 Introduction

Juvenile idiopathic arthritis (JIA) comprises a group of rare musculoskeletal disorders characterized by predominant chronic joint inflammation starting before the age of 16 (1). Recent studies have elucidated distinctions in pathogenesis, genetic predisposition and epidemiology among clinical JIA forms, such as oligoarthritis, polyarthritis, enthesitis-related arthritis, and others, varying across different regions (2–5). Assessment of disease activity remains pivotal in guiding treatment decisions for JIA. Currently, clinical assessment tools are widely utilized and validated for monitoring JIA activity in children (6, 7). Nonetheless, these tools encounter several limitations including various modifications developed for different JIA subtypes with diverse reference values for disease activity (7–10). Moreover, challenges also arise from factors such as young age (resulting in limited patient collaboration, age-related joint hypermobility, and an absence of self-reported joint pain) as well as transient childhood joint disorders (e.g., transient synovitis). Additionally, studies have revealed poor-to-moderate interrater agreement in the clinical arthritis assessment, along with various factors influencing physicians' evaluation of global JIA disease activity (11, 12). Consequently, pediatric rheumatologists are actively seeking more reliable and objective tools or biomarkers for assessing JIA activity in pediatric patients. Due to this, in recent years, musculoskeletal ultrasound (MSUS) has gained increasing interest among pediatric rheumatologists for evaluating chronic inflammation. The non-invasive, radiation-free, inexpensive, and highly patient-friendly nature of MSUS can help to delineate the extent of joint involvement in JIA.

Several previous studies on JIA patients have underscored the importance of detecting subclinical synovitis using MSUS for defining JIA subtypes and assessing active joint count (13–15). Recent efforts have focused on standardizing scanning protocols and synovitis evaluation across different joints in both JIA patients and healthy children, conducted by various study groups (16–19). However, longitudinal data on MSUS features in JIA patients throughout the disease course remain limited.

The aim of this study was to assess the frequency of subclinical synovitis observed via MSUS over a nine-month follow-up period in JIA patients. Additionally, it aimed to evaluate the consistency and correlation between clinical findings, standardized composite clinical score, and MSUS-detected during 9 months follow-up.

2 Materials and methods

2.1 Study design and study population

A prospective single-center study was conducted at the Hospital of Lithuanian University of Health Sciences enrolling all consecutive patients diagnosed with JIA (excluding systemic JIA) from January 2021 to March 2023. Inclusion criteria comprised: (1) diagnosis of JIA according to the International League of Associations for Rheumatology (ILAR) criteria (20); (2) age between 2 and 18 years. Exclusion criteria included: (1) systemic JIA form; (2) comorbid chronic diseases. Patients underwent regular follow-ups every three months for a nine-months period (M0, M3, M6 and M9), involving clinical examinations, laboratory tests, and MSUS assessments at each visit.

2.2 Data collection

Demographic data, including age and gender, disease characteristics, and treatment, were collected. Disease characteristic data included disease subtype according to ILAR categories, disease duration, presence/absence of rheumatoid factor (RF), antinuclear antibodies (ANA), and human leucocyte antigen B27 (HLA B27) results. The latest ophthalmological examination findings for signs of uveitis were reviewed. Moreover, signs of joint inflammation, such as swelling, pain, limited range of motion (LOM), and morning stiffness, were evaluated. All clinical signs were assigned a score of 0 (absent) or 1 (present) and quantified as the sum variable (ClinSUM) (total

score range 0–8) for each patient for subsequent analysis. Each variable was considered with the same weight (present or absent) trying to minimize the redundant scoring of severity (21). Clinical evaluation was performed by a pediatric rheumatologist (ASn) certified in joint examination by the Pediatric International Trials Organisation (PRINTO). JIA disease activity was assessed using the validated clinical juvenile arthritis disease activity scale of 10 joints (JADAS10) (22). Additionally, clinical outcome measures, such as active joint count (AJC), defined as a joint with presence of swelling or, if no swelling was present, of pain on motion, or limited range of motion, patient global assessment of disease activity (PaGA) using visual analogue scale of 10 (where 0 = no activity and 10 = maximum activity), and physician global assessment of disease activity (PhGA) using visual analogue scale of 10 (where 0 = no activity and 10 = maximum activity), were evaluated.

Inflammation markers including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were assessed at each visit. Data about medications used for JIA treatment were also collected.

Patients were categorized into an active disease (ACT) or remission (REM) groups based on the Wallace criteria (23) and JADAS10 scale using previously validated cutoffs (7).

2.3 Ultrasound assessment

At each visit, MSUS of 40 joints was performed by two proficient ultrasonographers: a radiologist with over 30 years of expertise in pediatric MSUS (RU), blinded to clinical examination, and a pediatric rheumatologist (ASn), who completed the European Alliance of Associations for Rheumatology (EULAR) intermediate course of pediatric MSUS and has six years of daily practice with pediatric patients. Gray scale (B-mode) and power Doppler (PD) ultrasonographic evaluations were performed using an Affinity 70G (Philips) and ACUSON Sequoia^{TM} (Siemens Healthineers) machines with a linear-transducers with frequency range from 5 to 18 Mega Hertz (MHz) for grayscale and up to 12.5 MHz for PD. A pulse repetition frequency of 500–900 MHz with a low-wall filter was used, adjusting the gain to eliminate signals on or below the bone surface. B-mode and Power Doppler images were acquired and scored from 0 to 3 for each of the 40 joints following the guidelines outlined by the Outcome Measures in Rheumatology Clinical Trials (OMERACT) group (17, 24, 25). Subclinical synovitis was defined as synovitis detected exclusively by MSUS.

In total, 1,240 joints were assessed using ultrasound every visit by two examiners. For analysis joints were grouped as follows: (1) small hand joints (SHJ), comprising MCP (metacarpophalangeal) and PIP (proximal interphalangeal) joints; (2) ankle (tibiotalar, talonavicular, subtalar joints); (3) all MTPs (metatarsophalangeal joints). Hips, knees, wrists, and elbows were rated separately.

Inter-rater reliability, particularly concerning the identification of grey scale or Doppler abnormalities, was highest in elbows, wrists and hips (kappa = 1), with the greatest disparity observed in MTP joints (kappa 0.890) (Supplementary Table S1).

2.4 Outcome and MSUS statistical analysis

Statistical analysis was performed using Microsoft Excel and IBM SPSS Statistics version 29.0 software (SPSS Inc., Chicago, IL, USA) for Windows. The Shapiro-Wilk test was used to assess the data normality. Continuous variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Qualitative data were expressed as counts and percentages (%).

The independent samples *t*-test was applied for normally distributed data to compare different follow-up visits, whereas the Wilcoxon signed ranks and Mann-Whitney *U*-tests analysed nonparametric data. Inter-rater reliability was evaluated using Cohen's kappa value, with cut-offs of below 0.20 indicating poor reliability, 0.21–0.40 indicating fair reliability, 0.41–0.60 indicating moderate reliability, 0.61–0.80 indicating good reliability, and 0.81–1 indicating excellent reliability. Spearman's correlation coefficients (r_s) assessed associations between presence/absence of subclinical synovitis signs in MSUS at the patient level, clinical data, and outcome measures. The correlation between the number of patients with clinical symptoms and the number of patients with signs of synovitis in MSUS was performed. A p-value < 0.05 was considered significant. No correlated variables were marked as NC in the tables.

2.5 Ethical consent

Permission to conduct the study was granted by Kaunas Regional Biomedical Research Ethics Committee (BE-2-100; 2020 Oct 26). Study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

3 Results

3.1 Patient characteristics and disease presentation

A total of 31 patients with JIA were included in the study, with a median age of 13.61 years (range 3–17 years). 87.1% of patients (27/31) were female. The median duration of JIA at the baseline visit was 21.6 months (min 0, max 98.7)). Almost half of the patients (45.2%) had the disease for more than one year, while only 9 patients (29%) had been ill less than 6 months (Table 1). According to ILAR JIA classification (20), 12 patients (38.7%) had oligoarthritis (extended or persistent), 10 patients (32.3%) RF negative polyarthritis, 1 patient (3.2%) RF positive polyarthritis, and 8 patients (25.8%) enthesitis-related arthritis. Antinuclear antibodies (ANA) were detected in two-thirds of the patients, and human leucocyte antigen B27 (HLA B27) in one-third of the patients (Table 1).

3.2 Clinical presentation and disease course

Regarding clinical arthritis presentation signs at the baseline visit, joint pain was reported by 67.7% of patients, while half of

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TABLE 1 Demographic and clinical features of the study cohort at baseline visit.

Characteristic		Total (n = 31)
Gender (n,%)	Female	27 (87.1)
	Male	4 (12.9)
Age at M0 in years, mean (SD)		13.61 (4.31)
Age of JIA diagnosis in years, mean (SD)		11.81 (4.74)
Disease duration in months, median (IQR)		10 (0–17.7)
Disease duration <6 months (n,%)		9 (29)
Disease duration from 6 months till 1year (n,%)		8 (25.8)
Disease duration >1 year (n,%)		14 (45.2)
Oligoarthritis (n,%)		12 (38.7)
RF negative polyarthritis (n,%)		10 (32.3)
RF positive polyarthritis (n,%)		1 (3.2)
Enthesitis related arthritis (n,%)		8 (25.8)
ANA positive (n,%)		19 (61.3)
HLA B27 positive (n,%)		10 (32.3)

ANA, antinuclear antibodies; AntiTNF, tumor necrosis factor inhibitors; HLA B27, human leucocyte antigen B27; n, number of patients; RF, rheumatoid factor; SD, standard deviation.

the children (54.8%) expressed complaints of joint swelling and LOM. Morning stiffness was present in only 6 patients (19.4%), and none of the children displayed signs of uveitis (Table 2). During the follow-up period, the majority of the symptoms resolved, with only 22.5% of patients experiencing joint pain, and joint swelling decreased to less than 20% (Table 2). Moreover, LOM and morning stiffness had completely disappeared.

3.3 Disease activity

According to JADAS10 cut-offs, half of the cohort (51.6%) was categorized having high disease activity during the baseline visit (Table 2). Only 8 patients (25.8%) achieved clinical remission according to both JADAS10 and Wallace criteria (23) for more than 6 months. Notably, patients with shorter disease duration at the M0 visit had higher disease activity (Table 3). By the M9 visit the remission group significantly increased to 80.6%, with 5 patients exhibiting low disease activity according to JADAS10, and only one patient was classified as having high JIA activity (Table 2). The median JADAS10 score between the M0 visit and M9 visit decreased from 7 to 0 ($p < 0.001$). Furthermore, the outcome measures such as PhGA and PaGA also decreased significantly during the 9 months follow-up period (Table 2).

Regarding conventional inflammation markers such as ESR and CRP, only 4 patients (12.9%) exhibited an elevation in CRP during the initial visit, which normalized in subsequent visits. There were no significant changes in ESR during all follow-up visits.

3.4 Treatment

The majority of the patients (71%) was undergoing treatment with methotrexate (MTX), 35.5% were prescribed tumor necrosis factor inhibitors (anti-TNF) at the time of inclusion in the study.

TABLE 2 Frequency of patients with pathological clinical findings, according to disease activity status, outcome measures and treatment at baseline and at the last visit.

Characteristic	M0 visit (n = 31)	M9 visit (n = 31)
Clinical features		
Joint swelling (n,%)	17 (54.8)	6 (19.4)
Joint pain (n,%)	21 (67.7)	7 (22.5)
Limited range of motion (n,%)	17 (54.8)	0
Morning stiffness (n,%)	6 (19.4)	0
Uveitis (n,%)	0	0
Disease activity (DA)		
Remission (n,%)	8 (25.8)	25 (80.6)
Low DA (n,%)	3 (9.7)	5 (16.1)
Moderate DA (n,%)	4 (12.9)	0
High DA (n,%)	16 (51.6)	1 (3.2)
JADAS10 median (IQR)	7 (1–12)	0 (0–1)*
PhGA median (IQR)	3 (1–5)	0 (0–0.5)*
PaGA median (IQR)	3 (0–5)	0 (0–1)*
ClinSUM median (IQR)	2 (0–3)	0 (0–1)
Treatment		
• None (n,%)	2 (6.7)	5 (16.1)
• NSAIDs (n,%)	13 (41.9)	1 (3.2)
• Prednisone (n,%)	1 (3.2)	0
• Intraarticular injections of steroids (n,%)	2 (6.5)	0
• MTX (n,%)	22 (71)	22 (71)
• Sulfa (n,%)	2 (6.5)	1 (3.2)
AntiTNF (n,%)	11 (35.5)	16 (51.6)
MTX + antiTNF (n,%)	7 (22.6)	12 (38.7)

AntiTNF, tumor necrosis factor inhibitors; DA, disease activity; IQR, interquartile range; JADAS10, juvenile arthritis disease activity score 10; PhGA, physician global assessment of DA; PaGA, patient/parent global assessment of DA; M0, baseline visit; M9, nine months from inclusion into the study; MTX, methotrexate; n, number; NSAIDs, non-steroidal anti-inflammatory drugs; Sulfa, sulfasalazine. Statistically significant differences between visits are marked with *. $P < 0.05$ was considered significant.

TABLE 3 Disease activity according to disease duration at baseline visit.

Total, n = 31	Disease duration		
Disease activity	Up to 6 mo	6mo—1y	More than 1y
High, n (%)	9 (29)	6 (19.4)	1 (3.2)
Moderate, n (%)	0	1 (3.2)	3 (9.7)
Low, n (%)	0	1 (3.2)	2 (6.5)
Inactive/REM, n (%)	0	0	8 (25.8)

y, years; mo, months; n, number; REM, remission.

22.6% of patients were receiving combined therapy with MTX and anti-TNF. Additionally, 41.9% of patients were using nonsteroidal anti-inflammatory drugs (NSAIDs). Despite the treatments, 74.2% of children still exhibited some degree of disease activity during the baseline visit. By the M9 visit, the prescription of anti-TNF increased to 51.6%, and NSAIDs consumption decreased to 3.2%, resulting in the significant increase of REM group (Table 2).

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3.5 Longitudinal assessment of clinical outcome measures and subclinical synovitis in MSUS

Several clinical outcome measures were evaluated at each visit for all patients. A notable positive correlation was found between the cumulative value of all clinical signs (ClinSUM) with MSUS-detected synovitis at M0, M3, and M6 visits (M0 $r_s = 0.678$; M3 $r_s = 0.736$; M6 $r_s = 0.716$; $p < 0.001$). However, at the M9 visit, when the majority of the patients were in clinical remission for more than 6 months, the correlation weakened ($r_s = 0.309$; $p < 0.097$). A similar trend was identified between JADAS10, AJC and MSUS examination results (Table 4).

Analysis of separate arthritis symptoms, such as joint pain and swelling, also resulted in significant positive correlations with MSUS in the first 3 visits (pain and MSUS: M0 $r_s = 0.757$; M3 $r_s = 0.557$; M6 $r_s = 0.559$; $p < 0.01$; swelling and MSUS: M0 $r_s = 0.477$; M3 $r_s = 0.753$; M6 $r_s = 0.667$; $p < 0.001$) and with no correlation at the M9 visit ($p = 0.453$, $p = 0.492$, respectively for pain and swelling) (Table 4). Interestingly, LOM showed no correlation with MSUS in any visit (Table 4).

Moreover, there was no association seen between MSUS and blood inflammation markers (ESR and CRP) throughout all follow-up visits (Table 4).

Despite the consistently strong correlation between PaGA and PhGA throughout the follow-up period ($r_s > 0.895$; $p < 0.001$), both outcome measures displayed moderate ($r_s = 0.647$; $p < 0.001$) to weak ($r_s = 0.377$; $p = 0.04$) correlations with MSUS (Table 4). Notably, the correlation of PaGA with MSUS remained statistically significant at the M9 visit ($r_s = 0.377$, $p = 0.04$), while PhGA showed no correlation ($p = 0.094$).

Subclinical synovitis was evident in 5.2% of all joints (Table 5) and in the majority of patients (80.6%) at the M0 visit (Table 6).

TABLE 4 Correlation of clinical symptoms, inflammation markers and patient- and physician-reported measures with MSUS synovitis.

	MSUS synovitis			
	M0 r_s	M3 r_s	M6 r_s	M9 r_s
ClinSUM	0.678*	0.736*	0.716*	0.309
Joint Pain	0.757*	0.557*	0.559*	0.142
Joint Swelling	0.477*	0.753*	0.667*	0.130
LOM	0.046	0.200	NC	NC
AJC	0.701*	0.720*	0.741*	0.386*
JADAS10	0.69*	0.617*	0.685*	0.354
ESR	−0.007	−0.199	−0.332	−0.170
CRP	0.336	0.638*	−0.119	−0.082
PhGA	0.597*	0.484*	0.647*	0.311
PaGA	0.562*	0.474*	0.588*	0.377*

The correlation between the number of patients with clinical symptoms and the number of patients with signs of synovitis in MSUS was performed. The absolute ESR and CRP values for correlation with synovitis in MSUS were used.

ACJ, active joint count; ClinSUM, sum of all clinical signs; cJADAS, clinical juvenile arthritis disease activity scale; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; LOM, limited range of motion; M0, baseline visit, M3, 3 months from inclusion into the study; M6, 6 months from inclusion into the study; M9, 9 months from inclusion into the study; NC, non correlated as there were no LOM registered for any patient; PaGA, patient/parent global disease activity; PhGA, physician's global disease activity; r_s, Pearson's correlation coefficient. $P < 0.05$ was considered significant. Statistically significant results are provided with*.

TABLE 5 Clinical and MSUS signs of inflammation in different joints at baseline visit and at the last visit.

Joints examined in total (n)	M0 visit				M9 visit			
	Joints with arthritis by physical examination only (%)	Joints with arthritis by MSUS only (%)	Joints with arthritis on both physical examination and MSUS (%)	Total joints affected (%)	Joints with arthritis by physical examination only (%)	Joints with arthritis by MSUS only (%)	Joints with arthritis on both physical examination and MSUS (%)	Total joints affected (%)
Elbows ($n = 60$)	1 (1.7)	4 (6.7)	0	5 (8.3)	0	8 (13.3)	0	8 (13.3)
Wrists ($n = 60$)	1 (1.7)	4 (6.7)	3 (5)	8 (13.3)	0	5 (8.3)	3 (5)	8 (13.3)
MCPs ($n = 300$)	1 (0.3)	4 (1.3)	1 (0.3)	6 (2)	0	4 (1.3)	0	4 (1.3)
PIPs ($n = 300$)	0	13 (4.3)	11 (3.7)	24 (8)	6 (2)	2 (0.7)	1 (0.3)	9 (3)
Hips ($n = 60$)	3 (5)	0	1 (1.7)	4 (6.7)	0	1 (1.7)	0	1 (1.7)
Knees ($n = 60$)	1 (1.7)	10 (16.7)	10 (16.7)	21 (35)	0	11 (18.3)	4 (6.7)	15 (25)
Ankles ($n = 60$)	4 (6.7)	10 (16.7)	2 (3.3)	16 (26.7)	2 (3.3)	7 (11.7)	1 (1.7)	10 (16.7)
Subtalar ($n = 60$)	0	5 (8.3)	0	5 (8.3)	0	3 (5)	0	3 (5)
MTPs ($n = 300$)	3 (1)	10 (3.3)	3 (1)	16 (5.3)	0	6 (2)	1 (0.3)	7 (2.3)
Total ($n = 1,240$)	14 (5.8)	64 (5.2)	31 (2.5)	105 (8.5)	8 (0.6)	47 (3.8)	10 (0.8)	65 (5.2)

MSUS, musculoskeletal ultrasound; MCPs, metacarpophalangeal joints; PIPs, proximal interphalangeal joints; MTPs, metatarsophalangeal joints; M0, baseline visit; M9, nine-month visit from inclusion into the study.

TABLE 6 Patient proportion in different visits according to disease activity and MSUS synovitis.

Disease activity	M0 visit MSUS synovitis, *n* (%)	M3 visit MSUS synovitis, *n* (%)	M6 visit MSUS synovitis, *n* (%)	M9 visit MSUS synovitis, *n* (%)
High	13 (41.9)	8 (25.8)	5 (16.1)	1 (3.2)
Moderate	4 (12.9)	5 (16.1)	6 (19.4)	2 (6.5)
Low	1 (3.2)	4 (12.9)	1 (3.2)	4 (12.9)
Inactive/REM	7 (22.6)	7 (22.6)	5 (16.1)	14 (45.2)
Total	25 (80.6)	24 (77.4)	17 (54.8)	21 (67.7)

MSUS, musculoskeletal ultrasound; M0, baseline visit; M3, 3 months from inclusion into the study; M6, 6 months from inclusion into the study; M9, nine-month visit from inclusion into the study.

During the follow-up period, the total number of joints with subclinical synovitis decreased to 3.8%; however, the proportion of affected patients remained high (67.7%), with the majority being in the REM group (Table 6). The joints most affected by subclinical synovitis on MSUS at the M0 visit were knees (16.7% of all knee joints) and ankles (16.7%), followed by subtalar joint (8.3%), elbows (6.7%), wrists (6.7%), proximal interphalangeal joints (PIP) (4.3%), MTPs (3.3%), and MCPs (1.3%) (Table 5). At the M9 visit, subclinical synovitis remained mostly in knees (18.3%), ankles (11.7%), elbows (13.3%), and wrists (8.3%).

Furthermore, the correlation between clinical examination and MSUS of different joints did not show consistent pattern. Correlation could not be calculated for some of the joints due to the absence of clinical signs of arthritis (Supplementary Table S2).

4 Discussion

Juvenile idiopathic arthritis (JIA), although rare, ranks among the most prevalent chronic rheumatological conditions affecting children (2, 26). Regular assessment of disease activity and potential treatment adjustments are necessary in any type of JIA. While various clinical tools are employed to evaluate JIA activity (7), an increasing body of evidence suggest implication of ultrasound (13, 27–30). In our prospective study with a 9-month follow-up, disease activity in 31 JIA patients was assessed through both clinical examination and MSUS. Several clinical outcome measures demonstrated a strong correlation with MSUS during the initial 6 months of the disease. However, at the 9-months mark, the majority of patients continued to display signs of inflammation in MSUS despite achieving clinical remission.

Subclinical inflammation signs in MSUS among JIA patients have been recognized for several years (14, 27–29, 31, 32). However, most studies have focused on newly diagnosed or short-term remission JIA cases (13, 14, 32), and the majority did not utilize validated MSUS definitions for pediatric population. In our study, we applied the OMERACT group definitions and criteria for MSUS in pediatric patients (17, 24, 25). Only a quarter of our study patients at the baseline visit could be classified as in clinical remission according to the Wallace criteria (23) and as having inactive disease according to JADAS10 cut-offs (7). Naturally, more patients with longer disease duration were classified as inactive. However, the majority still exhibited signs of synovitis in MSUS. Similar results were described in a few previous studies. In De Lucia et al.'s study, 22.7% of clinically inactive patients and 0.98% of scanned joints showed abnormal MSUS results (33). Loredo et al. observed 11.8% of JIA patients in remission displaying subclinical inflammation in MSUS over a year (34). Bugni Miotto e Silva at al. found a higher prevalence of subclinical synovitis in 36 JIA patients (median duration: 1.9 years), with 41.7% of patients and 3.1% of joints affected (29).

The relationship between clinically active JIA and inflammation signs in ultrasound is well-established. However, considerable portion of active JIA patients also demonstrated signs of subclinical synovitis in MSUS. In a recent study by Vega-Fernandez et al. up to 30% of joints of clinically active patients had signs of subclinical inflammation on MSUS (13). Several other studies reported a lower number of subclinical synovitis cases in active JIA group (32, 34, 35). The correlation of ACJ with MSUS signs of inflammation in active or newly diagnosed JIA is well-known and robust (34–36). In our patient cohort, half exhibited high disease activity according to JADAS10 cut-offs at the baseline visit, and most of them had signs of subclinical synovitis on MSUS. Moreover, inflammation signs on MSUS were observed in most patients classified as having JIA remission. Nevertheless, our study revealed a strong positive relationship between ACJ and MSUS at baseline and in earlier follow-up visits (M3 and M6). However, during the remission phase in the last visit (M9), this correlation weakened, indicating persistent sings of synovitis in MSUS. Similar findings were found in recent study by Vega-Fernandez et al. which evaluated active JIA patients after 3 months of follow-up (13).

The distribution of inflammation signs variations among the different joints has been noted in several previous multi-joint scanning studies (29, 31, 32, 35). The joints most prone to subclinical inflammation signs on MSUS are knees, ankles and wrists (29, 31, 32, 35). The same observation was made in our scanned joints complex, with knees and ankles standing out throughout all follow-up period. Moreover, the MSUS correlation with clinical signs in the knee joint was significantly moderate in baseline visit and 3 months after ($rs = 0.52$, $p = 0.003$ and $rs = 0.45$, $p = 0.012$, respectively), appending previous results by several studies (35, 37).

Clinical signs of arthritis serve as the core measures in JIA diagnosis, definition of JIA category and disease activity evaluation. From the first studies on MSUS in JIA patients, it has been noted that different clinical signs have different correlations

with MSUS. Magni-Manzoni et al. described poor correlation of MSUS with tenderness, pain on joint movement, or LOM. Only swelling showed a moderate to strong correlation with MSUS (14). In our study clinical signs like joint pain and swelling showed moderate to strong correlation with MSUS during the first 6 months of follow-up. Interestingly, we did not find any correlation of MSUS with LOM in any visit, emphasizing the importance of composite clinical evaluation of the patient.

Currently, the measurement of JIA disease activity involves several clinical outcome measures, some of which are integrated into joint tools such as various modifications of JADAS (cJADAS, JADAS10, JADAS27 etc.) (7). Several research groups have identified a significant correlation between JADAS and MSUS in active JIA patients (35). In our study, we also observed a significant positive moderate correlation between JADAS10 and inflammation signs in MSUS during the initial 6 months of the follow-up. However, no correlation was found at the M9 visit, when the majority of patients were in clinical remission, mainly due to persisting signs of synovitis in MSUS. Moreover, a recent study by Licciardi et al. described a significant correlation of JADAS27 with MSUS in the active JIA group but not in remission (35).

Conflicting results regarding the correlation between other clinical disease activity measurements, such as physician's and patient's/parent's global disease activity evaluation and MSUS have been observed in several studies. Bugni Miotto e Silva et al. analysed patients with a median remission time of 1.9 years and did not find relationship between MSUS detectable synovitis and clinical disease activity evaluation by PhGA or PaGA (29). Moreover, a recent study by Nguyen et al. underscores that clinical outcome measures do not uniformly change during the disease course (38). Despite an increased proportion of inactive JIA patients according to cJADAS and PhGA, little or no improvement was seen in PaGA of JIA activity (38). Interestingly, in our cohort of patients, the correlation between PaGA and MSUS remained positive at 9 months of follow-up despite clinical signs of inflammation disappearing, and no relationship was found between MSUS and PhGA. These findings suggest that the perception of disease activity by patients or parents may be more sensitive to subclinical inflammation within the body. A similar tendency of consistently elevated PaGA scores was evidenced in a prospective multicenter study encompassing over 1,000 JIA patients (39). Researchers identified older age and enthesitis-related JIA as potential risk factors for prolonged elevation of PaGA. Moreover, a longitudinal, population-based study conducted by Rypdal et al. delineated PaGA as a principal factor associated with higher JADAS10 values (40). Our study indicated that subclinical synovitis detected via MSUS could be one of the explanations for prolonged elevation of PaGA.

Our study has some limitations. Firstly, a small number of JIA patients was evaluated. However, given that JIA is a rare disease and considering the 9-months follow-up period, the study population is similar to the cohorts of some previous single-center studies (13, 29–31, 35, 41). A second limitation is the variety of JIA subgroups included, making the cohort less homogenous. However, previously validated clinical disease activity tool JADAS10 and separate cut-offs of this scale for oligo and polyarticular JIA were used to classify patients according to the disease activity level. This approach ensures that the full spectrum of non-systemic JIA manifestations included provides a wide view of the MSUS synovitis signs in different JIA groups in remission. Moreover, the evaluation of a variety of peripheral joints by MSUS (1,240 joints at each visit) contributes to some of the largest studies in pediatric population done so far (13, 28, 29, 33).

Strengths of this study include the evaluation of joints according to criteria tailored specifically for pediatric patients. Additionally, regular follow-up visits every 3 months throughout the 9-months period were conducted, whereas most studies only included two visits at different time points or one visit across different JIA activity groups (13, 28, 29, 34). Furthermore, the parallel evaluation of all children in all visits by two ultrasonographers on the same day in real-time adds an additional advantage in inter-rater reliability. Our study demonstrated that despite different specialties and experience in ultrasound, MSUS is a reliable imaging modality for evaluating inflammation in JIA patients when appropriate guidelines are followed.

Currently, the definition of remission or an inactive state of JIA does not include imaging results (7, 23). However, as more data emerges on the importance of MSUS, ultrasound findings should be considered alongside clinical assessments for a comprehensive evaluation of disease activity and could potentially be included as an additional criterion for defining JIA remission in the future.

In conclusion, our study indicates that despite being in clinical remission for 6 months or more, a significant number of JIA patients exhibit signs of subclinical inflammation in MSUS. The study highlights the importance of MSUS in assessing JIA patients in remission. Overall, ultrasound findings provide a valuable insight into disease activity and should be integrated into routine clinical practice for assessing and managing JIA patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Kaunas Regional Biomedical Research Ethics Committee, Lithuania. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

ASn: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing –

review & editing. AS: Data curation, Formal Analysis, Investigation, Writing – review & editing. RU: Investigation, Methodology, Writing – review & editing. RS: Supervision, Writing – review & editing. PJ: Data curation, Formal Analysis, Writing – review & editing. AB: Conceptualization, Supervision, Writing – review & editing. LJ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1414384/full#supplementary-material

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Bone mineral density determinants in adolescents and young adults with congenital adrenal hyperplasia

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Background: The effects of long-term glucocorticoid (GC) treatment on bone mineral density (BMD) in patients with congenital adrenal hyperplasia (CAH) remain controversial.
Objectives: This cross-sectional study aimed to evaluate BMD in relation to genotype, growth, vitamin D status, cumulative GC doses, and other relevant factors in youths with CAH.
Methods: Thirty-two patients with classical CAH (13 males; mean age 26.0 ± 7.1 years) were compared with 32 healthy controls matched by age and sex. BMD was measured using dual-energy x-ray absorptiometry, and statistical analyses, including the Mann–Whitney *U*-test and Spearman's correlation coefficient, were performed to evaluate differences and associations.
Results: Median whole-body and lumbar BMD *Z*-scores were similar between CAH patients and controls ($p = 0.27$ and 0.15, respectively). Low bone density was observed in 12.5% of CAH patients and 18.75% of controls ($p = 0.5$), and osteoporosis was confirmed in 12.5% of CAH patients and 0% of controls ($p = 0.04$). BMD did not correlate with cumulative GC doses, estradiol, renin, phosphate, sodium levels, or anthropometric parameters in CAH patients. There was no significant difference in BMD between severe and non-severe genotypes of CAH. However, a positive correlation was found between the whole-body BMD *Z*-score and growth velocity during infancy ($r = 0.776$, $p = 0.021$) in CAH patients. Vitamin D deficiency was noted in 56.25% of CAH patients, although vitamin D levels did not correlate with BMD or genotype. No history of bone fractures was reported among study participants.
Conclusions: CAH patients are at risk of developing osteoporosis, but in this study, BMD *Z*-scores were not associated with cumulative GC doses. The study did not identify an association between genotype and BMD. Poor growth during infancy was linked to decreased BMD in adulthood.

KEYWORDS

congenital adrenal hyperplasia, CAH, bone health, bone mineral density, transition age

1 Introduction

Osteopenia and osteoporosis are systemic skeletal diseases characterized by low bone mass, microarchitectural deterioration of bone tissue leading to increased bone fragility, and a consequent increase in fracture risk (1). Low bone density is usually the result of accelerated bone turnover due to estrogen deficiency, whereas in aging women and

men, vitamin D insufficiency and secondary hyperparathyroidism may further contribute to bone loss and are not frequent in adolescents and young adults. However, the etiology, diagnosis, and treatment of osteoporosis in adolescents and adults under 50 years of age remain poorly defined (2).

Along with heredity factors that affect genes and their polymorphisms and account for 50%–80% of the variation in bone mass and structure among individuals (3), endocrine, nutritional, and other risk factors, such as low levels of physical activity, delayed puberty or secondary amenorrhea, systemic inflammation, or long-term corticosteroid treatment, precipitate bone loss at a younger age (3).

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that results from impaired steroidogenesis in the adrenal cortex (4, 5). In 95% of cases, CAH is caused by mutation of the *CYP21A2* gene, which encodes the enzyme 21-hydroxylase (21OH) (6, 7). Owing to a deficiency in 21OH (21OHD), the synthesis of cortisol and, in severe forms, aldosterone is impaired. The treatment of CAH requires the replacement of glucocorticoid (GC) and mineralocorticoid (MC) therapy, balancing between adrenal insufficiency and androgen excess (8); therefore, in most cases, the therapeutic doses of GC are supraphysiological. Low BMD has been detected in 40%–60% of CAH cases (9–11); however, data on its association with GC type and dosage and other presumed risk factors are conflicting (12, 13).

