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Editorial

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Challenges in Japan's dual systems of support for pediatric and adult intractable diseases

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SUMMARY: Japan has developed two separate frameworks to support patients with chronic and rare diseases: the Specified Pediatric Chronic Diseases (SPCD) Program and the Designated Intractable Diseases (DID) System. Although both aim to provide medical and social assistance, they differ in age of eligibility, diseases covered, and administrative procedures. The SPCD Program provides support to individuals under 18 years of age (extendable to 20) with 858 eligible conditions, whereas the DID System, with 348 designated diseases, applies to all ages. These structural discrepancies create a critical policy gap when pediatric patients transition into adulthood. Those whose conditions are not listed under the DID System lose their eligibility for public subsidies, resulting in sudden financial strain and reduced social participation. Additional issues include inconsistencies in diagnostic criteria, limited access to transitional care facilities — currently established in only 12 prefectures — and insufficient family-centered support, especially for siblings acting as young carers. To achieve continuity of care and equity, Japan must harmonize disease definitions and transition criteria, introduce temporary relief measures for non-designated patients, increase the number of Transitional Care Support Centers in regions, and institutionalize family-inclusive assistance. Establishing a seamless policy framework over a patient's life will not only encourage patients' independence but also strengthen the sustainability of Japan's healthcare system in the face of an aging population.

Keywords: specified pediatric chronic diseases, designated intractable diseases, transitional care, subsidy for medical expenses, family support

1. Introduction

Rare and intractable diseases pose a significant global public health challenge, affecting an estimated 3.5 to 5.9% of the population, yet only around 5% of these conditions have effective therapeutic options. These diseases are characterized by a low prevalence, a complex diagnosis, and limited availability of treatment, which together impose substantial medical, psychological, and social burdens on patients and their families (*1,2*). Over the past decade, numerous countries have sought to improve care and support for rare and intractable diseases through the establishment of national programs and policy frameworks (*3-5*).

Until the 2010s, Japan lacked a comprehensive legal framework specifically addressing medical care and support for patients with intractable and rare diseases. This changed with the enactment of the Act on Medical Care for Patients with Intractable/Rare Diseases in 2014, which came into force on January 1, 2015. This legislation established a foundation for promoting research, improving the quality of medical care, and strengthening support for patients' independence (*6*). In the same year, a separate legal system — the Measures for Specified Pediatric Chronic Diseases — was introduced to provide medical and welfare support for minors, principally those under 18 years of age (*7*).

Although both systems aim to ensure medical and social support for patients with chronic and intractable conditions, they differ in the scope of diseases covered and operate independently without coordination. Consequently, when patients with Specified Pediatric Chronic Diseases (SPCD) reach adulthood, they may lose eligibility for public subsidies if their condition is not listed among the Designated Intractable Diseases (DID). This discontinuity often results in a sudden increase in personal medical expenses, placing greater socioeconomic strain on individuals already facing a long-term illness and limited opportunities for education or employment.

This editorial outlines the structures of Japan's two major support systems and discusses issues with them.

Table 1. Comparison between the Specified Pediatric Chronic Diseases (SPCD) Program and Designated Intractable Diseases (DID) System in Japan

Category	SPCD Program	DID System
Legal basis	Child Welfare Act	Act on Medical Care for Patients with Intractable/Rare Diseases
Eligible age group	Under 18 years (extendable to under 20 if continuous treatment is required)	All ages
Number of diseases covered (as of April 2025)	858 diseases	348 diseases
Subsidy for medical expenses	Income-based copayment ceiling applies	Income-based copayment ceiling applies
Transitional support	Transitional Care Support Centers established in only 12 prefectures	Not incorporated institutionally
Key features	Emphasizes support for independence and social participation	Emphasizes ensuring access to quality medical care

2. National programs for support of SPCD and DID

The SPCD Program targets diseases that develop during childhood, persist chronically, and may threaten life over the long term. These conditions typically require prolonged treatment and impose a significant financial and psychological burden on both patients and their families. Under this scheme, patients younger than 18 years are eligible for subsidies for medical expenses, assistive equipment, and programs to encourage independence. If continued treatment is deemed necessary, financial assistance may be extended until the age of 20. As of April 2025, 858 diseases are covered under this program (*8*).

The DID System provides support for diseases that are rare, lack an established treatment, require long-term management, have a defined diagnostic standard and severity classification, and affect a small patient population. Assistance includes subsidies for medical expenses, provision of devices to assist with daily life, and employment assistance, without any age restriction. As of April 2025, 348 diseases have been officially designated as DID (*9*).

3. Structural discrepancies and policy gaps

3.1. Discontinuity and inconsistency between the two systems

Table 1 summarizes the major structural differences between the two systems. One of the most serious issues lies in the discontinuity of support for medical expenses arising from the age limit of the SPCD Program and the differing lists of eligible diseases. Patients can receive subsidies under the pediatric system until age 20 at the latest; if, however, their condition does not fall under the DID System, then their subsidies are terminated upon reaching adulthood. As a result, patients who already face educational and occupational barriers due to chronic illness must also bear substantial medical costs. This policy gap threatens not only patients' health and financial stability but also their long-term social participation and the amassing of clinical and research knowledge regarding these diseases.

Another issue is the inconsistency in disease definitions and diagnostic criteria between the two systems. The SPCD Program list includes 858 diseases, while the DID System covers 348, and many diseases and criteria differ between the two. For patients and families without medical expertise, understanding which system applies can be confusing and may lead to under-utilization of available support. Harmonization of disease nomenclature and rationalization of overlapping categories are urgently needed.

3.2. Issues with transition and family support

Even when a transition from the pediatric to the adult support system is possible, additional barriers remain. The transition period coincides with the developmental shift from adolescence to adulthood, during which patients must often change both medical departments and institutions. Because each system requires certification by designated medical facilities, those diagnosed under the pediatric system may have to seek new hospitals or specialists once they transition to the adult system. To address this, Japan has begun establishing Transitional Care Support Centers, which facilitate coordination between pediatric and adult healthcare providers and promote self-management among patients. However, as shown in Figure 1, as of June 2025 only 12 of Japan's 47 prefectures have such centers, most of which are concentrated in metropolitan areas such as Tokyo and Osaka (*10*). The map was created using the National Land Numerical Information. Patients in rural regions therefore continue to face significant disparities in access to transitional support.

A further concern is the limited support available

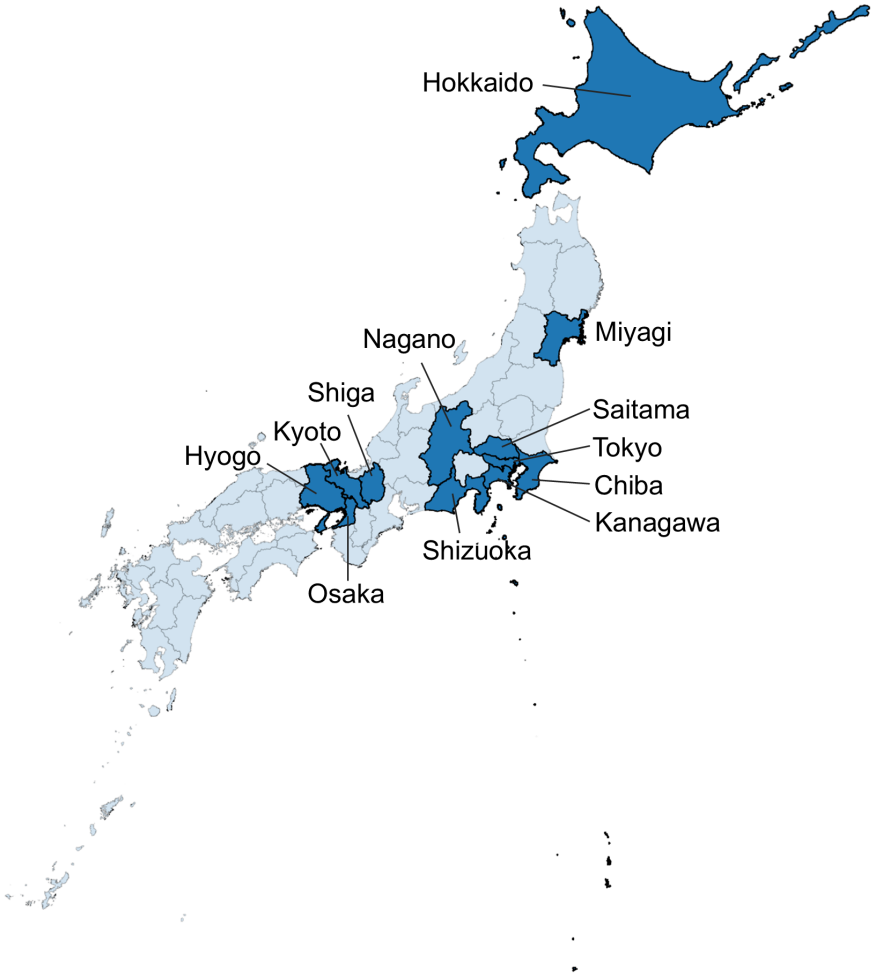

Figure 1. Prefectures that are home to Transitional Care Support Centers in Japan. Highlighted prefectures include Hokkaido, Miyagi, Saitama, Chiba, Tokyo, Kanagawa, Nagano, Shizuoka, Shiga, Kyoto, Osaka, and Hyogo. The figure was produced using the National Land Numerical Information, provided by the Ministry of Land, Infrastructure, Transport, and Tourism, Japan.

for families, and particularly siblings of affected children. In families with multiple children, siblings may serve as young carers, taking on caregiving responsibilities that can impose psychological burdens and interfere with schooling and daily life. Current frameworks provide little systematic assistance for these family members. Future policies should expand from patient-centered to family-inclusive support, addressing the holistic needs of households living with chronic pediatric diseases.

3.3. Policy implications and recommended actions

Based on the current situation, four policies are urgently required:

i) Clarification of the transition criteria between the SPCD Program and the DID System and standardization of disease nomenclature;

ii) Establishment of relief measures for SPCD patients whose conditions are not included among DID (*e.g.*, temporary extension of subsidies);

iii) An increase in the number of Transitional Care Support Centers nationwide and the creation of multiple regional hubs to reduce geographic disparities;

iv) Institutionalization of family-inclusive support, extending assistance to siblings and caregivers.

4. Conclusion

Patients with SPCD inevitably reach adulthood. Designing a policy framework that provides support over their course of their life is essential not only to promoting patients' independence and social participation but also to enhancing the long-term sustainability of Japan's healthcare system. Bridging institutional gaps and strengthening transitional care should therefore be considered a critical priority for Japan's rare and intractable disease policy.

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Review

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A scoping review of dietary interventions to treat obesity among Prader-Willi syndrome individuals

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SUMMARY: Prader-Willi syndrome (PWS) is a genetic disorder resulting from the absence of paternal 15q11-q13 alleles and is clinically characterised by pathological obesity, delayed satiety, hyperphagia, decreased muscle mass, and increased fat mass. Dietary management constitutes a key component in the prevention and treatment of obesity in individuals with PWS. This scoping study aimed to identify dietary interventions available for treating obesity among PWS individuals. A systematic search using the six stages of the scoping review methodology proposed by Arksey and O'Malley was conducted across four databases: PubMed, Scopus, EBSCOhost, and Cochrane Library. The inclusion criteria were full-text research articles published in English between 2017 and 2023, involving human participants with PWS, and reporting on dietary interventions for obesity management. Out of 100 articles retrieved, five studies were identified. Two studies described multidisciplinary programs integrating dietary and physical activity components, while three focused exclusively on dietary interventions. The outcomes varied by intervention and study design. Ketogenic diets and multidisciplinary programs with exercise often resulted in favourable weight and body fat reduction. However, strict diets like the modified Atkins faced adherence challenges and frequent weight regain. Multidisciplinary, supervised programs result in higher adherence and more effective weight management, with body mass index near normal. In conclusion, although research in this area remains limited, current evidence suggests that both dietary and multidisciplinary interventions have the potential to support obesity management in individuals with PWS.

Keywords: Prader-Willi syndrome, dietary intervention, obesity, weight management

1. Introduction

Prader-Willi syndrome (PWS) is a genetic disorder in which paternal alleles of the chromosome 15q11-q13 region are missing due to a genomic imprinting error or defect (*1-3*). PWS was first reported in 1956 by two Swiss endocrinologists, Andrea Prader and Heinrich Willi, with Alexis Labhart as an internist. The syndrome was estimated to occur once every 15,000 to 25,000 births (*4*). Two chromosomal abnormality tests can be used to validate the syndrome genetically. The first is fluorescent in-situ hybridisation (FISH), which only identifies PWS individuals with deletion and cannot distinguish between uniparental disomy (UPD) and an imprinting error. The second test is the DNA methylation analysis test (Methylation PCR), which identifies more than 95% of PWS genetic subtypes (*5,6*). There are three genetic subtypes: PWS deletion (60%), UPD (36%), and imprinting defect (4%) (*6*). During pregnancy, the physical characteristics of PWS start appearing in the third trimester. Lethargy of foetus movement; abnormalities in the flexion of the hands, feet, and elbows; excessive accumulation of amniotic fluid; and breech presentation are noted (*7*). In early infancy, growth retardation and severe hypotonia occur in infants with PWS due to poor suckling ability. Weak crying, genital hypoplasia, and depigmentation are also observed (*8*). Additionally, in late childhood and adolescence, individuals with PWS are characterised by a short structure, a narrow nasal bridge, almond-shaped eyes with mild strabismus, thin upper lips, scoliosis, obesity, hypogonadism, mild hypoplasia, and small hands and feet (*9*). The PWS population experiences four nutritional phases of eating behaviours and weight gain difficulties. Feeding difficulties and a lack of appetite are noticed in the early period (0–9 months). The second phase is divided into two sub-phases. In the first phase (2a), individuals with PWS gain weight without increases

in appetite or calorie consumption. In the second phase (2b), increases in appetite and calorie consumption are reported although the PWS individuals still feel full. The third phase lasts from the age of eight until adulthood. Hyperphagia will be apparent, and the patient rarely feels full. It is considered impossible to control appetite during the final nutritional phase (*10*).

According to de Lima *et al.* (2016), in comparison to those without the syndrome, individuals with PWS have specific dietary needs due to their reduced energy expenditure, constant food craving that leads to morbid obesity, complicated medical issues, and behavioural challenges. Children with PWS require an intake of 8-11 kcal/cm/day, whereas those who are typically developing require 11–14 kcal/cm/day (*11*). A balanced low-calorie diet consisting of approximately 30% fat, 45% carbohydrate (with at least 20 g of fibre per day), and 25% protein has been shown to bring significant improvements in body weight composition in individuals with PWS aged 2–10 years, compared with a standard energy-restricted diet (*12*). In individuals with PWS, weight gain starts before the onset of hyperphagia (*13*). As the prevalence of obesity rises, it elevates the intricacy and complexity of the issue (*13*). According to the report by de Lima *et al.* (2016), the main causes of early morbidity and mortality in people with PWS are obesity, as well as overweight-related illnesses (diabetes mellitus, cardiopathies, sleep disorders, and osteoarticular disorders) (*1*). Despite the lack of agreement on the most effective dietary strategies for preventing obesity among PWS individuals, the recommendation for a hypocaloric diet is widely acknowledged (*1*). Several publications have been produced on dietary interventions among PWS individuals in order to manage obesity (*6,10,14*), but no scoping reviews have been released. The current scoping review aims to identify the dietary interventions that have been utilised to treat obesity among PWS individuals.

2. Methodology

This scoping review was conducted to identify the dietary interventions that have been utilised to treat obesity among PWS individuals, following the six stages of the Arksey and O'Malley scoping review methodology (*15*): *i*) stating the research question, *ii*) identifying related literature, *iii*) selecting relevant studies *iv*), mapping out the data, *v*) outlining, arranging, and stating the results, and *vi*) proficient consultation.

2.1. Stage 1: Stating the research question

The most recent and relevant studies were employed to identify the research question. Consequently, the current scoping review aimed to answer the following research question: Which dietary interventions have been utilised to treat obesity among PWS individuals?

2.2. Stage 2: Identifying related literature

The present scoping review employed specific search terms and databases to identify the relevant literature. The following search terms were employed during the scoping review: (Dietary Treatment OR Dietary Intervention OR Dietary Approach OR Dietary Strategies OR Dietary Management OR Dietary Education OR Mediterranean Diet OR Low Caloric Diet OR Low Carb Diet) AND (Prader Willi Syndrome Children OR Prader Willi Syndrome Adolescent OR Prader Willi Syndrome Patients OR Labhart Willi Syndrome Children OR Labhart Willi Syndrome Adolescent OR Labhart Willi Syndrome Patients).

The inclusion criteria employed in this study were full-text research articles, human studies, English-language studies, and studies published between 2017–2023 that identified dietary interventions that have been utilized to treat obesity among PWS individuals. To identify relevant studies, a comprehensive search was conducted across four academic databases: Scopus, EBSCOhost, MEDLINE (PubMed), and CENTRAL. These databases were purposefully selected to provide broad coverage of research within the area of interest.

Studies that focused solely on hormonal treatments, pharmacological procedures, dietary supplement interventions, physical activity interventions, or surgical procedures without addressing dietary interventions were eliminated. Additionally, studies categorized as study protocols, conference abstracts, book chapters, case reports, reviews, and short communications were also excluded.

2.3. Stage 3: Selecting relevant studies

The study selection process was conducted autonomously by a single reviewer, who reviewed each article based on the inclusion criteria regarding the target population, study design, duration, intervention, outcomes of interest, and type of article. To minimize the risk of bias or omissions, the selected studies were then reviewed and verified by the two authors (Rajikan R and Abdul Hamid H). The PRISMA-ScR (Reporting Items for Systematic Review and Meta-Analysis Extension for Scoping Review) flowchart for the study selection process is shown in Figure 1 (*16*). After identifying the studies, the titles were evaluated to identify relevant studies to eliminate duplicate records. The abstracts were then assessed according to the eligibility criteria. Ultimately, the publications were selected after a full-text assessment.

2.4. Stage 4: Mapping out the data

Data extraction, the fourth stage of the Arksey and O'Malley process, refers to applying a descriptive-analytical method to chart the data. The data from the

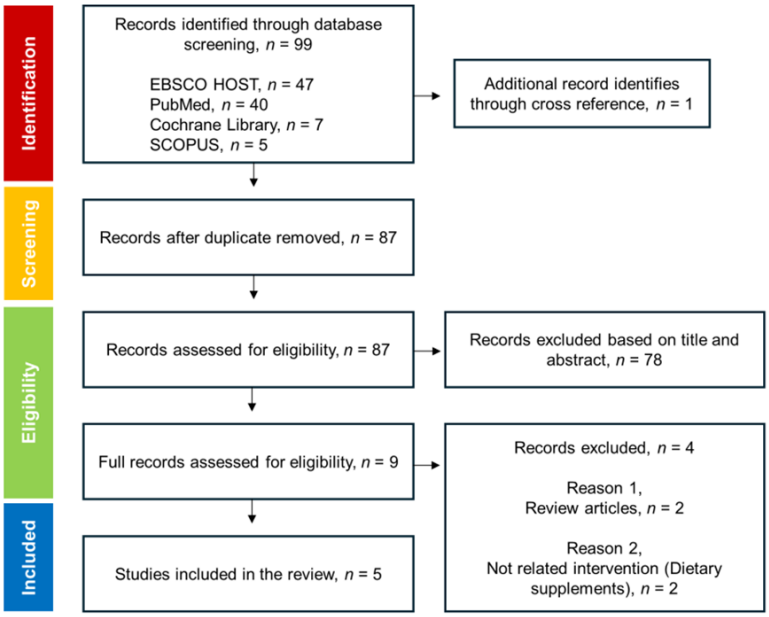

Figure 1. Reporting items for systematic review and meta-analysis extension for scoping review (PRISMA-ScR) flow chart diagram.

eligible studies were organized in a standardized table using Microsoft Excel. This highlighted fundamental components: English language, full-text articles, title, study objectives, participants, publication year, author(s), study duration, study design, intervention, and study outcomes and findings.

Although scoping reviews do not essentially require formal quality appraisal, we incorporated a basic quality appraisal to support clearer results interpretation. Studies evaluation focused on key domains such as authors (year) (ref), country, sample size and age (years), duration, study design, intervention, control group, adherence, and outcomes. Three reviewers independently performed the studies assessments, and differing opinions were discussed and resolved collaboratively. This approach provided a clearer vision of the evidence by reflecting detailed and nuanced information from the included studies. The appraisal highlighted notable strengths, including well-described interventions, duration and outcomes. However, a limitation is that, due to the heterogeneity in study designs and methodologies, the appraisal findings should be interpreted with caution and do not constitute a formal risk-of-bias evaluation. Nonetheless, these insights enriched our understanding and interpretation of the evidence without excluding any studies, ultimately strengthening the review's conclusions.

2.5. Stage 5: Outlining, arranging, and stating the results

The fifth stage of the Arksey and O'Malley framework (2005) is the creation of a result template by grouping comparable articles based on the inclusion criteria used in the review (review question and purpose), demonstrating quantitative and thematic analysis, as well as applying the scoping review PRISMA guidelines shown in Figure 1 below.

2.6. Stage 6: Proficient consultation

The scoping review outcomes were assessed by three experts in nutrition and dietetics from the Faculty of Health Sciences at the Universiti Kebangsaan Malaysia. Each expert independently evaluated the review based on the established inclusion and exclusion criteria, methodological rigor, and relevance to the research objectives to ensure the accuracy and reliability of the assessment. Any disagreement was resolved by the reviewers through discussion.

3. Results

3.1. Study and participants characteristics

The literature search was conducted across four electronic databases, and 100 publications were retrieved using the framework and inclusion criteria mentioned above. After duplications were removed, 87 records remained for screening. At this stage, 87 records were identified and evaluated for eligibility by reading each study's abstract, and nine articles were selected for full-text evaluation to determine their eligibility. A total of four records were excluded, with justifications. Two reviews and two non-relevant interventions (dietary supplement only) studies were excluded. Only five articles fulfilled the review's inclusion criteria, as shown in Figure 1. Participants' characteristics and studies' geographical contexts are highlighted in Table 1. Across the five studies, a total of

Table 1. The characteristics of PWS individual's dietary interventions that have been utilized to treat obesity included studies

Authors (Year) (*Ref*)	Country	Sample Size (*n*) & Age (Years)	Duration	Study Design	Intervention	Control Group	Adherence	Outcomes
Koizumi M, *et al.* (2020) (*3*)	Japan	*n* = 17 / (2–7 years)	Between November 1981 and March 2018	Retrospective Study	Energy-restricted diet (< 10 kcal/cm height/day) with macronutrient distribution of 13–20% protein, 50–60% carbohydrates, and 20–30% fat, supplemented with vitamins/minerals. Parents received education on PWS behavior, diet, and physical activity.	Comparison held among PWS subgroups (GH-treated group *vs.* GH-untreated group)	Adherence inferred from ongoing clinical follow-up; not explicitly reported. 4 of 7 subjects completed the diet; others dropped due to compliance challenges.	No significant weight reduction was shown among participants.
Felix G, *et al.*, (2020) (*4*)	USA	*n* = 7 / (6–12 years)	4 months	Feasibility Study	Modified Atkins diet with limited carbohydrates (10–15 g/day), customized protein and fat intake, vitamin and mineral supplementation, and hydration emphasis.	None	All included subjects	Weight loss was limited: one participant lost weight, while others maintained their weight. After the participants stopped following the intervention, weight regain was observed.
Teke Kısa P, *et al.* (2023) (*17*)	Turkey	*n* = 10 (median age 52.5 (47–77) months)	16.5 (11–52) months	Retrospective, cross-sectional descriptive study	A ketogenic diet consists of a macronutrient distribution of daily calories (75–85% from fat and 15–25% from protein + carbohydrates).	None	who completed ≥6 months with high adherence.	A favorable weight loss was observed among participants.
Bedogni G, *et al.* (2020) (*18*)	Italy	*n* = 45 / (22–30 years)	Between June 2001 and February 2013	Retrospective cohort study	A Mediterranean diet subtracting at most 500 kcal from TEE, one hour of aerobic exercise, and 3–4 km of walking.	None	Regular follow-up every 6 months, attendance to in/out-patient programs documented	A significant reduction in body weight after a follow-up period of 6 years. This weight loss is associated with a decrease in body fat mass (FM), total cholesterol, and low-density lipoprotein (LDL).
Hirsch HJ, *et al.* (2021) (*19*)	Israel	*n* = 34 / (4–19 years)	From 2008 to 2019.	Cross-sectional study	Caloric requirements are determined by height and BMI, with macronutrient ratios of 40–45% carbohydrates, 25–30% protein, 30% fat, and 30 minutes of daily treadmill activity.	PWS adults living at home with families, matched by age to PWS adults hostel residents	High adherence due to structured and supervised hostile environment	Participants demonstrated effective weight management with body mass index (BMI) values within or approaching the normal range.

113 participants with PWS were included, comprising both adult and paediatric populations. Of these, 55.7% of the participants were female, and 44.2% were male. The studies exhibited varied age ranges, with the first (2–7 years) and third (4–6.5 years) focusing on early childhood, whereas the second (6–12 years) encompassed middle childhood to early adolescence. The fourth study exclusively focused on young adulthood (22–30 years), whereas the fifth study encompassed the widest age range (4–19 years), spanning early childhood to early adulthood. Additionally, studies were conducted across various geographical locations, which were Japan, the USA, Italy, Turkey, and Israel. Moreover, the studies adopted a range of research approaches, comprising three retrospective studies, one clinical feasibility study, and one cross-sectional study, spanning multiple settings such as research institutions and hospitals. A notable variability in the intervention duration was observed, ranging from short-term interventions of four months to long-term follow-up assessments spanning up to 36 years.

3.2. Dietary interventions for obesity treatment

This comprehensive review identified two distinct dietary intervention approaches. Three articles focused exclusively on dietary interventions (*3,4,17*), while two examined multidisciplinary programs that combined dietary intervention and physical activity (*18,19*), as summarized in Table 1.

3.3. Exclusive dietary interventions

Three studies investigated dietary interventions for obesity management in PWS individuals with obesity. Koizumi *et al.* (*3*) conducted a retrospective analysis of the effect of nutritional intervention on patients aged 2 to 27 years with PWS who were treated at Osaka Women's and Children's Hospital from 1981 until 2018 (*3*). In the study, a caloric restriction program was implemented in growth hormone (GH) treated and untreated PWS individuals. Participants received less than 10 kcal/cm height per day, age-specific macronutrient ratios (13–20% protein, 50–60% carbohydrates, and 20–30% fat), as well as vitamin and mineral supplementation. Moreover, regular exercise and parents' continuing education on PWS-specific eating habits were recommended for all subjects. The results showed that the GH-treated group had higher muscle mass (73.1%) and lean body mass (76.8%), whereas the GH-untreated group had increased fat mass (35.8%) (*3*). These findings suggested that GH therapy in line with dietary control and regular physical exercise stimulates muscle anabolism and inhibits body fat accumulation in PWS individuals.

In a US-based pilot study (*4*), the modified Atkins diet (MAD) was trialled in seven individuals with PWS over four months. The diet involved 10–15 grams of net carbs per day, while protein and fat intake were adjusted to induce ketosis, alongside the prescription of multivitamins and minerals. Participants were also provided counselling on diet, measuring urine ketones, recipes, and meal samples. Among the four participants who completed the four-month diet trial, one achieved a weight loss of 2.9 kg, while the others maintained their weight. The body mass index z-scores of three of the four individuals improved. However, after the regimen was terminated, all subjects gained weight, including the patients who had lost weight (*4*). This highlights the limited sustainability of MAD without long-term adherence strategies.

A Turkish descriptive cross-sectional study (*17*) investigated the impact of a structured dietary intervention in a sample of 10 PWS participants aged 47–77 months old. Families attended a four-hour education session, and personalized eating plans were generated based on medical history, level of physical activity, and nutritional requirements. The intervention was conducted for 16.5 months and involved restricting the caloric intake to 600–1,800 kilocalories per day, of which 70–85% were obtained from fats, while 15–30% were obtained from proteins and carbohydrates, with carbohydrate consumption restricted to 20–60 g/d. Body weight according to the Standard Definition (SD) significantly reduced from 2.10 to 0.05 (p = 0.007) with a decrease in median BMI SD from 3.05 to 0.41 (p = 0.002). These results suggest that the structured dietary education program and individualized dietary intervention would be useful for weight control in PWS individuals.

3.4. Multidisciplinary interventions (diet and physical activity)

Two studies evaluated the impact of multidisciplinary interventions incorporating both diet and physical activity components in individuals with PWS. In a retrospective cohort study conducted in Italy (*18*), the effects of a long-term metabolic rehabilitation program on 45 obese PWS individuals aged ≥ 17 years were assessed. The intervention comprised a Mediterranean diet with an energy intake of 500 kcal less than total energy expenditure and a structured physical activity program involving five days of supervised physical activity. This included one hour of moderate aerobic activity and three to four kilometres of outdoor walking. Patients and caregivers were regularly counselled about diet and fitness. BMI reduced by 1.7 kg/m² and 2.1 kg/m² over three and six years, respectively, and weight decreased by 3.6 kg and 4.6 kg, respectively. Body fat percentage dropped by 2%, and total and low-density lipoprotein cholesterol decreased (*18*). The results indicate that following a Mediterranean diet in conjunction with a well-structured exercise program can considerably improve BMI, weight, body composition,

and lipid profile in obese subjects with PWS.

Similarly, a study from Israel by Hirsch *et al.* (*19*) examined the impact of multidisciplinary interventions on weight management among obesity in 34 children and adolescents aged 4–19 years old living in residential care homes with an average follow-up of 6.9 years. Participants were compared to age-matched controls living in family environments. Both cohorts participated in annual multidisciplinary clinics with similar dietary, exercise, and behavioural interventions. Individualized meal plans were developed by dietitians, and the calorie intake was between 800 and 1,500 kcal in subjects with a BMI below 23 kg/m². The macronutrient distribution was 40–45% carbohydrates, 25–30% protein, and 30% lipids. Treadmill exercise was tailored by weight into regular 30-minute bouts (*19*). The results indicated that of the 23 participants, four subjects who lived at the family home had a BMI exceeding 30 kg/m², compared to 17 of the 23 participants living at a care home. Moreover, most participants with a high BMI upon entering a care home lost weight and kept their normal weight status (*19*). The results suggest that multidisciplinary care home-structured intervention settings may offer more effective support for long-term weight control in PWS individuals compared to less structured home environments.

4. Discussion

A comprehensive strategy is essential for monitoring health and improving the quality of life of individuals with PWS. This includes dietary therapy, physical activity, hormonal treatments, and pharmacotherapy. PWS individuals' demands are subjective. Although recent advancements have enhanced our understanding of the genetic factors contributing to obesity in PWS, optimal weight management protocols remain a subject of ongoing debate. Ensuring that individuals with PWS adhere to a balanced diet from an early age is critical because this fosters physical well-being and helps them manage hyperphagia and obesity throughout their lives (*20,21*).

