

ISSN 2186-3644 Online ISSN 2186-361X

IRDR

Intractable & Rare Diseases Research

Volume 15, Number 3
August 2026



www.irdrjournal.com

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Intractable & Rare Diseases Research



ISSN: 2186-3644
Online ISSN: 2186-361X
CODEN: IRDRA3
Issues/Year: 4
Language: English
Publisher: IACMHR Co., Ltd.

Intractable & Rare Diseases Research is one of a series of peer-reviewed journals of the International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) Group and is published quarterly by the International Advancement Center for Medicine & Health Research Co., Ltd. (IACMHR Co., Ltd.) and supported by the IRCA-BSSA.

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Review

- 200-218** **Japanese intractable and rare dermatologic diseases: From the official skin-centered nanbyo core to an illustrative mechanism-based framework**
Yue Han, Tian-Ge Qin, Peipei Song
- 219-231** **Mitochondrial DNA A3243G variant-associated MELAS: Recent advances in clinical trials, therapeutic interventions, and disease-modifying strategies**
Kuan-Yu Chu
- 232-246** **Phenotypic variability of Fragile-X syndrome in Asian population: A systematic review**
Christina L Minar Napitupulu, Jeslyn Tenglawan, Agustini Utari, Tri Indah Winarni, Nydia Rena Benita Sihombing

Original Article

- 247-253** **Cutaneous lymphomas reported following the use of dupilumab, nemolizumab, tralokinumab, or lebrikizumab: A case investigation based on JADER database and disproportionality analysis**
Hiroyuki Tanaka, Toshihiro Ishii

Brief Report

- 254-259** **Generation and characterization of induced pluripotent stem cells from a patient with classic Fabry disease**
Guowei Li, Jing Luan, Zihan Li, Yali Yang, Chonghao Shao, Jing Wang, Zhenzhong Han, Quanlin Wang, Yuan Zhu, Che Yu, Yazhou Cui, Jinxiang Han
- 260-267** **Prenatal molecularly supported diagnosis of a fetus with urinary malformation caused by novel compound heterozygous variants in the *FAM149B1* gene**
Jinyu Liu, Yi Wu, Shixuan Xu, Yirui Qin, Guoqiang Li, Yiyao Chen, Lulu Li, Fang Liu, Shuyuan Li, Niu Li, Jian Wang

Correspondence

- 268-272** **Traditional Chinese medicine research on rare diseases: Publication trends and future perspectives**
Li-ming Chen, Xin-yi Chen, Bo-wen Liu, Ying Mu, Qi Kang, Huan-gan Wu
- 273-276** **Fulminant myocarditis and sudden death associated with thymoma-related autoimmunity**
Ying Liu, Xue Yin, Baitong Wang, Yuqing Zhao, Xianglin Chu, Liewen Pang, Chongbo Zhao, Jian Li, Yiming Wang, Sushan Luo

277-279	Skeletal involvement in Joubert syndrome: From low bone mineral density to severe secondary osteoporosis <i>Tatsiana Babina, Bogumiła Alicja Górczewska, Elżbieta Jakubowska-Pietkiewicz</i>
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Japanese intractable and rare dermatologic diseases: From the official skin-centered nanbyo core to an illustrative mechanism-based framework

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SUMMARY: Japan's designated intractable disease (nanbyo) framework combines a population threshold with unresolved pathogenesis or treatment, long-term care needs, objective diagnostic criteria, and severity-linked assistance with medical expenses. We conducted a targeted narrative review of official Japanese sources and biomedical literature through August 3, 2026. Evidence ranged from regulatory approvals and randomized trials to observational, case-report, and preclinical data, so therapeutic maturity was assessed separately from mechanistic plausibility. We distinguished the 12 official skin/connective-tissue entries from six purposively selected designated entries or disease groups with decisive cutaneous phenotypes or Japan-specific translational value. The resulting 18-entry framework is illustrative, not exhaustive. It highlights Japan-specific biology or evidence regarding xeroderma pigmentosum, acquired idiopathic generalized anhidrosis, DPP-4 inhibitor-related bullous pemphigoid, generalized pustular psoriasis, cold-medicine-related SJS/TEN, anti-MDA5 dermatomyositis, and Nakajo-Nishimura syndrome. Recently approved indications or uses include dupilumab for adult bullous pemphigoid, IL-36 receptor blockade, topical COL7A1 gene delivery, autologous gene-corrected epidermal sheets, MEK inhibition, IL-1 blockade, and topical mTOR inhibition; JAK-interferon strategies remain investigational. We contend that Japan's designation infrastructure could become an interoperable translational platform linking natural-history cohorts, endotype-defined enrollment, mechanism-aligned endpoints, and multinational adaptive trials.

Keywords: autoimmune blistering disease, epidermolysis bullosa, generalized pustular psoriasis, interferonopathy, precision medicine, orphan-disease policy

1. Introduction

Rare dermatologic disorders span immunology, genetics, oncology, toxicology, rheumatology, neurology, and metabolic medicine. This breadth is clinically consequential: the skin may be the dominant organ, an early diagnostic window, or only one organ affected by a lethal multisystem disease. Japan's designated intractable disease system provides an unusually coherent counterweight because it links disease definitions to public policy, longitudinal documentation, and organized research. As of April 2026, 348 diseases are designated under the national framework (1,2). The dermatologic value of this system is not limited to the 12 conditions displayed under the official skin and connective-tissue category (3); several other designated diseases have highly informative cutaneous phenotypes and are

managed jointly by dermatologists and other specialists.

The present review addresses three gaps in the existing literature. First, it explains what is specifically Japanese about nanbyo dermatology and why direct comparison with prevalence-based rare-disease frameworks in the United States and European Union is informative but incomplete. Second, it distinguishes the 12 official skin-centered entries from six selectively expanded designated entries or disease groups in an illustrative mechanism-based framework rather than claiming that they represent an exhaustive spectrum. Third, it links molecular architecture to clinical intractability, biomarker selection, trial endpoints, and recent therapeutic translation. A targeted narrative search strategy and explicit selection criteria are provided to make the scope and evidentiary status transparent. The central question is not whether this framework

constitutes a complete disease catalogue, but whether an official core plus a deliberately illustrative mechanistic layer can more clearly connect policy, disease biology, and trial design.

2. Methods and search strategy

This targeted narrative review searched PubMed, Web of Science, J-STAGE, and Ichushi-Web from database inception through August 3, 2026. Representative queries combined ('nanbyo' OR 'designated intractable disease' OR 'rare dermatology') with the name of, gene for, biomarker of, or therapy for each candidate entry or disease group; syntax was adapted to each database. Official and regulatory sources were searched separately, including the Ministry of Health, Labour and Welfare, the Nanbyo Information Center, the Pharmaceuticals and Medical Devices Agency (PMDA), the Japan Registry of Clinical Trials (JRCT), UMIN-CTR, and ClinicalTrials.gov. English- and Japanese-language sources were included. The final literature search and regulatory-status verification were completed on August 3, 2026.

Acceptable evidence consisted of official diagnostic and policy documents, practice guidelines, randomized trials, prospective or retrospective observational studies, post-marketing surveillance, and selected case reports or preclinical studies when they provided unique mechanistic or emerging therapeutic information. Evidence was classified descriptively as regulatory/approved, randomized clinical, observational or post-marketing, case report/series, or preclinical/mechanistic. When sources conflicted, current regulatory documents and peer-reviewed primary reports were prioritized; registry records were used to ascertain trial status and obtain results not yet available in a full publication. Duplicate reports, non-designated conditions without a decisive cutaneous phenotype or Japan-specific translational rationale, and sources that did not provide material information on the mechanism, diagnosis, treatment, or trial design were excluded. Because this was a targeted narrative review, no quantitative synthesis, PRISMA flow count, or formal risk-of-bias grading was undertaken.

Three units of analysis were distinguished: an official designated entry (an administrative unit), a disease group (clinically or genetically related conditions), and a mechanistic phenotype (a prognostically or therapeutically relevant subset within an entry). The 12 official skin and connective-tissue entries were retained without discretionary selection. The six expanded entries were purposively selected from designated diseases with prominent skin manifestations to illustrate complementary mechanistic and translational models. The three criteria - (i) a decisive cutaneous phenotype, (ii) molecular, epidemiologic, clinical, regulatory, or policy relevance to Japan, and (iii) a mechanism-aligned therapy or trial model with broader translational value -

were used to make the selection transparent rather than to generate an exhaustive or uniquely reproducible disease set. The candidate pool also included systemic sclerosis, systemic lupus erythematosus, and vasculitides; these were not selected because their inclusion would duplicate connective-tissue or vascular mechanisms already represented and because their selection would broaden the article beyond a tractable conceptual demonstration. Table 1 indicates the unit of analysis and rationale for each included expansion.

3. Scope and organizing principles

The official core used here contains 12 designated entries or disease groups: neurofibromatosis (NF), pemphigus, inherited epidermolysis bullosa (EB), generalized pustular psoriasis (GPP), Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), xeroderma pigmentosum (XP), congenital ichthyosis, Hailey-Hailey disease (HHD), pemphigoid including epidermolysis bullosa acquisita (EBA), acquired idiopathic generalized anhidrosis (AIGA), and pseudoxanthoma elasticum (PXE) (3). These entries remain the anchor because their diagnostic criteria and severity classifications are explicitly tied to Japan's designated-disease infrastructure.

The illustrative expanded layer includes Behcet disease, the polymyositis/dermatomyositis (PM/DM) designated entry with a focused discussion of anti-MDA5 dermatomyositis, tuberous sclerosis complex (TSC), cryopyrin-associated periodic syndrome (CAPS), Nakajo-Nishimura syndrome/proteasome-associated autoinflammatory syndrome (NNS/PRAAS), and STING-associated vasculopathy with onset in infancy (SAVI). These are representative choices rather than a complete list of designated diseases with important skin manifestations. Each was purposively selected to illustrate a complementary mechanistic or translational model, as summarized in Table 1. SAVI is especially timely because it was placed on the Japanese designated nanbyo list as disease no. 345 as of April 2025 (1). The official 12-entry core and six illustrative expanded entries or disease groups are summarized in Table 2. In this review, PM/DM is treated as an official administrative entry, anti-MDA5 dermatomyositis is treated as a mechanistic phenotype within that entry, CAPS is treated as a disease group, and NNS/PRAAS is treated as a disease spectrum.

4. What is distinctive about the Japanese nanbyo model?

4.1. A clinical-welfare-research framework rather than a prevalence-only label

In the United States, the Orphan Drug Act generally defines a rare disease as one affecting fewer than 200,000 people (4). In the European Union, orphan

Table 1. Transparency matrix for the six purposively selected illustrative expanded entries or disease groups

Expanded entry/disease group	Decisive cutaneous phenotype	Japan-specific dimension	Mechanism-aligned therapy or trial model	Illustrative role in framework	Analysis unit
Behcet disease	Oral/genital ulcers, erythema nodosum-like lesions, pathergy	Japanese cohort and prespecified trial-subgroup evidence	Apremilast and anti-TNF strategies	Geographic, neutrophilic, and multisystem inflammatory model	Disease
PM/DM entry (anti-MDA5 DM focus)	Palmar papules, ulcers, livedoid change	Japanese/East Asian cohort and prognostic evidence	Early combination/rescue therapy; JAK approaches investigational	Skin-to-lung prognostic warning model	Official entry plus mechanistic phenotype
Tuberous sclerosis complex	Facial angiofibromas, shagreen patches, periungual fibromas	Japan-specific topical drug development and clinical evaluation	Direct mTORC1 inhibition	Topical pathway-directed therapy model	Disease
CAPS	Urticaria-like neutrophilic eruption	Japanese nationwide cohort and post-marketing evidence	IL-1 blockade	Inflammasome-to-IL-1 treatment and surveillance model	Disease group
NNS/PRAAS	Pernio-like/nodular lesions and lipomuscular atrophy	Japanese founder biology	JAK-interferon targeting remains investigational	Japan-specific founder interferonopathy model	Disease spectrum
SAVI	Acral vasculitis, ulceration, tissue loss	Japan-specific policy and designation context	JAK or IFNAR-directed strategies remain investigational	Newly designated interferon vasculopathy model	Disease

Abbreviations: CAPS, cryopyrin-associated periodic syndrome; DM, dermatomyositis; IFNAR, interferon- α/β receptor; IL-1, interleukin-1; JAK, Janus kinase; MDA5, melanoma differentiation-associated protein 5; mTORC1, mechanistic target of rapamycin complex 1; NNS, Nakajo-Nishimura syndrome; PM, polymyositis; PRAAS, proteasome-associated autoinflammatory syndrome; PSMB8, proteasome subunit β type 8; RELIEF, phase 3 apremilast trial in Behcet syndrome; RP-ILD, rapidly progressive interstitial lung disease; SAVI, STING-associated vasculopathy with onset in infancy; TNF, tumor necrosis factor.

Table 2. Official 12-entry skin-centered core and six illustrative expanded designated entries or disease groups

Designated entry/ disease group	Scope	Disease-defining mechanism	Japan-specific dimension
Neurofibromatosis entry (NF1/NF2-related schwannomatosis)	Official skin-centered entry	NF1/NF2 tumor suppressor loss, RAS-MAPK or merlin pathway	Japanese clinical evidence: pediatric MEK-inhibitor study; NF2 retained at entry level
Pemphigus	Official skin-centered entry	Anti-Dsg IgG, desmosome disassembly, B/T-cell memory	Japanese prospective clinical evidence: rituximab and BTK-inhibitor studies
Inherited EB	Official skin-centered entry	Structural protein deficiency and chronic wound-fibrosis-cancer axis	Japan regulatory context: Vyjuvek approved following PMDA review
GPP	Official skin-centered entry	IL-36-neutrophil feed-forward inflammation	Japan-specific biology and clinical evidence: recurrent IL36RN variants and spesolimab experience
SJS	Official skin-centered entry	Drug/HLA-specific cytotoxic epithelial injury	Japan-specific pharmacogenetics: cold-medicine ocular phenotype and HLA-A*02:06
TEN	Official skin-centered entry	Extensive granulysin-mediated epidermal necrosis	Population-specific pharmacogenetic context
XP	Official skin-centered entry	Nucleotide excision repair failure and UV mutagenesis	Japan-specific biology and epidemiology: high incidence and XPA founder variant
Congenital ichthyosis	Official skin-centered entry	Lipid, cornification, or protease-regulatory defects	Global mechanism; Japan relevance is designation and genotype/endotype care
Hailey-Hailey disease	Official skin-centered entry	ATP2C1/SPCA1 calcium-manganese homeostasis failure	Global mechanism; no uniquely Japanese biology is claimed
Pemphigoid/EBA	Official skin-centered entry	BMZ autoantibodies, complement/type 2 inflammation, anchoring-fibril injury	Japan-specific prescribing and pharmacogenetic evidence: DPP-4 inhibitor-associated BP
AIGA	Official skin-centered entry	Putative immune- and stress-associated eccrine dysfunction	Japanese criteria, cohorts, and translational evidence
PXE	Official skin-centered entry	ABCC6-dependent ATP/pyrophosphate deficiency	Global systemic mechanism recognized through a skin-centered designated entry
Behcet disease	Illustrative expanded entry	HLA/ERAP1, neutrophils, Th1/Th17, endothelial injury	Japanese cohort and clinical-trial subgroup evidence
PM/DM entry (focus: anti-MDA5 DM)	Illustrative expanded entry	RNA-sensor autoimmunity, type I interferon, macrophage/endothelial injury	Japanese/East Asian cohort evidence for high-risk RP-ILD
Tuberous sclerosis complex	Illustrative expanded entry	TSC1/TSC2 loss and mTORC1 activation	Japan-specific drug development and regulatory context: topical sirolimus
CAPS	Illustrative expanded entry	NLRP3 inflammasome and IL-1 β excess	Japanese nationwide cohort and post-marketing evidence

Abbreviations: ABCC6, ATP-binding cassette subfamily C member 6; AIGA, acquired idiopathic generalized anhidrosis; ATP, adenosine triphosphate; ATP2C1, ATPase secretory pathway Ca²⁺ transporting 1; BMZ, basement membrane zone; BP, bullous pemphigoid; BTK, Bruton's tyrosine kinase; CAPS, cryopyrin-associated periodic syndrome; DM, dermatomyositis; DPP-4, dipeptidyl peptidase-4; Dsg, desmoglein; EB, epidermolysis bullosa; EBA, epidermolysis bullosa acquisita; ERAP1, endoplasmic reticulum aminopeptidase 1; GPP, generalized pustular psoriasis; HLA, human leukocyte antigen; IgG, immunoglobulin G; IL, interleukin; IL36RN, interleukin 36 receptor antagonist; MDA5, melanoma differentiation-associated protein 5; MEK, mitogen-activated protein kinase kinase; mTORC1, mechanistic target of rapamycin complex 1; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2; NLRP3, NLR family pyrin domain containing 3; NNS, Nakajo-Nishimura syndrome; PM, polymyositis; PMDA, Pharmaceuticals and Medical Devices Agency; PRAAS, proteasome-associated autoinflammatory syndrome; PSMB8, proteasome subunit β type 8; PXE, pseudoxanthoma elasticum; RAS-MAPK, rat sarcoma-mitogen-activated protein kinase; RNA, ribonucleic acid; RP-ILD, rapidly progressive interstitial lung disease; SAVI, STING-associated vasculopathy with onset in infancy; SJS, Stevens-Johnson syndrome; SPCA1, secretory pathway Ca²⁺/Mn²⁺-ATPase 1; STING1, stimulator of interferon response cGAMP interactor 1; TEN, toxic epidermal necrolysis; Th1/Th17, T helper 1/7; TSC1/TSC2, tuberous sclerosis complex 1/2; UV, ultraviolet; XP, xeroderma pigmentosum; XPA, xeroderma pigmentosum group A.

Table 2. Official 12-entry skin-centered core and six illustrative expanded designated entries or disease groups (continued)

Designated entry/ disease group	Scope	Disease-defining mechanism	Japan-specific dimension
NNS/PRAAS	Illustrative expanded entry	PSMB8 immunoproteasome failure, proteotoxic stress, type I interferon	Japan-specific founder biology
SAVI	Illustrative expanded entry	STING1 gain of function and type I interferon vasculopathy	Japan policy and designation context

Abbreviations: ABCC6, ATP-binding cassette subfamily C member 6; AIGA, acquired idiopathic generalized anhidrosis; ATP, adenosine triphosphate; ATP2C1, ATPase secretory pathway Ca^{2+} transporting 1; BMZ, basement membrane zone; BP, bullous pemphigoid; BTK, Bruton's tyrosine kinase; CAPS, cryopyrin-associated periodic syndrome; DM, dermatomyositis; DPP-4, dipeptidyl peptidase-4; Dsg, desmoglein; EB, epidermolysis bullosa; EBA, epidermolysis bullosa acquisita; ERAP1, endoplasmic reticulum aminopeptidase 1; GPP, generalized pustular psoriasis; HLA, human leukocyte antigen; IgG, immunoglobulin G; IL, interleukin; IL36RN, interleukin 36 receptor antagonist; MDA5, melanoma differentiation-associated protein 5; MEK, mitogen-activated protein kinase kinase; mTORC1, mechanistic target of rapamycin complex 1; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2; NLRP3, NLR family pyrin domain containing 3; NNS, Nakajo-Nishimura syndrome; PM, polymyositis; PMDA, Pharmaceuticals and Medical Devices Agency; PRAAS, proteasome-associated autoinflammatory syndrome; PSMB8, proteasome subunit β type 8; PXE, pseudoxanthoma elasticum; RAS-MAPK, rat sarcoma-mitogen-activated protein kinase; RNA, ribonucleic acid; RP-ILD, rapidly progressive interstitial lung disease; SAVI, STING-associated vasculopathy with onset in infancy; SJS, Stevens-Johnson syndrome; SPCA1, secretory pathway $\text{Ca}^{2+}/\text{Mn}^{2+}$ -ATPase 1; STING1, stimulator of interferon response cGAMP interactor 1; TEN, toxic epidermal necrolysis; Th1/Th17, T helper 1/T helper 17; TSC1/TSC2, tuberous sclerosis complex 1/2; UV, ultraviolet; XP, xeroderma pigmentosum; XPA, xeroderma pigmentosum group A.

designation usually requires a life-threatening or chronically debilitating condition affecting no more than 5 in 10,000 people, along with the absence of a satisfactory method or a demonstration of significant benefit (5). These definitions are primarily instruments for product development and regulatory incentives. Japan's designated intractable disease framework is not a prevalence-only taxonomy. Its formal conditions combine a patient population generally smaller than approximately 0.1% of the national population with an insufficiently elucidated pathogenesis, lack of an established treatment, a need for long-term care, and objective diagnostic criteria (1,2,6). Assistance with medical expenses is not automatic for every diagnosed patient: eligibility is linked to disease-specific severity criteria, with a mild-but-high-cost exception for patients whose repeated expenses exceed the prescribed threshold (2). Thus, nanbyo is both an epistemic category and a clinical-welfare-research framework.

This design has two potential benefits. First, the clinical survey form can be used to standardize core variables across institutions and enable disease-specific registries. Second, national policy research groups can iteratively revise diagnostic criteria, severity classifications, and care pathways. These are plausible institutional advantages; this review did not identify direct comparative evidence that designation itself improves trial efficiency or patient outcomes. However, the system also creates an administrative boundary problem because dermatologically important diseases may appear under rheumatologic, neurologic, pediatric, or autoinflammatory categories rather than under the skin category. The two-layer framework used here is an author-defined analytical solution that preserves the official core while recognizing cross-specialty skin biology. Drug-specific post-approval surveillance is

implemented through separate PMDA and company pharmacovigilance systems and should not be treated as an automatic consequence of nanbyo designation. The principal structural differences between the Japanese, US, and EU frameworks are summarized in Table 3.

4.2. Japan-specific epidemiology, pharmacogenetics, and disease recognition

Specificity to Japan is assigned here only when supported by one of three dimensions: population biology, Japanese cohort or clinical evidence, or a Japan-specific regulatory and policy context. Japanese clinical guidelines report an XP incidence of approximately 1 in 22,000 (8), which is substantially higher than estimates in many Western populations, while a splice-site founder variant in xeroderma pigmentosum group A (XPA) accounts for a large proportion of Japanese mutant alleles and is carried heterozygously by nearly 1% of the population (7,8). AIGA has been characterized largely through Japanese case series and national research efforts, making Japan a central source of disease definitions and therapeutic experience (9,10). Japanese studies also identified a clinically less inflammatory form of dipeptidyl peptidase-4 inhibitor-associated BP and an association with *HLA-DQB1*03:01* (11-13). These observations are particularly relevant in an aging population with high exposure to antidiabetic therapy.

Additional examples extend across immunology and pharmacogenetics. Recurrent IL36RN variants helped establish GPP as a distinct autoinflammatory disease in Japanese cohorts (14,15). Cold-medicine-related SJS/TEN with severe ocular complications has been found to be associated with HLA, including *HLA-A*02:06*, in contrast to the classic carbamazepine-HLA-B*15:02 signal emphasized in parts of Southeast Asia (16,17).

Table 3. Comparison of the Japanese nanbyo framework with US and EU rare-disease/orphan frameworks

Domain	Japan	United States	European Union
Primary concept	Population threshold plus unresolved pathogenesis or treatment, long-term care, objective diagnosis, severity, and public support	Population threshold for a rare disease under the Orphan Drug Act	Prevalence plus seriousness and lack of satisfactory therapy/significant benefit for orphan designation
Current threshold/list	Disease-specific designation; patients generally < approximately 0.1% of the national population	< 200,000 affected persons in the United States	<= 5 per 10,000 in the European Union
Direct patient support	Severity-linked medical-expense subsidy, with a mild-but-high-cost exception	Not intrinsic to rare-disease definition	Not intrinsic to orphan designation
Standardized clinical data	Disease-specific diagnostic criteria, severity classifications, and clinical survey forms	Variable by disease/registry	Variable by disease/registry
Research structure	National policy research groups and disease-linked surveys	NIH/FDA programs and disease registries	European Reference Networks, EMA, and registries
Dermatology-specific limitation	Skin-relevant diseases are distributed across administrative categories	Rare disease and dermatology classifications remain separate	Orphan designation is product-specific, not a clinical disease taxonomy
Post-approval surveillance	Separate PMDA/company pharmacovigilance; not automatic under nanbyo designation	FDA/company pharmacovigilance; separate from rare-disease definition	EMA/company pharmacovigilance; separate from orphan designation

Abbreviations: EMA, European Medicines Agency; EU, European Union; FDA, Food and Drug Administration; NIH, National Institutes of Health; PMDA, Pharmaceuticals and Medical Devices Agency; US, United States.