Vitamin D deficiency is known to be associated with low bone mineral density (BMD) in the general population (14) and is common (50%–80%) in CAH patients (10, 15); however, only a few studies have reported the association of vitamin D levels with BMD in CAH patients (16). In addition, it has previously been shown that early growth patterns are predictive of BMD status in later life (17, 18). In our previous study, we demonstrated that patients with classical CAH forms have different GC dose requirements during infancy dependent on the genotype and residual 21OH secretion and hypothesized that it may affect their early postnatal growth pattern (19). Therefore, in the present study, we aimed to analyze BMD in relation to genotype, growth, vitamin D status, cumulative GC doses, and other relevant factors in adolescents and young adult patients with classical forms of CAH in comparison with healthy controls.

2 Patients and methods

2.1 Subjects

All patients older than 14 years of age in the Lithuanian database of CAH patients were invited to participate in the study conducted by the Lithuanian University of Health Sciences (Kaunas, Lithuania) from 2018 to 2022. All patients included in the study were born before the establishment of the Newborn Screening Program for CAH in Lithuania (20). We included patients with classical CAH diagnosed through genetic testing and excluded those with other endocrine disorders that might affect bone density. Thirty-two adolescents and young adults (14–37 years of age) with CAH were recruited and compared with 32 healthy control subjects rigorously matched for age, sex, pubertal stage, and ethnicity. Eight patients each in the CAH and control groups were adolescents (14–18 years old). Puberty was evaluated according to the Tanner stage (21).

2.2 Physical examination

Anthropometric measures of height (cm) and weight (kg) were obtained from all participants. Height and sitting height were measured using a Harpenden stadiometer. The height standard deviation score (Ht-SDS) was calculated according to age and gender using Lithuanian National Children Growth Evaluation Chart references for all participants under 18 years of age (22). The sitting height and height (SitHt/Ht) ratio was calculated and adjusted for age and pubertal stage. The body mass index (BMI) SDS was calculated using Lithuanian National Children Growth Evaluation Chart references (22). A BMI between 25 and 29.9 kg/m^2 (>+1 SD and <+2 SD) was classified as overweight, and a BMI of 30 kg/m^2 or more (>+2 SD) was classified as obesity. Growth velocity (cm/year) was evaluated in patients with CAH across three distinct periods from birth to final adult height: (1) Infancy—from birth to 1 year of age; (2) Childhood—from 1 year of age until the appearance of the first signs of puberty; (3) Puberty—from the onset of puberty until final adult height was reached. Medical records of growth evaluation were available for 20 CAH patients (6 males and 15 salt wasters).

2.3 Biochemical and molecular diagnosis of CAH

The diagnosis of 21OHD was confirmed through mutation analysis of the *CYP21A2* gene. All patients were genotyped for confirmation of the diagnosis. *CYP21A2* gene and *CYP21A1P* pseudogene copy number analysis was performed using quantitative multiplex ligation-dependent probe amplification (MLPA) with SALSA® MLPA® probemix P050-C1 CAH [MRC-Holland, Amsterdam, The Netherlands; reference sample—SD039-S02 Reference DNA (MRC-Holland)]. The detection of sequence changes in the *CYP21A2* gene was performed using Sanger sequencing after selective long-range PCR with primers specific for *CYP21A2* and/or *CYP21A1P* (23).

The definition of CAH forms was based on the *CYP21A2* genotype, initial and follow-up plasma renin concentrations, and electrolyte status at diagnosis: severe *CYP21A2* mutations on both alleles or complete gene deletions/conversions, significantly elevated renin concentrations, or apparent salt loss at the time of CAH diagnosis were indicative of the salt-wasting (SW) form of CAH. Patients with severe *CYP21A2* mutations on one allele and mild mutations on another allele causing mild 21OHD with normal or only slightly elevated renin concentrations and normal electrolyte levels were diagnosed with the simple virilizing (SV) form of CAH. The stratification of common *CYP21A2* pathogenic variants by residual enzyme activity was based on Krone et al. and Concolino and Costella (24, 25) and is detailed in Table 1.

TABLE 1 Grouping of common *CYP21A2* pathogenic variants by residual enzyme activity.

Enzyme activity (%)	Phenotype	*CYP21A2* pathogenic variant	Grouping of mutations according to 21OH activity
0	Severe (classic)	Whole-gene deletion Large- gene conversion p.Gly111ValfsTer21 p.[Ile237Asn; Val238Glu; Met240Lys] p.Leu308PhefsTer6 p.Gln319Ter p.Arg357Trp	Null
<1		c.293-13A>G c.293C>G	A
2–11		p.Ile173Asn	B
~20–50	Mild (non-classic)	p.Pro31Leu p.Val282Leu p.Pro454Ser	C

21OH, 21-hydroxylase.

Genotypes were classified according to residual 21OH activity (Null, A, B, and C). For further analysis, CAH patients were divided into two subgroups according to genotype: the first subgroup ($n = 17$) included patients with mutations that caused 0% or close to 0% 21OH activity (null mutations in both alleles or null mutations in one allele and an A type mutation in another allele), and the second subgroup ($n = 15$) included other combinations of mutations associated with ≥1% of the residual 21OH activity.

2.4 Therapy

GC doses were expressed as the cumulative dose per body surface (mg/m^2/day). The median dose of GC was calculated as the actual cumulative corticosteroid dose during all treatment periods. All the GC doses used were converted into hydrocortisone (HC) dose equivalents for the purpose of normalization using anti-inflammatory equivalents [20 mg of hydrocortisone = 5 mg of prednisolone (PD) = 0.75 mg of dexamethasone (DEX)] (26). Five patients (15.6%) were treated with PD and six (18.8%) with DEX; the rest were treated with HC.

Treatment efficacy was assessed by serum 17-hydroxyprogesterone (17OHP, 12–32 nmol/L) and testosterone (T; normal T levels were evaluated according to the chronological age and sex in at least two of four annual measurements) and adrenocorticotropic hormone (ACTH; normal range 1.63–14.15 pmol/L) measurements. MC replacement was monitored by blood pressure and renin concentration (normal range 1.6–14.7 ng/L) and was maintained within the upper normal limit.

The vitamin D level was not evaluated routinely for the patients before this study and one-third of the patients periodically used supplements containing vitamin D, often at a dose of 600–1,000 IU/day. Information about the use of vitamin D supplements was not collected from the controls.

One female with SW CAH had nephrocalcinosis. All the studied subjects who had normal thyroid function did not use thyroid-function-affected medication. No patient used any additional medications that affected bone mineralization or structure (e.g., aromatase inhibitors to increase adult height) (2). There was no history of bone fractures in any of the study groups.

2.5 Laboratory investigations

Fasting blood samples were taken in all study subjects before GC and fludrocortisone administrations, between 8:00 and 9:00 am, from the antecubital vein catheter for measurements of 17OHP, T, ACTH, renin, 25OH-vitamin D, parathormone (PTH), alkaline phosphatase (ALP), calcium (Ca), phosphate (P), and sodium (Na).

Vitamin D deficiency was defined as a 25OH-vitamin D concentration below 50 nmol/L (20 ng/ml) and vitamin D insufficiency was defined as 25OH-vitamin D levels between 50 and 75 nmol/L (21–29 ng/ml) (27). The normal reference range of PTH levels was defined as 1.26–6.97 pmol/L. The normal reference range for ALP varies depending on age and sex; the normal range was defined as 30–120 U/L for participants (young adults) in our study.

Hyponatremia was defined as an Na concentration below 136 mmol/L.

2.6 Bone mineral density measurement

Whole-body dual-energy x-ray absorptiometry (DXA; Hologic, Marlborough, MA, USA) was used to measure total body BMD, and lumbar vertebra (L1–L4) BMD. Bone mineral density values were used to calculate BMD *Z*-scores according to chronological age. According to the International Society for Clinical Densitometry (ISCD) 2007 Pediatric Official Positions and current literature, in children, low BMD was defined as a *Z*-score of less than −2.0 SD at the lumbar spine and/or whole body adjusted for age, gender, and body size, as appropriate (28). For adults, low BMD was defined as a *T*-score of less than −1.0 and at least −2.5 SD, and osteoporosis was defined as a *T*-score less than of −2.5 SD at the femoral neck and lumbar spine (L1–L4) according to World Health Organization criteria (29).

2.7 Biochemical and hormonal assays

Concentrations of testosterone (nmol/L; Biosource, Belgium), 17-hydroxyprogesterone (nmol/L; DIAsource, Belgium), adrenocorticotropic hormone (pmol/L; DIAsource, Belgium), dehydroepiandrosterone sulfate (DHEAS; μmol/L, Stratec biomedical systems, Germany), renin (ng/L; DIAsource, Belgium), parathormone (pmol/L; DIAsource, Belgium), alkaline phosphatase (U/L; Beckman Coulter Prague, Czech Republic),

calcium (mmol/L; Beckman Coulter, Prague, Czech Republic), phosphate (mmol/L; Beckman Coulter, Prague, Czech Republic), sodium (mmol/L; Beckman Coulter, Prague, Czech Republic), and 25OH-vitamin D (nmol/L; Tosoh Corporation, Japan) were measured using an immunoradiometric assay.

2.8 Bioethics

The study was approved by the Kaunas Regional Ethics Committee of Biomedical Research (No BE-2–29, approved 23 April 2018). All procedures were carried out with the adequate understanding and written consent of the participants. For participants below 18 years of age, written consent from their parents or caregivers was also obtained. The investigation was carried out in accordance with the Declaration of Helsinki.

2.9 Statistics

Statistical analyses were performed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA). Given the non-parametric nature of our data, several statistical tests were employed to evaluate the relationships between variables.

The Mann–Whitney *U*-test was selected for comparing two independent groups (e.g., CAH patients vs. controls and SW CAH vs. SV CAH) due to its robustness when dealing with non-normally distributed data. Unlike parametric tests such as the *t*-test, the Mann–Whitney *U*-test does not assume normality in the data, making it appropriate for the small sample sizes and skewed distributions observed in our study. This test ranks all the values from both groups together and then assesses whether the ranks differ significantly between the groups, providing a *p*-value that indicates whether there is a statistically significant difference in the central tendencies of the two groups.

Spearman's correlation coefficient was used to assess the strength and direction of association between two continuous or ordinal variables. This non-parametric measure is particularly suited for our study as it does not assume a linear relationship between variables and is less sensitive to outliers than Pearson's correlation coefficient. Spearman's correlation ranks the values of the variables and then calculates the correlation based on these ranks, providing a correlation coefficient (r) that ranges from −1 to 1. A positive value indicates a direct relationship, whereas a negative value indicates an inverse relationship. The significance of the correlation is determined by the associated p-value, with $p < 0.05$ considered statistically significant in our analyses.

Continuous variables were presented as medians with interquartile ranges (IQR) or means with standard deviations (SD) depending on their distribution. Before selecting the appropriate statistical test, the normality of the data distribution was evaluated using the Shapiro–Wilk test. Given that many variables did not follow a normal distribution, non-parametric tests were generally favored.

3 Results

Twenty (60%) patients had the SW form of CAH and 12 patients (40%) had the SV form of CAH (Figure 1). In each study group, one patient (12.5%) was in Tanner stage 3, three (37.5%) were in Tanner stage 4, and four (50%) were in Tanner stage 5.

3.1 Bone mineral density and other clinical characteristics

CAH patient and healthy control characteristics are presented in Table 2. The median of whole-body and lumbar BMD *Z*-scores did not differ between the CAH and control groups ($p = 0.27$ and 0.15, respectively), but osteoporosis (whole-body BMD *Z*-score < −2 SD) was detected only in CAH patients (12.5% vs. 0%, $p = 0.04$, respectively). The frequency of low BMD, defined as a BMD *Z*-score between −2.5 and −1 SD, was detected in 18.75% of controls and 12.5% of CAH patients

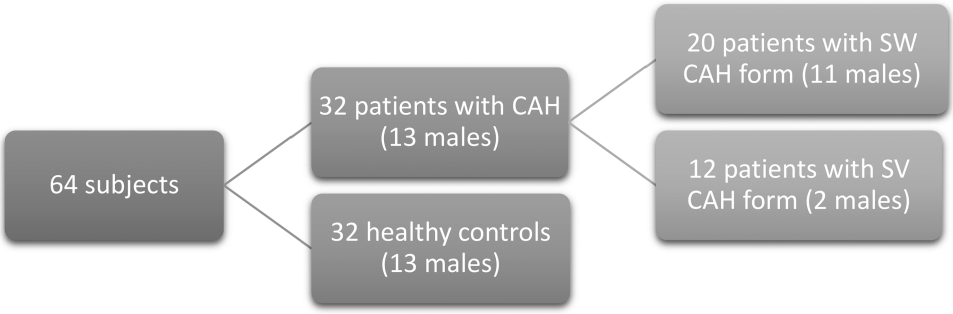

FIGURE 1
Descriptive scheme of the study subjects. The study subjects were 32 CAH patients and 32 healthy controls. CAH, congenital adrenal hyperplasia; SW CAH, salt-wasting CAH form; SV CAH, simple virilizing CAH form.

TABLE 2 Clinical characteristics of the CAH patients and matched controls.

Variables	SW males (n = 11)	SV males (n = 2)	SW females (n = 9)	SV females (n = 10)	SW (n = 20)	SV (n = 12)	All CAH (n = 32)	Controls (n = 32)
Age (years)	26.6 (16.9 to 29.2)	30.2 (28.9 to 30.45)	24.3 (20.8 to 28.4)	30.9 (20.9 to 36.5)	25.0 (17.1 to 29.2)***	30.9 (23.7 to 36.0)***	26.9 (17.9 to 31.6)	28.6 (17.1 to 31.3)
Whole-body BMD (Z-score)[a]	−0.9 (−1.45 to −0.17)*	−3.4 (−3.4 to −22.7)*	−0.95 (−1.2 to −0.45)	−0.4 (−2.2 to 0.2)	−0.95 (−1.3 to −0.32)	−0.65 (−2.35 to 0.13)	−0.9 (−1.45 to −0.15)	−0.6 (−1.3 to −0.15)
Lumbar BMD (Z-score)[a]	−0.5 (−0.8 to −0.1)	−1.75 (−2.9 to −1.75)	−0.3 (−0.8 to 0.5)	−0.35 (−1.07 to 0.3)	−0.4 (−0.8 to 0.05)	−0.55 (−1.5 to 0.1)	−0.45 (−0.9 to 0.03)	−0.4 (−0.85 to 0.1)
Median cumulative equivalent HC dose (mg/m^2/day)	16.6 (14.2 to 17.7)	13.5 (13.3 to 13.5)	16.8 (13.6 to 20.7)	14.4 (12.3 to 16.1)	16.6 (14.1 to 18.5)***	13.9 (12.6 to 15.2)***	15.4 (13.2 to 17.7)	
Median cumulative MC dose (μg/day)[a]	100 (87.5 to 125)		100 (50 to 100)		100 (50 to 125)			
BMI-SDS[b]	0.31 (−0.4 to 1.87)	2.28 (1.24 to 2.5)	1.84 (1.14 to 3.01)**	1.8 (1.7 to 2.63)**	0.93 (−0.03 to 2.17)	1.98 (1.57 to 2.82)	1.63 (0.3 to 2.4) ****	0.41 (−0.68 to 1.19)****
Ht-SDS[c]	−1.47 (−2.13 to −0.75)*	−2.11 (−2.4 to −2.11)*	−0.69 (−1.28 to 0.1)**	−1.13 (−3.29 to −1.13)**	−1.14 (−1.18 to −0.58)	−1.76 (−2.58 to −1.15)	−1.13 (−2.3 to −0.79)****	0.19 (−0.84 to 1.03)****
Vitamin D (nmol/L)	54.5 (31.4 to 80.5)	36.8 (36.8 to 38.6)	45.9 (35.5 to 76.7)	39.3 (29.0 to 59.7)	48.9 (0.41 to 0.55)	36.8 (29.1 to 56.8)	45.2 (30.6 to 70.3)	57.8 (40.9 to 69.4)
Calcium (mmol/L)	2.35 (2.19 to 2.45)	2.39 (2.39 to 2.39)	2.36 (2.23 to 2.4)	2.22 (2.17 to 2.28)	2.35 (2.2 to 2.43)	2.25 (2.2 to 2.32)	2.28 (2.2 to 2.42)	2.4 (2.32 to 2.46)
Phosphate (mmol/L)	1.1 (0.94 to 1.3)	1.25 (1.25 to 1.25)	1.12 (0.81 to 1.3)	1.12 (1.06 to 1.23)	1.1 (0.92 to 1.3)	1.17 (1.08 to 1.23)	1.15 (0.97 to 1.28)	1.04 (0.93 to 1.16)
Sodium (mmol/L)	138 (136 to 141)	136 (136 to 136)	139 (135.3 to 139)	137 (133.2 to 138.8)	138 (136 to 139)	136 (133.5 to 138.5)	138 (135.2 to 139)	139 (137 to 140)
T (nmol/L)[b]	19.1 (8.3 to 23.9)	9.7 (7.5 to 10.2)	3.78 (1.54 to 5.46)	1.74 (0.52 to 4.7)	14.4 (5.1 to 22.5)***	1.87 (0.84 to 6.48)***	7.08 (2.7 to 19.3)	1.99 (1.39 to 14.9)
17OHP (nmol/L)[b]	124.2 (20.3 to 420)	61.5 (4.1 to 70.6)	434 (258 to 472)	229 (20.7 to 410)	161 (35.2 to 520)	119 (20.6 to 329)	156 (20 to 410)	
Renin (ng/L)[b]	13.7 (4.0 to 29.4)	9.2 (4.5 to 10.2)	28.3 (7.5 to 57.8)	14.9 (9.1 to 21.8)	13.7 (5.6 to 31.4)	14.6 (9.0 to 19.8)	14.6 96.8 to 29.1)	
SHBG (nmol/L)	32.8 (25.5 to 40.6)	39.9 (27 to 39.9)	49.0 (32.5 to 74.5)	66.5 (38.7 to 179.0)	34.5 (27.3 to 47.0)	48.4 (34.5 to 127.1)	40.5 (29.3 to 49.0)	
TFM (kg)[d]	16.2 (13.9 to 18.9)*	32.4 (19.7 to 35.7)*	29.4 (18.5 to 32.4)**	25.2 (24.6 to 37.4)**	17.5 (14.6 to 23.5)***	25.2 (23.5 to 37.4)***	22.5 (16.2 to 27.1)	19.3 (16.8 to 24.7)
TFM (Z-score)[c]	0.4 (0.2 to 0.5)	1.25 (0.1 to 1.3)	0.8 (−0.2 to 0.82)**	0.3 (0.2 to 1.1)**	0.45 (0.12 to 0.8)	0.3 (0.15 to 1.2)	0.4 (0.2 to 0.8)****	0.05 (−0.5 to 0.27)****
VAT (grams)[c]	274 (252 to 359)*	750 (360 to 800)*	434 (197 to 450)**	543 (247 to 566)**	283 (249 to 387)	543 (266 to 662)	348 (252 to 501)	293 (201 to 358)
SAT (kg)[c]	16.0 (13.7 to 18.5)*	31.7 (19.3 to 32.6)*	28.9 (18.3 to 30.5)**	25.0 (24.3 to 36.6)**	17.3 (14.4 to 23.1)***	25.0 (23.3 to 36.8)***	22.3 (16.0 to 26.6)	18.9 (16.6 to 18.9)
Lean body mass (kg)[c]	43.3 (40.0 to 52.6)	56.5 (54.1 to 58.5)	41.1 (37.1 to 43.2)	41.0 (39.6 to 43.3)	43.3 (39.7 to 52.8)	43.1 (39.7 to 51.5)	43.2 (39.7 to 52.6)	44.3 (36.6 to 55.6)

CAH, congenital adrenal hyperplasia; SW, salt-wasting; SV, simple virilizing; M, males; F, females; HC, hydrocortisone; MC, mineralocorticoid; BMI-SDS, body mass index by standard deviation score; Ht-SDS, height by standard deviation score; BMD, bone mineral density; T, testosterone; 17OHP, 17-hydroxyprogesterone; TFM, total fat mass in kilograms; kg, kilograms; VAT, visceral abdominal tissue mass; SAT, subcutaneous adipose tissue; SHBG, sex hormone binding globulin.

Values are represented as median (25th–75th percentile).

*$p < 0.05$ between the SW and SV CAH males subgroups.

**$p < 0.05$ between the SW and SV CAH females subgroups.

***$p < 0.05$ between the SW and SV CAH subgroups.

****$p < 0.05$ observed between the CAH and controls subgroups.

[a]Adjusted for age, pubertal stage, body surface area.

[b]Adjusted for age, pubertal stage.

[c]Adjusted for age, gender.

[d]Adjusted for age, Ht-SDS.

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TABLE 3 Relationship between BMD and testosterone levels in the whole study group.

	Spearman's rho	*p*-value
Total BMD (g/cm^2)	0.295	0.042
Subtotal BMD (*Z*-score)[a]	0.422	0.003
Ribs BMD (*Z*-score)	0.486	0.001
Right arm BMD (g/cm^2)	0.519	0.001
Left arm BMD (g/cm^2)	0.455	0.001

[a]Subtotal BMD: total body BMD excludes head.

($p = 0.5$). CAH patients were significantly shorter than controls ($p < 0.001$), had a higher BMI-SDS ($p < 0.001$), and a higher total fat mass (TFM) *Z*-score ($p = 0.003$).

The median of vitamin D level was 45.2 (30.6–70.3) nmol/L in CAH patients and 57.8 (40.9–69.4) nmol/L in the control group ($p = 0.35$). Eighteen (56.3%) patients and 13 (40%) controls had vitamin D deficiency ($p = 0.21$). BMD Z-scores did not significantly correlate with vitamin D levels ($p > 0.05$). The median levels of PTH (2.08–5.78 pmol/L) and ALP (48–113) were within the normal range across all subject groups. Significant differences and correlations between variables were not detected between the groups. There was no history of bone fractures in CAH patients and the controls.

3.2 The relationship between bone mineral density and other clinical and genetic factors

We identified significant associations between whole-body BMD and the BMD of separate body segments with T levels in the whole study cohort (Table 3). We did not observe any significant correlations between BMD and other clinical or biochemical parameters [17OHP, T, estradiol, renin, DHEAS, sex hormone binding globulin (SHBG), vitamin D, calcium, phosphate and sodium levels, age, height and achieved final height, SitHt/Ht ratio, BMI-SDS, TFM, visceral abdominal tissue (VAT), subcutaneous adipose tissue (SAT), or lean body mass].

When analyzing the whole CAH patient group separately, we did not find significant associations between BMD and others variables (17OHP, T, estradiol, renin, DHEAS, SHBG, vitamin D, calcium, phosphate and sodium levels, age, height and achieved final height, SitHt/Ht ratio, BMI-SDS, TFM, VAT, SAT, or lean body mass). The final height of patients with SW and SV CAH was lower than controls. Patients did not achieve their target height.

Analyzing CAH patient subgroups separately, total BMD (g/cm^2) was significantly related to SAT mass ($r = 0.773$, $p = 0.005$) in males with the SW form, although the median of SAT mass was higher in the SV group than in the SW group [31.7 (19.3–32.6) kg vs. 16.0 (13.7–18.5) kg ($p = 0.013$), respectively].

In males with CAH, whole-body BMD *Z*-score and total BMD in g/cm^2 were directly associated with 17OHP concentration [$r = 0.74$ ($p = 0.006$) and $r = 0.59$ ($p = 0.026$), respectively]; total BMD in g/cm^2 was also significantly related to DHEAS levels ($r = 0.695$, $p = 0.012$), and the lumbar BMD *Z*-score was inversely related to SHBG levels ($r = -0.669$, $p = 0.017$). In females with the SW form of CAH, the lumbar BMD *Z*-score was inversely related to calcium levels ($r = -0.9$, $p = 0.037$).

Patients with the SW form of CAH were treated with significantly higher median cumulative equivalent HC doses than patients with the SV form of CAH [16.6 (14.1–18.5) and 13.9 (12.6–15.2) mg/m^2/day, respectively, $p < 0.05$] (Table 2), but cumulative equivalent HC doses were not related to BMD.

The correlation between genotype and expected phenotype and the positive predictive value of this cohort have been described previously (19). No significant differences were found in the BMD *Z*-scores or BMD (g/cm^2), but a significantly higher vitamin D concentration was identified in the Null/Null and Null/A group compared with the other group with the mildest genotype [64.6 (25.2–107) vs. 38.0 (21.7–70.3), respectively, $p = 0.003$]. We did not find any differences in the other analyzed clinical and biochemical parameters when comparing CAH patients carrying Null/Null and Null/A mutations with other genotypes.

We analyzed the association of BMD and linear growth in different periods of postnatal growth in CAH patients. A positive strong correlation was found between the whole-body BMD *Z*-score and growth velocity (cm/years) in infancy (from birth until the first year of life) ($r = 0.776$, $p = 0.021$) (Figure 2). No significant correlations were identified between BMD and growth velocity during childhood (from 1 year until puberty onset) or puberty (from the first signs of puberty until the achieved final height).

The sodium level for CAH patients was within target limits throughout the whole treatment period. Chronic hyponatremia was not observed in any patient. The median sodium concentration during the whole treatment period was 137 (136–138) mmol/L and did not differ between the CAH subgroups ($p = 0.53$). Sodium concentrations at the cross-sectional study visit, sodium concentrations at diagnosis, and sodium concentrations throughout the whole treatment period were not associated with BMD *Z*-scores and GC and MC doses.

4 Discussion

We identified osteoporosis in 12.5% of CAH patients, which was significantly more frequent than in healthy controls. In our study, the prevalence of osteoporosis was one of the lowest compared with other studies on CAH patients, in which the prevalence varied between 13% and 52% (29–34). Of note, only a few studies evaluated BMD in young CAH patients with the SW form of the disease (30–32, 34). One of the largest studies in the USA reported normal BMD in the majority of children with CAH (10).

Some studies have indicated that GC therapy is a possible contributor to decreased BMD and osteoporosis in CAH patients (4, 15, 33). However, in our study, BMD was not associated with cumulative doses of GC. A systematic review and meta-analysis of BMD in adults with CAH presented analyses of studies and the majority of them did not observe correlations between GC

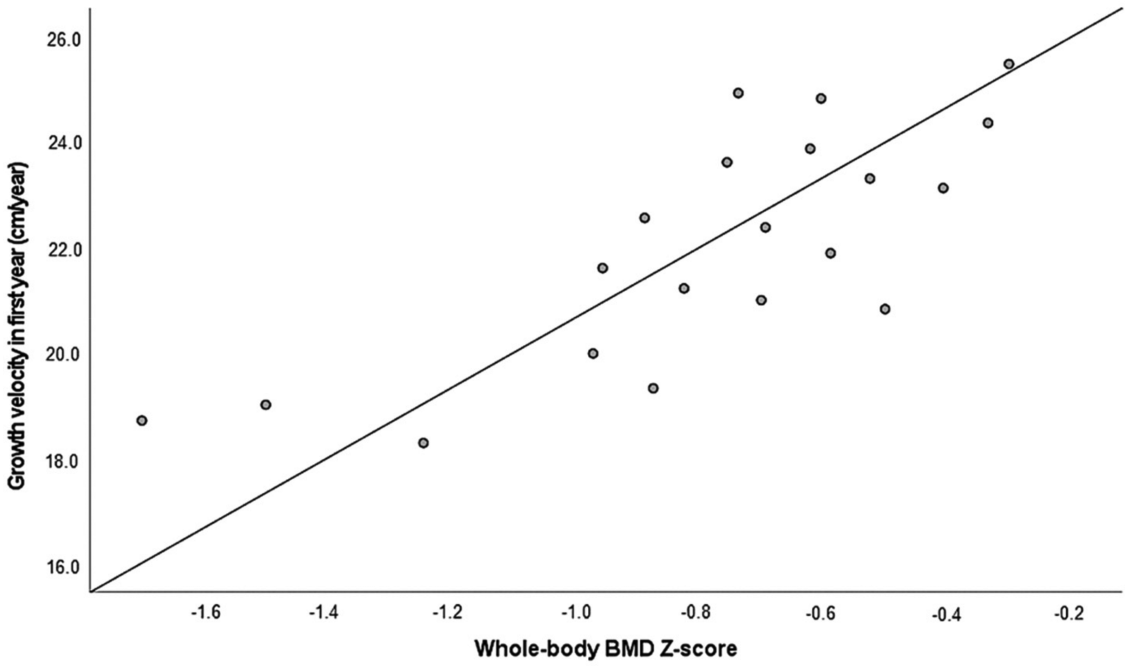

FIGURE 2
The whole-body BMD *Z*-score in association with growth velocity during the first year of life ($r = 0.776$, $p = 0.021$, $n = 20$). BMD, bone mineral density.

doses and BMD, regardless of the high variation in HC equivalent doses (9.66–22 mg/m^2/day) (35).

Similarly, in a study by Finkielstain et al., adult patients with low BMD (a *Z*-score ≤ 2) did not differ from patients with normal BMD in terms of age, sex, CAH phenotype, or GC doses; only 17OHP was lower ($p = 0.007$) in patients with low BMD (10).

Based on the relationship of gonadal and adrenal androgens with osteoblasts as stimulators of proliferation and differentiation in both males and females (36), we aimed to analyze this association in our study. We determined the correlation between T and BMD in all subjects (patients and controls) and a strong positive correlation between DHEAS and BMD in the male CAH group (37). Some studies found an association between the failure to have a physiological increase in DHEAS levels during adrenarche and low BMD (38). This relationship is based on the result of a GC overdose, which affected growth and osteoblastic function in these patients and might be the cause of low BMD.

In addition, recent research has focused on the role of adrenal androgens, particularly DHEAS, in bone health. Our study found a significant association between DHEAS levels and BMD in male CAH patients, consistent with recent findings that suggest adrenal androgens may play a role in bone density through their effects on osteoblast function. However, the complex interplay between GC therapy, androgen levels, and bone health suggests the need for further research to better understand these relationships and develop targeted interventions to preserve bone health in CAH patients (39).

In our study, we analyzed the relationship between BMD and growth in various periods of life. We determined a strong positive association between whole-body BMD and growth velocity during infancy. This finding confirmed the conclusion of previous studies that poor growth during early life is linked to a number of adverse health outcomes in adulthood, including decreased adult bone mass (17, 40).

In recent years, the impact of GC therapy on BMD in patients with CAH has been extensively studied, yet the results remain inconsistent. Although some studies suggest a negative impact of higher cumulative GC doses on BMD, others have not found a significant correlation. A recent meta-analysis by Falhammar et al. in (41) found that the relationship between GC doses and BMD in CAH patients is variable, with individual responses likely influenced by factors such as genotype, adherence to therapy, and early-life growth patterns (39).

Emerging evidence indicates that early-life growth is a critical determinant of BMD in adulthood. Several studies have shown that suboptimal growth during infancy, often due to adrenal crises or inadequate early GC therapy, is associated with lower BMD in later life. This association underscores the importance of optimizing early growth to prevent long-term skeletal complications. Our study aligns with these findings, as we observed a strong positive correlation between growth velocity during the first year of life and whole-body BMD in adulthood, highlighting the need for personalized GC dosing strategies during infancy to mitigate future risks (42).

Sweden's study analyzed a high number of patients with 21OHD and presented data showing a high incidence of fractures. Significantly higher frequencies of fractures were determined in patients born before the introduction of neonatal screening but not in those born afterward, which presumes the

association between late diagnosed CAH and a higher GC dose in infancy due to developed adrenal crisis. It could support our determined correlation between BMD and growth velocity during the first year of life. However, in the same study, the highest prevalence of fractures was observed in the SV phenotype and I172N genotype, whereas the I2 splice (determinant SW form) genotype did not show an increased prevalence (42), the delayed diagnosis of the SV form later in childhood and lower BMD in adulthood were not related to the reduced growth velocity during the early years.

In conclusion, our study provides valuable insights into the factors influencing bone mineral density in adolescents and young adults with congenital adrenal hyperplasia. Although no significant correlation was found between cumulative glucocorticoid doses and BMD, our findings highlight the importance of early-life growth patterns in determining adult bone health. The positive association between growth velocity during infancy and BMD underscores the need for optimized CAH management from early life to prevent long-term skeletal complications.

Furthermore, the observed relationship between DHEAS levels and BMD in male patients suggests that adrenal androgens may play a role in bone health, warranting further investigation. These findings have important clinical implications, suggesting that more personalized approaches to GC therapy, considering both genetic and early growth factors, may be beneficial in preserving bone health in CAH patients.