The primary purpose of this scoping review was to identify dietary interventions that have been applied to treat obesity among PWS individuals. The five studies included in the review explored dietary interventions and measures of effectiveness related to PWS (*3,4,17-19*). These included three exclusive dietary interventions and two multidisciplinary programmes combining diet, physical activity, and caregiver support. The study by Koizumi *et al.* involved age-appropriate dietary intervention of less than 10 kilocalories per centimetre, in addition to dietary supplements such as vitamins and minerals, which were applied for PWS children (*3*). The second study examined the effects of a MAD on PWS children (*4*), while the third study used ketogenic diets as an intervention for PWS children (*17*). The fourth study examined a multidisciplinary metabolic rehabilitation program composed of a Mediterranean diet and physical activity in terms of its potential effectiveness in assessing weight and body composition (*18*). Lastly, the fifth study involved long-term weight management in PWS individuals residing in care homes using a multidisciplinary metabolic rehabilitation program (*19*). Although the included studies provided valuable insights, the rarity of PWS resulted in only a small number of eligible studies, each with limited sample sizes and varied methodologies. This restricts the generalizability of the findings and hampers robust conclusions about long-term effectiveness and safety of dietary interventions. Future research with larger cohorts and standardized protocols is essential to strengthen the evidence base and improve applicability across diverse PWS populations.

Furthermore, considerable heterogeneity exists among the included studies, reflected in their diverse designs — retrospective, cross-sectional, and feasibility studies and intervention durations ranging from 4 months up to 36 years. Additionally, the studies employed differing outcome measures and varied dietary intervention protocols, complicating direct comparisons and synthesis. This variability highlights the pressing need for future investigations to adopt standardized study designs, consistent intervention frameworks, and uniform outcome assessments to enable clearer interpretation of intervention efficacy and safety in individuals with PWS.

Evidence for exclusive dietary strategies was mixed. In the study by Koizumi *et al.*, a hypocaloric diet implemented for weight control intervention alone did not yield statistically significant findings (*3*). Furthermore, PWS individuals in the treated group saw linear growth due to GH administration (*3*). The administration of GH may have played a role in the observed height rise, irrespective of the hypocaloric diet, as it directly affects bone growth and height development. Moreover, the hypocaloric diet supplied the necessary nutrients to support the action of GH. On the other hand, the results from other studies have proven the diet's effectiveness when paired with structured protocols (*1,11*). Notably, the investigations mentioned the exact amounts and types of dietary elements, educating PWS individuals and caregivers about the hypocaloric diet, provision of personalized dietary plans, and regular follow-up to confirm an appropriate and effective dietary approach (*1,11*). This underscores the critical need for a structured and comprehensive approach to maintain growth and weight control among PWS population. Future interventions should prioritize multidisciplinary collaboration of structured dietary protocols, GH therapy, caregiver education, and long-term studies of personalized weight and growth management among PWS individuals to ensure dietary strategies are both clinically effective and sustainable in real-world settings.

Next, the MAD has been implemented as a dietary intervention in weight management for PWS individuals (*4*). The first prospective clinical study that involved

a comprehensive support system for PWS individuals noted varying levels of low-carbohydrate diet adherence (*4*). Overall, weight loss was barely noticeable (*4*). Conversely, the supporting literature on a keto diet in other populations suggests that Cervenka *et al*. employed a more rigid dietary limit — 20 grams of net carbohydrates daily — without comparable emphasis on caregiver training or tailored dietary planning. The limited strategy used led to weight gain in some participants with an elevation of some biomarkers, such as cholesterol and LDL (*22*). This highlights that the approach used in the intervention demands further assessments and adjustments to enhance its efficacy in weight management outcomes among similar populations. Furthermore, it is essential to evaluate the risks associated with MAD to determine cardiovascular safety and therapeutic value.

As for the ketogenic diet, several studies revealed its effectiveness in reducing body weight among the normal population (*23-26*). Moreover, the results of the included study imply that a reduced-calorie, carbohydrate-restricted, well-balanced diet that incorporates strict observation can favourably influence weight reduction in individuals with PWS (*17*). Furthermore, the investigation of a reduced energy intake and a well-balanced diet for weight control in children with PWS indicated that dietary education, regular feedback, relying on caregiver-reported dietary recalls collected biannually, and altering the macronutrient allocation culminated in a remarkable mass reduction compared to conventional caloric restriction alone (*12*). Additionally, the study outcomes stated that a high-fibre diet was superior to a standard reduced-calorie diet (*12*). Hence, despite the promising outcomes shown in PWS populations when using the ketogenic diet, the effectiveness and safety of this dietary approach cannot be definitively determined due to the limited number of participants and the short length of the trials. Therefore, long-term clinical trials with large sample sizes are urgently needed before such restrictive dietary interventions are recommended to focus on future studies.

Restrictive dietary approaches such as Ketogenic and MAD showed efficacy in addressing obesity management among PWS individuals. Despite their potential, they introduce remarkable risks such as safety, long-term feasibility, and adherence. Evidence noted that ketogenic diet commonly reported side effects such as gastrointestinal upset, elevated cholesterol or triglycerides, liver enzyme abnormalities, and, although infrequent, complications like cardiomyopathy or pancreatitis (*27,28*). Restrictive diets must be cautiously assessed among the PWS population, where metabolic and hormonal imbalances and instability exist. Furthermore, adhering to low-carbohydrate dietary regimens poses significant challenges to maintain, due to compulsive food-seeking behavioural characteristic of PWS and hyperphagia. Regarding the MAD, clinical findings indicated notable adherence challenges, where participants commonly faced adherence issues and post-intervention weight regain (*20*). Caregiver burden is substantial as they must manage both behavioural supervision and medical follow-up, including blood tests for lipid profiles, and hepatic and renal function. In addition, nutritional markers are essential to minimize risks and ensure safety (*10*). This emphasizes that while restrictive dietary interventions may present therapeutic value, their practical implementation demands cautious long-term evaluation, personalized consideration, and comprehensive care team involvement. Future investigations are needed to emphasize not only therapeutic impact, but also the development of sustainable, and realistic diet protocols that are designed to accommodate PWS individual's physiological characteristic and eating behaviours.

From the perspective of long-term multidisciplinary dietary rehabilitation programs for people with PWS, an investigation by Bedogni *et al*. revealed a significant average weight reduction and a corresponding decrease in BMI after a follow-up period of six years (*18*). These results are consistent with those of a previous study on obesity in PWS individuals, and they highlight the important role of multidisciplinary dietary rehabilitation programs in weight control among people with PWS (*29*). In addition, based on their investigation, Bedogni *et al.* emphasized a logical decrease in the percentage of adipose tissue and an increase in muscle development, both of which are consistent with the expected results of an average weight reduction. We can perceive that people with PWS can achieve similar results with a continuous and appropriate dietary rehabilitation intervention. However, it is difficult to derive a cause-and-effect relationship from an observational study. Results obtained in a tertiary care centre may restrict broader applicability in other contexts. In addition, full body composition measurements were not available due to technical limitations, which could have affected the accuracy of the results. Future investigations are recommended to verify these findings using more robust designs and comprehensive assessment tools across diverse healthcare contexts.

The accomplishment of the dietary intervention among PWS individuals living in care homes can be attributed to the regulated environment, unfailing backing from a multidisciplinary group, and limited availability of energy-dense foods in comparison to the subjects who lived in the family home (*19*). Similarly, Kaufman *et al.* conducted a study of a PWS group living with their families, implementing diet restrictions, supervised exercise, and a structured environment, which led to significant weight reduction among PWS participants (*30*). The findings from both investigations highlight the significance of a controlled environment and multidisciplinary assistance in helping PWS individuals successfully manage their weight. This type

of investigation requires a larger sample size and a more thorough analysis of the social, environmental, or health factors that contribute to the observed findings. Further research is needed with larger populations and across different living conditions to disentangle the impact of related factors such as caregiver capacity, socioeconomic status, and access to health services. Moreover, the controlled conditions and multidisciplinary guidance provided in these PWS studies raise issues regarding validity, as such settings may not reflect exact real-life conditions. The effectiveness of dietary interventions cannot be generalized to different living conditions. Therefore, further research is necessary. In addition, a more holistic research framework that extends beyond obesity, such as behavioural, psychological, and metabolic health, could provide a more comprehensive understanding of the overall health of PWS individuals living in residential hostels and family care settings.

5. Strengths and limitations of the study

The strengths of the present review included the use of a systematic search strategy to locate publications demonstrating the dietary intervention strategies used to treat obesity in PWS patients. The risk of bias was reduced by screening duplicates and applying prior inclusion criteria. As PWS is a rare genetic disorder, this review is the first scoping study to comprehensively identify the dietary intervention strategies used to treat obesity among PWS individuals. To establish this, we conducted a systematic and extensive literature search across multiple databases — PubMed, Scopus, EBSCOhost, and the Cochrane Library — using relevant keywords related to PWS and dietary intervention, as detailed in the methodology section. This thorough search found no previous scoping reviews specifically addressing dietary approaches for obesity in this population, hereby underscoring the originality and significant contribution of the present study. Consequently, only a small fraction of the studies would likely have been missed. Nonetheless, certain limitations must be acknowledged. The small number of recent publications on PWS dietary intervention studies is due to the rarity of the syndrome, which occurs once every 15,000 to 25,000 births (*4*), and its complexity, as well as the challenges linked to recruitment, ethical considerations, resource constraints, and lack of awareness. While these challenges exist, ongoing efforts are being made by researchers, healthcare professionals, and advocacy organisations to better understand and manage PWS.

6. Conclusion

In summary, the current scoping review highlighted the dietary interventions, such as the ketogenic diet, as well as other approaches like multidisciplinary metabolic rehabilitation programs and living in a restricted environment, that have been used to treat obesity among individuals with PWS. However, it is important to note that the small number of eligible studies, along with small sample sizes and methodological differences due to the rarity of the syndrome, pose a challenge to the generalizability of findings and to establish more conclusive evidence on the long-term impact of dietary interventions in individuals with PWS. Although adherence to restricted diets can be challenging for people with PWS and their caregivers, significant results have been observed with a long-term multidisciplinary dietary program and a ketogenic diet *(17-19*). Further research in the field of dietary intervention, involving larger, more standardized samples, and methodologies are essential for developing systematic dietary approaches that can effectively contribute to the prevention and treatment of obesity in people with PWS. Specifically, there is a need for well-designed randomized controlled trials, standardized dietary protocols, and longitudinal studies to fill existing evidence gaps and better evaluate long-term outcomes and adherence challenges. This scoping review has laid the foundation for future research and underlined the importance of constant efforts to understand and implement effective dietary approaches for people with PWS.

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Review

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Mitochondrial DNA A3243G variant: Current perspectives and clinical implications

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SUMMARY: The mitochondrial DNA A3243G variant, located in the MT-TL1 gene encoding $tRNA^{Leu(UUR)}$, represents one of the most clinically significant pathogenic mitochondrial mutations. This point mutation accounts for approximately 80% of Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) syndrome cases and is the primary cause of Maternally Inherited Diabetes and Deafness (MIDD) syndrome. The clinical spectrum associated with this mutation ranges from asymptomatic carriers to severe multisystem disease with early mortality. The pathophysiology involves impaired mitochondrial protein synthesis leading to respiratory chain dysfunction, with phenotypic expression determined by heteroplasmy levels and tissue-specific energy demands. Understanding the complex inheritance patterns, genetic bottleneck effects during oogenesis, and heteroplasmy variations is crucial for comprehending the variable clinical presentations observed in affected families. Histological examination reveals characteristic features including ragged-red fibers, cytochrome c oxidase-deficient fibers, and abnormal mitochondrial proliferation. Current therapeutic approaches focus on metabolic support, antioxidant therapy, and management of specific complications, with L-arginine showing promise for stroke-like episodes. However, careful attention to drug safety profiles and potential mitochondrial toxicity is essential in treatment planning. Understanding the diverse clinical manifestations and implementing appropriate screening strategies are crucial for early diagnosis and optimal patient management. This review synthesizes current knowledge regarding the A3243G variant's pathophysiology, clinical features, diagnostic approaches, and therapeutic interventions.

Keywords: MELAS syndrome, point mutation, lactic acidosis, mitochondrial DNA (mtDNA), transfer RNA ($tRNA^{Leu(UUR)}$), heteroplasmy

1. Introduction

Mitochondrial DNA disorders encompass a heterogeneous group of inherited diseases that impair oxidative phosphorylation (OXPHOS) (*1*). Among more than 400 reported mtDNA variants, the A3243G transition in MT-TL1 is both prevalent and clinically important, with a population frequency estimated at 7.6–236 per 100,000 (*2,3*). Initially linked to Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS), A3243G is now recognized as the genetic basis for a wide array of phenotypes (*1*). The mutation disrupts $tRNA^{Leu(UUR)}$ structure, diminishing aminoacylation and mitochondrial protein synthesis (*4*), and is classically associated with MELAS and Maternally Inherited Diabetes and Deafness (MIDD) (*5*).

Phenotypic variability reflects heteroplasmy, tissue energy requirements, and nuclear background (*6*). Advances in molecular diagnostics, neuroimaging, and targeted therapy have improved detection and management of A3243G-related disease.

This comprehensive review examines the current understanding of the A3243G mitochondrial variant, focusing on its pathophysiology, clinical manifestations, diagnostic approaches, and therapeutic strategies, with particular emphasis on the two major associated syndromes: MELAS and MIDD.

2. Pathophysiology

2.1. Molecular mechanisms

The primary consequence of the A3243G mutation is impaired mitochondrial protein synthesis, particularly affecting the translation of mitochondrial-encoded respiratory chain subunits (*7*). The A3243G mutation occurs in the D-loop region of the *MT-TL1* gene encoding $tRNA^{Leu(UUR)}$, specifically disrupting the structure and

function of this crucial transfer RNA molecule. This mutation leads to the loss of post-transcriptional taurine modifications at the wobble uridine base (τm5U; 5-taurinomethyluridine), which are essential for accurate codon recognition and efficient translation (*8*).

The absence of τm5U modifications specifically impairs the recognition of UUG leucine codons while having minimal effect on UUA codon translation (*8*). This defect has profound implications for mitochondrial protein synthesis because the thirteen mitochondrial-encoded proteins contain varying numbers of UUG codons.

The ND6 subunit of Complex I contains the highest frequency of UUG codons among all mitochondrially-encoded proteins, making it particularly vulnerable to translational defects caused by the A3243G mutation (*9*). This explains why Complex I deficiency is the most consistent and severe biochemical abnormality observed in patients with this mutation.

The impaired UUG codon recognition leads to amino acid misincorporation during protein synthesis, resulting in the production of unstable, incorrectly folded respiratory chain subunits. These defective proteins cannot properly assemble into functional respiratory complexes and are rapidly degraded by mitochondrial quality control mechanisms (*10*). This results in deficiencies of complexes I, III, and IV of the electron transport chain, with complex I showing the most consistent and severe impairment (*11*). The A3243G mutation demonstrates dominant negative effects, where mutant tRNAs interfere with the normal processing of wild-type mitochondrial transcripts (*12*).

2.2. Bioenergetic consequences

The respiratory chain deficiency associated with the A3243G mutation leads to multiple bioenergetic abnormalities. Affected cells demonstrate reduced ATP synthesis, increased lactate production, and compromised maximal oxygen uptake capacity (*6*). Studies using induced neurons derived from A3243G patients have revealed heteroplasmy-dependent decreases in basal respiration, ATP-linked respiration, and spare respiratory capacity (*13*).

The mutation also triggers excessive reactive oxygen species (ROS) production, particularly in cells with high heteroplasmy levels. This oxidative stress contributes to mitochondrial membrane potential dissipation and promotes mitochondrial fragmentation through altered dynamics favoring fission over fusion (*13*). The combination of impaired ATP production and increased oxidative stress creates a cellular environment conducive to dysfunction and death, particularly in metabolically active tissues.

2.3. Tissue-specific vulnerability

Different tissues exhibit varying thresholds for mitochondrial dysfunction, correlating with their specific energy demands and reliance on oxidative phosphorylation (*14*). Tissues with high metabolic activity, such as skeletal muscle, cardiac muscle, and the central nervous system, are particularly susceptible to the effects of the A3243G mutation. The threshold effect of heteroplasmy levels determines the severity of clinical manifestations in each tissue type (*15*).

2.4. Factors contributing to phenotypic variability

The remarkable phenotypic variability observed in patients with the A3243G mutation results from multiple interconnected factors. Heteroplasmy levels represent the primary determinant of disease severity, with different tissues requiring varying thresholds of mutant mtDNA to manifest dysfunction. Skeletal muscle typically requires a 50–65% mutation load to develop symptoms, while cardiac and central nervous system manifestations may require higher thresholds of 60–90% (*16,17*).

Nuclear genetic background also significantly influences phenotypic expression. Polymorphisms in nuclear genes involved in mitochondrial biogenesis, DNA repair, and antioxidant defense can modify disease severity and age of onset. Additionally, environmental factors such as oxidative stress, infections, and metabolic demands can trigger clinical decompensation in previously asymptomatic carriers (*18*).

The tissue-specific energy demands and reliance on oxidative phosphorylation further explain the selective vulnerability observed in different organ systems. Post-mitotic tissues with high energy requirements, such as neurons, cardiac myocytes, and skeletal muscle fibers, are particularly susceptible to mitochondrial dysfunction compared to rapidly dividing tissues that can rely more heavily on glycolytic metabolism (*19*).

3. Genetic inheritance and transmission patterns

3.1. Maternal inheritance patterns

Unlike nuclear DNA disorders that follow Mendelian inheritance patterns, mitochondrial DNA mutations such as A3243G are exclusively maternally inherited. This unique inheritance pattern occurs because mitochondria are predominantly derived from the oocyte, with paternal mitochondria being actively eliminated during early embryogenesis (*20*). Consequently, all offspring of an affected mother are at risk of inheriting the mutation, while affected fathers cannot transmit the mutation to their children.

The maternal inheritance pattern has important implications for genetic counseling and family planning. Risk assessment must consider not only the presence of the mutation in the mother but also her heteroplasmy level and the potential for heteroplasmy shifts during transmission. The lack of Mendelian segregation patterns

can make genetic counseling challenging, as traditional risk calculations do not apply (*21*).

3.2. Heteroplasmy and its clinical implications

Heteroplasmy refers to the coexistence of both wild-type and mutant mitochondrial DNA within the same cell or tissue. The proportion of mutant mtDNA (mutation load) is the primary determinant of clinical phenotype severity. Importantly, heteroplasmy levels can vary significantly between different tissues within the same individual, explaining the variable organ involvement observed in A3243G patients (*22*).

Heteroplasmy levels also change over time, with most studies documenting a decline in blood mutation load with advancing age. This age-related decline in blood heteroplasmy can complicate diagnosis in older patients and may necessitate testing alternative tissues such as urine sediment or muscle biopsy for accurate genetic confirmation (*23*).

3.3. Mitochondrial genetic bottleneck

The mitochondrial genetic bottleneck represents a crucial mechanism that occurs during oogenesis and determines the heteroplasmy levels transmitted to offspring. During oocyte maturation, the mitochondrial population undergoes a significant reduction in number followed by subsequent amplification, creating an opportunity for stochastic changes in mutation load (*24*).

This bottleneck effect explains why a heteroplasmic mother can produce offspring with widely varying heteroplasmy levels, ranging from very low to very high mutation loads. The bottleneck occurs primarily during postnatal folliculogenesis rather than embryonic oogenesis, and involves replication of a small subpopulation of mitochondrial genomes (*25*). Understanding this mechanism is essential for accurate genetic counseling and risk assessment in families affected by mitochondrial disease.

4. Histological features

4.1. Skeletal muscle pathology

Skeletal muscle biopsy remains the gold standard for diagnosing mitochondrial myopathies (*26*). Characteristic histological findings include ragged-red fibers on modified Gomori trichrome staining, representing abnormal accumulations of mitochondria beneath the sarcolemma and between myofibrils. These fibers typically comprise 2-5% of total muscle fibers in A3243G patients (*27*).

Cytochrome c oxidase (COX) histochemistry reveals COX-deficient fibers, appearing as pale or negative-staining myofibers when compared with normal brown-staining fibers. Serial staining with succinate dehydrogenase (SDH) demonstrates preserved or enhanced activity in COX-deficient fibers, creating the characteristic dual-staining pattern that helps differentiate mitochondrial from other myopathies. Notably, the A3243G mutation may preserve COX staining better than other mitochondrial mutations, making SDH staining of blood vessel walls a valuable diagnostic clue (*26*).

Electron microscopy reveals ultrastructural mitochondrial abnormalities including proliferation, pleomorphism, and the presence of paracrystalline inclusions within damaged mitochondria. These changes are observed not only in skeletal muscle fibers but also in smooth muscle cells of arterioles and pericytes of capillaries, reflecting the systemic nature of mitochondrial dysfunction (*28*).

4.2. Vascular pathology

Vascular involvement represents a critical component of A3243G-associated pathology, particularly in MELAS syndrome. Histological examination reveals mitochondrial proliferation in smooth muscle cells of arterioles and capillary pericytes. These vascular changes contribute to the characteristic stroke-like episodes observed in MELAS patients and may explain the predilection for posterior cerebral involvement (*29,30*).

4.3. Central nervous system pathology

Neuropathological findings in A3243G-associated disorders include stroke-like lesions that predominantly affect the posterior cerebral regions, particularly the parietal and occipital cortices (*31*). These lesions cross vascular territories and show characteristics of cellular rather than vascular dysfunction, distinguishing them from typical ischemic strokes (*32*). Additional findings include cerebellar and cerebral atrophy, which typically occur only in severe disease and represent irreversible tissue damage (*33*).

5. Clinical features

5.1. MELAS syndrome

MELAS syndrome represents the most severe phenotypic expression of the A3243G mutation. The cardinal features include mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, though the clinical presentation is highly variable (*9*).

Stroke-like episodes occur in 95% of MELAS patients and typically manifest before age 40 years. These episodes are characterized by acute onset of focal neurological deficits, including hemiparesis, hemianopia, cortical blindness, and aphasia. Unlike typical strokes, these lesions do not follow vascular territories and may be reversible with appropriate treatment (*1*).

Seizures affect 88% of MELAS patients and may be the presenting symptom in some cases. The seizures can be focal or generalized and are often difficult to control with standard antiepileptic medications (*34*).

Encephalopathy, characterized by cognitive impairment, dementia, and psychiatric symptoms, develops in 90% of MELAS patients. This may include progressive intellectual decline, memory impairment, behavioral changes, and psychosis (*1*). Lactic acidosis, while present in 85% of cases, may be intermittent and is often most pronounced during acute episodes (*35*).

5.2. MIDD syndrome

Maternally inherited diabetes and deafness syndrome presents with a more restricted but characteristic clinical phenotype. The two cardinal features are diabetes mellitus and sensorineural hearing loss, though additional manifestations may develop over time (*36*).

Diabetes mellitus affects 90% of MIDD patients and typically presents as insulin-requiring diabetes with onset in adulthood. The diabetes is characterized by progressive β-cell dysfunction and may initially be misclassified as type 2 diabetes mellitus before the underlying mitochondrial etiology is recognized (*37*).

Sensorineural hearing loss occurs in 95% of MIDD patients and is typically progressive, bilateral, and more severe in higher frequencies (*38,39*). The hearing loss often predates the diagnosis of diabetes and may be more pronounced in males (*37*). Unlike MELAS, stroke-like episodes do not occur in MIDD syndrome, although other neurological manifestations may develop over time (*40*).

5.3. Systemic manifestations

Beyond the cardinal features of MELAS and MIDD syndromes, the A3243G mutation is associated with numerous systemic complications that may affect multiple organ systems.

Cardiac involvement manifests as cardiomyopathy in 25–30% of patients, typically presenting as hypertrophic or dilated cardiomyopathy. Conduction abnormalities are frequent and include various degrees of atrioventricular block, bundle branch blocks, and pre-excitation syndromes (*41*). Wolff-Parkinson-White syndrome has been specifically associated with MELAS patients carrying the A3243G mutation. These conduction disturbances may be progressive and can lead to sudden cardiac death if not appropriately managed (*42*).

Ocular manifestations are common and may be among the earliest signs of disease. Retinal dystrophy affects 45–75% of patients and represents the most common ocular manifestation of the A3243G mutation. The retinopathy typically presents as bilateral macular dystrophy with various degrees of retinal pigment epithelium atrophy and hyperpigmentation. Pattern dystrophy and perifoveal atrophy are characteristic findings that may precede systemic symptoms (*43*).

Table 1. Clinical Manifestations Associated with A3243G Mutation

Clinical Features	MELAS (%)	MIDD (%)	*Ref.*
Stroke-like episodes	95	0	(*1*)
Seizures	88	15	(*1,34*)
Encephalopathy	90	10	(*1*)
Lactic acidosis	85	30	(*1,35*)
Sensorineural hearing loss	75	95	(*37,39*)
Diabetes mellitus	35	90	(*36,37*)
Short stature	60	35	(*1,37*)
Exercise intolerance	80	45	(*1,39*)
Muscle weakness	75	30	(*1,6*)
Cardiomyopathy	30	25	(*14,42*)
Retinal dystrophy	45	75	(*5,43*)
Nephropathy	25	35	(*14,44*)

Renal involvement develops in 25–35% of A3243G carriers and may manifest as focal segmental glomerulosclerosis (FSGS), tubulointerstitial nephritis, or bilateral enlarged cystic kidneys (*44*). Renal involvement is often progressive and may lead to end-stage renal disease, particularly in patients with prominent systemic manifestations (*14*).

Gastrointestinal symptoms include chronic constipation, gastroparesis, and pseudo-obstruction. These manifestations likely result from mitochondrial dysfunction in smooth muscle cells of the gastrointestinal tract and may significantly impact quality of life (*45,46*).

Table 1 provides a comprehensive comparison of the clinical features observed in both syndromes, highlighting the distinct phenotypic patterns associated with each presentation.

6. Diagnostic approaches

6.1. Genetic testing

Molecular genetic testing for the A3243G mutation can be performed on various tissue types, with each offering distinct advantages and limitations. The choice of tissue depends on the clinical presentation, accessibility, and required sensitivity.

Skeletal muscle biopsy remains the gold standard for diagnosis, particularly in patients with prominent myopathic features. Muscle tissue typically harbors the highest heteroplasmy levels and provides the most reliable genetic confirmation. However, the invasive nature of muscle biopsy limits its use in some clinical scenarios.

Blood testing offers a non-invasive alternative but has limitations due to age-related decline in heteroplasmy levels. The mutation load in blood decreases with age, potentially leading to false-negative results in older patients (*47*).

Urine sediment analysis has emerged as a valuable

Table 2. Tissue-Specific Heteroplasmy Thresholds for A3243G Mutation

Tissue/Organ System	Threshold for Symptoms (%)	Diagnostic Utility	*Ref.*
Skeletal muscle	50–65	Gold standard	(*6*)
Blood	15–30	Age-dependent decline	(*45*)
Urine sediment	30–50	Stable, high sensitivity	(*43*)
Hair follicles	25–40	Accessible, moderate load	(*43*)
Buccal mucosa	20–35	Non-invasive option	(*15*)
Cardiac muscle	60–80	Functional correlation	(*42*)
Retina	45–70	Phenotype correlation	(*5,43*)
Kidney	55–75	Progressive involvement	(*14,44*)
Central nervous system	70–90	Severe manifestations	(*1*)

alternative, offering stable heteroplasmy levels that do not decline with age. This approach provides high sensitivity and specificity while remaining non-invasive (*43*).

6.2. Biochemical testing

Lactate elevation, both at rest and after exercise, represents a common biochemical abnormality in A3243G patients. However, lactate levels may be normal between acute episodes, limiting the diagnostic utility of single measurements. The lactate-to-pyruvate ratio may be more informative than absolute lactate levels.

Amino acid analysis may reveal elevated alanine levels, reflecting impaired pyruvate metabolism secondary to respiratory chain dysfunction.

6.3. Histological examination

Muscle biopsy with appropriate histological and histochemical studies remains crucial for diagnosis and phenotypic characterization. The combination of ragged-red fibers, COX-deficient fibers, and SDH enhancement provides strong supportive evidence for mitochondrial disease.

Electron microscopy, while not routinely necessary, can provide additional ultrastructural evidence of mitochondrial abnormalities and help exclude other myopathic conditions.

Table 2 summarizes the heteroplasmy thresholds and diagnostic utility of various tissue types for detecting the A3243G variant.

7. Therapeutic approaches

7.1. Metabolic support therapy

Current therapeutic strategies for A3243G-associated disorders focus primarily on metabolic support and symptom management, as no curative treatments are currently available.

Coenzyme Q10 (CoQ10) supplementation at doses of 3.4–10 mg/kg/day aims to enhance electron transport chain function and reduce oxidative stress (*48*). Clinical studies have demonstrated variable responses to CoQ10 therapy, with some patients showing improvement in exercise tolerance, reduction in lactic acidosis, and decreased frequency of stroke-like episodes (*49*). However, larger controlled studies have failed to demonstrate consistent beneficial effects across all patients, suggesting that individual responses may depend on specific genetic and phenotypic factors (*50*).

Idebenone, a synthetic CoQ10 analog, has been used at doses of 5–20 mg/kg/day and may offer advantages over CoQ10 due to its improved bioavailability and enhanced antioxidant properties (*51*).

Dichloroacetate (DCA) at doses of 25–50 mg/kg/day has been employed to reduce lactate levels by activating pyruvate dehydrogenase, though its long-term efficacy and safety profile require further evaluation (*52*).

B-vitamin supplementation, including riboflavin (50–200 mg/day), thiamine (100–300 mg/day), and nicotinamide (50–500 mg/day), is commonly used to support respiratory chain function, though evidence for clinical benefit remains limited (*53*).

7.2. L-arginine therapy

L-arginine supplementation represents one of the most promising therapeutic interventions for A3243G-associated disorders, particularly for the prevention and treatment of stroke-like episodes in MELAS syndrome.

Oral L-arginine at doses of 150-300 mg/kg/day may help prevent stroke-like episodes by improving endothelial function and nitric oxide availability (*7*).

Intravenous L-arginine at doses of 500 mg/kg during acute stroke-like episodes has shown promise in reducing the severity and duration of symptoms, though larger controlled trials are needed to establish definitive efficacy (*54*).

7.3. Supportive care and symptomatic management

Comprehensive management of A3243G-associated disorders requires a multidisciplinary approach addressing diverse systemic complications.

Cardiac monitoring and management are essential given the high frequency of cardiomyopathy and conduction abnormalities. Regular echocardiography and electrocardiographic monitoring should be performed, with prompt intervention for significant arrhythmias or heart failure (*41*).

Hearing assessment and management should include regular audiometric testing and early intervention with hearing aids or cochlear implants when appropriate (*44*).

Ophthalmologic surveillance for retinal dystrophy and other ocular complications should be performed annually (*55*).