Anti-MDA5 dermatomyositis is highly relevant in East Asian practice because rapidly progressive interstitial lung disease may overshadow prognosis despite limited muscle disease (18-20). NNS is a proteasome disorder initially identified in Japan that connects immunoproteasome failure to type I interferon biology (21,22). Together, these examples show that population genetics, prescribing patterns, environmental exposure, and national identification of cases can ascertain disease endotypes that are not fully captured by global averages.

5. Autoimmune blistering diseases

5.1. Pemphigus: Desmosomal autoimmunity, signaling, and immune memory

Mechanism. Pemphigus vulgaris and pemphigus foliaceus are defined by pathogenic IgG against desmoglein (Dsg) 3 and/or Dsg1, but acantholysis is not explained by steric interference alone. Antibody binding promotes the clustering and internalization of desmosomal cadherins, p38 MAPK activation, actin and keratin reorganization, and destabilization of desmosome turnover. The tissue distribution of Dsg1 and Dsg3 helps explain mucosal versus cutaneous phenotypes, whereas epitope spreading and non-Dsg antibodies may modify severity. Disease persistence reflects long-lived plasma cells, autoreactive memory B cells, and T-cell help; therefore, serum antibody decline and clinical remission

do not always occur synchronously.

A Japan-specific dimension and treatment evidence. Japan has provided prospective therapeutic evidence rather than relying only on retrospective experience. A multicenter study of 20 Japanese patients substantiated the use of rituximab to treat refractory pemphigus and noted a clinically meaningful reduction in steroids (23). A Japanese phase 2 study of the selective Bruton's tyrosine kinase inhibitor tirabrutinib showed that direct suppression of B-cell receptor signaling can induce remission in a subset of refractory patients while reducing corticosteroid exposure (24). FcRn blockade remains mechanistically attractive because it accelerates IgG catabolism without eliminating B cells; however, results of clinical development have been heterogeneous, and future studies should integrate pathogenic IgG kinetics, Dsg-specific B-cell assays, and treatment-free remission rather than relying on serum titers alone. The major innovation is a shift from nonspecific immunosuppression to staged control of B-cell activation, plasma-cell output, and IgG persistence.

5.2. Pemphigoid and EBA: Endotype heterogeneity at the dermal-epidermal junction

Mechanism. Bullous pemphigoid (BP) is driven primarily by autoantibodies to BP180/collagen XVII and BP230, whereas EBA targets type VII collagen. Antibody binding activates complement, Fc receptors, eosinophils,

neutrophils, proteases, and type 2 cytokine circuits. The resulting disease is not immunologically uniform. Some patients have complement-rich inflammation and urticarial erythema, some have eosinophil- and IL-4/IL-13-dominant pruritus, and others have relatively non-inflammatory blistering. EBA can be further divided into inflammatory and mechanobullous phenotypes, with trauma, scarring, and milia reflecting the direct compromise of anchoring fibrils.

A Japan-specific dimension, current therapy, and evidence limitations. DPP-4 inhibitor-associated BP often exhibits less erythema, distinct BP180 epitope recognition, and enrichment of *HLA-DQB1*03:01* (11-13). National database and registry studies provide evidence of a real drug-associated risk, and especially in susceptible patients soon after exposure. This phenotype indicates the need for medication history, HLA-informed research, and epitope-level serology rather than treating all BP as identical. In LIBERTY-BP ADEPT, sustained remission at week 36 required complete remission with oral corticosteroids discontinued by week 16, no subsequent relapse, and no rescue therapy. The endpoint was achieved in an estimated 18.2% of dupilumab-treated participants and 4.0% of placebo-treated participants (risk difference: 14.2 percentage points; 95% CI: 1.97–27.74; $p = 0.025$) (25,26). Dupilumab was subsequently approved for adult BP in the United States in June 2025 and in Japan in March 2026 (27,28). At the review cutoff, final numerical results were available from trial registries and regulatory sources rather than a full peer-reviewed efficacy report. FcRn inhibitors, complement inhibitors, and anti-eosinophil strategies remain investigational. Future Japanese trials should pre-specify inflammatory, type 2, drug-associated, and fibrosing/mechanobullous endotypes because a single aggregate endpoint may obscure pathway-specific benefit.

6. Discuss Genetic fragility, cornification, and keratinocyte adhesionsion

6.1. Inherited EB: From supportive care to local gene correction

Mechanism. Inherited EB consists of genetically heterogeneous disorders in which skin cleavage results from defects in keratins, plectin, integrins, laminin-332, collagen XVII, or type VII collagen. Recessive dystrophic EB is the most instructive translational model. COL7A1 deficiency reduces type VII collagen and anchoring fibrils, causing lifelong wounds, inflammation, microbial dysbiosis, progressive fibrosis, mitten deformities, and aggressive cutaneous squamous cell carcinoma. The malignant niche is sustained not only by mutation accumulation but also by chronic tissue damage, TGF- β -driven fibrosis, and an altered extracellular matrix. Effective therapy must therefore address structural correction, wound ecology, fibrosis,

pain, nutrition, and cancer surveillance in parallel.

Evidence from approved and investigational treatments. Topical beremagene geperpavec (B-VEC) delivers COL7A1 using a modified herpes simplex virus type 1 vector, and it achieved superior durable wound closure in a phase 3 inpatient-controlled trial (29). Vyjuvek Gel was approved in Japan in July 2025 following PMDA review, giving Japan access to the first locally applied gene therapy for dystrophic EB (30). This is a conceptual milestone: treatment can restore a missing structural protein in selected wounds without systemic gene transfer. The limitation is equally important - untreated skin and extracutaneous tissues remain genetically defective, and repeated application is required. *Ex vivo* autologous gene-corrected epidermal sheets, including prademagene zamikeracel, provide a graft-based strategy for chronic RDEB wounds; the product was approved by the US FDA as ZEVASKYN on April 28, 2025 (31,32). Unlike repeated topical B-VEC, this autologous cell-sheet approach entails harvesting, *ex vivo* gene modification, and grafting, creating distinct considerations for durability, procedural burden, and treated surface area. Japanese programs involving mesenchymal stromal cells and other regenerative products further reflect the country's distinctive regulatory experience with regenerative medicine. The next generation should combine molecular correction with anti-fibrotic and cancer-prevention strategies and should use digital wound imaging, pain, function, and squamous-cell carcinoma incidence as long-term endpoints.

6.2. Congenital ichthyosis: Genotype-specific barrier failure and immune activation

Mechanism. Congenital ichthyoses arises from defects in epidermal lipid transport, lamellar-body secretion, transglutaminase activity, protease regulation, or keratin/cornified-envelope assembly. The resulting barrier defect increases transepidermal water loss, alters skin surface pH and microbiota, and releases alarmins that activate innate and adaptive inflammation. This explains why erythroderma and pruritus may persist even when scale is partly controlled. The mechanism varies by genotype: TGM1 deficiency disrupts cornified-envelope cross-linking; ABCA12 deficiency impairs lipid transport in harlequin ichthyosis; NIPAL4 and PNPLA1 variants alter lipid processing; and protease imbalance contributes to syndromic forms.

Current management, investigational therapy, and limitations. The 2025 updated international management guidelines emphasize structured neonatal care, temperature and fluid control, ophthalmologic management, infection prevention, keratolysis, and systemic retinoids while acknowledging emerging biologic and small-molecule therapy (33,34). Recent cohorts suggest that patients with strong type 2 or IL-

17/IL-23 inflammatory signatures may improve with targeted biologics, although responses are variable and off-label (35). Gene replacement, mRNA, enzyme replacement, and lipid-substitution approaches remain experimental. The critical advance is to stop treating congenital ichthyosis as one disease: genotype, inflammatory endotype, and age-specific risk should determine both therapy and trial design.

6.3. HHD: Calcium homeostasis, proteostasis, and environmental amplification

Mechanism. HHD is caused by heterozygous ATP2C1 variants that impair the Golgi secretory-pathway Ca^{2+} / Mn^{2+} ATPase SPCA1. Disturbed calcium and manganese homeostasis consists of desmosomal protein processing, keratinocyte differentiation, mitochondrial function, and oxidative-stress control, producing suprabasal acantholysis. The genetic defect is constitutive, but disease expression is highly environmental: heat, sweat, friction, ultraviolet exposure, infection, and obesity amplify local damage. This genotype-environment interaction explains recurrent flexural erosions and the frequent mismatch between mutation status and clinical severity.

Current management and evidence limitations. Management remains individualized and evidence is predominantly from small series. Antiseptic and anti-inflammatory therapy, topical calcineurin inhibitors, botulinum toxin, low-dose naltrexone, laser or surgical ablation, and systemic agents may be used depending on the trigger pattern and extent (36). A 2025 report on topical ruxolitinib added JAK inhibition to the investigational landscape (37), but it should not be interpreted as established therapy. Mechanistically informed trials could shed light on inflammatory lesions, measure calcium-dependent adhesion and stress signatures, and separate lesion clearance from relapse prevention.

7. Autoinflammatory and neutrophilic dermatoses

7.1. GPP: The IL-36-neutrophil feed-forward loop

Mechanism and Japan-specific biology. GPP is a systemic autoinflammatory disease and not simply severe plaque psoriasis with pustules. IL-36 cytokines released by keratinocytes activate IL-36 receptor signaling in epithelial and immune cells, inducing release of CXCL chemokines, neutrophil recruitment, and further protease-mediated activation of IL-36. Loss-of-function variants in IL36RN remove a critical brake in this loop; CARD14 and AP1S3 variants can converge on NF- κ B and vesicular/autophagic pathways (38). Japanese studies identified recurrent IL36RN variants and helped establish the genetic distinction between GPP with and without plaque psoriasis (14,15). Infection,

pregnancy, drug withdrawal, and systemic stress can then trigger explosive flareups within a genetically or immunologically primed background.

Randomized trial evidence and limitations.

Spesolimab, an anti-IL-36 receptor monoclonal antibody, demonstrated rapid pustule clearance in the Effisayil 1 trial and established a direct path from mechanism to acute flareup control (39). Effisayil 2 subsequently showed that high-dose subcutaneous spesolimab delayed the first recurrent flareup over 48 weeks compared to a placebo (HR = 0.16, 95% CI 0.05–0.54) (40). Asian subgroup analyses and a 2026 Japanese expanded-access report substantiated its relevance and short-term safety in regional practice (41,42). Residual molecular inflammation after visible clearance suggests that clinical resolution does not necessarily equal pathway normalization. The remaining questions concern long-term durability, the optimal maintenance dose, cumulative safety, management in pregnancy or infection, and whether genotype or baseline IL-36 signatures predict response. Japan's long-standing recognition of GPP as a separate severe disease places it in a key position to lead longitudinal biomarker and maintenance-treatment studies.

7.2. CAPS: NLRP3 inflammasome disease and somatic mosaicism

Mechanism. CAPS consists of familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and neonatal-onset multisystem inflammatory disease. Gain-of-function NLRP3 variants promote inflammasome assembly, caspase-1 activation, and excessive IL-1 β release. The skin phenotype - recurrent urticaria-like neutrophilic eruption - is a visible marker of systemic inflammation that may include fever, arthralgia, sensorineural hearing loss, meningitis, and amyloidosis. Low-level somatic mosaicism is clinically important because conventional sequencing may miss pathogenic variants, and particularly in apparently mutation-negative or late-onset patients.

Evidence from a Japan-specific cohort and approved treatment. A 2024 Japanese nationwide survey refined the national phenotype, genotype spectrum, and treatment experience (43). IL-1 blockade with canakinumab can rapidly suppress a rash and systemic inflammation, and 2026 Japanese post-marketing surveillance added real-world safety and effectiveness data (44). Persistent hearing or neurologic damage despite inflammatory control underscores the need for early diagnosis. Dermatologists can reduce the diagnostic delay by recognizing that an urticaria-like eruption without typical itching, combined with a fever or cold sensitivity, may represent an inflammasome disease rather than chronic urticaria.

7.3. NNS/PRAAS and SAVI: A disease first found in

Japan and an emerging interferonopathy

Mechanism and a Japan-specific founder biology. NNS is a Japanese form of proteasome-associated autoinflammatory syndrome typically caused by the founder variant PSMB8 p.Gly201Val. Immunoproteasome dysfunction impairs protein degradation, produces proteotoxic and endoplasmic-reticulum stress, and activates type I interferon and JAK-STAT programs. Patients develop pernio-like or nodular skin lesions, recurrent fever, lipomuscular atrophy, and joint contractures (21,22). Identification of the disease was pivotal because it linked a protein-quality-control defect to interferon-mediated inflammation. Conventional corticosteroids and immunosuppressants are often incomplete. A Japanese registry indicates that there was a single-arm, open-label study on baricitinib with a target enrollment of five; the registry indicates that the study was suspended/terminated after the study period ended, so it should not be described as actively recruiting (45).

Mechanism, Japan regulatory context, and investigational evidence. SAVI results from gain-of-function STING1/TMEM173 variants that constitutively activate TBK1-IRF3 and type I interferon signaling. Acral vasculitic lesions, ulceration, and tissue loss coexist with potentially fatal interstitial lung disease. Its addition to the Japanese designated disease list in 2025 is important because early recognition may prevent irreversible pulmonary damage (1,46). JAK inhibitors have resulted in partial responses in interferonopathies, but infection, cytopenia, and incomplete lung control remain concerns (45,47). Anifrolumab and hematopoietic stem-cell transplantation are emerging experimental strategies but are not standard care (48). NNS and SAVI illustrate a broader innovation: rare skin lesions can act as pathway reporters with which to direct interferon-targeted therapy before multisystem injury becomes evident.

8. Severe cutaneous adverse reactions: SJS and TEN

Mechanism. SJS and TEN are hyperacute immune-mediated diseases in which drug-specific cytotoxic T cells and natural killer cells cause widespread keratinocyte death. HLA-restricted antigen presentation, T-cell receptor specificity, granulysin, perforin/granzyme, Fas-Fas ligand, and inflammatory cytokines interact in a rapidly amplifying network. Granulysin is especially important because blister fluid concentrations correlate with widespread cytotoxic injury (49). Once epithelial detachment and mucosal destruction accelerate, treatment is constrained by irreversible damage, infection, fluid loss, and delayed drug withdrawal.

Japan-specific pharmacogenetics and treatment limitations. Japan offers a distinct perspective on prevention and phenotype. Cold-medicine-related SJS/TEN with severe ocular complications has been associated with *HLA-A*02:06* and other population-specific signals

(16). This differs from the high-value *HLA-B*15:02* screening paradigm for carbamazepine in selected Asian populations, emphasizing that pharmacogenetic prevention is not wholly transferable between ethnic groups or drug classes (17). Acute management should integrate immediate culprit withdrawal, severity assessment, critical-care support, and early ophthalmologic intervention. Immunomodulatory evidence for cyclosporine, systemic corticosteroids, intravenous immunoglobulin, and TNF inhibition remains heterogeneous; therefore, future studies should stratify SJS and TEN by causative drug, treatment timing, HLA background, and epithelial injury biomarkers rather than pooling those diseases.

9. DNA repair failure and neurocutaneous tumor syndromes

9.1. XP: A Japan-enriched model of cumulative mutational injury

Mechanism and Japan-specific biology. XP is caused by defects in nucleotide excision repair or translesion synthesis. Failure to remove ultraviolet-induced cyclobutane pyrimidine dimers and 6-4 photoproducts produces extreme photosensitivity, early skin cancer, and, in selected complementation groups, progressive neurodegeneration. Japanese clinical guidelines estimate an incidence of approximately 1 in 22,000 (8), whereas the founder splice-site variant in XPA provides the principal population-genetic explanation for the high burden (7). This aspect makes XP one of the clearest examples of a genuinely Japan-specific rare dermatologic burden.

Current management and evidence limitations. The therapeutic logic differs from inflammatory disease. Rigorous ultraviolet avoidance, environmental measurement, vitamin D management, frequent skin and ophthalmologic surveillance, and early cancer treatment remain central (8). Once mutation burden accumulates, anti-inflammatory therapy cannot reverse it. Recent genotype-phenotype work has improved the understanding of XPA-associated neurodegeneration and it highlights the need for molecular diagnosis before neurologic decline (50). Future progress will require systemic approaches capable of protecting neural tissue, while dermatologic care should use digital lesion tracking and lifelong cancer prevention as formal outcomes.

9.2. NF and TSC: Pathway-directed neurocutaneous care

Mechanism, Japan-specific evidence, and treatment. NF1 is caused by loss of neurofibromin, a RAS GTPase-activating protein, leading to persistent RAS-MAPK signaling. Cafe-au-lait macules, axillary freckling, cutaneous neurofibromas, and plexiform neurofibromas provide a dermatologic entry point to a multisystem tumor-predisposition syndrome. MEK inhibition with

selumetinib shrank symptomatic inoperable plexiform neurofibromas; a Japanese pediatric phase 1 study established regional dosing and safety experience (51). More recent studies have extended MEK inhibition to cutaneous neurofibromas, using lesion count, volume, and patient-reported visibility or discomfort as endpoints (52,53). Because chronic therapy may be required, growth suppression must be balanced against cardiotoxicity, ocular toxicity, skin toxicity, and rebound after discontinuation.

Limitations in scope. The official Japanese NF entry also encompasses NF2-related schwannomatosis. Its predominant morbidity is neurologic and oncologic rather than cutaneous, although cutaneous schwannomas may contribute to recognition. Japan-specific skin-directed therapeutic evidence is substantially stronger for NF1, so the mechanistic and treatment discussion here is NF1-focused; NF2 is retained at the administrative-entry level rather than presented as equivalently covered.

Mechanism, Japan-specific development, and treatment. TSC, added here from outside the skin category, is caused by TSC1 or TSC2 loss and constitutive mTORC1 activation. Facial angiofibromas, shagreen patches, hypomelanotic macules, and periungual fibromas are often diagnostically decisive. Japan has been a leader in topical sirolimus gel, which directly targets mTORC1 in cutaneous lesions and reduces systemic exposure compared to oral therapy. A Japanese 2024 analysis further refined investigator-based scoring for facial angiofibromas (54). TSC shows how a visible lesion can serve as both a diagnostic biomarker and a pharmacodynamic readout for a systemic signaling disorder.

10. Sweat-gland dysfunction and ectopic mineralization

10.1. AIGA: A Japan-centered, putative immune- and stress-associated eccrine disorder

Mechanism and Japan-specific evidence. AIGA is characterized by widespread acquired anhidrosis or hypohidrosis without generalized autonomic failure or destructive sweat-gland disease. Many patients experience heat intolerance, cholinergic urticaria-like symptoms, or pain after exertion. Its pathogenesis remains incompletely defined. Proposed mechanisms include impaired acetylcholine signaling through the muscarinic M3 receptor, immune-mediated dysfunction in eccrine glands, reduced receptor expression, T-cell-predominant inflammation, and cellular stress. The clinical literature is disproportionately Japanese, and national criteria have helped distinguish AIGA from neuropathy, endocrinopathy, drug effects, and congenital hypohidrosis (9).

Current treatment and investigational biomarkers. Systemic corticosteroid pulse therapy can restore sweating in a subset of patients, and particularly when treatment is early, but relapse and nonresponse are common. A

2026 single-cell and molecular study identified cellular-stress and unfolded-protein-response signatures in affected eccrine units and linked those changes to treatment response (10). This finding broadens the disease model beyond simple receptor loss and creates testable biomarkers. Future trials should combine quantitative thermoregulatory sweat testing, regional sweat maps, symptom burden, heat-safety outcomes, and tissue endotypes.

10.2. PXE: The hepatic ATP-pyrophosphate-skin axis

Mechanism. PXE is caused predominantly by biallelic ABCC6 variants. Although the characteristic yellow papules and fragmented calcified elastic fibers are cutaneous, the primary metabolic defect is systemic: hepatic ABCC6-dependent ATP release is reduced, lowering circulating inorganic pyrophosphate, an endogenous inhibitor of mineralization. Progressive calcification affects the skin, Bruch membrane in the retina, and vasculature. This skin-liver-eye-vessel axis explains why dermatologic diagnosis must trigger ophthalmologic and cardiovascular surveillance.

Investigational treatment and evidence limitations. Therapeutic development now targets mineralization rather than skin appearance alone. Etidronate, oral pyrophosphate, magnesium or phosphate-modulating approaches, lansoprazole, and ENPP1-Fc replacement strategies are being clinically investigated (55-59). A 2025 preclinical study of liver-directed ABCC6 base editing indicates the possibility of correcting the systemic source defect (60). None of these approaches yet constitutes a universal disease-modifying standard. Trials require slow-progression endpoints, quantitative vascular and retinal imaging, and long follow-up, which makes international harmonization particularly important.

11. Systemic designated entries with decisive cutaneous phenotypes

11.1. Behcet disease: Neutrophilic inflammation at a geographic and genetic interface

Mechanism and Japan-specific cohort evidence. Behcet disease is included in the illustrative expanded framework because recurrent oral aphthae, genital ulceration, erythema nodosum-like lesions, acneiform eruptions, and pathergy are central to its recognition. HLA-B*51, ERAP1, innate immune activation, neutrophil hyperreactivity, Th1/Th17 polarization, endothelial injury, and microbiome triggers contribute to pathogenesis. The disease is prevalent along the historical Silk Road, and Japanese cohorts have been essential to phenotype and treatment studies. The key clinical issue is organ stratification: mucocutaneous disease can be disabling, but ocular, neurologic, vascular, or gastrointestinal disease determines irreversible

morbidity.

Current and investigational treatments. Apremilast reduced the oral ulcer burden in the international RELIEF trial, and a prespecified Japanese subgroup analysis of 39 patients confirmed regional efficacy and safety (61,62). Anti-TNF therapy remains crucial for severe ocular, vascular, neurologic, or intestinal involvement, while treatments involving the IL-1, IL-17, IL-6, and JAK pathways are under investigation. Cutaneous endpoints should not be isolated from organ activity because the same patient may transition between mucocutaneous and organ-threatening phases.

11.2. Anti-MDA5 dermatomyositis within the PM/DM entry: Skin findings as an early warning for lethal lung disease

Scope, mechanism, and biomarkers. The official PM/DM designated entry is broader than the anti-MDA5 subset. This review focuses on anti-MDA5-positive dermatomyositis because cutaneous signs may precede or outweigh muscle disease and because rapidly progressive interstitial lung disease is particularly important in Japanese and East Asian practice. MDA5 is a cytosolic RNA sensor; autoimmunity is associated with a type I interferon-rich state, macrophage activation, endothelial injury, and severe lung inflammation. Painful palmar papules, ulceration, livedoid changes, alopecia, and oral lesions should raise suspicion. Ferritin, anti-MDA5 titer, KL-6, oxygenation, and radiologic progression can help identify patients at risk of rapidly progressive interstitial lung disease.