Future research should focus on identifying specific factors that contribute to the variability in bone health outcomes among CAH patients and explore potential strategies to mitigate the adverse effects of chronic GC therapy on bone. This will be crucial in improving the overall quality of life for individuals with CAH as they transition into adulthood.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Kaunas Regional Ethics Committee of Biomedical Research (No BE-2-29, approved 23 April 2018). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

RN: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AV: Formal Analysis, Investigation, Writing – review & editing. RV: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Clinical characteristics and treatment efficacy in patients with primary severe IGF-1 deficiency treated with recombinant IGF-1

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Aim of the study: To evaluate the clinical characteristics and treatment efficacy of patients with severe primary IGF-1 deficiency (PSIGFD) using a recombinant IGF-1 (rhIGF-1).
Objectives of the study: To examine the clinical characteristics of patients with PSIGFD before starting treatment with a rIGF-1. To assess the height changes in patients with PSIGFD, before and after treatment with a rhIGF-1. To analyze the clinical characteristics, side effect frequency, and treatment efficacy with a rhIGF-1 analog in patients with PSIGFD.
Methods: A retrospective analysis was conducted on patients with PSIGFD treated with the rhIGF-1 (mecasermin). Data were collected from patients' medical records, focusing on the impact of treatment on their growth and monitoring any side effects.
Results: The study showed that treatment with rhIGF-1 positively affects growth rate, especially in the first years of treatment. However, the growth rate decreases over time. The change in height from the beginning to the end of the treatment was 0.76 ± 0.64 SD, with the first quartile at 0.29 SD and the third quartile at 1.14 SD. During the treatment period, patients' average body mass increased by 0.37 ± 1.35 SD, with the first quartile at −0.33 SD and the third quartile at 0.92 SD. Side effects occurred in 50% of patients, with 40% of patients treated with rhIGF-1 experiencing hypoglycemia during treatment.
Conclusions: Treatment with rhIGF-1 is effective in treating patients with PSIGFD, causing significant improvement in growth, but requires continuous monitoring and treatment adjustment.

KEYWORDS

primary severe IGF-1 deficiency, short stature, recombinant IGF-1 analog, mecasermin, side effect

Introduction

Insensitivity to growth hormone (GH), also known as primary insulin-like growth factor 1 (IGF-1) deficiency, is a rare pathological condition that causes significant growth disorders in children, leading to physical and psychological disabilities (1–3). IGF-1 (also known as somatomedin C) is a hormone (a protein composed of 70 amino acids) with a structure very similar to insulin. Its production in the liver is stimulated by GH. IGF-1 promotes systemic body growth and has a growth-promoting effect on

almost all body cells, especially skeletal muscles, cartilage, bones, liver, kidneys, nerves, skin, hematopoietic, and lung cells (4, 5).

The diagnosis of primary IGF-1 deficiency (PIGFD) encompasses a wide range of disorders resulting from molecular defects in the GH-IGF-1 axis. These defects can be related to genes encoding proteins that regulate GH binding or signal transmission, as well as IGF-1 synthesis, transport, or action. The location of the defect is associated with different phenotypic manifestations and a spectrum of biochemical anomalies (1, 6–8). The prototype of GH insensitivity, Laron syndrome, usually occurs due to defects in the GH receptor gene or the IGF-1 gene (9). Individuals with this syndrome are characterized by extremely short stature, normal or increased GH secretion, very low IGF-1 concentration, and low IGF-1 response to GH (1, 10).

PIGFD is usually diagnosed in childhood when growth retardation is noticed, and it requires prompt and effective treatment to improve growth and prevent metabolic complications. Recombinant IGF-1 (*mecasermin*) therapy has been shown to be an effective treatment for this condition, improving growth and metabolic parameters. At the start of treatment, a rapid and significant acceleration in growth rate is observed, but the long-term effect of the treatment and individual patient response can vary. In clinical practice, the effectiveness of *mecasermin* treatment is assessed based on changes in height and weight, as well as monitoring for side effects. One of the most common adverse effects is hypoglycemia, which is related to the impact of IGF-1 on glucose metabolism. Therefore, it is especially important to regularly monitor patients' glucose levels and adjust to each patient's individual reactions during treatment (6, 11).

However, there is no gold standard or unified clinical criteria for diagnosing primary severe IGF-1 deficiency, which complicates the management and treatment of this condition. This study aims to present cohort data and contribute to the limited global database on the outcomes of recombinant IGF-1 (rIGF-1) treatment. By providing detailed clinical characteristics and treatment outcomes, this research seeks to enhance the understanding of rIGF-1 therapy's efficacy and safety, and address the variability in clinical practice.

Methods

Study design

A retrospective study was conducted on patients with severe primary IGF-1 deficiency treated with rIGF-1 (*mecasermin*) at the Endocrinology Clinic of LSMUL Kaunas Clinics from 2017 to 2024. However, the search for patients meeting SPIGFD criteria was applied from 2012. Patients were diagnosed with severe primary IGF-1 deficiency if their height was extremely low, defined as less than −3.0 standard deviations (SD) from the mean for their sex and age, and their IGF-1 concentration was below the 2.5th percentile (or <−2 SD) for their sex and age, while their growth hormone (GH) concentration was normal (confirmed by GH stimulation tests). Normal GH concentration is considered then GH peak is ≥10 ng/ml during GH stimulation tests. An IGF-1 generation test was performed on all patients to evaluate GH sensitivity. The standard test involved a four-day procedure (Figure 1) (12, 13). Baseline IGF-1 concentration was determined, and then daily rhGH was injected subcutaneously in the evening for four consecutive days at a daily dose of 33 µg/kg body weight. The IGF-1 response was assessed by measuring IGF-1 concentration the next morning at 9 a.m., 12–15 h after the fourth rhGH injection. An increase of IGF-1 concentration less than 50% confirmed the diagnosis of severe primary IGF-1 deficiency. Data from a total of 10 patients (7 boys and 3 girls) were selected and analysed (Figure 2). Anthropometric, hormonal, and metabolic indicators were evaluated.

Data collection

The following data were collected from the outpatient health records of the patients: parents' heights, gestational age in weeks,

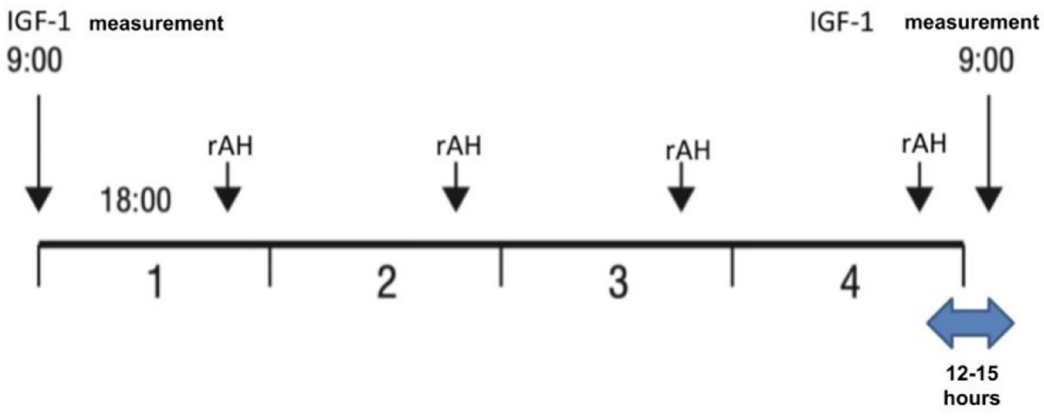

FIGURE 1
Performance of the IGF-1 generation test.

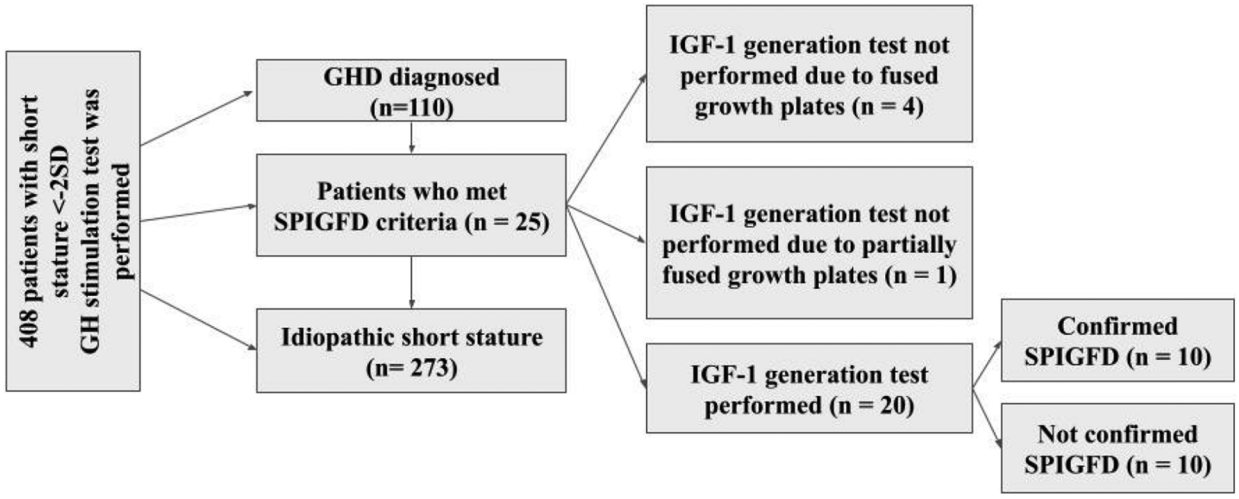

FIGURE 2
Selection of study patients from 2012 to 2023 years. GHD, growth hormone deficiency; SPIGFD, severe primary IGF-1 deficiency.

birth weight/height, height/weight at the start of rhIGF-1 treatment, height/weight at each visit until the end of rhIGF-1 treatment, bone age (BA) assessed before the treatment and on the most recent x-ray of the non-dominant hand, and body mass index (BMI) calculated at each visit. The standard deviations for these measurements according to age and sex were derived based on the National Growth Charts (0–18 years) (14). The mean treatment duration was 4.44 years, with a range of 2.49–6.91 years (5th–95th percentile).

General data of study subjects

The average age at the start of rhIGF-1 (mecasermin) treatment was 9.66 years (boys - 9.68 years, girls - 9.63 years); the youngest patient was 3.24 years old, and the oldest was 12.86 years old. The average height SDS (standard deviation score) at the start of treatment was −3.69 SDS. At the beginning of treatment, nine patients were at Tanner stage 1 of sexual development, and one patient was at Tanner stage 2. The characteristics of the study group are presented in Table 1. The initial dose of mecasermin was 40 μg/kg twice daily, which is the recommended starting dose. Subsequently, the dosage was gradually increased according to recommendations, with the highest dose used being 120 μg/kg body weight twice daily. All children were given the same adherence recommendations as provided by the drug supplier. The treatment monitoring was applied uniformly. Each patient was provided with a personal glucometer (Accu-Check Performa) for self-monitoring of blood glucose (SMBG). They were instructed to check their blood glucose levels 2 h after the rhIGF-1 injection and whenever they experienced symptoms indicative of hypoglycemia, such as sweating, dizziness, or

TABLE 1 Characteristics of study patients before starting rIGF-1 treatment.

Characteristic	Mean (n = 10)	Min	1st Quartile	Median	3rd Quartile	Max
Height (SDS)	−3.69	−5.48	−4.17	−3.40	−3.06	−2.93
Weight (SDS)	−3.30	−5.37	−4.02	−3.46	−2.60	−1.53
BMI (SDS)	−1.11	−2.29	−1.90	−1.18	−0.51	0.28
GH peak (mU/L)	39.32	23.3	32.6	33.3	51.5	55.9
Basal IGF-1 (nmol/L)	12.84	8.3	10.9	12.2	12.2	20.7
Prolactin (mU/L)	219.2	116	239	247	250	272
Fasting glucose (mmol/L)	4.40	3.56	3.84	4.32	4.59	4.86
Fasting insulin (mU/L)	5.37	3	5.1	5.1	5.3	6
HOMA-IR	1.05	0.47	0.87	1.02	1.04	1.3
ALP (U/L)	150.5	127	148	159	168	174
Cholesterol (mmol/L)	4.41	3.63	3.81	4.81	4.87	5.4
HDL (mmol/L)	1.42	1.15	1.22	1.37	1.41	1.91
LDL (mmol/L)	2.69	1.9	2.25	3.28	3.33	3.46
Triglycerides (mmol/L)	0.69	0.37	0.38	0.67	0.76	0.96

SDS, standard deviation score; BMI, body mass index; GH, growth hormone; HOMA-IR: homeostatic model assessment for insulin resistance; ALP, alkaline phosphatase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

weakness. Hypoglycemia was defined as any occurrence of typical clinical symptoms, confirmed by SMBG or laboratory-tested venous blood with glucose levels below 3.9 mmol/L. Severe hypoglycemic episodes were classified as those with glucose levels below 2.8 mmol/L, requiring hospitalization or assistance from another person. These readings were recorded in patient diaries and reviewed during follow-up visits. Follow-up visits were conducted every three months. During each follow-up visit, IGF-1 and fasting glucose concentrations were assessed. Every six months, thyroid-stimulating hormone (TSH), free thyroxine (FT4), and serum electrolyte concentrations were evaluated. Every 12 months, a cardiologist consultation with echocardiography and an otolaryngologist consultation with audiometry were performed to monitor for potential lymphoid organ hypertrophy due to rhIGF-1 treatment, and bone age (BA) was assessed.

Statistical analysis

Descriptive and analytical statistical methods were used. To compare the means of the same quantitative variable in two groups, when the distribution of the variable met the normal distribution, the Student's *t*-test was used. For comparing two paired samples that did not meet the normality condition, the non-parametric Wilcoxon signed-rank test was employed.

The data for parametric variables are presented as mean and standard deviation (SD), while the data for non-parametric variables (since the sample size is <30) are presented as median with the 1st and 3rd quartiles. To evaluate the association between qualitative variables, the chi-square (χ^2) test was applied. Differences were considered statistically significant when $p < 0.05$. The frequency and type of side effects were reported as percentages.

Bioethics

The study was approved by Kaunas Regional Ethics Committee of Biomedical Research (No BEC-MF-207, approved 23 February 2023). All procedures were carried out with adequate understanding and written consent of the participants and their parents or caregivers were obtained as well. The investigation was carried out in accordance with the Declaration of Helsinki.

Results

Growth outcomes

The height of the entire study group improved with mecasermin treatment. The average height before treatment was -3.69 ± 0.82 SDS. Upon completion of treatment or at the last visit for patients still undergoing treatment, the average height increased to -2.93 ± 1.26 SDS, $p < 0.01$. The average height SDS increase for all patients was 0.76 ± 0.64 (1st quartile 0.29 SDS, 3rd quartile 1.14 SDS) (Figure 3).

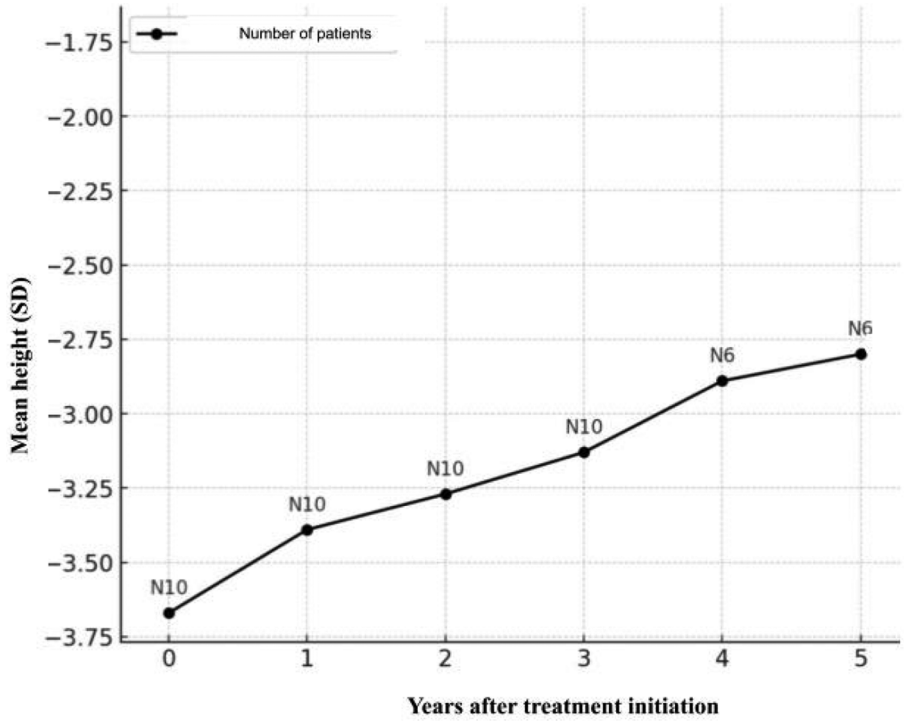

FIGURE 3
Average change in height (SD) per year from the start of rhIGF-1 treatment.

Patient No. 4 was confirmed to have a Noonan syndrome-like disorder with a pathogenic mutation in the *SHOC2* gene, and patient No. 8 was confirmed to have mitochondrial disease (a possibly pathogenic variant m.3761C>A in the MT-ND1 gene causing mitochondrial complex I deficiency). To compare if these genotypes resulted in a poorer response to treatment, the height SDS change for patients 4 and 8 was calculated to be 0.10 ± 0.21, while the average change in the other patient group was 0.88 ± 0.64. This shows a statistically significant difference in response to treatment between these two patients and the other group members, as confirmed by *t*-test results ($p = 0.03$).

Figure 4 is presented to visually show the individual growth trajectories of each patient from the start of treatment, highlighting the varied response to recombinant IGF-1 and emphasizing the importance of individualized treatment adjustments based on specific patient outcomes.

Weight outcomes

During the treatment period, the average body mass SDS of the patients increased by 0.37 ± 1.35 (1st quartile −0.33 SDS, 3rd quartile 0.92 SDS). This indicates that, on average, body mass SDS increased, but there were significant differences in BMI among the patients. The change in body mass SDS varied greatly between individual patients: some patients' body mass SDS increased, while others decreased. For example, one patient's body weight SDS increased by 0.66 over 1,197 treatment days, while another patient's body weight SDS decreased by −1.02 over 793 days. This suggests that the observed average change in body mass SDS could have occurred by chance, and no statistically significant relationship between treatment duration and change in body mass SDS was observed (Figure 5).

Side effects

Side effects of the treatment occurred in five patients (50%). Among the patients who experienced hypoglycemia, three (30%) had mild episodes characterized by weakness, which were promptly corrected with fast-acting carbohydrates. For one of them, hypoglycemia episodes were associated with physical exercise. However, one patient had a single hypoglycemic episode, classified as severe due to a glucose level below 2.8 mmol/L, but the patient only exhibited mild symptoms such as weakness and sweating. This episode was detected during a routine check, and the patient was hospitalized for monitoring, though no further complications occurred. All patients experienced only a single episode of hypoglycemia throughout the study. One patient (10%) had hyperlipodystrophic changes at the injection sites, and the same patient experienced lip and facial swelling as well as headaches. Hypertrophy of the lymphoid tissue of the pharyngeal tonsils occurred in one patient (10%). One patient (10%) had episodes of hyperglycemia up to 10 mmol/L, but no other symptoms of diabetes were observed. One female patient developed hirsutism (Farriman-Gallway score – 10) (15) after six

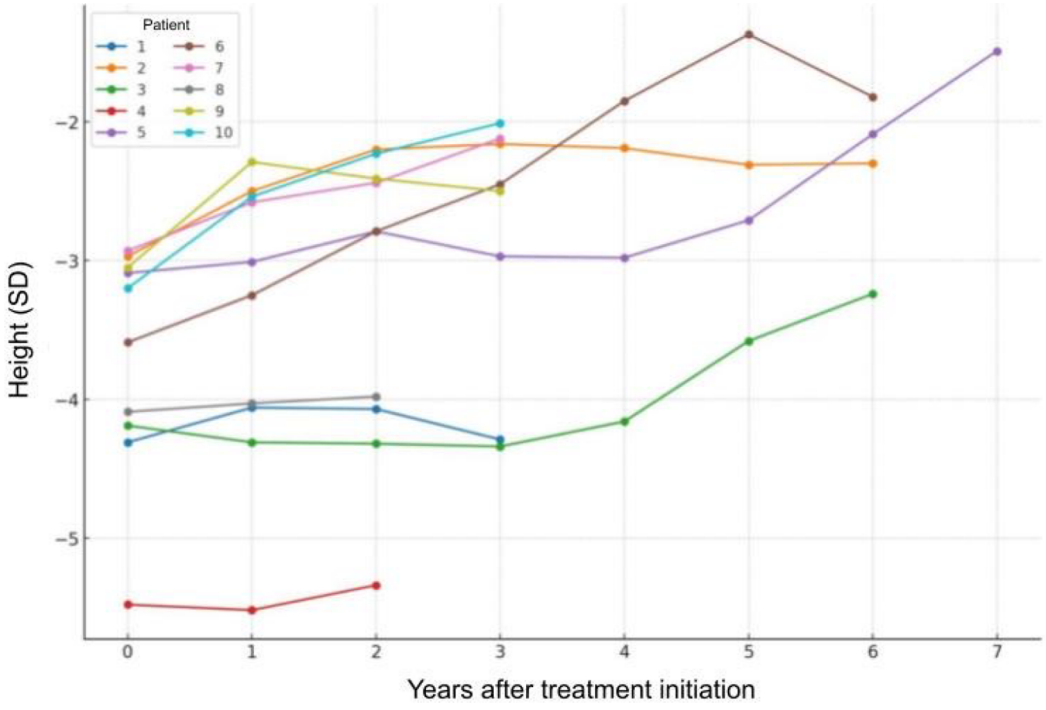

FIGURE 4
Individual growth trajectories of each patient from the start of rhIGF-1 treatment.

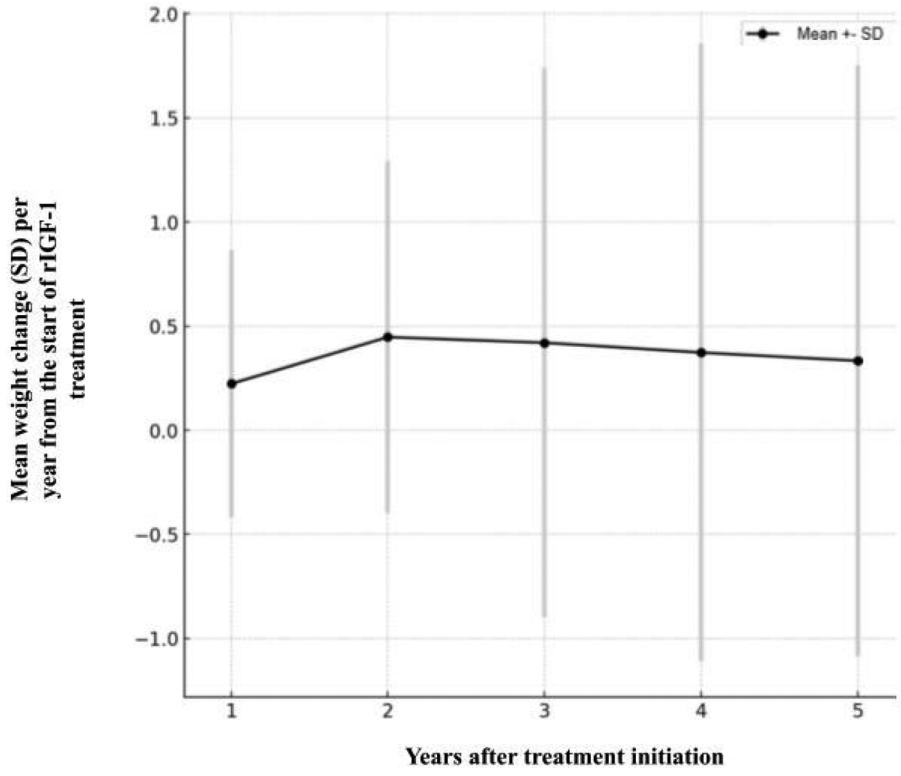

FIGURE 5
Average change in body mass (SD) per year from the start of rIGF treatment.

months of treatment. However, the relationship between this symptom and the treatment remained unclear.

Discussion

Our study demonstrated a consistent improvement in height SD among the treated patient group. After one year of treatment with mecasermin, the average height SD in our study group increased from −3.69 to −2.93, showing significant improvement compared to the baseline. This result is similar to those obtained in other studies, although some studies show greater variability. For instance, a study conducted in the USA (6) found that the average height change at the final analysis stage was +1.9 SD, ranging from +0.1 to +4.7 SD. In this study, nine patients improved their height by ≥ +2.0 SD, while twelve patients achieved only up to +1.7 SDS height increase, indicating an individual response to rhIGF-1 treatment (6, 11, 13).

A study conducted in Poland (12) shows that after 36 months of mecasermin treatment, the average height SD improved from -3.52 ± 0.82 to -2.25 ± 0.91, indicating a significant increase in height SD during the treatment period ($p < 0.01$). The average height gain was 1.45 ± 1.06 SD, demonstrating a stable and long-term treatment effect.

The results of the European Increlex® Growth Forum database also provide important insights. The main findings of the study show that treatment significantly improved height SD until the pubertal period, compared to the initial measurement. For example, among patients who reached the end of puberty, the average height SD increased from −3.7 to −2.6 in boys and from −3.1 to −2.3 in girls. This study showed a delay of about 1.5 years despite continuous rhIGF-1 treatment, and the growth spurt rate during puberty occurred later and was slightly lower than that of healthy control group children (16).

In five clinical studies with rhIGF-1 (Increlex®), significant height improvement was observed in children with SPIGFT. The initial height SDS of these patients was -6.9 ± 1.8. After the first year of mecasermin treatment, the height SDS improved to -6.1 ± 1.8, and continued to improve in subsequent years, reaching -5.6 ± 1.7, -5.3 ± 1.7, -5.1 ± 1.7, -5.0 ± 1.7, and finally stabilized around -4.9 ± 1.6. These data confirm the long-term efficacy of mecasermin therapy, consistently improving height indices over the treatment period. These results show that mecasermin effectively improves height SD in the long term, especially for patients who start treatment early in childhood (11).

The review of these studies shows that early and continuous mecasermin treatment can be very effective in improving height SD in children with SPIGFT, although treatment outcomes can vary greatly depending on the patient's baseline condition and the timing of treatment initiation (1, 6, 12).

The individual variability in treatment outcomes observed in our study indicates that genetic, clinical, and demographic factors significantly influence patients' response to mecasermin treatment. The impact of genotypes on therapeutic response was

particularly important. During our study, patients with Noonan-like syndrome (*SHOC2* gene mutation), mitochondrial pathology, and *EXT2* gene variant showed distinctive treatment responses. For example, patients with Noonan syndrome (*SHOC2* mutation) had a lower growth rate increase with rhIGF-1 treatment compared to other subjects with standard GH receptor gene deletions. Studies on patients with Noonan syndrome, who have pathogenic mutations affecting the RAS/MAPK pathway, have shown that genetic differences significantly influence treatment outcomes, confirming the impact of genotype on therapeutic response (17, 18).

Additionally, the pathogenic variant of the *EXT2* gene, associated with hereditary multiple osteochondromas syndrome, led to unpredictable growth changes despite the applied therapy (19). Moreover, mitochondrial pathology, which improves due to IGF-1 treatment, affected overall body energy production and metabolism, potentially modifying and reducing the effectiveness of mecasermin treatment (19). Such genetic features not only explain the clinical response variability to treatment but also highlight the need to individualize treatment strategies based on each patient's genotype. These insights emphasize the importance of personalized medicine, indicating that future clinical practices should apply individualized treatment strategies tailored to specific genetic profiles and associated pathologies (20–23).

IGF-1 (mecasermin) and GH treatments both enhance growth in children with deficiencies, but they operate through different mechanisms and have distinct efficacy and side effect profiles. IGF-1 directly supplements IGF-1 levels, bypassing GH stimulation, and is particularly effective for those with GH insensitivity or receptor mutations. In contrast, GH therapy boosts growth by increasing IGF-1 levels and is effective for a wider range of growth issues, including GH deficiency and idiopathic short stature, while also improving body composition and metabolic profiles (23).

Studies show that IGF-1 treatment initially results in significant growth, averaging 8.0–8.5 cm annually in the first year, but its effectiveness declines in subsequent years, often dropping below 6.0 cm in the second year. In comparison, children with GH deficiency can achieve growth rates of 9–14 cm per year with GH therapy. Despite these benefits, GH can cause side effects like edema and insulin resistance, while IGF-1 may lead to hypoglycemia. Long-term rhIGF-1 therapy is limited in achieving normal height in severe cases due to its negative impact on endogenous GH levels. These findings suggest that combined therapies with both rhIGF-1 and GH may improve growth outcomes more effectively than monotherapy, emphasizing the need for personalized treatment strategies based on individual genetic factors and responses (23).

Hypoglycemia is one of the most commonly reported adverse events associated with rhIGF-1 therapy. In our study, hypoglycemia was observed in several patients, with most episodes being mild and manageable with fast-acting carbohydrates. These findings align with previous research, such as data from the European Increlex® Growth Forum Database (Eu-IGFD), which also highlighted hypoglycemia as a frequent adverse event, particularly in younger patients or those with a prior history of hypoglycemia. Given that continuous glucose monitoring (CGM) was not utilized in this study, some hypoglycemic episodes, especially asymptomatic ones, may have gone undetected. Future studies should consider incorporating CGM to provide a more comprehensive assessment of glycemic fluctuations in children undergoing rhIGF-1 therapy. Additionally, based on our findings, it is advisable for clinicians to evaluate the sufficiency of current glucose monitoring protocols, particularly during periods of increased physical activity or illness. The occurrence of hypoglycemia underscores the importance of careful glucose monitoring and patient education to reduce risks during rhIGF-1 therapy. While most hypoglycemic events are mild, the potential for severe episodes necessitates vigilance, particularly in patients with additional risk factors such as Laron syndrome (24).

Conclusions

This study involving patients with SPIGF-1 deficiency treated with rhIGF-1 analog demonstrated that rhIGF-1 is an effective growth-promoting agent, especially in the first year of treatment. However, the long-term perspective shows a decrease in growth rate, necessitating continuous monitoring and treatment adjustments.

The study revealed significant aspects related to treatment initiation and side effects. Side effects like hypoglycemia required adjustments in treatment strategies, considering patients' age and prior health conditions. Optimal treatment efficacy is achieved through comprehensive patient monitoring and individualized dosing, emphasizing a personalized approach based on specific health characteristics and treatment response dynamics.

Further research with larger patient samples and longer follow-up is recommended to optimize treatment protocols and ensure maximum benefit. This thesis contributes to the scientific advancement in treating rare growth disorders and highlights the importance of improving clinical protocols for safety and efficacy.

Study limitations

This study has several limitations. SPIGFD is very rare condition and due to the low prevalence of the disease, the patient cohort was relatively small. This limited sample size may affect the generalisability of the study findings. Moreover, genetic analysis was not performed for all patients in our patients' group. The absence of genetic data may have impacted the ability to predict outcomes of targeted treatment. In addition, glycemia was not consistently observed using a continuous glucose monitoring (CGM) system. Utilizing CGM could have provided more detailed and accurate data on glycemic fluctuations and potential hypoglycemic episodes, leading to a better understanding of the side effects associated with the treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Kaunas Regional Ethics Committee of Biomedical Research. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

DD: Writing – review & editing, Writing – original draft, Visualization, Software, Formal Analysis, Data curation. RN: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Data curation, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Partnership of I-ACT for children (US) and European pediatric clinical trial networks to facilitate pediatric clinical trials

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Background/aims: Due to a lack of standard pediatric prescribing information, medicines are often used in a dosage form or for an indication that has not been investigated in children. Pediatric clinical trial research networks aim to facilitate the timely availability of innovative drugs for children by developing standardized trial facilitation and conduct processes. This paper aims to assess the (pre)feasibility duration and characteristics of a US-sponsored clinical trial, in collaboration with I-ACT for Children, for distribution across European sites via European clinical research facilitation networks.
Method: A transatlantic partnership between the Belgian Pediatric Clinical Research Network (BPCRN,) and I-ACT for Children conducted feasibilities in Europe for industry-sponsored early-stage pharmacological clinical trials between 2019 and 2022. The collaboration recorded time to event for key elements of feasibility, influences on successful feasibility, and benefits of collaboration.
Results: Trials were conducted across 17 European countries with 202 participating hospital sites. The initial phase, the pre-feasibility questionnaire had a 70% response rate from 142 sites, and sites took a median 38 days (IQR 20 days) to complete the questionnaire for five trials. All responses underwent a quality control, addressing inaccuracies in site capabilities and recruitment. The first trial's CDA and feasibility questionnaire were completed in roughly 2 months for 7 countries. Time to completion was affected by precontracted sites, limited scope of studies, changes in timelines, COVID-related disruptions, and a learning curve for collaboration.
Conclusion: Collaboration between European collaborative national networks and US-network I-ACT for Children has supported site identification of global pediatric clinical trials. This illustrates one method for the importance of early engagement with sponsors and implementation of effective communication systems.

KEYWORDS

pediatric, drug development, networks, metrics, global

Abbreviations
BPCRN, Belgian Pediatric Clinical Research Network; c4c, conect4children; CDA, confidentiality disclosure agreement; CRO, Clinical Research Organization; CT, clinical trial; CTU, clinical trial unit; EES, early engagement survey; EMA, European Medicines Agency; FDA, Food and Drug Administration; FQ, feasibility questionnaire; IMI, innovative medicines initiative; PI, Principal Investigator.