Renal function monitoring is important given the risk

Table 3. Therapeutic Approaches for A3243G-Associated Disorders

Therapeutic Agent	Dosage	Mechanism of Action	Evidence Level	*Ref.*
L-arginine (oral)	150–300 mg/kg/day	NO precursor, endothelial function	Moderate	(*7,51*)
L-arginine (IV)	500 mg/kg during SLE	Acute SLE management	Limited	(*7,51*)
Coenzyme Q10	3.4–10 mg/kg/day	Electron transport enhancement	Moderate	(*47,49,50*)
Idebenone	5–20 mg/kg/day	Antioxidant, CoQ10 analog	Limited	(*49*)
Dichloroacetate	25–50 mg/kg/day	Lactate reduction	Limited	(*50*)
Creatine monohydrate	0.3 g/kg/day	Energy metabolism support	Limited	(*46,50*)
Riboflavin (B2)	50–200 mg/day	Respiratory chain cofactor	Limited	(*50,53*)
Thiamine (B1)	100–300 mg/day	Pyruvate metabolism	Limited	(*46,50*)
Nicotinamide (B3)	50–500 mg/day	NAD + synthesis	Limited	(*50,53*)
Supportive care	Individualized	Symptom management	Strong	(*32,46*)

of progressive nephropathy, particularly in patients with systemic manifestations (*44*).

Seizure management may require specialized expertise, as patients with mitochondrial disorders may be sensitive to certain antiepileptic drugs and may require modified treatment approaches (*50*).

7.4. Emerging therapies

Research into novel therapeutic approaches for mitochondrial diseases continues to evolve, with several promising strategies under investigation.

Gene therapy approaches are currently in various stages of development, with the most advanced applications focusing on Leber Hereditary Optic Neuropathy (LHON). Lenadogene nolparvovec has completed Phase 3 clinical trials and demonstrated efficacy and good tolerability for LHON treatment (*56*). For mtDNA disorders like those caused by A3243G mutations, gene therapy approaches face unique challenges due to the need to target mitochondria specifically.

Mitochondrial-targeted genome editing represents a breakthrough therapeutic approach. Recent developments include mitochondrial-targeted platinum transcription activator-like effector nucleases (mpTALENs) that can selectively reduce mutant mtDNA loads in patient-derived stem cells. This technology has successfully achieved mutation loads ranging from 11% to 97% in iPSCs from A3243G patients, representing a significant advance in precision mitochondrial medicine (*57*).

Mitochondrial replacement therapy and advanced gene editing approaches are in early developmental stages but face significant technical and regulatory challenges. CRISPR-based mitochondrial genome editing systems are being developed to selectively eliminate mutant mtDNA while preserving wild-type copies (*58*).

Metabolic modulators aimed at bypassing defective respiratory chain complexes or enhancing alternative energy production pathways are in various stages of preclinical and early clinical development. These include compounds targeting specific aspects of mitochondrial dysfunction beyond traditional antioxidant approaches (*59*).

7.5. Safety considerations and drug interactions

Patients with mitochondrial diseases, including those with A3243G mutations, require careful consideration of drug safety profiles due to potential mitochondrial toxicity. Several classes of medications are known to impair mitochondrial function and should be used with caution or avoided entirely in this population (*60*).

Medications to avoid or use with extreme caution include aminoglycoside antibiotics (particularly in patients with hearing loss), valproic acid (risk of severe hepatotoxicity and status epilepticus), linezolid (can cause severe lactic acidosis), and certain chemotherapeutic agents. The aminoglycoside class is particularly problematic as these antibiotics can cause irreversible hearing loss in patients with certain mtDNA mutations (*60*).

Drugs requiring careful monitoring include statins (myopathy risk), metformin (lactic acidosis risk), and certain antiepileptic drugs. When these medications are necessary, patients should be closely monitored with regular clinical assessments and laboratory studies including creatine kinase and lactate levels (*60*).

L-arginine safety profile: While generally well-tolerated, high-dose L-arginine supplementation (9–30 g/day) can cause gastrointestinal disturbances including diarrhea and nausea and may slightly reduce blood pressure. Intravenous L-arginine overdoses can lead to life-threatening hyperkalemia and hyponatremia, necessitating careful dosing and monitoring (*61*).

CoQ10 and Idebenone are generally considered safe with minimal reported adverse effects. However, therapeutic doses higher than 10 μM may be required to restore mitochondrial respiratory chain enzyme activities to control levels (*62*).

Table 3 outlines the available therapeutic agents, their mechanisms of action, and the current evidence supporting their use in patients with A3243G mutations.

8. Prognosis and disease monitoring

Prognosis for patients with A3243G-associated disorders varies significantly based on the specific clinical phenotype, age of onset, and severity of systemic

involvement. MELAS syndrome generally carries a more severe prognosis than MIDD syndrome, with increased risk of early mortality due to stroke-like episodes and multisystem complications.

Regular monitoring should include: *i*) Cardiac assessment with echocardiography and electrocardiography, *ii*) Audiometric testing for hearing loss progression, *iii*) Ophthalmologic examination for retinal complications, *iv*) Renal function assessment, *v*) Neurological evaluation for cognitive decline or new symptoms, and *vi*) Exercise tolerance and functional capacity assessment.

9. Conclusion

Current evidence establishes that the A3243G mutation affects mitochondrial protein synthesis through impaired $tRNA^{Leu(UUR)}$ function, leading to respiratory chain dysfunction that preferentially impacts metabolically active tissues. The complex interplay between heteroplasmy levels, tissue-specific energy demands, genetic bottleneck effects, and nuclear-mitochondrial interactions determines the remarkable phenotypic variability observed in affected patients. The threshold effects observed across different tissues provide important insights for both diagnostic approaches and prognostic assessment. Recognition that skeletal muscle heteroplasmy levels as low as 50% can produce symptoms challenges earlier assumptions about mutation thresholds and emphasizes importance of tissue-specific testing.

Understanding the maternal inheritance patterns, heteroplasmy dynamics, and genetic bottleneck mechanisms is essential for accurate genetic counseling and family planning. The exclusive maternal transmission and variable heteroplasmy shifts during oogenesis create unique challenges for risk assessment that differ substantially from traditional Mendelian inheritance patterns.

The multisystem nature of A3243G-associated disorders necessitates comprehensive, multidisciplinary care approaches that address not only the primary neurological and metabolic manifestations but also important systemic complications affecting cardiac, renal, retinal, and gastrointestinal systems. Early recognition and proactive management of these complications can significantly impact quality of life and long-term outcomes. Additionally, careful attention to drug safety profiles and potential mitochondrial toxicity is crucial for optimal patient management.

Recent advances in mitochondrial-targeted gene editing and cellular reprogramming technologies offer unprecedented opportunities for developing precision therapies. Successful development of tools that can modulate heteroplasmy levels in patient-derived cells represents a significant step toward therapeutic interventions that target the fundamental genetic defect.

As our understanding of mitochondrial disease mechanisms continues to evolve, the A3243G variant will undoubtedly remain a critical model for advancing both fundamental knowledge and therapeutic development in mitochondrial medicine. Lessons learned from studying this mutation have broader implications for understanding the pathophysiology of mitochondrial dysfunction and developing rational therapeutic approaches for the entire spectrum of mitochondrial diseases.

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Mini-Review

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Advances in research on congenital and hereditary intestinal diseases: From molecular mechanisms to precision medicine

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SUMMARY: Congenital and hereditary intestinal diseases are a group of major disorders caused by gene mutations or embryonic developmental anomalies and are characterized by diverse clinical manifestations and complex management. This review systematically explores the molecular genetic basis and pathogenic mechanisms of common intestinal diseases, including familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome (PJS), Lynch syndrome (LS), Hirschsprung disease (HSCR), congenital short bowel syndrome (SBS), and cystic fibrosis (CF). It focuses on cross-disease commonalities in translational research frontiers such as gene-environment interactions, organoid-based precision medicine, the immune microenvironment, and metabolic and microbiome remodeling. The review also forecasts future directions, including gene therapy, targeted drugs, and other cutting-edge research advances.

Keywords: congenital intestinal diseases, hereditary gastrointestinal disorders, precision medicine, organoid models

1. Introduction

Congenital and hereditary intestinal diseases are key categories of conditions affecting human digestive health, and they are often closely related to key gene mutations, embryonic developmental abnormalities, or metabolic dysregulation (*1*). "Congenital" diseases typically refer to anatomical or functional abnormalities present at birth, stemming from disturbances during embryonic development. In contrast, "hereditary" diseases refer to pathological states caused by genetic material alterations (*e.g.,* DNA sequence mutations and chromosomal rearrangements) that may manifest at birth or later in life. Although distinct in definition, these two categories often overlap in clinical practice — some diseases have a clear genetic basis and also present clinical manifestations at birth. For instance, hereditary precancerous conditions caused by high-penetrance mutations, such as familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome (PJS), and Lynch syndrome (LS) (*2*), typically develop in adolescence or early adulthood. In contrast, Hirschsprung disease (HSCR) and congenital short bowel syndrome (SBS) often present as structural or functional abnormalities in the neonatal period (*3*).

These diseases impact patients' lives long term and impose a psychological burden on patients and their families (*4*). On one hand, some diseases have high morbidity or mortality rates; on the other hand, their heterogeneity and complexity lead to difficulties in diagnosis, limited treatment options, and a long-term reliance on comprehensive medical interventions. Therefore, in-depth analysis of the molecular pathogenesis of these common congenital/hereditary intestinal diseases not only aids in understanding their pathophysiological basis but also provides theoretical support for developing precise diagnostic tools and targeted therapeutic strategies.

2. Disease-specific mechanisms

2.1. Hereditary polyposis and cancer syndromes

2.1.1. FAP

FAP is an autosomal dominant syndrome driven by germline mutations in the *APC* gene, with its core pathogenic mechanism being constitutive activation of the Wnt/β-catenin signaling pathway (*5*). Recent studies have shown that *APC* truncation mutants can mediate aberrant overexpression of METTL3, which impairs tumor immune surveillance by m6A methylation of HIF1α mRNA (*6*). Upregulation of translation control factors like eIF3a is considered an important cooperative mechanism for sustained Wnt pathway activation in FAP,

suggesting the therapeutic potential for interventions at the translational level (*7*). More importantly, research has confirmed that morphologically normal colon epithelium in FAP patients already exhibits metabolic reprogramming and monoclonal evolution, revealing the early basis for intestinal field cancerization and the widespread tendency for carcinogenesis (*8*). Epigenetic analyses have further confirmed that although DNA methylation changes are subtler compared to LS, genome-wide methylation dynamics are still an integral part of FAP tumorigenesis (*9*). These findings not only deepen the understanding of FAP carcinogenesis initiation but also highlight the value of epigenetic drugs and immunotherapy as potential combination strategies.

2.1.2. PJS

PJS is caused by germline mutations in the *STK11/LKB1* gene, with its molecular pathological core being dysregulation of the AMPK/mTOR signaling pathway and loss of apicobasal cell polarity (*10*) . The LKB1 protein encoded by *STK11* is a key upstream activator of AMP kinase; its functional inactivation releases inhibition of the mTORC1 pathway, aberrantly promoting cell growth. The latest research has revealed its downstream effects, including activation of the CRTC2-IL-17 signaling axis and overexpression of interleukin-11 in polyp-specific fibroblasts, providing potential new targets for targeted therapy (*11,12*).

2.1.3. LS

LS is a high-penetrance autosomal-dominant, hereditary cancer predisposition syndrome. Its molecular basis lies in germline mutations in key DNA mismatch repair (MMR) system genes (*13*). An MMR functional deficiency leads to a Microsatellite Instability-High (MSI-H) state, accompanied by extensive DNA methylation changes, collectively driving tumorigenesis (*9,14*). Research on its pathogenic network has expanded to broader aspects, including potential lipid metabolism dysregulation triggered by *MLH1* variants (*15*), epigenetic reprogramming by the histone methyltransferase EZH2 suppressing anti-tumor immunity (*16*), and the potential promoting role of specific gut microbes in colorectal cancer development (*17*). The molecular mechanism of LS is no longer limited to mutation accumulation mediated by dMMR, but presents a more complex collaborative pathological network across dimensions like epigenetics, metabolic rewiring, and gut microbiota.

2.2. Congenital intestinal structural/Neurodevelopmental abnormalities

2.2.1. HSCR

The primary cause of HSCR is the impaired migration, proliferation, or differentiation of enteric neural crest cells (ENCCs) during embryogenesis. Besides *RET* as the main causative gene (*18*), genome-wide association studies have identified multiple susceptibility loci including *JAG1* and *HAND2* (*19*). At the molecular level, secretagogin affects ENCC migration *via* Lymphoid Enhancer Factor-1 (*20*), while the histone methyltransferase SMYD2 regulates cell behavior by modulating METTL3 expression affecting m6A methylation levels, revealing the important role of epigenetic regulation in HSCR (*21*).

2.2.2. SBS

Congenital SBS can be caused by mutations in genes regulating intestinal development, such as *FOXF1* associated with intestinal malrotation, and *CLMP* and *FLNA*, which are closely related to intestinal length development (*22*). Recent studies have found that defects in the immunoglobulin-like cell adhesion protein *CLMP* and the smooth muscle cell proliferation key regulator *SNRK* are the genetic basis for a human congenital SBS-like pathology (*23*).

2.3. CF

CF is caused by mutations in the *CFTR* gene. Its core pathophysiology is defective chloride channel function leading to impaired epithelial ion transport and thickened mucus (*24,25*). In the intestinal system, besides mechanical obstruction, activation of inducible nitric oxide synthase in inflammatory cells produces excess nitric oxide, slowing intestinal motility and contributing to ileus (*26*). Due to the disease's characteristics, patients often have genotype-associated intestinal inflammation (*27*). Table 1 systematically summarizes the key genes, core mechanisms, clinical management, and research frontiers for these six major congenital and hereditary intestinal diseases.

3. Common translational frontiers and precision medicine platforms

3.1. Organoid models and precision medicine

Patient-derived intestinal organoid models provide a revolutionary platform for disease research and individualized therapy. In CF, the forskolin-induced swelling (FIS) assay based on organoids allows precise characterization of *CFTR* function and effectively predicts patient response to modulators (*28,29*). In FAP, these organoids can be used to model tumorigenesis processes and screen intervention strategies (*30-32*). For SBS, preclinical studies have confirmed that transplantation of ileum-derived organoids into the colon can restore absorptive function, providing proof-of-concept for regenerative medicine (*33*).

Table 1. Comparative summary of congenital and hereditary intestinal disorders: From genes to clinical translation

Disease	Key Gene(s)	Core Pathogenic Mechanism	Main Clinical Manifestations	Diagnostic Methods	Clinical Management	Research Hotspots & Advances	*Ref.*
Familial Adenomatous Polyposis (FAP)	*APC*	Constitutive activation of the Wnt/β-catenin signaling pathway, leading to uncontrolled cell proliferation.	Hundreds to thousands of colorectal adenomas, inevitable progression to CRC; elevated risk of duodenal/thyroid cancer.	Colonoscopy, *APC* genetic testing, upper endoscopy surveillance.	Prophylactic colectomy (IRA/IPAA); lifelong endoscopic surveillance.	Interception Therapy: Wnt inhibitors (PORCNi), *APC* vaccines, chemopreventive agents (HAMSB).	(*5,45*)
Peutz-Jeghers Syndrome (PJS)	*STK11/LKB1*	Dysregulation of AMPK/mTOR pathway and loss of cell polarity, leading to hamartomatous polyp formation.	GI hamartomatous polyps, mucocutaneous pigmentation; high risk of intussusception; significantly increased cancer risk in multiple organs.	Clinical criteria, *STK11* genetic testing, video capsule endoscopy/enteroscopy.	Endoscopic polypectomy to prevent intussusception; multi-system cancer screening.	Targeted Therapy: mTOR inhibitors (*e.g.,* Everolimus), IL-11 inhibitors; gut microbiota-metabolite modulation.	(*11,12*)
Lynch Syndrome (LS)	*MLH1, MSH2, MSH6, PMS2*	Defective DNA mismatch repair (dMMR), resulting in Microsatellite Instability-High (MSI-H) and accelerated tumorigenesis.	Early-onset colorectal cancer; high risk of extracolonic cancers (endometrial, gastric, urothelial, *etc.*).	Tumor MMR protein IHC, MSI testing, germline genetic testing.	Personalized colonoscopy surveillance; prophylactic surgery; genetic counseling and family testing.	Precision Immunotherapy: PD-1/PD-L1 inhibitors; Prevention: Neoantigen vaccines.	(*39,40*)
Hirschsprung Disease (HSCR)	*RET*	Impaired migration, proliferation, or differentiation of enteric neural crest cells, leading to aganglionosis in the distal gut.	Functional intestinal obstruction in neonates, abdominal distension, constipation; can be complicated by HAEC.	Rectal suction biopsy, contrast enema, *RET* genetic testing.	Surgical resection of the aganglionic segment (*e.g.,* Swenson, Duhamel procedures).	Regenerative Medicine: Stem cell/enteric neural crest cell transplantation; Complication Management: HAEC mechanism research, 5-HT agonists.	(*18,19*)
Short Bowel Syndrome (SBS)	*CLMP*	Massive intestinal resection or congenital maldevelopment, resulting in critically reduced absorptive surface area.	Severe diarrhea, steatorrhea, malnutrition, dependence on parenteral nutrition (PN).	Clinical presentation, imaging, surgical history, nutritional assessment.	Enteral/parenteral nutrition support, dietary management.	Enhancing Intestinal Adaptation: GLP-2 analogs (Teduglutide, Glepaglutide); Surgical Innovation: STEP/LILT procedures; Regenerative Medicine: Organoid transplantation.	(*22,23*)
Cystic Fibrosis (CF)	*CFTR*	Dysfunctional *CFTR* chloride channel, leading to thick, dehydrated secretions and ductal obstructions.	Chronic lung disease, pancreatic insufficiency (steatorrhea), meconium ileus, malnutrition.	Sweat chloride test, *CFTR* genetic testing, newborn screening.	Lifelong multidisciplinary care (pulmonary, GI, nutrition); pancreatic enzyme replacement.	*CFTR* Modulator Therapy: Correctors/potentiators (*e.g.,* ETI triple therapy); Personalized Prediction: Organoid-based drug testing (FIS assay).	(*24,25*)

3.2. Microbiome and metabolic remodeling

The gut microbiome and its metabolites play crucial roles in disease progression. PJS patients exhibit gut microbiota dysbiosis characterized by enrichment of Veillonellaceae bacteria and reduced synthesis of short-chain fatty acids (SCFAs); levels of these metabolites negatively correlate with polyp burden (*34,35*). In LS, colibactin-producing Escherichia coli is associated with the risk of metachronous colorectal cancer and adenoma development (*17*). Moreover, the gut microbiota remodeling effects demonstrated by *CFTR* modulators and GLP-2 analogs in treating their respective diseases suggest the therapeutic potential of microecological intervention (*36,37*). Figure 1 illustrates the complex interactions between the microbiome, metabolism, and the immune system — a network shared as a pathological basis by many hereditary intestinal diseases.

3.3. Tumor immune microenvironment and immunotherapy

In-depth analysis of the immune microenvironment in precancerous lesions has laid the foundation for immune intervention. The immune signature of PJS polyps resembles that of colorectal cancer tissue, suggesting active immune editing (*38*). LS-associated dMMR/MSI-H tumors are highly sensitive to immune checkpoint inhibitors, marking the advent an era of precision immunotherapy (*39*). The highly expressed frameshift-derived neopeptides in this syndrome provide promising targets for preventive vaccine development (*40*). These findings collectively indicate that the timing for immune intervention could be significantly advanced to the precancerous stage, providing a rationale for 'interception therapy' for hereditary cancers.

3.4. Early detection and risk stratification strategies

Advances in endoscopic monitoring have significantly improved the detection rate of early lesions. For example, linked color imaging and chromoendoscopy can significantly enhance the identification of neoplastic lesions in LS (*41*). Genotype-based individualized monitoring schemes have become standard practice; for instance, colonoscopy screening intervals can be tailored based on the specific MMR gene mutation, which is a strategy that has proven cost-effective (*42*). Figure 2 systematically illustrates this integrated pathway: from initial genetic diagnosis to treatment plan validation based on functional platforms like organoids, culminating in dynamically optimized long-term comprehensive management.

4. Directions for future research

4.1. Gene editing and precision genomic medicine

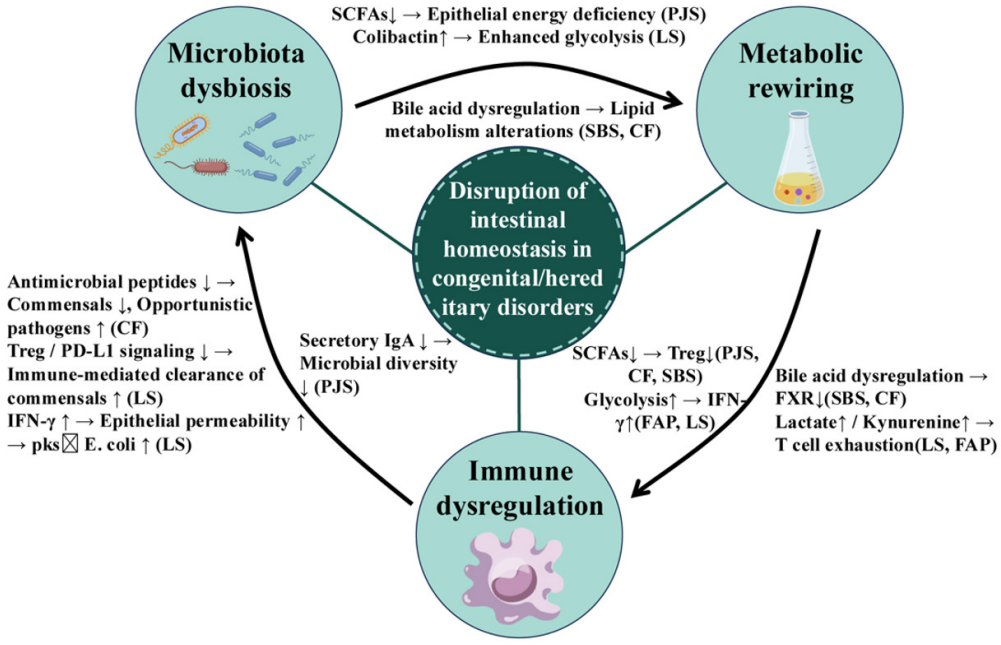

Figure 1. The core microbiota-metabolism-immune interactome in hereditary intestinal disorders. This diagram summarizes a shared pathophysiological network across different diseases. Microbiota Dysbiosis (*e.g.,* SCFA reduction in PJS, colibactin-producing E. coli expansion in LS) drives Metabolic Rewiring, which in turn shapes Immune Dysregulation (*e.g.,* Treg suppression and IFN-γ upregulation). Conversely, immune alterations reciprocally impact the microbiota *via* factors like antimicrobial peptides and secretory IgA. The dynamic interplay between these three core components creates a self-reinforcing cycle that ultimately leads to a Disruption of Intestinal Homeostasis, manifesting as impaired epithelial barrier function, chronic inflammation, and aberrant proliferation. Disease-specific examples (PJS, LS, FAP, CF, and SBS) of validated interactions are annotated. Arrows indicate the direction of "leads to" or "promotes".

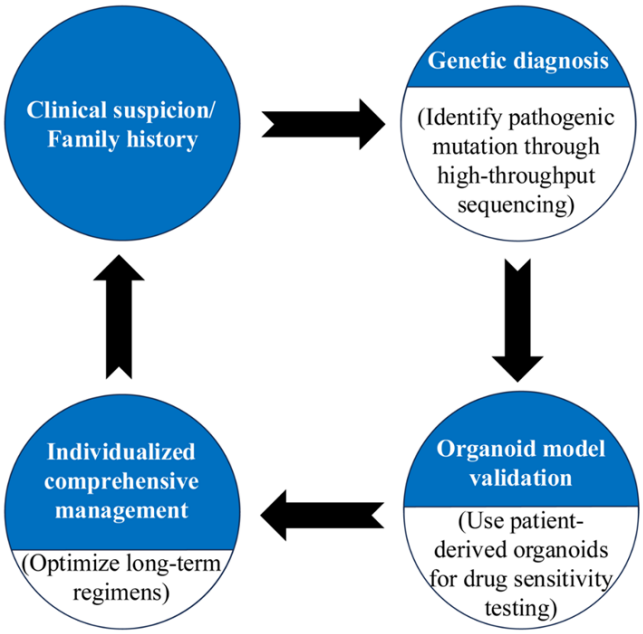

Figure 2. A clinical pathway for precision medicine for congenital and hereditary intestinal diseases. This schematic outlines an integrated, closed-loop pathway from clinical suspicion to individualized management. The journey begins with Clinical Suspicion/Family History, leading to a definitive Genetic Diagnosis through high-throughput sequencing. The pathway then integrates Multi-omic Risk Stratification and functional validation using Organoid Models (*e.g.*, drug sensitivity testing) to inform Individualized Comprehensive Management strategies, including targeted therapies, endoscopic surveillance, surgery, and nutritional support. This framework facilitates long-term, dynamically optimized care, representing a paradigm shift from fragmented interventions to a unified, proactive model of precision medicine.

Gene editing technologies like CRISPR hold promise for curing monogenic hereditary diseases. Future research needs to focus on developing efficient and safe *in vivo* delivery systems to correct pathogenic mutations in somatic cells, *e.g.,* repairing *CFTR* gene function in CF patients or correcting pathogenic variants in key genes like *RET* causing HSCR (*43,44*). The core bottlenecks for clinical translation are: the lack of efficient and safe *in vivo* delivery systems, urgently requiring development of novel vectors targeting epithelia of multiple organs like the gut and lungs; and the verification of long-term safety and controllability, necessitating thorough evaluation of off-target effects and immunogenicity in relevant animal models.

4.2. Immune prevention and neoantigen vaccines

Immune prevention for hereditary cancer syndromes is a highly promising direction. The abundant neoantigens generated by frameshift mutations in LS are ideal targets for developing preventive vaccines (*40*). Similarly, *APC* interception vaccines for FAP have entered the proof-of-concept stage and are intended to stimulate the immune system to clear early lesions expressing mutant APC protein (*45*). The key future challenge lies in overcoming the immune-tolerant microenvironment of precancerous lesions and verifying whether the immune response elicited by a vaccine can provide durable, broad tissue protection in long-term follow-up, thereby effectively preventing multi-organ tumors.

4.3. Regenerative medicine and tissue engineering

For structural or functional deficiency diseases, regenerative medicine aims to achieve fundamental functional reconstruction. In HSCR, the research focus is on how to reconstruct a functional enteric nervous system in the aganglionic segment through stem cell/enteric neural crest cell transplantation (*46*). For SBS, using organoid tissue engineering technology to construct bioengineered intestine with absorptive function is one ultimate solution for intestinal failure (*33*). Achieving these goals requires overcoming major challenges such as cell sources and functional integration post-transplantation (*e.g.,* neural connection and vascularization).

4.4. Microbiome engineering and metabolic intervention

As the role of the gut microbiome in disease progression becomes clearer, its precise modulation will become an important adjunct treatment strategy (*47*). Future approaches may involve designing synthetic microbial communities or engineered bacteria to supplement SCFAs deficient in PJS patients, degrading potential carcinogens in the LS gut, or modulating CF-associated intestinal inflammation (*17,35,48*). Current research is mostly still at the level of describing correlations between microbiota and disease. In the future, research must move towards causal mechanism verification and, based on this, it must design synthetic microbial communities

or engineered bacteria capable of targeted colonization and on-demand secretion of specific metabolites (*e.g.,* supplementing SCFAs deficient in PJS), achieving precise and dynamic remodeling of the gut microecology.

4.5. Multi-omics integration and artificial intelligence (AI)-driven precision management

Utilizing multi-omics data and AI technology to build computational models capable of ultra-early warning, individualized prognosis prediction, and dynamic treatment adjustment is an inevitable trend in the future (*1,49*). The key challenges in this direction are the standardization and sharing of multi-center, multi-omics data and the development of next-generation AI algorithms that can interpret high-dimensional complex biological networks, rather than merely identifying associations.

5. Conclusion

In recent years, research on common congenital and hereditary intestinal diseases has been undergoing a transition from a "single-gene model" to a "systems biology framework", integrating multi-level networks like immunity, metabolism, and the microbiome, thereby enhancing the ability to explain phenotypic complexity. The understanding of disease mechanisms has also expanded from local intestinal pathology to dynamic coupling between multiple organs and the tumor microenvironment, revealing broader intervention windows. Organoid models are being heavily integrated with AI algorithms and high-throughput screening technologies, creating new platforms for precision medicine and individualized therapy. At the same time, the management of long-term complications from the neonatal period to adulthood has promoted the clinical implementation of the "whole-life-cycle care" concept. Overall, future research and clinical pathways for these intestinal diseases will accelerate towards multi-omics integration, automated screening, intelligent intervention, and dynamic health prediction.

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Characteristics of rare diseases cases: A summary analysis of hospitalized patients at a hospital in Western China from 2015 to 2023

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SUMMARY: Rare diseases, characterized by low prevalence and high heterogeneity, impose a significant burden on patients and healthcare systems globally. Utilizing clinical data from the Hospital Information System's Patient Discharge Summaries (2015–2023), we analyzed all rare disease inpatient admissions at a major tertiary hospital in Western China. We examined demographic characteristics, classification of disease systems, medical costs, and readmission rate. Among 1086 inpatient admissions identified with rare diseases (mean age: 46.89 ± 18.99 years), diseases of the nervous system (39.69%), the blood and blood-forming organs and certain disorders involving the immune mechanism (18.32%), the musculoskeletal system and connective tissue (10.50%) constituted the top three disease system categories. The number of the top15 diseases accounted for 73.66% of the total number of patients. The top 3 diseases were POEMS syndrome (11.23%), optical neuromyelitis (10.22%), and Castleman disease (7.46%). Hospitalization costs were predominantly composed of diagnostic (ranged from 6.41% to 49.75%) and medication costs (ranged from 12.97% to 46.22%). The 10 highest readmission rates ranged from 42.86% to 95.90%. The rare diseases in this hospital had a large age span, diverse disease types, high hospitalization costs and large individual differences, which was representative to a certain extent, and can provide scientific basis for the diagnosis, treatment, and prevention of rare diseases in Gansu Province and even the northwest region of China.

Keywords: rare diseases, epidemiology, in-hospital analysis, Western China

1. Introduction

Rare diseases, or orphan diseases, are a broad category of illnesses with a very low prevalence (*1*). There are an estimated 6,000–7,000 rare diseases affecting approximately 300 million individuals, with approximately 80% being genetic in origin, and 50–75% being pediatric onset, 30% of whom have a lifespan of no more than 5 years (*2*). The global prevalence is estimated to be between 3.5% and 5.9%, impacting 263–446 million people (*3*). Due to variations in medical status, social security, and economic conditions, there is no unified global definition — prevalence thresholds range from 5 to 76 cases/100,000 people across regions, with a global average of 40 cases/100,000, and definitions by the World Health Organization (WHO), the European Union (EU), and the United States (US) differing accordingly, but not exceeding (*4-8*). Globally, 90% of rare diseases lack effective treatments, leading to delayed diagnosis, high misdiagnosis rates, and heavy burdens on patients and society, especially in developing countries (*9,10*). Despite variations in incidence rates across different countries and regions, rare diseases globally demonstrate an overall upward trend year by year, posing a serious threat to public health worldwide.