Japan-specific cohort evidence, current practice, and limitations. Japanese and East Asian cohorts report a particularly high burden of rapidly progressive lung disease. A 2026 Japanese multicenter study identified anti-MDA5-positive interstitial lung disease without prominent skin or muscle manifestations as a high-risk and potentially overlooked subgroup (18). This observation intensifies, rather than weakens, the dermatologic message: characteristic ulcers, palmar papules, or livedoid lesions can be an early warning, but their absence does not rule the disease out. Early combined glucocorticoid, calcineurin inhibitor, and cyclophosphamide therapy has been used in high-risk Japanese patients, while rituximab, plasma exchange, JAK inhibition, and other rescue strategies are substantiated by evolving but nonuniform evidence (19,20). The innovation for dermatology is prognostic: when present, specific skin lesions are visible indicators of systemic endothelial and interferon injury that should prompt urgent pulmonary evaluation.

12. Therapeutic translation and trial architecture in Japan

The most persuasive recent programs have a

corresponding mechanism and endpoint: B-VEC restores type VII collagen and is evaluated based on wound closure (29,30); spesolimab blocks IL-36 receptor signaling and is evaluated based on pustule or flareup control (39-42); selumetinib suppresses RAS-MAPK signaling and is evaluated based on tumor volume (51-53); canakinumab blocks IL-1 β and is evaluated based on inflammatory control (43,44); and topical sirolimus inhibits mTORC1 and is evaluated based on facial angiofibroma severity (54). Table 4 maps biomarkers, outcomes, and evidence maturity, and Table 5 summarizes recent Japan-relevant therapeutic evidence and jurisdiction-specific status.

Japan's regulatory and research environment consists of distinct but potentially linkable streams: nanbyo diagnostic criteria and clinical survey forms, specialist registries, PMDA review and pharmacovigilance, jRCT and UMIN trial records, and post-marketing surveillance by companies. Post-approval surveillance is not automatically initiated as a result of nanbyo designation. The approval of Vyjuvek Gel in July 2025 and the inclusion of SAVI in the designated list illustrate how regulatory and policy timelines can respond to molecular medicine (1,30). Administrative data, specialist registries, genetic laboratories, pathology repositories, and patient-reported outcomes are not always transferable. Rather than describing an existing national standard, we therefore propose a rare dermatologic condition model that maps genotype, autoantibody, endotype, lesion imaging, treatment exposure, and longitudinal function across institutions.

Ultra-small populations also require different statistical logic. Conventional parallel-group trials may be infeasible for NNS, SAVI, AIGA, or EBA. We propose Bayesian borrowing, adaptive randomization, within-patient controls, N-of-1 series, external natural-history controls, and platform protocols to increase information yield, provided that baseline progression and measurement error are well characterized. Japan should combine domestic depth with international scale: national cohorts can define precise phenotypes, while multinational networks provide sufficient sample size for confirmatory inference.

13. Cross-cutting mechanisms and conceptual innovation

Seven partially overlapping mechanistic architectures explain most of the intractability across this illustrative framework. First, constitutive structural or barrier failure produces repeated injury in EB, congenital ichthyosis, and HHD. Second, self-sustaining adaptive autoimmunity produces pathogenic antibody persistence in pemphigus, BP, and EBA. Third, innate immune and neutrophilic feed-forward loops drive GPP, CAPS, and much of Behcet disease. Fourth, cytotoxic or interferon-mediated vascular-epithelial injury creates rapid irreversibility in

Table 4. Mechanism-aligned biomarkers, endpoints, and evidence maturity across the illustrative framework

Entry/disease group	Biomarkers or confirmatory tests	Mechanism-aligned outcomes	Evidence maturity/status
Pemphigus	Anti-Dsg1/3 ELISA, DIF, pathogenic IgG and B-cell assays	PDAI remission, steroid-free remission, pathogenic IgG kinetics	DIF and anti-Dsg ELISA established; pathogenic B-cell assays exploratory
BP	BP180/BP230 serology, DIF, eosinophils, complement, epitope profile	Blister control, pruritus, steroid exposure, sustained remission	DIF/serology established; epitope and type 2 endotyping exploratory
EBA	COL7 serology, DIF/salt-split mapping, scarring phenotype	Blister control, wound healing, scarring, pain and function	Diagnostic mapping established; outcome evidence mainly observational
Inherited EB	Genotype, IF mapping, type VII collagen, digital wound imaging	Durable wound closure, pain/function, cSCC incidence	Genotype/IF mapping established; wound endpoints validated in randomized trials
GPP	IL36RN/CARD14/AP1S3, CRP, IL-36 signature	Pustule clearance, flare duration, flare-free survival	Genetic testing selected; IL-36 signatures exploratory; flare endpoints randomized
SJS/TEN	Drug causality, HLA, granulysin and epithelial-injury markers	Mortality, re-epithelialization, ocular and mucosal sequelae	Clinical causality and mortality endpoints established; biomarkers context-specific
XP	NER genotype/complementation, UV damage, tumor surveillance	New cancers, neurologic progression, adherence to photoprotection	Molecular diagnosis established; long-term outcomes observational
NF1	NF1 genotype, volumetric imaging, RAS-MAPK pathway markers	Plexiform/cutaneous neurofibroma volume, pain, visibility, function	Genotype and volumetric imaging established; cutaneous endpoints emerging
NF2-related schwannomatosis	NF2 testing, MRI tumor burden, audiology	Tumor control, hearing preservation, neurologic function	Genetic testing, MRI, and audiology established
TSC	TSC1/TSC2 genotype, lesion scoring, mTORC1 pathway markers	Facial angiofibroma severity, lesion burden, patient-reported visibility	Genotype and lesion scoring established; topical pathway endpoint randomized
AIGA	Thermoregulatory sweat test, sweat map, eccrine M3 and stress signatures	Recovered sweat area, heat tolerance, symptom burden, relapse	Sweat testing established; tissue stress signatures exploratory
PXE	ABCC6 genotype, plasma pyrophosphate, retinal/vascular imaging	Mineralization progression, visual and vascular events	Genotype/imaging established; pyrophosphate is a research biomarker
CAPS	NLRP3 variant/mosaicism, CRP/SAA, IL-1 activity	Complete inflammatory response, hearing/neurologic preservation	Genotype/CRP/SAA established; mosaicism testing specialized
NNS/PRAAS	PSMB8 genotype, interferon score, inflammatory and functional measures	Skin healing, steroid reduction, fever control, physical function	Genotype established; interferon score and functional endpoints exploratory
SAVI	STING1 genotype, interferon score, HRCT and pulmonary function	Skin healing, ILD stabilization, oxygen requirement, survival	Genotype established; interferon score and lung endpoints early/observational

Abbreviations: ABCC6, ATP-binding cassette subfamily C member 6; AIGA, acquired idiopathic generalized anhidrosis; AP1S3, adaptor related protein complex 1 subunit sigma 3; BP, bullous pemphigoid; BP180, bullous pemphigoid antigen 180; BP230, bullous pemphigoid antigen 230; CAPS, cryopyrin-associated periodic syndrome; CARD14, caspase recruitment domain family member 14; COL7, type VII collagen; CRP, C-reactive protein; cSCC, cutaneous squamous cell carcinoma; DIF, direct immunofluorescence; DM, dermatomyositis; Dsg, desmoglein; EB, epidermolysis bullosa; EBA, epidermolysis bullosa acquisita; ELISA, enzyme-linked immunosorbent assay; GPP, generalized pustular psoriasis; HLA, human leukocyte antigen; HRCT, high-resolution computed tomography; IF, immunofluorescence; IgG, immunoglobulin G; IL, interleukin; ILD, interstitial lung disease; IL36RN, interleukin 36 receptor antagonist; KL-6, Krebs von den Lungen-6; M3, muscarinic acetylcholine receptor M3; MDA5, melanoma differentiation-associated protein 5; MRI, magnetic resonance imaging; mTORC1, mechanistic target of rapamycin complex 1; NER, nucleotide excision repair; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2; NLRP3, NLR family pyrin domain containing 3; NNS, Nakajo-Nishimura syndrome; PDAI, Pemphigus Disease Area Index; PRAAS, proteasome-associated autoinflammatory syndrome; PSMB8, proteasome subunit β type 8; PXE, pseudoxanthoma elasticum; RAS-MAPK, rat sarcoma-mitogen-activated protein kinase; RP-ILD, rapidly progressive interstitial lung disease; SAA, serum amyloid A; SAVI, STING-associated vasculopathy with onset in infancy; SJS, Stevens-Johnson syndrome; STING1, stimulator of interferon response cGAMP interactor 1; TEN, toxic epidermal necrolysis; TSC, tuberous sclerosis complex; TSC1/TSC2, tuberous sclerosis complex 1/2; UV, ultraviolet; XP, xeroderma pigmentosum.

Table 4. Mechanism-aligned biomarkers, endpoints, and evidence maturity across the illustrative framework (continued)

Entry/disease group	Biomarkers or confirmatory tests	Mechanism-aligned outcomes	Evidence maturity/status
Behcet disease	Organ-specific activity, CRP, ulcer counts and pain	Oral/genital ulcer control plus ocular, vascular, neurologic and intestinal outcomes	Clinical organ outcomes established; biomarkers nonspecific
Anti-MDA5 DM	Anti-MDA5 titer, ferritin, KL-6, oxygenation, HRCT	RP-ILD survival, oxygen-free survival, lung stabilization, ulcer control	Autoantibody/HRCT established; prognostic thresholds cohort-based

Abbreviations: ABCC6, ATP-binding cassette subfamily C member 6; AIGA, acquired idiopathic generalized anhidrosis; AP1S3, adaptor related protein complex 1 subunit sigma 3; BP, bullous pemphigoid; BP180, bullous pemphigoid antigen 180; BP230, bullous pemphigoid antigen 230; CAPS, cryopyrin-associated periodic syndrome; CARD14, caspase recruitment domain family member 14; COL7, type VII collagen; CRP, C-reactive protein; cSCC, cutaneous squamous cell carcinoma; DIF, direct immunofluorescence; DM, dermatomyositis; Dsg, desmoglein; EB, epidermolysis bullosa; EBA, epidermolysis bullosa acquisita; ELISA, enzyme-linked immunosorbent assay; GPP, generalized pustular psoriasis; HLA, human leukocyte antigen; HRCT, high-resolution computed tomography; IF, immunofluorescence; IgG, immunoglobulin G; IL, interleukin; ILD, interstitial lung disease; IL36RN, interleukin 36 receptor antagonist; KL-6, Krebs von den Lungen-6; M3, muscarinic acetylcholine receptor M3; MDA5, melanoma differentiation-associated protein 5; MRI, magnetic resonance imaging; mTORC1, mechanistic target of rapamycin complex 1; NER, nucleotide excision repair; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2; NLRP3, NLR family pyrin domain containing 3; NNS, Nakajo-Nishimura syndrome; PDAI, Pemphigus Disease Area Index; PRAAS, proteasome-associated autoinflammatory syndrome; PSMB8, proteasome subunit β type 8; PXE, pseudoxanthoma elasticum; RAS-MAPK, rat sarcoma-mitogen-activated protein kinase; RP-ILD, rapidly progressive interstitial lung disease; SAA, serum amyloid A; SAVI, STING-associated vasculopathy with onset in infancy; SJS, Stevens-Johnson syndrome; STING1, stimulator of interferon response cGAMP interactor 1; TEN, toxic epidermal necrolysis; TSC, tuberous sclerosis complex; TSC1/TSC2, tuberous sclerosis complex 1/2; UV, ultraviolet; XP, xeroderma pigmentosum.

SJS/TEN, anti-MDA5 dermatomyositis, NNS/PRAAS, and SAVI. Fifth, cumulative or systemic biochemical injury drives XP and PXE. Sixth, tumor-suppressor loss produces pathway-dependent proliferation in NF and TSC. Seventh, organ-specific functional dysregulation and cellular stress provide a working model for AIGA, while acknowledging that its pathogenesis has yet to be completely elucidated.

This classification is clinically useful because it identifies the level of correction a treatment can plausibly achieve. Structural replacement is required for missing proteins such as COL7A1; immune or signaling blockade may control active disease without eliminating immune memory or future triggers; and preventive measures can limit new injury without reversing accumulated mutation or mineralization. Explicitly labeling an intervention as structural, immunologic, signaling, preventive, or symptomatic reduces therapeutic overstatement.

The proposed contribution of a Japanese rare dermatologic condition framework is therefore a linkage model rather than a longer disease list or a claim that designation already improves outcomes. The official 12-entry core can provide policy-defined cohorts, while population-specific biology, Japanese clinical evidence, and mechanism-aligned trials can be linked across administrative boundaries. The six-entry layer is illustrative and neither complete nor unique.

14. Unmet needs and research priorities

The first priority is natural history. AIGA, HHD, NNS, SAVI, EBA, and PXE lack trial-ready progression models. Prospective cohorts should define untreated variability, clinically meaningful change, and irreversible milestones. The second priority is biomarker validation.

Candidate markers - Dsg-specific B cells, BP180 epitope profiles, IL-36 signatures, interferon scores, sweat-gland stress states, pyrophosphate levels, and digital wound features - must demonstrate analytical validity and treatment responsiveness before they are used as enrichment or surrogate endpoints.

The third priority is equity and implementation. Genomic testing, advanced biologics, gene therapy, and repeated specialist follow-up may be concentrated at tertiary centers. National designation reduces the financial burden but does not automatically improve geographic access, the transition from pediatric to adult care, or the practical workload of wound care and photoprotection. Patient-reported function, caregiver time, heat safety, vision, pain, itching, stigma, and working should be treated as core outcomes rather than optional supplements.

The fourth priority is durability and late effects. Gene therapy, chronic MEK inhibition, long-term cytokine blockade, and JAK inhibition require surveillance for malignancy, infection, organ toxicity, reproductive effects, and rebound. We recommend prospectively linking the clinical survey infrastructure to treatment exposure and molecular data to support post-marketing science. International data standards are also essential because rare disease populations are not amenable to incompatible definitions, image formats, or outcome measures across countries. These are author-proposed priorities rather than established national standards and are summarized in Table 6.

15. Limitations

Several limitations should temper this work's interpretation. This is a targeted narrative review rather

Table 5. Selected therapeutic advances in Japan: Evidence level and jurisdiction-specific regulatory status

Entry / intervention	Mechanistic target	Evidence level	Status in Japan	Status in US/EU	Status verified as of
Pemphigus / rituximab	CD20-positive B-cell depletion	Prospective Japanese single-arm study plus randomized international evidence	Intravenous rituximab approved for refractory pemphigus vulgaris and foliaceus	US/EU: intravenous rituximab approved for moderate-to-severe pemphigus vulgaris	August 3, 2026
Pemphigus / tirabrutinib	BTK/B-cell receptor signaling	Japanese phase 2 single-arm study	Not approved for pemphigus; investigational oral therapy	US/EU: no pemphigus indication	August 3, 2026
Bullous pemphigoid / dupilumab	IL-4/IL-13 type 2 inflammation	Randomized phase 2/3 registry and regulatory results; final peer-reviewed efficacy report not identified at the cutoff	Subcutaneous dupilumab approved for adult moderate-to-severe BP in March 2026	US: subcutaneous dupilumab approved for adult BP on June 18, 2025; EU: BP is not an approved indication	August 3, 2026
Dystrophic EB / beremagene geperpavec (B-VEC)	Topical COL7A1 gene delivery	Phase 3 randomized intrapatient evidence	Topical gel approved for DEB in July 2025 following PMDA review	US/EU: topical gene therapy approved for DEB wounds	August 3, 2026
Recessive dystrophic EB / prademagene zamikeracel	Autologous gene-corrected epidermal sheets	Phase 3 randomized intrapatient evidence	Not approved	US: approved as ZEVA SKYN on April 28, 2025; EU: no marketing authorization identified	August 3, 2026
GPP / spesolimab	IL-36 receptor blockade	Randomized flare-treatment and flare-prevention trials	Intravenous 450-mg formulation approved for treatment of GPP flares; no Japanese subcutaneous flare-prevention indication identified	US/EU: intravenous flare treatment and subcutaneous flare prevention approved; eligible ages and formulations differ	August 3, 2026
NF1 / selumetinib	MEK inhibition	Prospective phase 1/2 and nonrandomized clinical evidence	Oral capsules approved for patients aged 3 years or older with symptomatic, inoperable plexiform neurofibromas	US/EU: oral selumetinib approved for symptomatic, inoperable plexiform neurofibromas in NF1; age/formulation ranges differ	August 3, 2026
TSC / topical sirolimus gel	mTORC1 inhibition	Randomized Japanese trials and observational evidence	Topical sirolimus gel approved for TSC-associated skin lesions	US/EU: no approved topical sirolimus gel for TSC skin lesions identified	August 3, 2026
CAPS / canakinumab	IL-1 β neutralization	Clinical-trial, nationwide, and post-marketing evidence	Subcutaneous canakinumab approved for CAPS	US/EU: subcutaneous canakinumab approved for CAPS	August 3, 2026

Abbreviations: BP, bullous pemphigoid; B-VEC, beremagene geperpavec; BTK, Bruton's tyrosine kinase; CAPS, cryopyrin-associated periodic syndrome; CD20, cluster of differentiation 20; COL7A1, collagen type VII alpha 1 chain; DEB, dystrophic epidermolysis bullosa; DM, dermatomyositis; EB, epidermolysis bullosa; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; EU, European Union; GPP, generalized pustular psoriasis; IFNAR, interferon- α/β receptor; IL, interleukin; JAK, Janus kinase; JRCT, Japan Registry of Clinical Trials; MDA5, melanoma differentiation-associated protein 5; MEK, mitogen-activated protein kinase kinase; mTORC1, mechanistic target of rapamycin complex 1; NF1, neurofibromatosis type 1; NNS, Nakajo-Nishimura syndrome; PDE4, phosphodiesterase 4; PMDA, Pharmaceuticals and Medical Devices Agency; PPI, inorganic pyrophosphate; PRAAS, proteasome-associated autoinflammatory syndrome; PXE, pseudoxanthoma elasticum; SAVI, STING-associated vasculopathy with onset in infancy; STAT, signal transducer and activator of transcription; TSC, tuberous sclerosis complex; US, United States.

Table 5. Selected therapeutic advances in Japan: Evidence level and jurisdiction-specific regulatory status (continued)

Entry / intervention	Mechanistic target	Evidence level	Status in Japan	Status in US/EU	Status verified as of
Behcet disease / apremilast	PDE4-mediated cytokine modulation	Randomized phase 3 evidence and prespecified Japanese subgroup	Oral apremilast approved for Behcet-related oral ulcers inadequately responding to topical therapy	US/EU: oral apremilast approved for adult Behcet-related oral ulcers	August 3, 2026
NNS/PRAAS / baricitinib- JAK inhibition	Type I interferon-JAK-STAT signaling	Small single-arm open-label study	No approved NNS/PRAAS indication; the Japanese JRCT study was suspended/terminated	US/EU: no NNS/PRAAS indication	August 3, 2026
SAVI / JAK- or IFNAR- directed strategies	Type I interferon signaling	Case-series and early clinical evidence	No approved SAVI indication for JAK- or IFNAR-directed therapy	US/EU: no approved SAVI indication	August 3, 2026
PXE / PPi-ENPP1 and mineralization strategies	Restoration of anti-mineralization activity	Randomized/early-phase and preclinical evidence	No approved disease-modifying PPi/ENPP1 therapy	US/EU: no approved disease-modifying PPi/ENPP1 therapy	August 3, 2026
Anti-MDA5 DM / combined and rescue therapy	Interferon, B-cell, T-cell, and macrophage pathways	Observational and case-series evidence	No pathway-specific approved regimen; combination/rescue strategies remain off-label	US/EU: no pathway-specific approved regimen	August 3, 2026

Abbreviations: BP, bullous pemphigoid; B-VEC, beremagene geperpavec; BTK, Bruton's tyrosine kinase; CAPS, cryopyrin-associated periodic syndrome; CD20, cluster of differentiation 20; COL7A1, collagen type VII alpha 1 chain; DEB, dystrophic epidermolysis bullosa; DM, dermatomyositis; EB, epidermolysis bullosa; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; EU, European Union; GPP, generalized pustular psoriasis; IFNAR, interferon- α/β receptor; IL, interleukin; JAK, Janus kinase; JRCT, Japan Registry of Clinical Trials; MDA5, melanoma differentiation-associated protein 5; MEK, mitogen-activated protein kinase kinase; mTORC1, mechanistic target of rapamycin complex 1; NF1, neurofibromatosis type 1; NNS, Nakajo-Nishimura syndrome; PDE4, phosphodiesterase 4; PMDA, Pharmaceuticals and Medical Devices Agency; PPi, inorganic pyrophosphate; PRAAS, proteasome-associated autoinflammatory syndrome; PXE, pseudoxanthoma elasticum; SAVI, STING-associated vasculopathy with onset in infancy; STAT, signal transducer and activator of transcription; TSC, tuberous sclerosis complex; US, United States.

Table 6. Priority research agenda, as proposed by the author, for rare Japanese dermatological conditions

Priority	Rationale	Near-term action recommended by the author	High-yield examples
Interoperable natural history	Administrative and specialist data are fragmented	Common data model linking survey forms, genotype, imaging, treatment, and PROs	AIGA, HHD, EBA, PXE, NNS, SAVI
Endotype-defined trials	Rare cohorts cannot absorb biological heterogeneity	Pre-specified antibody, cytokine, interferon, and drug-associated strata	BP, pemphigus, GPP, anti-MDA5 DM
Mechanism-aligned endpoints	Generic skin scores may miss target engagement	Wound closure, flare abortion, tumor volume, sweat recovery, ILD stabilization	EB, GPP, NF1, AIGA, SAVI
Durability and late effects	Advanced therapies may require lifelong exposure or surveillance	Linked post-marketing cohorts and treatment registries	Gene therapy, MEK/mTOR/JAK/IL-1 therapies
Patient-centered implementation	Financial designation does not eliminate access burden	Caregiver time, heat safety, pain, vision, work, transition, regional access	All diseases
International adaptive platforms	Domestic ultra-rare cohorts remain underpowered	Bayesian, platform, within-patient, and external-control designs	NNS, SAVI, EBA, AIGA, CAPS

Abbreviations: AIGA, acquired idiopathic generalized anhidrosis; BP, bullous pemphigoid; CAPS, cryopyrin-associated periodic syndrome; DM, dermatomyositis; EB, epidermolysis bullosa; EBA, epidermolysis bullosa acquisita; GPP, generalized pustular psoriasis; HHD, Hailey-Hailey disease; IL, interleukin; ILD, interstitial lung disease; JAK, Janus kinase; MDA5, melanoma differentiation-associated protein 5; MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic target of rapamycin; NF1, neurofibromatosis type 1; NNS, Nakajo-Nishimura syndrome; PROs, patient-reported outcomes; PXE, pseudoxanthoma elasticum; SAVI, STING-associated vasculopathy with onset in infancy.

than a systematic review; evidence was not quantitatively synthesized or formally risk-of-bias graded. Searches were limited to English and Japanese and to selected biomedical databases, creating language and database-coverage bias. The evidence base spans regulatory documents, randomized trials, observational or post-marketing studies, case reports, and preclinical work, so findings should not be interpreted as a uniform level of therapeutic efficacy.

The six expanded entries are illustrative and purposefully selected, not exhaustive. Other designated diseases with important skin manifestations - including systemic sclerosis, systemic lupus erythematosus, and vasculitides - could satisfy one or more criteria but were not selected because the framework was designed to illustrate complementary mechanisms within a tractable scope; their exclusion does not imply lesser dermatologic importance. The unit of analysis also varies among administrative entries, disease groups, and mechanistic phenotypes. Japanese official classifications and regulatory status are time-sensitive and may change after the August 3, 2026 cutoff. Finally, national data on registry completeness, regional access, and interoperability are limited; proposals for a common data model, adaptive platforms, and linked post-marketing cohorts are author recommendations.

16. Conclusion

Japanese nanbyo dermatology is distinctive because it combines a national care-and-research framework with population-specific disease biology. The 12 entries in the official skin-centered category remain a coherent core, but they do not represent the full dermatologic burden

of designated intractable disease. Adding six selectively chosen entries or disease groups - Behcet disease, the PM/DM entry with an anti-MDA5 dermatomyositis focus, TSC, CAPS, NNS/PRAAS, and SAVI - produces an 18-entry illustrative framework that is mechanistically informative but neither exhaustive nor the only defensible expansion.

