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Editorial

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Artificial intelligence applications in rare and intractable diseases: Advances, challenges, and future directions

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SUMMARY: Rare and intractable diseases affect an estimated 3.5% to 5.9% of the global population but remain largely underserved in terms of diagnosis and treatment, with effective therapies available for only about 5% of conditions. This paper presents an overview of recent advances in artificial intelligence (AI) applications targeting these challenges. In diagnostic support, AI has been utilized to analyze genomic data and facial images, enhancing the accuracy and efficiency of identifying rare genetic syndromes. In therapeutic development, AI-driven analysis of biomedical knowledge graphs has enabled the prediction of potential treatment candidates for diseases lacking existing therapies. Additionally, generative models have accelerated drug discovery by identifying novel targets and designing candidate compounds, some of which have progressed to clinical evaluation. AI has also facilitated clinical trial support by automating patient eligibility screening using electronic health records, improving recruitment efficiency for trials that often struggle with small, geographically dispersed patient populations. Despite these advancements, challenges remain in ensuring data quality, interpretability of AI outputs, and the standardization of infrastructure across institutions. Moving forward, international data-sharing platforms integrating diverse modalities — clinical, genomic and image — are expected to play a pivotal role in enabling reliable, scalable, and ethically responsible AI applications. These developments hold the potential to transform the landscape of rare disease diagnosis, treatment, and research.

Keywords: rare diseases, intractable diseases, artificial intelligence

1. Introduction

Although rare and intractable diseases are individually uncommon, they are estimated to affect approximately 3.5% to 5.9% of the global population, corresponding to 263 to 446 million people worldwide (*1*). Most of these diseases are chronic and progressive, severely impairing patients' quality of life and requiring long-term support from healthcare and welfare systems. To date, effective treatments have been established for only about 5% of rare diseases (*2*), leaving the majority of patients to live with uncertainty in diagnosis and treatment. One of the most pressing challenges in this field is the frequent occurrence of delayed and inaccurate diagnoses. A large-scale European survey reported that the average time from symptom onset to a confirmed diagnosis is approximately 4.7 years, with one in four patients requiring over five years (*3*). This so-called "diagnostic odyssey" often involves consultations with more than five physicians and multiple instances of misdiagnosis before an accurate diagnosis is reached.

The causes of diagnostic delay are multifaceted, including insufficient knowledge of rare diseases among clinicians, nonspecific symptoms, symptoms spanning multiple medical specialties, and limited access to specialized medical centers. Misdiagnosis not only delays appropriate treatment but may also result in unnecessary or harmful interventions, deterioration of trust between patients and healthcare providers, and wasteful use of medical resources. Furthermore, the economic burden associated with treatment is a critical concern. Orphan drugs developed for rare diseases tend to be extremely expensive, with an average annual treatment cost in the United States reaching approximately USD 219,000 (*4*).

Thus, the field of rare and intractable diseases faces substantial unresolved challenges in diagnosis, treatment, and financial support. In recent years, artificial intelligence (AI) technologies have attracted increasing attention as promising tools to address these challenges. Applications of AI in this domain have demonstrated utility in diagnostic support, patient screening, drug discovery, and literature analysis using natural language processing. This article highlights notable AI-based studies on rare and intractable diseases, reflects on the challenges of implementing AI models and the critical role of data platforms, and offers insights into future

developments in the field.

2. Applications of AI in rare and intractable diseases

2.1. Diagnostic support

Various AI-driven approaches have been explored to support the diagnosis of rare and intractable diseases. In recent years, notable advancements have been reported in fields such as genomic data analysis and AI-based medical imaging.

In the domain of genomic diagnosis, De La Vega *et al*. developed an AI-powered whole genome interpretation system aimed at improving the efficiency of genetic diagnoses in patients with rare diseases (*5*). This system integrates genomic variant data with clinical phenotypes to automatically rank candidate disease-causing genes. Validation using data from 119 patients demonstrated that in 92% of cases, the correct gene was ranked within the top two candidates. Moreover, for cases involving structural variants, the system successfully identified the causative gene as the top-ranked candidate in 17 out of 20 cases. However, the method has primarily been validated on known diagnostic cases, and its applicability to novel gene discovery and unresolved cases requires further investigation. Additionally, as the AI-generated outputs necessitate expert validation, the role of AI in diagnosis is best situated within a human-in-the-loop framework, emphasizing collaboration with clinicians.

In the field of medical imaging, AI has also been employed to support the diagnosis of rare diseases. A prominent example is DeepGestalt, a deep learning model developed by Gurovich *et al*. that analyzes facial photographs to suggest potential genetic syndromes. Trained on more than 17,000 facial images, the model can recognize over 200 syndromes (*6*). Clinical evaluations have reported a top-10 accuracy of 91%, indicating strong potential as a diagnostic support tool. In a study involving 25 patients with KBG syndrome, the correct diagnosis was included within the top five suggestions in 80% of cases (*7*). These findings highlight the utility of image-based AI in objectively capturing subtle morphological features characteristic of rare syndromes.

2.2. Prediction of therapeutic options

Currently, only approximately 5% of rare and intractable diseases have established and effective treatment options. To bridge this gap, increasing attention has been paid to drug repurposing — extending the indications of existing drugs — and the application of AI technologies to novel drug design.

One notable example is the development of TxGNN, a graph neural network-based AI model (*8*). This model successfully identified treatment candidates for over 17,000 diseases, including rare and ultra-rare conditions. TxGNN is the first AI model capable of predicting therapeutic indications for untreated diseases through zero-shot learning. Compared to conventional approaches, it demonstrated approximately 50% higher accuracy in candidate identification. By learning from comprehensive biomedical knowledge graphs, TxGNN systematizes what was previously a process reliant on serendipity or clinical experience, thereby facilitating the rapid identification of potential treatments for rare diseases. However, the therapeutic effectiveness of candidate drugs proposed by TxGNN requires experimental validation, and concerns remain regarding the completeness of the underlying knowledge graphs and potential data biases. Despite these limitations, this study represents a landmark example of AI's potential in the rare disease domain.

Additional studies have also explored AI-driven discovery of therapeutic candidates using large-scale biological data and knowledge bases. For instance, Cong *et al*. proposed a two-step machine learning approach that first clusters diseases based on gene expression patterns and then evaluates drugs that can reverse abnormal expression profiles (*9*). Using this method, 22 drug candidates were identified, including the HDAC inhibitor vorinostat. These agents showed potential therapeutic effects for rare inflammatory myopathies such as inclusion body myositis, polymyositis, and dermatomyositis.

These studies demonstrate how deep learning and knowledge graph analysis can uncover latent associations between diseases and drugs, offering opportunities for drug repurposing that may be overlooked by traditional methods. Nonetheless, challenges persist, including the high cost of experimental validation and the inherent data scarcity associated with rare diseases, which may limit model accuracy. Moving forward, a key focus will be on developing efficient strategies to prioritize and validate AI-generated therapeutic predictions.

2.3. Drug discovery

AI technologies are also increasingly being applied to drug discovery for rare and intractable diseases. Traditionally, drug development has been a time- and cost-intensive process, spanning from target identification to lead compound design and clinical evaluation. However, recent advances in generative AI models have begun to significantly accelerate these steps. A prominent example is the AI-driven drug discovery platform PandaOmics, which successfully identified a novel therapeutic target for idiopathic pulmonary fibrosis and designed a small-molecule compound targeting it within just a few years. This candidate has already progressed to a Phase IIa clinical trial, marking the first case in which both the disease target and the compound were discovered and designed entirely by AI and

subsequently advanced to human trials.

These developments suggest that generative AI can make identification of previously unknown targets feasible and enable rapid design of novel therapeutic candidates, even for diseases previously considered untreatable. Nevertheless, compounds proposed by AI must still undergo rigorous validation to confirm their efficacy and safety through conventional clinical trials. Therefore, while AI has potential to streamline the drug discovery pipeline, cautious and thorough evaluation remains essential. Despite these limitations, such pioneering examples highlight the transformative potential of AI in reshaping drug discovery strategies for rare and intractable diseases. Integration of AI into various stages of drug development is expected to become increasingly impactful in the years ahead.

2.4. AI applications in clinical trial support and patient recruitment

In the context of rare and intractable diseases, recruiting eligible participants for clinical trials poses a significant challenge due to the limited number of potential candidates and their wide geographical distribution. Traditionally, patient enrollment has been a time-consuming and labor-intensive process, often leading to delays or even discontinuation of trials. To address this issue, recent advances have focused on leveraging AI to improve the efficiency of patient identification and eligibility screening. In addition to structured data from electronic health records (EHRs), AI techniques such as natural language processing and machine learning have shown promise in extracting relevant information from unstructured clinical narratives and assessing trial eligibility.

For example, a study involving breast cancer patients demonstrated that an AI system could evaluate trial eligibility using EHR data with an accuracy of approximately 87.6% (*10*). Such AI-supported systems can automate the otherwise complex and manual process of matching patients to trial criteria, thereby reducing human errors and oversight. Moreover, these technologies can accelerate patient recruitment and reduce the time required to initiate trials. With continued advancements in EHRs standardization and AI model refinement, efficient execution of clinical trials for rare diseases is becoming increasingly feasible and practical.

3. Challenges and future perspectives in application of AI

Application of AI to rare and intractable diseases has produced promising advances in a variety of areas, including diagnosis, treatment, drug discovery, and clinical trial support. AI, with its capabilities in computational processing and information integration, has shown great potential in domains where conventional medical technologies and research approaches face limitations. However, practical implementation in clinical settings and broader societal adoption are hindered by a range of technical, ethical, and regulatory challenges. To ensure effective and sustainable AI deployment, it is essential to systematically address these issues and establish a clear vision for future development. This section outlines the major obstacles currently facing AI applications and discusses strategies for overcoming them, along with prospects for future advancement.

3.1. Overcoming data scarcity and learning bias

The performance of AI models is highly dependent on the quality and quantity of training data. In the field of rare diseases, the intrinsic "rarity" of each condition presents a major obstacle — available case numbers are extremely limited. This scarcity makes it difficult to compile large, high-quality datasets required for effective machine learning, increasing the risk of reproducibility bias, where model performance is skewed due to imbalanced training data in terms of disease type, ethnicity, or age group.

Additionally, structural inconsistencies across institutions — such as variations in EHR formats and discrepancies in diagnostic terminology — further complicate data integration. Under such constraints, models are prone to overfitting and may lack sufficient generalizability, compromising their reliability in real-world clinical applications. To mitigate these issues, strategies such as transfer learning, data augmentation, and the use of simulated data are increasingly employed. Synthesized datasets that emulate characteristics of rare diseases, as well as transfer learning from similar conditions using large existing databases, may help build robust models even in data-constrained environments.

3.2. Model transparency and clinical accountability

Clarifying the rationale behind AI-generated predictions and diagnoses is essential for clinical applications. Particularly when AI is used to assist in medical decision-making, a lack of transparency in the model's reasoning process can undermine clinicians' trust and reliability of their judgments. This challenge is especially prominent in deep learning-based systems, often referred to as "black-box" models.

To address this, the field has seen increasing interest in explainable AI. Techniques such as SHapley Additive exPlanations (*11*) and Local Interpretable Model-Agnostic Explanations (*12*) can provide visual explanations of feature contributions and justify predictions by comparing similar cases. Moreover, attention mechanisms and graph-based architectures with inherent interpretability have been proposed, which can further support integration into clinical workflows. These advances are expected to facilitate human-in-the-loop

collaboration by enabling physicians to better understand and verify AI-driven insights.

3.3. Future directions and perspectives

To enable AI to function effectively in the realm of rare and intractable diseases, a multifaceted approach is necessary — not only technological innovation but also supportive policy frameworks and social infrastructure. Among the most critical priorities is establishment of international data-sharing platforms. Given the inherently limited case numbers for rare diseases, data collection at a single institution or within a single country is insufficient. Instead, large-scale data integration through multi-institutional and multinational collaborations, using standardized data formats, is essential. These platforms are not merely repositories; they serve as information hubs connecting researchers, clinicians, and patients, and play a vital role in enhancing both diversity and statistical validity of data used for AI model development.

Furthermore, platforms capable of integrating multidimensional data — such as clinical records, genomic profiles, imaging data, and lifestyle information — are essential for building high-accuracy, generalizable AI models. In the future, such platforms are expected to serve as implementation infrastructures for AI-based diagnostic and treatment support systems in actual clinical practice. In addition, platforms designed to continuously collect real-world data will enable ongoing model refinement and feedback learning, contributing to sustainable performance improvement and overall healthcare quality. Thus, development and operationalization of such platforms will be a cornerstone in long-term evolution of AI-enabled precision medicine.

In conclusion, rare disease research inherently faces critical limitations — such as disease heterogeneity, extremely limited patient populations, and diagnostic complexity — that conventional medical technologies and research approaches often struggle to address. In this context, AI holds great promise due to its strengths in information integration and knowledge analysis. Application of AI to diverse data types — such as genomic, imaging, and natural language data — has led to tangible progress in improving diagnostic accuracy, identifying new therapeutic candidates, and enhancing patient recruitment in clinical trials. However, successful implementation of AI in real-world clinical practice still faces significant hurdles. These include data scarcity and bias, the need for model interpretability, and establishment of appropriate regulatory and ethical frameworks. Addressing these issues will require not only technological innovation but also structural efforts such as international collaboration and development of inclusive, patient-centered data infrastructures. In particular, construction and operation of international data-sharing platforms will be key to ensuring the reliability and generalizability of AI models in the rare disease domain. Such platforms, by enabling standardized collection and analysis of multidimensional data and supporting cyclical feedback between model development and real-world deployment, are expected to significantly accelerate practical adoption and long-term advancement of AI-driven medical support systems.

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Review

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Frailty in older adults: A systematic review of risk factors and early intervention pathways

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SUMMARY: Frailty is an independent risk factor linked to a higher likelihood of various diseases. With limited healthcare resources worldwide — especially in developing countries — the factors that contribute to frailty need to be understood across different populations and a universal model needs to be developed. This could help reduce the burden on healthcare systems and lessen the negative health effects of frailty. This review aims to summarize current evidence on the key factors influencing frailty and its impact on disease outcomes in different countries. The goal is to facilitate the development of strategies that can help prevent or even reverse frailty. Studies were included if they examined physical frailty using validated assessment tools in older adults and explored how various factors affect its development and progression. A comprehensive search of the PubMed database was conducted from March 1 to March 31, 2024 using the keywords "vulnerability" and "influencing factors." Studies published between January 1, 2001, and March 31, 2025 were considered. A total of 1,614 articles were initially identified, with 50 studies ultimately meeting the predefined inclusion and exclusion criteria. The findings indicate that frailty is influenced by a wide range of interrelated risk and protective factors, which in turn have various effects on different disease outcomes. These interconnected factors highlight both the complexity and the potential for targeted intervention. The review provides a comprehensive understanding of the factors associated with frailty in older adults across diverse settings and underscores the urgent need to develop a robust, evidence-based frailty model to facilitate the early identification, prevention, and possibly reversal of frailty.

Keywords: frailty, influencing factor, older adults, disease, frailty model

1. Introduction

An aging population is a common phenomenon experienced by every country around the world (*1*). China has over 3.623 billion people age 65 and above, making it the country with the largest aging population in the world. Similarly, Japan has an aging rate as high as 29.1, the highest in the world (*2*). This will pose challenges to healthcare in various nations and even lead to significant difficulties in international healthcare planning.

As the population ages, the problem of frailty of the elderly has gradually come into the public's view (*3*). Prior to 2001, positing of a phenotypical operational definition of frailty by Fried *et al*. (*4*) resulted in considerable progress in understanding and exploring the pathophysiology of frailty. They defined frailty as the display of three or more of five physiological deficits (muscle weakness, low gait speed, unintentional weight loss, exhaustion, and low physical activity), and their work has attracted the attention of academic researchers focused on frailty. Frailty is a geriatric syndrome defined as the gradual reduction in functional reserve and resilience, as well as impaired adaptive capacity across multiple physiological systems, that increases the vulnerability against stressors and leads to deterioration and adverse health outcomes in the elderly (*5*), such as falls (*6*), depression (*7*), delirium (*8*), hospitalization (*9*), and even death (*10*). The demands for healthcare from frail elderly individuals continue to increase, and this will pose a tremendous burden in terms of healthcare costs (*11*).

Given that older adults are frail and the condition coexists with other age-related diseases, clinical diagnosis and screening in primary care settings is critical (*12*). Globally, an array of assessment methods

has emerged to facilitate routine screening for frailty. Nevertheless, a universally recognized "gold standard" for frailty assessment remains elusive. The Fried Frailty Phenotype (FP), the FRAIL scale, and the Edmonton Frailty Scale (EFS) are among the tools used most widely (*13*). A systematic review reported on the prevalence of and factors influencing frailty in older patients with diabetes in China (*14*), and most studies have used FP and the FRAIL scale for screening. The global prevalence of hypertension among community-dwelling older adults in a study by Liu *et al.* also supports this view (*15*). However, due to the different geographical regions and the diversity of screening tools used, reaching a definitive conclusion about which tools are best is difficult.

With the increasing global focus on healthy aging, a growing group of researchers are considering frailty to be a potentially reversible condition that can be alleviated with various types of interventions. Thus, identifying factors influencing frailty could contribute to the implementation of interventions aimed at preventing or reversing frailty to reduce physical impairments and adverse health outcomes in the elderly. Many studies have reported risk factors associated with frailty. In general, in addition to age being recognized as the strongest factor related to frailty, there are still other risk factors associated with frailty that frequently appear in the literature on global aging populations, such as being female, unmarried, lack of exercise, and low income (*16-19*). Recently, an increasing number of studies have focused on psychological issues and identified psychological factors associated with frailty, such as depression and anxiety (*20,21*). These findings may provide a more comprehensive perspective for further improving measures related to the health management of older adults.

Most reviews limit their scope to specific regions or diseases when evaluating factors influencing frailty, which may lead to an overestimation or underestimation of these factors' impact on frailty. The aim of the current review is to address this gap. This paper not only systematically reviews factors associated with frailty but also summarizes frailty as an independent risk factor for various diseases. By objectively understanding the factors influencing frailty among older adults worldwide and exploring the interconnections between these factors, we can develop a universal frailty model that provides valuable guidance for healthcare professionals in preventing and managing frailty. This review aims to systematically identify and synthesize the key factors influencing frailty among older adults and to propose evidence-based pathways for early detection and intervention.

2. Research design and literature search strategy

2.1. Inclusion criteria and exclusion criteria

The inclusion criteria included: *i*) Study population: the definition of older persons may vary in different countries and regions but is usually based on age and related characteristics. This study was conducted in the Japanese context, so the median age for older adults was ≥ 65 years; *ii*) study content: assessment tools for frailty must be explicitly mentioned in the literature; *iii*) outcome indicators: prevalence of frailty and influencing factors; and *iv*) study type: retrospective, observational, prospective, cross-sectional, and longitudinal studies, with the language limited to English.

The exclusion criteria include: *i*) Only the abstract was published or the full text was not available; ii) physical frailty was not reported; and *iii*) duplicate publications.

2.2. Literature search strategy

The PubMed database was searched from March 1, 2025 to March 31, 2025. All literature published from January 1, 2001 to March 31, 2025 was included, with the language limited to English. Keywords ("vulnerability") and ("influencing factors") were used to conduct the search. Publications were identified among the literature that met the criteria. The publication selection process is shown in Figure 1.

2.3. Literature screening and data extraction

Data were extracted from each paper onto formatted spreadsheets in Excel files, including the first author and year of publication, country, type of study, sample size, age (mean or median and range), region of investigation, prevalence data, the frailty assessment tool used, and influencing factors. The second author subsequently checked for completeness again. Any disagreements were discussed until reaching a consensus.

3. Key findings based on a literature analysis

3.1. Factors influencing frailty were classified by country

A total of 1,614 publications were initially identified. After removing duplicates, 1,611 publications remained, the titles and abstracts of which were read and screened. Subsequently, the full text of 336 publications was screened. Of these, 50 papers were selected that met the eligibility criteria. Figure 1 is a flow diagram showing this process and detailing the reasons for exclusion. Of the publications included, data were collected in China (n = 15), the United States (n = 4), Japan (n = 4), and Spain (n = 4). The majority, 25 cross-sectional studies, were included in this review; 24 of the publications examined inpatients, 22 examined older adults in the community, and 4 examined outpatients.

Table 1 and Table 2 provide a summary of all included publications.

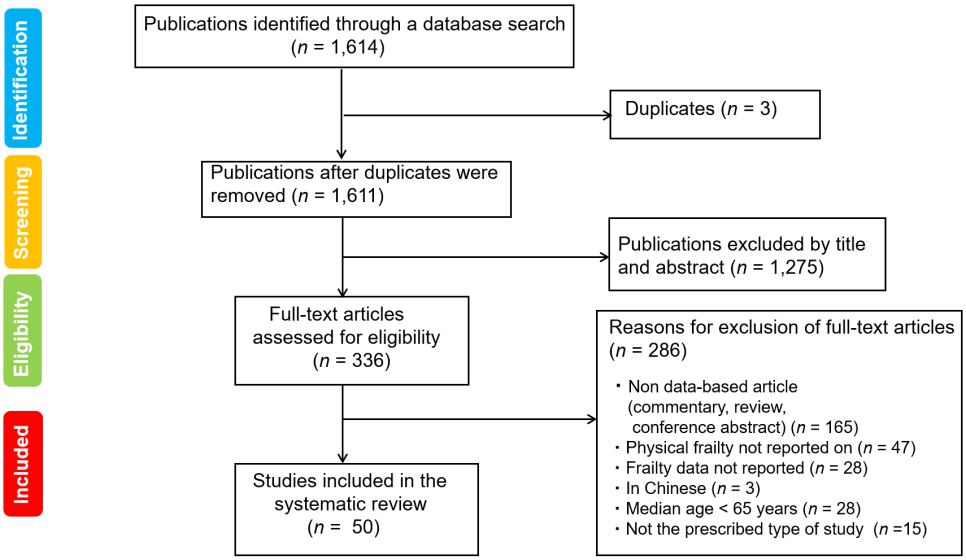

Figure 1. Flow diagram for identifying studies.

3.2. Factors influencing frailty were classified by frailty screening tool

The included studies involved a total of 11 frailty screening tools, with the FP, Frailty Index (FI), and FRAIL Scale being used most frequently. The development and refinement of instruments to assess frailty have been pivotal to advancing both frailty research and clinical use. These tools offer valuable insights into frailty from multiple perspectives, expanding from physical and psychological health to encompass functional and social domains. Despite these advances, however, the heterogeneity among frailty tools remains a significant challenge. Variations in their conceptual frameworks, scoring thresholds, and target populations contribute to inconsistencies in frailty prevalence estimates and in the strength of associations with adverse outcomes.

3.3. Factors influencing frailty were classified by type

A total of 29 studies on factors for frailty were included, and the factors associated with frailty varied across publications. This review identified 34 influencing factors. Thus, a vast number of factors affect frailty and these factors relate to our lives. Moreover, the relationships among the influencing factors are intricate and interwoven. Here, the factors influencing frailty are roughly divided into four categories for discussion (Table 1):

i) Physiological factors: age (*22-26*), sex (*17,19,20,22*), BMI (*27*), marital status (*20,28,29*), nutritional status (*25,30*), grip (*31*), muscle strength (*32*), oral health (*33*), dysphagia (*34*), disability (*16*), a history of falls (*16*), comorbidities (*25,29,35*), polypharmacy (*26*), abnormal excretion status (*24*), prealbumin (*24*), cortisol level (*36*), thyroid function (*37*), blood Gal-3 level (*38*), CRP (*19,31*), hemoglobin level (*39*), and ferritin (*26*).

ii) Psychological factors: depression (*40*), cognitive function (*16,22*), and anxiety (*20*).

iii) Socioeconomic factors: education level (*17,21,23*), economic status (*16,17,29*), and social services (*41*).

iv) Factors related to living conditions: living alone (*42,43*), smoking status (*44*), alcohol intake (*31*), exercise status (*20,23,29*), sleep status (*31,44*), activities of daily living (ADL) (*17,21*), and diet (*45,46*).