China is one of the most populous countries in the world, with a population of approximately 1.4 billion. There are about 20 million rare disease patients, with over 200,000 new patients diagnosed annually (*11*). A survey conducted in 93 hospitals across seven provinces revealed that a total of 405,589 patients with 952 types of rare diseases were registered as inpatients, among which at least half were congenital diseases (*12*). Over the past few years, the Chinese Government has paid greater attention to rare diseases and it has incorporated rare diseases in national health strategy and planning (*13-15*). It released the first (2018) and second (2023) national rare disease lists covering 121 and 87 diseases respectively, which established a national diagnosis and treatment network (expanded to 394 member hospitals

by February 2024), built the National Rare Diseases Registry System (NRDRS), and set up quality control centers and multidisciplinary expert teams (*16-20*). These measures are aimed at accelerating scientific and standardized development of rare diseases diagnosis and treatment, continuously improving public awareness, and exploring Chinese solutions for the overall goal of early detection, early diagnosis, treatment, management, medicine, and affordability of rare diseases.

Despite these efforts, significant challenges persist. "Poor drug accessibility" and "heavy economic burden" remain critical issues, particularly for rural patients (*21,22*). Diagnosis is extremely difficult due to the multidisciplinary nature of rare diseases: Some patients have to consult with 5 to 10 doctors, and it may take up to 30 years to receive an accurate diagnosis. Furthermore, many doctors have limited knowledge about rare diseases, leading to frequent misdiagnoses and missed diagnoses. Currently, approximately 44% of rare diseases in China are misdiagnosed, and 75% of rare diseases are treated in a non-standardized manner. According to findings of the "2020 Comprehensive Social Survey on Rare Diseases in China" conducted by the China Alliance for Rare Disorders, among the over 20,000 patients surveyed, 15.5% required 1 to 4 years to receive a correct diagnosis, while 5% needed between 5 and 20 years. The average time to diagnosis varied from 4 to 26 years. Moreover, 42% of patients had experienced misdiagnosis. This data objectively highlights significant challenges faced in diagnosis and treatment of rare diseases in China (*23*).

Due to the extremely low incidence of such diseases, the morbidity and prevalence of the population are difficult to estimate worldwide, leading to the real assessment of the status of disease diagnosis and treatment is difficult. Current epidemiological data on rare diseases in China are remarkably limited. China's Rare Disease Diagnosis and Treatment Guide, and data on the incidence/prevalence of 76 rare diseases (62.81%) were available for 121 rare diseases in China (*12*). This data scarcity is particularly acute in the less developed regions of Western China. Although rare disease researches have received increasing attention in China over the last few years, epidemiological and health economic studies remain exceptionally scarce in the underdeveloped regions of western China. Specifically, researches focusing on clinical profiles, inpatient burden, and healthcare utilization patterns of rare disease patients in Western Chinese hospitals are virtually absent. A regional distribution analysis of rare disease research in China revealed that the western region (12 provinces) contributed only 12.3% of the national research output, significantly lower than eastern (65.7%) and central (22.0%) regions (*24*). It even affects implementation of medical insurance policies and adjustment of drug catalogs for disease relief. Although countries around the world have explored establishment of rare disease registration systems and carried out related cohort studies, existing studies mainly focus on individual characteristics of rare diseases and drug use for rare diseases. In recent years, there have also been studies on the social psychological status of rare disease patients, but few studies on distribution characteristics of rare disease patients in hospitals, and there is a lack of reliable data support.

This study focuses on characteristics of inpatients with rare diseases in a tertiary hospital of a provincial capital in western China. As well as focus on analyzing the age and gender distribution of various diseases, epidemiological characteristics of the top fifteen disease types, and composition of hospitalization costs and readmission rates, *etc*. The objective of our study is to understand the current state of diagnosis and treatment for rare diseases in western China, thereby providing a scientific foundation for the prevention and control of rare diseases, the enhancement of diagnostic and treatment capabilities, and related policy research.

2. Materials and Methods

2.1. Data source

The Hospital Information System (HIS) is a computerized information system that integrates multiple functions, aiming to improve internal workflows and management efficiency in hospitals through information technology. The HIS system typically covers common modules of daily hospital operations such as outpatient management, inpatient management, pharmacy management, and others. All data in this study were extracted from the Patient Discharge Summary in the electronic medical record system of Gansu Provincial People's Hospital. The Patient Discharge Summary is a summary of the information generated during a patient's hospital stay, including diagnosis, surgery, procedures, blood transfusions, treatment outcomes, total hospital charges, fee categories, and payment methods.

China's first rare diseases list (121 diseases) was released on May 22, 2018 (*16*), followed by the second list (86 diseases) formulated according to the "Working Procedures for Drafting the List of Rare Diseases" and announced on September 18, 2023 (*17*). In order to gain a deeper understanding of the epidemiology, clinical diagnosis, and medical insurance status of rare diseases in China, National Health Commission of the People's Republic of China launched the registration and reporting system for case diagnosis and treatment information in 2019, and retrospectively reported cases diagnosed between January 1, 2015 and October 31, 2019 (*25,26*).

Therefore, we included hospitalization cases with discharge dates between January 2015 and December 2023. Using the tenth revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) for the 207 diseases listed in the

first and second batches of the national rare disease directories as matching criteria, we mapped the discharge diagnosis codes. Ultimately, 1,086 cases were included in this study and involved 11 categories of disease classification out of 22 disease classifications according to the 10th Revision of the International Classification of Diseases and Related Health Statistics, coded as shown in Additional file 1 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=273*).

This study received an ethics exemption from the Ethics Committee of Gansu Provincial Hospital. All patient identifiers were rigorously removed or anonymized during data extraction and processing prior to analysis.

2.2. Study variables

We extracted information from the Patient Discharge Summary. The information included the basic information of patients (hospitalization number, gender, age), diagnosis and treatment information (admission route, medical insurance payment type, major diagnosis name and code. Other diagnosis names and codes, length of stay, and cost information (total hospitalization cost, drug cost, nursing cost, diagnosis fee, medical service fee, *etc.*). We double-checked questionable information and incomplete data to ensure that case information is accurate. Medical records were excluded if any of the following conditions applied: *i*) length of stay (LOS) was zero, which was excluded to ensure analytical consistency, as they represent non-actionable admissions or data anomalies inconsistent with sustained inpatient care; *ii*) some key variables were missing or unclear, including age, sex, and primary diagnosis; *iii*) other cases with obvious logical errors and unmatched information. All research information was managed and analyzed by designated personnel to ensure patient information security.

2.3. Statistical analysis

Patients' age at admission was used for analysis of age distribution. The age group included 0–14 years, 15–44 years, 45–59 years, and 60~ years. The number of these four age groups were calculated.

Readmission rate is defined as the proportion of patients readmitted for the same or related condition within a specified time window (*e.g.*, 30 days, 90 days, or 1 year) relative to the total discharged population. In this study, given the low prevalence of rare disease patients, the time window is defined as any period within the study duration. Specifically: *i*) Numerator: The total number of repeat hospitalizations for each rare disease during the study period. For a given patient with ≥ 2 hospitalizations, each admission after the first is counted as a readmission (*e.g.*, 3 hospitalizations = 2 readmissions). *ii*) Denominator: The total number of hospitalizations (including initial admissions) for the corresponding rare disease during the study period.

Data organization and cleaning were performed in Microsoft Excel 2019. Statistical analysis was conducted using IBM SPSS Statistics v23 software (IBM Corporation, USA). Graphs were performed using GraphPad Prism (version 9.0.0). Continuous variables conforming to a normal distribution were described by the mean ± standard deviation (X ± SD), while those with a non-normal distribution were presented as median (P25–P75). Categorical variables were described as frequency and proportion. Student's *t*-test or Mann–Whitney *U* test was employed to compare continuous variables (for normally and non-normally distributed data, respectively), and Chi-square test or Fisher's exact test was used for categorical variables. Bonferroni correction for multiple comparisons was applied. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

By matching ICD-10 of the rare disease catalog, a total of 1086 hospitalized cases were finally included, of which 550 were males (50.64%) and 536 were females (49.36%), with a male-to-female ratio of 1.03:1. The age distribution ranged from 0 to 89 years old, with an average age of 46.89 ± 18.99 years old, including 46.57 ± 20.69 years old for males and 47.22 ± 17.09 years old for females. There was no significant difference in the mean age between males and females ($t = 0.570$, $p = 0.571$). These cases were mainly admitted and treated through the following three routes: outpatient admission in 925 cases (85.17%), emergency admission in 149 cases (13.72%), and transfer to other medical institutions in 12 cases (1.10%). The median length of stay for all patients was 7 days (4–11 days). From the type of medical insurance payment, there were 651 cases (59.94%) of self-funded medical insurance, 223 cases (20.53%) of basic medical insurance for urban and rural residents, 188 cases (17.31%) of commercial insurance, and 24 cases (2.22%) of provincial and municipal employee medical insurance. From the perspective of time distribution, the number of cases increased year by year from 2015 to 2020, decreased slightly from 2021 to 2022 and showed a significant rebound in 2023. Table 1 showed a full demographic description of the dataset.

3.2. The disease classification of cases

According to the ICD-10, 11 of 21 disease system classifications were involved in the study, covering 96 diseases, accounting for 46.38% (96/207) of the total number of diseases in China's rare disease catalog. The distribution of 1,086 cases according to the disease classification system is shown in Table 2. The distribution

of rare cases in each disease system is uneven, in order from the highest to the lowest proportion of covered cases: diseases of the nervous system (39.69%), diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism (18.32%), diseases of the musculoskeletal system and connective tissue (10.50%), Neoplasms (8.56%), endocrine, nutritional and metabolic diseases (6.63%), eye and adnexa diseases (4.24%), circulatory system diseases (3.96%), respiratory system diseases (3.87%), congenital malformations, deformations, and chromosomal abnormalities (3.78%), skin and subcutaneous tissue diseases (0.37%), digestive system diseases (0.09%). Among them, the top three disease categories covering the number of disease types are neurological diseases (27 diseases), endocrine, nutritional, and metabolic diseases (17 diseases), and congenital malformations, deformations and chromosomal abnormalities (12 diseases). The Chi-square test was used to analyze the differential distribution of male and female patients in each disease system, and the results showed that the distribution was statistically different ($\chi^2 = 31.688$, $p < 0.01$).

Based on age group categorization, the study cases were stratified by admission year and accumulated according to disease classification. The results showed that there were differences in distribution of disease types of the cases in each age group. Distribution of cases across the four age groups is as follows: 0–14 years old (77/1086), 15–44 years old (340/1086), 45–59 years old (475/1086), 60~ years old (194/1086). In the < 15 years old age group, the top three were nervous system diseases (46.75%), blood and hematopoietic organ diseases and some diseases of immune mechanism (23.38%), endocrine, nutritional and metabolic diseases (6.49%). The top three diseases in the age group of 15–44 years old were nervous system diseases (34.41%), blood and hematopoietic organ diseases and some diseases of immune mechanism (18.53%), malignant tumor/benign tumor/benign and malignant tumor (17.94%). The top three diseases in the 45–59 age group were nervous system diseases (42.95%), blood and hematopoietic organ diseases and some diseases of immune mechanism (22.32%), musculoskeletal system and connective tissue diseases (13.26%). Diseases of the nervous system (38.14%), musculoskeletal system and connective tissue (18.56%), and respiratory system (9.79%) accounted for the top three diseases in the age group 60 and above.

Table 1. Demographic and clinical characteristics of hospitalized cases with rare diseases according to subgroups

Variable	No.	%
Gender		
Male	550	50.64
Female	536	49.36
Age		
0–14	77	7.09
15–44	340	31.31
45–59	475	43.74
60~	194	17.86
Admission route		
Outpatient referral	925	85.17
Emergency	149	13.72
Referral	12	1.10
Payment type		
Self-funded healthcare	651	59.94
Urban and Rural Resident Basic Medical Insurance	223	20.53
Commercial insurance	188	17.31
Provincial and Municipal Employee Medical Insurance	24	2.22
Year		
2015	31	2.85
2016	43	3.96
2017	69	6.35
2018	90	8.29
2019	113	10.41
2020	169	15.56
2021	167	15.38
2022	136	12.52
2023	268	24.68

Table 2. Distribution of 1,086 hospitalized cases according to the disease classification system

ICD-10 Code	Disease Classification	Diseases No.	Cases No.	%	χ^2=31.688		$p < 0.01$
					Male	Female	Gender Ratio
G00-G99	Diseases of the nervous system	27	431	39.68	217	214	1.01
D50-D89	Diseases of the blood and rming organs and certain disorders involving the immune mechanism	9	199	18.32	73	126	0.58
M00-M99	Diseases of the musculoskeletal system and connective tissue	7	114	10.50	64	50	1.28
C00-D48	Neoplasms	4	93	8.56	51	42	1.21
E00-E90	Endocrine,nutritional and metabolic diseases	17	72	6.63	50	22	2.27
H00-H59	Diseases of the eye and adnexa	1	46	4.24	23	23	1.00
I00-I99	Diseases of the circulatory system	4	43	3.96	21	22	0.95
J00-J99	Diseases of the respiratory system	2	42	3.87	23	19	1.21
Q00-Q99	Congenital malformations, deformations and chromosomal abnormalities	12	41	3.78	25	16	1.56
L00-L99	Diseases of the skin and subcutaneous tissue	2	4	0.37	2	2	1.00
K00-K93	Diseases of the digestive system	1	1	0.09	1	0	/

It can be seen that neurological diseases are the main types of rare diseases in all age groups, but with age, musculoskeletal system and connective tissue diseases and respiratory diseases gradually become the main types of diseases. The results are shown in Figure 1.

3.3. Analysis of the top fifteen disease categories

The study cases were further analyzed according to the types of discharge diagnoses. The top fifteen disease categories are polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, skin changes (POEMS) syndrome (11.23%), optic neuromyelitis (10.22%), Castleman disease (7.46%), multiple system atrophy (7.09%), amyotrophic lateral sclerosis (6.54%), antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (4.24%), retinitis pigmentosa (4.24%), progressive muscular dystrophy (4.05%), systemic sclerosis (3.50%), hemophilia (3.41%), multiple sclerosis (3.31%), idiopathic pulmonary fibrosis (2.67%), idiopathic pulmonary arterial hypertension (2.30%), autoimmune encephalitis (1.75%), and Marfan syndrome (1.66%). With the exception of POEMS syndrome, optical neuromyelitis, systemic sclerosis, multiple sclerosis, and idiopathic pulmonary arterial hypertension, where the proportion of female patients exceeds that of males, the remaining ten disease categories show a higher proportion of male patients compared to females. The results are shown in Figure 2 and Figure 3.

An analysis was conducted on the age distribution of patients with the top fifteen disease categories, which are presented in Figure 4. The results showed that these patients account for 73.66% of all cases. Among them, all fifteen disease categories have patients within the 45–59 age group. Eight disease categories have patients under 15 years old, fourteen disease categories have patients within the 15–44 age group, and twelve disease categories have patients aged 60 or above. Specifically, progressive muscular dystrophy (61.36%) and hemophilia (35.14%) primarily affect individuals under 15 years old; Castleman disease (72.84%) and Marfan syndrome (72.22%) mainly occur in the 15–44 age group; ANCA-associated vasculitis (54.35%), idiopathic pulmonary fibrosis (65.52%), and idiopathic pulmonary arterial hypertension (48.00%) predominantly affect individuals aged 60 or above. For the remaining diseases, the highest proportion of cases is found in the 45–59 age group.

The results of the analysis on the composition of hospitalization costs for patients within the top fifteen disease categories are presented in Table 3. The findings revealed that for the majority of these conditions, hospitalization costs were predominantly composed of diagnostic and medication costs, with diagnostic costs accounting for between 6.41% and 49.75%, and medication costs ranging from 12.97% to 46.22%. Furthermore, for retinitis pigmentosa and Marfan syndrome, surgical costs represented the second largest component of the cost structure, accounting for 14.41% and 8.61%, respectively. For rare diseases involving neurological disorders, such as multiple system atrophy,

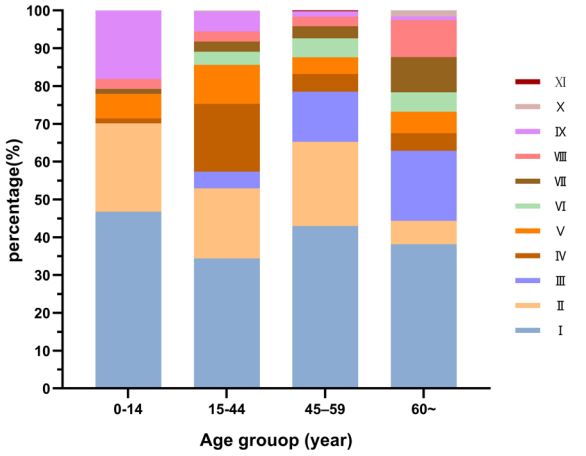

Figure 1. The distribution of cases across the four age groups.

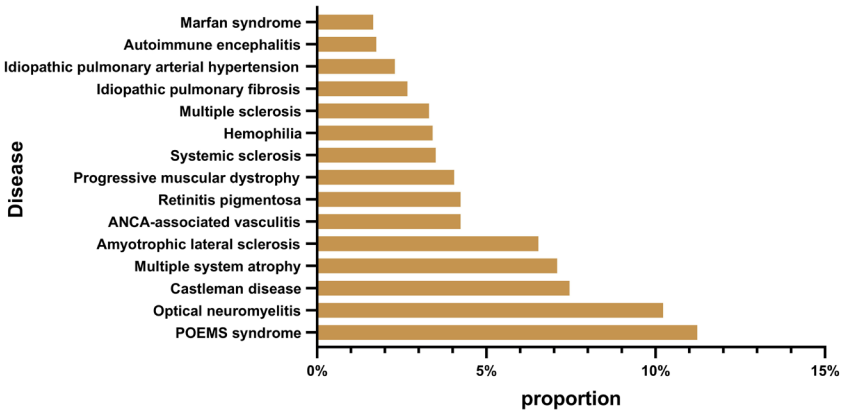

Figure 2. The proportion of the top fifteen diseases categories.

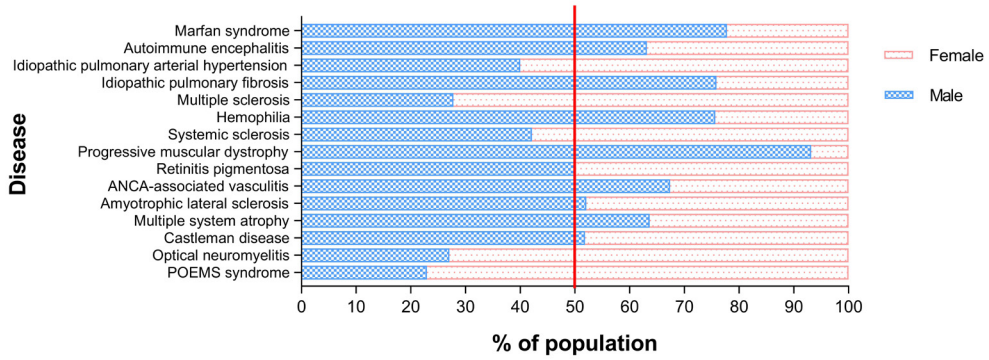

Figure 3. The gender distribution of cases in the top fifteen diseases categories.

autoimmune encephalitis, amyotrophic lateral sclerosis (ALS), and multiple sclerosis, rehabilitation treatment costs were relatively higher, at 2.23%, 1.63%, 1.12%, and 1.02%, respectively. About total hospitalization costs, the mean hospitalization costs for the top fifteen disease syndromes had the highest mean hospitalization cost at $11,559.06 ± 13,834.57, with a median cost of $3,595.89; Autoimmune encephalitis followed, with a mean hospitalization cost of $5,347.21 ± 7,175.00 and a median cost of $2,716.60; the lowest mean hospitalization cost was observed for Castleman's disease, at $863.15 ± 1,914.45, with a median cost of $373.42.

3.4. Readmission rate

Among the 96 disease categories involved in this study, 29 exhibited instances of readmission. The 10 diseases with the highest readmission rates, listed in descending order, were McCune-Albright syndrome, POEMS syndrome, Castleman disease, Langerhans cell histiocytosis, albinism, pulmonary alveolar proteinosis, ANCA-associated vasculitis, paroxysmal nocturnal hemoglobinuria, optical neuromyelitis, and Fanconi anemia. The readmission rate ranged from 95.90% to 42.86%, which is presented in Table 4.

4. Discussion

Gansu Provincial People's Hospital, a prominent tertiary A comprehensive hospital in northwest China, boasts a high patient volume, diverse patient demographics, and a broad spectrum of disease entities, with a significant proportion of complex and critically ill cases. The hospital's clinicians possess extensive and profound expertise in diagnosing and treating rare diseases, rendering the cases studied highly representative. This is also the first study conducted in Gansu on the characteristics of hospitalized patients with rare diseases, laying the foundation for research on rare diseases in northwest China and filling a gap in this field (by comprehensive literature search to confirm the

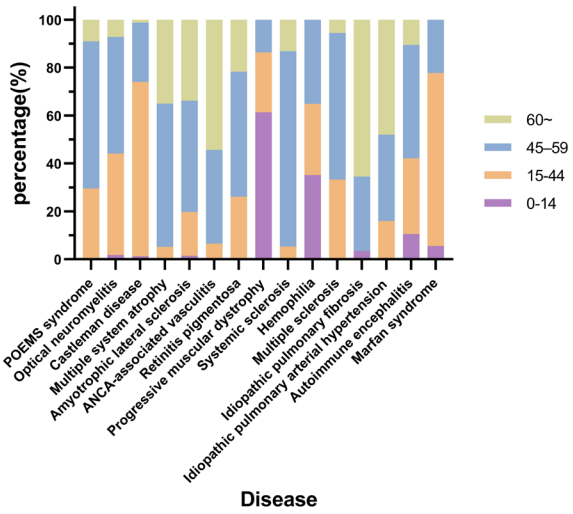

Figure 4. The age distribution of cases in the top fifteen disease categories.

novelty of our study). The results of our investigation indicated substantial heterogeneity in both composition of different rare diseases and demographic distribution of their affected populations. Consequently, our findings underscore the critical need for advancing precision of diagnosis and treatment across a wide range of medical specialties.

Our study found that neurological diseases emerged as the most common category (39.69% of cases), diseases of the blood and blood-forming organs, certain diseases involving immune mechanisms (18.32% of cases), and diseases of the musculoskeletal system and connective tissue (10.50% of cases). This is consistent with the characteristics of rare diseases, which are mostly genetic and affect multiple organs and systems, resulting in poor patient outcomes and long rehabilitation periods. In China, 106,746 hospitalizations for a rare disease were captured from 1 January 2014 to 31 December 2015, accounting for 0.69% of inpatients during the same period (*27*). The top 10 rare diseases with most cases on the TRDL 2017 were thalassemia, idiopathic pulmonary arterial hypertension, pulmonary Langerhans cell

Table 3. Analysis of the composition of hospitalization costs for patients with the top fifteen Diseases

Disease	No.	Average Hospitalization Cost per admission (USD)	Median hospitalization cost per admission (USD)	Nursing Costs (%)	Medical Service Costs (%)	Diagnostic Costs (%)	Surgical Costs (%)	Rehabilitation Costs (%)	Medication Costs (%)	Blood and Blood Products Costs (%)	Consumables Costs (%)	Other Costs (%)
POEMS syndrome	122	913.13 ± 1,569.44	363.46	1.12	12.71	37.34	1.56	0.03	25.90	1.36	0.00	1.90
Optical neuromyelitis	111	2,299.74 ± 2,021.12	1,509.58	2.75	8.08	32.68	0.27	0.56	46.22	0.12	0.00	2.69
Castleman disease	81	863.15 ± 1,914.45	373.42	0.63	9.71	49.75	1.28	0.04	23.07	3.89	0.00	0.00
Multiple system atrophy	77	2,414.77 ± 4,715.24	1,403.61	9.41	7.78	27.57	0.27	2.23	32.86	0.00	0.00	9.61
Amyotrophic lateral sclerosis	71	1,551.70 ± 1,265.60	1,332.00	2.24	6.84	37.96	0.93	1.12	22.49	0.00	0.00	14.57
ANCA-associated vasculitis	46	2,751.23 ± 6,081.99	857.46	2.49	6.99	28.24	2.56	0.34	29.36	2.17	0.00	0.97
Retinitis pigmentosa	46	1,207.94 ± 924.72	1,006.84	0.85	4.02	25.55	14.41	0.17	12.97	0.00	0.00	14.41
Progressive muscular dystrophy	44	1,235.98 ± 962.67	927.28	5.30	9.80	37.89	1.19	0.32	25.73	0.00	0.00	14.30
Systemic sclerosis	38	1,577.47 ± 2,112.03	620.41	4.20	9.72	42.00	1.03	0.07	36.57	0.59	0.00	0.00
Hemophilia	38	1,844.60 ± 1,778.23	1,378.55	1.36	8.48	30.45	3.11	0.22	22.62	10.85	0.00	5.76
Multiple sclerosis	36	2,049.26 ± 1,554.74	1,560.57	2.63	9.13	42.25	0.05	1.02	35.92	0.00	0.00	1.54
Idiopathic pulmonary fibrosis	29	2,960.66 ± 4,671.22	1,669.05	3.55	10.13	33.39	1.92	0.08	41.80	2.05	0.00	0.00
Idiopathic pulmonary arterial hypertension	26	2,062.04 ± 1,157.64	1,878.84	3.67	7.19	33.53	0.78	0.23	24.56	0.36	0.00	6.43
Autoimmune encephalitis	19	5,347.21 ± 7,175.00	2,716.60	8.95	7.70	21.03	0.23	1.63	43.71	0.44	0.00	7.77
Marfan syndrome	18	11,559.06 ± 13,834.57	3,595.89	3.16	2.41	6.41	8.61	0.51	14.64	3.37	0.00	15.94

histiocytosis, moyamoya disease, motor neuron disease, idiopathic pulmonary fibrosis, systemic sclerosis, hepatolenticular degeneration, coarctation of the aorta, and transposition of the great arteries (*28*). A study from a top-tier hospital in Shaanxi Province, which is located in the northwest region of China, found that patients with nervous system diseases, respiratory system diseases, and blood system diseases accounted for the highest proportions, at 74.12%, 7.39%, and 6.26% respectively (*29*). An analysis of characteristics of hospitalized rare disease cases in Zhejiang Province, located in the eastern region of China, found that diseases of the blood system, congenital malformations, and diseases of the nervous system ranked as the top three in terms of case numbers (*30*). In the southwestern region of China, the top three categories of rare disease inpatients were endocrine and metabolic diseases, neurological diseases, and hematological diseases (*31*). This may be related to regional differences, lifestyle habits, and the varying strengths in disease diagnosis and treatment among different hospitals. Studies have indicated that insufficient awareness of rare diseases among physicians is one of the contributing factors to delayed diagnosis and misdiagnosis of rare disease patients (*32*). Therefore, it is necessary to strengthen professional training for physicians in relevant specialties such as neurology, endocrinology, pediatrics, and hematology based on distribution of the rare disease spectrum in the region. Additionally, efforts should be made to establish expert teams for rare disease diagnosis and treatment, enhance research on clinical differential diagnosis and treatment, and optimize the multi-disciplinary diagnosis and treatment model for rare diseases, thereby improving diagnosis rate and treatment rate of rare diseases in the region.

Our study identified notable sex-based and age-related disparities in classification of rare diseases. Female patients exhibited a higher prevalence of blood and rming organs and certain disorders involving the immune mechanism, while male patients were more frequently diagnosed with musculoskeletal system and connective tissue diseases, respiratory, congenital malformations, deformations and chromosomal abnormalities, and neoplasms. These differences are consistent with previous research, which suggests that sex hormones, immune system variations, and genetic susceptibility contribute to sex-specific disease manifestations (*33,34*).

The age of diagnosis differed markedly among distinct disease groups. Although a substantial proportion of rare disorders were identified during adult years (45–59 years in our study), certain categories — particularly nervous system and congenital malformations, deformations and chromosomal abnormalities — showed a strong pediatric predominance. Distribution of the top fifteen diseases varies among different age groups. Neurological diseases are the predominant type

Table 4. Statistics on the top 10 diseases by readmission rate

Disease	No.	Readmissions No.	Readmission rate (%)
POEMS syndrome	122	117	95.90
Castleman disease	81	72	88.89
Langerhans cell histiocytosis	16	13	81.25
McCune-Albright syndrome	4	3	75.00
Pulmonary alveolar proteinosis	13	8	61.54
ANCA-associated vasculitis	46	29	63.04
Albinism	5	3	60.00
Optical neuromyelitis	111	65	58.56
Paroxysmal nocturnal hemoglobinuria	11	6	54.55
Fanconi anemia	7	3	42.86

of rare diseases across all age groups. As age increases, musculoskeletal and connective tissue diseases, as well as respiratory system diseases, gradually become the main types of diseases. This trend is consistent with progression and changes in diseases that occur with aging. It indicates that patients with rare diseases mainly affect the working-age population of young and middle-aged individuals, imposing a heavy burden on individuals, families, and social development in Gansu Province. This finding is somewhat different from conclusions reported in previous literature, which state that "the occurrence of rare diseases is closely related to genetics", "about 80% of rare diseases are caused by genetic defects", and "50% to 70% of rare diseases manifest at birth or during childhood". This discrepancy may be due to the fact that the sentinel hospitals selected for our study were general hospitals, which tend to have fewer pediatric patients compared to specialized children's hospitals. Additionally, Gansu Province, located in a less developed area of western China, faces challenges with public and primary healthcare workers' early prevention and recognition capabilities of rare diseases, which are not as advanced as those in more developed eastern regions. Consequently, many rare diseases are not identified and managed promptly at onset, leading to underdiagnosis and misdiagnosis. Therefore, we recommend sustained public health education initiatives to disseminate knowledge on the three-tiered prevention strategy for rare diseases, thereby enhancing population awareness and health literacy. This approach fosters a compassionate and socially supportive environment for affected individuals.

The severity of a disease, its treatment methods, length of hospital stay, and medication costs are all to some degree determined by type of disease. From the perspective of the global situation, treatments for rare diseases encompass medications, dietary adjustments, surgery, or rehabilitation with medical devices. Among these, pharmacological treatment is currently the primary mode of clinical diagnosis and treatment (*33*). We found that in the top fifteen diseases, inpatient costs were predominantly comprised of diagnostic fees and medication expenses, with the latter accounting for up to 46.22% of total cost. This finding is consistent with the current global situation, where rare diseases are characterized by complex etiologies, challenging diagnostic processes, extended treatment and rehabilitation periods, and a substantial economic burden on patients. Moreover, the same disease can result in significant variations in inpatient costs due to differences in patient age, disease progression, treatment methods, type of medical payment, and degree of recovery. Consequently, the standard deviation of the average inpatient cost is relatively high. On the other hand, the level of healthcare institutions, medical insurance policies, regional economic conditions, and the allocation of medical resources are also key factors that influence inpatient costs for rare diseases. A 2019 study on the economic burden of rare diseases in the United States found that inpatient medical service fees and prescription medication costs were the largest components, accounting for 32% and 18% of direct medical costs, respectively (*34*). A study on the economic burden of Epidermolysis Bullosa in Spain found that direct non-medical costs represented the largest proportion, primarily consisting of informal care costs. Among healthcare expenses, the cost of specialist visit was most significant, accounting for 5.72% of total costs, followed by nursing care, which accounted for 2.37% of total costs (*35*). In Shaanxi, China, an analysis of the top five cost components for inpatient care of rare disease patients at a tertiary hospital revealed that medication costs (ranging from 24% to 54%) and diagnostic fees (ranging from 18% to 44%) accounted for a significant proportion of total expenses (*36*). In Shanghai, China, the estimated annual average cost for inpatient care of 23 rare diseases is ¥9,846.77. Compared to the annual disposable income, the cost of inpatient treatment for rare diseases represents a significant proportion of annual disposable income for urban residents and nearly half of annual disposable income for rural residents (*22*). This also clearly indicates that medical expenses for rare diseases are heavy, especially for families from less developed rural areas.