Recent progress demonstrates the value of precision: anti-IL-36 receptor therapy for GPP, topical COL7A1 gene delivery and autologous gene-corrected epidermal sheets for dystrophic EB, MEK inhibition for NF1, mTOR inhibition for TSC, IL-1 blockade for CAPS, and emerging JAK-interferon approaches for NNS/PRAAS and SAVI. We contend that linking designation data to interoperable natural-history cohorts, endotype-defined enrollment, mechanism-aligned outcomes, and international adaptive trials could help Japan develop its current infrastructure into a more fully translational ecosystem. This is a policy and research recommendation rather than a demonstrated consequence of designation. The central message is that rarity should not be the endpoint of classification; it should be the starting point for biologically specific, patient-centered interventions.

Funding: This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (24K14216).

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

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- Received June 24, 2026; Revised August 10, 2026; Accepted August 16, 2026.
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- Released online in J-STAGE as advance publication August 18, 2026.

Mitochondrial DNA A3243G variant-associated MELAS: Recent advances in clinical trials, therapeutic interventions, and disease-modifying strategies

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SUMMARY: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, most often caused by the m.3243A>G mitochondrial DNA variant, represents one of the most clinically significant inherited mitochondrial disorders. This variant disrupts mitochondrial tRNA^{Leu(UUR)} function, leading to impaired mitochondrial protein synthesis and progressive multisystem dysfunction. This narrative review synthesizes recent clinical trials, therapeutic advances, and emerging disease-modifying strategies for m.3243A>G-associated MELAS, with emphasis on evidence published between 2024 and 2026, drawing on PubMed/MEDLINE, Embase, the Cochrane Library, ClinicalTrials.gov, and the EU Clinical Trials Register. Key advances include: *i*) regulatory approval of high-dose taurine supplementation (9–12 g/day), which achieved complete prevention of stroke-like episodes in 60% of participants in a phase III trial; *ii*) phase IIb data (company-reported) indicating that sonlicromanol (KH176) showed signals of improvement in cognition, mood, and fatigue, supporting progression to a phase III registrational trial (KHENERFIN, NCT06451757); *iii*) ongoing trials of zagociguat and TTI-0102 targeting vascular dysfunction and oxidative stress; *iv*) KL1333, a novel NAD⁺ modulator, in phase II evaluation (FALCON trial); and *v*) promising preclinical gene-based approaches—including mitochondria-targeted TALENs, DdCBE base editors, and mitoARCUS nucleases—demonstrating heteroplasmy shifting in patient-derived cells and animal models, though direct clinical applicability to m.3243A>G MELAS requires further investigation. The therapeutic landscape for MELAS is evolving from symptomatic care toward mechanism-based disease modification, led by taurine as the first regulatory-approved disease-modifying therapy and complemented by late-stage small molecules and mitochondrial genome-editing technologies.

Keywords: cysteamine, NAD, oxidative stress, genetic therapy, taurine

1. Introduction

1.1. Clinical significance of the m.3243A>G variant

Mitochondrial DNA (mtDNA) disorders comprise a heterogeneous group of inherited diseases that impair oxidative phosphorylation (OXPHOS), and the m.3243A>G transition in the *MT-TL1* gene is among the most prevalent and clinically important pathogenic variants (1). With an estimated population frequency of 7.6–236 per 100,000, the m.3243A>G variant accounts for approximately 80% of genetically confirmed MELAS cases and is the primary genetic cause of maternally inherited diabetes and deafness (MIDD) (2,3).

This variant disrupts the structure and function of mitochondrial tRNA^{Leu(UUR)}, specifically impairing

post-transcriptional taurine modification at the wobble uridine base ($\tau\text{m}^5\text{U}$; 5-taurinomethyluridine) (4). The resulting defect compromises codon recognition and mitochondrial protein translation, leading to respiratory chain dysfunction that preferentially affects metabolically demanding tissues, including the central nervous system (CNS), cardiac muscle, and skeletal muscle (5).

1.2. Phenotypic spectrum and clinical burden

Clinical expression of m.3243A>G-related disease is highly heterogeneous, ranging from asymptomatic carriers to severe multisystem disease with early mortality (6). MELAS represents the most severe phenotype and is characterized by stroke-like episodes (reported in 95% of patients), seizures (88%), encephalopathy with cognitive

decline (90%), and lactic acidosis (85%) (7). These recurrent stroke-like episodes, which typically occur before age 40 years, cause cumulative neurologic injury and progressive disability (8).

In contrast, the MIDD phenotype has a more restricted but characteristic presentation, with diabetes mellitus affecting approximately 90% of patients and progressive sensorineural hearing loss affecting approximately 95% (9,10). Additional systemic manifestations observed across the MELAS–MIDD spectrum include cardiomyopathy (25%–30%), retinal dystrophy (45%–75%), nephropathy (25%–35%), and gastrointestinal dysfunction (11,12).

1.3. Evolution of treatment paradigms

Historically, management of MELAS largely focused on symptomatic care, including seizure control, metabolic support, and prevention of complications (13). Over the past decade, however, the field has shifted toward mechanism-based interventions that target key pathophysiologic pathways implicated in m.3243A>G disease. Major advances include *i*) identification of the taurine modification defect as a central pathogenic mechanism, enabling the development of targeted taurine supplementation (14); *ii*) recognition of nitric oxide (NO) deficiency and endothelial dysfunction in stroke-like episode pathogenesis, supporting L-arginine/L-citrulline-based approaches (15); *iii*) development of novel small-molecule therapeutics, including ROS-redox modulators (sonlicromanol), NAD⁺ enhancers (KL1333), and soluble guanylate cyclase stimulators (zagociguat) (16,17); and *iv*) rapid progress in gene-based technologies designed to manipulate mitochondrial heteroplasmy using mitochondria-targeted nucleases and base editors (18,19).

In this review, I synthesize recent clinical trial data, therapeutic advances from 2024 to 2026, and emerging disease-modifying strategies for m.3243A>G-associated MELAS.

2. Review approach

2.1. Literature search and source selection

I conducted a comprehensive literature review of publications from January 2020 through February 2026, with an emphasis on developments reported in 2024–2026. I searched PubMed/MEDLINE, Embase, the Cochrane Library, ClinicalTrials.gov, and the EU Clinical Trials Register (CTIS). Search terms included "MELAS syndrome," "mitochondrial encephalomyopathy," "m.3243A>G variant," "A3243G mutation," "MT-TL1," "taurine therapy," "L-arginine," "sonlicromanol," "KL1333," "zagociguat," "gene therapy," "mitoTALEN," "DdCBE," "clinical trials," and "heteroplasmy."

2.2. Scope and evidence selection

I included *i*) phase I–III clinical trials evaluating therapeutic interventions for MELAS; *ii*) observational studies reporting treatment outcomes in patients with genetically confirmed m.3243A>G; *iii*) preclinical studies of gene-based approaches providing proof of concept, particularly those using patient-derived cells and demonstrating functional rescue; *iv*) reviews and consensus statements relevant to MELAS management; and *v*) English-language publications.

I excluded *i*) case reports including fewer than three patients; *ii*) studies focused exclusively on mtDNA mutations other than m.3243A>G without extractable m.3243A>G-specific data; and *iii*) *in vitro* studies that did not include validation in patient-derived cells.

2.3. Data synthesis

For each clinical trial, I extracted study design, sample size, patient population, intervention details (agent, dose, and duration), primary and secondary endpoints, efficacy findings, safety outcomes, and registration identifiers. For preclinical gene-based studies, I extracted the technology platform, target mutation, model system, magnitude of heteroplasmy shift, and evidence of functional restoration.

2.4. Evidence appraisal

I appraised preclinical studies for translational relevance based on the use of patient-derived cells or tissues and demonstration of correction of a disease-relevant functional phenotype.

3. Current and emerging therapeutic strategies

3.1. Taurine supplementation: First regulatory-approved disease-modifying therapy

3.1.1. Molecular rationale and mechanism

The discovery that the m.3243A>G variant disrupts taurine-dependent post-transcriptional modification at position 34 (the wobble position) of mt-tRNA^{Leu(UUR)} identified a specific molecular target for therapy (20). In healthy mitochondria, this uridine is modified to 5-aurinomethyluridine (τm⁵U), which is required for accurate decoding of UUG leucine codons; in m.3243A>G, τm⁵U hypomodification promotes mistranslation and destabilization of respiratory chain subunits (21).

Preclinical experiments using patient-derived cybrid cells showed that taurine supplementation (0.3 mM in culture media) improved oxygen consumption, restored mitochondrial membrane potential, and reduced oxidative stress (22), supporting proof of concept for targeting the taurine modification defect.

3.1.2. Phase III clinical trial data

A multicenter, open-label phase III trial by Ohsawa and colleagues enrolled 10 patients with recurrent stroke-like episodes and genetically confirmed m.3243A>G. Participants received high-dose taurine for 52 weeks: 9 g/day for body weight < 50 kg and 12 g/day for body weight ≥ 50 kg, administered in three divided doses with meals.

Primary endpoint findings included: *i*) Complete prevention of stroke-like episodes (100% responder rate): 60% (95% CI: 26.2%–87.8%); *ii*) The lower bound of the 95% CI (26.2%) exceeded the prespecified null threshold of 5%, supporting statistical significance.

Secondary endpoint findings included: *i*) ≥ 50% reduction in stroke-like episode frequency (50% responder rate): 80% (95% CI: 44.4%–97.5%); *ii*) Annual stroke-like episode relapse rate decreased from 2.22 ± 0.73 (pretrial) to 0.72 ± 0.62 during the evaluation period ($p = 0.001$); and *iii*) Annual focal neurologic deficit events decreased from 33 episodes (pretrial) to 8 episodes during the evaluation period.

Mechanistic validation was demonstrated by increased $\tau\text{m}^5\text{U}$ modification rates in peripheral blood leukocytes after treatment in five assessed patients ($p < 0.05$), indicating target engagement with oral taurine (23).

Safety findings indicated no severe adverse events attributed to taurine; long-term tolerability has been favorable, with preliminary observations of sustained benefit in patients treated for more than 9 years and no evidence of cumulative toxicity (22).

3.1.3. Regulatory status and implementation

Based on these results, Japan's Pharmaceuticals and Medical Devices Agency (PMDA) granted marketing authorization for taurine to prevent stroke-like episodes in MELAS in February 2019, representing the first disease-modifying therapy to receive formal regulatory approval for MELAS (in Japan; approved as タウリン 散98%「大正」 by Taisho Pharmaceutical Co., Ltd.; orphan drug designation; PMDA approval list: <https://www.pmda.go.jp/review-services/drug-reviews/review-information/p-drugs/0030.html>, accessed June 2026) (24). In clinical practice, the labeled approach is used for patients with genetically confirmed m.3243A>G and a history of recurrent stroke-like episodes, typically at 9–12 g/day divided three times daily with meals, with monitoring focused on clinical episode frequency and, where available, molecular or biochemical correlates of response.

3.2. L-arginine and L-citrulline: Targeting vascular dysfunction

3.2.1. Vascular hypothesis and nitric oxide deficiency

Stroke-like episodes in MELAS have been linked to endothelial dysfunction and impaired nitric oxide (NO) bioavailability in cerebral microvasculature (25). Reported abnormalities include reduced flow-mediated dilation and elevated endothelin-1, consistent with systemic endothelial dysfunction, and mitochondrial respiratory chain impairment in vascular endothelium may reduce NO synthase activity, contributing to vasoconstriction and microcirculatory disturbance (26).

3.2.2. Clinical evidence for L-arginine

L-arginine, a substrate for NO synthesis, has been evaluated for both acute treatment and chronic prophylaxis in MELAS (27). For acute episodes, a prospective multicenter study by Koga and colleagues proposed an intravenous regimen of 0.5 g/kg L-arginine hydrochloride (maximum 30 g) administered over 30–60 minutes, ideally within 6 hours of symptom onset (28); in 10 patients, headache improved in 90% at 2 hours post-infusion and nausea/vomiting improved in 80%, with additional improvements in impaired consciousness and visual symptoms and no severe treatment-related adverse events reported.

For chronic prophylaxis, oral L-arginine at 150–300 mg/kg/day in 2–3 divided doses was associated with prolongation of the interictal interval during a 2-year period ($p = 0.0625$), although 7-year follow-up indicated that benefit did not fully halt progression, consistent with the progressive nature of MELAS (28).

A pragmatic pharmacodynamic target has been proposed: maintaining plasma arginine concentrations above 167 $\mu\text{mol/L}$, which has been associated with reduced recurrence of stroke-like episodes and can be used to guide dose optimization (29).

3.2.3. L-citrulline and bioavailability considerations

L-citrulline, which is converted to L-arginine *via* the arginine–citrulline cycle, may offer advantages including better oral bioavailability, reduced first-pass hepatic metabolism, and more sustained elevation of plasma arginine (30). A phase I dose-finding study (NCT03952234) completed in 2019 established safety and tolerability; and subsequent phase II efficacy trials are planned (31).

3.3. Novel small-molecule therapeutics in clinical development

Key active and recently completed interventional studies in MELAS and the broader m.3243A>G spectrum—including trials of KL1333 (FALCON), zagociguat (PRIZM), sonlicromanol (KHENERGIZE/KHENEXT transitioning to KHENERFIN), TTI 0102 (TTI MITO 001), and L citrulline—are summarized in Table 1 (30–41).

Table 1. Active and recent clinical trials for MELAS and m.3243A>G spectrum disorders (2024–2026)

Trial Name	Intervention	Mechanism	Phase	Status	NCT Number	Primary Endpoints	Sponsor	n	Country/Region
Taurine Phase III	Taurine 9–12 g/day	rm ³ U modification restoration	III	Completed (approved Japan 2019)	UMIN000011908	Complete prevention of stroke-like episodes	Tohoku Univ./MELAS Research Group (Japan)	31	Japan
FALCON	KL1333 (NAD ⁺ modulator)	NAD ⁺ :NADH ratio normalization, mitochondrial biogenesis	II	Recruiting	NCT05650229	Fatigue severity, 6-minute walk test	Abliva AB	180 est.	US, AU, BE, CZ, DK, FR, DE, IT, NL, ES, UK
PRIZM	Zagociguat 15–30 mg/day	Soluble guanylate cyclase stimulation	IIb	Active, not recruiting (43 enrolled)	NCT06402123	Cognitive performance, fatigue	Tisento Therapeutics	43	US, AU, CA, DE, IT, UK
KHENERFIN	Sonlicromanol 100 mg BID	ROS-redox modulation	III	Recruiting (started Feb 1, 2026; est. completion Sep 30, 2028)	NCT06451757	Physical fatigue symptoms, balance, cognition	Khondrion BV	220 est.	US, DK, FR, DE, IT, NL, UK
TTI-MITO-001	TTI-0102 (cysteamine)	Antioxidant, mitochondrial protection	II	Recruiting	NCT06644534	Safety, efficacy, PK/PD parameters	Thiogenesis Therapeutics, Inc.	12 est.	France, Netherlands
L-Citrulline Dose-Finding	L-citrulline	NO precursor, superior bioavailability	I	Completed	NCT03952234	Safety, maximum tolerated dose	Baylor College of Medicine	10	United States

Data Sources: ClinicalTrials.gov and EU Clinical Trials Register; all registry data accessed June 2026. Abbreviations: BID, twice daily; NO, nitric oxide; PK/PD, pharmacokinetics/pharmacodynamics; ROS, reactive oxygen species.

3.3.1. Sonlicromanol (KH176): ROS-redox modulation

Sonlicromanol is a ROS-redox modulator; its active metabolite (KH176m/KH183) is intended to reduce oxidative stress associated with OXPHOS dysfunction (32) and modulate cellular redox balance. In the randomized, placebo-controlled 28-day phase IIb KHENERGIZE study in m.3243A>G MELAS spectrum disorders, the primary endpoint was not met, but multiple secondary endpoints improved (16,33). In the 52-week KHENEXT open-label extension, improvements were reported across cognition (Cognitive Failures Questionnaire), mood (Beck Depression Inventory), pain, fatigue, motor function (balance), and quality-of-life measures, with an acceptable safety profile and no serious adverse effects attributed to sonlicromanol (16,34).

These phase IIb findings supported progression to a phase III program, and the planned registrational study KHENERFIN (NCT06451757) commenced recruitment on February 1, 2026, with estimated completion September 30, 2028 (Sponsor: Khondrion BV; *n* = 220 estimated; US, Denmark, France, Germany, Italy, Netherlands, UK; ClinicalTrials.gov NCT06451757, accessed June 2026) (42). Sonlicromanol has also received orphan drug designation from the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for several mitochondrial indications (including MELAS).

3.3.2. KL1333: NAD⁺ pathway modulation and mitochondrial biogenesis

KL₁₃₃₃ is an orally bioavailable NAD⁺ modulator that increases the NAD⁺:NADH ratio through *NQO*₁ activation (43), with downstream engagement of the *SIRT1*/AMPK/*PGC*_{1α} axis to support mitochondrial biogenesis and oxidative metabolism (36). In fibroblasts derived from MELAS patients harboring m.3243A>G, preclinical studies reported increased ATP production, reduced lactate and reactive oxygen species (ROS) levels, improved mitochondrial mass and membrane potential, and increased maximal oxygen consumption, alongside activation of biogenesis-related signaling (36).

A phase 1a/1b study evaluated safety, tolerability, and pharmacokinetics in healthy volunteers and patients with primary mitochondrial disease (37). The FALCON phase II study (NCT05650229) is recruiting adults with primary mitochondrial disease, including MELAS–MIDD spectrum disorders, and evaluates fatigue severity, lower-extremity function (6-minute walk test), endurance, quality of life, and biomarkers (including fibroblast growth factor 21 [FGF21] and growth differentiation factor 15 [GDF15]) over 48 weeks of treatment.

3.3.3. Zagociguat: Soluble guanylate cyclase (sGC) stimulation

Zagociguat is an sGC stimulator designed to enhance

NO–cGMP signaling and address vascular dysfunction, providing a mechanistic complement to NO precursor strategies (38). The PRIZM phase IIb randomized, double-blind, placebo-controlled crossover trial (NCT06402123) enrolled 43 participants across the US, Australia, Canada, Germany, Italy, and UK (Sponsor: Tisento Therapeutics; ClinicalTrials.gov NCT06402123, accessed June 2026) and is evaluating 15 mg and 30 mg daily dosing versus placebo for 12 weeks in adults with genetically and phenotypically defined MELAS (39), with cognitive performance and fatigue severity as primary endpoints and imaging/biomarker and quality-of-life measures as secondary endpoints.

3.3.4. TTI-0102: Cysteamine prodrug (antioxidant strategy)

TTI-0102 is an enteric-coated, delayed-release cysteamine formulation intended to provide mitochondrial antioxidant effects (40). A randomized, double-blind, placebo-controlled trial (NCT06644534; Sponsor: Thiogenesis Therapeutics, Inc.; $n = 12$ estimated; France and Netherlands; ClinicalTrials.gov, accessed June 2026) is evaluating TTI-0102 versus placebo for up to 6 months in MELAS (41), using a 2:1 randomization and including an interim review after nine participants complete 3 months of treatment. Primary objectives include evaluation of efficacy, safety, and tolerability, as well as pharmacokinetics and pharmacodynamics at steady state.

A consolidated overview of major pharmacologic interventions—including taurine, L arginine/L citrulline, mitochondrial cofactors, and late stage small molecules—with their mechanisms, dosing regimens, evidence levels, principal clinical outcomes, and safety profiles is provided in Table 2 (13,20-41,43).

3.4. Gene-based therapeutic approaches: Heteroplasmy manipulation

The principal gene based therapeutic platforms under development for m.3243A>G related MELAS—including mitoTALEN/mpTALEN, mitochondria targeted zinc finger nucleases, DdCBE base editors, mitoARCUS, mitochondrial replacement therapy, and allotopic expression—are compared in Table 3 with respect to targets, mechanisms, development stage, key achievements, and remaining challenges (18,19,44-51).

3.4.1. Mitochondria-targeted TALENs (mitoTALEN/mpTALEN)

Mitochondria-targeted transcription activator-like effector nucleases (TALENs) have been engineered by adding mitochondrial localization sequences to enable selective cleavage of mtDNA (44), with the goal of preferentially eliminating mutant genomes and shifting heteroplasmy toward wild type. Yahata and

colleagues (2025) reported optimized mtDNA-targeted platinum TALENs (mpTALENs) enabling bidirectional heteroplasmy modification in patient-derived induced pluripotent stem cells (iPSCs) harboring m.3243A>G (18), including improvements intended to enhance specificity, reduce mtDNA copy-number depletion, and support controlled expression.

In that work, mpTALEN treatment yielded heteroplasmy modulation spanning approximately 11% to 97% mutant load across clones; when mutant load fell below pathogenic thresholds, differentiation capacity was restored, providing functional validation of the heteroplasmy-threshold concept (18). Key barriers remain, including delivery to the CNS, skeletal muscle, and heart; transient reductions in mtDNA copy number during treatment; and the possibility that repeat dosing may be needed over time.

3.4.2. CRISPR-free base editing: DdCBE and related editors

Because guide RNAs do not readily traverse mitochondrial membranes, conventional CRISPR-Cas approaches are not directly applicable in mitochondria (19); DdCBE (DddA-derived cytosine base editor) addresses this limitation using TALE DNA-binding domains fused to split halves of a deaminase to enable mtDNA base editing without guide RNAs or double-strand breaks. Recent work has focused on improving specificity and product purity, demonstrating correction of pathogenic variants in patient-derived systems, and refining delivery modalities (including mRNA-based approaches (45) and lipid nanoparticle formulations) to move toward clinical translation (46).

For m.3243A>G specifically, direct sequence correction requires A→G (adenine-to-guanine) base editing, which is not achievable with current DdCBE platforms that only enable C→T conversions; this represents a fundamental limitation for direct m.3243A>G correction. The ongoing development of mitochondrial adenine base editors (mitoABEs) is the critical enabling step (52), though published mitoABE tools remain at proof-of-concept stages and have not yet been applied to the *MT-TL1* locus in patient-derived cells.

3.4.3. Engineered meganucleases: mitoARCUS

mitoARCUS is an engineered meganuclease platform tailored for mitochondrial applications; preclinical work has demonstrated elimination of mutant mtDNA (including m.3243G) with resultant heteroplasmy shifts and functional improvements in cellular respiration consistent with the heteroplasmy reduction achieved (53). Current development efforts focus on optimizing delivery strategies and tissue targeting for *in vivo* use. Critically, all published mitoARCUS work for MELAS remains at

Table 2. Pharmacological interventions for m.3243A>G MELAS: mechanisms and evidence Levels

Intervention	Mechanism of Action	Dosing Regimen	Evidence Level	Key Clinical Results	Safety Profile
Taurine	Restoration of m ³ U modification in mt-tRNA ^{Leu} (UUR) [^]	9–12 g/day PO divided TID	Level I	60% complete stroke-like episode prevention; 80% ≥50% reduction	Well-tolerated; no severe AEs; 9+ years safety data
L-Arginine IV	Acute NO precursor, vasodilation	0.5 g/kg (max 30 g) IV over 30–60 min during episodes	Level II	90% headache improvement; 80% nausea/vomiting improvement	Safe for acute use
L-Arginine PO	Chronic NO precursor, prophylaxis	150–300 mg/kg/day PO divided BID–TID	Level II	Extended interictal phase; target plasma level >167 μmol/L	Well-tolerated long-term
L-Citrulline	NO precursor, superior bioavailability	Under investigation	Level III	Dose-finding complete; phase II planned	Phase I: acceptable safety
Sonlicromanol	ROS-redox modulation, antioxidant	100 mg PO BID	Level III	Improved cognition (CFQ), mood (BDI), pain, fatigue, balance	No serious AEs; well-tolerated
KL1333	NAD ⁺ pathway enhancement, <i>SIRT1/PGC-1α</i> /AMPK/ <i>PGC-1α</i> activation	Under investigation	Level II	Preclinical: ↑ATP, ↓lactate, ↑mitochondrial mass in MELAS cells	Phase I: well-tolerated
Zagociguat	Soluble guanylate cyclase stimulation, NO-cGMP pathway	15–30 mg/day PO	Level II	Ongoing (PRIZM trial recruiting)	Under evaluation
TTI-0102	Cysteamine prodrug, antioxidant	Under investigation	Level II	Ongoing (TTI-MITO-001 recruiting)	Under evaluation
CoQ10/Ubiquinol	Electron transport chain cofactor, antioxidant	300–600 mg/day PO	Level III	Variable individual responses; part of standard cocktail	Well-tolerated
Idebenone	<i>Synthetic CoQ10 analog, enhanced mitochondrial penetration</i>	900–2,250 mg/day PO	Level II	<i>Reduced cerebral lactate in small studies; inconsistent results</i>	<i>Generally well-tolerated</i>

Evidence Level criteria: Level I, peer-reviewed RCT or meta-analysis; Level II, open-label trial or comparative cohort; Level III, single-arm study, registry data, or company-reported data; Level IV, case series, preclinical study, or expert consensus. *Abbreviations:* AE, adverse event; BDI, Beck Depression Inventory; BID, twice daily; CFQ, Cognitive Failures Questionnaire; CoQ10, coenzyme Q10; IV, intravenous; NO, nitric oxide; PO, per os (oral); TID, three times daily; m³U, 5-taurinomethyluridine.