3.3.1. Physiological factors

Among the physiological factors, age was the most frequently reported, with 10 studies identifying a significant association with frailty. Next were sex and comorbidities, as show in Figure 2.

Age is a factor that was frequently found to be associated with the level of frailty and changes (*22-26*), although the direction of the association varied by publication. Wei *et al.* (*24*) indicated that the extent of frailty differed by age group in older adults; frailty often developed at the age 86 to 90 years, though other studies reported the opposite effect. Norazman *et al.* (*19*) reported that the frailty rate in the 60-69 age group is higher than that in the age 70 and older age group, and Kim *et al.* (*22*) found that as age increases, the rate of frailty also increases. This suggests that advanced age is a risk factor for frailty in older adults. However, more research is needed to explore the trajectory of age's impact on frailty.

Another factor frequently associated with frailty is sex (*17,19,20,22*). Different studies have reported that women are more likely to be frail than men. However, a

Table 1. Frailty status and risk influencing factors in various countries

Country	Lead Author, (Year) (*Ref.*)	Type of study	Participants	Prevalence of physical frailty	Frailty Screening tools	Influencing Factors
Mexico	Sánchez-García (2014) (*17*)	Cross-sectional, longitudinal	*n* = 1,933 Median age (SD) = 70.14 (7.15) years (Older group) Community-dwelling	Frail rate =15.7%	FP	1); 2); 3); 4); 8); 13); 15); 16); 17); 18); 19); 25)
USA	Peterson (2018) (*31*)	Longitudinal	*n* = 104 Median age (SD)= 68.9 (6.7) years (Older group) Community-dwelling	Frail rate =14%	FP	6); 10); 12); 32)
UK	Ramsay (2018) (*33*)	Cross-sectional and longitudinal	*n* = 1,622 Median age (SD) = 80.5 (5.4) years (Frail) Community-dwelling	Frail rate =19%	FP	21)
	Parsons (2019) (*45*)	Prospective	*n* = 1,660 Median age (SD)= 80.5 (5.4) years (Frail) Community-dwelling	Frail rate =17.1%	FP	14)
France	Steinmeyer (2020) (*39*)	Cross-sectional	*n* = 1,829 Median age (SD) = 82.44 (6.5) years Inpatients	Frail rate =48.82%	FP	33)
Germany	Henning (2023) (*30*)	Cross-sectional	*n* = 1,271 Median age (SD)= 75.64 (5.95) years Community-dwelling	Frail rate =14.8%	FI	5)
New Zealand	Teh (2021) (*42*)	Longitudinal	*n* = 459 Median age (SD) = 85.4 (1.8) years Community-dwelling	Frail rate =16%	FP	8)
Italy	Trevisan (2016) (*28*)	Observational	*n* = 1,887 Median age (SD) = 74.2 (7.0) years Community-dwelling	Frail rate =21.9%	Fried criteria	4)
	Delli Zotti (2022) (*21*)	Cross-sectional	*n* = 1,250 Median age (IQR) = 72 years (69-77 years) Inpatients	Frail rate =27.7%	FP	13); 15); 18)

SD, standard deviation; IQR, interquartile range; FP, Fried frailty phenotype; FI, frailty index; CHS, Cardiovascular Health Study; TFI, Tilburg frailty indicator. 1) age; 2) sex; 3) body mass index (BMI); 4) marital status; 5) nutritional status; 6) grip; 7) muscle strength; 8) living alone; 9) smoking status; 10) alcohol intake; 11) exercise status; 12) sleep status; 13) activities of daily living; 14) diet; 15) education level; 16) economic status; 17) social services; 18) depression; 19) cognitive function; 20) anxiety; 21) oral health; 22) dysphagia; 23) disability; 24) a history of falls; 25) comorbidities; 26) polypharmacy; 27) abnormal excretion status; 28) prealbumin; 29) cortisol level; 30) thyroid function; 31) blood galectin-3 (Gal-3) level; 32) C-reactive protein; 33) hemoglobin level; 34) ferritin.

Table 1. Frailty status and risk influencing factors in various countries (continued)

Country	Lead Author, (Year) (*Ref.*)	Type of study	Participants	Prevalence of physical frailty	Frailty Screening tools	Influencing Factors
	Damanti (2024) (*27*)	Cross-sectional, observational	*n* = 320 Median age (IQR) = 72 years (69-77 years) Community-dwelling	Frail rate =21%.	FI	3)
Romania	Maștaleru (2020) (*40*)	Retrospective	*n* = 411 Median age (SD) = 75.85 (6.35) years Inpatients	Frail rate =73.23%	FP	18)
Japan	Nishida (2021) (*34*)	Cross-sectional	*n* = 320 Median age (SD) = 77.3 (6.6) years Community-dwelling	Frail rate =14.1%	CHS	22)
China	Yang (2017) (*16*)	Cross-sectional	*n* = 1,494 Median age (SD) = 75.52 (9.28) years Inpatients	Frail rate =18%	FP	1); 3); 16); 18); 19); 23); 24); 26)
	Wei (2018) (*24*)	Cross-sectional	*n* = 587 Median age (SD) = 79.8 (9.34) years Inpatients	Frail rate =33.3%	Fried questionnaire	1); 7); 27); 28)
	Wang (2021) (*46*)	Cross-sectional	*n* = 780 Median age (SD) = 68.85 (2.64) years Community-dwelling	Frail rate =3.85%	FP	14)
	Lv (2022) (*23*)	Prospective	*n* = 3,836 Median age (SD) = 73.78 (6.451) years Inpatients	Frail rate =27.2%	FRAIL Scale	1); 3); 11); 15); 25); 26)
	Guan (2022) (*37*)	Cross-sectional	*n* = 487 Median age (SD)= 86 (2.9) years Community-dwelling	Frail rate =22.5%	FRAIL Scale	30)
	Fang (2022) (*41*)	Cross-sectional	*n* = 543 Median age (SD)= 70.99 (8.26) years Community-dwelling	Frail rate =24.9%	FRAIL Scale	17)

SD, standard deviation; IQR, interquartile range; FP, Fried frailty phenotype; FI, frailty index; CHS, Cardiovascular Health Study; TFI, Tilburg frailty indicator. 1) age; 2) sex; 3) body mass index (BMI); 4) marital status; 5) nutritional status; 6) grip; 7) muscle strength; 8) living alone; 9) smoking status; 10) alcohol intake; 11) exercise status; 12) sleep status; 13) activities of daily living; 14) diet; 15) education level; 16) economic status; 17) social services; 18) depression; 19) cognitive function; 20) anxiety; 21) oral health; 22) dysphagia; 23) disability; 24) a history of falls; 25) comorbidities; 26) polypharmacy; 27) abnormal excretion status; 28) prealbumin; 29) cortisol level; 30) thyroid function; 31) blood galectin-3 (Gal-3) level; 32) C-reactive protein; 33) hemoglobin level; 34) ferritin.

Table 1. Frailty status and risk influencing factors in various countries (continued)

Country	Lead Author, (Year) (*Ref.*)	Type of study	Participants	Prevalence of physical frailty	Frailty Screening tools	Influencing Factors
	Li (2023) (*44*)	Cross-sectional	*n* = 3,758 Median age (SD)= 68.81 (6.26) years Community-dwelling	Frail rate =2.37%.	FRAIL Scale	5); 9); 12)
	Ji (2023) (*38*)	Cross-sectional	*n* = 149 Median age (SD) = 72.04 (7.05) years Community-dwelling	Frail rate =21.48%	FP	31)
	Ma (2024) (*26*)	Cross-sectional	*n* = 110 Median age (SD) = 72.46 (10.43) years Community-dwelling	Frail rate =48.1%	FP	1); 2); 34)
	Guo (2024) (*20*)	Cross-sectional	*n* = 195 Median age (SD) = 71.52 (7.59) years Community-dwelling	Frail rate =85.13%	TFI	1); 2); 4); 11); 20); 25)
	Wei (2021) (*29*)	Cross-sectional	*n* = 391 Median age (IQR) = 73.3 years (65-91) Inpatients	Frail rate =28.4%	FRAIL Scale	1); 4); 11); 13); 16); 18); 25)
South Korea	Kim (2022) (*22*)	Prospective	*n* = 1,374 Median age (SD) = 75.9 (3.85) years Community-dwelling	Frail rate =10.9%	FRAIL Scale	1); 2); 18); 19); 26)
	Han (2022) (*43*)	Cross-sectional	*n* = 10,041 Median age (SD) = 66.91 (5.59) years Community-dwelling	Frail rate =6.0%	FRAIL Scale	8)
Malaysia	Norazman (2020) (*35*)	Cross-sectional	*n* = 301 Median age (SD) = 66.91 (5.59) years Community-dwelling	Frail rate =14.6%	FP	4); 7); 25)
	Norazman (2020) (*19*)	Cross-sectional	*n* = 301 Median age (SD) = 67.08 (5.536) years Community-dwelling	Frail rate =15.9%	FP	1); 2); 5); 7); 16); 32)

SD, standard deviation; IQR, interquartile range; FP, Fried frailty phenotype; FI, frailty index; CHS, Cardiovascular Health Study; TFI, Tilburg frailty indicator. 1) age; 2) sex; 3) body mass index (BMI); 4) marital status; 5) nutritional status; 6) grip; 7) muscle strength; 8) living alone; 9) smoking status; 10) alcohol intake; 11) exercise status; 12) sleep status; 13) activities of daily living; 14) diet; 15) education level; 16) economic status; 17) social services; 18) depression; 19) cognitive function; 20) anxiety; 21) oral health; 22) dysphagia; 23) disability; 24) a history of falls; 25) comorbidities; 26) polypharmacy; 27) abnormal excretion status; 28) prealbumin; 29) cortisol level; 30) thyroid function; 31) blood galectin-3 (Gal-3) level; 32) C-reactive protein; 33) hemoglobin level; 34) ferritin.

Table 1. Frailty status and risk influencing factors in various countries (continued)

Country	Lead Author, (Year) (*Ref.*)	Type of study	Participants	Prevalence of physical frailty	Frailty Screening tools	Influencing Factors
Turkey	Kocyigit (2024) (*25*)	Cross-sectional	*n* = 992 Median age (SD) = 73.2 ± 7.4 Outpatients	Frail rate =13.4%	FP	1); 5); 25)
Spain	Marcos-Pérez (2019) (*36*)	Cross-sectional	*n* = 252 Median age (SD) = 79.3 ± 8.8 Community-dwelling	Frail rate =34.9%	FP	29)
Brazil	Viana (2013) (*32*)	Cross-sectional	*n* = 53 Median age (SD) = 76.72 ± 5.89 Community-dwelling	Frail rate =15.1%	FP	7)

SD, standard deviation; IQR, interquartile range; FP, Fried frailty phenotype; FI, frailty index; CHS, Cardiovascular Health Study; TFI, Tilburg frailty indicator. 1) age; 2) sex; 3) body mass index (BMI); 4) marital status; 5) nutritional status; 6) grip; 7) muscle strength; 8) living alone; 9) smoking status; 10) alcohol intake; 11) exercise status; 12) sleep status; 13) activities of daily living; 14) diet; 15) education level; 16) economic status; 17) social services; 18) depression; 19) cognitive function; 20) anxiety; 21) oral health; 22) dysphagia; 23) disability; 24) a history of falls; 25) comorbidities; 26) polypharmacy; 27) abnormal excretion status; 28) prealbumin; 29) cortisol level; 30) thyroid function; 31) blood galectin-3 (Gal-3) level; 32) C-reactive protein; 33) hemoglobin level; 34) ferritin.

point worth noting is that studies (*19,22*) have found that the frailty rate among women is 2 to 3 times higher than that among men.

In addition, comorbidities are another factor related to frailty (*25,29,35*). A number of comorbidities can lead to frailty. A descriptive analysis by Wei *et al.* (*29*) indicated differences in the number and pattern of comorbid conditions between the frail group and the robust/prefrail group. The frail group had an average of 4.4 comorbidities, with the three most frequently reported comorbid conditions being hypertension, diabetes, and arthritis. In contrast, the robust/pre-frail groups had a mean of 3.3 comorbidities, and the most frequent were hypertension, hyperlipidemia, and arthritis. These findings indicate that proper prevention or management of comorbid conditions may delay the progression to frailty in this population.

3.3.2. Psychological factors

Depression and anxiety were factors influencing frailty (*20,40*). Both anxiety and depression share an overlapping symptomology, like functional impairment and sleep disturbance, leading to an increased risk of disability (*47*), and this may also be a consequence of increasing frailty. A previous study has indicated a clear bidirectional relationship between frailty and depression in older adults (*48*), but the association with anxiety is much less frequently explored. Anxiety was the only common factor influencing physical frailty, psychological frailty, and social frailty (*20*).

In the future, we should not only focus on the physical health of older adults but also pay greater attention to their mental well-being. Whether conducted in hospitals or communities, research has demonstrated an association between cognitive function and frailty (*16,22*). Yang *et al.* (*16*) and Sánchez-García *et al.* (*17*) further found that cognitive impairment is a risk factor for frailty. The correlation between influencing factors may even lead to depression. A review found that those with coexistent frailty and cognitive impairment had higher levels of depressive symptoms than peers (*49*). Further research is needed to explore potentially modifiable psychological factors, and this could lead to the development of supportive interventions.

3.3.3. Socioeconomic factors

Some studies found a risk effect of a low level of education on the rate of frailty (*17,23*), perhaps because people with a higher level of education usually have better living conditions, are more aware of their own healthcare, and pay more attention to disease prevention.

Similarly, economic status was found to affect frailty, the studies we included focused on aspects of employment and household income. Sánchez-García *et al.* (*17*) found that not having paid work is a protective

Table 2. Frailty as importance factor of various diseases

Various diseases	Authors, (Year) (*Ref.*)	Type of study	Participants and source of research subjects	Prevalence of physical frailty	Frailty Screening tools	Main findings
Colorectal Cancer (CRC)	Nina Ommundsen *et al*. (2014) (*52*)	Prospectively	*n* = 178 Median age (IQR) = 80 years (70-94 years) Hospital (In Norway)	Frail rate = 43%	GA	Frailty Is an Independent Predictor of Survival in Older Patients With Colorectal Cancer.
The liver transplant	Sinclair M *et al*. (2017) (*63*)	Prospectively	*n* = 587 Median age (IQR) = 60 years (53-64 years) Hospital (In USA)	Frail rate = 31.6%	FI	Physical frailty is a significant predictor of hospitalisation and total hospitalised days per year.
Myocardial infarction	Alonso Salinas GL *et al*. (2017) (*55*)	Prospectively	*n* = 234 Median age (SD) = 84.4 (5.8) years (frailty) Hospital (In Spain)	Frail rate = 40.2%	FI	Frailty was an independent predictor of the combination of death or nonfatal myocardial reinfarction, or major bleeding, and an independent predictor of readmission.
Kidney transplantation	Schopmeyer L *et al*. (2018) (*58*)	Prospectively	*n* = 139 Median age (SD) = 51.8 (14.5) years Hospital (In Netherlands)	Frail rate = 16.5%	GFI	Frailty and type of transplantation are independent factors associated with an increased risk of postoperative complications.
Chronic subdural hematoma	Shimizu K *et al*. (2018) (*62*)	Retrospective	*n* = 211 Median age (SD) = 83.5 (7.1) years Hospital (In Japan)	Frail rate = 50.2%	CFS	The evaluation of the presence of frailty on admission can be an important factor in the prediction of the prognosis of chronic subdural hematoma patients.
Elective laparoscopic cholecystectomy	Mastalerz K *et al*. (2018) (*69*)	Prospectively	*n* = 144 Median age (IQR) = 76 years (70-91 years) Hospital (In Poland)	Frail rate = 44.4%	CGA	Frailty was predictors of 1-year mortality.
Abdominal aortic aneurysm (AAA)	Morisaki K *et al*. (2019) (*59*)	Retrospective	*n* = 349 Median age (SD) = 79.1 (8.7) years (frailty) Hospital (In Japan)	Frail rate = 43.1%	FI	Assessing frailty in patients with AAA is useful for determining risk factors for 5-year overall survival and postoperative complications.
Glioblastoma in geriatric	Schneider M *et al*. (2020) (*70*)	Retrospective	*n* = 110 Median age (IQR) = 72 years (65-86 years) Hospital (In Germany)	Frail score = 0.18	FI	Frail patient as significant and independent predictors of 1-year mortality in geriatric patients with surgical treatment of glioblastoma.
Coronary artery bypass grafting	Kluszczyńska M *et al*. (2021) (*56*)	A cross-sectional	*n* = 180 Age (SD) = 69.3 (6.1) years Clinic (In Poland)	Frail rate = 28.6%	TFI	Frailty syndrome was a poor predictor of rehospitalization.

SD, standard deviation; IQR, interquartile range; FP, fried frailty phenotype; FI, frailty index; CHS, cardiovascular health study; TFI, tilburg frailty indicator; CFS, Clinical Frailty Scale; GA, geriatric assessment; GFI, Groningen Frailty Indicator; CGA, Comprehensive Geriatric Assessment; FSS, Frailty Scoring System; HFRS, Hospital Frailty Risk Score; G8, The G8 geriatric screening tool.

Table 2. Frailty as importance factor of various diseases (continued)

Various diseases	Authors, (Year) (*Ref.*)	Type of study	Participants and source of research subjects	Prevalence of physical frailty	Frailty Screening tools	Main findings
Asymptomatic aortic stenosis	Ramos M *et al*. (2021) (*71*)	Observational	*n* = 104 Age (SD) = 83.3 (8.8) years Clinic (In Spain)	Frail rate = 59.6%	FP	Frailty was independent factors for mortality, conferring an unfavorable short-term prognosis.
Hemodialysis (HD)	Li Y *et al*. (2021) (*67*)	Observational; prospectively	*n* = 150 Median age (IQR) = 69 years (64-75 years) Hospital (In China)	Frail rate = 34.7%	FP	Frailty was an independent indicator of all-cause mortality and emergency visits in elderly patients with HD.
Major hepatectomy forperihilar cholangiocarcinoma (PHCC)	Hosoda K *et al*. (2022) (*62*)	Prospectively	*n* = 87 Median age (IQR) = 72 years (59-88 years) (CFS score 3-9) Hospital (In Japan)	Frail rate = 50.6%	CFS	Frailty is a predictive factor for short- and long-term outcomes in patients who have undergone major hepatectomy for PHCC.
Surgery for Metastatic Spinal Column Tumors	Elsamadicy AA *et al*. (2022) (*64*)	Retrospective	*n* = 4,346 Median age (SD) = 66.8 (9.0) years Hospital (In USA)	Frail rate = 39.2%	HFRS	Intermediate frailty was found to be an independent predictor of unplanned 30-day readmission.
Advanced Non-Small Cell Lung Cancer	Jiménez Galán R *et al*. (2023) (*53*)	Retrospective	*n* = 101 Median age = 67 years Clinic (In Spain)	Frail rate = 31.7%	FSS	Frailty as an independent predictor of over all (OS) and progression-free survival (PFS).
Ovarian cancer (OC)	Anic K *et al*. (2023) (*65*)	Retrospective; Observational	*n* = 116 Median age (SD) = 70.9 (5.9) years Hospital (In USA)	Frail rate = 50.9%	G8	Preoperative frailty assessment with the G8 Score identified elderly women with OC recording a significantly higher rate of postoperative in-hospital complications.
Small Bowel Obstruction (SBO)	Laterza V *et al*. (2023) (*68*)	Prospectively	*n* = 424 Median age (IQR) = 85 years (82-89 years) Hospital (In Italy)	Frail rate = 33.9%	CFS	The presence of severe frailty could effectively predict an increased risk of in-hospital death.
Vascular cognitive impairment (VCI)	Zheng R *et al*. (2024) (*66*)	Retrospective	*n* = 431 Median age (SD) = 71.73 (6.41) years Hospital (In China)	Frail rate = 38.5%	FI	Frailty defined by the FI was effective for predicting the risk of mortality.
Hip fracture	Wang LX *et al*. (2024) (*57*)	Observational	*n* = 427 Median age (SD) = 80.28 (8.13) years Hospital (In China)	Frail rate = 30%	FI	Frailty serves as a reliable predictor of the probability of encountering severe adverse events while hospitalized for elderly individuals with hip fractures.

SD, standard deviation; IQR, interquartile range; FP, fried frailty phenotype; FI, frailty index; CHS, cardiovascular health study; TFI, tilburg frailty indicator; CFS, Clinical Frailty Scale; GA, geriatric assessment; GFI, Groningen Frailty Indicator; CGA, Comprehensive Geriatric Assessment; FSS, Frailty Scoring System; HFRS, Hospital Frailty Risk Score; G8, The G8 geriatric screening tool.

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Table 2. Frailty as importance factor of various diseases (continued)

Various diseases	Authors, (Year) (*Ref.*)	Type of study	Participants and source of research subjects	Prevalence of physical frailty	Frailty Screening tools	Main findings
Emergency laparotomy (EL)	Goh SSN *et al.* (2024) (*54*)	Retrospective	*n* = 233 Median age (SD) = 79 (*7*) years Hospital (In Singapore)	Frail rate = 26%	CFS	Frailty emerged as a pivotal factor influencing the postoperative trajectory of older adults undergoing EL in Singapore.
Gastric cancer	Miao X *et al.* (2024) (*61*)	A cross-sectional	*n* = 381 Median age (SD) = 69 (8.0) years Hospital (In China)	Frail rate = 70.3%	TFI	Early assessment to predict the occurrence of heterogeneous frailty trajectories is essential to improve the outcomes of elderly gastric cancer patients.

SD, standard deviation; IQR, interquartile range; FP, fried frailty phenotype; FI, frailty index;; CHS, cardiovascular health study; TFI, tilburg frailty indicator; CFS, Clinical Frailty Scale; GA, geriatric assessment; GFI, Groningen Frailty Indicator; CGA, Comprehensive Geriatric Assessment; FSS, Frailty Scoring System; HFRS, Hospital Frailty Risk Score; G8, The G8 geriatric screening tool.

factor for pre-frailty but increases the likelihood of frailty. Work status is usually related to education level, and especially in advanced age. Studies by researchers such as Yang *et al.* (*16*), Wei *et al.* (*24*), and Norazman *et al*. (*19*) have demonstrated that low income is a risk factor for vulnerability.

In addition, social services were suggested to affect changes in frailty. Findings from Fang *et al.* (*41*) suggested that the older adults who were unmarried, divorced, or widowed might perceive less social support. Sánchez-García *et al.* (*17*), however, found that use of healthcare services influenced the progression of frailty. Therefore, specific types of social services should be emphasized rather than all social services.