1 Introduction

Globally, due to a lack of standard pediatric prescribing information, clinicians often use medicines in a dosage form or for an indication that has not been adequately investigated in children (1). During the past 20 years, regulators have acknowledged the importance of studying drug safety and efficacy in a pediatric population through the Best Pharmaceuticals for Children (BCPA, US), the Pediatric Equity Act (PREA, US) and the EU Pediatric Regulation in 2007 (2–4).These legislations have had notable successes, resulting in more than 700 changes in US Food and Administration (FDA) product labels as well as doubling the amount of pediatric clinical trials being conducted in Europe (5–7). Notwithstanding these successes, many development-driven pediatric clinical trials in the last decade failed to achieve their intended goals on time (3). Although there are several factors that contributed to this outcome, a significant reason was the inability to recruit a sufficient number of patients within the required time frame to achieve meaningful results to require labelling (8, 9). Since the need for pediatric trials will be increasing further, especially in the case of pediatric rare diseases, the challenges will only become greater (10, 11).

To address the challenges inherent in conducting pediatric clinical trials, international networks can play a pivotal role by establishing and implementing a standardized process to support trial conduct through an overarching central network point and operations of a network on national or site level. This standardized approach would enhance efficiency by reducing administrative burdens and expediting trial timelines. Furthermore, these networks have the potential to optimize study design by providing input that sets realistic expectations for site and patient recruitment and ensures more successful and meaningful outcomes in pediatric clinical research. Networks can cover scattered geographical areas and group knowledge of jurisdictional as well as practical barriers (12–15). Overcoming these barriers is key to global interoperability focusing on intent-to-label trials and ultimately accessibility to adequate drugs for all children (16).

An established disease agnostic pediatric clinical trial research network is the Network for Advanced Clinical Trials (I-ACT) for Children, founded in 2017 in the US. The network was established through a grant from the FDA, memberships by biopharmaceutical companies, and philanthropy. The goal of the network is to facilitate the timely availability of innovative drugs for children, through a network of sites as well as expert facilitation regarding innovative methodologies in pediatrics. Examples include Bayesian statistics, adaptive trial designs, master protocols, decentralized trials, amongst others (14).

In response to gaps identified in the 5 and 10 year reviews of the EMA regulation, a multi-country pediatric clinical trial research network was setup with support from the Innovative Medicines Initiative (IMI2) public-private consortium, conect4children (c4c, www.conect4children.org) founded in 2018 in Europe (17, 18). The research network is focused on four main areas of services: strategic feasibility expert advice, an academy for education and training, support for managing data, as well as a network of over 200 sites following harmonized procedures coordinated through 20 National Hubs hosted by pediatric clinical research national networks and a central Network Infrastructure Office. The time limitation for the consortium extends until 2025, encompassing the 21 European beneficiaries and the 10 EFPIA partners involved in activities outlined within the grant agreement (No 777389). Engagement of c4c with other international networks has been ongoing from its initial stages, but operational collaboration is limited during the project (17, 18). A new legal entity, the conect4children Stichting has been established to ensure sustainability of this project's results, services and activities. A Mordor Intelligence Report, published in 2023, state that US continues to have the largest pediatric clinical output globally, followed by Europe. Both regions' output is expected to further increase, with a total compound annual growth rate of 14.5% in the coming five years (17).

Pediatric clinical trials must adhere to regulatory requirements, which differ between jurisdictions. Conducting separate trials in each continent is expensive and not always feasible. Connecting pediatric clinical trial networks across continents could improve efficiency by sharing resources and data while meeting quality standards, ensuring consistency and reliability of trial results. To explore the value of collaborating across multiple jurisdictions, a partnership was formed between I-ACT for Children, based in the United States, and European pediatric clinical research national networks that also collaborated in the conec4children project. The Belgian Pediatric Clinical Research Network (BPCRN) served as a contact person transatlantically with I-ACT for Children from 2018 to 2022, as an intermediary with the 20 other national collaborative networks as a proof-of-concept. This collaboration is unique in that it represents the first transatlantic partnership focused on clinical trials for both common and rare pediatric diseases.

This paper has three aims:

1. To provide an overview of the partnership between I-ACT for Children and the European pediatric national networks in c4c mediated by BPCRN.
2. To describe the partnership's experience of conducting early engagement surveys (EES) and site feasibility questionnaires (FQ) for pediatric clinical trials sponsored by industry
3. To provide a case study of collaboration between c4c and I-ACT for Children.

2 Methods

2.1 Partnership I-ACT for children and the European research networks

I-ACT for Children is a non-profit organization based in Maryland, USA, that operates as a neutral and independent entity. The organization has established site networks across multiple regions, including Australia, Latin America, Saudi Arabia, and a Canadian Network affiliation.

The Belgian Clinical Trial Network within the Belgian Pediatric Clinical Research Network the BPRCN, is based in Ghent, Belgium.

The conect4children (c4c) consortium was under the IMI2 grant restricted to conduct proof-of-viability studies from a handful of selected EFPIA partners and limited to 7 trials over these partners until April 2024. The sponsors that I-ACT is hired for, are mostly US-based industry partners that did not submit a trial request within the grant. Therefore, BPCRN, one of c4c connected networks, had to function as a contact person between I-ACT for Children and the other c4c-connected networks to facilitate pediatric clinical trials in Europe. These other c4c-connected networks include is the collaborative pediatric national networks of Italy (INCIPIT), The Netherlands (PEDMED-NL), Switzerland (SwissPedNet), Portugal (Stand4Kids), Estonia (ELAV), Austria (OKIDS), Belgium [Belgian Pediatric Clinical Research Network (BPCRN)], Greece (HELPNET), Poland (POLPEDNET), Norway (NORPEDMED), Germany (GermanNetPaet), Ireland (IN4KIDS), Sweden (SWEDPEDMED), Spain (RECLIP), Hungary (MCRN-Hungary), Finland (FINPEDMED), France (PEDSTART), Czech Republic (CzechPharmNet), Denmark (DANPEDMED), and has knowledge of submission for the UK. All the involved collaborative networks have been involved within Networking for National Networks (NNN) that could be used to communicate for I-ACT for Children studies conducted in Europe. More information and corresponding geographical figure of the collaborative pediatric national networks can be found on https://conect4children.org/national-hubs/.

All c4c networks have activities other than trial facilitations of intent-to-label trials, such as experts' advice and trainings, which will not be included within the scope of this paper. Both European Research Networks as well as I-ACT for Children focus on human studies within neonates, children and adolescents.

The Partnership's formation and development are described qualitatively. The nature and the content of each network, and their joint work were discussed during regular meetings with the coordinating stakeholders (BPCRN, I-ACT for Children, respective industry sponsors) during 2020–2022. To foster alignment of the I-ACT for Children and European pediatric clinical research national networks, an initial consultation was organized to familiarize both networks with leadership and operationally involved staff. This process included conducting virtual meetings with representatives from both networks to pinpoint their main challenges and needs. After these discussions, there was a phase of gathering data from the meetings, which led to the creation of collaboration conduct standardization as well as five trial projects to test the partnerships.

The five trials are from US-based sponsors that I-ACT for Children has been contracted to facilitate site identification and post-side identification optimalisation (site communications or recruitment optimalisations). All trials were rare disease indications. This study focuses on the metrics of the European facilitation by BPCRN together with the other European research networks, and does not include the parallel US-based facilitation performed by I-ACT for Children. However, we must note that US facilitation had the same milestones for completion as the European facilitation, and therefore follow a similar trend. Additionally, the questionnaires used in the five studies had a varying length, where early engagement or pre-feasibilities entailed 8–10 questions and feasibility questionnaires (requiring full protocol access) 10–24 questions. Both types of questionnaires were used for sponsors to be able to select sites for their trial. The focus of trial facilitation being on the pre-feasibility questionnaire without confidentiality disclosure agreement (CDA) and/or feasibility questionnaire (FQ) with CDA was at the liberty of the sponsor, which is a multifactorial decision (budget, experience with trial facilitation networks, initial outreach and timelines, complexity of the trial). The services of a trial facilitation network can also include input on how to improve site communication and recruitment in a third phase, shown qualitatively in Table 1 and quantitatively in Table 2.

Structured sponsor discussions, combined with insights from I-ACT for Children and European network feedback, have outlined trial stages, activities, and network roles. The multistakeholder feedback was collected during 2020–2022 and summarized according to relevance by both networks for a wider audience within Figure 2 and Table 3.

2.2 Experience with site identification

The experience of site identification is described both quantitively and qualitatively. A quantitively study was conducted on five pediatric industry trials that were supported by European pediatric collaborative clinical trial networks as subcontractors of I-ACT for Children, with the Belgian Network leading the effort from 2019 to 2022. The process of identifying the site was initiated based on the results of the EES and the completion of the FQ This step in identifying the site was part of the milestone-driven approach adopted by I-ACT for Children.

The process of tracking internal time was organized around two specific milestones. The data, which quantifies the duration of these milestones, is expressed in calendar days to facilitate comparison with other research networks. This data includes median and interquartile ranges (IQR) where more than 1 type of the trial was conducted. The first milestone is initial site outreach and conducting pre-feasibilities or EES. The second milestone is the conduct of CDA, FQ and where requested a summary per site. This milestone's duration was measured during a specific trial and is presented as the number of days required for completion. Since the CDA completion times were not tracked separately for each phase, the report provides a range of days spanning from the earliest CDA completion to the latest. Additionally, the report includes a total count of sites involved.

For countries participating in more than one trial, a correction was made to remove duplicate entries, ensuring that only unique countries are included in the results. However, the analysis does not cover a comparison between master CDAs and CDAs based on industry templates.

Both surveys (EES and FQ) were collected through REDCap (version 10 or higher, Vanderbilt University, Tennessee, USA). Quality control review was conducted within REDCap or MS Excel (version 2020 or higher). Quality summary reports were constructed in Microsoft Word (Microsoft office 2021) through a template developed by I-ACT for Children. Time estimations of summary reports were not standardly collected and were estimated based on calendar logging of the tasks.

TABLE 1 Services offered in the transatlantic partnership between BPCRN and I-ACT for children to industry sponsors, including the activities and roles of the network.

Stage of the clinical trial	Activity	Role of the network
Early Outreach	- Identifying countries with capable sites in the therapeutic domain and estimating the number of sites at the national level.	- Evaluating site accuracy and capability in real-time by country managers.
Early Engagement Survey (EES)	- Collecting preliminary information from site champions or established single points of contact - Examples include recruitment estimates, non-confidential protocol requirements, timelines, and direct contact information.	- Reviewing necessary questions and adjusting for national differences, facilitating troubleshooting directly in the national language. - When responses received, the responses are checked for accuracy and completed when missing data. - A comparison is made nationally or globally for realistic representation and a summary of the findings with advantages and disadvantages per site is provided.
Confidential Disclosure Agreement	- Securing confidentiality before sharing the protocol, either through a master-agreement or the template of each industry partner.	- Performing a first review of the agreement, adjusting to necessary national or site-level changes, and sending it to accurate and updated contacts at site level. - Performing a final review of accuracy before sending to the sponsor's contracting services.
Feasibility Questionnaire (FQ)	- Completing a lengthier questionnaire detailing infrastructure requirements, submission and start-up timelines, screening rates and failures, and multidisciplinary review sections.	- Reviewing of the questionnaire (for National adaptations) before dissemination. - Making detailed summary reports per site as well as recommendation of improvement points during the site initiation visit (SIV). - Sharing information directly to the sponsor, in either report style or full presentation, aside from in real-time updates of the trial progression.
Handover of sites to the Clinical Research Manager of the Sponsor	- Selecting, backing up, or declining a site, and handing it over to the sponsor's clinical research manager for validation and preparation for site opening.	- The network shares the information directly and provides a smooth handover so that each site is faced with only one trial study point of contact per phase.
Trial Start-up and Facilitations	- Overcoming secondary hurdles, such as unresponsiveness, submission delays, and recruitment difficulties during trial commencement.	- Supporting trial progress as a standby facilitator to both the site and sponsor due to personal peer-to-peer connections with the site.
Trial Communications	- Evaluating trial progress and providing updates to the global sponsor team, I-ACT for Children project manager, and respective subcontractor network leads.	- Sharing updates in real-time according to the sponsor's preferences, with a maximum of one working day delay.

The qualitative analysis examined internal communications to identify key differences and obstacles encountered across different continents. The selection of these data points was based on their relevance to the study's objectives and the feedback from both networks in regard to the trial facilitation [including the (pre) feasibility services and post site selection services]. Subsequently, detailed descriptions were formulated, which captured the essence of each facilitation activity and its outcomes in the trials. These descriptions were derived from direct observations, internal communications with sponsor, site and the research networks, and post-milestone feedback loops within the trial process. The descriptions were captured after every meeting and collected in the Tables 1, 3. Each entry in the Tables 1–3 was then cross-referenced with the primary data sources to ensure accuracy and completeness. The finalized tables provided a structured overview, categorized per facilitation types with applicable trials and their corresponding descriptions. The data was captured for Europe alone.

3 Results

3.1 The partnership

In 2019, the first steps were undertaken to standardize the collaboration. In agreement with c4c, the Belgian Pediatric Clinical Research Network was approved as the liaison between I-ACT for Children and the collaborative pediatric national networks. A confidential disclosure agreement was executed between both networks. The standardization of the collaboration, and the practical conduct of the trial facilitation is shown in Figure 1.

The activities of the network Partnership are summarized in Table 1.

Figure 2 summarizes the analysis of both the advantages and pitfalls of collaborating at the start of the partnership. The analysis revealed that one main advantage of collaboration is having a central point of contact for the global industry team, which can utilize the established infrastructure and expertise in each country. Consequently, the central point of contact of each respective network can share local knowledge and strategic advice with ease during the early stages of the outreach process. Pilot partnership enhance international collaboration, coupled with insights from PPP collaborations. Nevertheless, the analysis also identified some main pitfalls, including the three-year trial performance timeframe and the need to incorporate the learning curve of the collaboration within this period.

I-ACT for Children's infrastructure includes master CDA with their sites, which expedite the process for FQ requests. Since the master CDAs are pre-executed, they allow immediate progress to FQs needing protocol details without administrative delays. Traditionally, master CDAs were established between the site and

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TABLE 2 Metrics of 5 facilitated industry-sponsored trials through the European collaborative networks within Europe.

Trial	Therapeutic area	Timeframe	Number of countries involved within Europe	Number of Early Outreach European sites	EES completion (sites/%)[a]	EES completion in days [median, (IQR)]	CDA	FQ	Feasibility completion in days	Example of Trial Facilitation areas after feasibility
1	Neurology	May to September 2020	7	42	**29 (69%)**	30	**25**	**25**	**54**	Trial start-up and site communications.
2	Dermatology	March to May 2021	7	30	**25 (83%)**	35	/	/	/	UK-platform submission and national/site comparison upon collection.
3	Infectiology	July to August 2022	4	64	**40 (63%)**	56	/	/	/	Approaching sites outside of the established network.
4	Cardiology	March to November 2022	12	56	**42 (75%)**	42	/	/	/	Revisiting declined sites by the sponsor.
5	Neurology	August 2022	2	10	**6 (60%-**	28	/	/	/	Investigating duration of conflicting trial per site.
All			**32**	**202**	**142 (70%)**	38 (IQR 20)				

EES, early engagement survey; CDA, confidentiality disclosure agreement; FQ, feasibility questionnaire.
[a]including data cleaning, approaching incomplete surveys and summarizing findings.
Bold is the EES timelines, the most relevant metric of the table.

the Sponsor. Moving forward, I-ACT for Children will implement Master Site Agreements with site, sponsor and network, aiming to transcend the Master CDAs in upcoming collaborations. Within the European c4c consortium, a standardized CDA or cascading CDA has also been developed but due to grant constraints, meaning developed material that was being tested in the proof-of-viability separate studies within c4c could not be used within national network separate partnerships. Both networks work with a single point of contact at site level, also known as a site champion, who serves as the main contact person and advocates for trial facilitation through research networks. The site champion also keeps the national network hub updated on the site's progress and any challenges encountered.

In the EU, clinical trial sites are often categorized into university hospitals and regional hospitals, with considerable variation in size among them. It's crucial for sponsors to clearly communicate the nature and classification of these sites to facilitate successful trial management. However, this distinction is not always indicative of simplicity in contracting processes. In the US, although many university hospitals own smaller peripheral centers, leading to an assumption of streamlined contracting, yet the arrangement can complicate the process. When smaller centers have the autonomy to operate independently, they often achieve much faster contracting times. The primary advantage of such a system lies in the extensive referral network it provides, rather than simplification of contractual agreements.

3.2 Metrics of feasibility assessment

Five pediatric clinical trial studies were supported through this partnership, encompassing neurology (2/5), cardiology (1/5), infectiology (1/5), and dermatology (1/5) therapeutic areas. All trials involved rare diseases, defined as affecting less than 1 in 2,000 people, and included early outreach and engagement activities, with two trials featuring trial facilitation and summary of site advantages, difficulties and network recommendation, and one trial involving CDA and FQ completion.

The trials were conducted across 17 different European countries, involving a total of 202 hospital departments as trial sites. If not accounting for multiple trials within the same country, the total count would be 32 countries. The first phase of site identification, the early engagement questionnaire, pre-feasibility or EES, had a response rate of 70% of 142 sites. The completion of the EES questionnaire for the five trials had a median of 38 days (IQR 20 days). A quality check within RedCap was completed for all site responses, and corrections were principal investigator (PI) experience reported, inaccuracies of site capabilities based on previous experience working with the department/hospital site, as well as recruitment capabilities. For the first trial, the CDA and FQ process was conducted, with a completion within 54 days or roughly 2 months for 7 countries. One of the main delaying factors was the CDA completion, varying between sites from 1 day to 30 days.

Geographical area and activity metrics are included in Table 2. Case-examples of trial facilitation per activity are summarized in

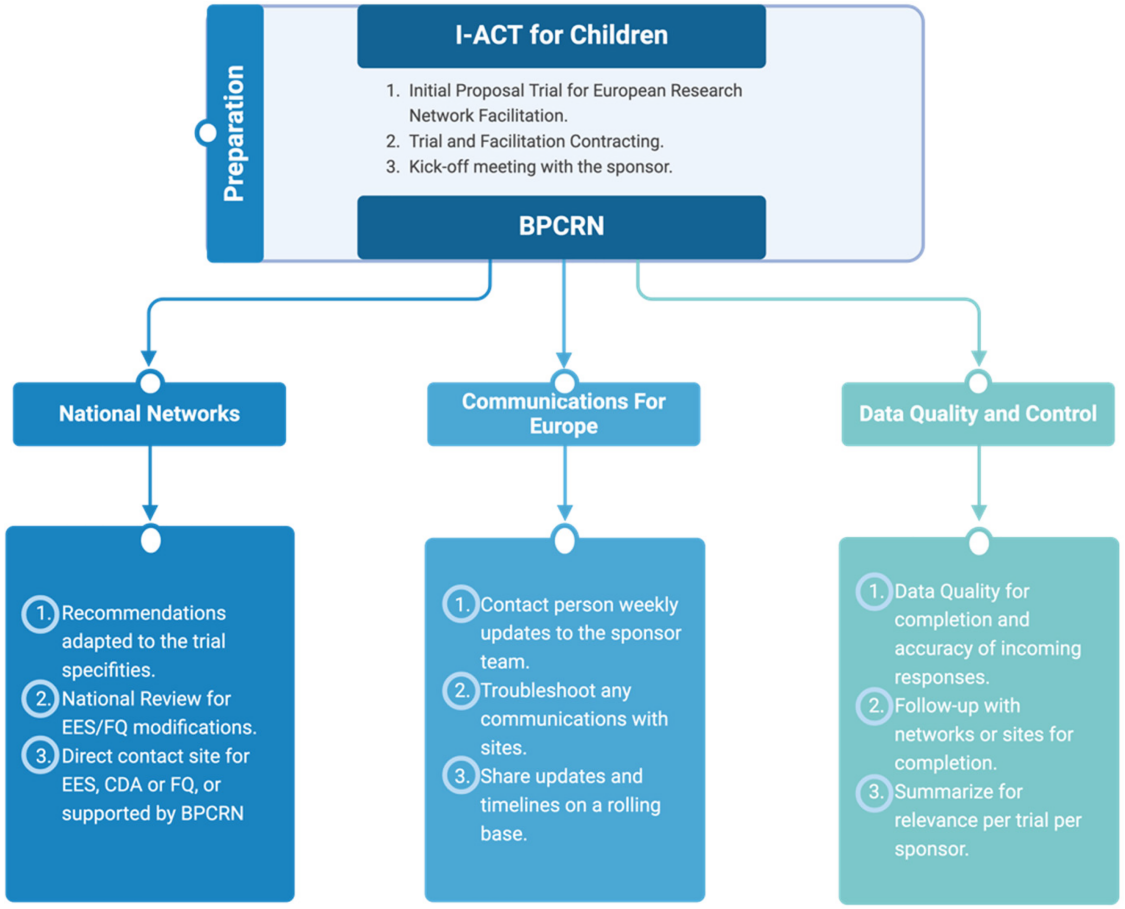

FIGURE 1
The standardization of the collaboration, and the practical conduct of the trial facilitation. EES, Early Engagement Survey; CDA, confidentiality disclosure agreement; FQ, feasibility Questionnaire; BPCRN, Belgian Pediatric Clinical Research Network. Created by Biorender (2024).

Table 3. The most frequently requested activities include Early Engagement Survey (EES) or pre-feasibility, CDA and Trial Facilitation. The EES phase brought its own set of challenges, as demonstrated in Trials 2 through 5. For instance, Trial 2 identified the need for direct, language-specific peer support to address issues like unresponsiveness. The influence of external factors, such as pandemic-induced regulatory adjustments, on Trials 3 and 4, necessitated innovative solutions like a "wave-model" for trial outreach and reorganization of the clinical trial unit. The "wave-model" approach implies that countries were not approached simultaneously to participate in the trials; instead, they were contacted in successive "waves" of prioritization based on each country's site response rate within a given wave. The qualitative analysis also revealed that some sites, as noted in Trials 3–5, initially declined participation due to various misunderstandings or constraints. However, leveraging personal connections resulted in a significant recovery, with a 28% re-inclusion rate for sites that had previously declined.

Moreover, for Trials 1–5 during the EES and FQ stages, it was recognized that over half of the completed questionnaires needed adjustments due to misinterpretations by the Principal Investigators (PI) or site team. Comparing similarly capable sites across countries highlighted the necessity for such adaptations, predominantly in areas like site availabilities and recruitment estimates. Comprehensive summary reports were then crafted, emphasizing the pros and cons, strategic recommendations, and evaluations relative to the sponsor's goals.

In addition to these specific cases, a qualitative analysis was undertaken, examining internal communications. This deep dive aimed to pinpoint the key distinctions and hurdles faced across various continents, thereby enriching the overall understanding and approach to future trial facilitations.

For two trials, a summary of the early engagement as well as the FQ analysis was necessary to give the sponsor an overview of specifities per site, foreseen challenges for site opening and the networks recommendation for inclusion. The experienced based time collection required 50 min per summary. The summary included benefits of the site, potential hurdles and suggestions for control during the visit, as well as a concluding recommendation to the trial team. The sponsor discussions confirmed the usefulness of having the site's information summarized in one or two pages, instead of the 10–24 pages included within the questionnaires.

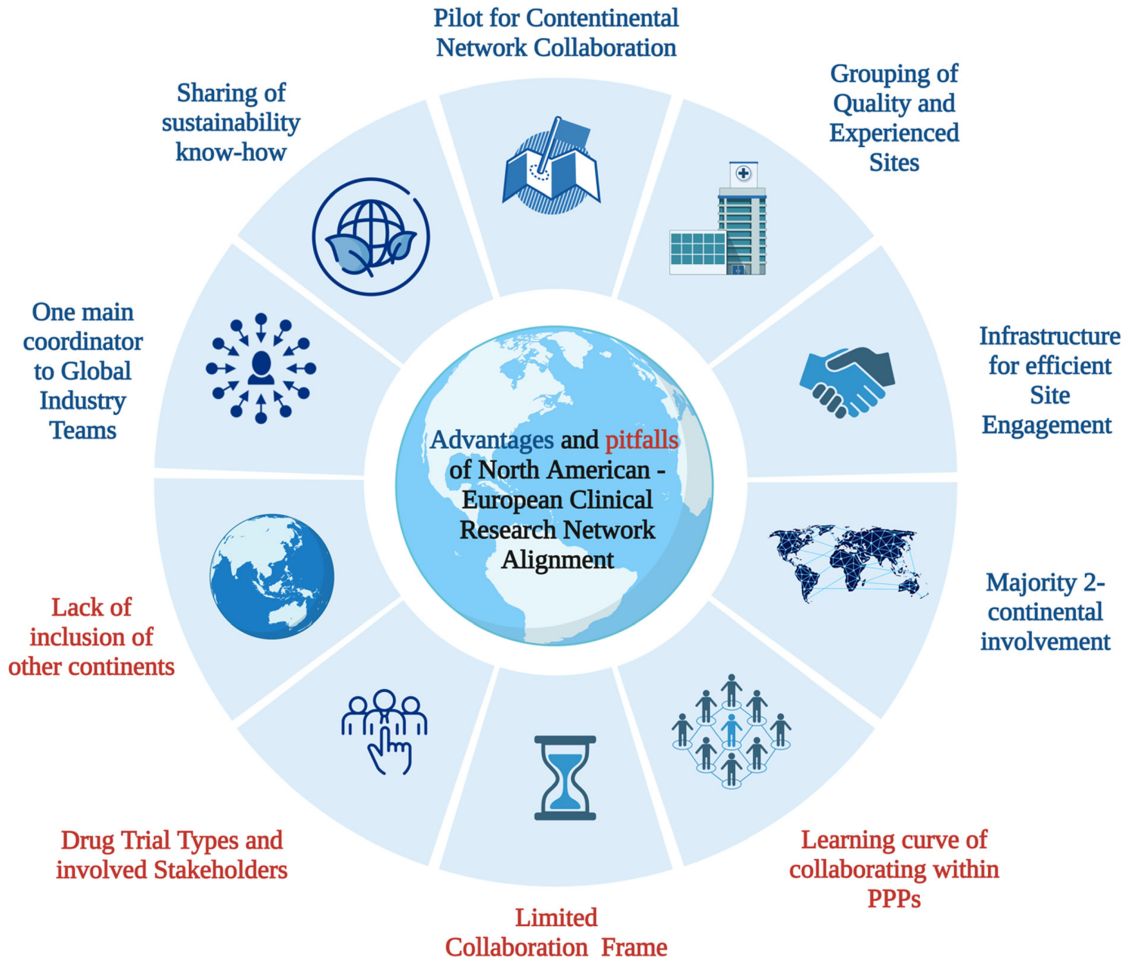

FIGURE 2
Overview of preliminary analysis: advantages and pitfalls of North America—European paediatric clinical research network alignment. CRO, clinical research organisation; PPP, public-private partnership. Based on template "Risk Factors of colorectal cancer', created by Biorender (2023).

4 Discussion

In the last four years, pediatric clinical research networks have partnered across the Atlantic to improve the site identification quality and efficiency with early promising metrics. Trials were conducted across 17 European countries with 202 participating hospital sites. The initial phase, EES, had a 70% response rate from 142 sites, and sites took a median of 38 days (IQR 20 days) to complete the questionnaire for five trials. The trial support activities, including EES, CDA and feasibility questionnaires, could be completed across 7 countries within 84 days. Factors such as precontracted sites, limited scope of studies, changes in timelines, COVID-related disruptions, and a learning curve for collaboration need to be considered when evaluating these metrics. Sponsors have confirmed additional benefits of transatlantic clinical trial facilitation, aside from the promising efficiency of site identification. These include the quality control and summarization of questionnaire findings, establishing personal connections with sites through peer-to-peer communication to increase willingness and understanding to participate in often lengthy questionnaires without reimbursement. Moreover, having a standby facilitator during the trial start-up has been requested often due to the high prevalence of unforeseen hurdles and communication difficulties.

The promising metrics are partly supported by the peer-to-peer connection networks have to the sites and the real-time evaluation of the capability and interest to participate per trial type. The availability standardized CDA processes is highly valued by sponsors, which is incorporated within the US network and was developed during the c4c project. The use of standardized or cascading CDA's in a European setting is available via through the c4c *Stichting* after grant completion (https://conect4children.eu/). Comparison of early-stage pharmacological clinical trials facilitation by networks is largely lacking in literature. The performative metrics of the c4c trials by Degraeuwe et al. (2023) showed feasibility completion within 30 days in 2 high-performative research networks in Belgium and the Netherlands, showing the value of expertise in the pediatric landscape as well as the benefit of standardized contracting (18). Moreover, *Tuft CSDD* reported site identification requiring at average 8 weeks

TABLE 3 Case studies on trial facilitation: impact on site outreach and Non-standard services provided.

Facilitation	Applicable trials	Description
Early Outreach	Trial 1–5	- Sponsors plan to approach countries with previous trial experience, preferably within the indication. - However, sponsor experience is often limited to adult physicians and clinical trial units. - A network's input on the most interesting **countries** and **site estimation** is crucial for strategically planning paediatric clinical trials.
EES	Trial 2	- **Peer-to-peer support** for low-experienced Principal Investigators (PIs). - A low-experienced PI at a smaller site was contacted in their local language to investigate a situation due to unresponsiveness to the EES. - The interpretation and barriers for completing the EES were resolved, and the site's odds were improved for inclusion in the trial.
EES	Trial 3–4	- **Pandemic regulations and fluctuations** influenced the site's capabilities to perform a trial. - A wave-model was constructed where trial outreach for different countries was compartmentalised within trial timelines through different outreach waves. - Sites received support to reorganize the clinical trial unit to continue performing trials.
EES	Trial 4	- Collaborative networks investigated the concept of **decentralized** trials to reach site opening, especially in low population density countries and/or pandemic situations.
EES	Trial 3–5	- Some sites declined due to **limited time or misinterpretation** of the non-confidential summary and/or wording of the questionnaire. - **Personal connections** with sites allowed revisiting responses, resulting in a 28% increase in **inclusion** of earlier sites that declined the trial request for one trial.
EES (& FQ)	Trial 1–5	- Question-response evaluation found that over **half of the completed questionnaires required adaptations** (one or multiple questions, missing data, unrealistic responses, amongst others) in interpretation by the PI/site team, which could be compared to similarly capable sites over different countries. - Majority of the **corrections** were based in site availabilities, recruitment estimates, and relevant experience, creating more realistic expectations for both site and sponsor. - **Summary reports** overviewed the advantages, disadvantages, strategic advice, and continent-wide evaluation of each trial recruitment goal in correspondence with the sponsor.
Other Trial Facilitations	Trial 1, 3, 4	- **Unresponsive site**: having contact details of multiple PIs and multidisciplinary contacts, a network was able to reactivate a site and plan a site initiation visit in a site where previous contact was troubled.
Other Trial Facilitations	Trial 2	- **Other paediatric disciplines**, such as Dermatology, which are usually not physically located within the paediatric departments, required an extension of contacts and knowledge of the interaction with the CTU per site.
Other Trial Facilitations	Trial 4	- Per discipline national coordinators or in some cases discipline **committees evaluated the trial request** upon early outreach for availability per country.
Other Trial Facilitations	Trial 1, 3, 4, 5	- **Communication clarifications** were needed when PIs were contacted through **multiple routes** (sponsor, networks, CRO's) causing confusion and hesitancy by the PI to participate. - A peer-to-peer personal phone call improved communication and reached a conclusion within one working day.

EES, early engagement survey; PI, principal investigator; FQ, feasibility questionnaire; CTU, clinical trial unit.

including both adult and pediatric studies, 50% higher than our findings. This metric does not account for national adaptations, quality control or summary report services.

Furthermore, during the pre-feasibility phase, the networks' expertise in the respective pediatric landscape allowed for recommendations to be made to increase country outreach selection and inclusion of quality sites. Responses were corrected and compared between sites and countries. For example, leveraging personal connections resulted in a significant recovery, with a 28% re-inclusion rate for sites that had previously declined. This resulted in increased quality of site and country for trial inclusion, including a theoretical higher recruitment of the respective studies. An important best practice for all in clinical trial facilitation is the implementation of a handover process, as sites may be contacted by multiple staff members from the sponsor or clinical research organization (CRO), which can cause confusion and reluctance to participate. Utilizing a trusted and familiar member of the research network to initiate contact with the site can prioritize the interests of both the site and sponsor/, and a smooth handover to the sponsor's/CRO's trial manager can establish a strong foundation for future communication and conduct of the clinical trial. In addition to these successful case-analysis in Europe through collaboration, c4c has developed internal standardized processes to address trial support across the trial lifecycle with multiple sponsors and across indications, which all pediatric clinical research networks have built on, implemented, and benefited from. I-ACT for Children has also established best practices that are exemplary for other countries and continents. These include the implementation of a master-CDA and centralization of regulatory authorities.

Moreover, recruitment has been identified by the EMA as a specific challenge in pediatric clinical trials (19). Currently, retention across the studies showed an average 20% loss over time and most clinical trial sponsors or CRO do not use the pre-existing networks or actively standardize and maintain the infrastructure. A call-to-action from regulators to use pre-existing established networks could be beneficial in aligning interest across and collaborations with multiple stakeholders. These networks can include overarching networks, as mentioned, or discipline specific networks such as the European Cystic Fibrosis Clinical Trial Network (ECFS-CTN; www.ecfs.eu/ctn) (20). The recruitment challenge will become more apparent when facing upcoming rare disease trials requiring scattered recruitment within pediatric and adult cases globally.