Table 3. Gene-based therapeutic approaches for m.3243A>G MELAS: technology platforms and translational status

Technology	Platform	Target	Mechanism	Development Stage	Key Achievements	Major Challenges
mitoTALEN/ mpTALEN	Mitochondria-targeted TALENs	Mutant mtDNA elimination	Selective cleavage of mutant genomes → heteroplasmy shift	Preclinical	Heteroplasmy modulation 11%–97% in m.3243A>G-iPSCs; restoration of differentiation capacity	Tissue delivery (CNS, muscle); mtDNA copy number management; repeated dosing requirements
mtZFN	Mitochondria-targeted zinc finger nucleases	Mutant mtDNA elimination	Obligate heterodimeric nucleases cleave mutant mtDNA	Preclinical	Heteroplasmy reduction in cybrids; restored respiration	Optimization of specificity; delivery systems
DdCBE	DddA-derived cytosine base editor	Point mutation correction (C→T editing)	Split deaminase + TALE binding → precise base editing without DSBs	Preclinical	Functional correction of m.4291T>C in patient cells; high specificity for target editing	Development of ABEs for A→G correction needed for m.3243A>G; off-target nuclear editing risk
mitoARCUS	Engineered meganuclease	m.3243G elimination	Designed nuclease with high specificity for m.3243G	Preclinical	Efficient heteroplasmy shift in patient cells; functional improvement	Delivery optimization; <i>in vivo</i> validation
MRT	Mitochondrial replacement therapy	Prevention of transmission	Nuclear transfer to enucleated donor oocyte	Clinical (reproductive)	8 babies born (UK, July 2025); prevention of high-level mutation transmission	Carryover mtDNA (0.5%–2%); reversion risk; not applicable to affected patients
Allotopic expression	Nuclear gene relocation	Protein replacement	MT-encoded genes relocated to nucleus; protein imported to mitochondria	Phase III (LHON only)	FDA/EMA approval for <i>ND4</i> (LUMEVOQ) in LHON	Not applicable to tRNA genes (<i>MT-TL1</i>); import mechanisms differ

Abbreviations: ABE, adenine base editor; CNS, central nervous system; DdCBE, DddA-derived cytosine base editor; DSB, double-strand break; iPSC, induced pluripotent stem cell; LHON, Leber hereditary optic neuropathy; MRT, mitochondrial replacement therapy; mtDNA, mitochondrial DNA; mtZFN, mitochondria-targeted zinc finger nuclease; TALEN, transcription activator-like effector nuclease.

the preclinical stage; no human therapeutic application has been reported. Key barriers to clinical translation include efficient delivery to post-mitotic tissues (CNS, skeletal muscle, and cardiac muscle), long-term stability of heteroplasmy shifts, and regulatory pathways for mitochondria-targeted nucleases.

4. Discussion

4.1. Paradigm shift from symptomatic to disease-modifying therapy

Over the past decade, mechanistic insight into m.3243A>G pathophysiology has fundamentally reshaped the therapeutic landscape for MELAS (5,13,24). The regulatory approval of high-dose taurine supplementation in Japan represents a milestone: it is the first disease-modifying therapy that targets an underlying molecular defect (taurine modification deficiency) rather than focusing solely on symptomatic management (14,20,23,24).

The phase III findings—showing complete prevention of stroke-like episodes in 60% of treated participants—set a new benchmark for clinically meaningful benefit in this population (23). Importantly, measurement of m^3U modification rates provides pharmacologic proof of target engagement and supports biological plausibility that correcting the molecular defect can translate into clinical benefit (21,23). Available long-term observations (including treatment beyond 9 years in some patients) further support the feasibility of sustained administration without clear evidence of cumulative toxicity, although rigorous long-term comparative data remain limited, underscoring the need for post-marketing surveillance studies (22).

At the same time, taurine is not a panacea for the multisystem burden of MELAS. The disease reflects convergent dysfunction across mitochondrial translation, bioenergetics, redox homeostasis, vascular signaling, and compensatory pathways such as mitochondrial biogenesis. This complexity provides a strong rationale for mechanism-based add-on therapies that address complementary nodes of pathophysiology, particularly for patients with persistent neurologic events, progressive myopathy, or significant systemic involvement despite optimized foundational care (5,15,32,36).

4.2. Multi-hit therapeutic model: Rationale for combination approaches

The pathophysiology of MELAS encompasses multiple interacting mechanisms, including mt-tRNA modification defects, respiratory chain dysfunction, oxidative stress, nitric oxide (NO) deficiency with endothelial dysfunction, and impaired mitochondrial biogenesis. This pathophysiology supports a proposed conceptual multi-hit therapeutic strategy in which therapies are layered

to target distinct mechanisms rather than relying on a single agent to address all clinically relevant pathways (5,15,32,43,54).

In my proposed tiered framework, the "current standard" layer includes evidence-based pharmacotherapy such as taurine to address the taurine modification defect (14,20,23), NO precursor strategies (L-arginine and/or L-citrulline) to mitigate vascular dysfunction (25-29), and selected cofactors commonly used in mitochondrial practice (e.g., coenzyme Q10, riboflavin, and L-carnitine) as supportive measures (13). Beyond this foundation, late-stage investigational agents provide plausible complementary benefits: sonlicromanol may mitigate redox imbalance and improve patient-centered outcomes (e.g., fatigue and mood) (32-35), while KL1333 may enhance mitochondrial biogenesis and energy metabolism by modulating NAD^+ pathways (36,37,43); zagociguat may augment NO-cGMP signaling through soluble guanylate cyclase stimulation, potentially complementing NO precursor therapy (38,39).

This tiered approach is intended to be practical: it mirrors how clinicians often escalate therapy when a patient's phenotype remains active despite baseline measures, while still grounding escalation in mechanistic plausibility and emerging trial evidence (32,37,39,43).

Combination strategies also reflect the clinical heterogeneity of MELAS. Stroke-like episodes may dominate in one patient, whereas myopathy, neuropsychiatric symptoms, cardiomyopathy, or endocrine manifestations may dominate in another. In this context, a flexible multi-hit model can serve as a clinical reasoning scaffold: it helps align therapy selection with the most salient pathophysiologic drivers and measurable outcomes for an individual patient rather than applying a "one-size-fits-all" regimen (6,7,9,11,54).

This tiered, mechanism-based approach is integrated into a proposed conceptual multi-hit therapeutic framework (presented here as a conceptual guide for clinicians, not a validated clinical algorithm), incorporating lifestyle measures, evidence-based pharmacotherapy, advanced small molecules, and emerging gene-based strategies.

4.3. Challenges in clinical trial design for MELAS

Several intrinsic features of MELAS complicate conventional trial design. First, disease heterogeneity is substantial: age at onset, organ involvement, clinical severity, and event frequency vary widely, and heteroplasmy levels differ across tissues and change over time (6,55). Second, patient numbers are limited by rarity and geographic dispersion, necessitating multicenter collaboration and careful enrollment criteria to preserve internal validity without making recruitment infeasible (37,39). Third, endpoint selection is difficult: stroke-like episodes are episodic and unpredictable, and short-duration studies may miss clinically meaningful changes

in a progressive disease with variable trajectories (23,37,39).

To address these constraints, current trials increasingly incorporate patient-reported outcomes, multidomain functional measures, and biomarker panels designed to capture signal across heterogeneous phenotypes (37,39). Adaptive and crossover designs can improve efficiency when sample sizes are small, although they introduce analytic complexity and require careful attention to carryover effects and event timing (38,40). The ongoing phase II programs discussed in this review illustrate this trend by pairing clinical outcomes (fatigue, cognition, function, quality of life) with biomarkers (*e.g.*, FGF21, GDF15) and imaging (*e.g.*, MRI/magnetic resonance spectroscopy [MRS]) to triangulate efficacy (37,39,56).

Another persistent limitation is the absence of head-to-head comparisons among foundational therapies (*e.g.*, taurine *vs.* L-arginine *vs.* combination regimens) and among investigational agents. Without comparative effectiveness data, treatment choices often rely on a synthesis of mechanistic rationale, limited clinical datasets, tolerability considerations, and patient-specific phenotype rather than definitive superiority evidence (13,23,32,38,43).

4.4. Precision medicine framework: Genotype-guided therapy selection

A precision medicine approach to MELAS requires integrating genotype, phenotype, and biomarkers to guide therapy selection and monitoring. Mutation-specific biology matters: taurine therapy is mechanistically aligned with the taurine modification defect associated with m.3243A>G and some related *MT-TL1* variants, whereas other mitochondrial genotypes may require different targeting strategies (14,20,23). Phenotype-based stratification is equally important. For example, a patient with frequent stroke-like episodes may warrant aggressive optimization of taurine plus NO pathway support, whereas a patient with prominent myopathy and fatigue may be a better candidate for therapies targeting mitochondrial biogenesis or redox balance as those agents mature in clinical development (5,57).

Biomarkers can support both baseline stratification and longitudinal monitoring, but their roles remain imperfect. FGF21 and GDF15 are among the most studied circulating biomarkers for mitochondrial disease burden and may correlate with severity (56); plasma arginine monitoring can guide NO precursor dosing and offers a practical pharmacodynamic target (29); tm^5U modification rate (where available) offers a uniquely direct measure of taurine target engagement (21,23). Serial heteroplasmy measurements can add context, but interpretation must account for tissue specificity and age-related dynamics, particularly the decline of blood heteroplasmy over time (58).

In practice, the goal is to move from empiric

escalation to data-informed adjustment—selecting therapies based on a patient's most actionable pathophysiologic drivers and using feasible clinical and biomarker endpoints to evaluate benefit. Achieving this will require prospective validation linking biomarker shifts to clinically meaningful outcomes, especially across diverse ages and phenotypes (37,39,56,57).

A structured summary of candidate genetic, biochemical, imaging, and functional biomarkers—including blood and urine heteroplasmy, FGF21, GDF15, plasma L arginine, lactate, tm^5U modification rate, brain MRS metrics, and exercise capacity measures—is presented in Table 4 to guide disease monitoring and assessment of treatment response (21,23,29,37,39,52,56-58).

4.5. Gene therapy: Transformative potential and remaining hurdles

Gene-based strategies offer the most direct route to addressing the root genetic defect by shifting heteroplasmy or editing pathogenic mtDNA. Proof-of-concept studies using mitochondria-targeted nucleases (including optimized TALEN-based approaches) demonstrate that heteroplasmy can be modulated in patient-derived systems and that reducing mutant load below pathogenic thresholds can restore cellular phenotypes, supporting the therapeutic premise (18,19,44).

However, formidable translational barriers remain. Effective delivery to clinically relevant tissues—particularly the central nervous system, skeletal muscle, and heart—is a primary challenge, and delivery platforms must balance efficiency with safety (44,51,53). Additional concerns include transient reductions in mtDNA copy number during nuclease activity, durability of heteroplasmy shifts over time in dividing tissues (18,44,59), and the need for rigorous evaluation of off-target effects and long-term genotoxicity (59). Regulatory pathways for mtDNA therapies must also contend with unique issues such as tissue-specific heteroplasmy thresholds and the complexity of defining validated surrogate endpoints (24,60).

Despite these constraints, progress from 2020 to 2026 has been rapid, and the direction of the field suggests that early human trials may become feasible within the next decade if delivery and safety hurdles can be addressed. For clinicians and investigators, these advances reinforce the importance of developing standardized outcome measures, biomarkers, and natural history datasets now, so that emerging genetic therapies can be evaluated efficiently and responsibly when they reach clinical testing (51,59,60).

5. Conclusions

The therapeutic landscape for m.3243A>G-associated

Table 4. Biomarkers for disease monitoring and treatment response assessment in MELAS

Biomarker Category	Specific Markers	Clinical Utility	Advantages	Limitations	Target Range/Interpretation	Validation Status/Clinical Applicability
Genetic	Blood heteroplasmy (m.3243A>G %)	Diagnosis, genotype confirmation	Noninvasive, widely available	Age-dependent decline; tissue discordance	Diagnostic threshold >10%-20%; severity correlation imperfect	Routine clinical use; Diagnosis (D), Monitoring (M)
Genetic	Urine epithelial cell heteroplasmy	Better phenotype correlation vs. blood	Noninvasive, more stable than blood	Inpatient variability; requires specialized testing	Generally higher than blood; better reflects disease burden	Specialist centre; Diagnosis (D), Monitoring (M)
Biochemical	FGF21 (serum)	Disease severity, treatment response	Strong diagnostic accuracy (AUC 0.81); correlates with myopathy	Elevated in other mitochondrial/muscle disorders; limited CNS specificity	Elevated in MELAS; decreasing levels may indicate treatment response	Specialist centre; Monitoring (M), Treatment response (T)
Biochemical	GDF15 (serum)	Disease severity, multisystem involvement	Highest diagnostic accuracy (AUC 0.89); correlates with IPMDS score	Nonspecific (elevated in inflammation, malignancy)	Elevated in MELAS; serial measurements track disease burden	Specialist centre; Monitoring (M), Prognosis (P)
Biochemical	Plasma L-arginine	Guide L-arginine/L-citrulline dosing	Direct pharmacodynamic marker for NO pathway therapy	Acute fluctuations with meals, stress	Target >167 $\mu\text{mol/L}$ for stroke-like episode prevention	Specialist centre; Monitoring (M), Treatment response (T)
Biochemical	Plasma/CSF lactate	Metabolic derangement	Widely available, real-time metabolic status	Variable with exercise, feeding; may be normal between episodes	Elevated during acute episodes; lactate:pyruvate ratio >20 suggestive	Routine clinical use; Monitoring (M)
Biochemical	tm^3U modification rate	Taurine therapy efficacy	Direct mechanistic biomarker; validates molecular correction	Specialized research assay; limited availability	Increased with taurine treatment; correlates with clinical benefit	Research setting only; Treatment response (T)
Imaging	Brain MRS (lactate peak)	Active stroke-like lesion	Noninvasive localization of metabolic dysfunction	Requires specialized MRI protocol and expertise	Elevated lactate peak at 1.3 ppm in active lesions	Specialist centre; Diagnosis (D), Monitoring (M)
Imaging	Brain MRS (NAA)	Neuronal integrity	Marker of neuronal loss/dysfunction	Nonspecific for mitochondrial disease	Reduced NAA in affected regions; marker of irreversible damage	Specialist centre; Monitoring (M), Prognosis (P)
Functional	6-minute walk test	Exercise capacity, functional status	Standardized, reproducible, clinical trial endpoint	Affected by comorbidities (cardiac, pulmonary, orthopedic)	Baseline varies; improvement ≥ 30 m considered clinically meaningful	Routine clinical use; Monitoring (M), Treatment response (T)
Functional	VO_{max} , anaerobic threshold	Whole-body mitochondrial capacity	Objective measure of OXPHOS function	Requires specialized equipment and protocols	Reduced in MELAS; improvement indicates enhanced mitochondrial function	Specialist centre; Monitoring (M), Treatment response (T)

Abbreviations: AUC, area under the curve; CNS, central nervous system; CSF, cerebrospinal fluid; FGF21, fibroblast growth factor 21; GDF15, growth differentiation factor 15; IPMDS, International Paediatric Mitochondrial Disease Scale; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NO, nitric oxide; OXPHOS, oxidative phosphorylation; tm^3U , 5-taurinomethyluridine; VO_{max} , maximal oxygen consumption.

MELAS has changed substantially over the past decade (5,13,24). The regulatory approval of high-dose taurine supplementation—showing complete prevention of stroke-like episodes in 60% of participants in a phase III study and demonstrating mechanism-based correction of the taurine modification defect—marks a decisive shift from purely symptomatic management toward disease-modifying therapy (20,23).

Multiple late-stage and emerging therapies are now expanding the mechanistic scope of intervention. Ongoing trials of novel small molecules—including sonlicromanol (a ROS-redox modulator advancing to phase III development) (32-35), KL1333 (an NAD⁺ pathway modulator in phase II evaluation) (36,37,43), zagociguat (an sGC stimulator in phase IIb) (38,39), and TTI-0102 (a cysteamine prodrug in phase II)—offer complementary approaches that target oxidative stress, mitochondrial biogenesis, and vascular signaling (40,41). The convergence of these programs supports a multi-hit treatment paradigm in which therapies are selected and layered to address the dominant pathophysiologic drivers in an individual patient (40,54).

Gene-based strategies may ultimately be the most transformative by directly addressing the genetic basis of disease through heteroplasmy manipulation. Mitochondria-targeted nucleases (including mitoTALEN/mpTALEN) (18,44), CRISPR-independent base editing platforms (e.g., DdCBE and related editors) (19,45,46,52), and engineered meganucleases (mitoARCUS) have demonstrated proof of concept in patient-derived systems, including meaningful heteroplasmy shifts with restoration of mitochondrial function (18,53). Major translational challenges remain—particularly safe and efficient delivery to the central nervous system and muscle and the need for rigorous long-term safety data (51,59)—but progress from 2020 to 2026 suggests a realistic pathway toward first-in-human studies within the next decade (60).

Looking ahead, I expect the greatest clinical gains to come from precision medicine strategies (57) that integrate genotype, phenotype, and biomarkers to guide therapy selection and monitoring (37,39,56). As additional disease-modifying therapies mature, patients and families affected by MELAS have reason for cautious optimism that the historically progressive course of this disorder may become increasingly modifiable through targeted, mechanism-based interventions (32,43,44).

This narrative review has several limitations. The evidence base for most therapeutic agents discussed remains early-stage: most small-molecule trials involve fewer than 50 participants, and many are open-label in design. Data for sonlicromanol's phase IIb outcomes include company-reported results (33,34) in addition to the peer-reviewed publication (16); TTI-0102 results (41) derive from a clinical trial registry entry for a 12-participant study. Several gene-based strategies (mitoTALEN, DdCBE, mitoARCUS) remain at preclinical stages with no human therapeutic application

reported to date. The multi-hit therapeutic model presented here is a proposed conceptual framework and has not been prospectively validated in clinical trials. Most clinical evidence derives from adult populations in Japan, Europe, and North America, limiting generalizability. Biomarker thresholds and treatment-response criteria require prospective validation. As a narrative synthesis, this review may be subject to selection bias, and comprehensiveness is not guaranteed.

Acknowledgements

The author acknowledges patients and families affected by MELAS syndrome whose participation in clinical research has advanced our understanding and therapeutic capabilities. The author also recognizes the international research community whose collaborative efforts have transformed treatment landscape for this rare disorder.

Funding: None.

Conflict of Interest: The author has no conflicts of interest to disclose.

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Received March 14, 2026; Revised July 4, 2026; Accepted July 7, 2026.

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Released online in J-STAGE as advance publication July 10, 2026.

Phenotypic variability of Fragile-X syndrome in Asian population: A systematic review

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SUMMARY: Fragile X syndrome (FXS) is the most common genetic cause of inherited intellectual disabilities. Individuals with full mutation of FXS exhibit physical and behavioral symptoms in addition to other comorbidities. The clinical features of FXS have been widely studied in Caucasians; however, they remain limited in the Asian population. This study aimed to characterize the spectrum and variability of physical and behavioral phenotypes in Asian populations. A total of 5,830 studies from the PubMed, ScienceDirect, Scopus, and Cochrane/CENTRAL databases were screened using the Covidence software. We identified FXS-specific research studies conducted in Asia that reported the clinical characteristics of individuals with FXS. This review summarizes 51 studies from different Asian regions. The frequently reported physical characteristics were large and prominent ears (72.63%), an elongated face (57.49%), and macroorchidism (45.21%). The three most prevalent behavioral characteristics were intellectual disability (ID), hyperactivity, and social withdrawal, reported in 99%, 77%, and 55% of all cases, respectively. Our findings show that the physical characteristics of FXS are variable in the Asian group but similar to those in other populations and are not recommended for early recognition. Individuals with intellectual disabilities, especially when combined with autism spectrum disorders and large prominent ears, are suggestive of further genetic testing for FXS.

Keywords: Fragile X syndrome, intellectual disability, phenotype, behavior

1. Introduction

Fragile X syndrome (FXS) is the most common genetic cause of inherited intellectual disability (ID) (1). An increase in the CGG trinucleotide repeat of over 200 in the promoter region of the *Fragile X Messenger Ribonucleoprotein 1 (FMR1)* gene results in the FXS phenotype (2). A number of CGG repeats between 5 and 44 is considered a normal allele, 45 to 54 as an intermediate allele, 55 to 200 as a premutation (PM) allele, and over 200 repeats is called a full mutation allele (3). Individuals with the full mutation (FM) allele have *FMR1* gene methylation, which results in transcription inhibition and a subsequent lack of Fragile X Messenger Ribonucleoprotein (FMRP). FMRP is an essential protein that affects mRNA translation and is involved in synaptic plasticity (4). FMRP, an RNA-binding protein, controls the translation of approximately 4% of fetal brain mRNA (5).

Full mutation of the *FMR1* gene results in ID and

phenotypic features in affected individuals, including a long face, large prominent ears, joint hypermobility, and macroorchidism (6). Other physical characteristics commonly described include a high-arched palate, strabismus, flat feet, and smooth skin. Additional health issues that may occur include gastrointestinal issues (such as gastric reflux, constipation, and loose bowel movements), neurological problems (such as hypotonia, motor incoordination, and seizures), cardiovascular abnormalities, obstructive sleep apnea, and abnormal growth patterns (such as macrocephaly and increased birth weight) (7).

Individuals with FXS also exhibit a broad spectrum of behavioral and psychological symptoms, in addition to physical symptoms and coincident medical conditions (8). There are variations in the behavioral symptoms that manifest in individuals with FXS (9). One of the most prevalent behavioral disorders seen in FXS is autism spectrum disorder (ASD). 30 to 50% individuals with FM FXS exhibit the full DSM-IV-TR criteria for autism,

while 60%-74% adhere to the classification for ASD (10). Other behavioral characteristics commonly found in FXS include impulsivity, tactile defensiveness, hand flapping, poor eye contact, gaze aversion, anxiety, hyperactivity, and deficiencies in social interaction (11).