While China’s medical security system for rare diseases has made notable progress — illustrated by 137 drugs granted priority review in Q1 2022 and an expansion of insurance coverage — high treatment costs remain insufficiently addressed (*37*). Nevertheless,

significant disparities persist when compared with the United States and European Union, particularly in drug development and approval timelines. Therefore, it is essential to increase focus on key population groups and specific disease types and to pay more attention to management of rare disease drugs and medical insurance policies. Establishing a multi-tiered medical security system and forming a co-payment mechanism involving multiple parties can effectively reduce hospitalization costs and alleviate the medical burden on patients. Additionally, less developed regions in the west of China should develop targeted medical insurance policies and drug management measures based on characteristics of rare diseases in their respective provinces. Meanwhile, efforts should be made to strengthen diagnosis and treatment training for primary care physicians at the grassroots level in the region and construction of an early referral network platform, so as to improve diagnosis rate and treatment rate of rare diseases.

As reported in our study, the number of hospital admissions was 169, 167, and 136 cases in 2020, 2021, and 2022, respectively, rising to 268 cases in 2023. This trend likely reflects delays in seeking or accessing healthcare services attributable to the COVID-19 pandemic. It is crucial to acknowledge potential impact of the pandemic on patterns of hospital admissions and disease reporting observed in our data. During peak periods of infection surges and associated public health restrictions, access to non-urgent and elective healthcare services was significantly reduced in many regions (*38-40*). Rare disease patients were further marginalised, particularly in access to regular health care, treatment (*41*). Consequently, trends observed in our data, particularly concerning admission cases or distribution of specific rare diseases diagnosed during 2020–2022, should be interpreted with caution, recognizing the exceptional circumstances that may have led to artificial depression or distortion of these metrics compared to pre or post-pandemic years.

This study had certain limitations. While the research, grounded in data from inpatients with rare diseases at a hospital in Gansu Province, offers valuable insights into the epidemiological features of a substantial portion of rare diseases within the province, it is inevitable that cases of rare diseases may be underreported, potentially leading to an underestimation of their true incidence. Firstly, this study only included information on inpatients with rare diseases, excluding outpatient records, which represent a significant proportion of the rare disease patient population. Secondly, diagnostic information utilized in this study was sourced exclusively from Patient Discharge Summary records. The accuracy of clinical diagnoses rendered by physicians and precise classification of ICD codes by medical record coders are pivotal in determining authenticity of rare disease case counts. Lastly, owing to inherent constraints of the study, we have only included Gansu Provincial People's Hospital as the sole medical institution, and were unable to obtain diagnosis and treatment data of rare diseases from all tertiary hospitals in the province. Consequently, it may inevitably exhibit a degree of selection bias and statistical bias. Despite these limitations, our findings contribute significantly to filling a data gap of rare diseases in Gansu Province. It provides important reference value for guiding medical institutions and health administrative departments in improving clinical competencies in diagnosing and treating, as well as optimizing and adjusting medical security policies for rare diseases.

5. Conclusion

Rare diseases are not only a public health problem, but also a social problem. Although national attention to rare disease communities has increased in recent years, accompanied by improved public awareness and deeper research in the field, uneven distribution of prevention and control efforts for rare diseases remains prevalent. This study focuses on the distribution characteristics of rare disease inpatients in a hospital located in western China. The demographic distribution characteristics of inpatients from 2015 to 2023, the classification of disease systems, the distribution of the top fifteen diseases, and the medical cost burden during hospitalization are described comprehensively. Our study provides important and foundational data support for development and improvement of rare disease prevention policies and medical services.

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Original Article

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Clinical and genetic characteristics of late-onset cobalamin C deficiency: A multicenter study in northern China

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SUMMARY: Late-onset cobalamin C (cblC) deficiency, an inherited metabolic disorder, is often misdiagnosed due to its heterogeneous clinical presentation. This study aims to characterize the clinical and genetic spectrum of late-onset cblC deficiency in a large northern Chinese cohort and proposes a novel clinical classification based on initial symptoms. A retrospective, multicenter study of 156 patients diagnosed between October 2012 and December 2023 was conducted. Clinical, biochemical, neuroimaging, and genetic data were analyzed. Patients were classified into six subtypes based on predominant initial symptoms, and genotype-phenotype correlations were explored. The cohort (95 males, 61 females) had a median onset age of 16 years (range: 2–65). Common symptoms included spastic paralysis (41.0%), mental and behavioral abnormalities (36.5%), and renal damage (28.8%). Genetic analysis identified 52 *MMACHC* variants, with c.482G>A (34.3%) and c.609G>A (17.6%) being most frequent. Elevated total homocysteine (tHcy) levels correlated with mental and behavioral abnormalities, renal damage, and anemia ($p < 0.05$). The proposed clinical classification identified six subtypes, with encephalopathy-dominant and encephalomyelopathy-dominant types being most prevalent. This study highlights the clinical heterogeneity of late-onset cblC deficiency and introduces a novel symptom-based classification system to aid diagnosis and management. Elevated tHcy levels and specific *MMACHC* variants are key biomarkers for disease severity. These findings underscore the importance of early intervention to improve outcomes.

Keywords: methylmalonic academia, hyperhomocysteinemia, late onset, *MMACHC* gene, Clinical manifestation

1. Introduction

Cobalamin C (cblC) deficiency (CCD; OMIM #277400), the most common inherited disorder of intracellular cobalamin metabolism, was first described by Mudd *et al*. over five decades ago (*1*). In 2006, Lerner-Ellis *et al.* identified *MMACHC* (OMIM #609831), the causative gene located on chromosome 1p34.1, which encodes a 282-amino acid protein (*2*). Dysfunction of *MMACHC* reduces intracellular levels of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), essential cofactors for methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (EC 1.16.1.8), respectively (*3*). Key biomarkers of CCD include elevated blood total homocysteine (tHcy), an increased propionylcarnitine/acetylcarnitine (C3/C2) ratio, and elevated urinary methylmalonic acid (MMA)(*4*). CCD manifests from prenatal stages to adulthood, with clinical severity

ranging from mild to life-threatening (*5*). Disease onset is categorized as early (< 1 year) or late (> 1 year), with late-onset patients typically exhibiting milder symptoms and better prognoses (*6*).

Early-onset CCD is characterized by severe manifestations, including feeding difficulties, growth retardation, lethargy, hypotonia, hydrocephalus, and maculopathy (*7-9*). In contrast, late-onset CCD presents with mental and behavioral abnormalities, cognitive impairment, epilepsy, spastic paralysis, myelopathy, peripheral neuropathy, ataxia, hematological abnormalities, renal damage, ocular involvement, and thromboembolic events (*10*). Although rare, the CCD incidence in Shandong Province, China, is approximately 1 in 4000, significantly higher than the 1 in 272,411 reported in some southern provinces such as Jiangsu Province (*11,12*). Recent increases in late-onset diagnoses suggest a higher prevalence than previously estimated. However, few studies have explored genotype-phenotype correlations, which could help predict disease onset and severity.

This study established a multicenter clinical cohort of late-onset cblC deficiency (LCCD) patients from eight major medical centers in northern China to analyze their clinical and genetic characteristics, providing critical insights for diagnosis and prognosis in this population.

2. Patients and Methods

2.1. Study participants

We included 156 patients with LCCD from Qilu Hospital of Shandong University, Ji nan Maternity and Child Care Hospital, Peking Union Medical College Hospital, XuanWu Hospital of Capital Medical University, Peking University First Hospital, Hebei Medical University Third Hospital, Beijing Tiantan Hospital and Lanzhou University Second Hospital between October 2012 and November 2023. The inclusion criteria for patients were as follows: *i*) hyperhomocysteinemia (HHcy), tHcy > 50 μmol/L; *ii*) biallelic pathogenic *MMACHC* variations confirmed; and *iii*) age of onset > 1 year. The exclusion criteria were as follows: *i*) HHcy caused by secondary or other genetic factors, and *ii*) incomplete clinical data or a lack of genetic diagnostic evidence.

2.2. Data collection

Clinical data, including demographics, age of symptom onset and diagnosis, family history, treatment regimens, and clinical outcomes, were collected. Additional data encompassed clinical manifestations, laboratory results, neuroimaging findings, electroencephalography (EEG), electromyography (EMG), and genetic testing results. Cognitive and executive functions were assessed using the standardized Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

2.3. Biochemical examination

Blood levels of acylcarnitines (including propionylcarnitine [C3] and acetylcarnitine [C2]) and amino acids were quantified using tandem mass spectrometry (MS/MS; Applied Biosystems, API 4000, California, United States) in dried blood spot samples (*13*). The C3/C2 ratios were calculated from these measurements. Urinary organic acids were analyzed by gas chromatography–mass spectrometry (GC–MS; Shimadzu Limited, QP2010, Kyoto, Japan) (*14*). Plasma homocysteine levels were measured *via* fluorescence polarization immunoassay (*15*). Additionally, routine blood and urine analyses were performed to assess electrolyte and glucose levels, liver and renal function, and folate and vitamin B_{12} concentrations.

2.4. Genetic testing

Whole-exome sequencing (WES) was performed on all 156 patients. Genomic DNA was extracted from peripheral blood leukocytes, and next-generation sequencing (NGS) was used to identify *MMACHC* variants. All variants were assessed for pathogenicity according to the *Standards and Guidelines for the Interpretation of Sequence Variants* published by the American College of Medical Genetics and Genomics (ACMG) in 2015.

2.5. Statistical analysis

Statistical analyses were performed using SPSS (version 27.0.0.0) and GraphPad Prism. The Shapiro–Wilk test indicated a non-normal distribution of the data; consequently, continuous variables are presented as medians (interquartile ranges), and group comparisons were performed using the nonparametric Mann–Whitney *U* test. Categorical variables are expressed as absolute frequencies and percentages, with comparisons made using the chi-square test or Fisher's exact test, as appropriate. A two-tailed p value < 0.05 was considered statistically significant.

2.6. Ethical approval

This study complied with the Ethics Guidelines and was approved by the Ethics Committee of Qilu Hospital of Shandong University. All patients or their parents (for patients younger than 18 years) signed informed consent forms prior to inclusion in the study. This study was conducted in accordance with the Declaration of Helsinki.

3. Results

3.1. Study population

The demographic, biochemical, and clinical features of the cohort are summarized in Table 1. This case series included 156 patients with late-onset cobalamin

Table 1. Clinical features of patients with different types of cblC deficiency

	Total	cblC-E	cblC-M	cblC-EM	cblC-R	cblC-N	cblC-O
No.	156	57 (36.5%)	27 (17.3%)	31 (19.9%)	11 (7.1%)	10 (6.4%)	20 (12.8%)
Sex ratio (male: Female)	95:61	32:25	17:10	16:15	7:4	9:1	14:6
Age at onset, years, median (IQR)	16.0 (12.0–24.0)	16.0 (9.5–25.5)	17.0 (13.0–24.0)	17.0 (14.0–23.0)	14.0 (9.0–21.0)	20.0 (16.0–25.8)	13.5 (10.8–24.5)
Time to molecular diagnosis, months, median (IQR)	12.0 (2.0–60.0)	24.0 (2.0–66.0)	12.0 (3.0–84.0)	12.0 (2.0–72.0)	24.0 (5.0–48.0)	2.0 (1.0–66.0)	5.5 (1.0–39.0)
Biochemical features							
Total plasma homocysteine level at presentation[a] (μmol/L) (n = 156) (IQR)	118.0 (95.4–185.0)	135.0 (102.7–188.8)	100.1 (77.2–136.5)	115.2 (91.7–193.4)	176.0 (89.1–293.0)	162.6 (109.5–198.4)	99.9 (73.5–164.1)
Methylmalonic acid level at presentation[b] (μg/mg creatinine) (n = 94) (IQR)	116.0 (63.8–191.6)	121.5 (83.5–204.7)	89.8 (43.6–141.9)	109.4 (57.6–187.8)	133.3 (35.1–1772.9)	209.7 (104.5–412.7)	85.1 (26.1–171.4)
C3/C2 ratio at presentation[c] (n = 94) (IQR)	0.5 (0.4–0.7)	0.6 (0.3–0.7)	0.4 (0.3–0.6)	0.5 (0.4–0.7)	0.5 (0.4–0.6)	0.8 (0.6–1.0)	0.5 (0.4–0.8)
Clinical manifestations							
Spastic paralysis	64/156 (41.0%)	3/57 (5.3%)	27/27 (100%)	31/31 (100%)	2/11 (18.2%)	0/10 (0.0%)	1/20 (5.0%)
Mental and behavioral abnormalities	57/156 (36.5%)	32/57 (56.1%)	2/27 (7.4%)	22/31 (71.9%)	1/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Renal damage	45/156 (28.8%)	15/57 (26.3%)	4/27 (14.8%)	9/31 (29.0%)	11/11 (100.0%)	2/10 (10.0%)	4/20 (20.0%)
Anemia	42/156 (26.9%)	15/57 (26.3%)	6/27 (22.2%)	9/31 (29.0%)	6/11 (54.5%)	1/10 (10.0%)	5/20 (25.0%)
Cognitive decline	34/156 (21.8%)	19/57 (33.3%)	3/27 (11.1%)	7/31 (22.6%)	2/11 (18.2%)	2/10 (20.0%)	1/20 (5.0%)
Limb weakness	31/156 (19.4%)	10/57 (17.5%)	1/27 (3.7%)	1/31 (3.2%)	0/11 (0.0%)	10/10 (100.0%)	9/20 (45.0%)
Ataxia	28/156 (17.9%)	3/57 (5.3%)	12/27 (44.4%)	8/31 (35.8%)	1/11 (9.1%)	1/10 (10.0%)	3/20 (15.0%)
Epilepsy	26/156 (16.7%)	18/57 (31.6%)	1/27 (3.7%)	7/31 (22.6%)	0/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Mental retardation	11/156 (7.1%)	10/57 (17.5%)	0/27 (0.0%)	0/31 (0.0%)	1/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Thrombus	8/156 (5.1%)	5/57 (8.8%)	2/27 (7.4%)	1/31 (3.2%)	0/11 (0.0%)	0/10 (0.0%)	0/20 (0.0%)
Visual impairment	8/156 (5.1%)	5/57 (8.8%)	1/27 (3.7%)	0/31 (0.0%)	2/11 (18.2%)	0/10 (0.0%)	1/20 (5.0%)
Pulmonary hypertension	5/156 (3.2%)	0/57 (0.0%)	0/27 (0.0%)	0/31 (0.0%)	2/11 (18.2%)	0/10 (0.0%)	2/20 (10.0%)

[a]Typical reference range, 5–15 μmol/L with slight variations in some tests. [b]Typical reference range, 0.2–3.6 μg/mg creatinine with slight variations in some tests. [c]Typical reference range, 0.02–0.20 with slight variations in some tests.

C deficiency (LCCD) due to pathogenic *MMACHC* variants, representing 144 families: 95 males (60.9%) and 61 females (39.1%). Age at symptom onset ranged from 2 to 65 years (median: 16 years). Patients were stratified into three age groups: young children (< 14 years), adolescents (14–17 years), and adults (≥ 18 years) (Figure 1A).

The interval between symptom onset and diagnosis varied from months to 28 years (median: 12 months). Diagnostic delays were significantly longer in the young children group compared to the other groups (Figure 1B). Two patients died: one at age 6 from severe pulmonary hypertension and another at age 23 due to metabolic cardiomyopathy precipitated by pregnancy and infection; neither had received regular treatment. Twenty-four patients were lost to follow-up, and 12 sibling pairs were identified. All patients were from 10 provinces in northern China.

3.2. Phenotypes and clinical features

Based on comprehensive clinical evaluation, we classified 156 LCCD patients into six distinct phenotypic subgroups according to their predominant initial symptoms: (1) encephalopathy-dominant (cblC-E, n = 57, 36.5%), (2) myelopathy-dominant (cblC-M, n = 27, 17.3%), (3) encephalomyelopathy-dominant (cblC-EM, n = 31, 19.9%), (4) renal dysfunction-dominant (cblC-R, n = 11, 7.1%), (5) peripheral neuropathy-dominant (cblC-N, n = 10, 6.4%), and (6) other symptom-dominant (cblC-O, n = 20, 12.8%) (Figure 2A).

The cblC-E subtype primarily presented with neuropsychiatric features, including mental/behavioral abnormalities (36.5%), epilepsy, and cognitive decline. cblC-M patients showed spinal cord involvement manifesting as spastic paralysis, while cblC-EM represented a combination of these neurological presentations. Renal impairment defined the cblC-R subtype, and cblC-N patients exhibited peripheral nerve dysfunction with limb weakness. Patients with other predominant initial symptoms were categorized as cblC-O (Figure 2A).

Beyond initial symptoms, disease progression occurred in the overwhelming majority of CCD patients. Figure 2B shows the frequency of overall clinical manifestations in the 156 LCCD patients. The most common manifestations were spastic paralysis (41.0%), followed by mental and behavioral abnormalities (encompassing social withdrawal, abnormal behavior,

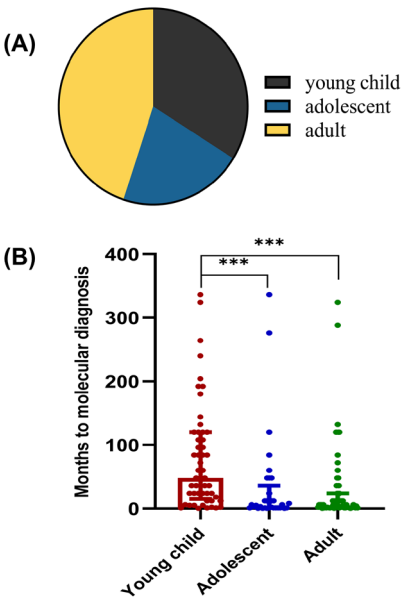

Figure 1. Patient characteristics. (A) Age stratification revealed three distinct groups: young children (< 14 years, n = 53, 34.0%), adolescents (14–17 years, n = 33, 21.2%), and adults (≥ 18 years, n = 70, 44.9%). **(B)** Diagnostic delays ranged from months to 28 years (median 12 months), with significantly prolonged intervals observed in young children versus older groups. ***p < 0.001.

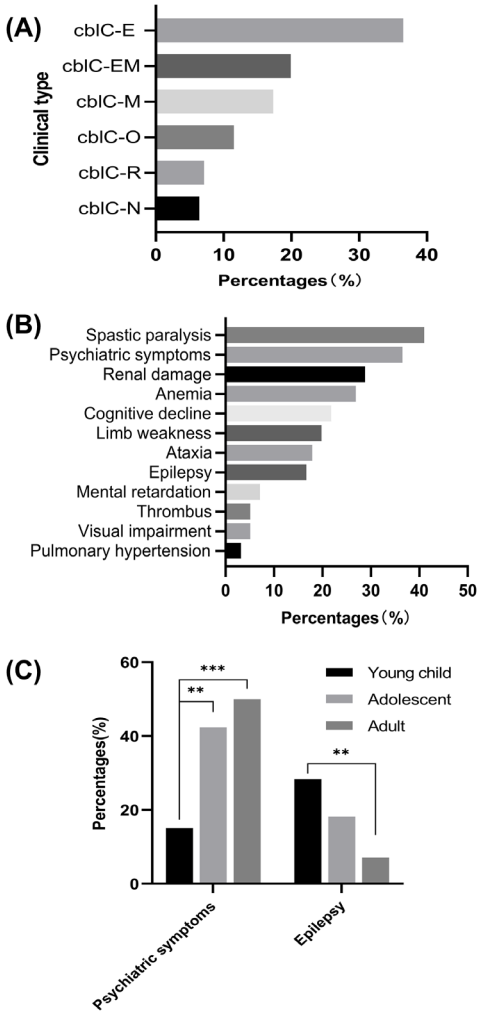

Figure 2. The phenotypes and clinical features of the patients. (A) Patients with cblC were divided into 6 types according to the symptoms at onset. **(B)** 156 patients had a total of 12 clinical signs. **(C)** Age-stratified analysis revealed significantly higher seizure frequency in children versus adults), while adults showed predominance of mental/behavioral abnormalities. **p < 0.01; ***p < 0.001.

raving, depression, and auditory hallucinations; 36.5%), renal damage (28.8%), anemia (26.9%), cognitive decline (21.8%), limb weakness (19.8%), ataxia (17.9%), epilepsy (16.7%), intellectual disability (7.1%), thrombotic events (5.1%), visual impairment (5.1%), and pulmonary hypertension (3.2%). Age-stratified analysis demonstrated higher seizure frequency in pediatric patients versus greater neuropsychiatric manifestations in adults (Figure 2C).

Patients were grouped based on the presence of spastic paralysis, mental/behavioral abnormalities, renal damage, anemia, cognitive decline, limb weakness, ataxia, or epilepsy. Factors including age of onset, diagnostic delay, and initial biochemical/metabolic data were analyzed (Table 2). Elevated tHcy levels correlated with both neuropsychiatric symptoms ($p < 0.05$) and renal impairment. Moreover, patients with limb weakness or epilepsy experienced a prolonged interval between symptom onset and diagnosis, whereas epilepsy patients had an earlier age of onset. Other clinical and biochemical features showed no significant differences. This phenotypic classification system provides a clinically meaningful framework for anticipating disease course and guiding management in LCCD patients.

3.3. Biochemical features

Homocysteine (Hcy) levels were measured in 156 patients at the first visit, and urine organic acid concentrations and plasma amino acid levels were recorded for 112 patients. Serum total homocysteine (tHcy) values were significantly increased in all patients (range: 53.0–447.7 μmol/L; median: 117.4 μmol/L). Additionally, urine methylmalonic acid (MMA) concentrations (range: 5.7–1213.9 μg/mg creatinine; normal: 0.2–3.6 μg/mg creatinine) and C3/C2 ratios (range: 0.20–2.39; normal: 0.02–0.20) were elevated in 112 patients. These biochemical findings supported a diagnosis of methylmalonic acidemia (MMA) combined with homocystinuria.

Age-stratified analysis demonstrated significantly higher tHcy levels in adults versus younger patients (*p* < 0.05; Figure 3A), whereas MMA levels, C3/C2 ratios, and other metabolic parameters showed no age-dependent variation (Figure 3A). Chi-square testing indicated significant differences in tHcy levels across groups, with lower levels observed in the cblC-M group (Figure 3B). Elevated tHcy correlated strongly with renal impairment (*p* < 0.05), neuropsychiatric manifestations, and anemia. In contrast, spastic paralysis was associated with comparatively reduced tHcy concentrations (Figure 3C).

3.4. Neuroimaging features

Brain MRI was performed on 119 patients. Results showed that 68 patients (57.1%) primarily exhibited cerebral atrophy and white matter lesions; a subset also presented with cerebral venous sinus thrombosis (Figure 4A). Spinal cord MRI was performed in 105 patients, with 31 (29.5%) exhibiting spinal cord atrophy and longitudinally extensive transverse myelitis (Figure 4B). Scoliosis was a common imaging abnormality among the patients.

3.5. Genotypes and degrees of heteroplasmy

Genetic analysis identified 52 distinct *MMACHC* variants (including 13 VUS) in 156 patients. Exon 4 harbored 57.7% (30/52) of these mutations (Figure 5A). The most frequent variant was c.482G>A, present in 107 (34.3%) alleles, followed by c.609G>A (17.6%), c.658_660del (7.1%), c.567dupT (4.8%), and c.80G>A (4.2%).

Compared to patients without the c.482G>A mutation, those with the mutation exhibited a lower incidence of epilepsy, mental retardation, visual impairment, and pulmonary hypertension, but a higher frequency of spastic paralysis and mental/behavioral abnormalities (Figure 5B). Additionally, the c.80G>A variant was consistently associated with earlier onset age, nephropathy, and pulmonary hypertension. Patients harboring c.80G>A typically presented with early onset, cblC-R subtype, renal damage, pulmonary hypertension, and mental retardation (Figure 5C).

The predominant variant combination was c.482G>A and c.609G>A, found in 29 patients (18.6%). Patients were stratified into four genotypic groups: c.482G>A alone, c.609G>A alone, both mutations, or other variants. Patients with c.482G>A had a significantly later age of onset than those with c.609G>A (Figure 5D). No significant differences in treatment response or biochemical markers (tHcy, MMA, C3/C2 ratio) were observed across the genotypic groups.

3.6. Long-term treatment and follow-up

All patients received acute-phase therapy with intramuscular hydroxocobalamin (OH-Cbl; 10 mg/day) or mecobalamin (1,000 μg/day), combined with metabolic adjuncts: L-carnitine (2 g/day), betaine (6–9 g/day), leucovorin (7.5 mg/day), and vitamin B6 (10–100 mg/day). Long-term treatment was then individualized based on patient condition.

While all patients showed initial clinical improvement — with neuropsychiatric symptoms resolving faster than motor deficits — while outcomes varied by subtype. Among the 156 patients, 24 (15.4%) were lost to follow-up, one patient died at age 6 years due to severe pulmonary hypertension, and one died at age 23 years from heart disease.

Patients with the cblC-E subtype consistently demonstrated a better prognosis than those with other subtypes; however, rigorous analysis is needed to confirm these results due to differences in treatment regimens and adherence. Treatment adherence issues

Table 2. Variable phenotypes and factors in 156 patients (symptoms in > 20 patients were analyzed)

Symptoms and signs		Cases *n*	Age at onset (years) Median (P25, P75)	Time from onset to treatment initiation (years) Median (P25, P75)	Blood tHcy (μmol/L) Median (P25, P75)	Urinary methylmalonic acid (μg/mg creatinine) Median (P25, P75)	C3/C2 Median (P25, P75)
Spastic paralysis	N	92	16.0 (10.3, 25.0)	12.0 (1.0, 48.0)	133.7 (98.2, 203.4)	121.5 (79.5, 204.7)	0.6 (0.4, 0.8)
	Y	64	17.0 (13.3, 22.8)	12.0 (3.0, 84.0)	112.5 (88.2, 158.2)	101.9 (46.2, 175.2)	0.5 (0.4, 0.7)
Z			-0.795	-1.235	-2.151	-1.411	-1.071
P			0.426	0.217	0.031	0.158	0.284
Mental and behavioral abnormalities	N	99	14.0 (10.0, 20.0)	12.0 (2.0, 72.0)	111.5 (90.0, 164.0)	111.7 (62.9, 182.5)	0.5 (0.4, 0.7)
	Y	57	18.0 (15.0, 26.0)	12.0 (2.0, 42.0)	139.0 (100.3, 233.5)	107.5 (70.1, 197.3)	0.6 (0.4, 0.7)
Z			-3.338	-0.783	-2.560	-0.237	-0.087
P			< 0.001	0.434	0.010	0.813	0.931
Renal damage	N	111	16.0 (12.0, 20.0)	12.0 (2.0, 48.0)	112.6 (88.0, 162.0)	111.7 (62.9, 185.8)	0.6 (0.4, 0.7)
	Y	45	19.0 (12.0, 26.0)	24.0 (2.0, 72.0)	146.3 (107.4, 248.2)	132.6 (79.2, 214.2)	0.5 (0.4, 0.7)
Z			-0.997	-0.909	-3.440	-0.752	-0.087
P			0.319	0.363	< 0.001	0.452	0.931
Anemia	N	114	16.0 (12.8, 24.3)	12.0 (2.0, 48.0)	115.2 (94.8, 165.6)	126.9 (66.9, 206.4)	0.5 (0.4, 0.7)
	Y	42	16.0 (12.0, 22.3)	12.0 (2.0, 51.0)	134.9 (94.1, 245.2)	102.2 (45.5, 155.8)	0.5 (0.4, 0.8)
Z			-0.540	-0.066	-2.054	-1.769	-0.907
P			0.589	0.947	0.040	0.077	0.365
Cognitive decline	N	122	16.0 (12.8, 24.0)	12.0 (2.0, 60.0)	115.5 (94.8, 180.5)	107.0 (58.1, 179.1)	0.5 (0.4, 0.7)
	Y	34	15.5 (12.0, 23.5)	7.0 (1.8, 60.0)	147.4 (94.5, 190.0)	165.7 (79.4, 236.6)	0.6 (0.3, 0.7)
Z			-0.069	-0.500	-0.766	-1.435	-0.374
P			0.945	0.617	0.444	0.151	0.709
Limb weakness	N	125	15.0 (12.0, 22.5)	12.0 (3.0, 84.0)	120.0 (96.0, 173.1)	107.0 (54.5, 172.4)	0.5 (0.4, 0.7)
	Y	31	18.0 (14.0, 26.0)	2.0 (1.0, 12.0)	109.9 (86.7, 211.4)	112.0 (77.5, 236.5)	0.6 (0.4, 0.8)
Z			-1.696	-3.042	-0.291	-1.652	-0.369
P			0.090	0.002	0.771	0.098	0.171
Ataxia	N	128	16.0 (12.0, 23.0)	12.0 (2.0,57.0)	119.0 (95.6, 184.6)	121.1 (67.0, 198.0)	0.6 (0.4, 0.7)
	Y	28	16.5 (14.0, 25.5)	6.0 (3.0, 69.0)	113.1 (82.3, 182.8)	85.0 (36.4, 147.6)	0.4 (0.3, 0.6)
Z			-0.583	-0.023	-0.455	-1.688	-2.386
P			0.560	0.982	0.649	0.091	0.017
Epilepsy	N	130	17.5 (13.0, 25.0)	11.0 (2.0, 48.0)	122.6 (96.0, 188.0)	121.5 (62.9, 198.0)	0.5 (0.4, 0.7)
	Y	26	12.5 (5.8, 17.0)	30.0 (12.0, 93.0)	106.1 (89.5, 146.9)	107.1 (66.3, 168.8)	0.6 (0.3, 0.8)
Z			-3.247	-2.326	-1.510	-0.451	-0.630
P			0.001	0.020	0.131	0.652	0.529

Notes: *n*, number; N, No; Y, Yes; *Z*, Mann–Whitney *U* Z-score; *P*, value was calculated by using a nonparametric unpaired Mann-Whitney *U* test; $P < 0.05$ was considered as statistically significant.