Furthermore, there is a growing demand for versatile networks that extend beyond traditional domains of pediatric clinical trials, including areas such as psychology, dermatology, and surgery. Additionally, there is a need for a wider range of trial types aside from drugs initially marketed for adults. The demand for conducting clinical trials involving ultra-rare diseases, vaccines, medical devices, drug repurposing, and academic-driven (intent-to-label) studies in children is increasing. National networks expertise can be beneficial to guide sponsors to select relevant and realistic drug trial types as well as improve racial and ethnic representation in pediatric clinical trials (21, 22). There is a continuing necessary investment into other areas of the world (23–25).

Greenberg et al. *(*2022) has noted that pediatric clinical research networks have been constructed similarly and with internal standardization, and should be globally interoperable in 5-years (13). To achieve global interoperability in pediatric clinical trials, there are several essential factors to consider. These include the widespread dissemination of network conduct and metrics, increased collaboration across continents, standardization of facilitation conduct and multi-stakeholder financial support. To prepare for the upcoming wave of pediatric clinical trials, it is also beneficial to include/promote additional national networks, such as those in Japan.

Limitations of this study include the timeframe of 3-years including 5 clinical trials, limiting the calibration of the metric results to other networks and/or reaching a higher learning curve of trial facilitation through networks by multistakeholder. As most of the trials are still ongoing, there is a lack of data available regarding recruitment and dropout rates during the later stages of the trials. Moreover, consultation of the sponsors included in the case study has been indirectly incorporated in Table 3 yet not directly documented. Furthermore, it was not possible to conduct a post-hoc analysis of recruitment and dropout rates at individual sites. Three out of five trials were conducted as rescue studies through research networks, which limited the ability to showcase success ratios of site selection and start-up timelines. It is worth noting that all the included trials originated from US-based pharmaceutical companies, and no service requests were received from a European-based setting.

We propose the utilization of the network directly to maximize time and budget efficiency in pediatric clinical trials. Implementing rescue strategies, which involve additional interventions to salvage trials, not only increase costs and burdens but also lead to frustration among the principal investigator (PI) and trial sites. These rescue strategies often result in repeated and consecutive requests for FQ assessments of the same protocol by various stakeholders, including pharmaceutical companies, CROs, and the network itself. Such repetitive requests negatively impact the quality of data delivered and can disrupt the smooth operation of clinical trial units.

5 Conclusion

Partnership between European collaborative national pediatric clinical research networks and the US-network I-ACT for Children has supported site identification of global pediatric clinical trials. This illustrates one method for the importance of early engagement with sponsors, promoting early metrics and implementation of effective communication systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ED: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft. CoH: Conceptualization, Supervision, Validation, Writing – review & editing. AD: Investigation, Supervision, Validation, Writing – review & editing. RF: Supervision, Writing – review & editing. CaH: Supervision, Validation, Writing – review & editing. LN: Validation, Visualization, Writing – review & editing. LP: Methodology, Project administration, Validation, Writing – review & editing. AR: Methodology, Resources, Supervision, Validation, Writing – review & editing. JV: Conceptualization, Supervision, Validation, Writing – review & editing. MT: Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Fostering the international interoperability of clinical research networks to tackle undiagnosed and under-researched rare diseases

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Clinical research is an essential component to advance diagnosis and therapeutic development. In 2022, the International Rare Diseases Research Consortium (IRDiRC) and the European Joint Programme on Rare Diseases (EJP RD) brought together key stakeholders from across the globe to discuss common themes in clinical research networks (CRNs) for rare diseases. Various topics were raised during discussions including current state of CRNs, the need for new CRNs, multi-stakeholder perspectives on value of CRNs, and ways to collaborate on a global scale. Communication and coordination between various groups, taking advantage of existing experiences, can expedite establishment and execution of complex collaborations that will be necessary for CRNs. In this perspective, we discuss opportunities and highlight key considerations for developing successful collaborative CRNs across the globe.

KEYWORDS

rare diseases, undiagnosed diseases, clinical research, clinical research networks, IRDiRC

Introduction

Rare diseases affect small and geographically dispersed patient populations. These often-complex disorders require multiple and interdisciplinary expert consultations to guide the implementation of the most suitable care pathway. Although individually rare, these disorders could collectively affect more than 400 million people worldwide making them a real challenge for healthcare systems and a global public health priority (1, 2). Despite the tremendous efforts of the rare disease community to accelerate research and care, most rare disease patients still lack a timely diagnosis and approved orphan therapies for their condition. Clinical research networks (CRNs) for rare diseases have been developed at the national or continental level and their scope may differ depending on field of research and geographical coverage (3). For example, European Reference Networks (ERNs) have been set up, in the European Union, to create reference points and compensate the problem of dispersed small patient populations, and support patients and multi-disciplinary teams to gain better understanding on specific rare disorders (4). CRNs have been established in the United Kingdom (UK), funded by the UK Health Department, to provide infrastructure to support high quality clinical research studies for the benefit of patients (5). In the United States, the Rare Diseases Clinical Research

Network (RDCRN) funded by the National Institutes of health (NIH), fosters collaborative research among scientists to better understand how particular rare diseases progress and to develop improved approaches for diagnosis and treatment (6). In Japan, the Initiative on Rare and Undiagnosed Diseases (IRUD), launched by the Japan Medical and Research Development Agency (AMED), is a clinical research program aiming to support the diagnosis of patients with undiagnosed disease via data sharing and to promote research into pathology that may lead to the development of new therapies (7). However, global collaborative efforts to connect CRNs are often lacking thus slowing their full potential in accelerating diagnosis and therapy development. Notably, these joint efforts could accelerate the collection and standardization of data on undiagnosed and under-researched rare diseases often characterized by very low prevalence and high complexity, and subsequently facilitate the implementation of drug development programs addressing better-defined patients` needs and expectations.

The International Rare Diseases Research Consortium (IRDiRC) is a global collaborative initiative, including public and non-for-profit funders, regulatory bodies, academics, patient umbrella groups and biopharmaceutical and diagnostic companies, aiming to tackle rare diseases through research and accelerate diagnosis and therapy development for rare diseases (8, 9). IRDiRC launched a Task Force in 2020 to analyze the structure and attributes of CRNs for rare diseases, including the key elements conducive to collaboration, and to identify the barriers and needs preventing their interoperability (10). The hurdles identified by the Task force were grouped into five categories addressing: funding limitation; lack of harmonization in regulatory and contracting process; need for common tools and data standards; need for a governance framework and coordination structure; and lack of awareness and robust interactions between networks. The task force also revealed that biopharmaceutical companies are poorly integrated into the CRNs (3).

Tackling these issues will require joint efforts from multiple stakeholders namely—patients, clinicians, researchers, industry, regulators, funders, policy makers—located in different geographical areas and working in different jurisdictions. Understanding the specificity of each network, defining common goals and the means to achieve them will be essential since CRNs for rare diseases do not all have the same mandate. An agreement on a global collaborative framework for CRNs could be a major steppingstone to the creation of a long-term roadmap supported by concrete actions and leveraging the experience of the existing networks.

Since IRDiRC member organizations represent all continents and due to its nature and commitments, IRDiRC can stimulate stakeholders' interactions and support the development of a roadmap for the international collaboration and interoperability of CRNs for rare diseases. Here, we present the perspective of IRDiRC on the topic.

Know your neighbor—the importance of mapping and networking of clinical research networks for rare diseases

CRNs for rare diseases present a wide diversity of mandates and activities from improving the diagnosis of rare disease patients to accelerating clinical trials, care management and therapy development (3). To define the framework of collaboration that can form the basis for CRNs' international interoperability and to implement joint actions, it is essential for CRNs to be aware of other existing networks, their structure, goals, and capacities. Such initial interactions can be facilitated by the organization of regular events dedicated to cross-CRN exchange aiming to share knowledge and identify collaborative pathways. The first international conference on CRNs for rare diseases was co-organized by IRDiRC and the European Joint Programme on Rare Diseases (EJP RD) (11) in December 2022 to gather experts from different continents and increase mutual knowledge on CRNs structure, activities and identify routes to stimulate cooperation and interoperability of these networks (12). IRDiRC, in partnership with the newly launched European Rare Diseases Research Alliance (ERDERA) (13, 14), is committed to support the organization of future conferences offering a forum to discuss the implementation of concrete actions and to stimulate the engagement of additional experts and organizations. As a global collaborative initiative of rare disease stakeholders, IRDiRC is well positioned to facilitate the international collaboration and interoperability of CRNs through its organization and activities as shown in Figure 1.

The international value of data

Undiagnosed and under-researched rare diseases are often characterized by very low prevalence and highly complex genotype–phenotype association. The limited knowledge on the etiology and the progression of these diseases, and the difficulty to capture regulatory-grade data for the initiation of drug development programs leave too many patients and families without consensus recommendations and approved medicinal products for managing and treating these diseases. Due to their complexity and extreme rarity, patients are—when possible—referred to CRNs to gain better disease understanding through consultation with multi-disciplinary teams. In this context, the multiplication of data sources using different nomenclature, the absence of concerted agreement on data collection, outcomes and endpoints selection, and the lack of interoperability of the systems, including the use of common consent forms, the standardization of data and the possibility to share data across networks, are major hurdles slowing down clinical research and limiting the opportunity for rare disease patients to gain access to clinical trials.

In the context where every data element is precious and can contribute to more accurate and faster diagnosis and treatment, the international dimension that CRNs can bring is of considerable importance. The collaboration between networks could facilitate and prioritize the harmonization and set-up of registries for under-researched rare diseases and accelerate the collection and validation of regulatory-grade data (e.g., biomarkers, clinical outcome assessments and endpoints) to expedite clinical trial readiness and inform drug development programs. Through concerted actions, CRNs for rare diseases could increase their legitimacy as expert partners for regulatory agencies to define the scientific context for data collection and use, including real world data to support multinational clinical studies and complex designs for clinical trials (15). In this context, concertation between regulatory agencies to accelerate the harmonization of the processes governing the capture of data, including real-world data and real-world evidence, and the conduct of clinical studies could greatly facilitate the implementation

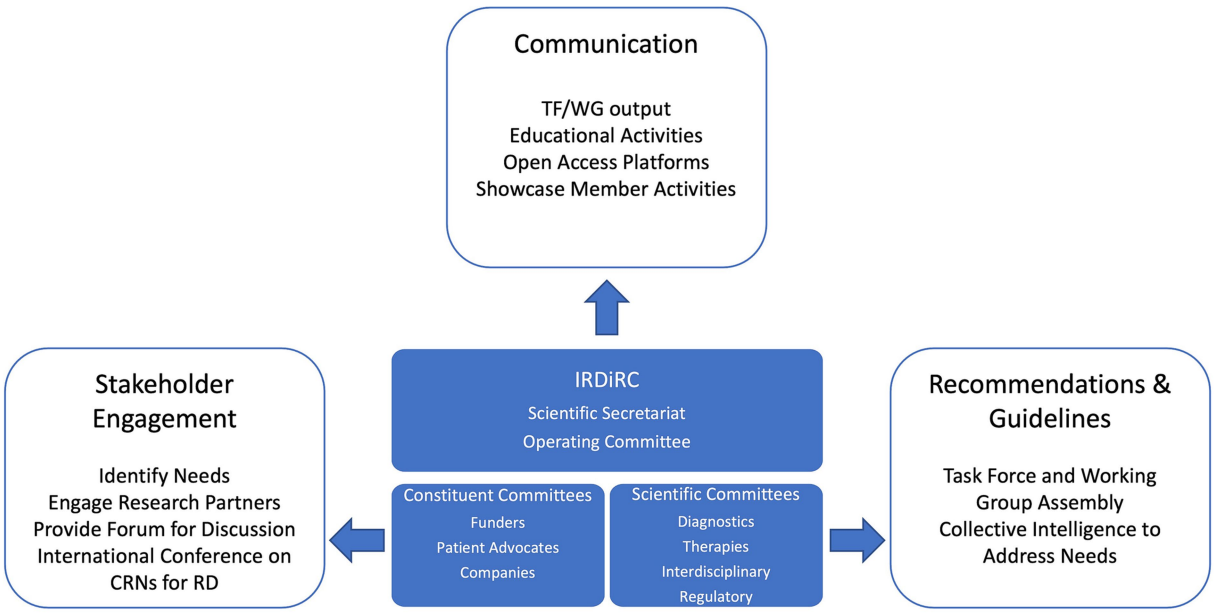

FIGURE 1
Mechanisms by which the International Rare Diseases Research Consortium (IRDiRC) can support the development and the collaboration of clinical research networks (CRNs) for rare diseases (RD) across the globe. By sharing collective intelligence in a non-competitive space, IRDiRC can rely on its constituent and scientific committees to foster worldwide multi-stakeholder engagement and can serve as a forum to discuss the development, the collaboration and the interoperability of CRNs for rare diseases. Continued monitoring and interactions with the global rare disease community connect IRDiRC with initiatives and organizations working toward the development and the interoperability of CRNs across the globe. Task Forces (TF) and Working Groups (WG) are the instruments used by IRDiRC to provide recommendations and guidelines that will help the community in developing collaborative models and tools to support CRNs interoperability. IRDiRC can rely on its network to communicate and disseminate the relevant material and information to advancing collaboration.

of drug development programs and the measure of medicine effectiveness (16). Furthermore, resources such as the EJP RD Virtual Platform (17, 18) and the Rare Disease Cures Accelerator-Data and Analytics Platform (19) that adhere to and promote FAIR (Findable, Accessible, Interoperable, Reusable) principles, and which have been designed and created to accelerate discovery and access to patient-level data, could greatly support CRNs in achieving their interoperability, data collection and access processes while preserving privacy of patients.

Connecting the dots through patient empowerment and partnerships

Patient empowerment and partnerships are cornerstones for CRNs as patient groups have the power to connect clinical teams around the world and guide them in respect to patients' needs and expectations. Moreover, internationally linked CRNs can be entry points and enhancers for under-researched rare diseases where no patient organizations exist and the need to connect different stakeholders is decisive. To support such engagement, funders may consider launching funding opportunities to support patient groups proposing research projects on under-researched rare diseases, connecting multiple clinical research teams linked to different CRNs, while building a multi-stakeholder community encompassing industry participation. Importantly, IRDiRC's Funders Constituent Committee or Board of Funders of EJP RD (and of the newly launched ERDERA) can be game changers in this area.

Rare diseases without frontiers

Analysis of existing CRNs demonstrated that depending on the regional or national priorities, and political context of their creation, CRNs may be more research or healthcare oriented (3). However, in the rare disease space clinical care and research are strongly integrated. In some cases, research and clinical care are considered a continuum, as clinical care and observations are essential to inform research which in turn will inform healthcare decisions. The discussions of the *International Conference on CRNs for Rare Diseases* (12) made clear that many regions or countries are still exploring how to create, or are preparing to launch, a CRN in their geographical area.

Following these lines, it is expected that the collaboration between CRNs for rare diseases should contribute to reduce inequities between geographies and support IRDiRC's vision to leave no one behind by promoting education and training. To reach this vision, sharing of knowledge among RD stakeholders, including expert health care providers involved in the joint assessment of the most complex patient cases, maximized data collection efforts and support in the initiation of clinical research studies in multiple networks should happen irrespectively of CRNs geography (20).

An additional consideration for the success of CRNs in conducting research is their interactions and collaboration with the biopharmaceutical industry. CRNs have a potential to improve the drug development process by delivering multiple activities related to clinical trials including the nonclinical proof-of-concept research, designing studies with a patient-centric approach, leading patient registries and natural history studies, and facilitating patient

recruitment into clinical trials. For this to become a reality CRNs need to build a pathway for industry to be considered as a research partner.

Conclusion

The creation of CRNs for rare diseases at the national and continental level has been shown to be essential to gain better understanding of specific rare diseases and accelerate clinical research, diagnosis and therapy development. However, for most of the under-researched rare diseases, the size of the patient population at the national or even continental level is not sufficient to generate critical mass of knowledge on the diseases and attract the interest of drug developers. The collaboration and the interoperability of CRNs for rare diseases should aim to overcome these hurdles. By connecting on a global scale, developing joint roadmaps for improved collection and access to standardized data, sharing expert knowledge, and benefiting from collective input and experience of patients irrespectively of their geography, socio-economic and cultural status, the CRNs can demonstrate the unprecedent power of cooperation for the benefit of all people living with a rare disease.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

GZ: Writing – original draft. C-HC: Writing – review & editing. SP: Writing – review & editing. DJ: Writing – review & editing. DP: Writing – review & editing.

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Conflict of interest

Author SP was employed by the Italfarmaco S.p.A.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A case report of *PGAP2*-related hyperphosphatasia with impaired intellectual development syndrome in a Chinese family and literature review

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Recently, mutations have been identified in six genes (*PIGA*, *PIGY*, *PIGO*, *PGAP2*, *PIGW* and *PGAP3*) encoding proteins in the Glycosyl phosphatidylinositol(GPI)-anchor-synthesis pathway in individuals with hyperphosphatasia with impaired intellectual development syndrome(HPMRS). Reports involving the rare pathogenic gene, post-GPI attachment to proteins 2 (*PGAP2*) are quite limited. In this study, we reported two patients with *PGAP2* variants related neurodevelopmental disorders from Asian population. The proband, onset of epileptic spasms at 5 months, concurrently with global developmental dalay, facial malformation and elevated alkaline phosphatase. His younger sister, onset of epileptic spasms at 2 months, having similar clinical features as the proband. Their phenotypes are consistent with *PGAP2* related diseases. The two missense variants [c.686C>T (p.Ala229Val) and c.677C>T (p.Thr226Ile)] in *PGAP2* gene found in this family were segregation with the disease, while c.677C>T (p.Thr226Ile) was a novel variant. All the two patients showed a positive response to ACTH treatment and high-dose pyridoxine. In summary, this study contributes to expanding the pathogenic variant spectrum of *PGAP2* related HPMRS, and provides new insights into the treatment.

KEYWORDS

PGAP2 variants, hyperphosphatasia with impaired intellectual development syndrome, epileptic spasms, facial malformation, ACTH treatment, pyridoxine

Introduction

Glycosylphosphatidylinositol (GPI) anchoring is a post-translational modification that attaches proteins to the plasma membrane, playing a role in protein sorting and transport. GPI anchoring proteins are highly conserved across eukaryotes, with mammals possessing over 150 GPI-anchored proteins (GPI-APs). Including receptors, adhesion molecules and enzymes (1). Defects in six genes involved in the GPI-anchor synthesis pathway-*PIGV* (MIM:610274), *PIGO* (MIM:614730), *PIGY*(MIM:610662), *PIGW*(MIM:610275), *PGAP3* (MIM:611801) and *PGAP2* (MIM:615187) -are associated with hyperphosphatase with impaired intellectual development syndrome [HPMRS (MIM 239300)](also known as Mabry syndrome) (1). HPMRS is a rare autosomal recessive genetic disorder, affecting both males and females, characterized by intellectual disability, hypotonia with poor motor development, speech disorder, and elevated serum alkaline phosphatas, reflecting a broad phenotypic spectrum of disorders that include ID and seizures (2–5).

Recent studies have identified that mutations in *PGAP2* are linked to HPMRS-3, which displays phenotypic overlap with the broader HPMRS category. HPMRS-3 is inherited in an autosomal recessive manner, with both homozygous and compound heterozygous variants reported, often in the children of consanguineous parents (2, 6–9). *PGAP2* encodes a Golgi/ER-resident membrane protein involved in fatty acid remodeling of GPI-APs in the Golgi, where it involved in reacylation with stearic acid, a saturated fatty acid (10, 11). Fatty acid remodeling is essential for ensuring proper association of GPI-APs with specific membrane domains known as lipid rafts or lipid microdomains (11, 12). Function study have indicated that *PGAP2* deficiency caused transport to the cell surface of lysoform GPI-APs that were easily cleavage by phospholipase D (2, 10, 13). However, reports on the *PGAP2* gene remain limited, with only 16 pathogenic variants in 25 patients documented (2, 6, 8, 9, 13–15).

In this study, we report a Chinese family with two children diagnosed with recessive HPRMS-3 through genetic testing. Both siblings are compound heterozgous for two *PGAP2* missense variants (c.686C>T (p.Ala229Val) and c.677C>T (p.Thr226Ile). To our knowledge, this is the first report about *PGAP2*-related neurodevelopmental disorders in East Asian population. The study was accompanied by a comprehensive literature review about the genotypes, phenotypes of the condition. Given the limited number of reported cases to date, an expanded clinical delineation of HPMRS-3 and insights for targeted therapeutic interventions would be greatly enhanced by the identification and longitudinal assessment of additional cases.

Materials and methods

Subjects

A 4 year and 5 month old boy (proband, II-1) and his younger sister (II-2) were admitted at Guiyang Maternal And Child Health Care Hospital. Tiro whole exome sequencing (trio-WES) was performed on the proband and his parents. The younger sister was also enrolled in this investigation to elucidate the genetic inheritance pattern. This study was approved by the Medical Ethics Committee of the Guiyang Maternal and Child Health Care Hospital and the written informed consent from genealogy parents was obtained.

Whole exome sequencing and data analysis

The clinical whole exome sequencing (WES) of the proband and their parents was performed to determine their genetic etiology. Genomic DNA was extracted from whole blood samples for library preparation using Hieff NGS OnePot Pro DNA Library Prep Kit for ILLUMINA (Yeasen, China). The xGen Exome Research Panel probes (IDT, USA) were utilized to capture the exon region following the manufacturer's recommendations. The raw data was sequenced on NovaSeq6000 platform (Illumina). Reads were then aligned to the hg19 human reference genome (GRCh37) using BWA MEM (v0.7.17). Subsequently, PCR and optical duplicate marking were performed using Genome Analysis Toolkit GATK(v4.1.4.0) and local realignment around indels and base quality score recalibration were carried out by GATK (v3.8.1). Finally, the variants were identified using GATK HaplotypeCaller 1 (v3.8.1). Variants were annotated by Annovar and the pathogenicity of candidate variants wase valuated according to American College of Medical Genetics and Genomics(ACMG) guidelines (16, 17).

Sanger sequencing

Sanger sequencing was performed on the two siblings and their parents. The primers design was accomplished using primer3 and synthesized by Beijing Tsingke Biotech Co., Ltd. (Beijing, China). The forward and reverse primer sequences were as follows: Forward 1: 5'-AAGCTGCAGAGTGATCAGACAG-3'; Reverse 1: 5'-GAAGGCCAGAATGG TATCGTGT-3'; Forward 2: 5'-ATCCAC AGGCGGTCAT GAGT-3'; Reverse 2: 5'-AGCCTTGGCCTAC ACCCTTC-3'. Polymerase chain reaction(PCR) was performed using a ABI2720 (Applied Biosystems). The amplified products were sequenced using ABI3730 (Applied Biosystems).

Results

Clinical overview of the pedigree

The proband, a male infant, presented with clusters of epileptic spasms at the age of 5 months. The patient had no significant medical or perinatal history and exhibited global developmental delay. Family history: The younger sister displayed similar manifestations. Physical examination revealed thick eyebrows, hypertelorism in both eyes, collapsed nasal bridge (Figure 1A), and hypotonia in the limbs. The results of routine blood tests, liver and kidney function tests, electrolyte levels, blood amino acids, acylcarnitine levels, and urine organic acid analysis were within normal limits. However, there was an elevation in serum alkaline phosphatase (ALP) levels to 789 U/L. Brain MRI revealed a thin corpus callosum, slightly enlarged lateral ventricles, and mildly widened extracerebral spaces in bilateral fronto-temporo-parietal lobes (Figure 1D). VEEG monitoring showed bilateral posterior head dominant hypsarrhythmia with burst suppression, bilateral posterior head dominant fast wave rhythmic discharge during sleep episodes, as well as clusters of spasms (Figures 2A,B). The administration of ACTH significantly reduced seizures; however, there was a recurrence of epileptic spasms and the emergence of atypical absence seizures. Despite the ineffectiveness of various antiseizure medications (ASMs), the gradual addition of high-dose pyridoxine provided relief from seizures. At 2 years and 10 months old, he exhibited impaired independent sitting, active grasping, and speech abilities. Subsequent repeated examinations revealed a significant increase in his ALP levels (789–3380 U/L).

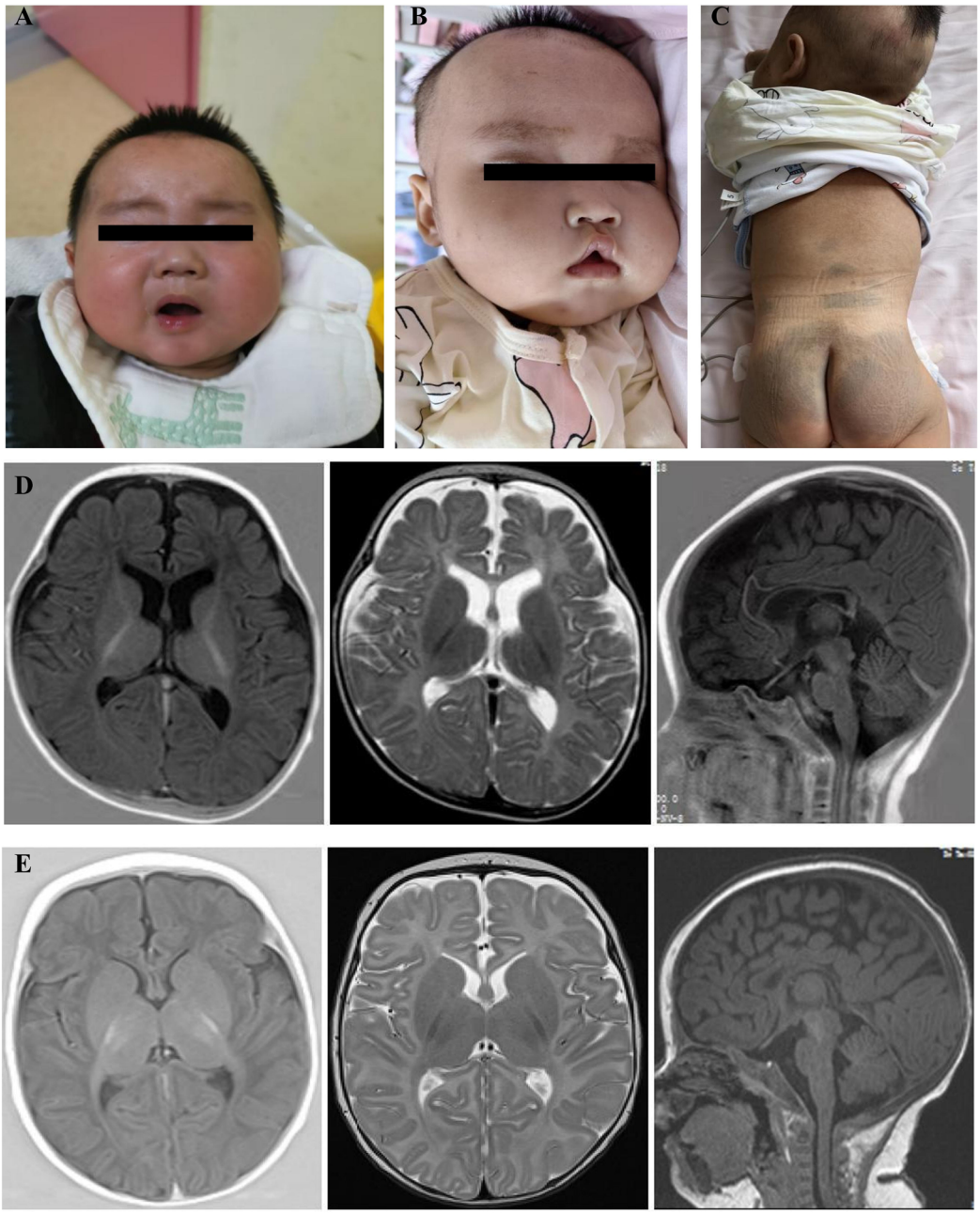

FIGURE 1
Facial features and brain MRI results of the proband and his younger sister. **(A)** Facial features of the proband at 19 months of age: thick eyebrows, hypertelorism in both eyes, and collapsed nasal bridge. **(B)** Facial features of the proband's younger sister at 2 months of age: thick eyebrows, collapsed nasal bridge, tented upper lip, and downward-facing corners of the mouth; **(C)** Large Mongolian patches on the dorsum and rump of the proband's younger sister **(D)** Proband, 5-month-old cranial MRI: thin corpus callosum, slightly enlarged lateral ventricles, mildly widened extracerebral spaces in bilateral fronto-temporo-parietal lobes, transverse axial T1W, T2W, and sagittal T1W. **(E)**Proband's younger sister, 2-month-old cranial MRI: hypoplastic corpus callosum, transverse axial T1W, T2W, and sagittal T1W.

The proband's younger sister, a female infant, presented with clusters of epileptic spasms at the age of two months. Physical examination revealed thick eyebrows, collapsed nasal bridge, tent-shaped upper lip, large patches of Mongolian spots on the back, buttocks and trunk (Figures 1B,C), and low muscle tone in the limbs. There was no significant history during perinatal period. Global developmental delay was observed. Family history showed similar manifestations in the elder brother. Blood routine tests, liver and kidney function tests, electrolyte levels, blood amino acids and acylcarnitines as well as urinary organic acid levels were normal; ALP level was 619 U/L. Brain MRI revealed hypoplastic corpus callosum (Figure 1E). VEEG monitoring indicated hypsarrhythmia and spasms (Figures 2C,D). ACTH treatment significantly reduced epileptic spasms but seizures

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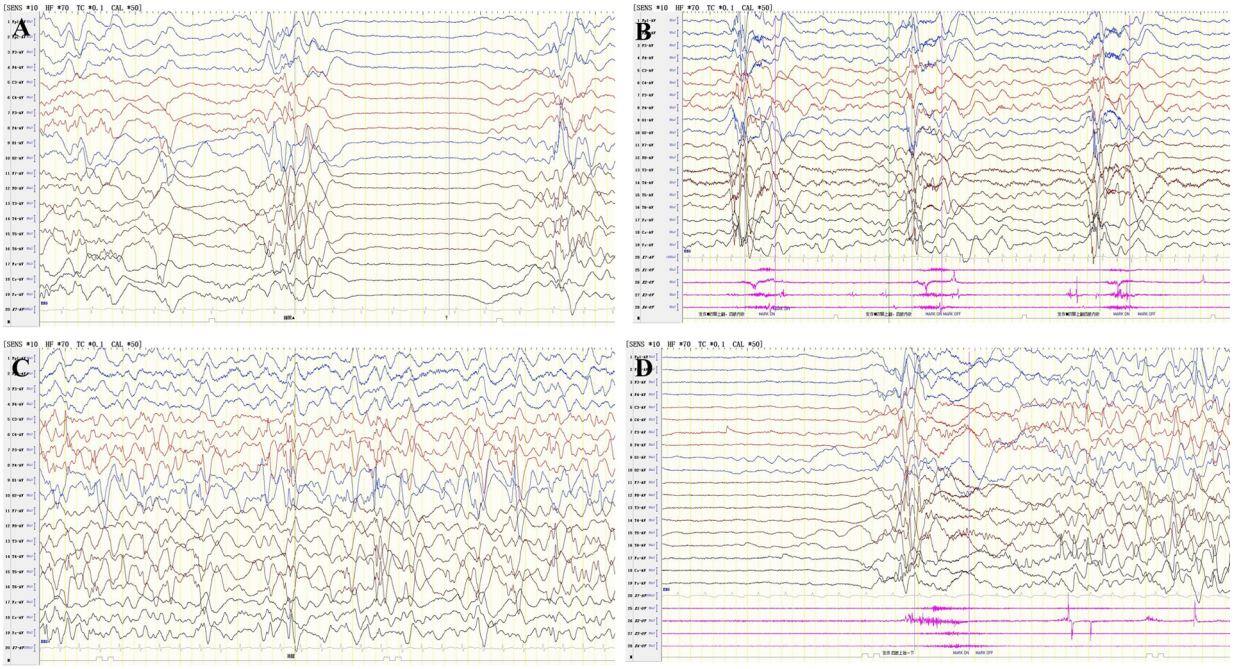

FIGURE 2
VEEG results of the two patients. VEEG of 5 months of age of the proband, **(A)** Interictal period: hypsarrhythmia with burst suppression; **(B)** Ictal period: clusters of epileptic spasms. VEEG of 4 months of age of the proband's younger sister, **(C)** Interictal period: hypsarrhythmia; **(D)** Ictal period: epileptic spasms.

recurred until high-dose pyridoxine was added to alleviate symptoms gradually over time. The patient is currently five months old with stable head control but poor sound/object tracking ability; he is unable to turn over or grasp objects actively.

The two children have no other siblings. No other family history of neurological disorders, behavioral issues, or seizures was reported in this family. Both parents were healthy and nonconsanguineous.