3.3.4. Factors related to living conditions

As shown Figure 2, living alone, exercise status, and ADL were among the factors related to living conditions. An equal number of studies indicated that all three are significantly associated with frailty. In the study by Song *et al*. (*43*), objective social isolation was a factor associated with worsening of the stages of frailty. That said, frailty can also lead to social isolation (*50*). Frailty and social isolation are interrelated, forming a cause-and-effect relationship, and may even jointly influence other factors. A study found that physical frailty and social isolation were associated with falls in older adults (*51*).

A study carried out in a Mexican community (*17*) concluded that there was an association between frailty and limitations on ADL in the older adults who were part of the sample. Results of the studies included in the current review are consistent with such an association, as the association is stronger in frail people compared to pre-frail people. Limitations on ADL further impact exercise. Exercise, without a doubt, is one of the factors influencing frailty. Lack of exercise is a risk factor for frailty (*23*); therefore, regular exercise can effectively prevent its onset. The optimal timing and type of exercise are topics worth contemplating, along with whether such exercise is suitable for various groups of people.

3.4. Classify the factors influencing frailty by disease type

Frailty plays an important role in various diseases and is also an independent predictor, especially in terms of prognosis, hospitalization, postoperative complications, and mortality rates, as shown Table 2. In this review, a large number of evidence-based studies found that frailty could be a predictive factor for adverse outcomes, including prognosis (*52-54*), hospitalization or rehospitalization (*55-57*), postoperative complications (*58*), and mortality rates (*59*), which means that screening for frailty is very important in a clinical setting.

The evaluation of the presence of frailty upon admission can be an important factor in predicting the

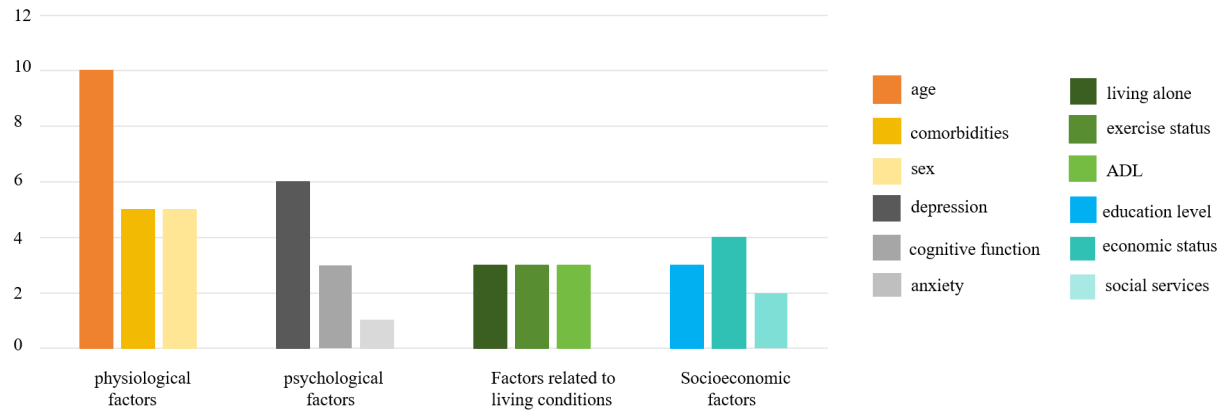

Figure 2. Top three frailty factors by category in included studies.

prognosis for patients with chronic subdural hematoma (*60*), gastric cancer (*61*), and those undergoing major hepatectomy for perihilar cholangiocarcinoma (PHCC) (*62*). Frailty was also an important predictor of hospitalization and readmissions for liver transplantation (*63*), after coronary artery bypass grafting (*56*), and metastatic surgery (*64*).

For postoperative patients, complications undoubtedly pose the greatest challenge. However, frailty is a risk factor that significantly increases the likelihood of postoperative complications, as demonstrated in studies by Schopmeyer *et al.* (*58*), Morisaki *et al.* (*59*), and Anic *et al.* (*65*). Therefore, screening for and intervening in cases of frailty in advance is essential, as this can help to further reduce the threat that postoperative complications pose to our lives.

Frailty as a predictor of mortality has widely been noted in different populations: patients with vascular cognitive impairment (*66*), patients on hemodialysis (*67*), patients with small bowel obstruction (*68*), patients undergoing elective laparoscopic cholecystectomy (*69*), geriatric patients with glioblastoma (*70*), and patients with asymptomatic aortic stenosis (*71*). Thus, independent of study design, country, and setting, frailty could be a prognostic factor for clinicians to predict mortality and frailty screening could help clinicians establish a comprehensive prognostic tool for predicting mortality in patients with various diseases and facilitate early intervention to alleviate frailty syndrome to reduce mortality rates.

Surprisingly, frailty is not only a predictor of adverse outcomes but also a significant factor in triggering cancer. Park *et al.* (*72*) established that an aged immune system promotes tumor growth, regardless of the age of the tumor or its surrounding stroma. Specifically, hematopoietic aging drives emergency myelopoiesis, and targeting IL-1R1 signaling during early tumor development to attenuate this process abrogates the protumorigenic effect of aging on tumor control. This shows that frailty can lead to changes in our bodies and even cause unforeseeable harm to our lives. This finding should prompt us to pay closer attention to the presence of frailty and encourage deeper reflection on its implications.

3.5. Multi-factor interaction and integration model

In recent years, research on frailty has shifted from examining isolated risk factors to investigating the complex interplay among multiple determinants. Increasingly, frailty is recognized not as the outcome of a single pathological process but as the dynamic result of interacting physiological, psychological, social, and lifestyle factors. Although traditional linear models are useful in identifying statistically significant predictors, they often fail to capture synergistic or antagonistic relationships between variables. To address this limitation, and as shown in Figure 2, we developed a model that better reflects the multifactorial and often non-linear nature of frailty.

As shown in Figure 4, by synthesizing findings from the literature and translating them into a practical tool for early detection and intervention, we offer a framework that enables healthcare providers and older adults to identify personalized pathways based on individual circumstances. Ultimately, these approaches may contribute to a more nuanced understanding of frailty and facilitate the development of personalized, multidisciplinary management strategies for older populations.

4. Discussion

4.1. Toward integrated models of frailty

An increasing body of evidence from studies conducted between 2001 and 2025 demonstrates a clear shift in frailty research from single-variable analyses to multifactorial and integrative modeling approaches. These models aim to capture the complex, non-linear, and often bidirectional relationships among determinants of frailty. As shown in Figure 2, frailty is

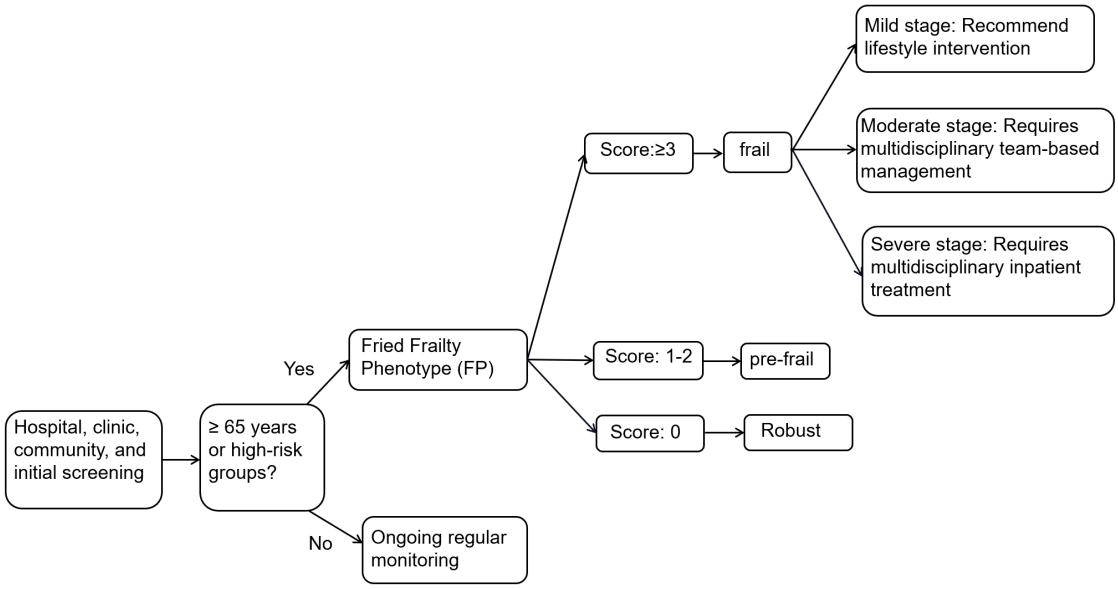

Figure 4. Model of early monitoring and intervention pathways for frailty.

a multifactorial condition influenced by physiological, psychological, behavioral, and social determinants. Notably, some studies have developed comprehensive models to elucidate how different domains interact to influence frailty progression and adverse outcomes. For example, the World Health Organization (WHO) recently published guidelines on the implementation of a new Integrated Care for Older People (ICOPE) framework, which emphasizes the integration of intrinsic capacity — including cognition, mobility, and sensory function — with environmental factors such as living conditions and access to care, forming a practical, person-centered conceptual structure (*73*). Machine learning (ML) has further enriched the understanding of frailty as a multifactorial construct. Studies using ML have proved useful in identifying individuals who became frail over time. One such study highlighted factors that may be useful in the early detection of frailty (*74*).

Importantly, there is a growing consensus on the utility of multidimensional, integrative models in both research and clinical settings. These models offer a more accurate and personalized understanding of frailty development and its implications for disease risk, care planning, and healthcare policy. Despite the promising insights yielded by interactive modeling, several challenges remain. Issues such as data heterogeneity, inconsistent variable definitions, and limited external validation hinder the generalizability of findings.

However, as shown in Figure 3, our model places greater emphasis on early screening using tools like the FP, which may be more feasible for implementation in primary care settings with limited resources. Our approach advocates for a tiered screening-to-intervention model that prioritizes early detection and scalable intervention — consistent with the emerging evidence supporting the reversibility of frailty in its initial stages. Future research should continue to integrate longitudinal datasets, real-time monitoring technologies, and advanced analytics to further refine these models.

Ultimately, such models will contribute to a more nuanced understanding of frailty and facilitate the development of personalized, multidimensional management strategies for older adults. Moreover, it can be more effectively translated into clinical and community-based screening and intervention strategies, facilitating earlier detection and management of frailty. This is particularly important given the current paucity of research in Asian populations. To address the global challenge posed by an aging population and the escalating burden of frailty on public healthcare systems, future studies should prioritize the development of a comprehensive, culturally adaptable, and globally applicable frailty model. Such a model would facilitate more equitable and effective healthcare responses across diverse regions.

4.2. The limitations of and gaps in the research

Only literature in English was included in this review, and there may be a language bias. Current studies have established a relatively consistent understanding of the determinants of frailty; however, the conclusions regarding its impact vary across different studies. Despite these mixed results, our overall findings help to elucidate the factors influencing frailty and highlight the disparity in how it affects separate groups of individuals in different ways.

In particular physiological factors seem to provide some insight into how an individual's frailty will progress over time, a finding which has important implications

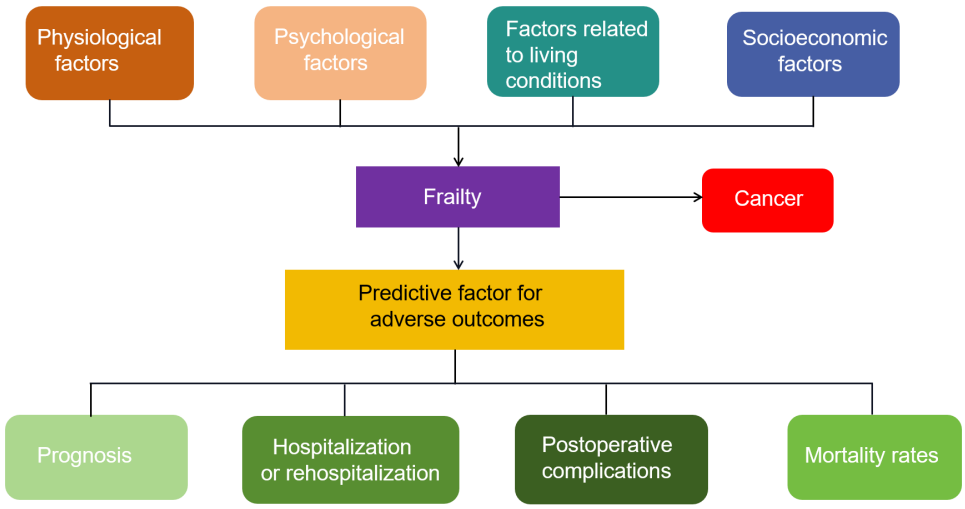

Figure 3. A multifactorial and non-linear model of frailty.

for public health policy as well as individuals and their caregivers. A major issue is the shift from viewing frailty as a static condition to understanding it as a dynamic and systemic process.

Future research should focus on developing a comprehensive yet easily interpretable frailty intervention model. A crucial first step toward achieving consistency may be the validation of a gold-standard frailty measurement tool, enabling more accurate and comparable research findings. Most studies are cross-sectional, hampering our ability to infer causal relationships. Researchers should also emphasize longitudinal studies to explore risk factors associated with frailty trajectories, as these insights may shape future strategies for frailty prevention and intervention.

4.3. Policy recommendations and future research directions

With the global acceleration of population aging, frailty — an integrated syndrome involving physiological, psychological, and social dimensions — has become a major public health issue affecting the quality of life and health outcomes of older adults. Current policies addressing frailty are mostly confined to the medical domain, lacking cross-sector collaboration and long-term integrated strategies. Based on a multilayered understanding of the factors influencing frailty, comprehensive health promotion policies should be devised. These should incorporate health management, chronic disease control, psychological support, nutritional interventions, and social participation to build an interdisciplinary and cross-sectoral frailty intervention system. Specifically, policies should promote the inclusion of frailty assessment and management in contracted primary care and encourage community healthcare centers to establish regular physical function screening, cognitive assessments, and mechanisms of monitoring nutritional status. At the same time, efforts should be made to enhance the tie between home-based and institutional care, to optimize the design of long-term care insurance systems, and to improve the quality of health management for patients with chronic conditions, and especially those with multimorbidity. In addition, social participation and promotion of mental health should be emphasized. Local governments and civil society organizations should be encouraged to enhance social connections among older adults through volunteer services, learning programs, and mutual support groups to reduce loneliness and depression. Moreover, the government should strengthen the digital infrastructure and promote the integration of frailty-related data on big data platforms to enable early detection and intervention. These measures could reduce the incidence of frailty, delay functional decline in older adults, and alleviate the socioeconomic burden.

Although existing studies have preliminarily identified several related factors — such as malnutrition, chronic inflammation, cognitive impairment, and insufficient physical activity — our understanding of the interrelationships, causal mechanisms, and dynamic progression of these factors remains limited. Future research should focus on the following areas: First, enhancing longitudinal cohort studies to explore the key pathways in the onset and progression of frailty using long-term follow-up data, and to identify reversible or modifiable nodes for intervention. Second, ML, multi-omics analysis, and systems modeling should be used to construct multifactorial models to predict frailty, enabling risk stratification and individualized assessment. Third, greater attention should be paid to specific populations, such as the very old, those living alone, and older adults in rural or ethnic minority areas, to uncover the unique risks and protective factors related to frailty. Fourth, interventional studies should promote a shift from single-dimension approaches to integrated, multidimensional

interventions, investigating the synergistic effects of diet, exercise, pharmacological strategies, and psychological strategies, and their scalability and cost-effectiveness should be validated through real-world research. Fifth, interdisciplinary collaboration should be encouraged by integrating perspectives from biomedicine, behavioral science, sociology, and healthcare economics to advance the concept of "precision frailty management." Such research will provide a scientific foundation for the early identification of and precise intervention in frailty, contributing to the development of a prevention-oriented active aging strategy.

5. Conclusion

This review has found that there are many factors affecting frailty among the elderly worldwide, the most notable of which are physiological factors. When dealing with the elderly in hospitals, clinics, and communities, healthcare professionals should enhance the monitoring of physical, psychological, and social factors and implement effective interventions to reduce the incidence of frailty to some extent. Currently, research on frailty in the elderly mainly focuses on investigation of current conditions. The hope is that future studies will conduct quality research to verify methods that can better reduce frailty. Future research should also pay attention to the potential reversibility of frailty in its early stages, and healthcare policies should encourage the routine use of validated screening tools, such as the FP, in primary care and community health settings. Early identification enables timely, targeted interventions that may slow or even reverse the progression of frailty, thereby reducing long-term healthcare burdens.

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Review

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Traditional Chinese medicine for intractable and rare diseases: Research progress and future strategies

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SUMMARY: Rare diseases have become a global public health challenge due to their low prevalence, difficult diagnosis, and limited treatment options. Intractable diseases are more common but often involve complex mechanisms, treatment with limited efficacy, and high medical costs, placing a heavy burden on patients and healthcare systems. In recent years, traditional Chinese medicine (TCM) has demonstrated unique advantages in the treatment of intractable and rare diseases and has gradually become an important complementary treatment. The current work is a systematic review of the progress of clinical and experimental research on TCM in typical rare diseases such as amyotrophic lateral sclerosis (ALS), systemic lupus erythematosus (SLE), mitochondrial encephalomyopathy, aplastic anemia (AA), and Wilson's disease (WD). It focuses on the multi-target therapeutic mechanisms of key Chinese herbal compound formulas, including immune regulation, antioxidative stress, and neuroprotection. The core TCM theories of "syndrome differentiation", "different treatments for the same disease" and the "same treatment for different diseases" are also discussed in the context of personalized medicine. In recent years, China has continuously promoted the development of TCM through a series of national plans and supportive policies, such as the 14th Five-Year Plan for TCM development, funding for key special projects, expedited approval pathways, and expanded coverage by medical insurance. These efforts have provided strong support for the clinical translation of TCM and technological innovation in the field of intractable and rare diseases. Notwithstanding the encouraging advances, the field of Chinese medicine continues to grapple with numerous challenges. In the future, the enhancement of mechanistic studies and quality multicenter clinical trials needs to be promoted while further enhancing policy support and international collaboration to substantiate the scientific basis and clinical value of TCM in the prevention and treatment of intractable and rare diseases.

Keywords: traditional Chinese medicine (TCM), intractable diseases, rare diseases, herbal compound formulas, multitarget mechanisms

1. Introduction

Rare diseases are an emerging public health priority, which are umbrella terms for diseases with very low prevalence. To date, between 6,000 and 8,000 distinct rare diseases have been identified, approximately 80% of which are of genetic origin and 50-75% of which manifest in childhood (*1,2*). There is no universal definition of rare diseases and their prevalence varies in different regions of the world. The European Union Orphan Drug Regulation defines a rare disease as a condition affecting < 50 per 100,000 of the European population (*3*), the American Orphan Drug Act defines a rare disease as a condition affecting < 200,000 people in the U.S. (*4*), and the most recent definition of a rare disease in China, published in 2021, defines it as a condition with an incidence of less than 1 in 10,000 live births, a prevalence of less than 1 in 10,000 individuals, or fewer than 140,000 people affected nationwide (*5*). In China, rare diseases affect approximately 20 million people, with over 200,000 new cases reported annually (*6,7*). In 2010, the Shandong Rare Disease Prevention and Control Association was established, becoming the first provincial-level academic organization for rare diseases in China. With the increasing national attention to rare diseases, the project for "China Rare Disease Prevention Research and Demonstration" (No. 2013BAI07B00) under the National Science and Technology Support Program of the 12th Five-Year Plan (*8*) and the project for a "Clinical Cohort Study of Rare Diseases" (No. 2016YFC0901500) under the 13th Five-Year Plan (*9*) have been launched. These

initiatives aim to promote the diagnosis and treatment of rare diseases and the development of orphan drugs. On October 24, 2018, the China Alliance for Rare Diseases was established in Beijing and built the China Rare Diseases Comprehensive Cloud Service Platform, which has registered about 780,000 cases of rare diseases as of November 2023, covering 31 provinces, municipalities, autonomous regions, and 502 hospitals across the country (*10*).

In addition to rare diseases, many conditions with a relatively high prevalence but complex diagnosis and poor treatment outcomes are collectively referred to in traditional Chinese medicine (TCM) as "intractable and rare diseases". These conditions pose similar challenges in clinical practice and are managed through pattern differentiation. China is the birthplace of TCM, and its extensive clinical use has established TCM's significant role in the field of herbal medicine. In recent years, the market share of Chinese herbal medicine has reached as high as 32.4% of the pharmaceutical market in China (*11*). In contrast, the number of traditional medicines produced account for about 2.2% of total pharmaceuticals production in Japan (*12*). The Chinese Government places great importance on the development of TCM and has introduced a series of supportive policies. The "14th Five-Year Plan for the Development of TCM" (*13*) and the "Implementation Plan for Major Projects in the Revitalization and Development of TCM" (*14*) have provided strong support for the industry's growth. Notably, the 14th Five-Year Plan emphasizes enhancing specialty disciplines in TCM and enhancing the capacity to diagnose and treat major and complex diseases. Moreover, Chinese medicine has long gained experience in diagnosing and treating rare and complex diseases, forming a systematic and mode of "Pattern Identification and Treatment". It offers unique advantages in managing multi-system damage and chronic progressive conditions. Recent research has increasingly highlighted its potential value in rare disease treatment, and especially through mechanisms such as immune regulation, modulation of antioxidative stress, and neuroprotection.

The aims of this review are to systematize the progress of research on Chinese medicine in the treatment of several typical intractable and rare diseases and to explore the prospects and challenges of its use to treat rare diseases by combining modern medical mechanisms and traditional theories.

2. Research on TCM in rare diseases and analysis of compound TCM Formulations

TCM has been increasingly explored in the treatment of various rare diseases. The following section highlights representative case studies, focusing on the clinical use of and animal experiments on selected TCM formulas and their components as well as the underlying pharmacological mechanisms from a modern perspective (Table 1).

2.1. Amyotrophic lateral sclerosis (ALS)

ALS is a rare and fatal neurodegenerative disease that primarily affects the upper and lower motor neurons in the motor cortex, brainstem, and spinal cord. It leads to progressive, painless muscle weakness, and patients typically die from respiratory failure within 3 to 5 years (*15*). At present, commonly used western medicines such as riluzole and edaravone have limited efficacy in slowing disease progression. Riluzole requires long-term administration and imposes a significant financial burden, while edaravone is an intravenous preparation that is inconvenient to use and that demonstrates suboptimal therapeutic efficacy.

In contrast, TCM has unique advantages in the treatment of ALS. It can effectively alleviate clinical symptoms, delay disease progression, enhance quality of life, and is associated with fewer obvious adverse effects (*16*). Huoling Shengji Decoction, developed based on TCM syndrome differentiation, has demonstrated promising therapeutic potential in both clinical and experimental studies. A randomized controlled trial (RCT) confirmed that Huoling Shengji Decoction has a positive therapeutic effect on patients with ALS (*17*). In animal experiments, this formula has also been shown to prolong the survival time of SOD1-G93A transgenic mice, protect motor neurons, and suppress neuroinflammation (*18*). The therapeutic effects of Huoling Shengji Decoction are believed to arise from the synergistic interaction of its herbal constituents, which collectively modulate oxidative stress, immune responses, and neuronal apoptosis. *Astragalus membranaceus* significantly enhances the scavenging capacity of nitric oxide free radicals, reduces NO-induced cytotoxicity, and improves the recovery rate of leukocyte levels (*19*). Astragalus polysaccharides, a key extract of *Astragalus membranaceus*, also possess strong antioxidant properties (*20*). *Epimedium brevicornum* has neuroprotective effects by modulating the expression of apoptosis-related factors and neurotrophic factors (*21*). Notably, the cannabis plant is also believed to have therapeutic potential. Medicinal uses of cannabis were documented as early as in Qianjin Yaofang and Bencao Gangmu. Modern studies have further shown that Δ^9-tetrahydrocannabinol, the active component of cannabis, has neuroprotective effects. In 2004, Raman *et al*. reported that Delta(9)-tetrahydrocannabinol could delay the onset of motor dysfunction and prolong survival in an ALS mouse model (*22*). Additionally, the endogenous cannabinoid system has been shown to have neuroprotective effects in SOD1-G93A transgenic mice through the activation of CB1 and CB2 receptors (*23*).