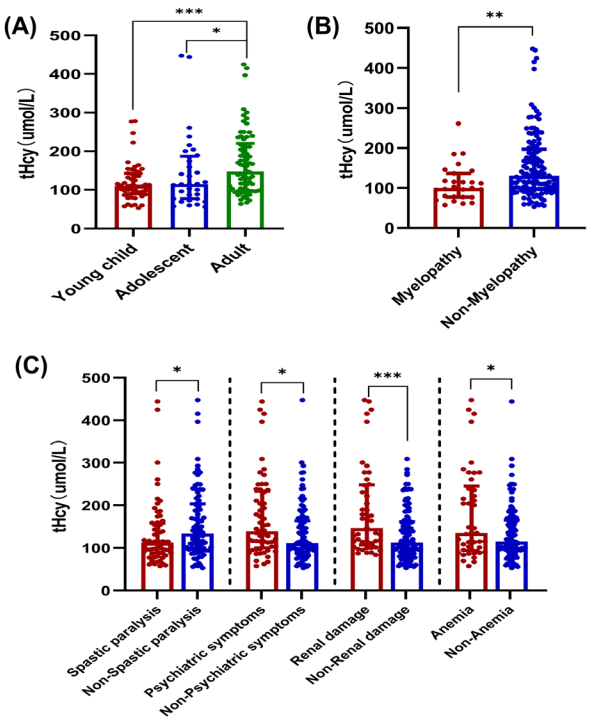

Figure 3. The biochemical features of the patients. (A) Adults demonstrated significantly elevated tHcy levels compared to younger patients. **(B)** The myelopathic subgroup (cblC-M) exhibited markedly lower tHcy concentrations versus other subtypes. **(C)** Hyperhomocysteinemia correlated strongly with renal impairment, neuropsychiatric manifestations, and anemia, while spastic paralysis associated with reduced tHcy levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$..

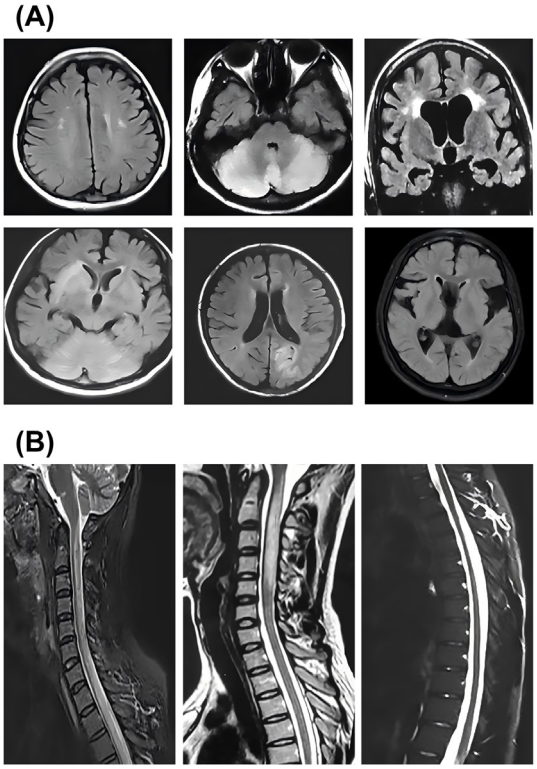

Figure 4. Neuroimaging Findings. (A) Brain MRI of some patients were characterized primarily by cerebral atrophy and white matter lesions. **(B)** Spinal cord MRI of some patients exhibited spinal cord atrophy and longitudinally extensive transverse myelitis.

affected 28% of patients, particularly with intramuscular regimens.

4. Discussion

Cobalamin C deficiency (CCD), caused by *MMACHC* mutations, is the most common form of methylmalonic acidemia (MMA) combined with homocystinuria (*16*). Late-onset cblC deficiency (LCCD) is rare and diagnostically challenging due to heterogeneous clinical manifestations (*5,17*). While approximately 90% of reported cases involve early-onset disease, fewer than 300 LCCD cases have been documented (*18,19*). Although neonatal screening based on elevated C3 and reduced methionine levels in dried blood spots has improved early detection, neurological and visual symptoms often progress despite timely intervention (*20,21*). In contrast, late-onset patients exhibit rapid biochemical and clinical improvement with prompt treatment, underscoring the critical importance of early diagnosis (*10*).

In this study, we analyzed 156 LCCD patients (61 females, 95 males) from northern China. The interval between symptom onset and diagnosis ranged from days to 28 years, with only 43.6% diagnosed within 12 months. Earlier symptom onset was associated with prolonged diagnostic delays, a known risk factor for poor outcomes (*22*). Notably, siblings with identical *MMACHC* variants exhibited variable ages of onset and prognoses, highlighting the disease's phenotypic variability.

The most common clinical manifestations in our cohort were spastic paralysis, mental/behavioral abnormalities, renal damage, and anemia. To date, most reports on LCCD are descriptive cohort studies and case reports. Currently, no consensus classification system exists for this disease, and few cohort studies have attempted a systematic or stratified analysis of its clinical presentations. In this context, the symptom-based classification system we propose represents a paradigm shift.

We classified patients into six subtypes based on initial symptoms, with encephalopathy-dominant (cblC-E) and encephalomyelopathy-dominant (cblC-EM) being most prevalent. Notably, among patients with the cblC-E subtype, those presenting with psychiatric abnormalities predominantly carried the c.482G>A mutation. The cblC-EM subtype, which is not uncommon in this disease, typically presents with concurrent encephalopathy and myelopathy in its early stages. This specific subgroup consequently exhibited a more favorable prognosis. In contrast, the myelopathy-dominant (cblC-M) subtype was associated with a potentially less favorable clinical outcome. Furthermore,

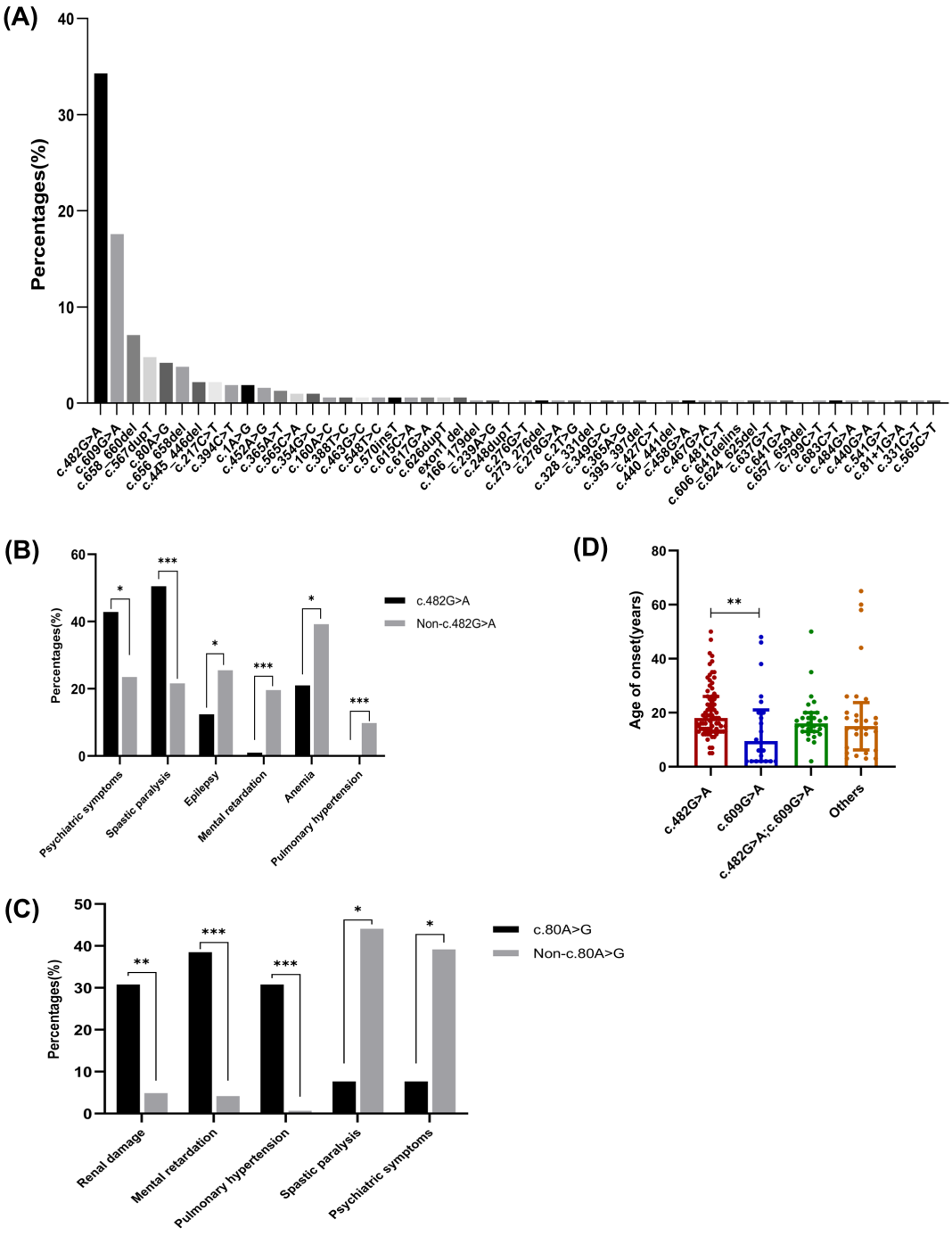

Figure 5. Genetic Characteristics and Phenotypic Correlations. (A) 52 distinct variants were identified among 156 patients. **(B)** Patients with c.482G>A mutation presented a lower incidence of epilepsy, mental retardation, anemia, visual impairment and pulmonary hypertension, along with a greater frequency of spastic paralysis, mental and behavioral abnormalities and anemia. **(C)** Patients with the c.80A>G mutation had the following characteristics: early onset age, renal damage, pulmonary hypertension and mental retardation. **(D)** The age of onset of patients with c.482G>A were significantly older than those with c.609G>A. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

the renal dysfunction-dominant (cblC-R) subtype demonstrated a higher prevalence of the c.80G>A variant and was characterized by an earlier age of onset. The peripheral neuropathy-dominant (cblC-N) subtype was the least prevalent among our cohort. Affected patients frequently presented with peripheral neuropathy, which predominantly manifested as axonal damage with limited potential for full recovery. Myelopathy—characterized by spastic paralysis, abnormal gait, and limb weakness—was exclusively observed in late-onset patients (*16,19,23*). Age-stratified analysis revealed epilepsy was more common in younger patients, while mental/behavioral abnormalities predominated in adults.

Megaloblastic anemia was the primary hematological abnormality, consistent with prior findings (*24*). Unlike early-onset patients, late-onset patients showed less

ocular involvement, with no clear evidence of retinal degeneration. However, poor visual acuity was noted in nine patients, though its direct association with CCD remains unclear. Ophthalmological evaluations should be incorporated into diagnostic protocols to clarify this relationship. CCD can present acutely or insidiously, with periods of stability interrupted by triggers such as infection, fever, strenuous exercise, or vegetarian diets (*25*). In our cohort, common triggers included pregnancy, surgery, infections, and dietary changes. Given its multisystemic involvement, CCD should be considered in patients with unexplained neuropsychiatric symptoms, metabolic crises, or multi-organ dysfunction.

Elevated plasma tHcy and urinary MMA levels remain reliable diagnostic markers. In our study, all patients exhibited significantly increased tHcy and MMA levels, supporting their utility in diagnosing CCD. Patients with high tHcy levels were more likely to exhibit renal damage and neuropsychiatric symptoms, while those with the cblC-M subtype had lower tHcy levels. Interestingly, younger patients had lower tHcy levels than adults, though no significant correlation was found between tHcy levels and clinical severity.

Neuroimaging revealed cerebral atrophy, white matter lesions, spinal cord atrophy, and chronic spinal cord injury as common findings (*26-28*). MRI findings may help assess CCD severity (*29*). Electromyography (EMG) indicated neurogenic damage in nearly half of tested patients. Electroencephalography (EEG) abnormalities were observed in 38 of 53 patients, predominantly in the cblC-E subtype. Recent studies suggest EEG may reveal early neuronal dysfunction in CCD (*30,31*), though no disease-specific EEG patterns were identified.

To date, over 100 different *MMACHC* mutations have been reported (*32*). While c.482G>A is most frequent in European CCD patients (*10*), c.609G>A predominates in Chinese patients, accounting for 55.4% of known mutations (*33,34*). In our cohort, c.482G>A was most frequent (34.3%) and associated with spastic paralysis and milder phenotypes. Other common variants included c.609G>A and c.658_660del, consistent with Chinese population reports. The c.609G>A mutation — typically linked to early-onset disease — was prevalent in our cohort, likely due to a founder effect (*35*). Patients with c.80G>A frequently presented with renal damage and pulmonary hypertension, highlighting the need for cardiovascular and renal monitoring in these cases (*36-38*). Morel *et al.* reported c.394C>T is common in South Asian and Middle Eastern populations with late-onset disease (*39*); however, only six patients with this mutation were included here. The wide clinical heterogeneity of CCD likely stems from *MMACHC* variants, though environmental factors, ethnicity, and diet may influence phenotypic expression.

The therapeutic agents for cblC include cobalamin, levocarnitine, betaine, folic acid and vitamin B. Intramuscular HoCbl is the only cobalamin form known to benefit CCD patients; cyanocobalamin (CNCbl) and methylcobalamin (MeCbl) are not recommended (*40,41*). High-dose cobalamin (approximately 0.33 mg/kg/day), L-carnitine, and betaine are used acutely to reduce tHcy and correct metabolic status (*41,42*). Maintenance therapy dosages are adjusted based on plasma tHcy and urinary MMA levels. Most LCCD patients respond well, showing significant biochemical, clinical, and neuroimaging improvement. However, delayed or non-standardized treatment can cause sequelae or death. In our cohort, cblC-E patients exhibited marked improvement in cognitive decline and mental/behavioral abnormalities. In contrast, spinal cord injury responded poorly, and treatment non-adherence was associated with symptom recurrence and adverse outcomes. Treatment response varied by genotype: patients with c.482G>A showed better metabolic control and clinical improvement with cobalamin therapy. However, poor treatment adherence was common, with many experiencing symptom exacerbation or death due to irregular medication. These findings underscore the need for rigorous follow-up to evaluate long-term outcomes and optimize strategies. Despite the discovery of CCD over 50 years ago, treatment advancements remain limited, relying heavily on clinical experience rather than robust trial evidence. Frequent intramuscular HoCbl administration poses significant practical challenges (*43*). Ongoing research into gene and cell therapies may offer future alternatives (*44,45*).

Our Study has several limitations: *i*) Retrospective data collection may have introduced incomplete datasets; *ii*) Lack of standardized treatment guidelines resulted in management variability; *iii*) Regional bias may limit generalizability, 100 of the 156 patients were recruited from one center. Despite these limitations, our multicenter analysis provides valuable insights into the clinical and genetic spectrum of LCCD, enhancing recognition and management among clinicians.

In conclusion, this multicenter study establishes the first symptom-based classification system for LCCD, identifying six distinct clinical subtypes with specific genotype-phenotype correlations. The c.482G>A variant (34.3%) is associated with spastic paralysis and a milder disease course, while c.80G>A predicts renal and pulmonary complications. Elevated tHcy levels serve as key biomarkers for neuropsychiatric and renal manifestations. Despite management strategies, diagnostic delays (median 12 months) and treatment adherence remain critical challenges. These findings enable tailored monitoring and emphasize the need for standardized management protocols to improve outcomes in this metabolically complex disorder.

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Factors associated with diagnostic delays in Peruvian patients with rare diseases

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SUMMARY: Rare diseases affect fewer than 1 in 2,000 individuals. Patients often encounter barriers to specialist care and prompt diagnosis, hindering effective disease management and access to appropriate treatments. This study aimed to identify determinants of diagnostic delay among patients with rare diseases affiliated with Peruvian associations in 2024. A descriptive cross-sectional design was employed in 2024, enrolling patients with rare diseases or their caregivers from Peruvian associations. Data collection utilized an expert-validated survey encompassing sociodemographic characteristics, medical history, and diagnostic challenges. The primary outcome was diagnostic delay, defined as the interval from symptom onset to confirmed diagnosis. Data analysis included descriptive and inferential statistical methods. A total of 236 participants responded, with the majority being women (61.4%). A diagnosis was received within a year of symptom onset for 54.7% of participants, and 46.2% reported difficulties accessing healthcare. Major barriers identified included prolonged wait times for appointments or treatment (52.3%) and geographic limitations impeding access (37.6%). The median diagnostic delay was longer for women (63.1 months) compared to men (26.9 months). Limited access to healthcare was associated with an average delay of 21.8 months, whereas consulting more than ten general practitioners was associated with a 42.6-month delay. In summary, over half of the patients with rare diseases in Peru included in this study received a diagnosis within one year. However, the most significant delays were observed in non-genetic rare diseases. Key contributors to prolonged diagnostic timelines included limited access to healthcare and consultations with multiple general practitioners.

Keywords: rare diseases, delayed diagnosis, risk factors, developing countries

1. Introduction

A rare disease (RD) is traditionally defined as a condition affecting fewer than 1 in 1,500 to 1 in 10,000 individuals (*1,2*). The European Union (EU) defines a rare disease as any genetic or acquired condition that is life-threatening or chronically disabling (*3*). Rare diseases are low-prevalence, highly complex conditions that can pose life-threatening risks or lead to long-term disability. While most of these diseases have a genetic origin, some arise from autoimmune, toxic, or infectious factors. The clinical manifestations of rare diseases are highly varied and may include rare cancers, unusual physical traits, neurodevelopmental disorders, and congenital anomalies (*3,4*).

According to the World Health Organization (WHO), there are approximately 7,000 rare diseases that collectively affect around 7% of the global population (*5*). The European Organization for Rare Diseases (EURORDIS) estimates that there are between 6,000 and 8,000 rare diseases affecting 6% to 8% of the population within the European Union (*4*). In Peru, it is estimated that more than 2.5 million people are living with a rare disease (*6*).

Individuals with rare diseases face significant diagnostic challenges that impede timely access to appropriate treatments and limit research opportunities. Additional barriers include limited access to specialized healthcare centers, high medical costs, and insufficient social and financial support. Furthermore, the general lack of awareness regarding these conditions can lead to social isolation and inadequate information about the disease (*4*). The diagnosis of rare diseases remains problematic due to the vast diversity of conditions, their often poorly defined nature, and the limited availability of information on their overall burden (*7*).

An important issue with rare diseases is that their low prevalence results in limited awareness among healthcare professionals. Symptoms are often confused with those of more common conditions, leading to

delays in diagnosis and reduced accuracy. Diagnostic confirmation frequently requires specialized testing, which — especially in low- and middle-income countries — is only available in a limited number of centers (*8*).

Reducing the diagnostic odyssey is crucial for the effective management of rare diseases, influenced by factors such as income level and healthcare system performance. In Spain, the average time to diagnosis is estimated at 6.18 years. More than half of the patients (56.4%) experience diagnostic delays of over one year, which can be categorized into three main groups: 19% wait between 1 and 3 years, 16.7% between 4 and 9 years, and 20.9% experience delays exceeding 10 years (*9*).

The objective of this study was to identify the factors contributing to diagnostic delays in Peruvian patients with rare diseases.

2. Patients and Methods

2.1. Study design

This study is a descriptive, cross-sectional analysis. Data were collected in 2024 through surveys distributed electronically by representatives of 14 Peruvian rare disease patient associations and the Peruvian Federation of Rare Diseases. The survey included both closed- and open-ended questions, allowing for the collection of both quantitative and qualitative data regarding patient experiences and access to healthcare services, from the onset of symptoms to the time of diagnosis.

2.2. Participants

Inclusion criteria: Patients were eligible if they: *i*) provided informed consent, *ii*) had a confirmed diagnosis of a rare disease established in a Peruvian healthcare facility (clinical, biochemical, or molecular), and *iii*) were able to complete the questionnaire either directly or with the support of a caregiver.

Exclusion criteria: We excluded participants who had incomplete questionnaires, unverified diagnoses, or who were unable to recall essential information regarding the diagnostic timeline (*e.g.*, approximate dates of first symptoms, first medical consultation, or final diagnosis).

2.3. Variables

The following variables were collected: age, sex, age at diagnosis, specific diagnosis, educational level, monthly family income, place of origin, and religion. Additionally, the time elapsed from symptom onset to diagnosis and the number of general practitioners and specialists consulted were recorded. We identified the initial healthcare facility (where the patient first sought care) and the final facility (where the diagnosis was ultimately made). These facilities were then classified by their level of care complexity (Levels I, II, or III) according to the RENIPRESS (*Registro Nacional de Instituciones Prestadoras de Servicios de Salud*, National Registry of Health Service Provider Institutions) database. We identified the patient's health system affiliation. Patients were categorized as belonging to: the Ministry of Health (*Ministerio de Salud*, MINSA), which covers the uninsured population; the Social Security system (*Seguro Social de Salud*, EsSalud), which covers formal employees; the private sector; or the Armed Forces health system. Perceived access to the healthcare facility where the diagnosis was made was categorized as either easy or difficult. The perceived difficulty in accessing healthcare was assessed as a subjective patient-reported measure. It was evaluated across three dimensions: waiting time for medical consultation, geographic distance to specialized care, and structural barriers within the healthcare system. Participants rated the degree of difficulty based on their personal experience.

The method of diagnosis was classified as clinical, laboratory-based, imaging, or molecular. Patient perceptions regarding the complexity of the diagnostic examination were also recorded. Finally, diseases were subclassified as genetic or non-genetic based on their etiology, and diagnoses were categorized as either clinical or clinically supported by additional testing.

2.4. Data collection method

Data were obtained using an online survey that comprehensively covered all study variables. To reduce potential response bias and maintain the integrity of self-reported information, participants (patients or caregivers) completed the survey independently and at their own convenience.

2.5. Sample size

The sample size included 236 patients or caregivers, calculated using the following formula (*10*):

n = [EDFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1) + p*(1-p)]; where
N: Population size
p: Expected proportion
z: 1.96

2.6. Statistical methods

A descriptive analysis was conducted by calculating measures of central tendency (mean or median) and dispersion (standard deviation or interquartile range) for quantitative variables, while absolute frequencies and percentages were calculated for qualitative variables. Differences in means or medians were assessed through bivariate analysis using the student's *t*-test, Mann–Whitney

U test, ANOVA, or Kruskal–Wallis test, as appropriate. Additionally, an exploratory multiple linear regression analysis was performed to evaluate diagnostic delay as a continuous variable (in months), reporting β coefficients and 95% CIs, and a multiple logistic regression model was also fitted using a ≥ 12-month delay as a dichotomous outcome (reporting adjusted odds ratios). Both analyses were exploratory, and the sample size was not powered to detect small effects. Data processing was carried out using Stata v.18 and Microsoft Excel. Statistical significance was set at $P < 0.05$, and 95% confidence intervals (CI) were calculated for all estimators.

2.7. Ethical Approval

The study was reviewed and approved by the Institutional Review Board of Universidad Ricardo Palma (Nº PG 012 2024-A). The survey ensured participant anonymity and involved no foreseeable risks. Although personal identification was not possible, the confidentiality of both participants and the data collected was strictly maintained. The study followed the ethical principles outlined in the Declaration of Helsinki, the Belmont Report, and the Code of Ethics of the Peruvian Medical Association, upholding autonomy, beneficence, non-maleficence, and justice.

3. Results

3.1. Participants

A total of 241 patients or caregivers participated in the survey, all of whom provided informed consent. Five participants were excluded due to incomplete responses or inability to recall key dates required to estimate diagnostic delay.

3.2. Demographic characteristics

The sample consisted of 145 women (61.4%), with a median age of 23 years. Most participants resided in Lima (71.6%). Among the participants, Catholicism was the most prevalent religion (84.7%). In terms of educational attainment, 20.8% had not any grade of education, while 25.4% had completed an undergraduate degree.

The median age at diagnosis was 12 years, and the median diagnostic delay — from symptom onset to confirmed diagnosis — was 12 months.. Patients reported a median of three consultations with general practitioners and two consultations with specialists. The median monthly household income was 2,000 Peruvian soles (Table 1).

3.3. Characteristics of the process at the onset of symptoms

The analysis of participants revealed that the majority, 54.7% (n = 129), received their diagnosis in less than 1 year, while the remaining 45.3% (n = 107) were diagnosed after more than 1 year. Concurrently, there was a near-even split regarding access to their health center: a slight majority of participants, 53.8% (n = 127), reported having easy access, compared to 46.2% (n = 109) who reported having difficult access. Most patients consulted fewer than nine general practitioners (92.4%) and fewer than nine specialists (94.5%). The first facility visited was most often a Ministry of Health (MINSA) hospital (31.8%) or an EsSalud hospital (25.5%). Based on the RENIPRESS classification of institutional complexity, 24.1% of patients received care at a level III-1 facility (Table 2).

3.4. Diagnostic characteristics

Most diagnoses were made at national institutes (31.4%) and EsSalud hospitals (26.7%). In terms of institutional complexity, level III-2 facilities predominated (53.3%). Most cases were diagnosed through clinical evaluation combined with laboratory tests (50.4%) or additional imaging studies (23.7%). Additionally, when assessing the use of advanced diagnostic tests, a significant majority of participants, 69.1% (n = 163), reported they had not undergone such testing, whereas 30.9% (n = 73) confirmed the use of advanced diagnostic procedures, with biopsy (45.2%) and exome/genome sequencing (16.4%) being the most used methods. Among imaging modalities, magnetic resonance imaging (33.3%) and

Table 1. General characteristics of patients with rare diseases affiliated with patient associations in Peru

Variable	Median	IQR
Age (years)	23	36.15
Socioeconomic level (Monthly income in soles)	2,000	2500
Age at diagnosis (years)	12	29
Diagnosis time (months)	12	44
Number of doctors they saw	3	4
Number of specialists they saw	2	2
Sex	*n*	%
Female	145	61.4%
Male	91	38.6%
Origin		
Lime	169	71.6%
Province	67	28.4%
Religion		
Catholic	200	84.7%
Evangelical	15	6.4%
Jehovah's Witness	6	2.5%
Others	15	6.4%
Level of education		
None	49	20.8%
Incomplete primary education	27	11.4%
Completed primary education	12	5.0%
Incomplete secondary education	19	8.1%
Completed secondary education	29	12.3%
Incomplete tertiary education	41	17.4%
Complete undergraduate degree	59	25.0%

IQR, Interquartile Range.

Table 2. Diagnostic journey of patients with rare diseases from Peruvian patient associations

Variable	*n*	%
Difficulty		
Waiting time for a medical appointment	57	52.3%
Distance	41	37.6%
Structural	11	10.1%
General practitioners who visited		
≤ 9 doctors	218	92.4%
≥ 10 doctors	18	7.6%
Specialists who visited		
≤ 9 specialists	223	94.5%
≥ 10 specialists	13	5.5%
Establishment that came for the first time		
MINSA Hospitals	75	31.8%
EsSalud Hospitals	55	23.3%
Health Posts	29	12.3%
Private hospitals	25	10.6%
National Institutes	22	9.3%
Multi-specialty clinic	16	6.8%
Hospital of the Armed Forces and Police	8	3.4%
Private practice	6	2.5%
The level of complexity they first went to		
Level of care I-1	7	3.0%
Level of care I-2	8	3.4%
Level of care I-3	31	13.1%
Level of care I-4	9	3.8%
Level of care II-1	16	6.8%
Level of care II-2	54	22.9%
Level of care II-E	10	4.2%
Level of care III-1	57	24.1%
Level of care III-2	40	16.9%
Level of care III-E	4	1.7%

ultrasound (25.9%) were the most frequently employed (Table 3).

3.5. Frequency of rare diseases

In the study population, the most prevalent diagnosis was systemic lupus erythematosus (25.4%), followed by hemophilia A (7.6%) and Ehlers–Danlos syndrome (5.1%). Other notable diagnoses included myasthenia gravis (4.7%), sensorineural hearing loss (3.8%), and cystic fibrosis (3.4%). However, most conditions had a low frequency (≤ 2.5%) (Supplementary Figure S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=277*).

3.6. Characteristics of the process at the onset of symptoms

Women experienced a significantly longer time to receive a definitive diagnosis compared to men (63.1 *vs.* 26.9 months). A paradoxical trend was observed regarding educational attainment: those with completed undergraduate degrees took a longer time for diagnosis. Participants with no schooling reported a median diagnostic delay of 6 months, whereas those with undergraduate completed degrees reported a median delay of 36 months (Table 4).

Table 3. Diagnostic setting, complexity level, and diagnostic tools in patients with rare diseases in Peru

Variable	*n*	%
The establishment where diagnosis was made		
National Institutes	74	31.4%
EsSalud Hospitals	63	26.7%
Private Hospitals	43	18.2%
MINSA Hospitals	42	17.8%
Private practice	6	2.5%
Hospital of the Armed Forces and Police	5	2.1%
Multi-specialty clinic	3	1.3%
The Level of complexity at which the diagnosis was made		
Level of care I-1	6	2.5%
Level of care I-3	3	1.3%
Level of care I-4	2	0.9%
Level of care II-1	8	3.4%
Level of care II-2	27	11.4%
Level of care II-E	17	7.2%
Level of care III-1	43	18.2%
Level of care III-2	125	53.0%
Level of care III-E	5	2.1%
Type of diagnostic test		
Clinical evaluation + clinical laboratory tests	119	50.4%
Clinical evaluation + clinical laboratory tests +imaging tests	56	23.7%
Clinical evaluation + imaging tests	52	22.0%
Clinical evaluation	7	3.0%
Others	2	0.9%
Type of laboratory diagnosis		
Biopsy	45	45.2%
Exome/Genome Sequencing	12	16.4%
Karyotype	9	2.8%
Genetic panel	3	4.1%
Neonatal screening	2	2.8%
Others	12	16.4%
Type of diagnostic imaging		
Magnetic resonance imaging	35	33.3%
Computed tomography	23	21.9%
Radiography	11	10.5%
Electromyography	11	10.5%
Ultrasound	10	9.5%
Auditory evoked potentials	6	5.7%
Echocardiogram	5	4.8%
Electroencephalogram	1	0.9%
Others	3	2.9%

No significant differences in the time to diagnosis were observed based on place of origin, religion, or perceived ease of access to healthcare. However, significant differences were noted among participants who experienced barriers such as long wait times for medical appointments and those who reported distance-related obstacles (Table 4).

An increase in patient age at diagnosis was associated with a 3.1-month delay in the time to diagnosis. Additionally, patients who consulted ten or more general practitioners experienced a delay of 142.6 months in receiving a diagnosis. Lastly, the diagnosis of non-genetic rare diseases was delayed by 54 months compared to genetic rare diseases (Table 5).

Greater difficulty in accessing healthcare services was linked to an increased risk of delayed diagnosis.