Genetic analysis and literature review

Trio whole exome sequencing identified two missense variants in *PGAP2* gene [NM_001256240.2:c.686C>T (p.Ala229Val) and NM_001256240.2: c.677C>T (p.Thr226Ile)] in the proband, resulting in a compound heterozygous state, with the father and mother being heterozygous carriers of c.686C>T (p.Ala229Val) and c.677C>T (p.Thr226Ile) respectively. Sanger sequencing in the younger sister of the proband confirmed the existence of the two variants (Figure 3A). The c.686C>T (p.Ala229Val) is present in population databases at a very low frequency (rs753497329, max frequency is 0.004% in South Asian in gnomAD v4.0.0), the variant has been reported in a patient with *PGAP2*-related HPMRS (15). The c.677C>T (p.Thr226Ile) has been identified in only 1 heterozygote carrier within the gnomAD v4.0.0 database, and no patient with the variant has reported. Both the two variants are predicted to be deleterious by three in silico predictive software packages (PolyPhen-2, Mutation Taster, and SIFT). Moreover, both variants are located in the transmembrane domain (Figure 3B). Variant curation using ACMG/AMP guidelines suggests that the two variants are all classified as Variants of Uncertain Significance (PM2_Supporting + PM3_Supporting + PP1 + PP3 + PP4) (16).

PGAP2 plays a critical role in the lipid remodeling steps of GPI-anchor maturation, essential for stable expression of GPI-anchored proteins at the cell surface (10). *PGAP2* gene encodes 16 different RNA transcripts, with 8 of which encode different isoforms and 8 that are noncoding RNAs. Among these, isoform 8(NM_001256240.2), which consists of 254 amino acids, is considered the biologically active isoform (2, 8, 15). This isoform has more than 90% homology with other mammalian orthologs (2). The UniProtKB/Swiss-Prot (18) annotate that it has five alpha-helix domains embedded in the Golgi membrane (Figure 3). To date, including cases from our patients, only 17 variants of this gene have been documented (Figure 3B, Table 1), predominantly characterized as missense mutations. Out of these, only two variants were truncating variants(c.103del [p.Leu35Serfs*90, and 2T > G (Met1?)] (9, 14) (Figure 3B, Table 1).

Discussion

HPMRS-3 exhibits an autosomal recessive inheritance pattern, and individuals who are heterozygous carriers of variants typically do not display any symptoms. However, Yonatan et al. observed that heterozygous carriers may present with a mild phenotype

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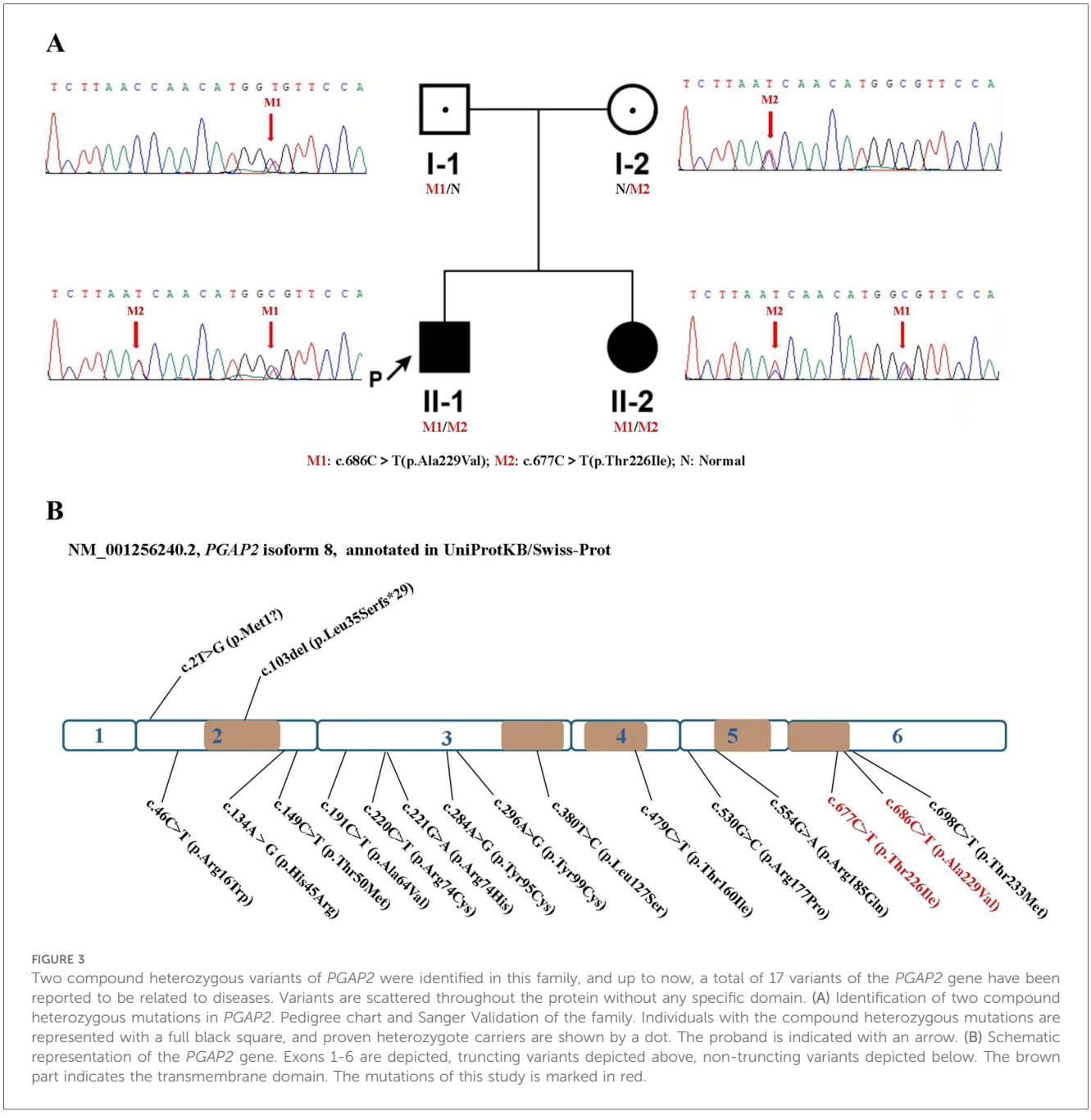

FIGURE 3
Two compound heterozygous variants of *PGAP2* were identified in this family, and up to now, a total of 17 variants of the *PGAP2* gene have been reported to be related to diseases. Variants are scattered throughout the protein without any specific domain. (A) Identification of two compound heterozygous mutations in *PGAP2*. Pedigree chart and Sanger Validation of the family. Individuals with the compound heterozygous mutations are represented with a full black square, and proven heterozygote carriers are shown by a dot. The proband is indicated with an arrow. (B) Schematic representation of the *PGAP2* gene. Exons 1-6 are depicted, truncting variants depicted above, non-truncting variants depicted below. The brown part indicates the transmembrane domain. The mutations of this study is marked in red.

characterized by slightly elevated serum levels of alkaline phosphatase and significant learning disabilities (7). We report two patients with HPMRS-3, who were diagnosed both clinically and genetically. In our cases, analysis of WES data revealed compound heterozygous mutations c.686C>T (p.Ala229Val) and c.677C>T (p.Thr226Ile) in *PGAP2* gene. The two variants are all classified as Variants of Uncertain Significance according to ACMG guidlines. Currently, it is difficult to classify the two variants as likely pathogenic or pathogenic variants because there have been few pedigrees hurboring the same variants reported, and missing functional experiments. However, using the ClinGen Bayesian classification framework (available at https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines), the variant got 5 points, which is very close to the criteria (6 points) for likely pathogenic variants. The two sibings' phenotypes matched the phenotype of *PGAP2*-related HPMRS, and the autosomal recessive pattern of segregation was observed in this family. After comprehensive consideration, the two patients can be diagnosed with *PGAP2*-related HPMRS-3.The c.677C > T (p.Thr226Ile) variants, identified as a novel disease locus, was initially reported to expand the genetic variation spectrum associated with HPMRS-3 and enhance our comprehension of its genetic basis.

When considering the location distribution of the missense mutations in *PGAP2*, approximately 1/3 are situated in the transmembrane domain, while the rest are found either within

TABLE 1 Clinical and genetic characteristics of reported patients with *PGAP2*-related disorders.

Patient ID	Gender	Mutation	Central nervous system phenotypes				Auxiliary examinations			Facial features	Other findings	Reference
			DD/ID	epilepsy	Seizure semiology	Seizure control Yes/not	EEG	Brian CT/MRI	ALP			
Ⅱ-1	M	c.677C > T (p.Thr226Ile); c.686C > T (p.Ala229Val)	Globle developmental daley	Yes	epileptic spasms	Yes, positive response to ACTH treatment and high-dose pyridoxine.	interictal: hypsarrhythmia with burst suppression, ictal:clusters of spasms	MRI: thin corpus callosum, slightly enlarged lateral ventricles, mildly widened extracerebral spaces in bilateral fronto-temporo-parietal lobes	elevated, 789-3,380 U/L	thick eyebrows, hypertelorism in both eyes, and collapsed nasal bridge	hypotonia	This study
Ⅱ-2	F	c.677C > T (p.Thr226Ile); c.686C > T (p.Ala229Val)	Globle developmental daley	Yes	epileptic spasms	Yes, positive response to ACTH treatment and high dose pyridoxine.	interictal: hypsarrhythmia, ictal:epileptic spasms	MRI:hypoplasia corpus callosum	elevated, 619 U/L	thick eyebrows, collapsed nasal bridge, tented upper lip, and downward facing corners of the mouth	Large Mongolian patches on the dorsum and rump, hypotonia	This study
Patient 1	F	c.2T > G (p.Met1?); c.221G > A (p.Arg74His)	severe psychomotor retardation	Yes	epileptic spasms	Yes, positive response to ketogenic diet	hypsarrhythmia and suppression-burst	NA	elevated, 1,496-2,780 U/L	a flat occiput and pectus excavatum	low birth parameters, chest deformities in neonatal period; severe hypotonia, chronic fever, respiration insufficiency.	Jezela-Stanek et al. (14). Pronicka et al. (19).
individual A	F	c.46C > T (p.Arg16Trp); c.479C > T (p.Thr160Ile)	postnatal development, started to walk at the age of 18 months, her initial speech development was normal	Yes	Febrile seizures, tonic-clonic seizures	Yes, responded well to valproic acid	NA	NA	elevated, 2,107–2,448 U/L	broad nasal bridge, tented upper lip	-	Krawitz et al. (13)
individual B	M	c.380T > C (p.Leu127Ser); c.380T > C (p.Leu127Ser)	severe psychomotor developmently delay	Yes	Myoclonic, tonic-clonic seizures	Yes, responded well to AEDs	multifocal sharp waves	MRI: hypoplasia corpus callosum	elevated, 2,022 U/L	median cleft palate, wide palpebral fissures, a short nose with a broad nasal bridge, a tented upper lip, and a small jaw	Distal tapering of fingers and mild nail, hypoplasia of the fifth digit, hirschsprung disease, atrial septal defect, sensorineural hearing loss, microcephaly, scoliosis, severe muscular hypotonia	Krawitz et al. (13)

(*Continued*)

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TABLE 1 Continued

Patient ID	Gender	Mutation	Central nervous system phenotypes				Auxiliary examinations			Facial features	Other findings	Reference
			DD/ID	epilepsy	Seizure semiology	Seizure control Yes/not	EEG	Brian CT/MRI	ALP			
patient 1	F	c.103del (p.Leu35Serfs*29); c.134A > G (p.His45Arg)	Globle developmental daley, intellectual disability, speech delay	Yes	absence seizures	Yes, absence seizures were well-controlled on Ethosuximide; after pyridoxine and folinic acid supplementation, the patient continued to make developmental progress	runs of 4–6/s spike-wave complexes over the posterior half of the head in wakefulness and sleep, facilitated by eye closure, and photic stimulation at 16 Hz.	MRI:normal	elevated, 1,300–1,832 U/L	no dysmorphic features	hypotonia, precocious adrenarche, esotropia, myopic astigmatism	Messina et al. (9)
Patient 1 (IV:4)	M	c.191C > T (p.Ala64Val); c.191C > T (p.Ala64Val)	intellectual disability, severely developmental regression	Yes	epileptic spasms	NA	no interictal epileptiform discharges, frequent spasms in ictal	NA	NA	NA	poor hearing, microcephaly	Naseer et al. (6)
Patient 2 (IV:3)	F	c.191C > T (p.Ala64Val); c.191C > T (p.Ala64Val)	developmental delay, growth retardation, intellectual disability	Yes	epileptic spasms	NA	no interictal epileptiform discharges, frequent spasms in ictal	NA	NA	NA	poor hearing, microcephaly	Naseer et al. (6)
family MR043a (IV-1)	F	c.296A > G (p.Tyr99Cys); c.296A > G (p.Tyr99Cys)	severe intellectual disability, severe motor development delay	No	-	-	NA	CT: atrophy and increased gyration	elevated, 4,455 U/L	no major dysmorphisms	pronounced muscular weakness and hypotonia, strabismus, sleep disorders	Hansen et al. (2). Reuter et al. (20)
family MR043a (IV-2)	F	c.296A > G (p.Tyr99Cys); c.296A > G (p.Tyr99Cys)	severe intellectual disability, severe motor development delay	No	-	-	NA	CT: atrophy and increased gyration	elevated, 4,375vU/L	no major dysmorphisms	pronounced muscular weakness and hypotonia, strabismus, sleep disorders	Hansen et al. (2). Reuter et al. (20)
family MR043b (IV-4)	F	c.296A > G (p.Tyr99Cys); c.296A > G (p.Tyr99Cys)	severe motor development delay, speech delay	Yes	absence epilepsy	NA	NA	CT: brain atrophy	NA	no major dysmorphisms	severe muscular hypotonia, muscle biopsy identified muscle atrophy	Hansen et al. (2). Reuter et al. (20)
family MR5(V:4)	M	c.530G > C (p.Arg177Pro); c.530G > C (p.Arg177Pro)	intellectual disability	No	-	-	NA	MRI: normal	NA	normal	anemia	Hansen et al. (2).
family MR5(V:5)	M	c.530G > C (p.Arg177Pro); c.530G > C (p.Arg177Pro)	intellectual disability	No	-	-	NA	MRI: normal	NA	normal	anemia	Hansen et al. (2).

(*Continued*)

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TABLE 1 Continued

Patient ID	Gender	Mutation	Central nervous system phenotypes				Auxiliary examinations			Facial features	Other findings	Reference
			DD/ID	epilepsy	Seizure semiology	Seizure control Yes/not	EEG	Brian CT/MRI	ALP			
family MR5(V:6)	F	c.530G > C (p.Arg177Pro); c.530G > C (p.Arg177Pro)	intellectual disability	No	-	-	NA	MRI: normal	NA	normal	anemia	Hansen et al. (2).
family MR5(V:7)	F	c.530G > C (p.Arg177Pro); c.530G > C (p.Arg177Pro)	intellectual disability	No	-	-	NA	MRI: normal	NA	normal	anemia	Hansen et al. (2).
IV-3	M	c.554G > A (p.Arg185Gln); c.554G > A (p.Arg185Gln)	mild intellectual disability, speech difficulties	Yes	Past history of febrile seizures	NA	normal	CT: normal	elevated, 499 IU/L at 23 yrs of age	normal	mood problems, depression	Perez et al. (7)
IV-5	M	c.554G > A (p.Arg185Gln); c.554G > A (p.Arg185Gln)	developmental delay, Moderate intellectual disability	Yes	Past history of seizures without fever	NA	normal background activity, generalized spike and wave interictal discharges during wakefulness	MRI: normal	elevated, 673 IU/L at 17 yrs of age	normal	behavioral problems, aggression	Perez et al. (7)
IV-6	F	c.554G > A (p.Arg185Gln); c.554G > A (p.Arg185Gln)	mild intellectual disability, speech difficulties	No	-	-	NA	NA	elevated, >1,000 IU/L at 10 yrs of age	normal	mood problems, depression	Perez et al. (7)
IV-7	F	c.554G > A (p.Arg185Gln); c.554G > A (p.Arg185Gln)	developmental delay, mild intellectual disability, speech difficulties	No	-	-	NA	Normal spinal MRI	elevated, 1,318 IU/L at 10 yrs of age	normal	enuresis	Perez et al. (7)
Family 1 (VI:4)	M	c.698C > T (p.Thr233Met); c.698C > T (p.Thr233Met)	Globle developmental daley	Yes	Unexplained major motor seizures	seizures had resolved without medication in old age	NA	NA	elevated, 2,440 IU/L	the characteristic facial gestalt remains characteristic throughout life, it generally becomes less pronounced in older patients	hypotonic, brachytelephalangy	Thompson et al. (8). Mabry et al. (21)
Family 1 (VI:16)	M	c.698C > T (p.Thr233Met); c.698C > T (p.Thr233Met)	Globle developmental daley	Yes	Unexplained major motor seizures	seizures had resolved without medication in old age	NA	NA	elevated, 1,628 IU/L	The characteristic facial gestalt remains characteristic throughout life, it generally becomes less pronounced in older patients	hypotonic, brachytelephalangy	Thompson et al. (8).Mabry et al. (21)

(*Continued*)

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TABLE 1 Continued

Patient ID	Gender	Mutation	Central nervous system phenotypes				Auxiliary examinations			Facial features	Other findings	Reference
			DD/ID	epilepsy	Seizure semiology	Seizure control Yes/not	EEG	Brian CT/MRI	ALP			
Family 2 (III-8)	F	c.698C > T (p.Thr233Met); c.698C > T (p.Thr233Met)	developmental disability	NA	NA	NA	NA	NA	NA	NA	NA	Thompson et al. (8).
Family 2 (IV-2)	F	c.698C > T (p.Thr233Met); c.698C > T (p.Thr233Met)	developmental disability	NA	NA	NA	NA	NA	NA	NA	NA	Thompson et al. (8).
TF001_1	M	c.220C > T (p.Arg74Cys); c.220C > T (p.Arg74Cys)	Global developmental delay	NA	NA	NA	NA	NA	elevated, NA	NA	nystagmus	Froukh et al. (22)
TF001_4	M	c.220C > T (p.Arg74Cys); c.220C > T (p.Arg74Cys)	Global developmental delay	NA	NA	NA	NA	NA	elevated, NA	NA	nystagmus, aganglionic megacolon	Froukh et al. (22)
proband	M	c.149C > T (p.Thr50Met); c.686C > T (p.Ala229Val)	global developmental delay	Yes	focal seizures with impaired awareness	Treatment with high-dose pyridoxine showed partial benefit, ketogenic diet treatment.	progressive background activity disorganization, multifocal epileptic discharges.	MRI: mild hypoplasia of the inferior cerebellar vermis and corpus callosum and mild white matter reduction.	elevated, 1,005–1,769 U/L	normal	visual impairment	Saracino et al. (15)
patient	M	c.284A > G (p.Tyr95Cys) [along with *PGAP3* c.259G > A (p.Val87Met), heterozygote]	Global developmental/ intellectual disability, delayed speech, delayed age at walking/no walking,	Yes	NA	NA	NA	NA	elevated, 3–4 times the upper limit of normal	short nose with broad tip	autistic features, hypotonia	Thompson et al. (23)

NA, not assessed; F, female; M, male; DD, developmental delay; ID, intellectual disability; EEG, electroencephalogram; MRI, magnetic resonance imaging; CT, computed tomography; ALP, alkaline phosphatase.

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the Golgi lumen or in the cytoplasmic region (Figure 3B). Due to the limited number of mutations identified, it remains challenging to draw comparisons regarding the differences in clinical phenotypes that may arise from distinct mutation sites, as the sample size is too small to conduct a meaningful statistical analysis.

The core manifestations of HPMRS include hyperphosphatasia, seizures, and developmental disability, all of which were observed in both of our patients. Notably, persistent hyperphosphatasia distinguishes this disorder from other similar conditions and is consistently present in all patients with HPMRS. In our two patients, the levels of ALP ranged from 617 U/L to 3380 U/L. Seizures occur frequently throughout the course of the disease, manifesting in various forms such as absence, epileptic spasms, tonic-clonic, myoclonic, and febrile seizures (2, 6, 8, 9, 13, 14) (Table 1). The age of onset varies, ranging from the neonatal period to childhood (2, 6, 8, 9, 13–15). Epileptic spasms dominated the two children in our family during infancy, and atypical absence seizures occurred in the later stage of the proband. In the previously reported cases, epileptic spasms were infrequent, and some patients did not have seizures, however, epileptic spasms were typically the initial and predominant symptom in our two patients. As the third core features of HPMRS, severe or moderate psychomotor retardation was manifested in all patients. Most of them had no speech skills, and more than half of them could walk with or without a support (2, 6, 8, 9, 13–15). In our study, the patients exhibited global developmental delay, particularly evident in language and motor domains.

Other variable features such as microcephaly, hearing loss, strabismus, autism, behavior problem, and abnormal craniofacial features have also been reported (6, 7, 9, 13, 23) (Table 1). Mild facial deformities were observed in the patients from our two family. Their common expression is thick eyebrows, collapsed nasal bridge, at the same time there are hypertelorism in both eyes and a tent-shaped upper lip etc, which reported by the previous literature.Notably, distinct facial dysmorphisms have been documented in literature for several patients with HPMRS-3, including a girl exhibiting a broad nasal bridge and a tented upper lip, as well as a boy presenting with cleft palate, wide palpebral fissures, a short nose with a broad nasal bridge, a tented upper lip, and micrognathia (13). The observed facial dysmorphisms in HPMRS-3 patients resemble those seen in HPMRS patients with different genetic backgrounds (5, 24, 25). However, it is noteworthy that these facial dysmorphisms may attenuate with age (8, 13). It should be emphasized that genetic analysis is imperative for definitive diagnosis of HPMRS-3 and the presence of facial dysmorphisms is not obligatory.

Currently, there is no specific treatment available for HPMRS-3. Some patients achieve seizure control through monotherapy or combination therapy involving ASMs, pyridoxine and folinic acid, or ketogenic diet (9, 13, 14) (Table 1). In certain cases, seizures decrease in frequency over time and may even resolve without medication in older age (8). A case report described a girl with HPMRS-3 who experienced epileptic spasms and exhibited positive response to a ketogenic diet for drug-resistant epilepsy (14). In our Chinese family cohort, both cases mainly manifested epileptic spasms, accompanied by hypsarrhythmia of EEG, which were in line with the characteristics of infantile epileptic spasms syndrome (IESS), and thus were treated with ACTH. The positive responses of these two patients to the ACTH treatment further supported the notion that ACTH is the preferred treatment modality for epileptic spasms caused by various etiologies. For HPMRS-3 presenting with epileptic spasms, ACTH is a potentially effective anti-seizure medicine.

A study demonstrated that supplementation of pyridoxine and folinic acid can effectively correct low levels of PLP and 5-MTHFR in the cerebrospinal fluid of HPMRS-3 patients, suggesting the potential benefit of pyridoxine in managing refractory seizures in HPMRS-3 (9). Subsequently, in 2024, a patient diagnosed with HPMRS-3 also showed partial benefit with high-dose pyridoxine (15). Other studies have also indicated the potential benefits of pyridoxine supplementation in managing intractable seizures in HPMRS patients with other different genetic backgounds (15, 26–29). In our patient, pyridoxine supplementation was administered concomitantly with ASMs, resulting in well-controlled epilepsy but ineffective neurodevelopment. This indicates that further observation on a larger sample size is required to determine the efficacy of using pyridoxine for treating this disease.

Conclusion

In conclusion, this case report represents the first documentation of *PGAP2*-related Hyperphosphatasia with impaired intellectual development syndrome in the Asian population, thereby expanding our understanding of the genetic and phenotypic spectrum of the disease. The patients in this family exhibited the typical clinical features of HPMRS, including elevated alkaline phosphatase (ALP), global developmental delay, seizures, and facial dysmorphisms. The diagnosis of HPMRS3 was confirmed through genetic testing, which revealed compound heterozygosity for variants in the *PGAP2* gene. The c.677c > T(p.Thr226Ile) variants expands the spectrum of PGAP2 gene variants associated with HPMRS3. The administration of ACTH and high-dose pyridoxine demonstrated efficacy in controlling seizures in the two patients, surpassing most previous cases. This finding holds significant clinical implications for the treatment of this disease. However, it should be noted that case reports and systematic studies related to *PGAP2* remain limited, warranting further research on the underlying pathogenic mechanisms and the development of standardized treatment strategies for *PGAP2*-related disorders.

Data availability statement

The data presented in the study are deposited in the Sequence Read Archive (SRA) data repository under the accession numbers SRR31480536, SRR31480535, and SRR31480534, which correspond to the proband, father, and mother, respectively.

Ethics statement

The studies involving humans were approved by Guiyang Maternal and Child Health Care Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

YP: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing, Supervision. BR: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing, Conceptualization, Writing – original draft. LC: Formal Analysis, Investigation, Methodology, Project administration, Writing – review & editing, Conceptualization. QL: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing.

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Conflict of interest

BR, LC were employed by the company Shanghai Nyuen Biotechnology Co., Ltd.

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Application of targeted high-throughput sequencing as a diagnostic tool for neonatal genetic metabolic diseases following tandem mass spectrometry screening

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Background: Tandem mass spectrometry (MS/MS) is a crucial technique for detecting inborn errors of metabolism (IEM) in newborns. However, the high false positive rate poses challenges in diagnosing specific types of diseases. Therefore, this study aimed to evaluate the role of targeted next-generation sequencing (NGS) in the accurate diagnosis of positive samples identified through MS/MS screening.

Methods: A cohort study of 260,915 newborns was conducted from January 2018 to June 2023 in Ganzhou City, southern China. Heel blood samples were collected within 72 h of birth and subjected to MS/MS analysis. Infants with positive MS/MS results underwent targeted NGS to confirm the diagnosis and identify genetic variants.

Results: Among 1,265 suspected cases with positive MS/MS results, 73 were confirmed by NGS, and 12 were identified as carriers of recessive diseases. The overall incidence rate was 1 in 3,574, effectively ruling out 94.2% (1,192/1,265) of the MS/MS false-positive. We found 76 variants in 18 genes associated with 15 types of IEM. Among these, 64.47% (49/76) were pathogenic, 10.53% (8/76) were likely pathogenic. Remarkably, 7.89% (6/76) were identified as novel variants. Variants in *SLC22A5* (NM_003060.4) gene was most prevalent, accounting for 41% (77/188), with hotspot variants including c.51C > G, c.1400C > G, and c.338G > A.

Conclusion: Targeted NGS technology can serve as a crucial diagnostic tool for neonatal genetic metabolic diseases following MS/MS screening. Additionally, we identified IEM variant hotspots and some novel variants in our region, which are the underlying causes of disease in patients with IEM.

KEYWORDS

genetic diagnosis, inborn errors of metabolism, newborn screening, targeted sequencing, tandem mass spectrometry

1 Introduction

Newborn screening (NBS) is a successful public health project that employs advanced testing techniques to detect some serious inherited metabolic diseases in newborns. This allows for early diagnosis and treatment before clinical manifestations occur, thereby preventing irreversible damage in children. According to statistics, genetic diseases occur in 3–5% of live births (1). Since Guthrie and Susi (2) first reported the bacterial inhibition test for phenylketonuria (PKU) screening in 1963, NBS has gained global recognition and is now a crucial tool in reducing neonatal morbidity and mortality. The implementation of NBS not only provides immediate health benefits for children diagnosed and treated early but also enables their participation in social activities and alleviates the burden on families. Traditional biochemical screening is currently the mainstream NBS method, including tandem mass spectrometry (MS/MS), electrophoresis technology, enzymology, immunology, and electrophoresis technology-high-pressure liquid chromatography (3). MS/MS technology is characterized by its high efficiency, sensitivity, and convenience, enabling early disease diagnosis (4). However, the spectrum of diseases tested by blood MS/MS is limited, and different diseases can result in elevations of the same metabolites, blood MS/MS testing has limited usefulness in accurate disease diagnosis. Moreover, metabolites can be influenced by various factors such as diet, underlying diseases, and preterm birth, potentially leading to false-positive and false-negative results, requiring further diagnosis (5).

In recent years, with the development of DNA sequencing technology, the focus on inborn errors of metabolism (IEM) screening technology has shifted from the metabolite level to the genetic level. Next-generation sequencing (NGS) technology was employed to discover the genetic factors of thousands of genetic diseases. Therefore, NGS is valuable for genotyping and detecting the genetic factors of IEM. By comprehensively assessing IEM based on the quantification of metabolites and genetic variants, NGS can effectively improve the accuracy of IEM screening, compensating for the limitations of MS technology (6).

In this study, we analyzed data from the NBS program with MS/MS over the past 6 years. Target NGS of genes in a custom panel was employed as a second critical step to diagnose high-risk infants identified by MS/MS, aiming to provide a definitive genetic diagnosis and determine IEM's genetic characterization. This work has enhanced the quality of NBS programs, providing a more accurate diagnosis for children with IEM and consequently enabling more precise targeted therapy.

2 Materials and methods

2.1 Study design and participants

From January 2018 to June 2023, a total of 260,915 newborns underwent screening for IEM at the Ganzhou Maternal and Child Health Hospital in Jiangxi Province, China. Among these, 1,265 infants tested positive and received genetic diagnoses through NGS. Subjects were all newborns who had completed 72 h after birth and had been fed adequately at least eight times. Other inclusion criteria were complete medical history. Additionally, newborns were excluded if they were undergoing emergency surgery or external blood transfusion. The clinical characteristics of newborns with suspected IEMs were all fully understood by a single physician. The clinical data included sex, major clinical features, and outcomes of IEM. The confirmatory tests vary depending on the disease, including genetic testing or blood biochemical indices testing, enzyme activities testing and urine organic acids analysis, etc. Pretest counseling was performed by physicians. The study was approved by the ethics committee of the Ganzhou Maternal and Child Health Hospital (2020001). The legal guardians of the participating infants gave their written informed consent for their children to be included in the study.

2.2 MS/MS screening method

Heel blood samples were collected from newborns, dripped on filter paper (Schleicher & Schue11 903, Wallac OY Turku, Finland), and dried naturally at room temperature. Dried blood spots were pretreated using a non-derivative MS/MS kit per the manufacturer's instructions (Fenghua, China) and then analyzed using a MS/MS system (Acquity UPLC-TQD, MA). Newborns with abnormal amino acid or carnitine (free carnitine and acylcarnitine) indices were recalled for recollection of heel blood (filter paper dried blood spot specimens) for rescreening. Additionally, mothers of newborns with positive results of free carnitine (CO) and 3-hydroxyisovaleryl-carnitine (C5OH) were recalled for re-examination to rule out maternal origin. If both screens were MS/MS-positive, the newborn was suspected of IEM.

2.3 NGS

Blood samples of patients and any participating family members were collected, and genomic DNA was extracted using the QIAamp DNA Mini Kit (Hilden, Germany) following the manufacturer's protocol. The coding exons of target genes were captured using an Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, CA, USA), and libraries generated from enriched DNA were sequenced using the Illumina NovaSeq 6,000 platform (Illumina Inc., San Diego, CA, USA) in the paired-end mode. The average on-target sequencing depth for exome sequencing was 90X. The sequencing reads were aligned to the human reference genome (UCSC GRCh37/hgl9) using the Burrows-Wheeler Aligner. Variant filtering was performed with the PhenoPro (7) phenotype-scoring algorithm. Detected variants were confirmed by PCR and subjected to direct automated sequencing using a 3500XL Genetic Analyzer (Applied Biosystems) per the manufacturer's specifications. The variant's pathogenicity was determined using the criteria established by the American College of Medical Genetics and Genomics (8).

3 Results

3.1 General results of NBS

A total of 260,915 newborns underwent MS/MS screening and 1,265 infants (687 male and 578 female) tested positive. The positive results were mainly divided into abnormal amino acid and abnormal

acylcarnitine markers. There were amino acid abnormalities, such as increased phenylalanine (Phe) and citrulline (Cit) (15.3 and 9.4% positivity rates, respectively), and carnitine abnormalities, such as decreased CO and increased isovaleryl-carnitine (C5) and C5OH (21.8, 9.3, and 8.1% positivity rates, respectively). Additionally, 3.7% of positive infants had simultaneously elevated or reduced indicators for certain amino acids or acylcarnitines. Following clinical and genetic diagnoses, 73 cases of IEM in newborns were diagnosed, with an overall incidence rate of 1 in 3,574 (Table 1). There cases were related to 12 IEM diseases, including 3 cases of fatty acid metabolic disease (39/73, 53.4%), 3 cases of amino acid metabolic disease (23/73, 31.5%), and 6 cases of organic acid metabolic disease (11/73, 15.1%). The highest incidence rate was that of primary carnitine deficiency (PCD, 1/7,248), followed by that of phenylketonuria (PKU, 1/15,348) and citrine deficiency (CD, 1/43,486). Additionally, 5 cases of PCD were confirmed in mothers of newborns. The overall detection rate of IEM screening in the 260,915 newborn screening population was 1/3345. Figure 1 shows the workflow of NBS.