Estimates of the global prevalence of FXS from a meta-analysis by Hunter *et al.* (2014) were 1:7,000 for males and 1:11,000 for females (12). The prevalence of FM FXS is estimated to be 1:5,000 males and 1:4,000–8,000 female individuals among the population in Europe and North America (2). A 20-year retrospective population study of over 2000 unrelated individuals with intellectual disabilities in South Africa revealed that FM among African participants was high (5.7%) (13). Some Asian countries, including China and Taiwan, are estimated to have a lower prevalence of individuals with FXS than Western countries (14). In Indonesia, the prevalence of FXS is 0.9-1.9% in the ID population and 6.15% in the ASD group (15). In Thailand, the frequency of FXS among Thai boys with developmental delays is approximately 7% (16). Meanwhile, the predicted prevalence of FXS individuals in Japan is 1 in 10,000, which is below the expected prevalence in Caucasian populations and among the subgroup of Mediterranean and Pakistani populations (1 in 4,000 males) (17).

Although the clinical features of FXS have been widely studied in Caucasians, information on Asians has not been frequently reported (7). A study of two healthcare systems in the USA consisting of 3.8 million people discovered that the estimated underdiagnosis rate of FXS ranged between 63.60–71.94%. Additionally, more than 80 percent of patients with FXS self-reported as white (Caucasian) (18). The disparity between the general population and clinical practice, as well as the homogeneous population, indicates the need to identify challenges in obtaining a diagnosis, especially in diverse populations.

The limited availability of genetic testing facilities and qualified professionals is one of the major challenges related to diagnostic techniques in South Asia (19). The clinical variability of FXS makes diagnosis challenging. FXS has a subtle physical phenotype, particularly at birth and during infancy. Most children with FXS are not diagnosed until they are around 3 years old (20,21).

Considering the importance of recognizing and potential variability in clinical characteristics of individuals with FXS, this study aimed to characterize the spectrum and variability of physical and behavioral phenotypes in Asian populations, thereby supporting recognition and diagnosis of FXS in resource-limited settings.

2. Literature search and study selection

2.1. Study selection process

This study was a systematic review of the physical

and behavioral phenotypes of individuals with FXS. The reporting in this systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Figure 1) (22). However, the review protocol was not preregistered in an international systematic review registry. Four literature databases (PubMed, ScienceDirect, Scopus, and Cochrane/CENTRAL) were systematically searched for relevant studies. The search consisted of main terms "fragile x syndrome", "physical", "behavior", and word variations. When available, both controlled (MeSH) terms and free-text words were used. The full search strategies are detailed in Supplementary Table S1 (<https://www.irdrjournal.com/action/getSupplementalData.php?ID=312>). The results were imported and de-duplicated using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia, available at www.covidence.org). The final database search was completed on April 2, 2025, yielding 5,830 studies.

We assessed the titles and abstracts prior to selecting papers for inclusion. The inclusion criteria included FXS-specific research studies that identified clinical characteristics of FXS. This study included populations originating from Asia. Initially, we ruled that only studies written in English would be included in the review. However, during the selection process, we identified crucial studies from Asian countries that were not published in English-language journals. After careful consideration, non-English articles were translated using DeepL and independently cross-checked by reviewers familiar with clinical terminology. Studies in the form of case reports, animal studies, and studies that did not emphasize the occurrence of FXS, as well as those that did not specify any physical or behavioral phenotypes, were excluded. Subsequently, studies that reported only premutation occurrences were excluded.

A total of 231 studies remained for full-text review. Our search covered the publication period between 1980-2025. Each article was screened by two reviewers (C.L.M.N. and J.T.), and any disagreements were resolved through discussion with a third reviewer (N.R.B.S., A.U., or T.I.W.). The same procedure was applied to screen full-text reviews.

2.2. Quality assessment

Reviewers assessed the methodological quality of each eligible study using standard criteria commonly applied to primary research in various fields. No established quality appraisal tool specifically addresses phenotype-focused observational studies of rare genetic disorders. Therefore, we adapted elements from the Kmet criteria and previous systematic reviews to develop a pragmatic six-item assessment tool that emphasizes diagnostic confirmation, participant selection, outcome validity, and methodological rigor (23,24).

A six-item assessment list in which points were given

to each criterion (high = 2, low = 1, unsure = 0) is shown in Table 1. The points given to each study were divided by the total score of 12 to obtain summary scores for each study, and the quality was classified as high (score > 80%), good (70–80%), adequate (50–70%), or limited (< 50%) (23,25). Studies with scores below 50% were excluded. The reviewers resolved disagreements through discussion. If an agreement could not be reached, a third reviewer resolved disagreements. The quality assessment step resulted in 55 included studies.

2.3. Data extraction

Four studies were excluded before data extraction. The reasons for exclusion were as follows: *i*) one study had female PM carriers as the study subjects; *ii*) two studies

were part of an included study of a larger cohort; and *iii*) data from one study were deemed inadequate for extraction by the third reviewer. Potentially overlapping cohorts were identified by comparing the study location, recruitment period, investigators, and participant characteristics. In cases of suspected overlap, the study with the most comprehensive dataset was retained. We attempted to conduct a backward reference search and contacted the authors for data clarification. For instance, a backward search of a cohort report (15) led to additional data from six different original studies (26-31).

Data extraction was performed for the 51 included studies. The first reviewer extracted the data, and the second reviewer confirmed its accuracy. Data were extracted into standardized formats for the following entries: study title, country, research setting, number of

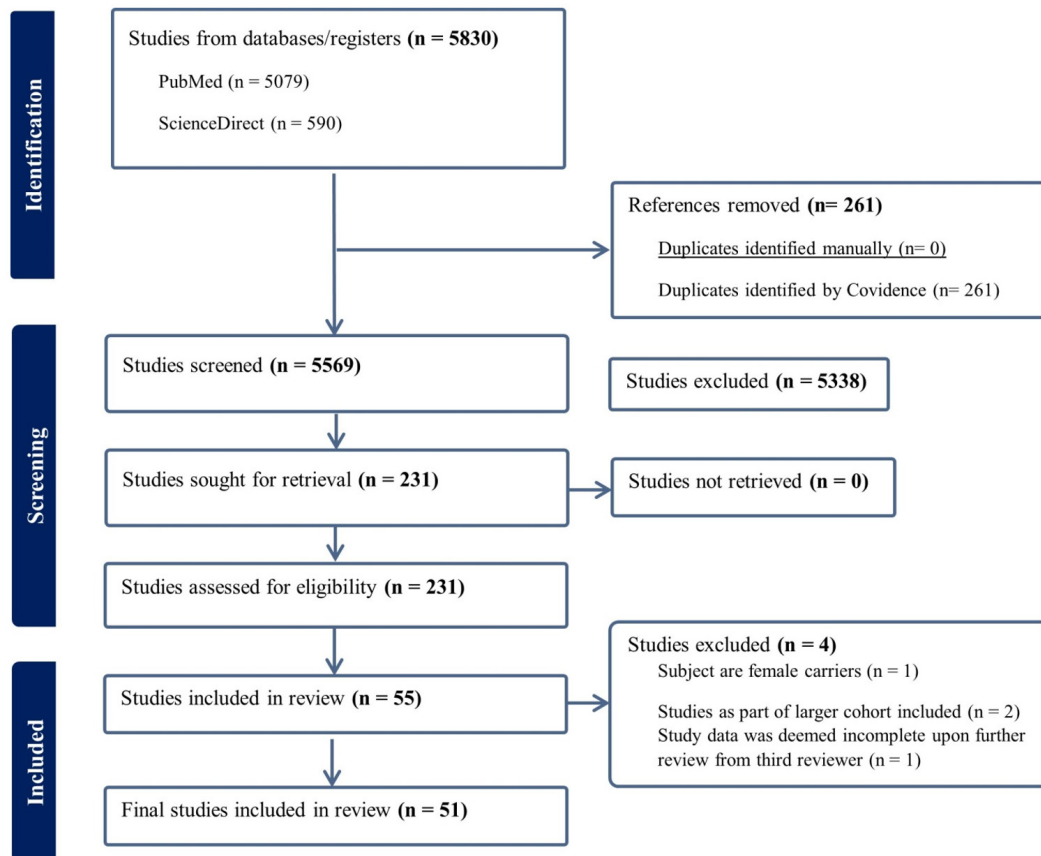


Figure 1. PRISMA flow diagram.

Table 1. Criteria for assessing the quality of the study included

Items	Answer
1. Molecular and cytogenetic diagnosis: Fragile site analysis, PCR, Southern blotting, TP-PCR	High/Low/Unsure
2. Was there a clear statement of the aims of the study?	High/Low/Unsure
3. Was the research design appropriate for addressing the aims of the research?	High/Low/Unsure
4. Was the data analysis sufficiently rigorous?	High/Low/Unsure
5. Patient selection (appropriate and clear inclusion/exclusion criteria)	High/Low/Unsure
6. Objectivity of outcomes/data validity: Physical and behavioral features are evaluated based on reliable references; being examined by experts	High/Low/Unsure

participants, number of individuals with FXS, sex, age, diagnostic testing, and outcomes, including physical and behavioral characteristics.

2.4. Data analysis and interpretation

Physical and behavioral characteristics were reported as percentages based on total number of participants in the included studies. Phenotypic prevalence was calculated using the number of individuals with whom a given characteristic was reported. Characteristics that were not explicitly assessed or reported were treated as missing rather than absent. Related or similar terms were combined into the most appropriate terms for the Fragile-X phenotype, such as the terms "intellectual disability" and "developmental delay" became "developmental delay/intellectual disability", due to a similar condition, only with a different age of diagnosis (32). Details of the combined terms are provided in Supplementary Table S2 (<https://www.irdrjournal.com/action/getSupplementalData.php?ID=312>).

Behavioral characteristics were categorized based on the Aberrant Behavior Checklist-Community (ABC-C) (33), which included five groups of behaviors:

hyperactivity, lethargy/social withdrawal, inappropriate speech, irritability, and stereotypic behavior. Physical and behavioral phenotype classification was discussed and decided by a Fragile-X expert (TIW). Two terms, "facial dysmorphism" and "physical signs" were removed because the terms could not be specified.

3. Study and clinical characteristics reported in Asian population

3.1. Included study characteristics

This review summarizes 51 studies conducted between 1984 and 2023. These studies were acquired from different areas, which were classified according to their distinct regions in Asia, including East Asia (China, Hong Kong, Japan, South Korea, and Taiwan), Southeast Asia (Indonesia, Singapore, and Thailand), South Asia (India, Pakistan, and Sri Lanka), and Western Asia/Middle East (Kuwait, Iran, Israel, Saudi Arabia, and Türkiye), as shown in Figure 2. The inclusion criterion for the methodological quality assessment was set at 50%. Included studies had quality ratings of strong in 44 studies, good in two studies, and adequate in five studies.

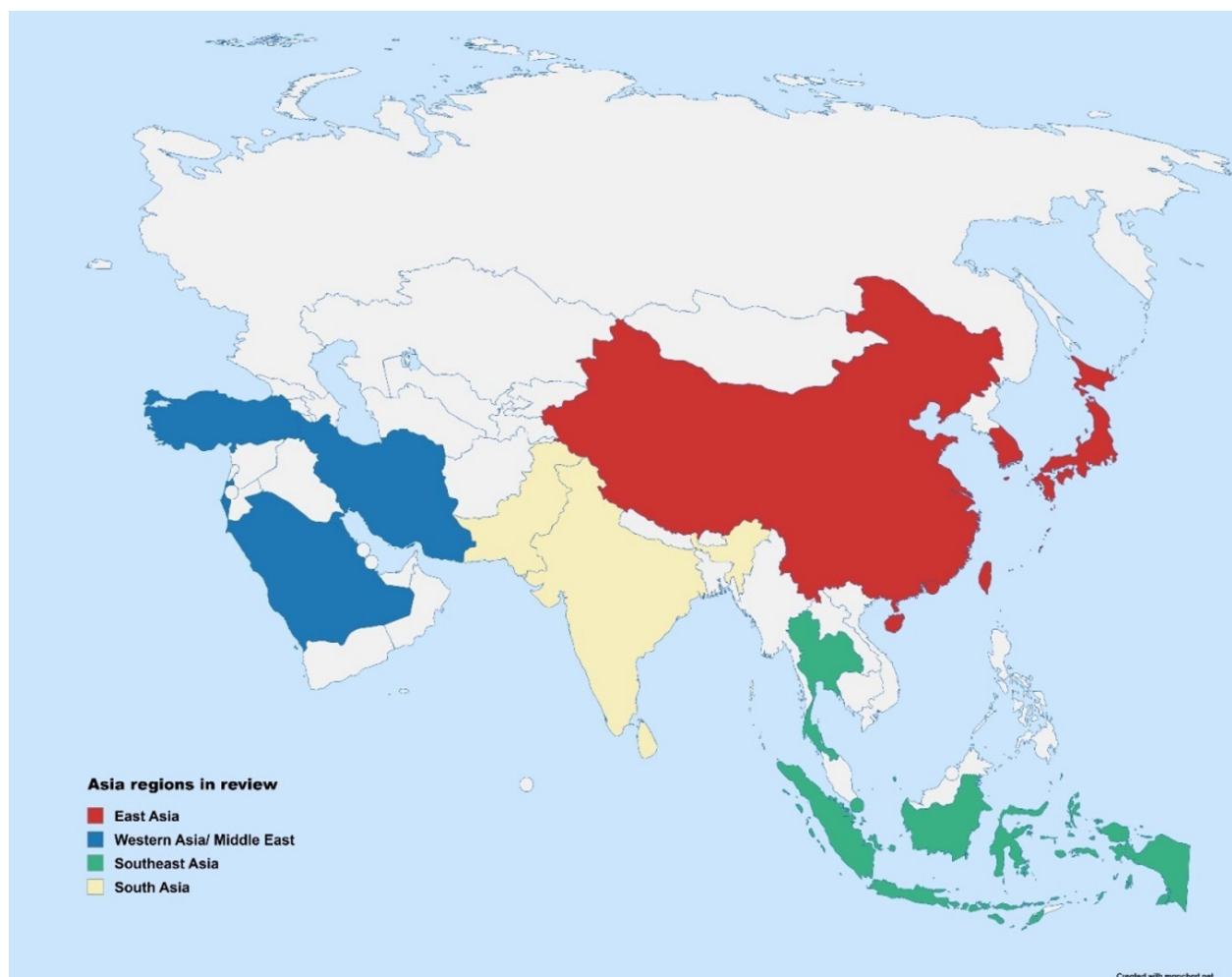


Figure 2. Included regions and countries in this review, showing 4 Asia regions and 16 countries.

The majority of the study population came from tertiary healthcare centers (70.6%), followed by those who underwent institutional screening (21.6%). Meanwhile, three studies combined both settings, and one study was based on province-wide population screening (34). Some studies described only a single characteristic, either a physical or behavioral trait. Details of included studies are shown in Table 2 (7,15,34-82).

3.2. Demographic and overall characteristics

Of the 24,173 participants identified across all included studies, 1,097 were diagnosed with FXS (4.54%). All diagnosed individuals were assessed for behavioral characteristics. Since not all studies described the individual's sex, the known numbers of male and female individuals with FXS were 700 and 87, respectively. 24 of the 51 articles mentioned the age of diagnosis/detection, with the majority ($n = 15$) diagnosed in childhood. Irrespective of physical or behavioral characteristics, the most observed feature was developmental delay/intellectual disability (98.04%). Some characteristics were reported infrequently (less than 1%). Table 3 highlights main findings of the included studies.

3.3. Physical characteristics

Physical features were described in 34 of the 51 articles (66.67%; Table 4). A total of 403 individuals were counted for physical characteristics, of which 380 were male, and 23 were female. The three most prevalent physical characteristics were large and prominent ears (72.63%), elongated face (57.49%), hyperextensible metacarpophalangeal (MCP) joint (48.06%), and macroorchidism (45.21%). Other features, such as a broad forehead, prognathism, and a high-arched palate, were reported less frequently. The "eye and periorbital problems" group included long palpebral fissure, strabismus, periorbital puffiness, hypertelorism, and other ophthalmological findings (see Supplementary Table S2, <https://www.irdrjournal.com/action/getSupplementalData.php?ID=312>).

3.4. Behavioral characteristics

All studies described at least one behavioral characteristic (Table 5). Intellectual disability (ID) was reported in 98.04% of all cases, the most prevalent finding in FXS, followed by hyperactivity (77.61%) and lethargy/social withdrawal (55.37%). ADHD became a distinct list, which included terms such as "attention deficit", "unspecified ADHD", "inattentive", "hyperactive-impulsive", and "combined ADHD", and was described in 12 studies, yielding a prevalence of 54.32%. There were 19 studies exploring autism spectrum disorders,

with a prevalence of 38.00%.

3.5. Associated medical findings

Some reported comorbidities included sleep problems (26.87%), which were reported in 11 studies, and epilepsy/seizures (21.18%), which were described in three studies. Meanwhile, a few reports mentioned gastrointestinal problems (48,65), connective tissue disorders (15), and other conditions with few individuals (only reported in one or two patients), such as schizophrenia (47), tetralogy of Fallot, Sotos syndrome-like, medulloblastoma, hypothyroidism, conductive hearing loss, precocious puberty, primary amenorrhea, and rectal cancer in one study (7).

4. Phenotypic variability and clinical implications

4.1. Comparison to non-Asian population

This review revealed that DD/ID and behavioral abnormalities (*i.e.*, hyperactivity and lethargy/social withdrawal) as the main features of FXS described across all studies. Meanwhile, physical characteristics were reported more heterogeneously. The most commonly described physical feature was large prominent ears, and the other features were reported in less than half of the studies. We compared our findings with those of other populations, such as the Caucasian/North American cohort of Lachiewicz *et al.* (83) and Brazilian/South American populations from Romero's study (84). Figure 3 shows a comparison heatmap between the three populations.

Large prominent ears and an elongated face were the most commonly identified craniofacial characteristics, with different findings. Across the three populations, large prominent ears were reported consistently, suggesting this feature as a global physical finding in individuals with FXS. Meanwhile, an elongated face was significantly more prevalent in the Caucasian cohort than in the Asian and Brazilian cohorts. A broad forehead was less commonly found in the Asian population. Other physical features commonly described were hyperextensible joints and flat feet. Notably, all Caucasian patients presented with hyperextensible joints, compared with approximately half of the Asian and Brazilian populations. The physical phenotype of children with FXS evolves with age and becomes comparable to the classic phenotype in the final stage of adolescence (85). Heulens *et al.* (2013) confirmed that typical facial characteristics of FXS include a long, narrow face and prominent ears. The remarkably narrow midface may explain the apparent prominence of the ears. A long face develops earlier than previously assumed and becomes increasingly apparent with age (86). Various findings on craniofacial features suggest that reliance on facial dysmorphology alone is insufficient for clinical

Table 2. Included studies in this review

Author, Year (<i>Ref.</i>)	Country	Quality assessment results (%)	Research settings	No. of FXS Individuals	No. of FXS Male	No. of FXS Female	Age of diagnosis
Alanay, 2007 (35)	Turkey	91.67	Tertiary healthcare service	24	24	0	2-17 y (mean 8.5 y)
Anvari, 2022 (36)	Iran	100.00	Tertiary healthcare service	12	9	3	25–80 y
Aoi, 1989 (37)	Japan	91.67	Institutional screening	1	1	0	43 y
Arinami, 1986 (38)	Japan	91.67	Institutional screening	13	13	0	19–69 y (38 y)
Arinami, 1987 (39)	Japan	91.67	Institutional screening	2	0	2	31 and 33 y
Bastaki, 2004 (40)	Kuwait	100.00	Tertiary healthcare service	20	20	0	NA
Chandrasekara, 2017 (41)	Sri Lanka	100.00	Institutional screening	11	11	0	5–18 y (mean 10.4 y)
Charalsawadi, 2017 (7)	Thailand	100.00	Tertiary healthcare service	56	56	0	2–16 y
Chaudhary, 2014 (42)	Saudi Arabia	91.67	Tertiary healthcare service	9	9	0	3–27 y
Chen, 2015 (43)	China	100.00	Tertiary healthcare service	5	5	0	NA
Dean, 2019 (44)	India	100.00	Tertiary healthcare service	18	18	0	1–26 y (mean 9.6 y)
Demirhan, 2003 (45)	Turkey	100.00	Tertiary healthcare service	14	9	5	6 m–12 y
Fazeli, 2022 (46)	Iran	83.33	Tertiary healthcare service	2	0	2	NA
Gabis, 2011 (47)	Israel	83.33	Tertiary healthcare service	28	23	5	Mean 14.2 y
Gabis, 2018 (48)	Israel	83.33	Tertiary healthcare service	117	92	25	2–52 y (mean 17 y)
Gu, 2006 (49)	China	91.67	Tertiary healthcare service	5	5	0	18–60 y
Guruju, 2009 (50)	India	91.67	Institutional screening	25	25	0	Female (>16 y; <i>n</i> = 4); Male (> 16 y; <i>n</i> = 17; 12–17 y; <i>n</i> = 3; < 12 y; <i>n</i> = 5)
Hong, 1999 (51)	South Korea	91.67	Tertiary healthcare service	5	5	0	Mean 3.8 y
Horiguchi, 2003 (52)	Japan	58.33	Institutional screening	56	NA	NA	NA
Horiguchi, 2005 (53)	Japan	58.33	Tertiary healthcare service	9	9	0	< 3 y (<i>n</i> = 3); 4–6 y (<i>n</i> = 3); > 20 y (<i>n</i> = 3)
Hou, 1998 (54)	Taiwan	100.00	Combined (both tertiary healthcare center and institutional screening)	233	NA	NA	NA
Hou, 2023 (55)	China	100.00	Tertiary healthcare service	3	3	0	2–62 y
Iqbal, 2000 (56)	Saudi Arabia	66.67	Tertiary healthcare service	26	24	2	NA
Jain, 1998 (57)	India	83.33	Tertiary healthcare service	29	29	0	3–28 y (mean 10.28 y)
Kabakus, 2006 (58)	Turkey	100.00	Tertiary healthcare service	8	8	0	5–18 y (mean 8.8 y)
Kanwal, 2015 (59)	Pakistan	100.00	Combined (both tertiary healthcare center and institutional screening)	13	10	3	NA
Ke, 2005 (60)	Taiwan	91.67	Tertiary healthcare service	12	10	2	2–7 y (mean 4.3 y)
Limprasert, 2000 (61)	Thailand	91.67	Tertiary healthcare service	27	27	0	8 m–13.6 y (mean 7.9 y)

Note: m = month(s); NA = not available; y = year(s).

Table 2. Included studies in this review (continued)

Author, Year (<i>Ref.</i>)	Country	Quality assessment results (%)	Research settings	No. of FXS Individuals	No. of FXS Male	No. of FXS Female	Age of diagnosis
Luo, 2009 (62)	China	83.33	Tertiary healthcare service	4	4	0	NA
Madokoro, 1989 (63)	Japan	75.00	Combined (both tertiary healthcare center and institutional screening)	2	2	0	31 m, 7 y
Matsuishi, 1987 (64)	Japan	83.33	Tertiary healthcare service	2	2	0	2.5 y, 3.5 y
Mei, 2023 (65)	China	100.00	Tertiary healthcare service	42	39	3	1 y 9 m–13 y 6 m
Moon, 1993 (66)	South Korea	100.00	Tertiary healthcare service	10	10	0	2–10.2 y
Nagarathinam, 2021 (67)	India	91.67	Tertiary healthcare service	3	3	0	22 y, 15 y, 11 y
Nanba, 1995 (68)	Japan	91.67	Tertiary healthcare service	2	2	0	6 m–49 y (mean 10.7 y)
Okazaki, 2021 (69)	Japan	91.67	Tertiary healthcare service	7	7	0	6–20 y
Pandey, 2002 (70)	India	100.00	Tertiary healthcare service	3	3	0	16 y, 18 y, 27 y
Ruangdaraganon, 2000 (71)	Thailand	91.67	Tertiary healthcare service	9	9	0	NA
Saha, 2001 (72)	India	100.00	Institutional screening	7	6	1	16–18 y
Seki, 1994 (73)	Japan	58.33	Tertiary healthcare service	3	3	0	NA
Sharma, 2003 (74)	India	58.33	Institutional screening	65	57	8	2–51 y
Shen, 1997 (34)	China	1997.00	Regional screening	7	4	3	NA
Sihombing, 2021 (15)	Indonesia	100.00	Institutional screening	43 (22 for physical characteristics)	17*	5*	3–25
Tan, 2000 (75)	Singapore	100.00	Institutional screening	6	6	0	5.6–15.5 y (mean 10.5 y)
Tirosh, 1992 (76)	Israel	100.00	Tertiary healthcare service	7	7	0	6–21 y (mean 10 y)
Tuncbilek, 1999 (77)	Turkey	100.00	Tertiary healthcare service	5	5	0	2–13 y (mean 7.5 y)
Verma, 1994 (78)	India	100.00	Tertiary healthcare service	20	20	0	< 10 y (<i>n</i> = 13); > 10 y (<i>n</i> = 7)
Winarni, 2022 (79)	Indonesia	100.00	Institutional screening	49	32	17	Mean: 32.9 ± 14.62 y (M); 33.4 ± 13.98 y (F)
Wong, 1992 (80)	Hong Kong	100.00	Tertiary healthcare service	2	2	0	NA
Xu, 1984 (81)	China	83.33	Tertiary healthcare service	3	3	0	NA
Zhang, 2022 (82)	China	91.67	Tertiary healthcare service	13	12	1	age of FXS positive mostly 6–18 y (53.8%; <i>n</i> = 7); followed by 1–6 y (46.2%; <i>n</i> = 6)

Note: m = month(s); NA = not available; y = year(s).

recognition of FXS, particularly in younger children and Asian populations. However, the presence of large, prominent ears alongside other hallmarks of FXS may prompt initial suspicion for further evaluation.