2.2. Systemic lupus erythematosus (SLE)

Table 1. Case studies and analysis of compound Chinese herbal formulas

System category	Disease	Study type	TCM compound	Main herbal medicine or key ingredients	Effects	*Ref.*
Nervous system	Amyotrophic lateral sclerosis	RCT	Huoling Shengji Decoction	*Astragalus membranaceus, Epimedium brevicornum*	Neuroprotection, regulation of apoptosis and trophic factors	(*17-21*)
		Animal study	Cannabis	Delta (9)-tetrahydrocannabinol	Neuroprotection, alleviation of excitotoxicity	(*22*)
Immune-related	Systemic lupus erythematosus	RCT	Shenqi Dihuang Decoction	*Astragalus membranaceus, Rehmannia glutinosa*	Immunomodulation, anti-inflammatory effects	(*28-30*)
		Animal study	Arsenic trioxide	Arsenic trioxide	Immunosuppression, anti-inflammatory effects	(*33*)
		Clinical study	Kunxian Capsule	Tripterygium wilfordii polyglycoside	Anti-inflammatory and immunosuppressive effects	(*34-35*)
Metabolic-related	Mitochondrial encephalomyopathy	Retrospective study	Xinnaoxin Capsule	Salidroside, *Lycium barbarum* polysaccharides	Antioxidation; improvement of mitochondrial function	(*39-41*)
Hematologic system	Aplastic anemia	RCT	Huangqi Injection	*Astragalus membranaceus*	Hematopoietic support, immune enhancement	(*49*),(*51*)
		Animal study	Danggui Buxue Decoction	Angelica polysaccharide	Replenishment of blood, immune regulation	(*50-52*)
		Clinical multi-center study	Pai-Neng-Da Capsule	*Astragalus membranaceus,* panaxadiol saponins	Hematopoietic support, modulation of immune function	(*53-55*)
Digestive system	Wilson's disease	RCT	Gandou Decoction	Curcumin, *Rhubarb* root, Berberine, Coptisine	Antioxidative effects, copper detoxification	(*62-66*)
		Animal study	Gandouling	Berberine, Coptisine, Aloe-emodin, Catechin	Anti-inflammatory and antioxidative effects	(*65-71*)

SLE is a diffuse connective tissue disease characterized by autoimmune inflammation. Its clinical manifestations are diverse and complex, primarily marked by the presence of multiple autoantibodies in serum and the involvement of multiple organs and systems (*24*). SLE significantly reduces patients' quality of life and imposes a substantial public health and economic burden (*25*). The mortality rate and risk of irreversible organ damage are considerably higher than those of the general population. Patients frequently require long-term medication; however, the use of conventional drugs is limited by adverse effects such as infections, metabolic disturbances, osteoporosis, and retinopathy (*26*).

TCM has a long history in the treatment of SLE and is considered one of the advantageous approaches in the field of rheumatology. A systematic review and meta-analysis of 14 RCTs with a total of 1,002 participants evaluated the efficacy and safety of combining Shenqi Dihuang Decoction with conventional Western medicine in the treatment of lupus nephritis (*27*). Results indicated that compared to Western medicine alone, the combination therapy significantly improved clinical efficacy, vascular endothelial growth factor levels, complement C3 levels, the erythrocyte sedimentation rate, and SLE Disease Activity Index scores. *Astragalus membranaceus* has demonstrated immunomodulatory and anti-inflammatory effects. One study found that astragalus improved pregnancy outcomes and alleviated renal pathological damage in a murine model of SLE by suppressing Th17 cell differentiation and reducing the expression of IL-17A and RORγt (*28*). Extracts of *Rehmannia glutinosa* have been shown to reduce the expression of inflammatory cytokines such as IL-2, IFN-γ, IL-6, and IL-10, thereby mitigating immune-mediated tissue damage associated with SLE (*29*). A clinical trial involving 52 patients with SLE showed that benefits in the treatment group, which received standard therapy combined with *Rehmannia glutinosa* and *Astragalus membranaceus*, included a reduction in the glucocorticoid dosage, lowering of 24-hour urinary protein levels, and alleviation of adverse effects such as insomnia, hot flashes, spontaneous sweating, and obesity (*30*). The cytokine IFN-γ plays a key role in the pathogenesis of SLE (*31,32*). Arsenic trioxide (As_2O_3), a TCM, has immunomodulatory effects in both MRL/lpr mice and human SLE patients by downregulating IFN-γ gene expression *via* epigenetic mechanisms (*33*). In addition, Kunxian Capsule , a novel TCM formula listed as a "Key Scientific and Technological Achievement" during China's 9th Five-Year Plan, has been used clinically to treat lupus nephritis for over ten years, and it may alleviate renal damage and T-cell infiltration in lupus nephritis by blocking the JAK1-STAT1 signaling pathway (*34*). Tripterygium wilfordii polyglycoside, a major component of Kunxian Capsule, has therapeutic effects on SLE by inhibiting the IL-17 signaling pathway and suppressing Th17 cell differentiation, thereby modulating immune responses and reducing disease activity (*35*).

2.3. Mitochondrial encephalomyopathy

Mitochondrial encephalomyopathy is a multisystem disorder caused by mitochondrial dysfunction due to mutations in mitochondrial DNA or nuclear DNA (*36*). Clinically, it is classified into four major subtypes: mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes, myoclonic epilepsy with ragged red fibers, Kearns-Sayre syndrome, and mitochondrial neurogastrointestinal encephalomyopathy (*37*). The main clinical manifestations include hearing loss, ptosis, optic atrophy, and exercise intolerance (*38*). Currently, treatment strategies focus on supportive care, including improvement of energy metabolism, antioxidant therapy, free radical scavenging, and mitochondrial support. However, the overall efficacy of these treatments remains limited.

In recent years, several studies of TCM have suggested potential benefits in the treatment of mitochondrial encephalomyopathy. Xinnaoxin Capsule, as a foundational formula, combined with conventional "cocktail" therapy, has shown clinical potential in alleviating neurological symptoms and exercise tolerance, significantly enhancing treatment efficacy and long-term prognosis (*39*). These effects may be attributed to the neuroprotective properties of its key herbal components, such as salidroside and *Lycium barbarum* polysaccharides. Experimental studies have demonstrated that salidroside can have neuroprotective effects by downregulating complement component C3 expression (*40*). *Lycium barbarum* polysaccharides have shown neuroprotective effects in Parkinson's disease models, possibly by improving mitochondrial function (*41*). Nevertheless, systematic studies on TCM treatment for mitochondrial encephalomyopathy remain limited, with current evidence largely derived from case reports or small-scale clinical observations (*42,43*).

2.4. Aplastic anemia (AA)

AA is a bone marrow failure syndrome caused by multiple etiologies and is characterized primarily by hematopoietic stem cell damage, fatty degeneration of the bone marrow, and peripheral pancytopenia (*44*). In Western countries, treatment mainly relies on immunosuppressive agents and hematopoietic stem cell transplantation, while androgens are used only as adjunctive therapy and are rarely used (*45,46*). In China, however, a combined therapeutic strategy involving immunosuppressive agents, androgens, and kidney-tonifying TCM is often used in clinical practice (*47,48*).

In recent years, multiple domestic clinical trials have reported on the efficacy of a Huangqi Injection (prepared from the single herb *Astragalus membranaceus*)

combined with androgens in the treatment of AA. A meta-analysis showed that the combination therapy group had superior improvement in hematopoietic function and peripheral blood cell counts compared to the androgen monotherapy group, with an overall response rate approximately 50% higher than that of the control group (*49*). In addition to injectable formulations, oral compound Chinese herbal prescriptions are also widely used in the treatment of AA. For instance, Danggui Buxue Decoction (consisting of *Angelica sinensis* and *Astragalus membranaceus*) is frequently used in clinical practice. Animal studies have shown that Modified Danggui Buxue Tang can ameliorate immune-mediated AA models by modulating T cell differentiation and inhibiting the Jak/Stat signaling pathway (*50*). As the core component of both formulas, *Astragalus* is considered to be an immunoenhancing biological response modifier that promotes hematopoiesis by increasing the CD4/CD8 ratio and reducing negative regulatory factors such as IL-2 and TNF-α (*51*). The active constituent extracted from *Angelica sinensis* - Angelica polysaccharide - has also displayed significant hematopoietic effects in experimental studies, possibly by regulating the Treg/Th17 cell balance and inhibiting mitochondrial apoptosis in bone marrow cells (*52*).

Moreover, a multicenter clinical study demonstrated that the efficacy of Pai-Neng-Da Capsule combined with cyclosporine and androgen was 88.1%, which was significantly higher than that in the control group (77.8%) (*53*). The primary constituent of Pai-Neng-Da-Capsule is panaxadiol saponins, which have been demonstrated to possess both the capacity to replenish blood and to regulate immunity (*54,55*).

2.5. Wilson's disease (WD)

WD is an autosomal recessive genetic disorder caused by mutations in the ATP7B gene, leading to dysfunctional copper metabolism and subsequent accumulation of Cu^{2+} in the liver, brain, cornea, and other organs. This accumulation results in liver damage, neurological symptoms, and multi-organ dysfunction (*56*). The liver is the earliest organ affected, and hepatic fibrosis can occur in the early stages of the disease. Therefore, early intervention is crucial to prevent the progression to cirrhosis and hepatic failure (*57*). Currently, copper-chelating agents and liver transplantation are the main therapeutic approaches (*58*). However, Western medications such as D-penicillamine, trientine, zinc preparations, and dimercaptopropanol, though capable of achieving a negative copper balance, are often associated with serious adverse effects including nephrotoxicity, dermatologic reactions, bone marrow suppression, and AA, which affect long-term treatment adherence (*59*).

TCM has been widely used to treat WD (*60*), with Gandou Decoction and Gandouling being the most studied. These medicines show promise as adjuvant therapies. A RCT indicated that Gandou Decoction combined with conventional copper-chelating therapy significantly improved dysfunctional balance in patients and their TCM syndrome scores, and the combination markedly increased 24-hour urinary copper excretion (*61*). In copper-loaded rat models, aqueous extracts of Gandou Decoction were shown to reduce serum ALT levels and alleviate histological liver damage by modulating oxidative stress and the Wnt/β -catenin signaling pathway (*60*). One of its active components is curcumin, which can partially restore the expression of mutated ATP7B protein and promote functional copper excretion (*60*). *Rheum palmatum* root and its active compounds possess antioxidant, antifibrotic, and anti-inflammatory properties (*62-64*). The active components of *Coptis chinensis*, berberine and coptisine, exhibit antifibrotic effects (*65,66*).

In the study of antifibrotic mechanisms, Gandouling has demonstrated action through multiple signaling pathways. It has significant anti-WD effects *via* anti-inflammatory and antioxidant activities (*67,68*). It also blocks the Wnt-1/β -catenin signaling pathway by binding to Wnt-1, thereby inhibiting hepatic stellate cell (HSC) activation and alleviating hepatic fibrosis in WD (*69*). Its effective constituents of berberine (*65*), coptisine (*66*), aloe-emodin (*70*), and catechin (*71*) have all shown antifibrotic effects.

3. Theoretical basis and concepts of TCM in treating ALS

In recent years, TCM has been increasingly emphasized by the academic community as a complementary alternative therapy for intractable and rare diseases. TCM is supported by clinical and experimental evidence. Here, ALS is used as an example to describe the theoretical basis and concepts of TCM in treating intractable and rare diseases from 3 various aspects (Figure 1).

ALS is a progressive neurodegenerative disorder characterized by the degeneration of motor neurons. Currently, there is no curative treatment available. The clinical presentation at disease onset is often heterogeneous and may mimic other neurological conditions, frequently resulting in delayed diagnosis (*15*). To date, modern medicine has achieved only limited progress in ALS treatment. The U.S. Food and Drug Administration has approved four pharmacologic agents for ALS: Riluzole, Edaravone, Tofersen (Qalsody), and AMX0035 (Relyvrio) (*72-74*). However, Relyvrio was voluntarily withdrawn from the market in April 2024 following its failure to meet endpoints in a phase III clinical trial (*75*). Overall, these treatments have demonstrated only modest benefits, primarily in slowing disease progression for a few months in select patient populations.

3.1. Treatment based on syndrome differentiation

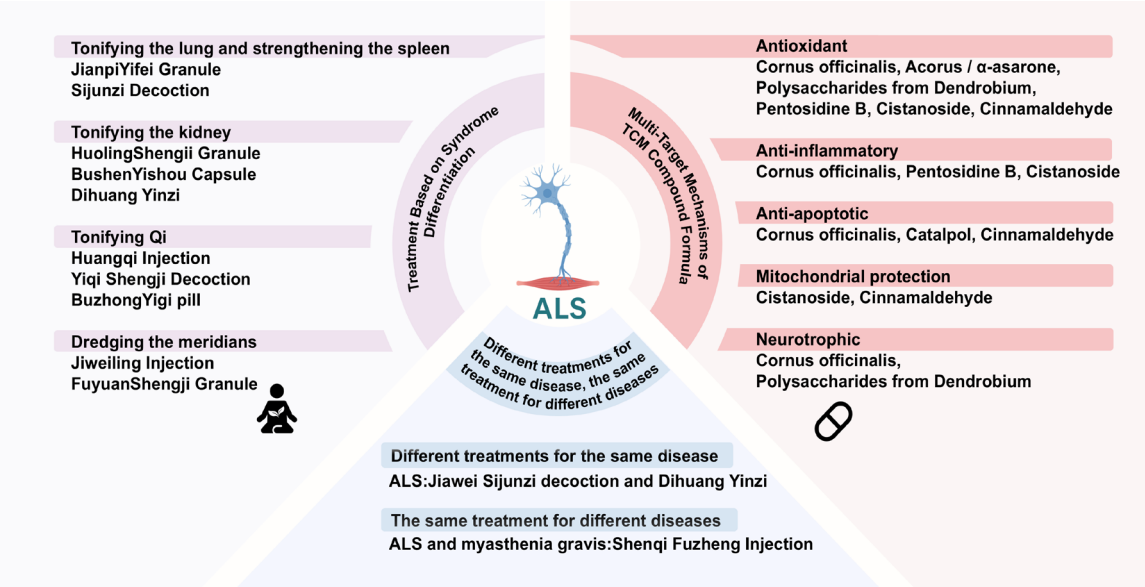

Figure 1. Theoretical basis and principles of TCM for ALS. TCM, traditional Chinese medicine; ALS, amyotrophic lateral sclerosis.

The London staging system offers a simple and practical method for assessing disease progression by categorizing ALS into five stages based on functional clinical milestones: Stage 1 is defined by the initial involvement of a single functional region, manifesting as weakness, muscle atrophy, spasticity, dysarthria, or dysphagia, functional regions are classified as bulbar, upper limb, lower limb, or diaphragmatic; Stage 2a denotes the formal diagnosis of ALS, while Stage 2b indicates the involvement of a second functional region; Stage 3 corresponds to the involvement of a third functional region; Stage 4a is characterized by the need for gastrostomy and Stage 4b by the initiation of non-invasive ventilation; and Stage 5 reflects the requirement for tracheal intubation, tracheostomy, or is defined by death (*76*). Interestingly, the disease trajectory reflected in the London staging system aligns with the TCM understanding of disease transmission and organ involvement, providing a potential framework for integrating TCM perspectives into the clinical management of ALS.

The primary syndrome differentiation-based therapeutic strategies for ALS in TCM include tonifying the kidney, strengthening the spleen and lung, tonifying "Qi," and dredging meridians. Kidney deficiency symptoms commonly observed in ALS often manifest in the lower limbs, such as lumbago, knee pain, and muscle atrophy. In a small clinical trial, use of Huoling Shengji Granule for 12 weeks resulted in comparable functional outcomes to riluzole but significantly improved TCM syndrome scores (*17*). Patients using Dihuang Yinzi also displayed significant improvement in bulbar paralysis and muscle fibrillations of the lower limbs (*77*). In ALS patients, progressive weakness of the diaphragm and other respiratory muscles leads to a decline in pulmonary ventilation function, thereby impairing gas exchange and resulting in hypoxemia and hypercapnia (*78*). Jianpi Yifei granules alleviated symptoms such as fasciculations, dysarthria, dysphagia, weak voice, and weak cough, particularly enhancing motor function in the upper limbs - an area where Riluzole fails to provide effective improvement (*79*). A clinical study indicated that patients treated with a combination of Riluzole and Jiawei Sijunzi Decoction displayed delayed disease progression, and especially in the sub-item evaluations of dyspnea and fatigue; the treatment group demonstrated superior outcomes compared to the Riluzole-only control group (*80*). Numerous studies have demonstrated a correlation between "Qi" and immune function (*81-83*). In ALS, abnormalities in the immune system are also thought to be associated with disease progression (*84,85*). A clinical experiment showed that Yiqi Shengji Decoction combined with acupuncture could alleviate symptoms of ALS in SOD1-G93A mice and slow disease progression (*86*). Buzhong Yiqi Tang is a traditional formula for tonifying "Qi." The formula was found to enhance locomotor activity, prolong survival, and have neuroprotective effects through anti-neuroinflammatory and antioxidant effects in an animal model of ALS (*87*). TCM theories contend that meridians are low hydraulic resistance channels that facilitate the transmission of various chemical substances and physical energies (*88,89*). Guided by the theories of extraordinary meridians and collateral disorders, a series of clinical studies led by Jinliang Chen's team demonstrated that Jiweiling injection has significant therapeutic effects. In a study involving 710 patients, the treatment group received a Jiweiling injection alone or in combination with oral Jiweiling capsules (*90*). The overall efficacy against neurodegenerative diseases such as ALS was as

high as 86.34%, which was significantly higher than that in the riluzole group. Jiweiling markedly alleviated the major clinical symptoms and signs of ALS.

3.2. Multi-target mechanisms of TCM compound formula

ALS is characterized by complex pathological mechanisms involving oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, autophagy impairment, and neuroinflammation (*91,92*). Conventional Western pharmacotherapy predominantly targets individual pathways, frequently resulting in suboptimal efficacy. Conversely, traditional Chinese herbal formulas, which are characterized by their composition of numerous bioactive components, have the capacity to induce multi-target and multi-pathway regulatory effects.

Dihuang Yinzi, a classic herbal formula consisting of 12 medicinal ingredients, is commonly prescribed in clinical practice to manage ALS. This formula exemplifies the multi-target therapeutic strategy of TCM. Cornus officinalis extract significantly reduces indicators of oxidative stress and attenuates neuronal cell damage: in a rat stress model, administration of cornus officinalis reduced the levels of reactive oxygen species (ROS), malondialdehyde, and pro-inflammatory cortisol, while up-regulating the expression of SOD, CAT, and BDNF in brain tissue and regulating the Bax/Bcl-2 ratio (*93*). Acorus and its active compound α-asarone protect hippocampal neurons by suppressing PERK-mediated endoplasmic reticulum stress and reducing ROS accumulation (*94*). Polysaccharides from Dendrobium exhibit potent antioxidant effects and promote neurotrophic factor expression (*95*). In microglial-neuronal co-cultures, pentosidine B significantly inhibited LPS-induced neuroinflammatory responses: decreased the release of inflammatory factors such as NO, TNF-α, IL-1β, and IL-6, and reduced ROS by inhibiting the TLR4/MyD88/NF-$_k$B pathway and NADPH oxidase activity (*96*). Cistanoside, a major active compound from Cistanche tubulosa, has potential therapeutic effects on neurodegenerative diseases, including ALS, through multiple mechanisms such as preserving mitochondrial function, scavenging ROS, and suppressing neuroinflammation (*97*). Catalpol is a core bioactive compound derived from Rehmannia glutinosa. Catalpol reduces neuronal apoptosis and promotes motor function recovery after spinal cord injury by inhibiting CHOP/GRP78-mediated endoplasmic reticulum stress and modulating the Caspase3/Bax/Bcl-2 pathway (*98*). Lv *et al*. found that cinnamaldehyde pretreatment effectively reduced oxidative damage, maintained mitochondrial membrane potential, reduced ROS generation and cytochrome c release, and inhibited the caspase-mediated apoptotic pathway by up-regulating Bcl-2 and down-regulating Bax (*99*).

3.3. Different treatments for the same disease and the same treatment for different diseases

TCM emphasizes the therapeutic principles of "different treatments for the same disease" and the "same treatment for different diseases". In treating ALS, TCM adopts distinct therapeutic approaches based on syndrome differentiation: patients with spleen "Qi" deficiency are treated with Jiawei Sijunzi decoction to tonify "Qi" and strengthen the spleen (*100*), while those with yin and yang deficiency receive Dihuang Yinzi (*77*). The use of different essential formulas for the same disease exemplifies the principle of personalized treatment based on pattern differentiation in TCM.

Conversely, Western-defined diseases such as ALS and myasthenia gravis may be classified under the same TCM syndrome pattern such as spleen-kidney yang deficiency, and thus can be treated with similar herbal formulas like Shenqi Fuzheng Injection (SFI). Animal experiments also further proved the correctness of the approach involving the Same Treatment for Different Diseases. SFI significantly delays disease onset, prolongs survival, and protects motor neurons in a mouse model of ALS by reducing oxidative stress and activating the Nrf2 antioxidant pathway (*86*). SFI also effectively reduces the severity and duration of transient worsening in myasthenia gravis patients receiving high-dose steroid therapy, with minimal adverse effects (*101*).

4. Research approaches and techniques

Currently, researchers are use multi-scale approaches and techniques to elucidate the mechanisms by which multi-component TCM formulas act on rare and intractable diseases (Table 2). Multi-omics studies of Huanglian Jiedu decoction have shown that it may ameliorate Alzheimer's disease-like pathology by modulating gut microbiota, lipid metabolism, and inflammatory pathways. Its mechanisms include suppressing gut dysbiosis and related Aβ deposition, alleviating neuroinflammation, and reversing cognitive impairment (*102*). Network pharmacology allows for the construction of "herb-active compound-target-disease" interaction networks. One example is research on *Astragalus membranaceus* in the treatment of lupus nephritis, which revealed approximately 200 shared key genes between the herb and the disease and which identified the PI3K/AKT/mTOR signaling pathway as the core mechanism (*103*). Animal experiments play an important role in validating mechanisms and evaluating the efficacy of TCM. Lycium barbarum polysaccharides demonstrated significant anti-inflammatory effects in a mouse model of Sjögren's syndrome by effectively reducing glandular inflammation (*104*). In addition, the clinical translation of TCM therapeutic strategies is steadily progressing. A typical example is an ongoing multicenter phase II clinical trial evaluating the efficacy

Table 2. Research pathways and technical approaches

Path/Technology	Content	Examples of use in TCM
Multi-omics	Multi-level biological networks regulated by TCM	Huanglian Jiedu decoction for the treatment of Alzheimer's disease
Network pharmacology	Compound-target-disease interaction network	Astragali radix for the treatment of lupus nephritis
Animal models	Rare diseases using disease-specific mouse models	Lycium barbarum polysaccharide for the treatment of primary Sjögren's syndrome
Multicenter clinical trial	Phase II clinical trial	Shenrong granules for the treatment of ALS

and safety of Shenrong granules (*105*) in the treatment of ALS, marking a significant step toward evidence-based validation of TCM interventions in the management of rare diseases.