3.2 Results of IME genetic diagnosis

Among the 1,265 infants suspected of having IEM, we performed genetic diagnosis utilizing targeted NGS technology. Following genotyping and interpretation, 73 cases were confirmed as IEM (Supplementary Table S1), comprising 46 cases of compound heterozygosity and 27 cases of homozygosity. Additionally, 12 cases were identified as carriers of recessive disorders (Supplementary Table S2). Specifically, among 73 infants with IEM, MS/MS testing suggested some forms of IEM in 49 cases (49/73, 67.1%). The result in P59 via NGS was inconsistent with that of MS/MS. The results of NGS revealed the homozygosity of *SLC25A13* c.851_854del (p.M285Pfs), classified as CD. In the remaining 48 infants (48/73, 65.8%), the genetic result was consistent with that of MS/MS. Additionally, MS/MS revealed that 24 infants (24/73, 32.9%) suffered from certain kinds of IEM, and then disease types were identified by NGS. Among 24 infants, 17 (P40–P56) showed an increase in Phe and Phe/Tyr by MS/MS detection and 13 were cases of phenylalanine hydroxylase deficiency, while four were cases of tetrahydrobiopterin deficiency by NGS. Three infants (P70–P72) showed an increase in C3 and C3/ C2 by MS/MS detection and two were cases of methylmalonic acidemia and one was a case of propionic acidemia by NGS. Four infants (P63-P66) were detected by MS/MS with C_5OH increasing; one was a case of biotinidase deficiency and three were cases of 3-Methylcrotonyl-CoA carboxylase deficiency by (3MCC) NGS. Results from confirmatory biochemical tests were employed to verify the genetic findings (Supplementary Table S3). The consistency was observed in the outcomes of 73 cases of genetically confirmed IEM abnormalities. Overall, 72 true positive cases and one false negative case were identified through NGS, and 94.2% (1,192/1,265) of the false positivity results were excluded (Supplementary Table S4).

3.3 Analysis of genetic variation

Among 1,265 infants with suspected IEM, 76 variants involving 18 IEM-related genes were detected by NGS. Approximately 64.47% (49/76) of the variants were classified as pathogenic, 10.53% (8/76) were likely pathogenic, and 18.42% (14/76) were categorized as being of uncertain significance, based on the ACMG guidelines and criteria (Supplementary Table S5). The annotation results indicated that 71.1% (54/76) were missense variants, 7.9% (6/76) were frameshift variants, 10.5% (8/76) were splice variants, 6.6% (5/76) were nonsense variants, and 3.9% (3/76) were inframe variants. Additionally, 7.89% (6/76) were novel variants which has not yet been included in the Human Gene Mutation Database, the 1,000 Genomes Project and the Exome Aggregation in the Consortium. These six novel variants were located

TABLE 1 The incidence and spectrum of 260,915 newborns in the screening program.

Types of diseases	Cases (*n*)	Accounting for patients (%)	Incidence
Fatty acid metabolic disease	39	53.4	
Primary carnitine deficiency	36	49.3	1/7,248
Short-chain acyl-CoA dehydrogenase deficiency	2	2.7	1/130,458
Medium chain acyl CoA dehydrogenase deficiency	1	1.4	1/260,915
Amino acid metabolic disease	23	31.5	
phenylalanine hydroxylase deficiency	13	17.8	1/20,070
Citrin deficiency	6	8.2	1/43,486
Tetrahydrobiopterin deficiency	4	5.5	1/65,229
Organic acid metabolic disease	11	15.1	
3-Methylcrotonyl-CoA carboxylase deficiency	3	4.1	1/86,972
Glutaric acidemia type I	3	4.1	1/86,972
Methylmalonic acidemia	2	2.7	1/130,458
Biotinidase deficiency	1	1.4	1/260,915
Propionic acidemia	1	1.4	1/260,915
Isovaleric acidemia	1	1.4	1/260,915
Total	73	100%	1/3,574

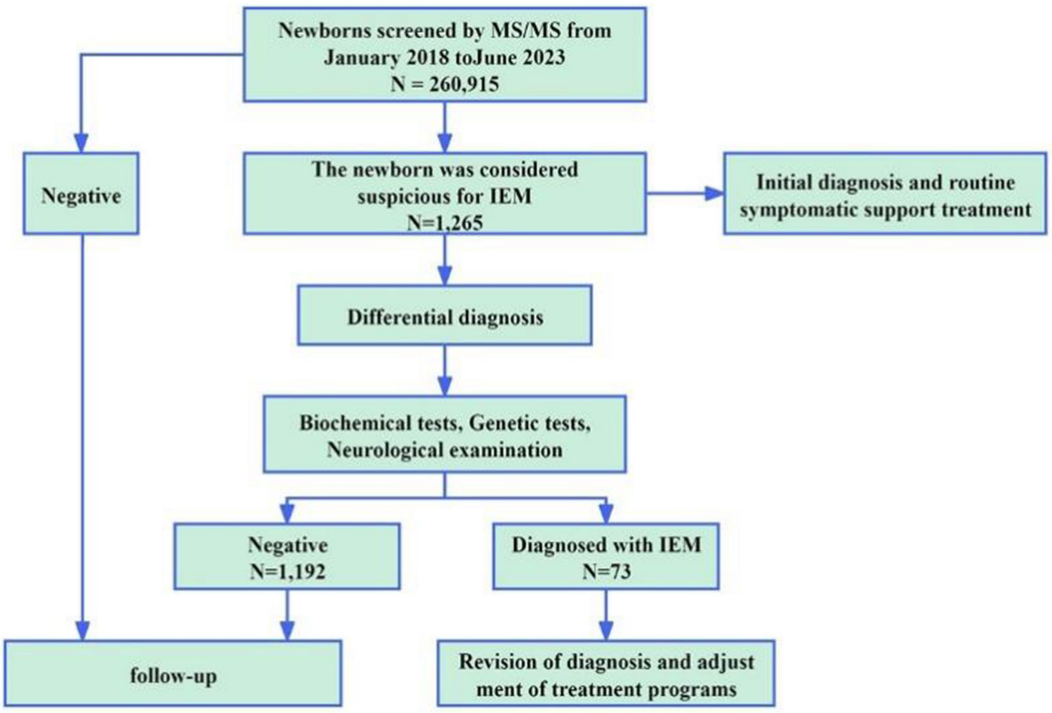

FIGURE 1
Screening and diagnosis of IEM.

in four genes, including c.547G > T (p.E183Ter) and c.948delA (p.E316Ter) in *PAH* (NM_000277.1) associated with PKU, c.628G > A (p.E228K) and c.79A > C (p.T27P) in *ACADS* (NM_000017.4) causing short-chain acyl-CoA dehydrogenase deficiency, c.1364G > C (p.R455P) in *SLC25A13* (NM_014251.3) causing CD, and c.493A > C (p.T165P) in *MCCC1* (NM_020166.5) causing 3MCC. These results broadened our understanding of the IEM diseases. Additionally, our findings revealed that variants of the *SLC22A5* gene were the most prevalent, accounting for 41% (77/188) of all identified variants. In 40 cases of primary carnitine, c.51C > G in NM_003060.4 is one of the most common variant, accounting for 36.4% of all variants (28/77) and affecting 55% (22/40) of patients, followed by c.1400C > G (17/77, 22.1% and 17/40, 42.5%). Additionally, variants of the *PAH* gene were also common, accounting for 18.1% (34/188). The most common variant was c.728G > A in NM_000277.3, accounting for 26.5% (9/34) of all variants and affecting 41.2% (7/17) of patients, followed by c.611A > G (5/34, 14.7% and 4/17, 23.5%). Additionally, variants of *SLC25A13* were also common, accounting for 8.0% (15/188). The most common variant was c.852_855del in NM_014251.3, which accounted for 66.7% (10/15) of all variants and affecting 66.7% (6/9) of patients. These variants are pathogenic. These results reflected the variant characteristics of IEM diseases in Ganzhou and provided important information for the clinical diagnosis of other samples in the future.

4 Discussion

IEM is a group of diseases that affect the growth and development of newborns and children and even lead to death. Their occurrence is associated with genetic defects in the biosynthesis process of the skin, protease, receptor, carrier, and membrane pump, which the body needs to maintain normal metabolism (9). IEM often leads to progressive and irreversible nerve damage and physical and mental disability, posing a major threat to families and society. In this study, 260,915 neonates were screened for IEM using MS/MS technology, and 12 diseases were detected, with an overall incidence rate of 1 in 3,574. Compared with other regions of China such as Taiwan (10) (1/7,030) and Liuzhou (11) (1/3,733), the overall incidence rate is higher. Hence, performing early IEM screening and accurate diagnosis in this area is of particularly importance.

MS/MS has proven to be a reliable method suitable for clinical use, offering many advantages such as high efficiency, sensitivity, and convenience (4). However, biochemical screening has limitations and *in vivo* metabolism is influenced by many factors, leading to the existence of false positives and lowering the positive predictive value. In this study, 1,265 infants were positive in the MS/MS screening, and 73 cases were ultimately diagnosed by NGS, indicating a 94.2% false-positive rate. The simultaneous increase or decrease of several indicators of amino acids or acylcarnitines in the positive results of MS/MS screening can be influenced by various factors such as gestational age at birth, certain diseases, medications, diet and maternal factors, of which can lead to transient or secondary metabolic disorders (5). Per our findings, C5 was common in acylcarnitine-positive indicators in MS/MS; however, only one case of isovaleric acidemia was diagnosed via NGS. False-positive cases should be followed up on. It was reported that there was the presence of isomers in metabolites, including isovalerylcarnitine, tervalerylcarnitine, and 2-methylbutyrylcarnitine, that are difficult to distinguish by MS/MS (12). It is critical to further clarify the nature of

the disease, implement targeted therapy, and exclude false alarms to reduce the unnecessary economic, physical, and mental burden on children and their families (13).

In recent years, an increasing number of genetic detection techniques have been employed in the field of NBS (14, 15). Most studies have indicated that the application of targeted NGS technology advanced NBS diagnosis and treatment and reduced the diagnostic delay (16). In this study, we designed an NGS-based genetic diagnostic panel for IEM. All children underwent identification using the NGS panel and received a definitive diagnosis. Among the identified IEM cases, PCD was the most frequently diagnosed, accounting for 49.3% (36/73) of the total. PCD, also known as the carnitine transport disorder or carnitine uptake disorder, is a fatty acid beta-oxidation disorder resulting from a variant of *SLC22A5* that encodes the carnitine transporter OCTN2 located in the cell membrane (17, 18). PCD is an autosomal recessive inherited disease with an incidence of approximately 1/300–142,000, varying across different countries and ethnic groups (19). The incidence of PCD in NBS in this area is 1/7,248, making it one of the highest incidence areas in China, comparable to Liuzhou (11), Quanzhou (20), and other areas. The most reliable and rapid method for early PCD diagnosis is the MS/MS detection of the CO level (21). However, the CO results of MS/MS screening can be affected by various factors, including the maternal CO level, prematurity, and inadequate intake, and other fatty acid oxidation defects (22). In this study, we employed targeted NGS to advance the differential diagnosis of children with suspected PCD, thereby eliminating false positives resulting from these factors. Several pathogenic variants of *SLC22A5* (NM_003060.4) were found, including c.51C > G, c.1400C > G, c.428C > T, c.338G > A, and c.760C > T, with c.51C > G and c.1400C > G having the highest frequency. It has been reported that c.760C > T (p.R254X) and c.1400C > G (p.S467C) are the two most common variants in patients with PCD (20). However, in this study, the main variants observed were c.51C > G (p.F17L) and c.1400C > G (p.S467C). Previous studies have reported a low variant frequency of c.760C > T (p.R254X) in asymptomatic neonates (23). This finding is consistent with the results of the present study.

PKU is the most common disease of abnormal amino acid metabolism and has the second-highest incidence in this study (1/15,348). This incidence is close to the prevalence rate of live births (1/15,924) in China (24). The incidence of PKU varies considerably between geographical regions, with China having the highest incidence among Asian countries (25). Its pathogenesis is associated with the variants in *PAH,* which encodes the phenylalanine lightening enzyme (26). If patients are not treated promptly, severe and irreversible mental impairment, growth retardation, psychological behavior, acquired microcephaly, systemic skin hypopigmentation, and musty sweat odor may occur (27). The use of MS/MS to detect the Phe concentration and the Phe/Tyr ratio in newborns enable the early detection of PKU in children. However, this method cannot distinguish between different phenotypes; therefore, it may not be suitable for timely and appropriate treatment (28). Thus, the key to treating PKU lies in further clarifying the exact type of PKU. This study demonstrates the effectiveness of targeted NGS technology in eliminating false positives in MS/MS screening and identifying the PKU type and genotype. This enables accurate targeted therapy for infants with specific types of PKU. Consistent with previous reports, early diagnosis and treatment contributed to favorable outcomes for patients with PKU (29). There is a high degree of variability in *PAH* (NM_000277.3), as two of the first variants found were c.1315 + 1G > A and c.1222C > T (p.Arg408Trp) (30). Within a few years, many new variants were discovered, and two of these new variants c.547G > T (p.E183Ter) and c.948delA (p.E316Ter) were also found in this study. According to ACMG and the available evidence, these new variants were classified as pathogenic. To date, over 800 variants in *PAH* have been identified, encompassing more than 100 different types of variants in children with PKU in China (31). It is noteworthy that there were variations in hotspot variants in *PAH* among different regions and populations. According to the results of large-sample research conducted in mainland China (26), the most common variant sites included c.728G > A, c.611A > G, c.331C > T, c.1238G > C, and c.442-1G > A, with c.728G > A having the highest variant frequency. The variant characteristics of *PAH* in this study were consistent with the results of large-cohort studies conducted in mainland China, including eastern China (18) and Nanjing (32). However, the hotspot variant c.158G > A detected in this study is uncertain and requires further validation.

CD, which is inherited in an autosomal recessive manner, is the most common disorder of the urea cycle. It is caused by pathogenic variants of *SLC25A13* (33) and results in a broad spectrum of phenotypes ranging from life-threatening hyperammonemia in neonates to adult-onset hyperammonemia with mild symptoms or no manifestations at all. The detection of neonatal blood amino acids (Cit, Cit/Arg) by MS/MS has a high sensitivity for the early diagnosis of CD children. However, an increasing number of case reports have found that the clinical manifestations and laboratory abnormalities of CD patients are varied and transient (34). This study identified six cases of CD through targeted NGS. One case was detected due to abnormal levels of CO, while the initial results for Cit or Cit/Arg were within the normal range. Therefore, while highly biochemical indicators are not strictly necessary, a combination of clinical manifestations and genetic analyses is essential for making an accurate diagnosis (35). With an incidence rate of 1/43,486, it ranked third in our study. A previous study indicated that the incidence rate in southern China is significantly higher than that in northern China, with provinces at lower latitudes having significantly higher incidence rates than those at higher latitudes (36). In *SLC25A13* (NM_014251.3), c.852_855del, c.1638_1660dup, c.615 + 5G > A, and c.1751-5_1751-4ins were the most common variants in China, accounting for 82.9% of all variants (37). In our study, we observed that c.852_855del was the most prevalent, accounting for 66.7% of cases. These findings are in line with results of previous studies (38).

Although targeted NGS technology has demonstrated many advantages in clinical applications, its high cost compared with MS/MS technology may limit its widespread use in resource-limited areas (39). Furthermore, targeted NGS primarily focuses on detecting known potential targets and exhibits limitations when addressing complex genomic variants, such as structural and copy number variations (40). This limitation could lead to the omission of certain disease-associated variants, potentially impacting clinical decision-making.

5 Conclusion

In summary, targeted NGS technology can serve as a crucial diagnostic tool for neonatal genetic metabolic diseases. Its combination with MS/MS technology proves effective and suitable for clinical screening and diagnosis. Additionally, we identified IEM variant hotspots and some novel variants in our region. These variants

are the cause of IEM in certain patients, helping to elucidate the etiology of the disease at the genetic level.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Ganzhou Maternal and Child Health Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

GL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. QG: Data curation, Software, Writing – original draft. ZL: Investigation, Methodology, Software, Writing – original draft. HC: Data curation, Formal analysis, Methodology, Project administration, Supervision, Writing – original draft. XT: Data curation, Project administration, Supervision, Writing – review & editing. JC: Conceptualization, Data curation, Methodology, Writing – review & editing. JH: Methodology, Project administration, Software, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1461141/full#supplementary-material

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A gut instinct for childhood leukemia prevention: microbiome-targeting recommendations aimed at parents and caregivers

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Childhood leukemia accounts for 30% of all pediatric cancer cases with acute lymphoblastic leukemia (ALL) being the most common subtype. Involvement of the gut microbiome in ALL development has recently garnered interest due to an increasing recognition of the key contribution the microbiome plays in maintaining the immune system's homeostatic balance. Commensal gut microbiota provide a first line of defense against different pathogens and gut microbiome immaturity has been implicated in ALL pathogenesis. Several environmental factors such as nutrition, mode of delivery, breastfeeding and, early social or livestock contacts are known to alter the composition of the gut microbiota. Variations in these factors influence the risk of childhood leukemia onset. This review aims to elucidate the risk factors influencing microbial composition in the context of childhood ALL. The link between gut microbiome diversity and childhood ALL offers the opportunity to develop risk-reducing strategies that can be communicated to a broad target population of (future) parents and caregivers for childhood leukemia prevention. Here, we summarize evidence on how promoting a diverse gut microbiome in newborns through simple measures such as increasing social contacts early in life may decrease the risk of developing ALL in these children later on.

KEYWORDS

childhood leukemia, risk factors, gut microbiome, prevention, recommendations, public health

1 Introduction

Cancer is the second most frequent cause of death among children in developed countries and acute lymphoblastic leukemia (ALL) is the most common subtype accounting for 30% of childhood cancer cases (1). ALL incidence peaks at 2–4 years of age (Figure 1A) and is increasing steadily (2). Around 80% of childhood ALL cases are characterized by proliferation of abnormal B-cell progenitors characterized as B-cell precursor ALL (BCP-ALL) (3). Although the 5-year survival rate of children with BCP-ALL has improved significantly, there are several adverse side effects associated with treatment (4). Today, there are about half a million childhood cancer survivors living in Europe and two-thirds of them suffer from acute and late treatment-related toxicities, accounting for a large proportion of deaths (4, 5). For instance, chemotherapy treatment for childhood ALL can affect all organs and cause acute and persistent organ damage (4). Common acute adverse effects include opportunistic infections, mucositis, neuropathy, thromboembolism, bone toxicities, endocrinopathies, hypersensitivity, pancreatitis, nephrotoxicity, thrombosis, and hyperlipidemia (3, 4). Long-term toxicity stemming from the treatment such as cognitive impairment, osteonecrosis, secondary cancers, infertility and, depression, can be severe and alter the socioeconomic participation of at least half of those affected (6). The increasing childhood cancer incidence in Europe highlights the need to shift the current paradigm from therapy to prevention (7). Preventive strategies could circumvent traumatic and toxic treatments, associated life-long health sequelae, and the experience of relapse or treatment resistant leukemia subtypes, which occur in about 20% of cases (8). In addition, adopting preventive strategies could significantly reduce the cumulative public health burden incurred by an increasing number of adult cancer survivors that suffer from the aftereffects of treatment and a decreased life quality (5).

Childhood ALL is frequently triggered by genetic mutations somatically acquired before birth (Figure 1B) (3). The most common event is the translocation t(12,21)(p13;q22) generating the *ETV6::RUNX1* fusion gene (3). Secondary oncogenic gene alterations are necessary for overt leukemia and are likely driven by modifiable environmental and lifestyle factors, therefore making ALL in principle a preventable disease (9). Several factors known to increase the risk of developing ALL also have a strong impact on the composition of the gut microbiota (Figure 2).

Indeed, a potential causative involvement of the microbiome in the development of ALL is increasingly recognized (10). This is particularly relevant in the context of Greaves' delayed infection theory of childhood leukemia development proposing that exposure to common infectious agents may trigger leukemia in genetically predisposed children (11). The delayed infection theory highlights the role of typical common childhood infections, including respiratory or gastrointestinal pathogens, which might influence immune system dysregulation in genetically susceptible children (11). There are reports of BCP-ALL space-time clusters associated with different specific pathogens: adenovirus for 13 patients in the Fallon cluster, streptococcal fever for 8 same-school patients in the Niles cluster, and influenza A H1N1 swine flu virus for seven patients in the Milan cluster (12–15). Furthermore, Christoph et al. identified virus sequences corresponding to common human pathogens, such as *Anelloviridae, Herpesviridae*, and *Parvoviridae* family in 11 B-ALL cases analyzed by whole genome sequencing (16). In addition, a study in UK observed peaks of BCP-ALL ~6 months after seasonal influenza epidemics. According to Greaves (as illustrated in Figure 1B), if the exposure of a child's immune system to infectious triggers is delayed due to lack of social contacts or an exaggerated hygiene, the eventual immune response may be dysregulated and lead to the progression of pre-leukemic cells (3).

The gut microbiome and immune system co-develop during early infancy and childhood. During this period the gut microbiome plays a major role in shaping host's immunity (10). Commensal microbiota provide a first line of defense against different pathogens, and gut microbiome immaturity due to genetic or lifestyle factors results in untrained immunity which could promote the switch toward overt leukemia in genetically predisposed children (10). *In vivo* mouse models demonstrated that the lack of commensal microbiome alone could be sufficient to promote leukemia in genetically predisposed mice, simply by inducing a microbiome disruption via early-life antibiotic treatment (2). Infections combined with an altered microbiome composition may not only provide sufficient proliferative stimuli for pre-leukemic cells to induce the acquisition of secondary oncogenic driver mutations but also drive a pro-inflammatory and immunosuppressive hematopoietic niche supporting a leukemia-favorable microenvironment (3, 10).

The gut microbiome and the immune system develop simultaneously starting *in-utero*. Different prenatal and postnatal factors influence this process (Figure 3) (17). During pregnancy, placental transmission of bacterial-derived metabolites originating from maternal diet and microbiota initiate the priming and development of the immune system, emphasizing the importance of a healthy maternal nutrition in preventing ALL (17, 18). Although essential components of both innate and adaptive immunity already develop at the prenatal stage, they predominantly evolve after birth alongside with the diversification of the microbiome (17, 19). At birth, the mode of delivery directly determines the most abundant type of bacteria present in a newborn's intestine (17). Gut microbiome of vaginally delivered children mainly consists of gram-negative bacteria capable of synthesizing lipopolysaccharides, which are known to be effective stimulators of innate immunity (20). In contrast, the microbiome of cesarean section (C-section) delivered babies is mainly composed of opportunistic pathogens circulating in the hospital's environment (21). These initial variations in microbial composition can strongly impact the innate lymphoid cell maturation. However, any defects in essential microbiota can be mitigated by immediate parent/caregiver-baby skin-on-skin contact or maternal vaginal/fecal microbiota transplantation to the baby, known as vaginal seeding (20–22). During lactation, breastfeeding provides specific prebiotics (such as inulin and human oligosaccharides) that stimulate the growth of commensal microbiota and thereby affect immune responses, particularly those of the innate arm (23). Eventually, the gut microbiome matures due to social and livestock interactions, infections and possibly active immunization via vaccination, resulting in an increased microbial diversity, which

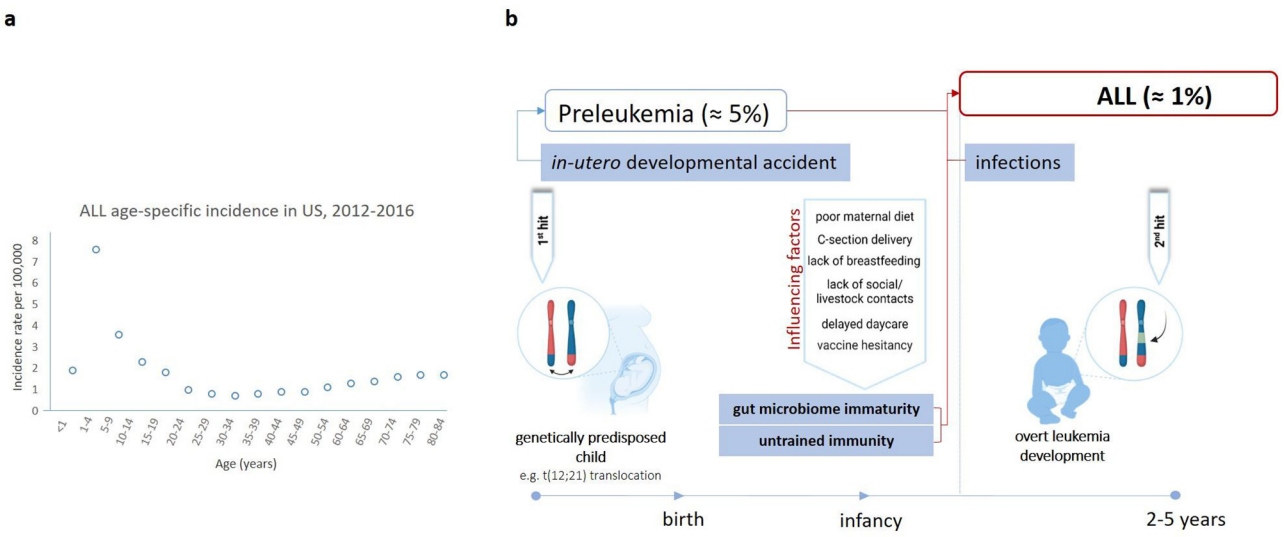

FIGURE 1
ALL age distribution and the "two hit and delayed infection model" of ALL pathogenesis. **(A)** ALL age distribution in the USA from 2012 to 2016, with a peak at 1–4 years. Incidence per 100,000 cases is shown (2). **(B)** The "two-hit theory" suggests that ALL occurs in a two-step process (3). The first one involves a genetic mutation, e.g., the $t_{(12,21)}$ translocation, which arises *in-utero* predisposing the child to leukemia (~1–5% of healthy newborns) (9). However, the acquisition of additional mutations is crucial for leukemia onset (3). These mutations are primarily driven by oncogenic deletions occurring in ~1% of genetically predisposed children likely due to a dysregulated immune response upon exposure to one or more common infectious agents (3). This is especially critical for those children who were raised in an exaggerated hygienic environment, pointing to a potential association between the gut microbiome and ALL development (10). Hence, other factors affecting gut microbiome diversity, including maternal diet, mode of delivery, breastfeeding, vaccination, social, and livestock contacts are also thought to be implicated (10, 24). Figure was created on BioRender.com.

has a profound impact on both adaptive and mucosal immunity (17, 24).

In this review, we summarize the current knowledge on childhood leukemia risk factors that are known to influence the immune system and the microbial constitution of the child's gut. We discuss evidence on how factors such as childbirth mode, breastfeeding, commercial milk substitutes, early life social and livestock contacts, and vaccination may influence microbiome composition and increase or reduce the risk of ALL development. We also discuss how parents or caregivers can compensate for lack of microbial seeding in newborns after birth. Based on the collected evidence, we eventually provide simple recommendations that can be communicated to parents and caregivers to reduce the individual risk of childhood leukemia.

2 Fetal stage

2.1 Maternal nutrition and ALL risk

Robust evidence indicates that microbiome colonization already starts *in utero* (19, 25). Maternal diet influences fetal development by affecting epigenetic, DNA synthesis and repair processes. Furthermore, maternal diet affects fetal immune establishment and may potentially impact leukemia initiation (25). Maternal diet may influence the infant gut microbiome composition through vertical microbial transmission via vaginal delivery and breastfeeding, contributing to the infant's immune development (26). A study of mother-infant couplets recruited in the New Hampshire Birth Cohort investigating the association between maternal diet components and fetal microbiome confirms the influence of maternal diet on the infant gut microbiome as stratified by the delivery mode (26). The large multiethnic case-control California Childhood Leukemia Study (CCLS), concluded that higher maternal diet quality, rich in one-carbon nutrients and vitamin supplements before and during pregnancy correlated with a lower risk for ALL in offspring (OR = 0.88, Cl 0.78–0.98) and similarly a reduced risk for acute myeloid leukemia (AML) (OR = 0.76, Cl 0.52–1.11) (26). A recent meta-analysis on maternal diet and ALL risk indicated an inverse relationship between ALL risk and maternal consumption of fruits (OR 0.71; 95% Cl 0.59–0.86), as opposed to coffee intake (OR 1.45; 95%, Cl, 1.12–1.89) (27). This could be explained by the fact that fruits are a source of vitamins, minerals, and folate, all known to be implicated in DNA methylation and repair (25, 27) adjusted for maternal educational attainment and gestational diabetes, but not for socio-economic or maternal health status. In this study, adjustments were done for maternal educational attainment and gestational diabetes, but not for maternal health conditions or socioeconomic status. Similarly, several studies associate an elevated childhood leukemia risk to a diet low in vitamin A and minerals (particularly selenium) and report a direct association of reduced ALL risk and maternal diet containing eggs, seafood, fish and poultry meat, with the exception of red or processed meat (28–30). Eggs, fish and poultry are respectively rich in choline, folate and omega-3 fatty acids,

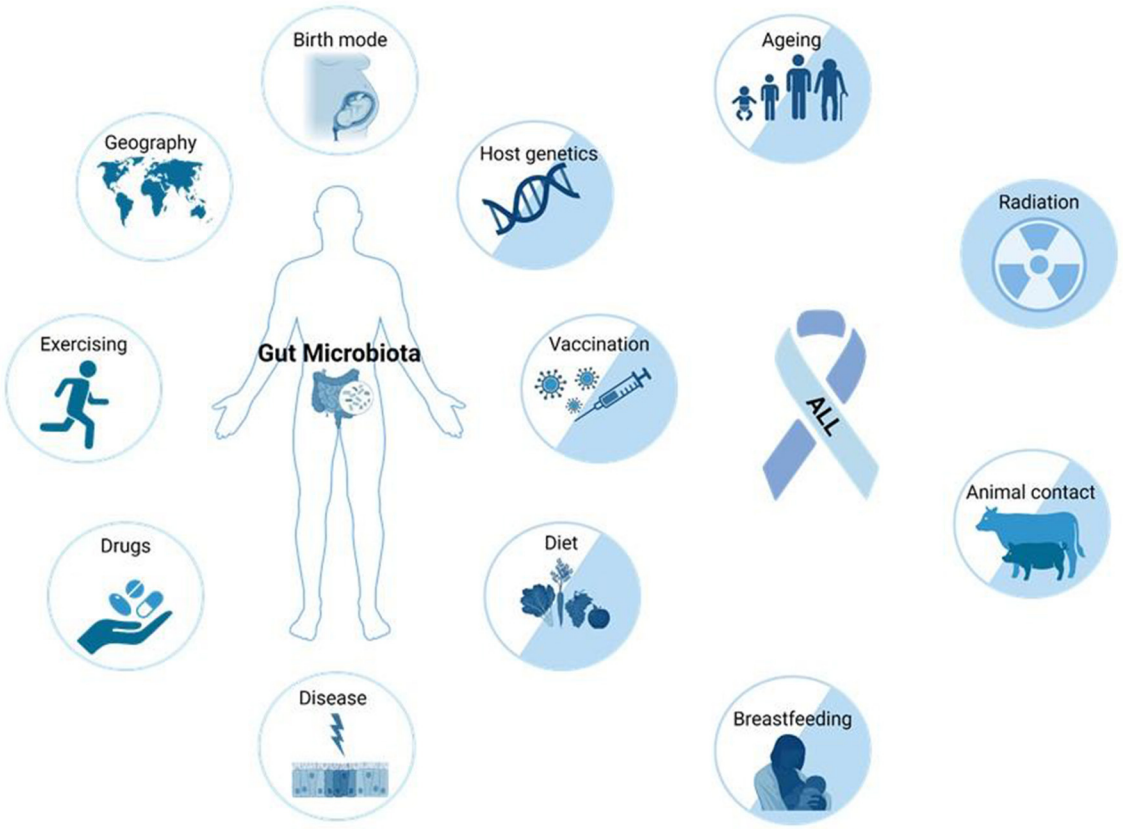

FIGURE 2
Factors that can influence the composition and function of the human gut microbiota, and alter ALL risk. Many external (drugs, exercise, geography, birth mode, vaccinations, diet, breastfeeding, and animal contact) and intrinsic factors (host genetics, disease, and aging) impact microbiome composition. The etiology of ALL is not yet fully understood, but causal connections to many factors accepted to influence the microbiome are also known to impact the risk of ALL development (factors represented by two-colored circles) (10). Figure was created on BioRender.com.

which play an essential role in histone modification, anti-inflammatory processes and/or DNA methylation, reducing the likelihood of epigenetic alterations that may lead to chromosomal aberrations predisposing for ALL. Caffeine intake may inhibit DNA topoisomerase II, potentially giving rise to chromosomal aberrations described in childhood ALL (22, 31, 32). These findings apply to other food/drinks that contain DNA topoisomerase II inhibitors (Table 1). Moreover, studies within the NewGeneris cohort have examined maternal caffeine intake and an increased frequency of micronuclei in neonatal blood, linking dietary caffeine exposure to chromosome instability, genome rearrangements, and mutagenesis. However, chromosomal aberrations have not been analyzed directly.

Herbal tea intake in pregnancy seems to uphold protective properties against ALL occurrence, potentially due to its low caffeine content and richness in flavonoids engaged in anti-proliferative and antioxidant processes (33, 34). Tobacco and alcohol intake evidently remain a threat due to their interference respectively with caffeine and folate metabolism (25, 35).