Table 4. Sociodemographic and clinical factors associated with diagnostic delay in patients with rare diseases in Peru

Variable	Mean ± SD	CI (95%)	*p*-value
Sex			
Male (*n* = 91)	26.92 ± 55.80	15.3–38.5	0.0034
Female (*n* = 145)	63.12 ± 118.36	43.7–82.6	
Origin			
Lima (*n* =169)	49.76 ± 105.14	33.8–65.73	0.4422
Province (*n* = 67)	47.65 ± 88.22	26.1–69.1	
Religion			
Catholic (*n* =200)	48.11 ± 99.36	34.25–61.96	0.3522
Non-Catholic (*n* = 36)	55.03 ± 107.53	18.64–91.41	
Access to the health center			
Easy (*n* = 127)	33.55 ± 79.06	19.67–47.44	0.0048
Difficult (*n* = 109)	67.35 ± 119.49	44.86–89.95	
Level of complexity of the establishment visited for the first time			
Level of care I-II (*n* = 108)	36.30 ± 82.46	20.57–52.03	0.0353
Level of care III (*n* = 128)	60.02 ± 112.60	40.32–79.71	
Establishment that came for the first time			
State (*n* = 187)	46.96 ± 93.87	34.03–59.89	0.1937
Private (*n* = 49)	63.73 ± 137.3	13.36–114.10	
Level of complexity of the diagnostic establishment			
Level of care I-II (*n* = 57)	62.03 ± 115.68	31.33–92.73	0.1338
Level of care III (*n* = 179)	45.06 ± 95.08	31.04–59.09	
Establishing a diagnosis			
State (*n* = 187)	43.08 ± 93.29	29.62–56.54	0.0346
Private (*n* = 49)	72.37 ± 122.36	37.22–107.52	
Diseases			
Genetics (*n* = 123)	44.61 ± 82.02	29.97–59.25	0.2345
Non-genetic (*n* = 113)	54.11 ± 117.45	32.22–76.01	
Type of exams			
Clinical examination (*n* = 7)	56.85 ± 80.18	-17.29–131.01	0.4188
Clinical examination + complementary tests (*n* = 229)	48.93 ± 101.13	35.76–62.10	
Level of education	Median	IQR	0.0006*
None	6	13	
Incomplete primary education	7	16	
Completed primary education	9	52	
Incomplete secondary education	15	20.7	
Completed secondary education	12	16	
Incomplete tertiary education	24	126	
Completed undergraduate degree	36	78	
Difficulty			
Distance	24	42	0.4811*
Structural	12	178	
Waiting time for a medical appointment	24	62	

*Mann-Whitney *U* test. IQR, Interquartile Range.

Likewise, patients who had consulted more than ten general practitioners had more than a fivefold increased risk of receiving a diagnosis more than twelve months after symptom onset (Supplementary Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=277*).

4. Discussion

Timely diagnosis of rare diseases is critically important due to its impact on the quality of life for both patients and their families. In this study, a diagnostic delay of 12 months or more was observed in 45.3% of cases.

The female predominance noted in this study aligns with previous reports from Spain, which documented similar proportions ranging from 56% to 58.8% (*9,11*). This predominance may be explained by the high proportion of patients with systemic lupus erythematosus in the sample, a condition known to have a female prevalence of up to 90% (*12*).

Most patients in this study were from the department of Lima (71.6%). This high frequency, compared to other regions of the country, could be attributed to potential underreporting in those areas, as clinical suspicion and definitive diagnosis of rare diseases are often more challenging outside the capital. This challenge may be partly due to the lower concentration — or even absence — of rare disease specialists in regions beyond Lima (*13*). However, a prior study conducted in Peru on economic evaluations reported that 70% of patients came from departments outside Lima. This finding may have been influenced by the small sample size in that study (*14*).

Published data indicate that between 71.9% and 80% of patients with rare diseases have a genetic etiology,

Table 5. Association between diagnostic delay in rare diseases and factors related to healthcare access, income level, hospital type, and etiology, Peru

Variable	Coef. β	*p*-value	95% CI
Age (years)	-0.083	0.098	-1.82–0.16
Socioeconomic level (Monthly income in soles)	-0.0003	0.509	-0.001–0.001
Age at diagnosis (years)	3.182	**< 0.001**	2.04–4.33
Number of doctors they saw	-1.834	0.618	-9.07–5.40
Number of specialists they saw	6.154	0.14	-2.03–14.34
Sex			
Male	-21.01	0.086	-45.01–2.99
Religion			
Not Catholic	-10.26	0.494	-39.77–19.24
Place of residence			
Province	11.667	0.349	-12.85–36.18
Access to the health center			
Difficult	21,860	0.052	-0.17–43.89
General practitioners visited			
≥ 10 physicians	142.62	**< 0.001**	72.15–213.10
Specialists who visited			
≥ 10 specialists	-16.50	0.690	-97.82–64.82
Establishment that came for the first time			
Private	13.542	0.484	-24.52–51.60
Level of complexity of the establishment visited for the first time			
Level of care I and II	-93.208	0.433	-32.69–14.05
Establishment where the diagnosis was made			
Private	-14.514	0.455	-52.76–23.73
Level of complexity of the establishment where the diagnosis was made			
Level of care I and II	-12.884	0.470	-47.98–22.22
Type of exams			
Clinical examination + tests complementary	24	0.454	-39.23–87.45
Diagnosis			
Non-genetic	54	**< 0.001**	25.96–82.01

with 69.9% presenting exclusively in childhood (*15*). The study found that 52.1% of the participants had genetic diseases. This discrepancy could be due to the sample size in the current study, which reflects a higher proportion of non-genetic rare disease diagnoses, including infectious, immunological, degenerative, or proliferative conditions (*3*). Another possible explanation is the lack of implementation of technologies in Peru, such as tandem mass spectrometry for expanded newborn screening, and genomic testing methods like next-generation sequencing and chromosomal microarray analysis (*16-18*). The existing gap in the diagnosis of rare diseases largely stems from the wide range of genetic tests currently available, which enable early and accurate identification of numerous genetic conditions. These tools have revolutionized diagnostic processes by facilitating timely and precise detection. In contrast, non-genetic rare diseases pose greater diagnostic challenges, as most lack specific tests for direct identification, often leading to delays in diagnosis and treatment (*19*).

The median time to diagnosis was 12 months, though the range was quite broad. In contrast, studies conducted in Spain and across the European Union reported mean diagnostic delays of 6.18 and 4.7 years, respectively (*9,20*). This discrepancy may result from the clinical heterogeneity of rare diseases. In our setting, some patients likely exhibit more evident clinical manifestations, facilitating earlier recognition and diagnosis, in line with the differences observed across this group of disorders (*21*). Conversely, individuals with milder or atypical presentations may experience significant delays in diagnosis (*22*). Another possible explanation is the presence of autosomal dominant inheritance diseases, which tend to manifest more frequently in multiple family members, allowing for earlier identification and evaluation of patients (*3*).

Patients who perceived easier access to health centers had a shorter average time to diagnosis compared to those who reported difficulties in accessing care. This finding aligns with existing evidence indicating that barriers such as limited appointment availability, geographic distance, or structural constraints can significantly contribute to diagnostic delays (*23*). This may be due to the lower density of specialists and subspecialists in our area, which hinders access to health services and contributes to delays in care.

A significant difference in diagnosis time was also observed between individuals who faced challenges obtaining medical appointments and those who dealt with geographic distance issues. These obstacles prevent timely evaluations, thereby extending the interval between symptom onset and diagnostic confirmation (*24*).

We found a paradoxical pattern regarding educational

level: participants with a completed undergraduate degree exhibited longer diagnostic delays. This may be explained by the fact that individuals with lower socioeconomic resources — who often have lower educational attainment — are more likely to seek care within the public healthcare system, where access to molecular diagnostic services is more consolidated (*15*).

Regarding the frequency of visits to general practitioners, a diagnostic delay of over one year was more frequently noted among patients who consulted more than ten general practitioners. This finding is consistent with previous reports indicating that when patients see specialist physicians more than ten times, it is associated with significant diagnostic delays (OR = 5.19; 95% CI: 2.6–5.15) (OR = 5.19; 95% CI: 2.6–5.15) (*11,20*). This situation may be explained by the fact that patients consulting more specialists often have a subtle disease presentation. This makes clinical suspicion challenging and prompts families or patients to seek multiple medical opinions. Similarly, the time to diagnosis does not appear to be shortened when patients undergo a combination of clinical evaluations and complementary tests. This contrasts with a study conducted in Spain, where genetic testing — while effective in confirming diagnoses — was associated with a longer time to diagnosis (OR = 1.3; 95% CI: 1.2–1.5) (*11*). Additionally, this difference may be attributed to the limited training or experience that general practitioners typically have in managing rare diseases compared to specialists. This gap in clinical knowledge may hinder the early recognition of uncommon signs and symptoms, prolonging the diagnostic process.

When examining the type of rare disease, individuals with non-genetic conditions reported a significantly longer time to diagnosis compared to those with genetic disorders. Consistent with previous studies conducted in Spain, individuals with nervous system diseases were found to have a higher risk of diagnostic delay (OR = 1.4; 95% CI: 1.0–1.8), whereas those with ocular and adnexal conditions had a lower risk (OR = 0.7; 95% CI: 0.5–0.9) (*11*). This discrepancy may be due to the absence of specific diagnostic tests for many of these diseases and the variability in their phenotypic manifestations (*2*). Furthermore, despite significant advances in disease diagnosis, their impact remains limited unless effectively integrated into the academic training and continuing education of healthcare professionals. Proper recognition of rare diseases requires ongoing updates and effective dissemination of scientific knowledge to medical personnel (*25,26*).

This study has certain limitations inherent to addressing a topic that remains underexplored. First, potential inconsistencies in the collected data may arise from the lack of standardized clinical documentation, variability in diagnostic criteria across healthcare facilities, or patients' difficulty in accurately recalling the time to diagnosis or the tests performed. Additionally, the low frequency of individual pathologies limited disease-specific analyses and restricted the ability to establish robust associations between diagnostic and therapeutic factors. Moreover, recruitment was carried out through patient associations, which may introduce selection bias. Individuals engaged in these organizations are often more informed about their condition and actively involved in advocacy activities, whereas those who are not part of such networks may have different levels of disease awareness or healthcare-seeking behaviors. Consequently, diagnostic delays and knowledge indicators reported in this study may not fully represent the broader population of affected individuals. Finally, diagnostic times were based on self-reported information, introducing the possibility of recall bias, as participants may not accurately remember the sequence or timing of consultations and diagnostic procedures. As this is a descriptive study, the findings should be viewed as an initial approximation, intended to serve as a foundation for future research aimed at enhancing our understanding of these processes in the context of rare diseases.

In the specific context of Peru, the population affected by rare diseases remains largely unidentified, posing a significant challenge for patient outreach and the collection of representative data (*6*). Moreover, the absence of consolidated registries may have influenced sample selection and affected the accuracy of the results. Similarly, the limited awareness and training among healthcare professionals regarding these conditions could have contributed to underreporting or misdiagnosis. A study conducted in Peru on the level of knowledge about rare diseases revealed that more than half of the participants demonstrated an impoverished understanding (*2*).

This research shows that diagnostic delays are common for individuals with rare diseases in Peru and highlights how health-system organization shapes patients' diagnostic journeys. The absence of unified registries, limited specialist awareness, and uneven access to molecular testing all lead to prolonged diagnostic pathways.

Improving primary-care training, establishing structured referral networks, expanding tele-genetics support, and creating a national registry are realistic steps that could shorten diagnostic delays and promote fair access to specialized care. Emphasizing these measures would meaningfully enhance early detection and overall care for people living with rare diseases in Peru.

Despite its limitations, the main strength of this study lies in its reflection of patient perceptions and its status as the first to quantify diagnostic delays while identifying modifiable factors that could optimize earlier detection.

5. Conclusion

Nearly half of the patients included in the study received a definitive diagnosis more than twelve months after

symptom onset, with such delays being more frequent in rare, non-genetic diseases. No significant associations were found between time to diagnosis and demographic or structural variables, including socioeconomic status, sex, religion, place of residence, type of healthcare facility, level of complexity, or type of diagnostic test. These findings suggest that barriers to timely diagnosis are multifactorial and may be influenced by factors that have yet to be identified or systematically studied.

However, a trend toward longer diagnostic delays was observed among patients who reported difficulties accessing health centers and those who consulted more than ten general practitioners. These findings highlight the need to improve access to specialized services and to strengthen the training of healthcare professionals in the recognition and management of rare diseases, particularly at the primary care level, in order to reduce diagnostic delays and optimize care for this vulnerable population.

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Clinical and genetic study of a family with epidermolysis bullosa simplex caused by a novel *KRT5* gene mutation c.987C>G (p.Asn329Lys)

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SUMMARY: This study investigated the association between the novel *KRT5* gene mutation c.987C>G (p.Asn329Lys) and the clinical phenotype of epidermolysis bullosa simplex (EBS), to provide a basis for the molecular diagnosis and genetic counseling of EBS. Clinical data were collected from a 20-year-old female patient. Whole-exome sequencing was performed on the proband, 5 affected family members, and 2 healthy family members, with mutations verified by Sanger sequencing. Functional prediction was conducted using SIFT, PolyPhen-2, and MutationTaster, while conservation analysis was performed using ConSurf and NCBI CDD databases. The pathogenicity of the mutation was evaluated according to the 2015 ACMG guidelines. Results showed that the proband and all affected family members carried the heterozygous *KRT5* gene mutation c.987C>G (p.Asn329Lys), while healthy members did not, consistent with autosomal dominant co-segregation. This mutation was not recorded in databases such as gnomAD, indicating it is a novel mutation. Functional prediction showed SIFT score 0.00 (damaging), PolyPhen-2 score 1.000 (PROBABLY DAMAGING), and MutationTaster classification as "Deleterious". Conservation analysis confirmed that the 329th amino acid is located in the highly conserved Filament domain (ConSurf score 0.92, CDD E-value = 1.04e-158). The ACMG classification determined it as "Pathogenic". Affected family members exhibited a mild phenotype characterized by "friction-induced blisters, seasonal dependence, and scarless healing". The *KRT5* gene mutation c.987C>G (p.Asn329Lys) is a novel pathogenic mutation for EBS. Its unique phenotype enriches the genotype-phenotype spectrum of EBS and has important reference value for clinical practice.

Keywords: epidermolysis bullosa simplex, *KRT5* gene, novel mutation

1. Introduction

Epidermolysis bullosa (EB) is a group of rare hereditary skin fragility disorders characterized by increased fragility of the skin and mucous membranes. The core pathogenesis involves mutations in genes encoding key proteins of the epidermal-dermal junction structure, leading to decreased resistance of the skin to mechanical damage, with clinical manifestations including recurrent blisters, erosions, and wound healing abnormalities (*1*). EB is caused by mutations in genes encoding keratins, desmosomes, hemidesmosomes, or other intraepidermal or dermal-epidermal adhesion filaments, which are characterized by poor cell adhesion, lack of tissue repair or barrier function, resulting in varying degrees of blister and ulcer formation (*2,3*). Based on the ultrastructural changes of the skin and the level of blister formation (from top to bottom), EB is classified into four main types: epidermolysis bullosa simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB (KEB). Among them, EBS is the most common, accounting for approximately 70% of all EB cases (*4*). Epidermolysis bullosa is clinically and genetically heterogeneous, with inheritance patterns of autosomal dominant (AD) or autosomal recessive (AR) (*5*). EBS exhibits significant genetic heterogeneity, and identified pathogenic genes include *KRT14*, *KRT5*, *etc*. Among them, the *KRT5* gene encodes type II cytokeratin 5, a key structural protein in epidermal basal cells, which forms heterodimers with type I cytokeratin 14, assembles into an intermediate filament network, and anchors to the basement membrane through hemidesmosomes, providing mechanical stability to the epidermis (*6,7*).

KRT5 gene mutations are one of the main causes of EBS, most of which are dominant-negative missense mutations inherited in an autosomal dominant manner. The location of *KRT5* mutations is closely related to the severity of clinical phenotypes (*7*). Currently reported

pathogenic mutations in *KRT5* are mostly concentrated in the highly conserved rod domain, but discovery of new mutations still helps to further clarify the genotype-phenotype relationship of EBS. Significant progress has been made in clinical and genetic research on EB in recent years, and application of molecular diagnostic techniques has laid the foundation for accurate typing and genetic counseling. This study identified a novel mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene through clinical and genetic analysis of a family with typical EBS phenotypes, and clarified its pathogenicity and clinical significance by combining functional prediction, conservation analysis, and family co-segregation verification.

2. Research design and data collection

The proband was a 20-year-old female who had recurrent blisters all over her body since birth, especially in friction-prone areas such as hands, feet, and extensor surfaces of joints (Figure 1). The blisters showed obvious seasonal dependence — frequent attacks in summer (when the temperature was above 30°C, 3–5 blisters per week) and significant reduction in winter (1-2 blisters per month). Skin lesions could heal spontaneously without scarring or pigmentation. Family survey showed that there were 5 affected individuals in 3 generations of the family (maternal grandmother, mother, younger brother, uncle, and cousin), all showing similar phenotypes.

Laboratory examination results were as follows: Physical examination revealed multiple blisters and broken surfaces on both feet, and scattered blisters on both lower limbs, without scarring or pigmentation. Direct immunofluorescence (DIF) showed negative IgG, C3, IgM, and IgA in the intercellular space of the epidermis and basement membrane, ruling out autoimmune bullous diseases. Skin biopsy showed hyperkeratosis, extensive subepidermal clefts and blisters, and sparse mononuclear cell infiltration in the superficial dermis. Transmission electron microscopy showed intraepidermal basal layer blisters and clefts, basement membrane and hemidesmosomes at the dermal edge, and a large number of tonofilaments arranged as homogenized masses, consistent with the ultrastructural characteristics of EBS. (Figure 2).

To clarify the pathogenic genotype, peripheral blood samples were collected from the proband, 5 affected family members, and 2 healthy members (father and aunt) (Figure 3). Genomic DNA was extracted for whole-exome capture and sequencing. Candidate variants were verified by Sanger sequencing in family members. Functional prediction was performed using SIFT, PolyPhen-2, and MutationTaster tools, while conservation analysis was conducted using ConSurf platform (parameters: HMMER E-value = 0.0001, MAFFT alignment) and the NCBI CDD database. Pathogenicity of the variants was evaluated according to the 2015 ACMG guidelines.

3. Key research findings

Whole-exome sequencing results showed that the proband had a heterozygous missense mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene, which was located in the 1A exon, resulting in the substitution of

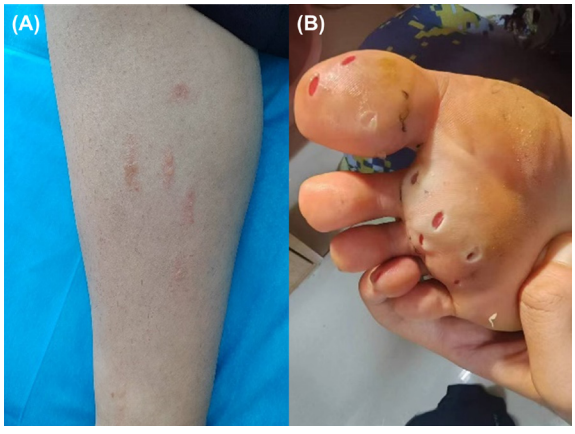

Figure 1. The patient's clinical manifestations. (A) Small blisters can be seen on the skin of the lower extremities after friction; **(B)** There are numerous blisters on both feet, and some of them are ruptured.

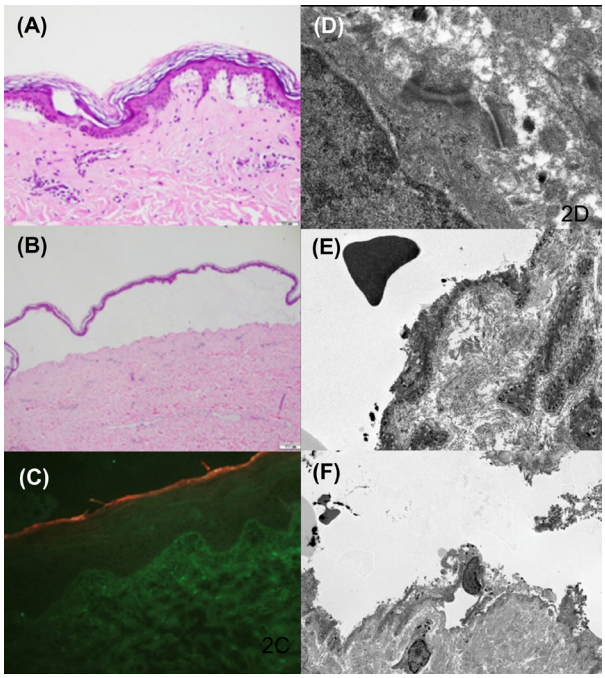

Figure 2. The patient's skin biopsy pathology, immunopathology and transmission electron micrographs. (A) Extensive cleavage can be observed beneath the epidermis. There is a sparse infiltration of mononuclear cells in the superficial layer of the dermis, and no obvious eosinophils are found. **(B)** Bulla formation can be seen beneath the epidermis. **(C)** By direct immunofluorescence, IgG, C3, IgM and IgA are negative among epidermal cells and in the basement membrane. **(D)** The basement membrane and complete desmosomes are visible at the dermal margin (DPx15.0k) **(E)** A large number of tonofilaments are arranged in a homogenized mass (DP3.0k). **(F)** Blisters and clefts are seen within the basal layer of the epidermis (DPx1.0k).

asparagine (N) with lysine (K) at the 329th amino acid (Figure 4). Sanger sequencing verification showed that all 5 affected family members carried this heterozygous mutation, while 2 healthy members (father and aunt) did not, completely consistent with the autosomal dominant co-segregation pattern (segregation ratio 1:1, $\chi^2 = 0.02$, $p = 0.89$). No record of this mutation was found in large-scale population databases such as gnomAD or in published literature, suggesting that it is the first reported novel mutation internationally.

Bioinformatics and conservation analysis further supported the pathogenicity of this mutation: In terms of functional prediction, SIFT tool scored 0.00 (determined to damage protein function), PolyPhen-2 scored 1.000 (determined as "PROBABLY DAMAGING"), and MutationTaster determined it as "Deleterious". The consistent results of the three tools strongly suggested that the mutation is harmful. Conservation analysis showed that the 329th amino acid had a conservation score of 0.92 (close to the full score of 1, belonging to a highly conserved site) calculated by the ConSurf platform. NCBI CDD database analysis confirmed that this site is located in the highly conserved Filament domain (167–480 amino acids) of the KRT5 protein, with an E-value of 1.04e–158 (approaching 0), indicating that it is subject to strong functional constraints during evolution and is the core functional region for keratin heterodimer assembly and intermediate filament network formation.

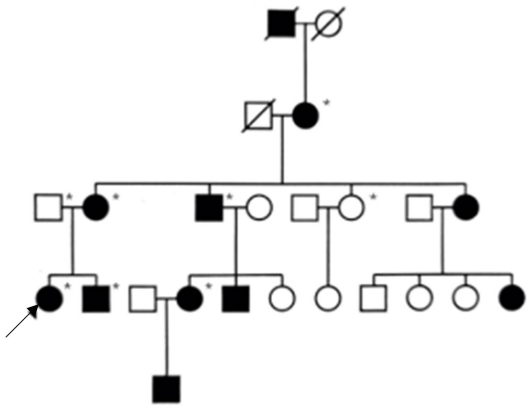

Figure 3. Patient's pedigree.

According to the 2015 ACMG guidelines for variant pathogenicity classification, this mutation met multiple pathogenicity criteria: It belonged to strong evidence (PS1) because the mutation was located in a known pathogenic functional domain (Filament domain) with no record of benign variants in this region; It belonged to moderate evidence (PM2) as it was not recorded in large-scale population databases and was an extremely rare variant; It belonged to supporting evidence (PP3) as multiple bioinformatics tools consistently predicted it to be harmful; It belonged to supporting evidence (PP4) as the patient's phenotype was completely consistent with EBS and family co-segregation was verified. Based on the above evidence, the mutation was clearly determined to be "Pathogenic" (Table 1).

Clinical phenotype analysis showed that all affected family members exhibited mild EBS characteristics. The impact of the disease on quality of life was mainly

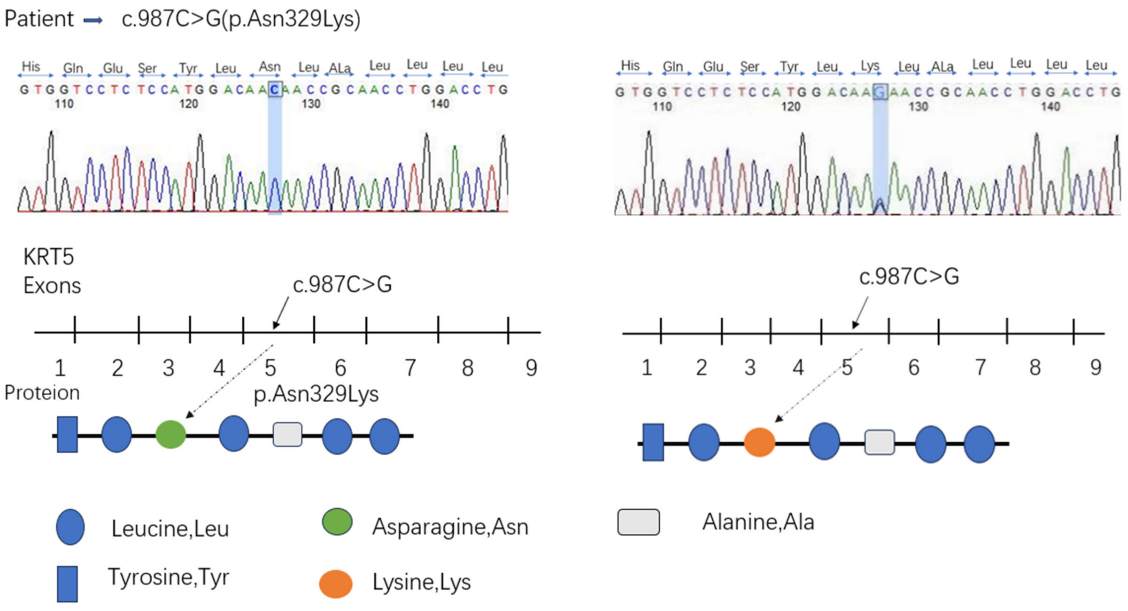

Figure 4. A missense mutation, specifically c.987C>G (p.Asn329Lys), was identified in the coding region of the keratin 5 (*KRT5*) gene in all patients. This mutation results in the substitution of polar uncharged asparagine with positively charged lysine at amino acid position 329 of keratin 5, thereby disrupting the native charge balance and hydrogen bond network. Such alterations impair keratin filament assembly and may lead to protein misfolding, consequently weakening the mechanical strength and barrier function of the skin and ultimately contributing to disease pathogenesis.

Table 1. ACMG evidence codes applied to evaluate KRT5 mutation pathogenicity in an epidermolysis bullosa simplex family

Evidence Type	Code	Criteria	Application to our mutation
Strong (P)	PS1	Located in a known pathogenic domain with no benign variants reported	Met: Mutation in *KRT5* Filament domain (core functional region with no benign variants)
Moderate (PM)	PM2	Absent/extremely rare in large population databases (*e.g.*, gnomAD)	Met: Not recorded in gnomAD or other databases (extremely rare)
Supporting (PP)	PP3	Consistent prediction of harmfulness by multiple bioinfor-matic tools	Met: SIFT (0.00), PolyPhen-2 (1.000), MutationTaster all predicted damage
Supporting (PP)	PP4	Phenotype matches the disease; variant co-segregates with phenotype in family	Met: Typical EBS phenotype; 5 affected family members carried the mu-tation, healthy members did not

reflected in social avoidance (such as refusing to participate in activities involving skin exposure like swimming due to skin appearance) and work restrictions (such as inability to engage in heavy physical labor), with no life-threatening complications.

4. Discussion

In this study, a novel mutation c.987C>G (p.Asn329Lys) in the *KRT5* gene was identified in an EBS family. Its pathogenicity was confirmed through multi-dimensional evidence, and a unique genotype-phenotype association was revealed, which holds significant implications for the clinical practice of EBS.

In terms of the pathogenic mechanism of the mutation, the Filament domain of the KRT5 protein contains four α-helical regions (1A, 1B, 2A, 2B). These regions assemble with KRT14 protein through hydrophobic interactions to form a stable intermediate filament network, which is crucial for maintaining the mechanical strength of the epidermis (*7*). The c.987C>G mutation identified in this study is located in the 1A helical region, causing the substitution of asparagine (a neutral amino acid) with lysine (a positively charged amino acid) at position 329. This change may affect protein function through two mechanisms: first, it disrupts the hydrophobic core structure of the α-helix, interfering with the formation of KRT5-KRT14 heterodimers; second, it alters the local charge distribution, undermining the stability of the intermediate filament network and reducing the resistance of basal cells to mechanical stress. This is consistent with the observation of "disordered arrangement of tonofilaments into homogenized masses" under transmission electron microscopy. Additionally, the 329th amino acid is highly conserved (all asparagine) across 12 species, further confirming its key role in maintaining keratin function and reinforcing the pathogenicity of the mutation.

Regarding genotype-phenotype correlation, the mild phenotypic characteristics caused by this mutation are closely related to its location and functional impact. Compared with classic mutations in the initial region of the 1A helix (such as p.Arg125Cys, which causes severe phenotypes with annual blister counts > 100), this mutation is located in the middle of the helix, exerting weaker interference on heterodimer assembly, thus resulting in a milder phenotype. This provides a new perspective for explaining the heterogeneity of EBS mucosal phenotypes.

In terms of clinical significance, the discovery of this novel mutation enriches the mutation spectrum of EBS, and its unique clinical phenotype offers important insights for clinical diagnosis and genetic counseling. In the early 1990s, prenatal diagnosis was performed using electron microscopy and/or fetal skin biopsy with IFM after 17 weeks of gestation (*8*). The main drawbacks of this technique include the possibility of sampling errors, the risk of miscarriage, and the emotional distress associated with terminating an affected fetus at an advanced stage. Prenatal diagnosis can also be accomplished by examining chorionic villus cells (usually around 10 weeks) or amniocentesis (usually around 16 weeks) (*9*). The accuracy of predicting postnatal EB diagnosis is over 98% (*10*). Many studies have attempted to isolate fetal 10lls from maternal blood. The successful implementation of this technology would enable prenatal diagnosis of EB from maternal blood samples as early as 6–7 weeks (*11*).

At the diagnostic level, for patients presenting with "season-dependent blisters, scarless healing, and mucosal involvement", priority should be given to detecting variations in the 1A helical region of the *KRT5* gene to improve the efficiency of molecular diagnosis. At the genetic counseling level, it is necessary to clearly inform family members about the autosomal dominant inheritance pattern of this mutation (50% risk of inheritance in offspring) and combine it with prenatal diagnostic technologies (such as chorionic villus sampling at 10 weeks of gestation) to provide a basis for family planning decisions. If the fetus carries the mutation, measures such as avoiding friction and high-temperature environments and wearing soft clothing after birth can reduce blister formation and significantly improve quality of life. For families

wishing to completely avoid genetic risks, prenatal testing and appropriate genetic counseling are integral to the management of EB patients and families at risk (*12*). Preimplantation genetic diagnosis (PGD) is a feasible option, and based on the clear mutation site in this family, the accuracy of PGD can be guaranteed.

The current treatment options for EB are mainly symptomatic, aiming to prevent mechanical damage, provide wound care, treat infectious complications, and address the external manifestations of the disease. To date, there is no cure for EB (*13*). In addition to basic symptomatic treatments, there are several emerging therapeutic approaches, such as gene therapy, cell therapy, protein replacement therapy, antisense oligonucleotides, and PCT interpretation (*14*). Emerging gene therapy research holds promise for the future. Current therapeutic research on EB includes the use of gene-corrected patient-specific iPS cells, gene editing technologies, and polymer-mediated DNA delivery systems (*15-17*). For epidermolysis bullosa (EB), the goal of gene therapy is to restore the function of skin structural proteins and enhance the mechanical strength of the skin. This can be achieved through various approaches, including gene addition, gene replacement, and gene editing technologies. The selection of gene vectors includes viral vectors, retroviral vectors, adeno-associated viral vectors (AAV), non-viral vectors, liposomes, and polymer vectors (*18*).