In addition to these conventional methods, emerging tools such as computational modeling and phenomics are currently being introduced. Machine learning algorithms can be used to analyze the clustering of TCM components based on cellular phenotypes, thus revealing their multi-targeting mode of action (*106*). Transcriptome-based linkage profiling, which utilizes 102 TCM components for gene expression profiling, provides a phenotype-oriented research tool for mechanisms of the effects of TCM (*107*). Comprehensive research techniques from computational prediction and high-throughput screening to traditional component separation and systems biology modeling are gradually revealing the multi-targeted and networked effects of TCM in the treatment of rare and complex diseases.

5. Discussion

In 2018, China released its first Catalogue of Rare Diseases, covering 121 rare diseases, including neurodegenerative, metabolic, and hematologic disorders (*108*). TCM has gradually demonstrated clinical value and potential in the treatment of multiple rare diseases (*26,110-112*). Diseases listed in the catalogue, such as ALS, SLE, AA, and WD, have become key focus areas for TCM research and clinical interventions. TCM, based on the theoretical framework of syndrome differentiation and combined with modern pharmacological mechanisms, possesses advantages of multi-component, multi-target, and multi-pathway interventions. It has displayed a certain level of efficacy and safety in the treatment of these rare diseases.

In recent years, several Chinese herbal compound preparations have been approved for clinical use in rare diseases through "expanded indications" or the expedited "green channel" regulatory mechanism. Gandouling Capsule, used to treat WD, has been approved by the National Medical Products Administration; Kunxian Capsule, Tripterygium Glycoside Tablets, and Kunming Shanhaitang Tablets, commonly used in the treatment of SLE and rheumatoid arthritis, are also being gradually incorporated into clinical pathways for rare autoimmune diseases. These medications reflect the potential and translational value of TCM. The integrative potential of TCM is also being increasingly recognized. A cohort study in Taiwan involving 1,188 patients with SLE and chronic kidney disease found that those who used 17 types of Chinese herbal compound prescriptions had significantly lower rates of progression to renal failure and all-cause mortality compared to non-users (*112*). Another cross-sectional survey conducted in Shanghai indicated that over 90% of ALS patients had received TCM interventions (*113*).

China currently faces multiple challenges in the development of orphan drugs. A study reported that between 2013 and 2022, a total of 481 clinical trials related to rare diseases were conducted in China, which was significantly fewer than in the United States, Europe, and Japan during the same period (*114*). This gap is largely attributed to the absence of a comprehensive patient registry, clinical research infrastructure, and an inclusive framework for reimbursement of rare disease treatment. Moreover, many rare diseases, such as mitochondrial disorders, inherited metabolic conditions, and rare genetic dermatological diseases, remain unexplored territories in TCM research and require urgent scientific attention.

In contrast, the United States leads globally in the development of therapies for rare diseases such as ALS, supported by the FDA's Orphan Drug Designation program, the ClinicalTrials.gov registry, and a robust national patient database system (*4,115,116*). Japan classifies rare and intractable diseases under the umbrella term "Nanbyo" (intractable and rare diseases) and has established the Nanbyo Information Center and a national disease registry to facilitate multi-center clinical studies. The Ministry of Health, Labour and Welfare also supports the development of targeted therapies through the Database of Drug Development for Rare Diseases database and a rare disease biobank (*117,118*). The Japanese Government has made significant investments in healthcare expenditures for intractable diseases. According to the "Act on medical care for patients with intractable diseases". patients diagnosed with "designated intractable diseases" are typically required to cover only 10% of their medical expenses, substantially alleviating

their financial burden (*119*). Of the 165 rare disease drugs approved for marketing in China, approximately 70% have been included in the national medical insurance catalog as of February 2024. Seventeen of these drugs are fully reimbursed, while the remaining 95 drugs are partially reimbursed (*120*). Although reimbursement efforts have intensified in recent years, the long-term treatment costs remain relatively high, imposing a considerable financial burden on patients.

To enhance the international competitiveness of TCM in the treatment of rare diseases, China has continued to strengthen its collaboration with the World Health Organization (WHO) in the field of traditional medicine. In 2024, the Chinese government pledged USD 5 million to support the WHO Traditional, Complementary, and Integrative Medicine Programme, aimed at facilitating the implementation of the WHO Traditional Medicine Strategy 2025–2034 (*121*). In December of the same year, China hosted the World Traditional Medicine Conference in Beijing, where representatives from 85 countries adopted the "Beijing Declaration", calling for strengthened evidence-based research and international cooperation to integrate traditional medicine into global health systems (*122*).

In summary, TCM has begun to establish a foundation for clinical translation in selected rare diseases and, supported by national policies, international collaboration, and scientific practice, holds promise for further development. However, advance mechanistic studies need to be conducted and the number of high-level, randomized multi-center trials needs to be increased to enhance TCM's reputation and academic recognition globally in the treatment of rare and intractable diseases.

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Brief Report

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Evaluation of pharmacotherapy in sickle cell disease in an Afro-Colombian community: A cross-sectional analytical study in San Basilio de Palenque, Bolívar

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SUMMARY: Sickle cell disease (SCD) is an orphan and extremely rare condition in Colombia and worldwide. However, a significant number of cases were identified in San Basilio de Palenque, Bolívar, enabling a pharmacotherapeutic follow-up study. This population represents a genetic bottleneck with limited admixture, making it crucial for further genetic and clinical research. Despite being largely unexplored due to lack of awareness and state neglect, SCD persists in this community. This study aimed to characterize and follow up pharmacotherapeutically on patients with SCD and traits. An observational, cross-sectional analytical study was conducted in 20 patients, assessing sociodemographic factors, pharmacotherapeutic follow-up, and pharmaceutical interventions. Results showed that 75% of patients were female, and 40% were homozygous. The most commonly used medications included folic acid, analgesics (paracetamol, tramadol, naproxen, codeine, ibuprofen, morphine), L-glutamine, and enalapril. Pain from vaso-occlusive crises and hemolytic episodes was the main reason for analgesic use. Notably, 62% of homozygous patients were not receiving baseline treatment with hydroxycarbamide, increasing their risk of complications. Addressing this gap through pharmaceutical interventions was one of the study's key contributions. In conclusion, this research highlights the need for a multidisciplinary approach to optimize treatment and improve the quality of life of affected patients. Given its genetic significance, San Basilio de Palenque represents a unique setting for further studies on SCD.

Keywords: hemoglobinopathy, sickle cell disease, pharmacotherapeutic follow-up, mutation, pharmaceutical intervention

1. Introduction

Sickle cell disease (SCD) is an orphan and extremely rare condition worldwide that originated in Africa as a protective mechanism to counteract malaria (*1,2*). Recognized as a public health problem by the World Health Organization since 2008, worldwide there are 300,000 children with hemoglobinopathies where 83% present SCD. In Colombia, it affects 20,000 children each year, especially in regions such as Bolivar, Valle del Cauca and Atlántico (*2*). The migration of the population from Africa to northern Colombia brought with it the genetic inheritance of SCD, being San Basilio de Palenque the place where part of that population settled. Currently there are about 3,500 people, of which it has not been determined exactly how many present both the condition and the trait. This information should be managed by regulatory entities such as the district administrative department of health (DADIS). However, the lack of knowledge contributes to the fact that SCD continues to grow along with the population due to the lack of diagnostics and timely education in the community; through pharmaceutical care, The sociodemographic characterization of the population was initially carried out and subsequently the pharmacotherapeutic follow-up of patients was carried out through the DADER method, which allows to have a pharmaceutical report that includes the patient's history, prescribed medications, clinical information, therapeutic results and recommendations to the patient; this allows to follow up any patient, in any health care setting, in a systematized, continuous and documented manner (*3,4*).

Considering the above, this study aimed to characterize and conduct a pharmacotherapeutic follow-up of patients with SCD in the town of San Basilio de Palenque (Bolívar), given their unique genetic heritage. The significant presence of SCD in this rural Colombian population makes it a compelling subject for the

scientific community and local authorities. Additionally, this study sought to implement adherence strategies to optimize treatment and improve patients' quality of life.

2. Patients and Methods

The low prevalence of SCD, a rare disease, significantly limited the sample size and made patient selection difficult. To address this limitation, we opted for non-probabilistic convenience sampling in the corregimiento of San Basilio de Palenque. The inclusion criteria focused on patients diagnosed with SCD and sickle cell trait, while those who did not give informed consent or had other hemoglobinopathies were excluded. The research was divided into three stages.

2.1. Stage 1: Patient selection and sociodemographic characterization

The first stage was the sociodemographic characterization of the population of the corregimiento of San Basilio de Palenque. Once the diagnosis of hemoglobinopathy was confirmed, an informed consent form was issued, which allowed patients to participate or not in the study; in the case of pediatric patients, their parents or guardians were responsible.

Those who agreed to participate in the study were given a comprehensive survey. This survey included a range of questions, from basic demographic information to more specific queries about the participant's condition and treatment. The thoroughness of the survey ensured that we gathered a comprehensive set of data for our study.

Patients with sickle cell traits were included in the genetic diagnosis, because they presented symptoms similar to homozygous patients, and it was decided to carry out pharmacotherapeutic follow-up.

2.2. Stage 2: Pharmacotherapeutic follow-up

This service was approached comprehensively, addressing both patients' health problems and their prescribed medications, with a focus on assessing the necessity, effectiveness, and safety of pharmacotherapy. Consequently, the next phase of this study involved presenting and offering pharmaceutical care to the characterized population using the DADER pharmacotherapeutic follow-up method, a validated approach developed at the University of Granada to identify, prevent, and resolve drug-related problems and negative outcomes associated with medication (*5*). Patients were given the option to accept or decline participation. Once enrolled, the first interview was scheduled to establish their pharmacotherapeutic history, with clinical documentation playing a crucial role. This process provided insight into the management of SCD by hematologists overseeing patient care. Following this, each clinical case was analyzed to evaluate the patient's current condition, identify specific needs, and address both medication-related and non-medication-related problems.

2.3. Stage 3: Pharmaceutical interventions

Upon completion of the data collection stage, a multidisciplinary group composed of hematologists, psychologists, some DADIS officials, and pharmaceutical chemists established interventions according to the criteria of each professional. These interventions were executed through a strategic action plan. Subsequent interviews confirmed that this approach significantly improved the quality of life of the patients.

2.4. Ethical considerations

The study adhered to strict confidentiality and ethical guidelines. Participants provided informed consent and were assured of minimal risk. Results were handled confidentially and used solely for research purposes.

All guidelines as per declaration of Helsinki and good clinical practice guidelines were followed

2.5. Statistical analysis

After completing the data collection stage, a database was created using Microsoft Excel to carry out descriptive statistics through tabulations and graphs. Subsequently, the Python programming language was used with the Matplotlib tool to generate Figure 1 and Figure 2.

3. Results and Discussion

In Colombia, about 20,000 children are born annually with SCD which highlights the importance of studies such as this one, especially in specific populations such as that of the township of San Basilio de Palenque, Bolivar due to its genetic inheritance. The lack of accurate data on the prevalence of SCD in Colombia by the governmental entities in charge of generating case reports represents a significant obstacle for the implementation of effective public policies aimed at this population. This lack of information, added to the generalized lack of knowledge about the condition on the part of patients increases the risk that numerous cases go undiagnosed and unreported to the health system. A literature study conducted in 2017 suggests that the prevalence of sickle cell hemoglobinopathy could reach 12% in Afro-Colombian communities (*3*).

Table 1 shows the sociodemographic characteristics of the patients included in the study, with a total of 20 people during the period 2022-2023, of which 75% were female and 25% male, given that SCD is a genetic disease determined by a specific mutation and not by factors related to sex or gender. The distribution found

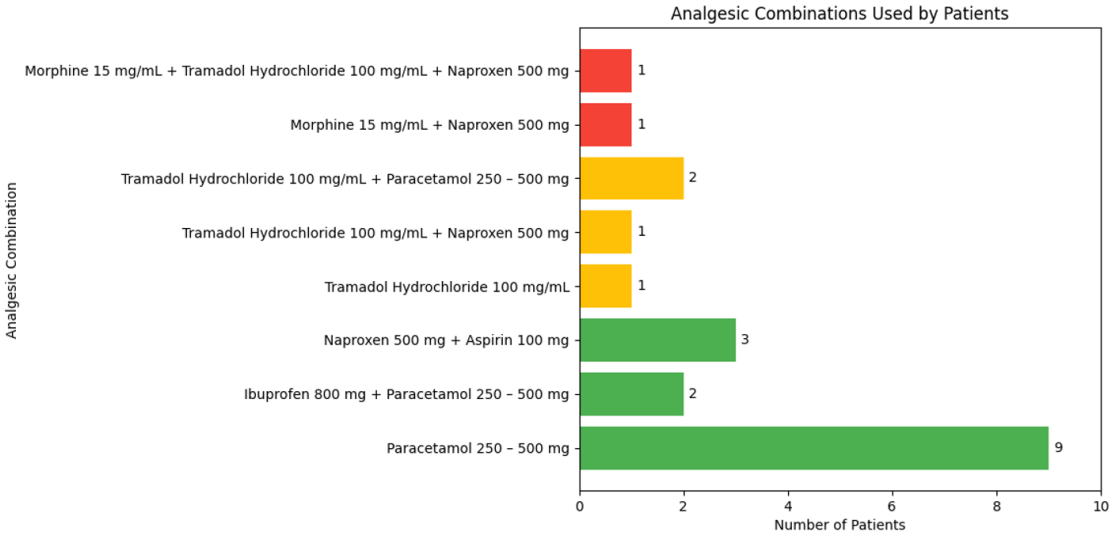

Figure 1. Medications used by patients to attenuate pain during crises. Green: Non-opioid analgesics (NSAID), Yellow: Weak opioids and NSAID and Red: Strong opioid and NSAID.

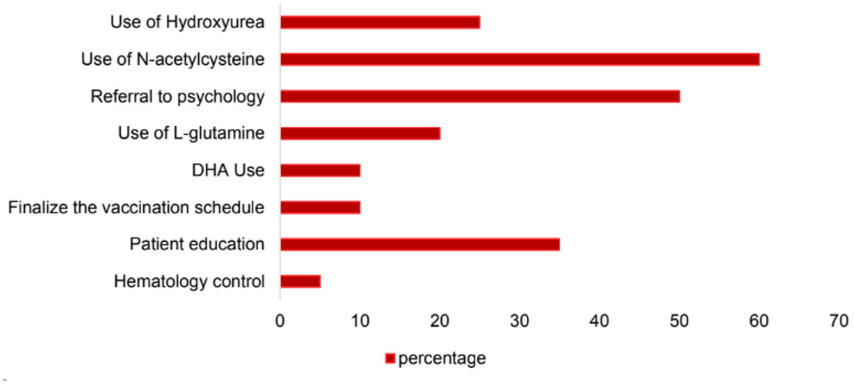

Figure 2. Multidisciplinary interventions implemented in the population of San Basilio de Palenque, Bolivar.

in this sample is random, as for the characteristics of hemoglobinopathy 40% were homozygous, that is, they presented the mutation in both alleles and 60% were heterozygous, mutation in one allele. Among the common diseases in the home it was found that 100% of patients have had respiratory complications and 80% gastrointestinal problems.

According to the results found, for the youngest patients, still in kindergarten (10% of the total), information was prioritized for the caregivers since they are the ones who spend the most time with them. In the case of children in primary school (30%) and high school (50%), visual resources such as animated diagrams were used to explain the disease clearly and concisely, as well as the preventive measures to follow to avoid episodes of crisis through simple steps such as keeping hydrated, constant hand washing, avoiding exposure to the sun, avoiding exposure to high temperatures, reducing physical activity in the event of changes such as changes in the color of the cornea to a more yellow color or the skin, notifying a responsible adult in the event of painful abdominal palpation or swelling of the upper and lower limbs, and emphasizing the importance of adherence to pharmacological treatment (*6*).

On the other hand, the DADER method categorizes DRPs into three main groups: unmet needs, efficacy and safety problems. Table 2 shows the DRPs found in the community under study, where 62% of homozygous patients at the beginning of the study did not receive the basic drug, and as regards to safety, it was found that they consumed painkillers indiscriminately to cope with the discomfort of crisis episodes and comorbidities.

Considering the above, it is necessary to take into account the treatment guidelines for SCD established by the Spanish Society of Hematology and Hemotherapy, where the basic treatment is Hydroxyurea 500 mg (HU). This is an antineoplastic drug that inhibits the M2 subunit of ribonucleotide reductase, blocking DNA synthesis and restructuring. It is used as a pharmacological inducer of fetal hemoglobin in SCD patients, inducing the synthesis of nitric oxide (NOS) and decreasing arginase in red blood cells and plasma,

leading to an increase in nitric oxide production, which is important for vasodilation. Also included is Folic Acid 1 or 5 mg, with the aim of stimulating the bone marrow to produce red blood cells at a faster rate, as these cells have a short lifespan in SCD (*7*).

Table 3 refers to the main reasons for which patients are admitted to hospital centers. In this case, the frequency of crises at the beginning of the study was five, and at the end, two. For headaches, six patients presented pain at the beginning and two at the end. To visualize significant changes, the patient should structure new habits in their lifestyle.

Upon obtaining these results, pain management was analyzed using the World Health Organization analgesic ladder, which consists of three steps, each tailored to different levels of pain intensity. The first step involves the use of non-opioid analgesics, such as paracetamol, for the relief of mild to moderate pain. When pain intensifies, the ladder recommends the second step, which combines weak opioids with other medications for improved control. Finally, for severe pain, strong opioids such as morphine are used.

As shown in Figure 1, patients from the district of San Basilio de Palenque were identified at the first step of the analgesic ladder; eight patients were taking paracetamol in 250 mg and 500 mg doses as their primary analgesic, either alone or in combination with 600 mg of ibuprofen. At the next level, three patients were identified as using weak opioids, such as tramadol hydrochloride 100 mg/mL, often in combination with other analgesics such as naproxen 500 mg or paracetamol 250 mg and 500 mg.

Figure 1 illustrates the use of analgesics and their combinations, ranging from the minimum level, such as the use of paracetamol 250 mg, to the highest combinations involving strong opioids, such as morphine 15 mg/mL + tramadol hydrochloride 100 mg/mL + naproxen 500 mg.

The above indicates that the patients used the three analgesic options allowed according to the intensity of the pain. For this reason, a specialized pharmaceutical professional in the field of pharmaceutical care was invited to guide the patients and caregivers on the proper use of analgesics and the potential consequences of their prolonged use over time, as in the case of opioids, which initially cause tolerance but over time can lead to dependence. Similarly, the prolonged use of paracetamol can cause liver damage because it undergoes extensive hepatic metabolism through three main pathways: conjugation with sulfate and glucuronic acid, and oxidation mediated by cytochrome P450 2E1. The drug follows the conjugation pathways, generating inactive metabolites that are excreted through the renal route. However, a small fraction is transformed into N-acetyl-p-benzoquinone imine (NAPQI), a hepatotoxic reactive metabolite, which causes liver damage (*8*).

Figure 2 shows the most frequent pharmacological interventions, with a high percentage of patients receiving hydroxyurea and N-acetylcysteine. The use

Table 1. Sociodemographic characterization of patients in San Basilio de Palenque, Bolivar

Characteristics	Percentage
Sex	
Female	75%
Male	25%
Age	
2–30	60%
31–58	40%
Characteristics of hemoglobinopathy	
Homozygous	40%
Heterozygotes	60%
Level of schooling	
Nursery	10%
Primary	30%
High school	50%
Technologist	10%
Common diseases	
Gastrointestinal	80%
Respiratory	100%

Table 2. Identification of problems related to medications in patients included in the study in the town of San Basilio de Palenque, Bolivar

PRM ID	Description
NEED (Untreated health problems)	62% of homozygous patients (mutation in two alleles) were not receiving Hydroxycarbamide 500 mg base treatment at the beginning of the study.
SAFETY (Self-medication)	65% of patients used paracetamol 250 and 500 mg consistently, 15% used weak opioid analgesics such as tramadol hydrochloride 100 mg/mL, and 15% used potent opioids such as morphine 15 mg/mL.

Table 3. Frequency of crises and headaches in patients immersed in the study at the beginning and at the end of the study

Measure	Start of the Study		End of the Study	
Frequency of Crisis	With Crisis	No Crisis	With Crisis	No Crisis
	5	3	2	6
Headache	In Pain	Painless	In Pain	Painless
	6	6	2	10

of L-glutamine was also significant, although to a lesser extent. These data suggest that pharmacological management is a mainstay in the treatment of this condition.

Pharmacotherapeutic monitoring allowed an assessment of the individual status of each patient. Such assessment was communicated to other health professionals; and this allowed for improving the quality of life of patients. In Figure 2, such multidisciplinary interventions are presented, as is the case of the hematology team which authorized access to Hydroxycarbamide 500 mg to patients adjusting the dose according to weight. HU is a key drug to prevent vaso-occlusive crises in patients with SCD. A 2009 study showed that, after implementing HU in 30 patients, mainly men, the number of transfusions and crises was significantly reduced, thus decreasing hospitalizations (*9*).

Complementary treatments for SCD include antioxidants. L-glutamine is an essential amino acid for the synthesis of NAD, a coenzyme involved in oxide reduction reactions in the body. When oxidative stress occurs in red blood cells, L-glutamine consumption increases to maintain glutathione levels (*10*). In the case of N-acetyl cysteine, it is transformed into L-cysteine, which in turn increases glutathione and decreases the oxidative stress of the red blood cell by bibliographic documentation (*10*).

Patients with SCD are more vulnerable to bacterial and viral infections due to a condition called functional asplenia, which is the loss of spleen function, affecting their ability to fight infections. In addition, they have alterations in other parts of their immune system. For this reason, the infections they develop are usually more severe and require more aggressive treatment. To prevent these infections, it is recommended that all patients be vaccinated and antibiotics such as penicillin administered, especially to children under five years of age (*11*). Under this premise, the pediatric patients in this study were vaccinated through the support of territorial entities such as DADIS and companies that financed the study.

Based on the observed results and the physiology of SCD, it was considered necessary to take into account the process of accelerated red blood cell production, known as erythropoiesis, which increases the body's demand for folate. To compensate for this deficiency and reduce anemia-related symptoms, patients with SCD should take regular folic acid supplements at doses of 1 to 5 mg (*12*). L-glutamine at 5 g is also used as a supplement, as lymphocytes — key cells of the immune system — primarily rely on glutamine for their function. In addition to serving as an energy source, glutamine helps protect these cells from damage caused by oxidative stress. Therefore, it plays a key role in preventing vaso-occlusive and hemolytic crises in these patients.

Clinical research has positioned HU as a reference treatment for SCD. A local study conducted in Barranquilla between 2012 and 2013 with 129 patients evidenced that the use of HU 500 mg compared to Folic Acid 1 mg was associated with a significant decrease in the frequency of seizure episodes in patients (*13*).