Males with FXS often present with macroorchidism,

which is also associated with age (87). Macroorchidism becomes more prominent after puberty, and as a result, it is underdiagnosed in younger or prepubertal individuals and might not be found in early screening. In our study, this feature was identified only in 45.21% of males,

Table 3. Most commonly described characteristics across all included studies

Characteristics	No. of individuals	Prevalence (%)
Physical characteristics*		
Large, prominent ears	284/391	72.63%
Elongated face	188/327	57.49%
Hyperextensible metacarpo-phalangeal joint	124/258	48.06%
Broad and/or prominent forehead	80/161	49.69%
Macroorchidism	137/303	45.21%
Flat feet	33/148	22.30%
Behavioral Characteristics**		
DD/ID	1,050/1,071	98.04%
Hyperactivity	312/402	77.61%
Lethargy/social withdrawal	227/410	55.37%
ADHD	88/162	54.32%
Inappropriate speech	105/197	53.30%
Other medical conditions		
Sleep problems	18/67	26.87%
Epilepsy/seizure	36/170	21.18%

*from 403 individuals with described physical features. **from 1,097 individuals with described behavioral features.

Table 4. Physical characteristics

Physical Characteristics*	No. of individuals	Prevalence	No. of studies describing the features
Large, prominent ears	284/391	72.63%	30/51
Elongated face	188/327	57.49%	22/51
Macroorchidism	137/303	45.21%	21/51
Hyperextensible metacarpo-phalangeal joint	124/258	48.06%	12/51
Large Hands and Feet (including macrocephaly, increased growth)	65/110	59.09%	10/51
Broad and/or prominent forehead	80/161	49.69%	9/51
Prognathism	79/122	64.75%	9/51
High arched/cleft palate	51/145	35.17%	8/51
Eye and periorbital problems	44/139	31.65%	7/51
Flat feet	33/148	22.30%	6/51
Single transverse palmar crease	16/65	24.62%	5/51
Slender/tall stature	36/52	69.23%	3/51
Short stature	15/15	100.00%	2/51
Hypotonia	4/30	13.33%	2/51
Dry skin	15/20	75.00%	1/51
Prader-Willi like symptoms	4/29	13.79%	1/51
Low set ears	7/20	35.00%	1/51
Gynecomastia	5/20	25.00%	1/51

*from 403 individuals with described physical features.

Table 5. Behavioral characteristics and other medical conditions

Characteristics	No. of individuals	Prevalence	No. of studies describing the features
Behavioral characteristics*			
Developmental delay/intellectual disability	1,050/1,071	98.04%	48/51
Hyperactivity	312/402	77.61%	24/51
Lethargy/social withdrawal	227/410	55.37%	23/51
Autism spectrum disorders	95/250	38.00%	19/51
ADHD (attention deficit, unspecified, inattentive, hyperactive-impulsive, combined)	88/162	54.32%	12/51
Irritability	99/209	47.37%	12/51
Stereotypic behavior	93/226	41.15%	12/51
Inappropriate speech	105/197	53.30%	11/51
Depression or anxiety (including specific phobia, grimace & giggling, post-traumatic stress disorder)	26/53	49.06%	4/51
Other medical conditions			
Sleep problems	18/67	26.87%	11/51
Epilepsy/seizure	36/170	21.18%	3/51

*from 1,097 individuals with described behavioral features.

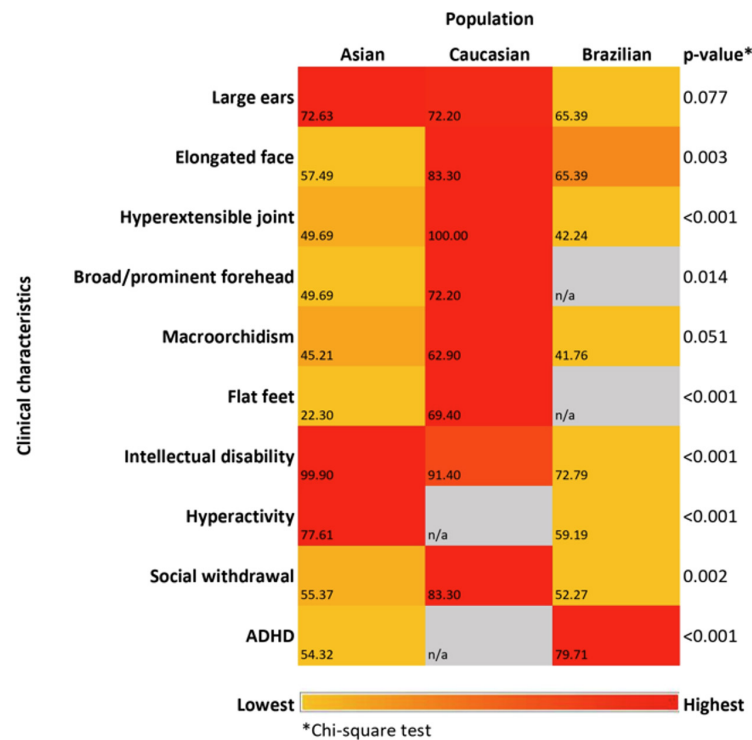


Figure 3. Heatmap comparison of the main clinical characteristics of FXS individuals between the Asian, Caucasian, and Brazilian populations.

which is less than that observed in the Caucasian cohort and another study by Merenstein *et al.*, which revealed more than 80% in the post-pubertal group of FXS males (88). This proportion may not represent the true number, since this study did not specify male individuals who have reached puberty.

Almost all FXS cases had ID/DD in our study, which was comparable to the Caucasian cohort and more commonly reported than in the Brazilian population. Most studies included in this review applied the Hagerman checklist when examining FXS clinical features. Notably, a review by Hagerman *et al.* stated that physicians generally rely on behavioral characteristics to recognize FXS (89). This finding suggests that the Asian dataset found more severe clinical features on the checklist or that milder cases are less frequently referred for genetic evaluation.

In this study, hyperactivity was more frequently reported as a distinctive behavioral phenotype in the Asian population with FXS, than in the Brazilian population. Conversely, attention deficit was more prevalent in the Brazilian cohort. These two features are often termed ADHD; however, in both populations, hyperactivity and attention deficit (ADHD) were described distinctly. A previous study showed that hyperactivity and attention problems are frequent behavioral signs of FXS (90). Based on the ABC-C criteria, hyperactivity and social withdrawal were the most common groups of behavioral characteristics in our study, followed by inappropriate speech, irritability,

and stereotypic behavior. Our study revealed that 38% of individuals with ASD were Asian, comparable to the known prevalence of FXS with ASD from a study in the Caucasian population (91). Approximately 60% to 90% of people with FXS exhibit symptoms related to autism, including avoidant eye gazing, hand flapping, repetitive activities, and verbal perseverations (92). Hatton *et al.* showed that autistic behavior in children with FXS develops slowly but significantly and periodically (93), while Hernandez *et al.* reported that it remained consistent (94).

4.2. Factors associated with the variability in clinical characteristics

4.2.1. Genetic factors

Various studies have been conducted to determine the genetic factors, specifically the correlation between protein levels and patient phenotypic characteristics. Insufficient or low levels of FMRP are essential in the pathogenesis of FXS (95). Size and methylation mosaicism are common in FXS. A previous study found that 82% of FXS, especially in males, carry mosaic FM/PM alleles (96). Some individuals have both "size" and "methylation" mosaicism, where some cells carry methylated alleles and others carry unmethylated alleles (with a CGG repeat number that spans from the premutation to full mutation range). Unmethylated alleles can be transcriptionally involved and overexpressed,

possibly leading to the production of FMRP (97). Therefore, patients with FXS in the mosaic may exhibit a milder phenotype (98). These mechanisms show that the resulting neurodevelopmental features are present globally across all ethnicities (7).

4.2.2. Variability in females with FXS

The phenotypic features of FXS are determined by biological sex and exhibit incomplete penetrance and variable expressivity (95). Consequently, females with FXS are often underdiagnosed. Our data consisted of 16 (31.38%) studies with female participants, half of whom reported physical characteristics. Some observed shyness and social withdrawal/lethargy in their studies (39,79), while another study reported hyperactivity in females with FXS (45). This finding shows a remarkable underrepresentation of females with FXS. This might be due to the fact that females with the full mutation also show the physical and behavioral characteristics the same as males with FXS, but with less severity. The clinical presentation of X-linked neurodevelopmental disorder genes in females is influenced by various factors, including the gene's escape from X Chromosome Inactivation (XCI) or skewing, variation type, and inheritance pattern (99). A preliminary cohort study in females with FXS showed that the overall cognitive profile was within the borderline or low-average range, and the cognitive score, specifically nonverbal abilities, correlated with overall adaptive behavior (100).

4.2.3. Variation in age and developmental trajectories

The age at diagnosis is a critical factor in identifying clinical characteristics of FXS (82). Our cohort consisted of patients of various age ranges, the youngest being 6 months (45) and oldest 51 years (74). When reporting phenotypes, some studies might observe more features than others, as seen in the various degrees of clinical feature descriptions. For example, macroorchidism was only described in less than half of all studies, although all studies described male patients. Similarly, hyperactivity, as one of the hallmarks of behavioral features in FXS, was reported in approximately half of the cohort data. Both features are associated with age, sex, and life course. A study showed that males exhibit hyperactivity more frequently than females, and irritability, stereotypic behavior, and hyperactivity were mildly improved in FXS males and females, lethargy slightly increased in males, and inappropriate speech remained consistent in males (79). According to a study by Bailey, Jr *et al.* (2009), the average age of FXS diagnosis was 35–37 months for boys and 41.6 months for females in the US (101). In Australia, the mean age of FM FXS searching for a diagnosis was 4.3 years (males) and 9.4 years (females) (102). Because early physical signs of FXS are often subtle, clinicians should consider genetic testing for

any children aged two to three presenting with ID/DD or speech delay (89).

4.2.4. Social factor and healthcare accessibility

Aside from biological factors, home environment factors were revealed to contribute to the general cognitive outcomes, specifically to language and attention capability in females with FXS (103). Environmental variables significantly influenced the autistic behavior. Variations in the environment, such as family, home, and educational settings, may improve or worsen behavioral and psychiatric issues associated with FXS, in addition to FMRP impacts (104).

Research indicates that families with FXS tend to be more positive, with fewer instances of behavioral problems in children and adults and less criticism (105). In boys with FXS, the quality of the family environment predicted adaptive behavior. Families with a boy with FXS are more inclined to improve the environment with appropriate stimuli and implement early treatments to adjust their home life to their needs (106). Although genetic factors may have played a role, the initial phenotypic involvement of FXS and subsequent improvement followed by combination therapy is noteworthy in young children with FXS. Early therapy for FXS can result in significant improvements (107).

4.2.5. Diagnostic pathway and differences in study settings

The variations between our cohorts need to be contextualized within the accessibility of healthcare services and the diagnostic approach. A definitive diagnosis may be delayed in young children due to the lack of a distinct phenotype, which can cause families to undergo a diagnostic odyssey and defer application of particular treatments (43). Our cohort revealed differences in the diagnostic pathway, ranging from screening for intellectually disabled individuals in institutions to studies from referral genetic centers. Social determinants of health, such as referral infrastructure, insurance coverage, and availability of social support, influence the diagnostic odyssey in individuals with DD/ID. In regions with limited resources, unavailability of first-tier genetic testing resulted in only that individuals with more pronounced clinical features would be reported (108). Even in the United States, up to 40% of families with females affected by FXS and 25% of those with affected males had another child before the first child received a diagnosis (101). Furthermore, significant variations in behavioral features were observed, such as the high prevalence of hyperactivity in our cohort compared to the Brazilian cohort, which may reflect provider-level diagnostic biases or differing center's capacities for comprehensive evaluation, rather than true biological factors (109).

We also explored the diagnostic testing methods used for the detection of FXS. Nevertheless, not all studies explained how the full mutation alleles were detected or the size of CGG repeats. Chromosomal analysis was utilized in some studies to diagnose FXS, especially in earlier publications. Historically, the molecular diagnosis of FXS relied on a combination of PCR and Southern blotting analysis to characterize full mutations (110). Triplet-primed PCR combined with high-resolution melting (HRM) and capillary electrophoresis analysis has become an alternative to the gold standard (111). More recently, long-read sequencing and optical genome mapping (OGM) have been introduced for molecular diagnosis of FXS (98). The availability of straightforward and precise testing will significantly improve diagnosis and management of individuals and families with FXS.

4.3. Study limitations

Common limitations acknowledged across the included studies were the diverse study populations and incomplete outcome data on clinical characteristics. A modified quality assessment tool may introduce the potential for subjective assessment and reduced reproducibility. The classification of each reported feature based on its associated traits is challenging because of the significant heterogeneity among studies. During data extraction, we realized a lack of systematic phenotypic assessment; thus, the prevalence estimates for behavioral features may be affected by reporting bias. To address these limitations, we listed all the phenotypes from the included studies and applied the appropriate denominator for each feature to provide a comprehensive overview of the Asian population.

We compared our cohort with the Caucasian/North American and Brazilian/South American populations to explore possible variations in FXS phenotypes. The primary limitation of this analysis is the difference in cohort sizes, particularly in the Caucasian cohort. The relatively small size of the Caucasian cohort limits the statistical power to explore subtle phenotypic differences and increases risk of errors. Furthermore, the prevalence of hyperextensible joints in the Caucasian study must be interpreted cautiously due to possible selection bias. Future studies with more balanced sampling might be beneficial to validate clinical feature variations between populations.

5. Conclusion and future directions

The challenges in the early clinical assessment of FXS are strongly correlated with age at diagnosis. The distinctive behavioral and physical characteristics of FXS typically appear gradually and become prominent as development progresses, especially in late childhood and adolescence, enhancing clinical recognition. However, delayed clinical detection poses an important

issue, as it can limit the possibility of early intervention. Early initiation of treatment increases likelihood of behavioral improvement in children with FXS. Some studies have explored the clinical suspicion framework for individuals with ID/DD in various global settings, all emphasizing need for FXS testing in (particularly) males with ID/DD, with presence of family history and clinical history related to FXS (112-114). The differences lie in the level of diagnostic testing, with more recent studies suggesting FXS testing as a second-tier test due to its low diagnostic yield (114). Our study in an Asian cohort suggests that clinicians may continue to work cautiously towards familial ID/DD with ASD and mild, age-related phenotypes (*i.e.*, large ears and elongated face, macroorchidism, hyperactivity, and social withdrawal).

Future research should prioritize a shift from ID-based detection to earlier, developmentally focused screening, particularly in children with unexplained developmental delays, autism, or ADHD. Age- and sex-stratified analyses are essential for clarifying developmental trajectories and better characterizing female phenotypes, which remain underrepresented. We emphasize that understanding clinical characteristics is critical for healthcare providers and individuals who may encounter these findings while suspecting an FXS diagnosis. Clinicians should be aware that individuals with FXS are prone to a variety of medical issues, not just typical disorders such as ID, ADHD, and ASD (4). Furthermore, increasing parental and caregiver knowledge of the clinical hallmarks of FXS starting in newborns may allow for earlier identification of possible consequences. This is especially important for children who exhibit developmental delays that fall outside the expected milestones. Several regions have limited access to advanced testing services, typically due to affordability and access constraints. In such cases, molecular testing may be more effective and efficient when prioritized for patients with indicative clinical symptoms.

Funding: This study was supported by the International Publication Grant of the Institute of Research and Community Services (LPPM), Universitas Diponegoro, Indonesia (grant no. 609-42/UN7.D2/PP/VIII/2023).

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

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- Received April 21, 2026; Revised July 13, 2026; Accepted August 4, 2026.
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- Released online in J-STAGE as advance publication August 11, 2026.

Cutaneous lymphomas reported following the use of dupilumab, nemolizumab, tralokinumab, or lebrikizumab: A case investigation based on JADER database and disproportionality analysis

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SUMMARY: Reports of cutaneous lymphomas (CL) following dupilumab administration are increasingly being documented, most frequently in patients with atopic dermatitis (AD). However, information regarding the association between CL and other biologic agents (nemolizumab, tralokinumab, or lebrikizumab) used for the treatment of AD is limited. To date, no large-scale pharmacovigilance study has systematically evaluated this issue in Japan. Therefore, we conducted a case investigation and disproportionality analysis using the Japanese Adverse Drug Event Report (JADER) database. Reports registered between April 2004 and August 2025 were analyzed. Both frequentist and Bayesian disproportionality analyses were performed using the reporting odds ratio (ROR) and information component (IC). In the primary analysis, including all reports in the JADER database, the ROR for dupilumab was 257.64 (95% confidence interval [CI], 164.50–404.39), and the IC was 4.52 ($IC_{025} = 3.89$). For nemolizumab, the ROR was 169.13 (95% CI, 61.78–463.00), and the IC was 2.29 ($IC_{025} = 0.95$). Reports of CL following tralokinumab or lebrikizumab administration were rare (one and zero cases, respectively) and did not meet the criteria for ROR calculation due to the small number. For sensitivity analysis, disproportionality analyses were restricted to reports with available data on age and sex, submitted by physicians, and cases involving no other suspected drugs. Under these conditions, safety signals based on both the ROR and IC were detected only for dupilumab. The safety signals identified in this study are exploratory in nature and require confirmation in future epidemiological studies.

Keywords: atopic dermatitis, pharmacovigilance, biologic agents, cutaneous T-cell lymphoma, safety signal

1. Introduction

Atopic dermatitis (AD), a chronic inflammatory skin disease, is characterized by pruritic eczematous lesions with a relapsing–remitting course encountered frequently in routine clinical practice (1).

Dupilumab was the first biologic agent approved for the treatment of moderate-to-severe AD by the U.S. Food and Drug Administration (FDA) in 2017. It exerts its therapeutic effect by binding to the interleukin-4 receptor alpha (IL-4R α), thereby inhibiting the signaling pathways of interleukin-4 (IL-4) and interleukin-13 (IL-13) (2).

Cutaneous lymphomas (CL) comprise a rare group of malignancies, with an incidence of 0.64 per 100,000 person-years reported in studies from the U.S., with documented racial differences in incidence (3). Although the precise incidence in Japan remains unknown, estimates based on a nationwide survey of CL cases conducted by the Prognosis Statistics Committee of the Japanese Skin Cancer Society suggest an incidence of

more than 0.4 per 100,000 person-years (4).

In recent years, cases of CL diagnosis or worsening following the initiation of dupilumab therapy, particularly among patients treated for AD, are on the rise. Analyses of the FDA Adverse Event Reporting System (FAERS) have identified safety signals for cutaneous T-cell lymphoma (CTCL) associated with dupilumab use (5,6). A report suggests that AD itself may be a risk factor for the development of CTCL (7).

Nevertheless, information regarding the association between CL and other biologic agents used for the treatment of AD, including nemolizumab, tralokinumab, and lebrikizumab, remains limited (8,9). Moreover, to date, no large-scale pharmacovigilance studies have systematically evaluated this issue in Japan. Nemolizumab is an anti–interleukin-31 receptor A (anti–IL-31RA) antibody first approved and launched in Japan in August 2022, and analysis of data in the Japanese Adverse Drug Event Report (JADER) database may therefore provide novel insights into the safety profile of this agent as well as other biologics used for the

treatment of AD.

Therefore, in this study, we conducted a case investigation and disproportionality analysis using the JADER database to evaluate the association between biologic agents for AD and CL.

2. Materials and Methods

2.1. Data source

Data registered in the JADER database between April 2004 and August 2025 were obtained from the official website of the Pharmaceuticals and Medical Devices Agency (PMDA) (<http://www.info.pmda.go.jp/fukusayoudb/CsvDownload.jsp>). The JADER database consists of four datasets: patient demographic information (demo), drug information (drug), adverse event information (reac), and medical history information (hist).

In this study, four biologic agents approved for the treatment of AD, namely dupilumab, nemolizumab, tralokinumab, and lebrikizumab, were selected as the target drugs. Adverse events recorded in the "reac" dataset are coded using Preferred Terms (PTs) based on the Medical Dictionary for Regulatory Activities, Japanese version (MedDRA/J).

Adverse events related to CL were defined according to previously published pharmacovigilance studies (10), with minor modifications to incorporate clinically relevant MedDRA/J PTs applicable to the present study. Specifically, the following PTs listed in MedDRA/J version 28.1J were included and defined as CL: "Cutaneous lymphoma (PT code: 10079945)", "Cutaneous T-cell lymphoma (PT code: 10011677)", "Cutaneous T-cell lymphoma stage I (PT code: 10011680)", "Cutaneous T-cell lymphoma stage II (PT code: 10011681)", "Cutaneous T-cell lymphoma stage III (PT code: 10011682)", "Cutaneous T-cell lymphoma stage IV (PT code: 10011683)", "Cutaneous T-cell lymphoma refractory (PT code: 10011679)", "Cutaneous T-cell lymphoma recurrent (PT code: 10011678)", and "Cutaneous B-cell lymphoma (PT code: 10085518)".

From all cases registered in the JADER database, those where both the target drugs and the predefined adverse events were recorded were extracted as a "case investigation". The following variables were analyzed: sex, age, drug involvement, indications, reporter of the adverse event, and clinical outcomes. In the JADER database, patient age is generally recorded in 10-year intervals (e.g., 10s, 20s, 30s); however, in some cases, age is recorded using specific categories such as "neonate", "infant", "child", "adolescent", "adult", or "elderly".

Indications were classified as AD or others; when multiple indications were reported, cases were classified as AD if this condition was among the reported indications. Adverse event reporters were categorized

as physicians or others; when multiple reporters were listed, reports were categorized as physician-reported if a physician was among the reporters. Drug involvement was categorized as suspected drugs, drug interactions, or concomitant drugs. In this study, only cases where the target drugs were reported as suspected drugs were included in the analysis. Cases involving multiple suspected drugs were included in the primary analysis for each reported suspected drug but were excluded from the sensitivity analysis. Clinical outcomes of CL cases were classified as poor, when defined as unrecovered, death, or with sequelae, or classified as favorable, when defined as recovery or in remission. Lacking data were categorized as "Missing or Unknown".