Gene editing technology CRISPR/Cas9: The CRISPR/Cas9 technology identifies specific DNA sequences through guide RNA (gRNA), and the Cas9 enzyme cleaves the DNA double strand to achieve precise gene editing. In EB research, CRISPR/Cas9 has been used to repair mutations in the *COL7A1* gene and restore its normal function (*19*). ALENs and ZFNs: These technologies achieve precise DNA cleavage and repair by specifically recognizing DNA sequences. TALENs and ZFNs have high specificity in gene editing, but their large molecular weight limits their application in viral vectors (*20*). In a 2006 study by Mavilio *et al*., the skin structure of patients was successfully repaired by introducing the *LAMB3* gene into the patients' epidermal stem cells (*21*). In studies on dystrophic EB, AAV vectors have been used to deliver the *COL7A1* gene into patients, and some patients have shown improved skin healing (*22*). However, this form of gene therapy also presents complex issues and risks, involving the technical development of gene vectors, carcinogenic potential, future risk of malignant tumors, and the duration of therapeutic effects. It is worth noting that there is currently no approved gene therapy for EB. The mutation in this study is located in the highly conserved Filament domain, and its clear functional localization provides a potential target for targeted intervention. For example, local gene repair strategies may alleviate symptoms by restoring the stability of keratin intermediate filaments.

The limitation of this study is the lack of *in vitro* functional experiments to directly verify the impact of the mutation on protein structure. In the future, mutant expression vectors can be constructed to observe the interaction with KRT14 and the assembly of intermediate filaments, thereby further clarifying the pathogenic mechanism.

In conclusion, the *KRT5* gene mutation c.987C>G (p.Asn329Lys) is a novel pathogenic mutation for EBS. The related research provides new evidence for understanding the genetic heterogeneity of EBS and has direct guiding value for clinical diagnosis and genetic counseling.

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Letter

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Early screening for respiratory and cardiac complications in pediatric mucopolysaccharidosis IVA: Insights from a case

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SUMMARY: Mucopolysaccharidosis type IVA (MPS IVA) is a rare genetic disorder characterized by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase, leading to significant growth and developmental challenges, increased morbidity, and reduced life expectancy. We report the clinical characteristics and genetic basis of MPS IVA in an 11-year-old male patient, emphasizing the critical role of early diagnosis and intervention. The combination of enzyme activity testing and genetic testing screening for suspected clinical cases may shorten the diagnosis time and reduce the difficulty of diagnosis. Early screening for respiratory and cardiac complications in confirmed cases is beneficial for reducing patient mortality.

Keywords: mucopolysaccharidosis type IVA, pulmonary hypertension, mitral regurgitation, delay diagnosis, optimize the diagnostic process

Mucopolysaccharide IVA (MPS IVA) is an autosomal recessive lysosomal storage disorder caused by a deficiency of N-acetylglucosamine 6-sulfate esterase. The estimated incidence rate of MPS IVA in China is 1.57–4.95 per 100,000 people (*1*). The clinical manifestations of MPS IVA patients are diverse and involve multiple systems, so the diagnosis of this disease cannot be based solely on symptoms without other more direct evidence. The occurrence of cardiac complications may be insidious and lead to early death in MPS IVA patients (*2*).

Here, we report a male adolescent patient with a history of growth delay for over ten years. Due to early reliance on clinical symptoms and enzyme activity testing for screening, the diagnosis was delayed but ultimately diagnosed as MPS IVA through exome sequencing. And due to the lack of early screening for cardiac complications and ineffective treatment, he ultimately died. This study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki, and the Medical Ethics Committee of Shandong Provincial Hospital affiliated to Shandong First Medical University approved all procedures in this report (LCYJ:NO.2019-147). Informed consent was obtained from the patient's guardian.

The patient was an 11 year and 6-month-old boy who presented to the Pediatric Endocrinology Department of Shandong Provincial Hospital for treatment on January 22, 2025. He had a history of growth retardation for up to ten years, and in the past 2–3 years, his motor ability had gradually deteriorated, resulting in his inability to walk. He developed acute upper limb weakness 10 days before admission. The patient's parents and sister are both in good health.

On physical examination, the patient exhibited coarse facial features, macrocephaly (head circumference 58 cm), severe pectus carinatum, and generalized short stature (height 90 cm, Standard Deviation Score (SDS)-8.34; Body Mass Index (BMI) 24.69 kg/m^2). Neuromuscular assessment revealed hypotonia, hyperextensible joints, shortened metacarpals, and complete loss of ambulatory function. A grade 2/6 systolic murmur was auscultated at the cardiac apex, and a 1 × 2 cm benign anal polyp was noted. Vital signs included tachycardia (Heart rate 120 bpm) and tachypnea (Respiratory rate 23/min), with stable blood pressure (Blood pressure 111/70 mmHg) (Supplementary Figure S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=276*).

Echocardiography displayed left atrial and ventricular dilation, moderate-severe mitral regurgitation, patent foramen ovale (0.3 cm left-to-right shunt), and pulmonary hypertension (pulmonary arterial systolic pressure PASP 36 mmHg). Hand radiography displayed that the patient had shortened metacarpals, osteopenia, dysplastic epiphyses, and delayed bone age (equivalent to 6–7 years) (Supplementary Figure S2, *https://*

www.irdrjournal.com/action/getSupplementalData.php?ID=276).

Two heterozygous variations of the Galactosamine (N-acetyl)-6-sulfatase (*GALNS*) gene were detected in blood samples, which are associated with mucopolysaccharidosis type IVA. The gene is *GALNS* NM_000512.5, including one pathogenic variant located at chromosome chr16:88907448 at c.374C>T p.Pro125Leu. Currently, a total of 573 GALNS variants have been reported in all publications (Supplementary Figure S3, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=276*).

Initial management included supplemental oxygen and coenzyme Q10 supplementation, which was discontinued due to possible adverse effects (blurred vision and hearing decline). The patient rapidly progressed to acute respiratory failure, characterized by refractory hypoxemia (SpO_2 < 70%), hypercapnia, and oliguria. Despite aggressive intervention — diuretics, sodium bicarbonate, corticosteroids, and repeated cardiopulmonary resuscitation — the patient developed cardiopulmonary arrest and expired on January 26, 2025.

The patient's growth retardation was first observed in infancy. The patient had clinical features of mucopolysaccharidosis such as delayed growth and development, bone and joint deformities, and rough facial features. Therefore, in 2017, the activity tests of alpha glucosidase, beta glucuronidase, and iduronidase were performed at the first visit without any abnormalities. But no further screening was conducted on other types of mucopolysaccharide diseases, and no genetic testing was performed. When readmitted in 2025, both physical and laboratory examination results indicated the presence of cardiac complications in the patient, and a significant increase in Pro-B-type natriuretic peptide (proBNP) levels suggested the possibility of long-term cardiac overload (*3*). The results of echocardiography also confirm this point. So heart failure has become the main cause of deterioration and even death in patients.

Delayed diagnosis and early misdiagnosis are common in mucopolysaccharide diseases, including MPS IVA, with an average delay of 9.42 months and an average misdiagnosis of 4.56 times (*4*). There are two main reasons for delayed diagnosis. One reason is that pediatricians or orthopedic doctors lack experience with this disease, and the clinical symptoms of multiple subtypes of mucopolysaccharidosis overlap (*5*). The second reason may be limited by testing capabilities of different medical institutions. However, early diagnosis during the initial or asymptomatic period may have a significant impact

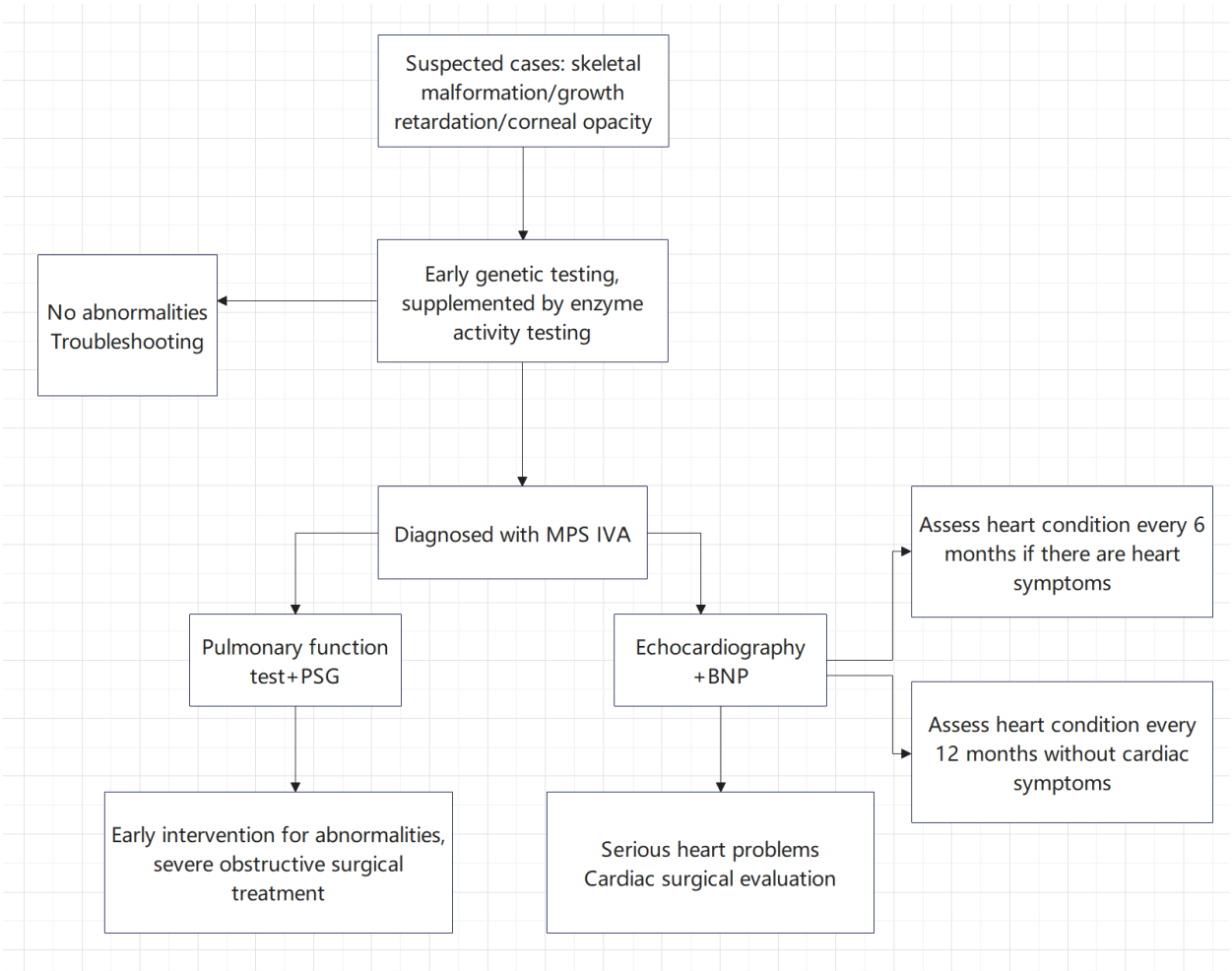

Figure 1. Mucopolysaccharidosis type IVA (MPS IVA) improved diagnostic process diagram.

on the final treatment outcome. So early diagnosis and intervention may have different outcomes.

Simultaneously conducting genetic testing while detecting enzyme activity can compensate for the subjective experience of doctors and the shortcomings of enzyme activity testing, while also benefiting genotype phenotype databases for better diagnosis, treatment, and evaluation of prognosis. Early gene detection may not only improve the identification of MPS, but also accurately calculate the incidence rate of MPS (*6*).

From the situation of this case, we believe that early screening for cardiac and respiratory complications after diagnosis is also very necessary. Respiratory failure and cardiac complications are the two leading causes of death in this disease, and studies have shown that early enzyme replacement therapy has a better improvement effect on the heart (*7*). Early respiratory intervention can significantly reduce mortality (*8*). When pediatricians or orthopedic surgeons encounter children with bone and joint deformities or growth retardation, it is recommended to undergo early genetic testing supplemented by enzyme activity testing for early diagnosis. Early screening for respiratory and cardiac complications after diagnosis and early intervention treatment are needed to maximize treatment effectiveness (Figure 1).

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Letter

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AI-driven enhancements in rare disease diagnosis and support system optimization

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SUMMARY: Rare diseases are characterized by an extremely low prevalence, high phenotypic heterogeneity, and complex pathogenesis. This combination of factors presents significant challenges, including prolonged diagnostic delays, lack of standardized care, and difficulties in pathological interpretation. The integration of artificial intelligence (AI) offers a transformative approach to overcoming these barriers. In recent years, researchers worldwide have been actively exploring the use of AI to diagnose and manage rare diseases. Key advances include few-shot learning algorithms designed to tackle data scarcity, clinically validated foundation models that enhance diagnostic consistency across institutions, and multimodal AI frameworks that integrate imaging, genomic, and phenotypic data to improve diagnostic accuracy. In addition, there is growing recognition that AI can enhance diagnostic efficiency and thereby optimize support systems for rare diseases. As challenges such as AI model interpretability and data equity are addressed, AI is expected to make significant strides in the diagnosis and treatment of rare diseases.

Keywords: rare disease, artificial intelligence, diagnosis, support system

Rare diseases are characterized by a low prevalence, high phenotypic heterogeneity, and complex pathogenesis. Patients with these diseases face prolonged diagnostic delays averaging 4–5 years (*1*), insufficient standardization of care, and challenges in pathological interpretation. The lack of precise diagnostic and therapeutic technologies is a critical barrier to improving patient outcomes. The further integration of artificial intelligence (AI) in healthcare has the capacity to overcome data scarcity ("the curse of dimensionality") (*2*), integrate multimodal clinical data, and enhance diagnostic and therapeutic precision. The accelerating advancement of AI is driving its growing use in the diagnosis and treatment of rare diseases. Our goal in this Letter is to summarize recent progress and to offer actionable insights for clinical practice.

Innovation in diagnostic algorithms for few-shot learning scenarios

The scarcity of annotated data for rare diseases is a primary bottleneck that restricts the development of diagnostic models. The recently introduced RetiZero model, a knowledge-rich vision-language foundation model pre-trained on over 400 rare and common fundus diseases, demonstrates remarkable adaptability with minimal data (*3*). When fine-tuned with only five images per category, it achieved exceptional area under the curve (AUC) scores (0.967, 0.859, 0.942) across three clinical datasets, significantly outperforming existing models. RetiZero integrates a masked autoencoder (MAE) with contrastive language-image pre-training (CLIP) to enable robust feature learning from minimal data, offering a powerful paradigm for scenarios with limited data.

Further demonstrating the versatility of few-shot learning in rare disease diagnosis, Alsentzer *et al.* introduced SHEPHERD, a geometric deep learning model that leverages a biomedical knowledge graph and simulated patient data for phenotype-driven genetic diagnosis (*4*). SHEPHERD was trained on over 40,000 synthetic patients with 2,134 rare diseases. When evaluated on a real-world, independent cohort from the Undiagnosed Diseases Network (UDN), it achieved a top 1 accuracy of 40% in causal gene discovery from EXPERT-CURATED gene lists and effectively retrieved "patients like me." Its ability to generalize across clinical sites and novel diseases underscores the potential of knowledge-guided, few-shot learning frameworks to overcome data scarcity in genomics and accelerate the diagnosis of rare genetic conditions.

Diagnostic and therapeutic applications of clinical-grade foundation models

The development of foundation models has overcome the limitations of "data dependency" in diagnosing rare

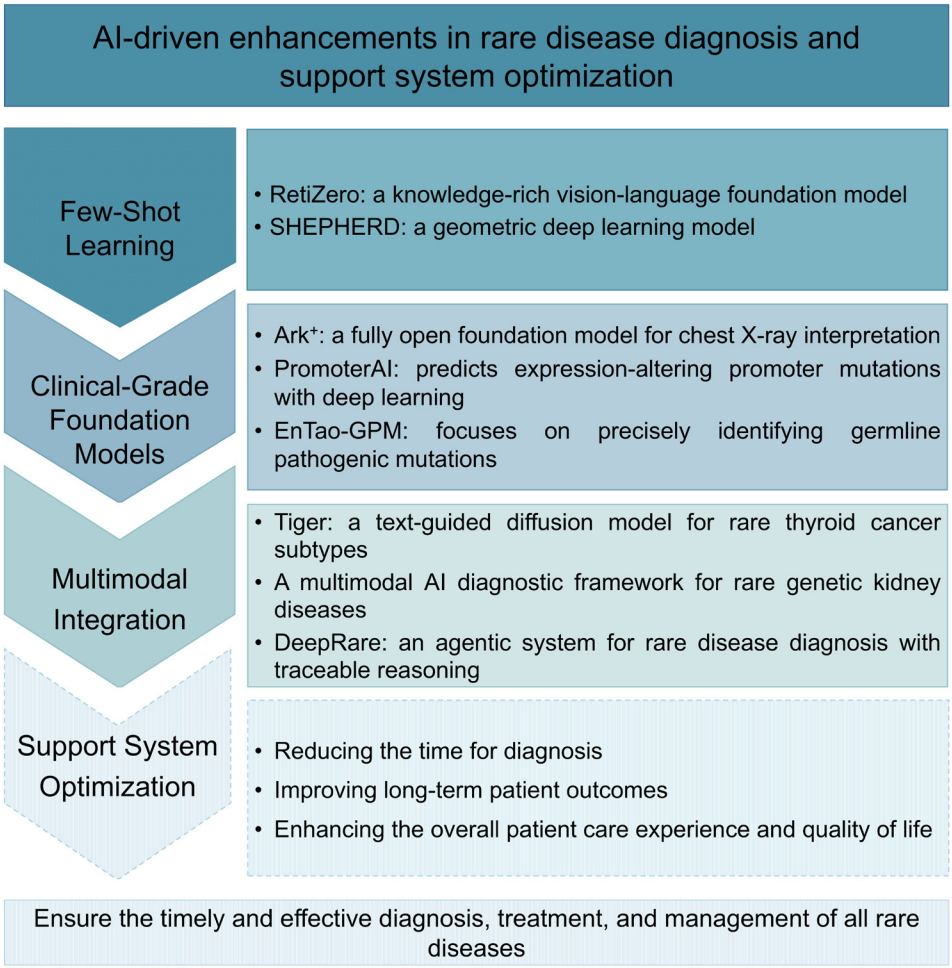

Figure 1. AI-driven enhancements in rare disease diagnosis and support system optimization. AI, artificial intelligence.

diseases. These models enable consistent interpretations across different clinical centers and disease types. Ark$^+$ is a fully open foundation model for chest X-ray interpretation, pre-trained by cyclically accruing and reusing knowledge from heterogeneous expert labels across six public datasets (*5*). It demonstrates strong generalizability and excels in few-shot learning to diagnose rare thoracic conditions, effectively addressing long-tailed disease distributions. Its capability for zero-shot transfer and federated learning enables robust, privacy-preserving diagnoses across institutions, making it a powerful, clinically adaptable tool for diagnosing both common and rare diseases.

Another example is PromoterAI, a deep neural network developed by Jaganathan *et al*. (*6*). First, it was trained on large-scale functional genomics datasets to learn the "regulatory syntax" underlying transcriptional and epigenetic programs. Then, it was fine-tuned using a curated set of rare promoter variants associated with outlier gene expression. By accurately detecting expression-altering variants, PromoterAI expands the clinical utility of genome sequencing and increases the yield of diagnoses for rare genetic diseases.

Similarly, Lin *et al*. developed the EnTao-GPM DNA Foundation Model, which focuses on precisely identifying germline pathogenic mutations (*7*). Through cross-species targeted pre-training, fine-tuning on the ClinVar database and the Human Gene Mutation Database, and integration with large language models to generate interpretable clinical reports, the model significantly improves the accuracy of mutation classification (achieving an AUC of 0.963 for single nucleotide variants). The model serves as a powerful tool for shortening the diagnostic process for rare diseases and guiding personalized treatment.

Precision diagnosis through multimodal data integration

The diagnosis and treatment of rare diseases frequently require integrating multi-source data, including medical imaging, genomic data, and clinical phenotypes. The ability of AI to seamlessly integrate diverse data sources has been found to enhance diagnostic accuracy. Dai *et al*. developed a text-guided diffusion model (the Tiger Model) for rare thyroid cancer subtypes (*8*). This model integrates ultrasound imaging features with clinical text descriptions. The generated synthetic images were validated by physician Turing tests, achieving a realism rate of 92.2%. This approach improved the AUC for

subtype classification from 0.7364 to 0.8442, thereby laying the foundation for the development of customized treatment strategies.

Chen *et al*. proposed a multimodal AI diagnostic framework for rare genetic kidney diseases (*9*). This framework integrates genomic data, human phenotype ontology (HPO) terms, and imaging features. The researchers discussed the potential of AI approaches to address significant underdiagnosis and shorten the diagnostic odyssey for these conditions, thereby improving diagnostic precision.

Zhao *et al*. developed the DeepRare system (*10*), which features a three-tier architecture of "central host–specialized agent servers–heterogeneous web-scale medical sources," supporting multi-source data input and traceable reasoning. The system's performance was evaluated on 6,401 cases from multiple global centers, achieving an average Recall@1 score of 57.18% with HPO term input alone. This was further enhanced to 70.6% in multi-modal scenarios. The system has been implemented in numerous domestic and international institutions.

Optimization of rare disease support systems

There is growing recognition that AI can enhance diagnostic efficiency and thereby optimize support systems for rare diseases. This perspective has been discussed at numerous academic conferences, including the Second "Hai Shang" Rare Disease Forum, held in Shanghai, China, on November 14-15, 2025, which focused on the theme of "AI-Powered Diagnosis, Protection, and Humanistic Care in Rare Diseases."

One can reasonably expect improved diagnostic efficiency — driven by AI — to systematically propel the optimization of rare disease support systems and the implementation of humanistic care, thereby establishing a virtuous cycle where "diagnosis and treatment, support, and humanistic care" mutually reinforce one another (Figure 1). First, AI-assisted diagnosis reduces the time patients spend on their "diagnostic odyssey," which is expected to reduce unnecessary testing and ineffective treatments. This approach thus has the potential to alleviate financial burdens on both patients and the healthcare system, while also helping to preserve scarce, quality medical resources. In addition, the precise identification of pathogenic variants by AI provides a critical pre-symptomatic intervention window for hereditary rare diseases, thereby improving long-term patient outcomes. Finally, AI tools support primary care physicians by providing expert-level diagnostic guidance, minimizing misdiagnoses caused by limited experience. This, in turn, reduces patient suffering and enables more personalized treatment regimens, ultimately enhancing the overall patient care experience and quality of life.

In conclusion, as challenges related to the interpretability of AI models and data equity are addressed, the field of AI is poised to make significant advances in the diagnosis and treatment of rare diseases. The goal is to move from a focus on "precision" to a more comprehensive approach, aiming for "universal benefit." This will involve the development of tools and methodologies that ensure timely and effective diagnosis, treatment, and management of all rare diseases.

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IRDR
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2. Submission Types

Original Articles sshould be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables. Supplementary Data are permitted but should be limited to information that is not essential to the general understanding of the research presented in the main text, such as unaltered blots and source data as well as other file types.

Brief Reports definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

Reviews should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of a maximum of 10 figures and/or tables and 100 references. Mini reviews are also accepted, which should not exceed 4,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 50 references.

Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 30 references.

Communications are short, timely pieces that spotlight new research findings or policy issues of interest to the field of global health and medical practice that are of immediate importance. Depending on their content, Communications will be published as "Comments" or "Correspondence". Communications should not exceed 1,500 words in length (excluding references) and should be limited to a maximum of 2 figures and/or tables and 20 references.

Editorials are short, invited opinion pieces that discuss an issue of immediate importance to the fields of global health, medical practice, and basic science oriented for clinical application. Editorials should not exceed 1,000 words in length (excluding references) and should be limited to a maximum of 10 references. Editorials may contain one figure or table.

News articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

Letters should present considered opinions in response to articles published in Intractable & Rare Diseases Research in the last 6 months or issues of general interest. Summaries of research results and sharing of experiences in clinical practice and basic research (findings based on case reports, clinical pictures, etc.) can also be published as Letters. Letters should not exceed 800 words in length and may contain a maximum of 10 references. Letters may contain one figure or table.

3. Editorial Policies

For publishing and ethical standards, *Intractable & Rare Diseases Research* follows the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals issued by the International Committee of Medical Journal Editors (ICMJE, *https://icmje.org/recommendations*), and the Principles of Transparency and Best Practice in Scholarly Publishing jointly issued by the Committee on Publication Ethics (COPE, *https://publicationethics.org/resources/guidelines-new/principles-transparency-and-best-practice-scholarly-publishing*), the Directory of Open Access Journals (DOAJ, *https://doaj.org/apply/transparency*), the Open Access Scholarly Publishers Association (OASPA, *https://oaspa.org/principles-of-transparency-and-best-practice-in-scholarly-publishing-4*), and the World Association of Medical Editors (WAME, *https://wame.org/principles-of-transparency-and-best-practice-in-scholarly-publishing*).

Intractable & Rare Diseases Research will perform an especially prompt review to encourage innovative work. All original research will be subjected to a rigorous standard of peer review and will be edited by experienced copy editors to the highest standards.

Ethical Approval of Studies and Informed Consent: For all manuscripts reporting data from studies involving human participants or animals, formal review and approval, or formal review and waiver, by an appropriate institutional review board or ethics committee is required and should be described in the Methods section. When your manuscript contains any case details, personal information and/or images of patients or other individuals, authors must obtain appropriate written consent, permission and release in order to comply with all applicable laws and regulations concerning privacy and/or security of personal information. The consent form needs to comply with the relevant legal requirements of your particular jurisdiction, and please do not send signed consent form to *Intractable & Rare Diseases Research* to respect your patient's and any other individual's privacy. Please instead describe the information clearly in the Methods (patient consent) section of your manuscript while retaining copies of the signed forms in the event they should be needed. Authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013, *https://wma.net/what-we-do/medical-ethics/declaration-of-helsinki*). When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Reporting Clinical Trials: The ICMJE (*https:// icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html*) defines a clinical trial as any research project that prospectively assigns people or a group of people to an intervention, with or without concurrent comparison or control groups, to study the relationship between a health-related intervention and a health outcome. Registration of clinical trials in a public trial registry at or before the time of first patient enrollment is a condition of consideration for publication in *Intractable & Rare Diseases Research*, and the trial registration number will be published at the end of the Abstract. The registry must be independent of for-profit interest and publicly accessible. Reports of trials must conform to CONSORT 2010 guidelines (*https://consort-statement.org/consort-2010*). Articles reporting the results of randomized trials must include the CONSORT flow diagram showing the progress of patients throughout the trial.

Conflict of Interest: All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

Submission Declaration: When a manuscript is considered for submission to *Intractable & Rare Diseases Research*, the authors should confirm that 1) no part of this manuscript is currently under consideration for publication elsewhere; 2) this manuscript does not contain the same information in whole or in part as manuscripts that have been published, accepted, or are under review elsewhere, except in the form of an abstract, a letter to the editor, or part of a published lecture or academic thesis; 3) authorization for publication has been obtained from the authors' employer or institution; and 4) all contributing authors have agreed to submit this manuscript.

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Peer Review: *Intractable & Rare Diseases Research* operates a single-anonymized review process, which means that reviewers know the names of the authors, but the authors do not know who reviewed their manuscript. All articles are evaluated objectively based on academic content. External peer review of research articles is performed by at least two reviewers, and sometimes the opinions of more reviewers are sought. Peer reviewers are selected based on their expertise and ability to provide quality, constructive, and fair reviews. For research manuscripts, the editors may, in addition, seek the opinion of a statistical reviewer. Every reviewer is expected to evaluate the manuscript in a timely, transparent, and ethical manner, following the COPE guidelines (*https://publicationethics.org/files/cope-ethical-guidelines-peer-reviewers-v2_0.pdf*). We ask authors for sufficient revisions (with a second round of peer review, when necessary) before a final decision is made. Consideration for publication is based on the article's originality, novelty, and scientific soundness, and the appropriateness of its analysis.

Suggested Reviewers: A list of up to 3 reviewers who are qualified to assess the scientific merit of the study is welcomed. Reviewer information including names, affiliations, addresses, and e-mail should be provided at the same time the manuscript is submitted online. Please do not suggest reviewers with known conflicts of interest, including participants or anyone with a stake in the proposed research; anyone from the same institution; former students, advisors, or research collaborators (within the last three years); or close personal contacts. Please note that the Editor-in-Chief may accept one or more of the proposed reviewers or may request a review by other qualified persons.

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The manuscript must be accompanied by a cover letter prepared by the corresponding author on behalf of all authors. The letter should indicate the basic findings of the work and their significance. The letter should also include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been published previously or is not under consideration for publication elsewhere. The cover letter should be submitted in PDF format. For an example of Cover Letter, please visit: (Download Centre: *https://www.irdrjournal.com/downcentre*)

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6. Manuscript Preparation

Manuscripts are suggested to be prepared in accordance with

the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals", as presented at *https://www.ICMJE.org*.

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (*e.g*. DNA). Single words should not be abbreviated.

Title page: The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; 5) author contribution statements to specify the individual contributions of all authors to this manuscript, and 6) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose").

Abstract: The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. For articles that are Original Articles, Brief Reports, Reviews, or Policy Forum articles, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For Communications, Editorials, News, or Letters, a brief summary of main content in 150 words or fewer should be included in the manuscript. For articles reporting clinical trials, the trial registration number should be stated at the end of the Abstract. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Three to six key words or phrases that do not occur in the title should be included in the Abstract page.

Introduction: The introduction should provide sufficient background information to make the article intelligible to readers in other disciplines and sufficient context clarifying the significance of the experimental findings.

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Acknowledgments: All funding sources (including grant identification) should be credited in the Acknowledgments section. Authors should also describe the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. If the funding source had no such involvement, the authors should so state.

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Examples are given below:

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Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. Biosci Trends. 2010; 4:56-60.

Example 2 (Sample journal reference with more than 15 authors):

Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

Example 3 (Sample book reference):
Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

Example 4 (Sample web page reference):

World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. *http://www.who.int/whr/2008/whr08_en.pdf* (accessed September 23, 2022).

Tables: All tables should be prepared in Microsoft Word or Excel and should be arranged at the end of the manuscript after the References section. Please note that tables should not in image format. All tables should have a concise title and should be numbered consecutively with Arabic numerals. If necessary, additional information should be given below the table.

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Supplemental data: Supplemental data might be useful for supporting and enhancing your scientific research and *Intractable & Rare Diseases Research* accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g*., Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

7. Online Submission

Manuscripts should be submitted to *Intractable & Rare Diseases Research* online at *https://www.irdrjournal.com*. Receipt of your manuscripts submitted online will be acknowledged by an e-mail from Editorial Office containing a reference number, which should be used in all future communications. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail at *office@irdrjournal.com*

8. Accepted Manuscripts

Proofs: Galley proofs in PDF format will be sent to the corresponding author *via* e-mail. Corrections must be returned to the editor (*office@irdrjournal.com*) within 3 working days.

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