Figure 3 shows the family tree of an extended family from San Basilio de Palenque, which reveals a pattern of inheritance of SCD through multiple generations. The genealogical tree presented shows the inheritance patterns of the genetic condition under study, differentiating between homozygous and heterozygous individuals. Conventional genetic symbology is used: circles for females, squares for males, and colors to represent the characteristic of the hemoglobinopathy (red: homozygous; red and white: heterozygous; white: non-carrier). The numbers assigned to each allow identification of the patients included in the pharmacotherapeutic follow-up. Family relationships are represented by lines (solid: siblings; dotted: partners). Marital separations (transverse line).

Finally, the family tree of this family from San Basilio de Palenque provides valuable insight into the transmission of SCD through multiple generations. The extensive family network clearly identifies the autosomal recessive inheritance pattern and reveals the disease's clinical heterogeneity. In addition, the inclusion of unaffected relatives allows for the estimation of the mutant allele's frequency in the population and the assessment of the impact of modifying factors on phenotypic expression. That allows us to perform an analysis of past generations, confirming the origin of the homozygous or heterozygous characteristic, which in the present time allows the health professional to perform genetic counseling to the population to prevent the condition from continuing to spread and to present alternatives to those suffering from the condition that allows them to lead a relatively normal life.

4. Conclusion

The research highlights the need for a multidisciplinary approach to optimize treatment and improve the quality of life for affected patients, as evidenced by the monitoring of analgesic use, which can help prevent future medication dependency and the progressive deterioration of organs involved in the process. On the other hand, the study successfully characterized and conducted pharmacotherapeutic follow-up of patients with SCD and sickle cell traits, which allowed for a positive impact on patients and their families in the comprehensive management of their diagnostics. Moreover, due to its genetic significance, San Basilio de Palenque represents a unique setting for future studies on SCD.

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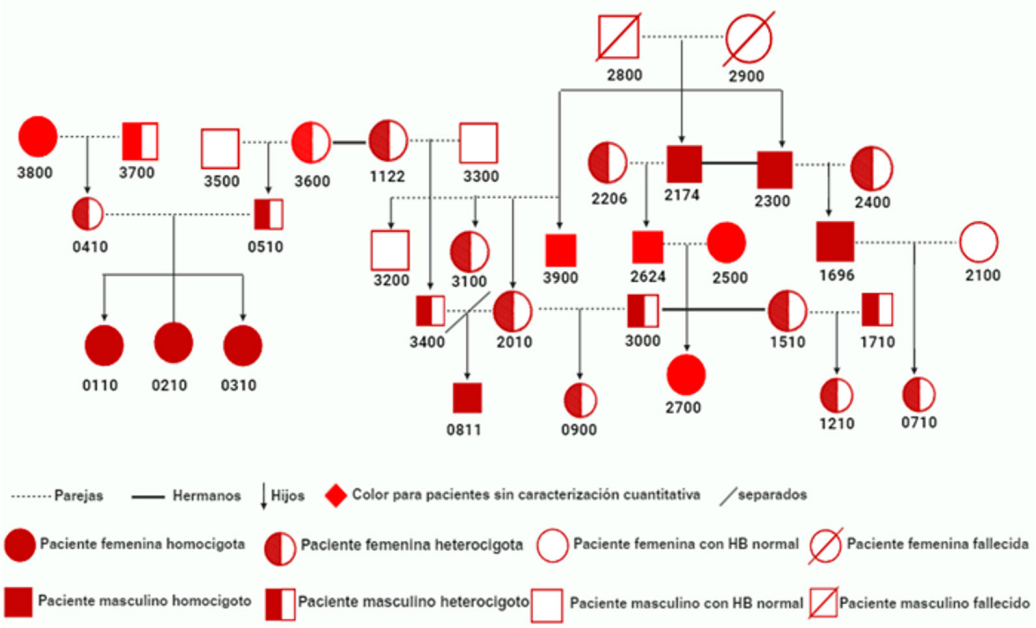

Figure 3. Family tree constructed using the data provided by the families as a result of pharmacotherapeutic monitoring.

Therapeutics research group, released funds for transportation, stationery, food, and supplies for the proper execution of the project's objectives.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Brief Report

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Clinical and genetic analysis of ulnar-mammary syndrome caused by a novel *TBX3* mutation in a Chinese boy

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SUMMARY: Ulnar-mammary syndrome (UMS) is caused by *TBX3* mutation and is a disorder characterized by altered limb, breast, tooth, hair, apocrine gland, and genital development. The clinical and genetic data of a 5.5th boy with UMS were carefully analyzed. Clinical biochemical data, pituitary MRI, and whole exome gene detection were analyzed. The impact of the mutation and stability of *TBX3* on the mRNA structure was analyzed by the M-fold program. Three-dimensional protein structures were calculated and analyzed. The patient presented with a hypoplastic left fifth finger, an absence of interphalangeal creases, a large space between the fourth and fifth fingers, no bending ability of the fifth finger, absent nipples, high palates, a flat nasal bridge, a micropenis, micro-testes, short stature and reduced axillary sweating. Pituitary magnetic resonance imaging (MRI) revealed pituitary gland hypoplasia with a thin pituitary stalk and loss of a strong signal in the posterior pituitary. A novel variant (c.1142_1146) in the *TBX3* gene was detected in the proband and further verified by DNA sequencing. M-fold results revealed that the variant altered the mRNA structure and stability of the *TBX3* gene. Clinical, genetic, and biochemical studies confirmed that the congenital normal idiopathic hypogonadotropic hypogonadism was associated with pituitary hypoplasia. After half a year of treatment with human chorionic gonadotropin (HCG), the micropenis was significantly improved. After 3.5 years of treatment with recombinant human growth hormone, the body height was largely improved. One novel variant of the *TBX3* gene was confirmed in an UMS patient, which enriched the spectrum of *TBX3* genotypes.

Keywords: ulnar-mammary syndrome, *TBX3*, micropenis, HCG, hGH

1. Introduction

Ulnar-mammary syndrome (UMS; MIM #181450), an autosomal-dominant disorder, is caused by mutations in *TBX3* (*1*). Despite the fact that such ulnar deficiencies may scarcely occur in 1 out of 25,000 births (*2*), the exact incidence of UMS is still unknown. Asymmetrical ulnar ray defects with shortening of the fifth digit or complete absence of the ulna radius, combined with hand defects, hypoplasia of the breast (areola and nipple), aphobia, subfertility with gonad deficiency, genital deviation, short stature, dental anomalies, cardiac defects, and obesity were observed.

The disorder displays obvious interfamilial and intrafamilial changes in phenotype. To date, twenty-two *TBX3* pathogenic variants with considerable insertions or deletions have been reported (*1,3-11*). UMS has some overlapping features with certain other syndromes. The main syndromes that overlap with UMS include the allelic disorders of acro-dermato-ungual-lacrimal-tooth syndrome (MIM #103285) (*12*) and limb-mammary syndrome (MIM #603543) (*13*); both are caused by TP63 gene mutations. Other overlaps exist with scalp-ear-nipple syndrome (MIM #181270), which is caused by *KCTD1* gene mutations (*14*). Genetic examination of UMS is therefore crucial for obtaining an accurate diagnosis.

We have carefully reviewed the literature using PubMed and WANFANG MED ONLINE. In this study, a novel variant in the *TBX3* gene was identified in a boy, which was the 4th reported case in China, with three previously reported cases (*15-17*). The deteriorating property of such mutation was verified by bioinformatic analysis. A follow-up study was completed to determine the prognosis of the patient after hormonal treatment. UMS clearly showed abundant variability in its mutational heterogeneity, phenotypic presentation, and ethnic diversity, as evidenced here in the report.

2. Patient and Methods

2.1. Ethical approval

The Institutional Human Ethics Review Board at Shandong Provincial Hospital affiliated to Shandong First Medical University approved this study (LCYJ:NO. 2019-147). The legal guardians of the participant were given written information to obtain signed consent to participate in the study. This study conforms to the provisions of the Declaration of Helsinki.

2.2. Patient

In 2021, a 5.5-year-old male patient visited the outpatient department of Paediatric Endocrinology, Shandong Provincial Hospital, for micropenis and retarded body growth. The parents of this patient were physically healthy and nonconsanguineous. The clinical evaluation, baseline and dynamic hormonal levels, and genetic analyses were obtained from the patient with signed consent from the parents.

2.3. Clinical observations

The following hormones were measured in the serum samples: follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estrogen, testosterone, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), and insulin-like growth factor-1 (IGF-1). Gonadotrophin-releasing hormone (GnRH) stimulation tests (those involving intravenous injection of GnRH and LH and FSH at baseline and +30', +60', +90' after GnRH injection), and growth hormone releasing hormone (GHRH) stimulation tests (those involving intravenous injection of GHRH and blood sample collection for GH determination at baseline and +30', +60', +90', +120', +150' after GHRH injection) were conducted with standard procedures. All hormones were measured by chemiluminescent methods (Roche, Basel, Switzerland) following the manufacturer's instructions. Blood electrolyte levels, routine blood tests, and qualitative urine calcium levels were measured in the hospital laboratory. Additionally, magnetic resonance imaging (MRI), bone age, and funduscopic examination were also carried out in the hospital.

2.4. Genome sequencing

Peripheral venous blood (3-5 mL) was collected from the proband and his parents. Peripheral blood DNA was sequenced using whole exome sequencing (WES). The exons from patient genomic DNA were fragmented, ligated, amplified, and purified following the manufacturer's protocol, and then examined with the SeqCap EZ Med Exome Enrichment Kit (Roche NimbleGen) according to the manufacturer's protocol. The exons and flanking regions of all known genes were captured. After postcapture amplification and purification, the Illumina HiSeq system was used to construct the DNA library.

The sequence data were aligned to the human genome reference 19 (hg19) by NextGene V2.3.4 to secure good coverage and depth of the mean reading of the target regions. Conserved nucleotide bases and amino acids, frequency of the normal populations (1000 Genomes Project, ExAC, dbSNP DNA and locus specific databases), predictions of the biological functions, and data from The Human Gene Mutation Database (HGMD) and Clinvar and Online Mendelian Inheritance in Man (OMIM), were obtained using NextGene V2.3.4. Variants were screened according to the published rules. Pathogenicity variants were interpreted by the American College of Medical Genetics (ACMG) guidelines for the interpretation of sequence variants published in 2015 using the Human Genome Variation Society (HGVS) nomenclature.

Sanger sequencing was used to verify the variants in the proband revealed by WES, and to test the co-segregation of variants in the family. Genome sequencing was completed in collaboration with Berry Genomics Co.

2.5. Bioinformatic analysis

Bioinformatics tools are wildly used for predicting and understanding the effects of genetic variants on the structure, stability and function of proteins and mRNA stability (*18*). Therefore, in this study, the disease-causing potential of the genetic variants was extensively analyzed using the silico methods.

To determine the changes in RNA thermodynamic stability caused by mutations, the changes before and after mutations were compared. The secondary structure of RNA influences the expression of genes by changing the stability of RNA or transcript, and the efficiency of translation. The mutation may alter the sequence of the mRNA and the secondary structure of RNA. Changes in the RNA structure or thermodynamic stability may affect the rate of mRNA translation into proteins. In this study, variations in the *TBX3* mRNA secondary structure and stability were predicted by the M-fold server (*http://unafold.rna.albany.edu/?q=mfold/RNA-Folding-Form*). The input sequences consisted of two different lengths of mRNA fragments, i.e 75 bases and 150 bases with the variant of interest centered in the middle of the mRNA fragments. Most of the predicted stable structures with minimum δG values were chosen for further calculation of the minimum free energy (δδG) of the mutant mRNA and wild-type mRNA (δδG= δGmutant- δGwild-type). The greater the positive δδG value, the lower the stability of the mutated mRNA relative to the wild-type mRNA. Our previous inputs consisted of short-length RNA fragments, as the complexity of potential structures increases exponentially with longer sequences, leading to

a decrease in prediction accuracy (*18*).

We demonstrated the spatial structure of the *TBX3* protein and the affected protein regions after generation of frameshift mutations. Prediction of three-dimensional protein structures based on the three-dimensional structure of mutant *TBX3* was achieved using I-TASSER software (*19*) (*https://zhang lab.ccmb.med.umich.edu/I-TASSER/*). The PyMOL Viewer software was used to visualize the effects of altered residues on the protein structure models.

3. Results and Discussion

UMS was first reported by McKusick in 1975 (*20*). A variety of abnormalities have been reported in addition to limb and apocrine defects (*21*) over the last few years. Various postaxial limb defects exist, such as hypoplastic distal phalanges in digit V or absent digits III-V with radial shortening of the ulnae.

When the condition is more severe, the hand, ulna, radius, and humerus are all absent. UMS patients may also exhibit dorsal hypoplasia/aplasia of the breast, absence of axillary hair, reduced or absent perspiration, short stature, obesity, delayed puberty, dental abnormalities, hypopigmentation of the nipples and areola, genital hypoplasia, cardiac defects, anatomical pituitary anomalies and scoliosis (*8,15,16,22,23*).

In this report, the patient had normal mental and nutritional status and a height of 107.8 cm (P3-10). He had a hypoplastic left fifth finger with no interphalangeal creases, and a wide space between the fourth and fifth fingers, and the fifth finger could not bend. He also had absent nipples, high palates, micropenis, flat nasal bridge, irregularly arranged teeth, a micropenis (1.5 cm × 1 cm), micro-testis (< 1 mL), and reduced axillary sweating (Figure 1). His parents had no similar symptoms.

Evaluation of hormone levels showed reduced testosterone (TO) (< 0.03 ng/mL), LH (< 0.1 mIU/mL) and FSH (0.76 mIU/mL) levels. The following parameters were used: normal IGF-1 (55 ng/mL, reference ranges: 45-305 ng/mL); TSH (2.14 μIU/mL, reference ranges: 0.7-4.17 μIU/mL); free T4 (15.95 pmol/L, reference ranges: 11.45-17.63 pmol/L); and prolactin (12.87 ng/mL, reference ranges: 4.04-15.2 ng/mL). After intramuscular injection of 1,000 U human chorionic gonadotropin (HCG) every week for approximately half a year, the level of TO (2.20 ng/ml) was increased, that of FSH (0.17 mIU/ml) decreased, and that of LH did not change.

MRI of the pituitary of this 5.5-year-old patient (taken on 2021-06-06) revealed that the upper edge of the pituitary was concave, the height of the adenohypophysis was approximately 3 mm, and the picture showed a thin pituitary stalk and a loss of high signal in the posterior pituitary, suggesting a clear pituitary gland hypoplasia.

A novel variant in the *TBX3* gene was observed by WES in the proband and confirmed by Sanger sequencing (*15*). A heterozygous *TBX3* variant NM_005996.4:exon6:c.1142_1146dup(p.P383 the Rfs*231) was identified (Figure 2). The score was PVS1_Strong+PM2+PM6, which was regarded as a pathogenic mutation according to ACMG Guidelines (*24*). Sanger sequencing confirmed that this new variant was not transmitted from the parents of this patient.

TBX3, located on chromosome 12q24.21, is an ancient and evolutionarily conserved T-box transcription factor. It plays an important role in the control of developmental signal systems (*25*) involved in critical structure formation of organs, such as the mammary glands, heart, lungs and limbs (*26*). All organs are developed on the highly conserved T-box DNA-binding domain, which is generally encoded by exons 1-3 and a part of exon 4 (*27*). The T-box is expressed in specific tissues of the developing embryo and is required for tissue differentiation. There is a close relationship between *TBX3* and *TBX5* on chromosome 12. For the differentiation of radial limbs, *TBX5* expression is essential, while *TBX3* controls ulnar limb development. A mutation in *TBX5* causes Holt-Oram syndrome, which is characterized by radial longitudinal deficiency and cardiac defects (*28*). UMS is believed to be mainly caused by *TBX3* haploinsufficiency. Patients with UMS are predicted to have mutations that disrupt the transcriptional regulation or render the proteins susceptible to degradation by nonsense mediated decay. To date, *TBX3* mutations and UMS clinical manifestations have little genotype-phenotype correlation (*3,5*).

This patient had a classical high palate, micropenis, flat nasal bridge, broad hands, and multiple pituitary hormone deficiencies. The mutation lies within exon 6 of the *TBX3* gene. As a result, amino acid 383 was changed from proline to arginine, which was terminated after amino acid 231, and may still retain some function or may be eliminated by nonsense-mediated RNA decay. In addition to this patient, other individuals with UMS with mutations downstream of the T domains were identified. Meneghini *et al.* (*7*) hypothesized that the presence of an intact T-box domain was most likely allowed for residual DNA-binding activity, leading to a milder clinical phenotype. However, other researchers (*3,8*) have clearly shown a correlation between classical UMS phenotypes and mutations preserving this T-box domain. Recently, the ability of the C-terminal domain of *TBX3* to interact with mRNAs and to regulate alternative splicing has been reported. Mutations found in UMS patients with truncated *TBX*3 5′ of the T-box domain were shown to dominantly interfere with the function in inhibiting splicing (*29*). *TBX3* mutations included two categories: those located within the location 5-prime of the T-box, or within the T-box, and mutations located 3-prime of the T-box. In this study, the mutation was located at the 3′ end, downstream of the T-box domain.

No other variants in *TBX3* were observed to modify

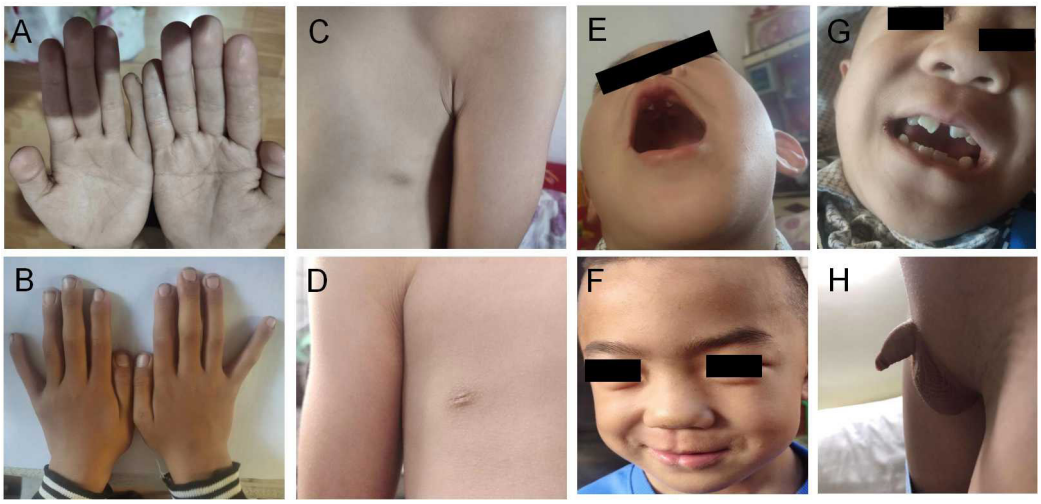

Figure 1. The clinical features of patient. (A, B) Hypoplastic left fifth fingers with absent interphalangeal creases, wide space between the fourth and fifth fingers, and the fifth finger cannot be bent. **(C, D)** Absent nipples. **(E)** High palates. **(F)** Flat nasal bridge. **(G)** Irregularly arranged teeth, and **(H)** Micropenis.

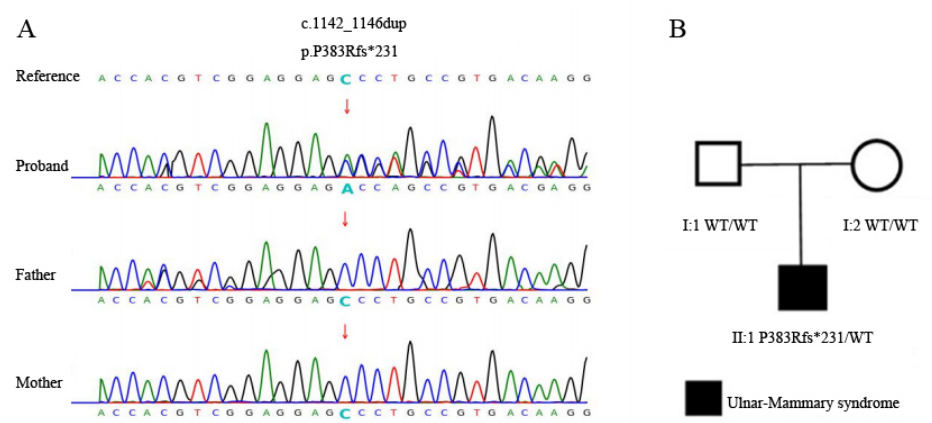

Figure 2. The genetic analysis. (A) *TBX3* gene mutation analysis of the patient (GenBank accession number: NM_005996.4). **(B)** The pedigree of this family.

the phenotype, and the factors causing phenotypic variability in this patient could not be identified. The phenotypic variability found in UMS families may be caused by the different degree of changes in *TBX3* function during embryonic development (*30*). It is also possible that other genetic variants may affect a similar function, causing UMS during development, which may contribute to variations in severity and affected organs.

The effect of c.1142_1146dup on mRNA structure and stability was evaluated by the M-fold server to predict substantial alterations in mutated mRNAs compared with the wild-type mRNAs. This mutation changed the mRNA sequence and the secondary structure of the transcript. After mutation, the original multiloop was changed, and a new hairpin loop was formed. The overall and partial structures of the wild-type and mutant mRNAs are shown in Figure 3. The greater the optimal energy is, the less stable the RNA. The variant increased the optimal energy and decreased the mRNA stability (Figure 4A, B). The three-dimensional protein structure model of the P383Rfs*231 mutant *TBX3* protein (green showing the affected area) is shown in Figure 4C.

This patient was followed up closely since his diagnosis. During the follow-up period, HCG treatment was given for 6 months (NaCl 1ml + HCG 1000 iu im qw). The micropenis was improved from 1.5 × 1 cm to 4 × 3 cm. After 3.5 years of treatment with recombinant human growth hormone (rhGH 0.15 u/kg ih qn), his height improved from 107.8 cm (P3-10) (5.5 years) to 135.2 cm (Near P50) (9 years). Additionally, thyroid hormones, blood electrolytes, IGF-1, ACTH, routine blood test, and qualitative urine calcium were monitored regularly and maintained within the normal range.

All the allelic variants reported in the literature and

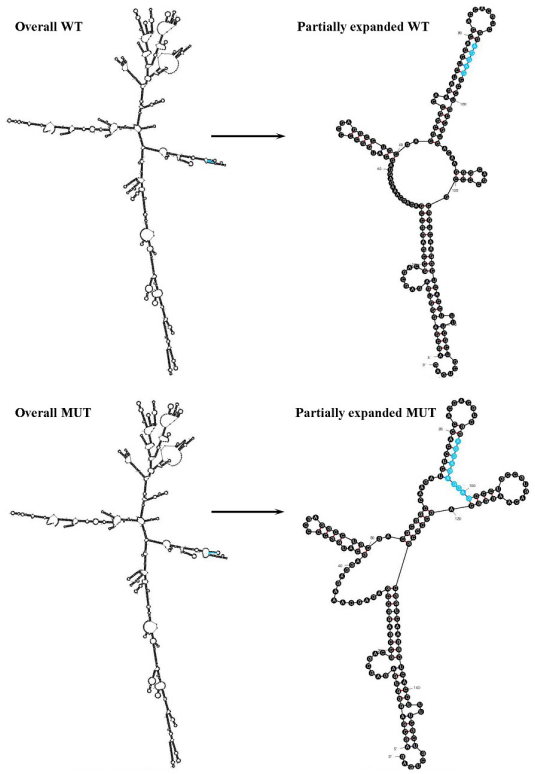

Figure 3. The analysis of the Mfold RNA secondary structure. Blue indicates the wild/mutated bases. The overall wild-type (WT) and mutant-type (MUT), and partially expanded WT and MUT structures are placed up and down for comparison, respectively.