Furthermore, dietary carcinogens including nitrosamines, ingested by the mother can pass through the placenta and expose the fetus to pre-leukemic transformative stimuli and increase the risk of childhood leukemia development (36). The NewGeneris Cohort study assessed the transplacental transmission of biomarkers of dietary exposure to carcinogens, such as oxidative fat metabolites, acrylamide, PAHs and nitrosamines (Table 2) in 1151 newborn cord blood samples. This was done by quantifying the levels of reactive metabolites bound to either hemoglobin or DNA (36). This technique primarily focuses on detecting adducts, which are formed when reactive metabolites bind to hemoglobin or DNA, as biomarkers of exposure to genotoxic compounds. Hemoglobin and DNA adducts were analyzed using high resolution mass spectrometry, whereas dioxin was measured via a commercially available validated bioassay (Dioxin Responsive Chemical Activated LUciferase gene eXpression or short "DR CALUX" bioassay). Indeed, most of the newborns were exposed to (pre)carcinogens and a successive analysis of this study found a significant association between the numbers of micronuclei in the cord blood lymphocytes and the level of exposure to maternal dietary carcinogens (36). Micronuclei are cytogenetic biomarkers whose frequency correlates to carcinogen-induced cancer risk. The study above highlights potential molecular

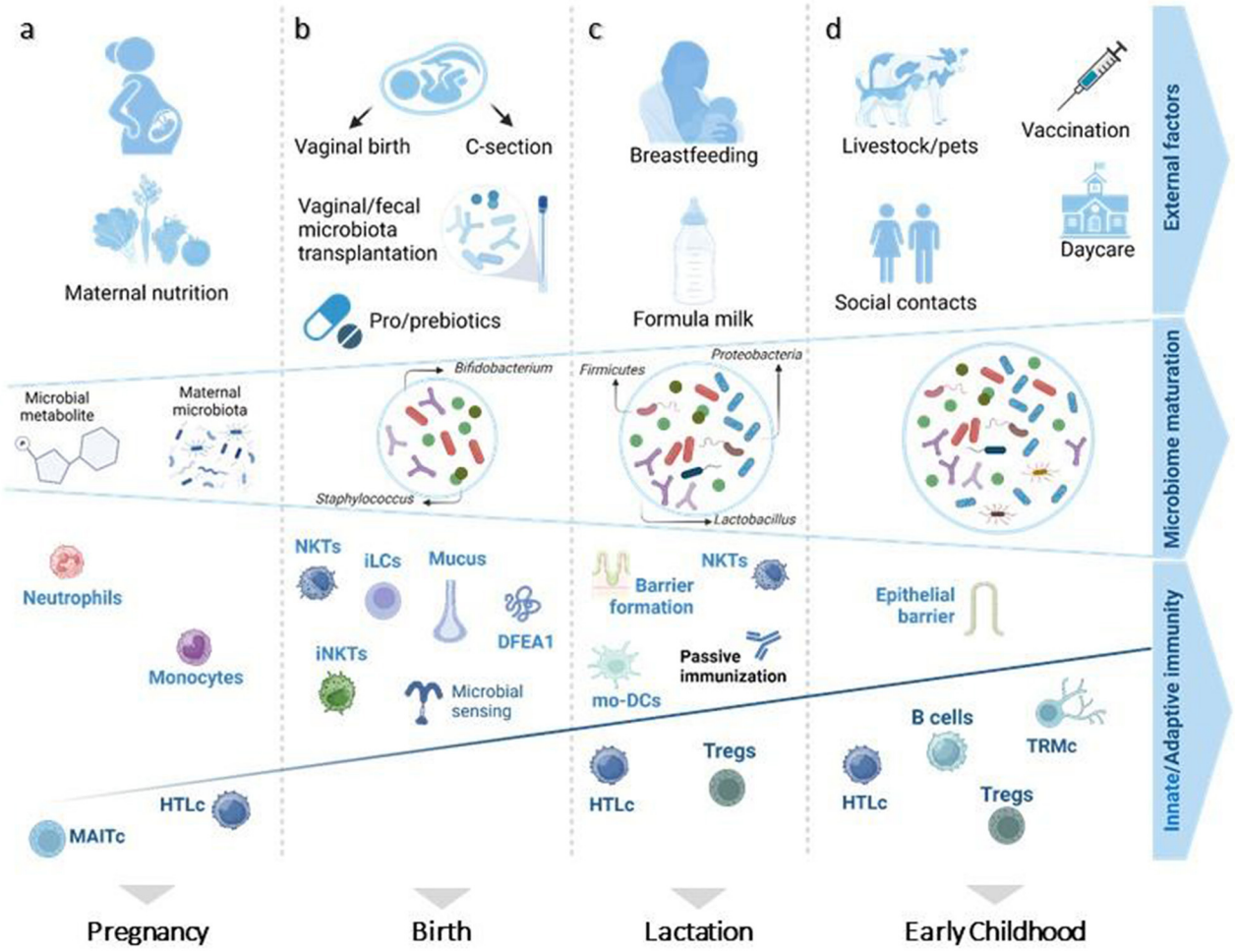

FIGURE 3
Prenatal and postnatal factors influencing microbiome diversity and immune system maturity of children. **(A)** *In-utero*, the presence of microbial-derived metabolites and maternal microbiota educates the immune system on how to confront postnatal microbes, affecting especially innate immune system cells (monocytes and neutrophils). **(B)** At birth, a neonate's immune system relies on maternal protection for its first encounter with living pathogens. **(C)** Breastfeeding plays an essential role in sustaining this protection by aiding in epithelial barrier formation and passive immunization. **(D)** Afterwards, increased microbiome diversity is followed by further evolvement of mucosal and adaptive immunity. Figure adapted from Kalbermatter et al. (17). MAITc, mucosal-associated invariant T-cells; HTLc, helper T lymphocyte cells; NKTs, natural killer T cells; iLCs, innate lymphoid cells; Tregs, regulatory T cells; iNKTs, invariant natural killer cells; DFEA1, alpha defensin 1; mo-DCs, monocyte-derived dendritic cells; TRMc, tissue -resident memory T cells. Figure was created on BioRender.com.

mechanisms that may contribute to *in-utero* carcinogen-induced leukemia (36).

Taken together, ALL is a multifactorial disease, whose development relies on an interplay between genetic and several environmental factors, including maternal exposure to dietary carcinogens. Current studies suggest that reducing exposure to dietary carcinogens could potentially mitigate genetic predispositions to ALL (Table 2). However, further research needs to be done to better understand the connection between maternal nutrition during pregnancy and the causative genetic factors. This should be analyzed in carefully controlled large mother-newborn cohorts, including for instance, dietary interventions. Investigating the impact of maternal antioxidant intake, or Mediterranean diet on ALL risk could provide valuable insights. In addition, mechanistic studies are necessary to clarify the role of maternal folate and omega-3 fatty acids in regulating DNA methylation and maintaining normal epigenetic marks in developing fetal cells.

3 Birth to infancy

3.1 Mode of delivery, prebiotics, vaginal seeding, skin-to-skin care, breastfeeding, and ALL risk

Mode of birth delivery represents a major contributor of microbiome colonization in newborns directly after birth (37). Vaginal delivery is considered as one of the most important early microbiome colonizing factors (38). This is supported by the fact that children delivered vaginally show a higher T-cell reactivity

(lasting up to age of 2 years) and a more diverse gut microbiota composition compared to C-section delivered children (39). However, three large US case-control population-based studies of C-section delivery and childhood ALL reveal no strong association (40, 41). A similar finding was reported by the United Kingdom Childhood Cancer Study (UKCCS) (42). Other studies were able to identify a direct link between mode of delivery and overt childhood leukemia (38, 43). In a Californian registry-based case-control study, stratification of cases was done according to the major leukemia subtypes (38). Analysis of the correlation between C-section and childhood leukemia risk was based on a logistic regression model adjusted for accepted influencing leukemia risk factors such as breastfeeding, gestational age, household income and Hispanic ethnicity (38). Further stratified analyses revealed that a strong association exists C-section delivery and childhood ALL among Hispanic mother and child dyads (OR, 2.34; 95% CI, 1.23–4.46) (38).

TABLE 1 DNA topoisomerase II inhibitors present in food and environment (31).

Substance	Source of exposure
Benzene metabolites 4,4′-biphenol Catechol Hydroquinone p-benzoquinone	Gasoline stations Exhaust fumes
Catechins	Tea, wine, and chocolate
Dipyrone, baygon, thiram	Insecticides
Flavonoids Genistein Quercetin	Soy, legumes, apples, and berries
Senna	Anthraquinone laxative
Thiram	Agricultural laxative

It is commonly accepted that vaginal delivery accounts for a large mother-to-neonate microbial transmission (44). However, the conflicting evidence on C-section delivery and ALL risk makes a link between delivery-related microbiome alterations and ALL uncertain. Furthermore, the reduced share of maternal microbiome during C-section delivery can still be compensated for after birth by other mother-to-child microbial transmission routes like skin contact, breastfeeding or even prebiotic use and vaginal microbiome seeding (22, 23, 45). In terms of childhood leukemia prevention, inulin and human milk oligosaccharides (HMO) are considered as useful prebiotics due to their beneficial effect on stimulating the growth of benign commensal gut bacteria. Benign commensal gut bacteria reduce oxidative stress and decrease gut colonization by *Fusobacterium*, a bacterium known to display pro-cancer properties (23).

Vaginal seeding, a newly emerged concept, consists of mainly oral administration of vaginal fluid to newborns delivered via C-section, aiming to compensate for the lack of microbiome exchange in absence of a vaginal birth (46). This method of maternal bacteria transfer has been recommended by researchers due to a rising prevalence of C-section births (46). In the first study of vaginal seeding, published in 2016, Dominguez-Bello et al., showed that exposing C-section born children to their mother's vaginal fluid could enrich the gut microbiome similarly to the vaginally delivered counterparts (46). A more recent observational study including a larger number of newborns delivered via C-section mode that underwent vaginal seeding also reports a comparable microbiome composition between vaginally and C-section delivered babies (22). So far, there are no reported adverse side effects due to this intervention, including transmitted infections. However, as with vaginal deliveries, vaginal seeding carries the risk of hepatitis B virus, hepatitis C virus, HIV or herpes simplex virus (HSV) transmission (47). Considering these risks, we recommend more conventional alternatives of infant microbiome seeding such as skin-to-skin contact or breastfeeding.

TABLE 2 Newborn exposure to carcinogens via maternal dietary intake during pregnancy.

Carcinogen	Daily dietary maternal intake	Cord blood level	Source (food containing high levels)
Oxidative fat metabolites (DNA adducts)	Omega 6 fatty acids 11.3 (1.2–77.0) g	34.2 (0.5–324.7) 10-9 nucleotides	Vegetable oils (soya, rape, and sunflower) Fatty meat Eggs
Acrylamide (hemoglobin adducts)	22 (1–135) µg	14.4 (4.4-124.8) pmol/g Hb	Coffee Crackers Biscuits Crisp bread Deep fried potato products (french fires, crisps)
Polycyclic aromatic hydrocarbons (DNA adducts)	80 (42–717) ng	8.4 (0.6–116.6) 10-8 nucleotides	Smoked and processed meat and/-or fish Barbecued/grilled meat
Nitrosamines (DNA adducts)	N-nitrosodimethylamine 82 (2–547)ng	0.40 (0.08–3.03) 10-8 nucleotides	Smoked meat Smoked fish Processed meat Preserved fish
Dioxin/PCBs (plasma)	77.2 (5.1–945.5) pg†	0.13 (0.01–104) pg/ml†	Full fat milk and dairy products Fatty meat Fatty fish

Data collected from NewGeneris database (36). †2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxic equivalency quotients.

The share of maternal microbiome to the child across six maternal and four infant body sites was calculated employing a fast-expectation maximization microbial source tracking algorithm (45). Breastfeeding was the predominant contributor of infant microbiome colonization (31.6%). However, a newborns' microbiota development benefitted from contact with other maternal sites including skin (25.7%), saliva (18.6%), nasopharynx (9.4%), feces (4.1%), and vagina (3.5%) (45). Importantly, 58.5% of infant microbiota can originate from any of the maternal transmission sources and the limited maternal microbial share due to a C-section delivery can easily be compensated (45).

Additional evidence attests to the essential role of skin-to-skin contact in building up a healthy newborn immunity by equipping the baby with beneficial microbes including *Staphylococcus epidermis* which prevents potential pathogen colonization and exerts anti-inflammatory properties (48). Newborns experiencing skin-to-skin contact during the 1st h after birth revealed a greater share of maternal microbiota and consequently a more diverse microbiome compared to neonates without skin-to-skin contact (49). Taken together, immediate after birth skin-to-skin care, either with the infant's mother or a caregiver, is highly recommended (50). Additionally, several studies report a likelihood for the mother to initiate and carry on the breastfeeding practice after having experienced immediate skin-to-skin contact with their babies (51). That is associated with an elevated level of oxytocin, which is a vital lactation hormone that not only improves bonding and reduces stress levels, but also facilitates the process of milk release during breastfeeding (52).

Maternal antibodies, passively transferred to neonates through breastfeeding, provide a crucial protection against pathogens during early life (53). In a recent study it was found that antibody-mediated protective immunity can be obtained from the commensal microbiome of pregnant mice via breastfeeding (54). Studies examining breast milk composition discovered that puerperium-stage milk (<1 week after birth) consists of ∼70% immune cells which drop down to 0–2% in the postpartum period (>2 weeks after birth) (55, 56). Furthermore, profiling of mature-stage milk revealed three previously unknown and unique epithelial lactocyte subpopulations found to play a pivotal role in immune defense and intestinal development (55). Bogeart et al., described not only decreased microbial transmission resulting from the C-section births but, a more significant impact of breastmilk related to a higher amount of *Rothia mucilaginosa* presence found in the fecal microbiota of C-section born children (45). This bacterium has been shown to positively influence the gut microbiota by altering its composition. In mice studies, *R. mucilaginosa* increased the abundance of beneficial bacteria such as *Firmicutes* and *Lactobacillus* while reducing harmful bacteria like *Bacteroidetes* (57). These changes enhance gut health by promoting nutrient absorption and metabolic balance. This metabolic activity also produces short-chain fatty acids and other metabolites that have systemic effects, influencing gut health and maintaining immune balance by interacting with immune cells and cytokines (57). Preliminary studies suggest that some *R. mucilaginosa* exhibits anti-inflammatory properties. For instance, its abundance was found to be negatively correlated with pro-inflammatory markers such as interleukin IL-8 and IL-1β in a cohort of adults with bronchiectasis (58). This may help in modulating inflammation by interacting with Toll-like receptors (TLRs) and other immune pathways (58). In addition, the findings from Bogeart et al., reiterate that breastfeeding may partially make up for the reduced infant microbial seeding upon C-section delivery and breastmilk represents the biggest contributor to newborn gut microbiome (31.6% of microbial content) compared to other after-birth transmission routes (45). Large meta-analysis studies from 2015 and 2021 suggest that continuation of breastfeeding for at least 6 months may result in a decrease of childhood leukemia incidence by 14–20% (59). However, there is noticeable differences in childhood leukemia rates and breastfeeding practices between high-income (HIC) and medium-low income countries with lower breastfeeding rates in HIC. Clearly, maternal socioeconomic status influences the choice to breastfeed and its duration. Aiming to rule out the socioeconomic differences, an analysis was carried out focusing only on 12 studies conducted in the HIC countries. The result unveiled a statistically significant inverse correlation between childhood leukemia and breastfeeding for over 6 months (OR, 0.84; 95% CI, 0.78–0.91) (59). Breastfeeding offers a low-cost and usually accessible public health measure for childhood leukemia prevention. However, breastfeeding practice faces many barriers, including lactation problems, infant behavior, early return to work, socioeconomic status, lack of social support and self-efficacy, and unsupportive childcare (60). Hence, for the mothers unable to breastfeed, immediate skin-to-skin contact, early life social, and livestock contact, vaccination, as well as daycare attendance provide alternative and effective stimuli for an early priming of the infant's immunity.

It is calculated that infants receive a load of up to 1 million immune cells in every feeding (61). Human milk, in comparison to commercial sources, consists of maternal immune cells and prebiotics (e.g., HMOs) (56). For instance, HMOs offer an essential protection in the context of an immature immunity and represent the third largest component of breastmilk. By contrast, commercial milk only contains traces of this complex sugar (61). For over a decade, market statistics show a drastic increase of babies fed with commercial milk formula instead of human milk (62, 63). A three-paper series published in 2023 in Lancet raises the concern of challenged breastfeeding practice due to highly predatory tactics used by formula milk industry (64). In one of the series, they outline the long-term benefits of breastfeeding in fighting disease for both mothers and the newborns by providing an immune boost that cannot be reproduced by commercial milk substitutes (65). Examining the relationship between exclusive breast milk and pure milk powder in preventing leukemia, a large retrospective case-control study of children diagnosed with leukemia vs. healthy controls found that the consumption of commercial milk powder instead of breastmilk might significantly increase the incidence of overt childhood leukemia (66). Whereas, analysis of the association between duration of breastfeeding and childhood leukemia incidence predictably indicated a slightly reduced leukemia risk upon >6 months of breastfeeding (67). Although a better understanding of the biological mechanisms between breastfeeding and risk of childhood leukemia is needed, existing data indicates a protective effect of breastmilk against leukemia development.

4 Early childhood

4.1 Social and/or livestock contacts and ALL risk

Early life microbial colonization is essential for the maturation of immune system and originates from the maternal microbiota (37). Although, microbial colonization might commence *in utero*, it is a persistent natural process and its largest share happens after birth. Hence, daycare attendance could help maintain a nurtured microbiome and facilitate immune system maturation through early exposure to common infections, which in turn may reduce the risk of ALL development (68). In a Danish childcare database study the ALL risk for children attending childcare is estimated to be reduced by 32% (69). However, these findings are not supported by a second recent Danish cohort study in which, childcare attendance shows no significant reduction in the context of ALL risk reduction (68). Since the enrolment age was 2–14 years in the later study, it could be inferred that immune modulation following microbiome alterations is critical to ALL risk during the 1st years of life (68).

The "delayed infection" theory postulates that early infections decrease the risk of childhood ALL. Early infections have shown to be beneficial in priming the immune system (3). Epidemiological studies on exposure to infectious agents and immune challenges by proxy in infancy (<1 year of age) support a protective effect of early infections against ALL (3). Taking this into account, we suggest early life livestock contacts, siblingship, or daycare attendance as they may provide the necessary after-birth microbiome colonization and subsequently protection against ALL.

In line with the "delayed infection" theory, a small ALL cluster was identified in Milan among seven children following AH1N1 swine flu (12). No evidence of previous exposure to ionizing and non-ionizing radiation or other leukemic causative chemicals was uncovered. Since none of the children attended nursery during the 1st year of life and six out of seven were firstborn, it is probable that lack of exposure to infections during early life became the reason of these ALL cases (12, 70). Interestingly, a German nationwide, population-based assessment of the influence of COVID-19 pandemic on the incidence of BCP-ALL childhood cancers revealed a general increase in 2020, which dropped below average in 2021 (71).

Additionally, a large-scale epidemiological study conducted after Germany's reunification showed a 25% higher childhood ALL incidence in the former East Germany as compared to only 1% increase in West Germany and the rest of Europe, over the same period of 6 years (72). In contrast to West Germany, nearly all the babies in the eastern part had to attend state nursery centers, a practice which was discontinued after reunification (72). Hence, cessation of universal daycare attendance after reunification may in part account for this shift (72). Further supportive evidence on this stems from a recent meta-analysis of 7,847 leukemia cases and 11,667 controls by the Childhood Leukemia International Consortium (73). The consortium demonstrated that regular contact of children (<1 year of age) with livestock, poultry and pets reduced the risk of ALL development significantly (livestock: OR = 0.65, 95% CI: 0.50, 0.85) (73).

4.2 Vaccination and ALL risk

Acquiring infections during the 1st year of life strengthens the immune system due to antibody production (74). "Trained immunity" during early life resulting from vaccination is a newly emerging concept which could impact childhood leukemia prevention as it primes the immune system and boosts immunity (75). For instance, the Bacille Calmette-Guerin (BCG) vaccination is thought to shape the innate immune response by production of trained natural killer cells (NK), macrophages and monocytes (76). So far, many epidemiological meta-analyses investigating the association between early life vaccination and ALL risk have been carried out, but only BCG vaccination has shown a protective effect in terms of childhood ALL prevention (74, 76). In a retrospective study, R. Rosenthal was the first to describe the role of BCG vaccination in childhood ALL incidence declension in 1972 (77). Since then, other observational studies suggest the valuable effect of BCG vaccination in reducing leukemia incidence (75, 78). However, no firm conclusion can be made as the outcome of these studies were often inconsistent. A lack of assessment of other ALL influencing factors including environmental stimuli (microbes/etiological agents), social contacts or mother-to-child interaction may account for the variable outcome. Notably, countries with an active BCG vaccination policy report the lowest childhood leukemia incidence (74, 77). The protective association between BCG vaccine and childhood leukemia rate was also observed while comparing ALL cases among children living in either East or West Germany before and after the reunification. In the former East, opposite to West Germany, BCG vaccination was mandatory (in addition to daycare attendance), until reunification (76). Accordingly, data showed a lower childhood leukemia incidence in the former East Germany with 3/100,000 children as compared to 3.7/100,000 in former West Germany, but reunification eventually canceled out this disparity (76). The influence of different vaccines, such as rubella, measles, mumps, diphtheria-tetanus-pertussis (DTaP) poliomyelitis, hepatitis B (HBV), and BCG in affecting the incidence of childhood ALL has also been explored in a large meta-analysis study (74). Results showed no evidence of reduced leukemia risk for all the other vaccines, whereas analysis of early vaccination (<3 months of age) with the BCG vaccine revealed a statistically robust protection from ALL (74). These associations are supported by numerous studies reporting on BCG vaccination of newborns and leukemia incidence in Austria, Chicago, Quebec and Germany (74).

The hypothalamus-pituitary-adrenal axis hypothesis by Schmiegelow proposes that early life infections lead to an increase in plasma cortisol levels and may consequently facilitate the elimination of preleukemic cells (69). Vaccine administration can also increase plasma cortisol, which might account for how vaccination may prevent childhood ALL development (79). Additionally, in line with Greaves' delayed infection theory, exposure to vaccines' pathogen-associated molecular patterns

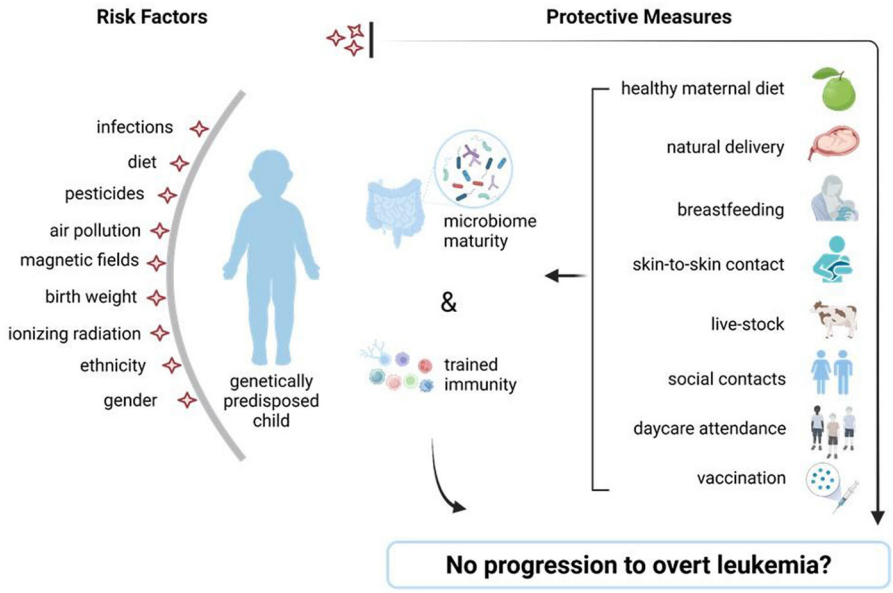

FIGURE 4
Risk factors for childhood leukemia and potential protective measures. Schematic view of accepted childhood leukemia risk factors **(left)** and preventive measures **(right)** which assure intestinal diversity and a mature immunity, able to prevent the switch from preleukemia toward overt leukemia, in genetically predisposed children. Figure was created on BioRender.com.

(PAMPs) stimulates innate immune response, which could mimic common infections acquired due to nursery attendance (74). However, further research is necessary to elucidate this concept, as this does not explain why reduction of leukemia risk appears to be limited to only the BCG vaccine.

5 Discussion and recommendations for the target population

Microbiome-targeted interventions are an emerging area of research for preventing ALL in genetically predisposed children and several prevention strategies, including diet modulation and/or early-life microbial exposure are currently under investigation. Epidemiological and experimental studies demonstrate that a healthy gut microbiome holds great potential in protecting against childhood leukemia development (2, 10, 23, 25). Several environmental factors (prenatal and especially postnatal) reviewed above may provide the adequate gut microbiome diversity that could aid in the protection against ALL development through the establishment of strong early life immunity (Figure 4) (4, 17, 20, 23, 24, 46, 69, 72, 80).

As reported by several epidemiological and experimental studies, diet during pregnancy may strongly influence maternal and fetal microbiome and provide the bacteria needed for "priming" of the offspring's immunity (25, 26, 29, 30).

C-section births have generally increased over the past two decades, potentially reducing microbial exchange at birth. While interventions like vaginal/fecal microbiome seeding remain still debatable, we strongly advocate early skin-to-skin mother-to-infant/and, or guardian-to-infant contact to promote microbial transfer and subsequent early immune priming, as supported by epidemiological studies. Early skin-to-skin contact is an important determinant in shaping a strong immunity. Therefore, an uninterrupted skin-to-skin mother or caregiver-infant contact for at least the 1st hour after birth, particularly for the non-vaginally born babies is recommended. Similarly, early social and livestock contact may offer an additional strategy toward childhood leukemia prevention (45, 46, 48–50, 72, 73).

Bogaert et al., demonstrates that breastmilk is the major source of newborn microbiota and can greatly compensate on its own for the lack of maternal microbial transmission after a C-section delivery. Hence, if feasible for the mother both physically and mentally, we encourage this practice as well as the implementation of breastfeeding-friendly measures at the workplace and focus on maternal mental wellbeing (45, 55, 56, 59).

Additionally, epidemiological studies show that vaccination and early exposure to infections or pathogens have a protective role against childhood leukemia, as well as asthma and allergies. Hence, overtly sterile environments and limited interaction with siblings or pets may serve as a trigger for dysregulated immune responses later on and negatively influence the onset of leukemia among genetically predisposed children (74–77, 79).

To advance these prevention strategies (dietary adjustments and early-life microbial exposure) further studies are needed to clarify the mechanisms linking gut microbiome alterations to ALL. For families with a genetic predisposition, adopting a healthy diet, avoiding unnecessary antibiotic use, and exploring evidence-based probiotic therapies could be practical steps to support microbiome health and potentially reduce ALL risk (1, 10, 11, 23, 80, 81). However, consulting healthcare professionals before starting any intervention is essential to ensure safety and efficacy.

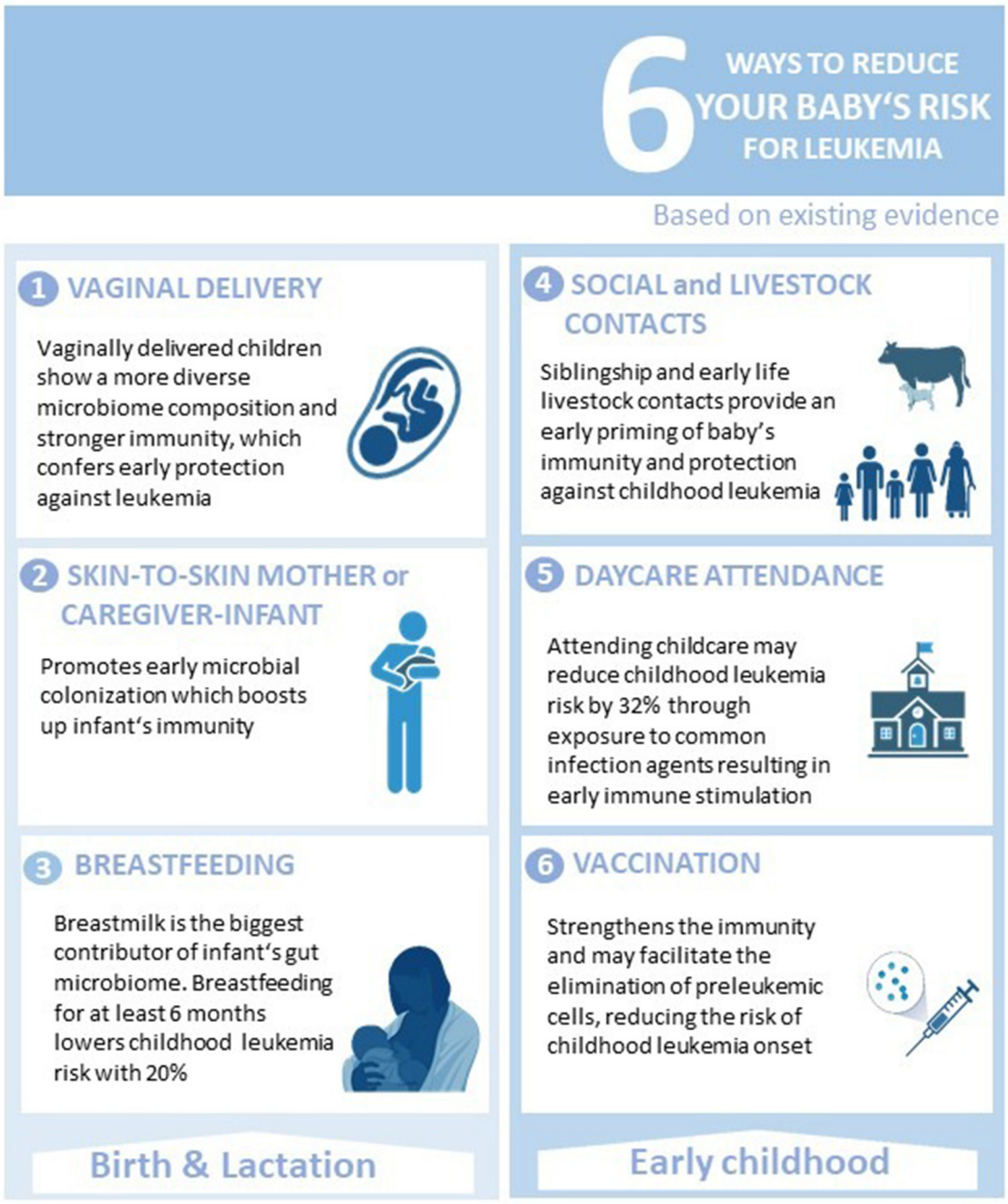

FIGURE 5
Simple measures toward childhood ALL prevention. Six ways parents and/or other caregivers can contribute in reducing the risk of childhood ALL development. Figure was created on BioRender.com.

6 Conclusions

Growing evidence suggests that early childhood ALL is in principle a preventable disease and the key to its prevention is nurturing early life gut microbiome and immune maturation that can be achieved effectively and safely through a healthy maternal diet, breastfeeding, vaginal mode of delivery, early skin-to-skin mother/caregiver-to-infant contact, nursery attendance, vaccination, and early life social and livestock contacts to facilitate exposure to common infection agents (Figure 5).

Author contributions

EK: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft. VJ: Writing – review

& editing. PS: Writing – review & editing. NR: Writing – review & editing. RB: Writing – review & editing. LB: Writing – review & editing. FC: Writing – review & editing. EG: Writing – review & editing. ND: Writing – review & editing. SJ: Investigation, Writing – review & editing. AB: Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. AP: Investigation, Supervision, Writing – review & editing. GK: Funding acquisition, Resources, Supervision, Writing – review & editing. UF: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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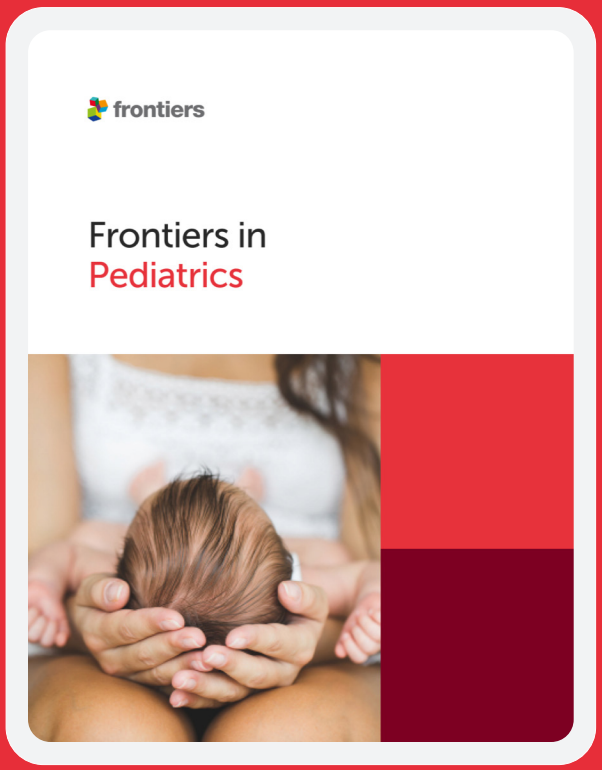

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