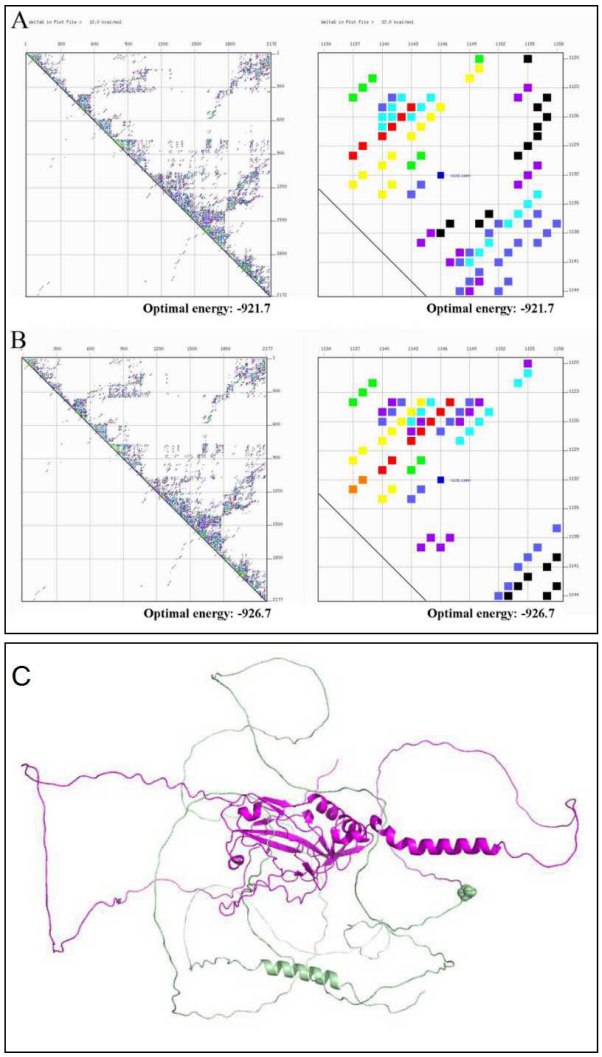

Figure 4. The analysis of the wild-type and mutant-type mRNA stability, and 3-D modelling of wild-type and P383Rfs*231 mutant *TBX3* protein. The differences in the alignment of wild-type (purple) and mutant (green) *TBX3* protein in 3-D modelling.

HGMD were reviewed to better understand the genotype-phenotype correlations in *TBX3*-related disorders (Supplemental Table S1, *https://www.irdrjournal.com/supplementaldata/250*). To date, 38 mutations in *TBX3* have been identified and most of these mutations are missense or nonsense mutations (17/38 or 44.74%), followed by deletions, insertions, and splicing mutations. However, *TBX3* mutation locations have not been related to any clinical disorders yet.

In conclusion, the other three previously reported cases in China (*15-17*) are mainly case reports with literature review, but do not include any bioinformatic analysis. This current work is the first UMS case with bioinformatic analysis in China. Further evidence is provided to demonstrate the variability in mutational heterogeneity, phenotypic expression, and ethnic diversity involved in this specific phenotype of UMS. In addition, UMS may be associated with dwarfism with special facial characteristics and dysplasia of the external genitalia, sweat glands, and mammary glands. *TBX3* is a pathogenic gene of UMS.

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Pathonign variants in recessive disorders: How extremely hypomorphic variants can be pathogenic and benign depending on the allele in trans

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SUMMARY: In recessive monogenic diseases, individuals with a single pathogenic variant are typically asymptomatic and symptomatic disease is only observed in patients with two pathogenic variants. Assuming that disease only occurs where protein concentrations or activity are below 50% of normal (since in recessive diseases, most carriers are asymptomatic) some hypomorphic variants could be deleterious in association with a LoF variant, but nevertheless yield > 50% protein activity/concentration when homozygous. These types of variants would be very weakly eliminated by natural selection, if at all, and thus their frequency in the population could increase by genetic drift. Thus the population frequency criterion often used to qualify variants as benign would be misleading. One such variant may be c.5603A>T (p.Asn1868Ile), in *ABCA4* (which causes Stargardt disease-1). This variant is pathogenic in trans with a null or missense variant but not when homozygous. We refer to these variants using the blend word "pathonign", since they are simultaneously pathogenic and benign in the population.

Keywords: monogenic disorder, hypomorphic allele, disease-causing variant, benign variant, phenotypic severity, recessive disorder

1. Introduction

In recessive monogenic diseases, individuals with a single pathogenic variant are typically asymptomatic and symptomatic disease is only observed in patients with two pathogenic variants (*1*). For some genes however, particular variant combinations can give rise to specific disease expression profiles. For example, we recently highlighted the Goldilocks situation that can arise for Mendelian diseases where the presence of two loss of function (LoF) variants is lethal prenatally, and symptoms are only observed in individuals with a LoF-hypomorphic variant combination (*2*) as in the case of recessive diseases linked to aminoacyl-tRNA synthetases (*2*) and thrombocytopenia-absent radius syndrome (*3*). In some cases therefore, phenotypic severity depends on the combined activities of pathogenic variants. While it is clear that some LoF variants are lethal when homozygous, conversely, extremely hypomorphic variants could be benign when homozygous and pathogenic only in association with a more severe variant. In this article, we briefly outline the mechanisms and consequences of this Schrödinger cat-like effect and its implications for patient care, with reference to *ABCA4* variants and Stargardt disease-1 (STGD1).

2. The importance of the variant in trans: A theoretical illustration

In recessive disorders, assuming that disease only occurs where protein concentrations or activity are below 50% of normal (since in recessive diseases, most carriers are asymptomatic) some hypomorphic variants could be deleterious in association with a LoF variant, but nevertheless yield > 50% protein activity/concentration when homozygous (Figure 1A). In theory for example, a hypomorphic variant producing a protein with 25% activity will be pathogenic in association with a null variant, but not when homozygous (50% activity) or with a > 25% functional allele in trans. The distribution of these variants in the population may depend on (or reflect) the clinical threshold of the corresponding disease, which is not necessarily 50%, as illustrated in Figure 1.

One such variant may be c.5603A>T (p.Asn1868Ile), in *ABCA4*, which is pathogenic in trans with a null or

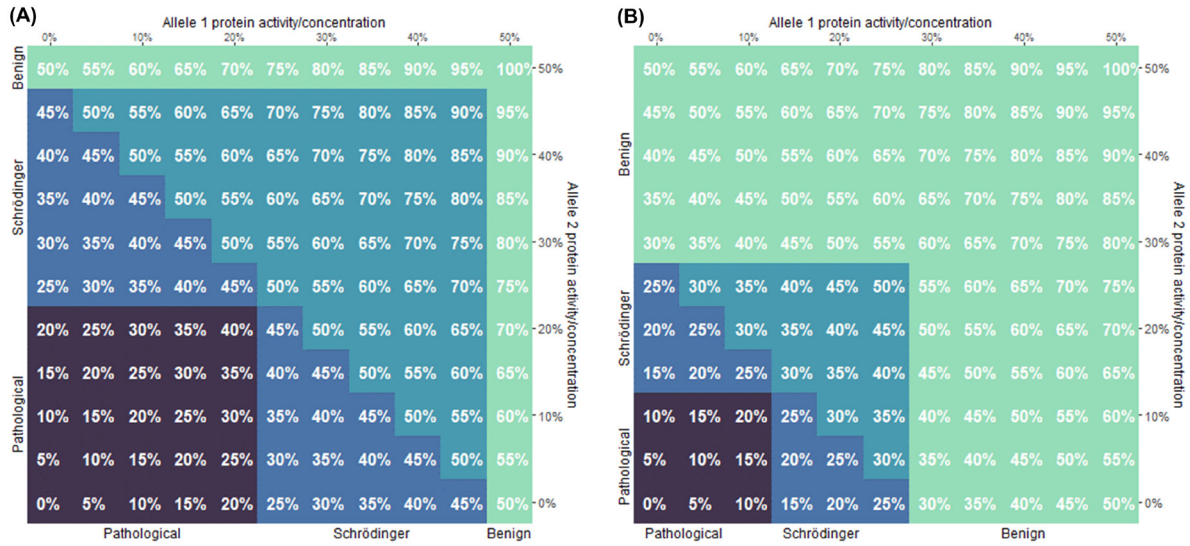

Figure 1. Tile plots of the combined activity (not frequency) of hypothetical variants in which the disease threshold is (A) 50% and (B) 30% protein activity/concentration. The tiles are colored in *dark blue* when the variants are pathological when homozygous and when combined with a hypomorphic variant, *light green* when the variants are always benign, and *blue* when the variants' pathogenicity is defined by the variant in trans (what we call pathonign variants): pathogenic (*denim blue*) when the combined activity from the two variants is below the disease threshold, but benign (*light blue*) when the combined activity from the two variants is above the disease threshold.

missense variant but not when homozygous (except if it is associated with a deleterious variation in cis). Pathogenic variations in the *ABCA4* gene cause STGD1, the most common cause of Mendelian recessive retinal dystrophy. c.5603A>T (p.Asn1868Ile) is a common variant (minor allele frequency, 5.8% in gnomAD) and has been implicated in 50% of cases of Stargardt disease-1 previously thought to be monoallelic (which represent 25% of cases of STGD1), with a milder phenotype and later onset (*4*,*5*). It is notable that this association was only identified because Zernant *et al*.'s study was large enough (> 600 patients) to significantly identify its overrepresentation in STGD1 patients (*4*). Patel *et al*. have also recently suggested that this type of variant may also be implicated in some cases of Knobloch syndrome (*6*).

These examples highlight both the existence of extremely hypomorphic variants and their potential phenotypic expression in patients in association with a more pathogenic variant. Presumably, these types of variants would be very weakly eliminated by natural selection, if at all, and thus their frequency in the population could increase by genetic drift. The population frequency criterion often used to qualify variants as benign (*7*) would be misleading in these cases, as would the presence of homozygous occurrences in databases (there are 2,989 homozygous occurrences of p.Asn1868Ile in GnomAD for example). In patients, these variants should occur more often than in the general population, and be associated with an extremely deleterious variant (either null or missense), and probably milder phenotypes. Proof of pathogenicity would require functional studies with a null allele in trans, because the homozygous state would not be pathogenic. The fact that p.Asn1868Ile is classified by AlphaMissense as likely benign (*https://alphamissense.hegelab.org/results*), suggests that pathogenicity prediction is insufficient. Note that this concept differs from those of risk alleles or low/reduced penetrance alleles in dominant disorders (*8*-*11*), because in recessive disorders, alleles cannot be considered in isolation and "penetrance" always depends on the variant in trans. In our theoretical example, the variant would be 0% penetrant when isolated or homozygous but 100% penetrant when associated with a LoF variant.

3. Conclusion

In conclusion, we think clinicians should be aware that in some cases, notably in trans of very deleterious variants, the second allele may have the features we describe theoretically here. We refer to these variants as pathonign variants, since they are simultaneously pathogenic and benign in the population.

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Perspective

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Precision grading of surgical strategies for small bowel Crohn's disease: An R0–R3 individualized framework based on lesion severity and functional preservation

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SUMMARY: Small bowel Crohn's disease (SBCD) presents unique surgical challenges due to segmental lesions and the need to balance radical resection with bowel function preservation. Current guidelines lack standardized surgical classifications, leading to variable outcomes. This study proposes a four-tier surgical strategy (R0-R3) tailored to lesion severity and functional preservation. R0 involves complete resection for localized mild lesions (creeping fat, no fibrosis) with ≥ 3 meters of residual bowel, using wide resection margins and anti-TNF-α therapy postoperatively. R1 preserves mild (non obstructive fibrotic) lesions and resects moderate to severe segments, with imaging surveillance support. R2 combines resection of severe lesions (fibrotic strictures/obstruction) with strictureplasty or partial preservation of moderate lesions to avoid short bowel syndrome. R3 employs temporary stoma creation for extensive complex lesions or high-risk patients, deferring definitive surgery until stabilization. This framework emphasizes individualized decision-making, prioritizing anatomical clearance, bowel conservation, and postoperative biologics to reduce recurrence. Compared to traditional approaches, the R0-R3 system enhances flexibility in managing heterogeneous SBCD, particularly in extensive disease. Future validation through multicenter trials and biomarker-driven predictive models is recommended to optimize long-term outcomes and quality of life. This strategy aligns with personalized surgical trends, addressing gaps in current guidelines by integrating lesion severity, functional prognosis, and staged interventions.

Keywords: Small-bowel Crohn's disease, Surgical stratification strategy, Individualized surgery

1. Introduction

Crohn's Disease (CD) is a chronic inflammatory disease that affects the entire gastrointestinal tract, with typical symptoms including abdominal pain, diarrhea, and internal fistulas, significantly impacting patients' quality of life (*1,2*). Small Bowel Crohn's Disease (SBCD) is characterized by segmental distribution and heterogeneity of lesions (radiologically defined as ≥ 2 non-contiguous lesions on CT/MR enterography) (*3*), posing dual challenges of anatomical complexity and functional preservation during surgical intervention. Unlike colonic Crohn's disease, the surgical strategy for small bowel CD requires a greater emphasis on balancing complete lesion resection and small bowel function preservation (*4*).

In recent years, the widespread use of biologics has significantly improved the medical treatment of CD. However, surgery remains an inevitable therapeutic option for most patients with small bowel CD, especially when medical treatment fails or complications arise. ECCO guidelines recommend ileocecal resection or segmental bowel resection when medical treatment fails or in cases of acute complications (such as bowel obstruction, perforation, or complex fistulas), while stoma surgery is recommended for extensive lesions (*5*). ECCO guidelines also recommend strictureplasty as the preferred option for multiple strictures (evidence level: b), but there is still no consensus on the surgical grading standards for SBCD (*5*). ACG guidelines emphasize surgical conservatism, particularly in young patients and those at high risk for short bowel syndrome, advocating for ileocecal valve preservation and minimal resection (*6*). Although the above guidelines provide directional suggestions for CD surgical strategies, they lack systematic classification and standardization for specific surgical strategies for small bowel CD. Especially in cases of extensive small bowel lesions, the choice of surgical strategy varies greatly among individuals, leading to high

postoperative recurrence rates and difficulty in ensuring quality of life. Moreover, current guidelines often adopt a fixed surgical pathway without individualizing based on lesion severity, presenting considerable limitations in clinical practice.

To address this issue, based on the clinical experience of our Inflammatory Bowel Disease Center, we propose a four-tier surgical strategy (R0-R3) based on lesion characteristics (Figure 1). First, we classify small bowel Crohn's disease lesions into three categories: mild, moderate, and severe, representing creeping fat without fibrosis, fibrotic stenosis without complete obstruction, and severe stenosis with obstruction, respectively. On this basis, we propose this four-level surgical strategy (R0, R1, R2, R3) to achieve a precise balance between complete lesion resection and intestinal function preservation, providing a more individualized surgical intervention plan for patients with different types of lesions. Through this strategy, we aim to address the shortcomings of current guidelines in surgical management of small bowel CD and further optimize surgical outcomes and long-term quality of life. In the future, we will further verify scientific validity and practicality of this strategy through multicenter clinical studies.

2. R0 strategy: Localized lesion, complete resection

2.1. Surgical indications

The R0 strategy is suitable for a single small bowel lesion or a lesion localized to a single segment of the small intestine, as confirmed by CTE/MRE evaluation and postoperative macroscopic examination showing no skip lesions, and without extensive fibrosis or fistula formation; the remaining normal small bowel after resection should exceed 3 meters.

2.2. Surgical approach

Resection range: The proximal and distal resection margins of the affected segment should be at least 2 cm, ensuring the removal of potential lesions.

Anastomosis method: Either antiperistaltic or isoperistaltic side-to-side anastomosis can be used, effectively reducing risk of anastomotic stricture (*7,8*).

Postoperative management: Early use (within 4 weeks after surgery) of anti-TNF-α agents to consolidate therapeutic effects and prevent anastomotic recurrence (*9,10*).

2.3. Strategy advantages

The R0 strategy maximizes lesion clearance, has a lower postoperative recurrence rate, and provides better long-term quality of life compared to other strategies. It is suitable for patients with a short disease course and localized mild lesions.

3. R1 strategy: Extensive lesions, partial resection

3.1. Surgical indications

The R1 strategy is indicated for cases with skip lesions of mild, moderate, or severe severity, such as fibrotic strictures in some intestinal segments without complete obstruction. The mildly affected bowel segment shows creeping fat but retains bowel elasticity.

3.2. Surgical approach

Resection range: Resection is performed on moderate to severe lesions, while mildly affected bowel segments are preserved as much as possible. Localized release can be performed for intestinal segments with creeping fat hypertrophy.

Anastomosis method: Side-to-side anastomosis is used to reduce intestinal tension and minimize risk of stricture.

Postoperative follow-up: Regular imaging monitoring, such as MR enterography or small bowel CTE, is recommended to evaluate the progression of residual lesions.

3.3. Strategy advantages

This strategy preserves intestinal function while minimizing the impact of severe lesions on bowel motility and absorption.

4. R2 strategy: Coexisting moderate and severe lesions, combined resection

4.1. Surgical indications

The R2 strategy is indicated mainly for moderate and severe lesions, where some bowel segments exhibit fibrotic stricture or localized obstruction. Imaging and endoscopic evaluations reveal alternating moderate and severe lesions, making surgical resection challenging.

4.2. Surgical approach

Resection range: Primarily targeting moderate to severe lesion segments while preserving relatively healthy bowel. When resecting moderate lesions in one stage carries a high risk of short bowel syndrome, partial preservation of moderate lesions can be considered. During surgery, bowel clamps can be used to physically expand the fibrotic segment, similar to endoscopic balloon dilation. If the patient's general condition is favorable, strictureplasty can be performed on the moderately stenotic bowel segment.

Anastomosis method: Side-to-side anastomosis

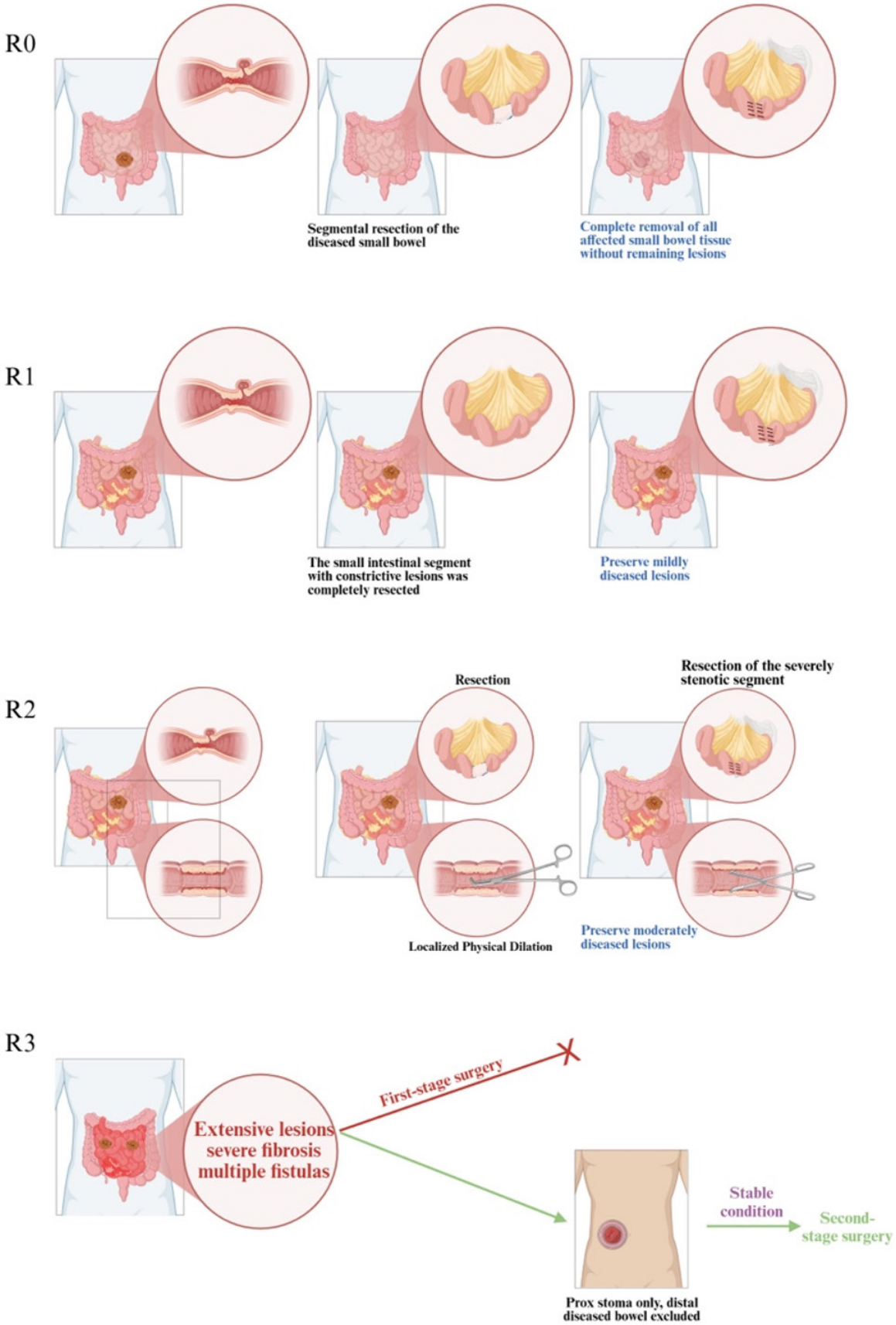

Figure 1. Precise classification of surgical strategies for small bowel Crohn's disease. When the patient's general condition is good, R0 surgery is preferred, followed by R1 and R2 approaches. When the patient's general condition is poor, intestinal surgery should be performed in two stages: first, the R3 strategy is employed to alleviate clinical symptoms and restore enteral nutrition, and after stabilization, an R0/R1/R2 surgery is considered.

is performed during digestive tract reconstruction to prevent postoperative anastomotic stricture. In cases with multiple anastomoses, creating a proximal stoma at an appropriate location can reduce the risk of anastomotic leakage.

Postoperative management: Combined anti-inflammatory and immunomodulatory treatment is used to prevent the progression of residual lesions.

4.3. Strategy advantages

The R2 strategy effectively removes severe lesions while minimizing loss of intestinal function. Postoperative maintenance with biologics can reduce risk of recurrence.

5. R3 strategy: Temporary stoma — Extensive complex lesions

5.1. Surgical indications

The R3 strategy is indicated for extensive and complex lesions with severe fibrosis and multiple fistulas, where resection carries a high risk of short bowel syndrome. It is also appropriate for patients with poor general condition and severe malnutrition, where single-stage resection is poorly tolerated.

5.2. Surgical approach

Surgical method: Perform a proximal stoma, leaving the distal diseased bowel segment unutilized. Once the patient's condition stabilizes, choose an R0, R1, or R2 strategy for second-stage surgery (*11*).

Postoperative management: Focus on nutritional support and stoma care. Resection of the diseased bowel segment can be considered after the patient's general condition stabilizes.

5.3. Strategy advantages

R3 surgery effectively alleviates symptoms caused by acute or extensive lesions and reduces surgery-related mortality. The second-stage surgery is highly flexible and can be adjusted according to the patient's recovery status.

6. Conclusion

For small bowel Crohn's disease, the four-tier surgical strategy (R0, R1, R2, R3) provides a scientific and standardized approach to surgical treatment. The advantage of this strategy lies in its precise selection of surgical methods based on severity of lesions, distribution characteristics, and individual patient differences, thus avoiding the limitations of traditional "one-size-fits-all" surgery. By clearly defining indications and surgical key points of each strategy, surgeons can flexibly address complex lesions in practice, especially in dealing with moderate to severe and extensive complex lesions, demonstrating enhanced practicality and flexibility.

The primary goal of CD intestinal surgery is to alleviate the patient's clinical symptoms. The long-term goal is to maximize duration of disease remission, while the ultimate goal is to achieve lifelong clinical non-recurrence for CD patients. This tiered strategy aligns with the current trend of personalized surgical treatment for CD, standardizing surgical practice and reducing risk of postoperative recurrence and short bowel syndrome caused by inappropriate surgical strategies. Future research should focus on long-term efficacy of different strategies and postoperative quality of life. Multicenter randomized controlled trials are needed to further validate the scientific and practical value of the tiered surgical approach. Leveraging big data and biomarker research to develop preoperative predictive models will facilitate more accurate preoperative surgical planning, advancing CD surgical strategies to a higher level.

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Letter

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Vimseltinib: A novel colony stimulating factor 1 receptor (CSF1R) inhibitor approved for treatment of tenosynovial giant cell tumors (TGCTs)

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SUMMARY: A tenosynovial giant cell tumor (TGCT) is a rare benign neoplasm arising from the tendon sheaths, bursae, or synovial lining of joints and is characterized by locally aggressive growth and the potential for recurrent disease. Surgery is still the main form of treatment for a TGCT, but these neoplasms, and most notably the diffuse type, exhibit a high proclivity for recurrence, thus highlighting the unmet clinical need for novel therapeutic modalities. At the same time, a subgroup of patients deemed ineligible for surgery are confronted with limited therapeutic alternatives, further underscoring the urgent need for innovative treatment paradigms. On February 14, 2025, the US Food and Drug Administration approved a new colony-stimulating factor 1 receptor (CSF1R) inhibitor, vimseltinib, for the treatment of symptomatic TGCTs in adult patients for whom surgical resection would likely result in severe functional limitations or serious complications. As the second-in-class CSF1R inhibitor approved for TGCTs, vimseltinib exhibits enhanced selectivity for CSF1R over pexidartinib, the first-in-class agent, suggesting potential translational benefits in safety profiles. The clinical utility of vimseltinib is anticipated to be further elucidated by real-world evidence and expanded clinical evaluations.

Keywords: CSF1R, TGCT, pexidartinib, vimseltinib, hepatotoxicity

A tenosynovial giant cell tumor (TGCT) is a non-malignant neoplasm originating from the tendon sheath of joints, bursae, or joint synovia (*1*). TGCT is a rare disease, with the highest incidence occurring in individuals ages 25 to 50, at a rate of approximately 43 per 1 million people (*2,3*). TGCT is classified into two types: localized and diffuse. The growth of the tumor can damage surrounding tissues and structures, causing pain, swelling, and restricted joint movement. Surgery is the primary treatment for TGCT, but recurrence is common, and especially in patients with the diffuse type (*3*). Persistent recurrence can lead to joint and surrounding tissue damage and degeneration, potentially resulting in severe disability. For a small subset of TGCT patients who are not eligible for surgery, therapeutic options are very limited, highlighting an urgent need for new treatment strategies.

On February 14, 2025, the US Food and Drug Administration approved a new colony stimulating factor 1 receptor (CSF1R) inhibitor, vimseltinib, for the treatment of symptomatic TGCTs in adult patients for whom surgical resection would likely result in severe functional limitations or serious complications (*4*). Due to chromosomal translocation, TGCT cells overexpress the *CSF1* gene, leading to excessive production of CSF1 (*5*). This protein recruits CSF1R-expressing cells, such as macrophages and other inflammatory cells, that make up the bulk of a TGCT (*1*). CSF1R inhibitors suppress CSF1R kinase activity, reducing the recruitment and activation of macrophages (*1*). By blocking downstream signaling pathways, these inhibitors suppress tumor cell proliferation, survival, and migration while inducing tumor cell apoptosis. The first CSF1R inhibitor, pexidartinib, was approved in 2019 for the treatment of TGCT and it exhibited significant antitumor activity with an overall response rate (ORR) of 38%, including a complete response rate of 15% and a partial response rate of 23% (*6*). However, its potential hepatotoxicity has limited its clinical use (*7*). In terms of specificity, pexidartinib inhibits not only CSF1R but also other tyrosine kinase receptors, such as FLT3, KIT, and PDGFR (*7*). In contrast, vimseltinib is a more selective CSF1R inhibitor, possibly leading to a better safety profile with fewer adverse reactions (*7*).

The results of a multicenter, randomized, double-blind, placebo-controlled, phase 3 clinical trial (MOTION, NCT05059262) showed that the drug vimseltinib demonstrated significant efficacy in

patients with TGCTs for whom surgical resection may have caused worsening functional limitation or severe morbidity (*8*). After patients received oral administration of vimseltinib at a dose of 30 mg twice a week during a 24-week treatment cycle, the ORR reached 40% (*8*). Of the patients, 85% of responders had a duration of response (DOR) of ≥ 6 months, and 58% of responders had a DOR of ≥ 9 months (*8*). At the 25-week assessment, the vimseltinib group had statistically significant improvements in functional outcomes compared to the placebo group (*8*). The most common adverse reactions are increased aspartate aminotransferase, periorbital edema, fatigue, rash, increased cholesterol, peripheral edema, facial edema, decreased neutrophils, decreased leukocytes, pruritus, and increased alanine aminotransferase (*4,8*).

Currently, multiple small molecule inhibitors targeting the CSF1R are in the clinical development stage, and some of them have entered phase III clinical trials, where they have demonstrated encouraging efficacy. For example, pimicotinib resulted in an overall response rate of 68% in patients with TGCTs in initial clinical trials (*9*). Meanwhile, antibody drugs targeting CSF1R (such as emactuzumab and cabiralizumab) are also undergoing clinical trials (*1*). A point worth noting is that the clinical value of these drugs has gone beyond the field of oncology, and exploratory studies in fields such as inflammatory diseases and bone-related diseases are underway. With the in-depth development of translational medicine research, these drugs are expected to achieve breakthroughs in precision treatment in multiple fields and provide innovative solutions for more refractory diseases.

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Letter

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Primary rectal malignant melanoma with schistosomiasis

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SUMMARY: Primary rectal malignant melanoma with schistosomiasis is extremely rare. To date, only a few cases have been reported in the literature. Due to its high mortality rate, most patients with rectal malignant melanoma die within five years of diagnosis. However, the etiology and optimal treatment strategies remain controversial. A 79-year-old female patient presented with intermittent hematochezia for 2 months. Digital rectal examination, computed tomography (CT) scan, and colonoscopy revealed a fleshy mass measuring 3 cm in diameter in the rectum. A biopsy confirmed a preoperative diagnosis of malignant melanoma of the rectum, and a radical rectal resection was performed. Histopathological examination of the surgical specimen confirmed malignant melanoma, and numerous *Schistosoma japonicum* organisms were identified within the tumor. The patient subsequently received Dabrafenib and Trametinib therapy and remained disease-free for 5 years postoperatively, with no evidence of recurrence. This case highlights the potential treatment strategies for this rare carcinoma and underscores the need for further investigation into the relationship between schistosomiasis and melanoma.

Keywords: primary rectal malignant melanoma, schistosomiasis, *Schistosoma japonicum*, rectal cancer, rare tumor, targeted therapy

Rectal malignant melanoma is a rare and aggressive neoplasm that primarily arises in melanocyte-rich tissues such as the skin, eyes, and meninges. However, it can also occur in the gastrointestinal tract. Melanomas account for 1-3% of gastrointestinal malignancies, and the majority are metastases from other primary sites (*1*). Therefore, the diagnosis of primary malignant melanoma should be made only when no other suspicious primary lesions are found. Primary rectal malignant melanoma can be easily misdiagnosed based solely on clinical or radiological examinations (*2*). Consequently, histological examination is essential to confirm the diagnosis. Schistosomiasis is caused by parasitic flatworms called schistosomes, which typically infect the intestinal tract, urinary tract, and liver (*3*). In this case, *Schistosoma japonicum* was identified within the tumor, and several studies have suggested that intestinal schistosomiasis might contribute to the development of intestinal tumors (*4*). However, whether schistosomiasis infection could be a potential risk factor for malignant melanoma remains unclear.

A 79-year-old female patient presented with intermittent hematochezia for 2 months. She reported no nausea, vomiting, abdominal pain, diarrhea, or weight loss. Her appetite and food intake were normal. Physical examination revealed no abnormal pigmentation of the skin, oral mucosa, or ocular regions. However, digital rectal examination identified a fleshy mass approximately 4 cm above the anal verge, occupying nearly one-third of the rectal lumen. Blood was observed on the examining glove following the procedure. Abdominal computed tomography (CT) scan (Figure 1A) and colonoscopy (Figure 1B) were performed, revealing a lesion originating in the rectum and extending proximally for approximately 3 cm. A biopsy was performed, and the tumor exhibited contact-induced bleeding. Histological examination of endoscopic biopsy samples was highly suggestive of malignant melanoma of the rectum. Following multidisciplinary discussion in the gastrointestinal (GI) division, a radical anterior resection with total mesorectal excision and sigmoid-rectal anastomosis was performed. Written informed consent was obtained from the patient for publication of this case report and the accompanying images. Microscopically, the tumor was composed of epithelioid melanoma cells with prominent nucleoli (original magnification: 400×, Figure 2A). Notably, a large number of *Schistosoma japonicum* organisms were identified within the tumor (Figure 2B). Immunohistochemical analysis showed tumor cell positivity for Melan-A (original magnification:

400×, Figure 2A), S100 protein (Figure 2B), HMB-45 (Figure 2C), and vimentin. Genetic analysis revealed a V600 mutation in the BRAF gene. Consequently, postoperative treatment included Dabrafenib and Trametinib, along with praziquantel for anti-schistosomal therapy. The patient recovered well after surgery and remained alive and disease-free 5 years postoperatively, with no evidence of recurrence.

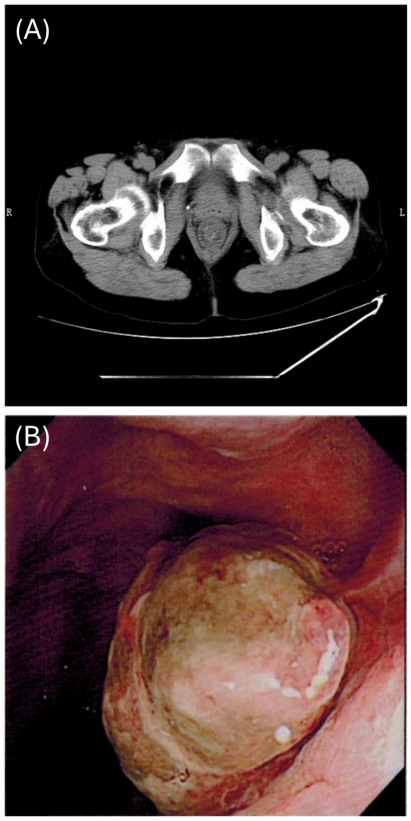

Figure 1. (A) Abdominal computed tomography scan showing a malignant rectal lesion, and (B) Colonoscopic view of the rectal tumor.

Currently, surgical resection remains the most effective treatment for this malignancy. However, due to the abundant vascular and lymphatic supply of the gastrointestinal tract, which facilitates early metastasis, the tumor is often not amenable to complete resection at the time of diagnosis, resulting in a high mortality rate from widespread metastases (*5*). The prognosis of primary malignant melanoma of the rectum remains extremely poor, with a 5-year survival rate of only 10-30% (*6*). Prognostic factors include patient age, disease stage, tumor location, lymph node involvement, depth of invasion, and the timeliness of diagnosis. Some studies have reported favorable outcomes when the depth of tumor invasion is less than 4 mm (*7*). Although melanomas are generally considered resistant to radiotherapy and conventional chemotherapy, approximately 10-20% of patients respond to certain chemotherapeutic agents, including dacarbazine, temozolomide, and cisplatin. For patients with mutations in the c-KIT gene or the BRAF V600 mutation, targeted therapies are indicated. These include c-KIT inhibitors such as imatinib, and BRAF inhibitors such as vemurafenib, dabrafenib, and trametinib (*8*).

Although the etiology of primary rectal malignant melanoma remains unclear, numerous studies have demonstrated that intestinal schistosomiasis can contribute to the development of colorectal cancer. In a study by Mingchai (*9*), 289 out of 454 colorectal cancer patients (63.7%) from schistosomiasis-endemic regions were found to have concomitant schistosomiasis.

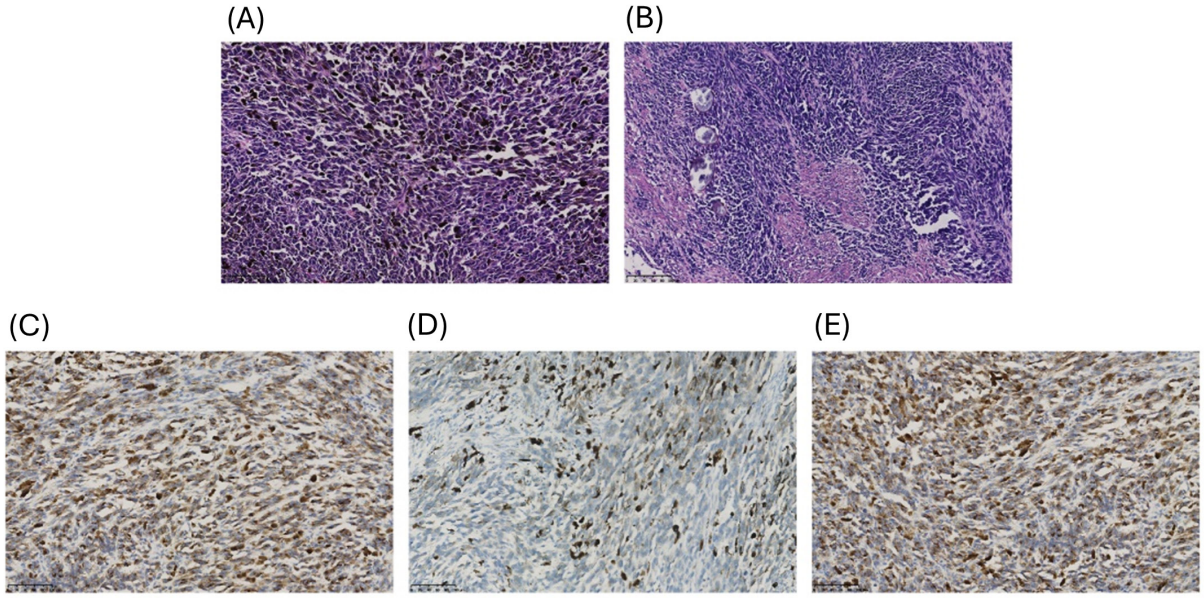

Figure 2. (A-B) H&E staining of tumor tissue at 400× magnification, highlighting epithelioid melanoma cells with prominent nucleoli (A) and the presence of *Schistosoma japonicum* within the tumor (B); (C-E) Immunohistochemical staining of tumor cells at 400× magnification, demonstrating expression of (C) Melan-A, (D) S100, and (E) HMB-45.

This suggests a potential association between schistosomiasis and colorectal carcinoma in endemic areas. Other studies have also proposed Schistosoma as a significant risk factor for colorectal cancer. Wang Z *et al*. (*4*) reported that schistosomiasis may promote colorectal tumorigenesis by influencing the polarization of tumor-associated macrophages toward the M2 phenotype, which plays a key role in tumor progression. Schistosoma eggs embedded in the submucosa can trigger chronic inflammation, polyp formation, mucosal atypical hyperplasia, and ultimately carcinoma. Interestingly, inflammatory features have been observed in melanoma tissues across all clinical stages (*10*). In this case, *Schistosoma japonicum* was identified within the rectal malignant melanoma. This raises the hypothesis that schistosomiasi may contribute to the tumor microenvironment in rectal melanoma. However, further studies are required to clarify this potential relationship.

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Letter

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A case of mitochondrial diabetes mellitus with successful therapeutic response following the initiation of imeglimin

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SUMMARY: Mitochondria are present in cells throughout the body and play a crucial role in energy production. They contain their own DNA, and mutations in this DNA can lead to a reduction in pancreatic beta cells and decreased insulin secretion, contributing to the development of diabetes. Insulin therapy has been considered a rational treatment, as the primary issue is impaired insulin secretion, but it primarily serves as a coping mechanism. Recently, however, imeglimin – a drug believed to influence various mitochondria-mediated processes – has been introduced and is expected to offer therapeutic benefits for mitochondrial diabetes. Here, we report a case of successful glycemic control following the addition of imeglimin in a patient with mitochondrial diabetes mellitus. After starting imeglimin, the patient's blood glucose levels stabilized, and he continues treatment. While the molecular target of imeglimin remains unknown, it is possible that the drug may offer significant benefits for patients with mitochondrial diabetes mellitus.

Keywords: mitochondrial diabetes mellitus, imeglimin, intractable disease

Mitochondria are present in the cells throughout the body and play a crucial role in energy production, among other functions. Mitochondrial diseases refer to a group of disorders caused by mitochondrial dysfunction. In Japan, mitochondrial diseases are classified as designated intractable diseases. Mitochondria have their own DNA, mutations in this DNA can lead to a reduction in pancreatic beta cells and decreased insulin secretion, where they contribute to diabetes (*1*). Mitochondrial diabetes was first reported in 1992 as being associated with the 3243A>G mutation in the mitochondrial gene (*2*). Mitochondrial dysfunction reduces insulin sensitivity, and insulin resistance is thought to contribute to the impaired glucose tolerance observed in mitochondrial diabetes (*3,4*). Pathology is also associated with a high rate of sensorineural hearing loss and increases the risk of complications such as cardiomyopathy and encephalopathy, making early diagnosis and appropriate treatment crucial. Insulin therapy has been considered a reasonable treatment for the disease, as it primarily involves impaired insulin secretion; however, it serves only as a coping therapy. Recently, imeglimin, a drug that reduces the accumulation of dysfunctional mitochondria and may help maintain β-cell function and achieve effective glycemic control in type 2 diabetes, has been introduced (*5*) and is expected to offer therapeutic benefits for mitochondrial diabetes. In this study, we report a case of successful glycemic control in a patient with mitochondrial diabetes mellitus treated with imeglimin.

The patient in this case is a man in his 40s. In his 20s, the patient was diagnosed with diabetes and visited a nearby hospital. He was started on oral diabetes medications but discontinued them on his own without further follow-up. In his mid-30s, his health deteriorated, and he visited the nearby hospital again. At that time, his blood glucose was consistently over 450 mg/dL, and his HbA1c was 12.6%. At the first visit, the prescribed insulin degludec, glimepiride, anagliptin, voglibose, followed by observation. The patient was found to have no neuropathy or diabetic retinopathy and was monitored for approximately 6 months. A slight increase in HbA1c was observed; therefore, empagliflozin was introduced. While HbA1c stabilized, the patient began complaining of hearing loss. Genetic testing confirmed moderate sensorineural hearing loss and the 3243A>G mutation, diagnosing him with mitochondrial diabetes mellitus, which is classified as an intractable disease. A discrepancy between HbA1c and blood glucose levels was observed, prompting a detailed blood glucose evaluation using FreeStyle Libre Pro®. The results indicated that the falsely high HbA1c levels were due to diurnal variations in blood glucose. It was determined that blood glucose levels should be evaluated not only by HbA1c but also by glycoalbumin (GA). Considering the patient's medication adherence and other factors,

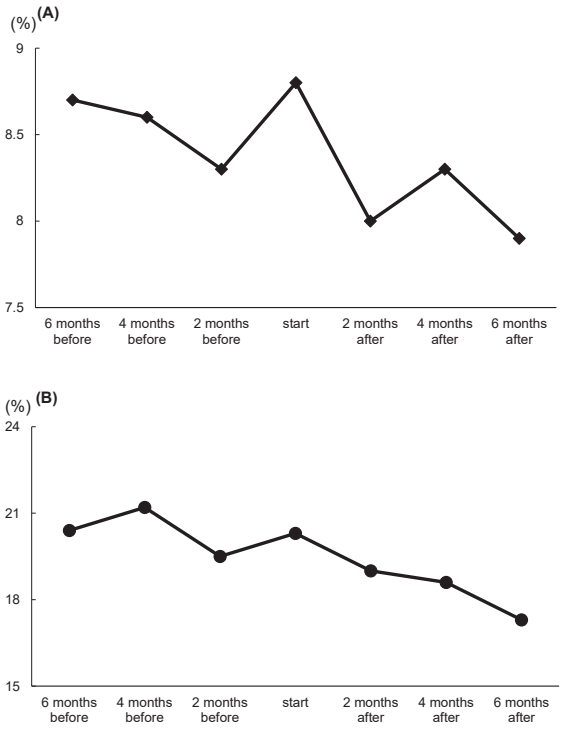

Figure 1. Blood glucose levels were assessed by (A) HbA1c and (B) GA. HbA1c, hemoglobinA1c; GA, glycoalbumin.

insulin degludec was adjusted to units, and sitagliptin, empagliflozin, mitiglinide, and voglibose were prescribed from 9 months prior to starting imeglimin. Despite these adjustments, the patient's blood glucose levels remained unstable and elevated. Therefore, imeglimin 2,000 mg/day was added to the regimen. Figure 1 shows the trends in HbA1c and GA from 6 months before to 6 months after starting imeglimin. Six months after starting imeglimin, HbA1c decreased from 8.8% to 7.9% and GA from 20.3% to 17.3%. Side effects, including nausea and abdominal discomfort, were noted but improved, and the prescription was continued. Consent for reporting was obtained from the patient.

This case involves a patient with mitochondrial diabetes mellitus who was started on imeglimin, resulting in stabilized blood glucose levels and a favorable therapeutic effect. Most patients require insulin therapy owing to a decrease in pancreatic beta cells and insulin secretion. Early initiation of intensive insulin therapy is important to reduce the burden on the remaining pancreatic beta cells. Mitochondrial dysfunction can also lead to lactic acid accumulation, increasing the risk of lactic acidosis. Therefore, strenuous exercise is restricted, and the use of biguanides is generally considered undesirable (*6*), making it challenging to address insulin resistance in mitochondrial diabetes.

Imeglimin is a hypoglycemic agent with both pancreatic and extrapancreatic roles. Although the molecular target of imeglimin is still unknown, various studies have been conducted on its expression. Imeglimin has been reported that enhancing mitochondrial function by increasing basic mitochondrial respiration (*7*). Furthermore, imeglimin may be beneficial for patients with mitochondrial diabetes because of its ability to reduce mitochondrial oxidative stress and increase ATP production (*8*). However, imeglimin is a new drug that was recently approved in 2021 (*7*), and this study did not evaluate the benefit of long-term continuous administration or improvement of symptoms, such as hearing loss, other than glycemic control. Therefore, further investigation is needed to evaluate the usefulness of imeglimin in mitochondrial diabetes mellitus. Finally, as the mechanism of action of imeglimin is still unknown and information about the drug is limited to the package insert and interview form, we hope that this case study provides valuable insights for future interventions by healthcare professionals treating patients with mitochondrial diabetes mellitus.

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