2.2. Statistical analysis

Descriptive statistics were used to summarize the results. Disproportionality analysis was conducted to evaluate the association between drugs and adverse events. In accordance with previous reports, both frequentist and Bayesian disproportionality analyses were performed using the reporting odds ratio (ROR) and the information component (IC), respectively (11). A positive signal was defined as a lower limit of the 95% confidence interval (CI) of the ROR greater than 1 and a lower limit of the 95% CI of the IC (IC₀₂₅) greater than 0. A sensitivity analysis was performed using a restricted dataset comprising physician-reported cases with complete data on sex and age, and without concomitant suspected drugs to evaluate the robustness of the disproportionality analysis under more stringent reporting conditions while minimizing potential information bias, reporter bias, and confounding from concomitant suspected drugs. All data were analyzed using EZR ver. 1.70 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (12).

2.3. Ethical considerations

This study adhered to the principles outlined in the Declaration of Helsinki and complied with the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The analysis was based exclusively on secondary use of publicly accessible data and did not require direct interaction with, or intervention in, human participants. Accordingly, formal ethical review and approval were waived by the institutional ethics committee. All data obtained from the JADER database were anonymized by the responsible regulatory authorities before being made available for research purposes.

3. Results

The JADER database used in this study comprised a total of 1,002,922 cases. Among these, dupilumab, nemolizumab, tralokinumab, and lebrikizumab were

registered as suspected drugs in 1,196, 232, 18, and 11 cases, respectively. In addition, 108 cases reported CL as an adverse event. The time trend of reports related to CL is shown in Figure 1A.

Among the 108 cases in which CL was reported, dupilumab was the most frequently reported suspected drug, accounting for 25 cases (23.1%) (Figure 1B). Nemolizumab use was associated with four cases, and tralokinumab use with one case; however, the case associated with tralokinumab use was the same as one of the cases associated with nemolizumab use, indicating that two suspected drugs were reported in a single case. No case of lebrikizumab use in association with CL was identified.

Because dupilumab is approved for multiple

indications, we examined the indications for which it was prescribed. Among the 1,196 cases in which dupilumab was registered as a suspected drug, 522 cases (43.6%) were treated for AD (Figure 1C). Amid these 522 cases, 19 (3.6%) were associated with reports of CL (Figure 1D).

The characteristics of the 25 cases where CL was reported following dupilumab administration—including sex, age, indication, reporter of the adverse event, the presence or absence of other suspected drugs, and clinical outcomes—are summarized in a Sankey plot (Figure 1E). In one case, another suspected drug, upadacitinib hydrate, was also reported.

In the primary disproportionality analysis using all reports in the JADER database, dupilumab showed an

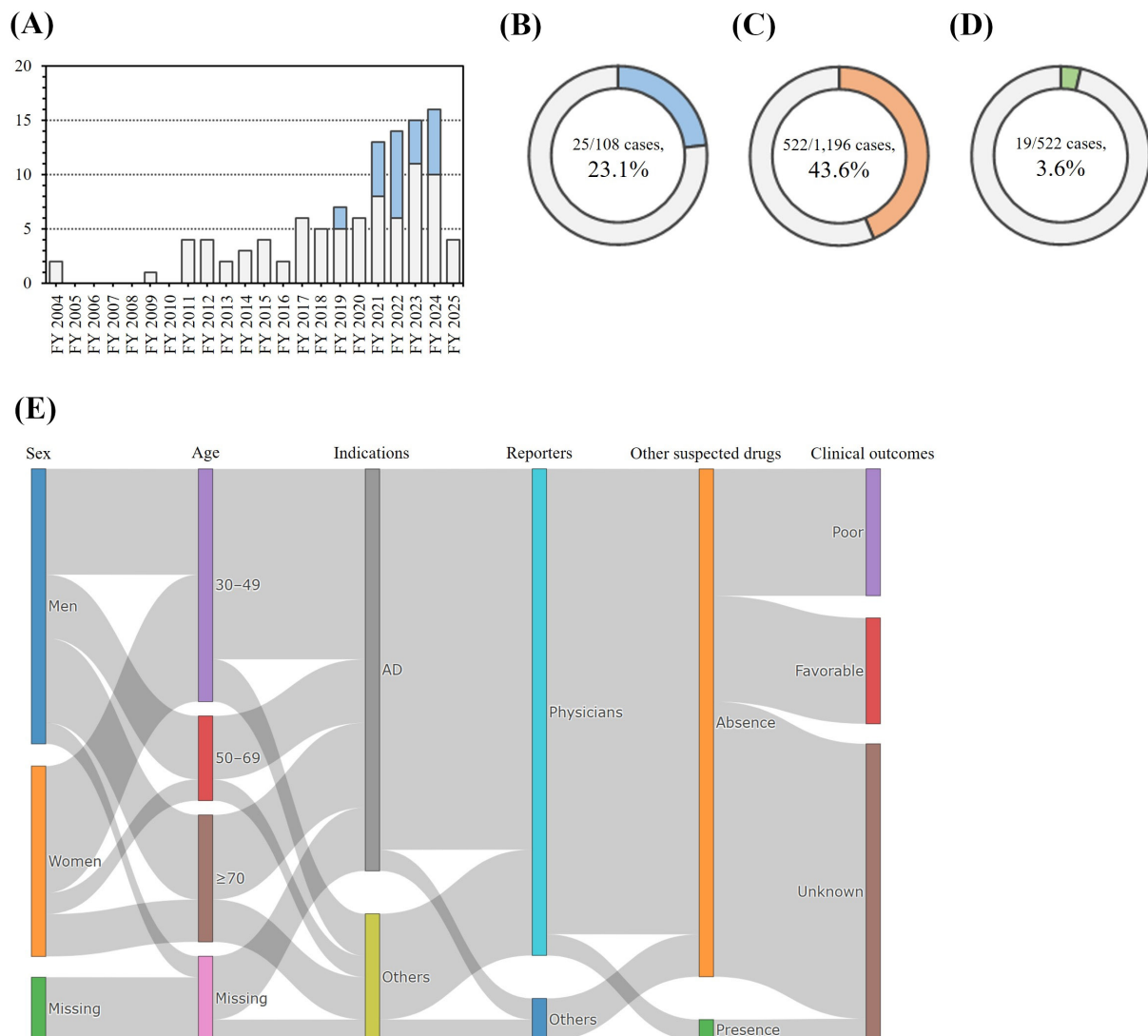


Figure 1. Characteristics of cutaneous lymphoma cases associated with dupilumab use. Panel (A) shows the time trend of reports related to cutaneous lymphoma; reports associated with dupilumab use are shown in blue. Panel (B) shows the number and proportion of cases in which dupilumab use was reported as a suspected drug among the 108 cutaneous lymphoma cases. Panel (C) shows the number and proportion of cases with atopic dermatitis as the indication among those in which dupilumab use was reported as a suspected drug. Panel (D) shows the number and proportion of cases in which cutaneous lymphoma was reported among patients treated with dupilumab for atopic dermatitis. Panel (E) presents a Sankey plot summarizing the characteristics of the 25 cases where cutaneous lymphoma was reported after dupilumab treatment, including sex, age, indication, reporter type, presence of other suspected drugs, and clinical outcomes.

ROR of 257.64 (95% CI, 164.50–404.39) and an IC of 4.52 ($IC_{0.25}=3.89$). Nemolizumab demonstrated an ROR of 169.13 (95% CI, 61.78–463.00) and an IC of 2.29 ($IC_{0.25}=0.95$). In contrast, CL was rare following administration of tralokinumab or lebrikizumab (1 and 0 cases, respectively), and the number of reports was insufficient to meet the criteria for calculation of ROR or meaningful IC estimation (Table 1).

As a sensitivity analysis, disproportionality analysis was restricted to reports with available data on age and sex, submitted by physicians, and without concomitant suspected drugs. Under these conditions, 19 of the 25 dupilumab-associated CL reports and 2 of the 4 nemolizumab-associated CL reports remained eligible for analysis. A safety signal based on both the ROR and IC was detected only for dupilumab. Although an ROR-based signal was also observed for nemolizumab, the IC signal was attenuated and no longer met the predefined criteria for signal detection (Table 2).

4. Discussion

This study conducted a case investigation and disproportionality analyses of reports of CL following treatment with four biologic agents approved for AD.

Dupilumab was the most frequently reported suspected drug associated with CL, accounting for 25 cases. Four cases were reported in association with nemolizumab use, and one case with tralokinumab use; however, one of the nemolizumab-associated cases overlapped with the single tralokinumab-associated case, indicating that two suspected drugs were reported in a single case. In contrast, no cases of CL were reported in association with lebrikizumab use. Because dupilumab is approved for multiple indications in addition to AD, including asthma and chronic rhinosinusitis with nasal polyps, interpretation of the observed signal requires

consideration of the treated population. In the present study, 19 of the 25 reported CL cases associated with dupilumab occurred in patients treated for AD, whereas no cases were reported in patients treated for asthma or chronic rhinosinusitis with nasal polyps. Several reports included other recorded indications that were not listed as approved indications in the package insert, such as eosinophilic gastrointestinal disorders, CTCL, or rash. These findings suggest that the observed signal was primarily driven by reports from the AD population, although indication data were unavailable or heterogeneous in a subset of cases. Furthermore, a literature search in PubMed and Ichushi-Web, as of February 5, 2026, identified seven case reports from Japan in which CL developed or became clinically apparent following dupilumab treatment (13–19). Although the JADER database does not provide detailed clinical information comparable to those available in published case reports, the inclusion of 25 cases in the analysis is considered informative as real-world pharmacovigilance data from Japan. Identification of four cases following nemolizumab treatment warrants attention.

In the primary analysis, including all available reports, safety signals for CL were detected for dupilumab and nemolizumab based on both ROR and IC. In contrast, reports of CL following tralokinumab or lebrikizumab treatment were limited (one and zero cases, respectively), and the number of reports was insufficient to meet the minimum criteria required for ROR calculation. In addition, dupilumab was approved earlier and has been more widely used in clinical practice in Japan than several other biologic agents included in this study. Therefore, the larger number of reports associated with dupilumab may partly reflect longer post-marketing exposure, greater cumulative patient exposure, and increased clinical awareness

Table 1. Disproportionality analysis for all available data

	Case	Non-case	ROR (95% CI)	IC ($IC_{0.25}$)
Dupilumab	25	1,171	257.64 (164.15–404.39)	4.52 (3.89)
Nemolizumab	4	288	169.13 (61.78–463.00)	2.29 (0.95)
Tralokinumab	1	17	NA	NA
lebrikizumab	0	11	NA	NA

Notes: NA, not applicable due to an insufficient number of reports. Abbreviations: ROR, reporting odds ratio; IC, information component; 95% CI, 95% confidence interval; $IC_{0.25}$, lower limit of 95% confidence interval of CI.

Table 2. Sensitivity analysis for physician-reported cases with complete sex and age data and without other suspected drugs

	Case	Non-case	ROR (95% CI)	IC ($IC_{0.25}$)
Dupilumab	19	699	299.41 (178.47–502.32)	4.20 (3.48)
Nemolizumab	2	213	81.78 (19.98–334.74)	1.55 (–0.16)

Abbreviations: ROR, reporting odds ratio; IC, information component; 95% CI, 95% confidence interval; $IC_{0.25}$, lower limit of 95% confidence interval of CI.

rather than differences in the underlying risk of CL. Differences in reporting dynamics related to approval timing and market penetration, including potential Weber effect-related influences (20), should therefore be considered when interpreting disproportionality signals across agents. As a sensitivity analysis, a disproportionality analysis was restricted to reports with available age and sex information, submitted by physicians, and without concomitant suspected drugs. Under these more stringent conditions, safety signals for dupilumab were consistently detected using both the ROR and IC, suggesting a degree of internal consistency across different analytical conditions. Although the ROR-based signal for nemolizumab persisted, the IC-based signal was no longer observed, indicating uncertainty regarding the stability and robustness of this signal across analytical assumptions. Differences in the pharmacological mechanisms of these agents may provide contextual information for interpreting these findings. Furthermore, time-to-onset (TTO) analysis was not performed because only a limited number of reports contained sufficiently complete temporal information. In addition, for diseases such as CL, the reported adverse event onset date may reflect the timing of diagnosis rather than the actual biological onset of disease, thereby limiting the interpretability of TTO analyses based on spontaneous reporting data.

Dupilumab targets the IL-4 α and inhibits IL-4 and IL-13 signaling, whereas tralokinumab and lebrikizumab selectively block IL-13, resulting in a more skin-focused modulation of type 2 inflammation. IL-4 plays a central role in the initiation and amplification of type 2 immune responses by promoting Th2 differentiation and IgE class switching, while IL-13 is more prominently involved in downstream tissue inflammation and epidermal barrier dysfunction (21). Both IL-4 and IL-13 have been implicated in the formation of a Th2-dominant, tumor-supportive tumor microenvironment in CTCL (22,23). However, whether blockade of these cytokines directly promotes lymphoma development or progression is unclear (24). Many reported cases of CTCL following dupilumab treatment have been interpreted as the unmasking or clinical manifestation of pre-existing subclinical disease rather than de novo tumor induction, and findings of this study are consistent with this interpretation (25,26). In addition, early-stage CTCL may clinically resemble AD and therefore initially be misdiagnosed prior to initiation of biologic treatment. Consequently, confounding by indication and diagnostic uncertainty may have contributed to the observed reporting signals. Similarly, fewer CTCL reports associated with IL-13-targeting antibodies such as tralokinumab and lebrikizumab should not be interpreted as evidence of absence of risk, particularly given the limited post-marketing exposure in Japan. Accordingly, the apparent scarcity of reports for these agents should be interpreted cautiously and does not

preclude the possibility of rare or delayed adverse events becoming evident with longer-term or broader clinical use.

IL-31 is a cytokine involved in Th2 inflammation and the neuroimmune axis, and expression of the IL-31 receptor (IL-31RA/OSMR β) has been observed in CTCL lesions (27). However, previous studies have not demonstrated a consistent association between IL-31 levels or receptor expression and CTCL disease severity or progression (28,29). This suggests that IL-31/IL-31RA signaling may primarily reflect the inflammatory and pruritic immune microenvironment rather than tumor biology itself. The safety signal detected for nemolizumab may represent one aspect of CTCL-associated immune responses; however, evidence for a direct tumor-promoting effect remains limited at present (30). Nevertheless, further studies are needed to evaluate long-term safety.

This study has several limitations inherent to spontaneous reporting databases. First, disproportionality measures such as the ROR and IC reflect reporting imbalances within a spontaneous reporting database rather than true incidence or relative risk, and therefore do not establish causality. As with all pharmacovigilance studies based on spontaneous adverse event reports, the findings reflect reported events rather than true incidence and may be influenced by time-dependent reporting dynamics, differences in market duration, and reporting awareness, including the Weber effect (20). Under-reporting and selective reporting could also have affected reporting patterns over time; therefore, the true incidence of CL could not be estimated from the JADER database. Second, channeling bias could not be fully excluded, as certain biologic agents may be preferentially prescribed to patients with more severe or long-standing AD, who may differ systematically from patients receiving other treatments. However, because detailed information regarding AD severity was not available in the JADER database, the potential influence of disease severity on treatment selection and baseline CTCL risk could not be directly evaluated. Third, confounding by indication was a concern, as AD itself, particularly in severe cases, may be associated with an increased risk of CTCL (7). In addition, early-stage CTCL may clinically mimic AD and remain unrecognized prior to initiation of biologic treatment, raising the possibility that some reported cases represented the unmasking of pre-existing disease rather than treatment-induced lymphoma. Fourth, because the JADER database is based on spontaneous adverse event reporting, duplicate reports and follow-up reports referring to the same case may have been included. Although potential duplicate cases can be partially identified using demographic and temporal information, complete and reliable duplicate removal was not feasible in the present study because of a substantial amount of missing data. Therefore, the possibility of residual

duplicate reports could not be excluded. Overall, the observed safety signals should be interpreted with caution and should not be regarded as evidence of a causal relationship.

In conclusion, safety signals identified in this study should be considered exploratory, and further epidemiological and clinical studies are required to more clearly evaluate these associations.

Funding: None.

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

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Received February 20, 2026; Revised May 14, 2026; Accepted June 16, 2026.

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Released online in J-STAGE as advance publication July 8, 2026.

Generation and characterization of induced pluripotent stem cells from a patient with classic Fabry disease

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SUMMARY: Fabry disease is an X-linked lysosomal storage disorder caused by pathogenic variants in the α -galactosidase A (α -Gal A, *GLA*) gene. The disease exhibits substantial clinical heterogeneity, with renal injury representing one of its most prominent manifestations. Due to the scarcity of human renal specimens and the inability of conventional animal models to recreate patient-specific pathological features, the precise mechanism underlying renal-predominant Fabry disease remains poorly understood. In this study, we successfully established and comprehensively characterized a urine-derived induced pluripotent stem cell (iPSC) line from a 35-year-old male patient with classic Fabry disease with a typical renal-dominant phenotype. The patient carried the *GLA* c.1080_1082delTGG (p.Gly361del) variant. Non-integrating episomal reprogramming was used to generate monoclonal iPSCs, which were further validated for pluripotency, trilineage differentiation ability, genomic stability, and exogenous vector clearance. The established iPSC line stably retained the patient-specific pathogenic variant, exhibited full pluripotent properties, and showed no genomic abnormality or residual episomal integration. Therefore, this well-characterized renal-phenotype-specific iPSC line provides a reliable cellular platform for investigating the mechanisms of progressive Fabry disease nephropathy and can facilitate future targeted drug screening.

Keywords: induced pluripotent stem cell, reprogramming, Fabry disease, nephropathy

1. Introduction

Fabry disease (OMIM #301500) is an X-linked lysosomal storage disorder resulting from loss-of-function variants in the *GLA* gene located on chromosome Xq22 (1). Clinically, Fabry disease is classified into classic and late-onset phenotypes, distinguished by residual α -galactosidase A activity and age of symptom onset. Deficient α -galactosidase A activity leads to progressive accumulation of globotriaosylceramide (GL-3/Gb3) in multiple organs, causing highly variable clinical phenotypes (2). Renal impairment is a major adverse event and one of the leading causes of mortality in patients with the disease (3).

Despite advances in disease awareness, the molecular mechanisms driving renal-specific injury and phenotypic

heterogeneity have yet to be completely elucidated, hindering the development of precise therapeutic strategies (4). The major obstacles include limited availability of human renal biopsy samples and the poor simulation accuracy of traditional animal and cell models, which fail to recreate individual genetic backgrounds and patient-specific renal pathology (5).

Patient-derived induced pluripotent stem cells (iPSCs) faithfully preserve individual genetic information and possess unlimited self-renewal and multilineage differentiation capacities (6). Urine-derived cells (UDCs) allow non-invasive, high-compliance sampling and have become an ideal resource for rare disease modeling, especially in pediatric patients (7). However, iPSC resources specifically generated from patients with familial renal-predominant Fabry disease remain

extremely limited.

Here, we established and validated an iPSC line from UDCs obtained from a patient with classic Fabry disease, *i.e.*, typical childhood-onset proteinuria and end-stage renal disease, who carried the *GLA* c.1080_1082delTGG (p.Gly361del) variant. This characterized cell line will serve as a valuable tool for exploring the progressive mechanism of renal injury and developing novel therapeutic strategies for refractory Fabry nephropathy.

2. Materials and Methods

2.1. Patient and samples

This study involved a 35-year-old male patient with clinically diagnosed classic Fabry disease who presented with predominant renal manifestations and a family history of the disease. The patient initially developed proteinuria at the age of 12 as the first clinical symptom and underwent a kidney transplant to treat progressive renal failure. Genetic analysis confirmed a pathogenic in-frame deletion variant *GLA* c.1080_1082delTGG (p.Gly361del). Biochemical assays showed significantly decreased plasma α -Gal A activity (0.29 μ mol/L/h; reference: 1.5–3.0 μ mol/L/h) and markedly elevated plasma GL-3 levels (90.82 ng/mL; reference: < 5 ng/mL). Urine samples were collected with informed consent. This study was approved by the Ethics Committee of Shandong Biomedical Technology Center.

2.2. Culture of urine-derived epithelial cells

Urine-derived epithelial cells were cultured in renal epithelial cell growth medium (REGM; Lonza, Switzerland) supplemented with 50% DMEM, 5% FBS (Gibco, USA), 1 $\times$ NEAA, and 1 $\times$ glutamine (Gibco, USA). Medium was refreshed every two days. Cells were passaged at a ratio of 1:3–1:4 after digestion with 0.25% trypsin-EDTA (SparkJade, China).

2.3. Reprogramming and culture of iPSCs

A total of 1.6×10^7 UDCs were nucleofected with the episomal vectors pEP4-EO2S-ET2K and pCEP4-miR302-367 using the Nucleofector 2b Device (Lonza, Switzerland). Transfected cells were seeded on 0.5%

Matrigel-coated plates (Corning, USA) and cultured in mTeSR1 medium (STEMCELL Technologies, Canada). Starting on day 10 post-transfection, small molecules including CHIR99021, thiazovivin, PD0325901, and SB431542 (Selleck, China) were supplemented. Putative iPSC colonies were manually selected between days 10–20.

Established iPSCs were maintained in mTeSR1 medium at 37 °C with 5% CO₂ and passaged every three days at a 1:6 ratio using 0.5 mM EDTA-DPBS (SparkJade, China). All cellular validation experiments were performed using stable iPSCs from passage 22.

2.4. qPCR analysis of pluripotency markers

Total RNA was isolated using TRIzol (Invitrogen, USA) and reverse-transcribed with SPARKscript II RT SuperMix (SparkJade, China). qPCR was performed on a LightCycler[®] 480 II system (Roche, Switzerland) with SYBR Green Master Mix and custom primers (Table 1). Cycling parameters: 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s.

2.5. Alkaline phosphatase (ALP) staining

ALP activity was detected using a BCIP/NBT staining kit (Beyotime Biotechnology, China) according to the manufacturer's instructions. Fixed cells were incubated in working solution in the dark and imaged under bright-field microscopy.

2.6. Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, USA), and blocked with goat serum (SparkJade, China). Primary antibodies against OCT4 (Immunoway, USA; #YM8272), SSEA-4 (#4755), and TRA-1-81 (#4745) (Cell Signaling Technology, USA) were incubated at 4 °C overnight. After secondary antibody incubation and DAPI counterstaining (SparkJade, China), images were acquired using an Olympus FV4000 confocal microscope (Olympus, Japan) at 20 $\times$ magnification.

2.7. Single nucleotide polymorphism (SNP) array analysis

Genomic stability was assessed by SNP array analysis

Table 1. Primer sequences for pluripotency-related genes

Target genes	Size (bp)	Sequence (5'-3')	
		Forward	Reverse
<i>OCT4</i>	164	CCTCACTTCACTGCACTGTA	CAGGTTTCTTTCCCTAGCT
<i>SOX2</i>	151	CCCAGCAGACTTCACATGT	CCTCCCATTTCCCTCGTTTT
<i>NANOG</i>	237	AAGGTCCCGGTCAAGAAACAG	CTTCTGCGTCACACCATTGC
<i>GAPDH</i>	152	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT

on the Illumina Asian Screening Array (ASA) (Illumina, USA) using genomic DNA extracted from iPSCs and parental UDCs (TIANGEN, China).

2.8. Mycoplasma detection

Mycoplasma contamination was ruled out using a commercial qPCR detection kit (Beyotime Biotechnology, China). Samples with Ct > 35 in the FAM channel and valid VIC amplification were considered to be negative.

2.9. Short tandem repeat (STR) analysis and Sanger sequencing

Genomic DNA from iPSCs clone LJ3212 and parental UDCs line LJ3181 was subjected to STR profiling and Sanger sequencing to confirm genetic identity and variant retention.

2.10. Confirmation of exogenous vector integration

PCR was amplified using vector-specific primers (Table 2) and SparkHiFi Max Master Mix (SparkJade, China). The thermal program consisted of 98 °C for 30 s, 30 cycles of

98 °C for 10 s, gradient annealing for 10 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. Transfected UDCs served as positive controls. PCR products were separated on 2.5% agarose gels and imaged using a ChemiDoc XRS+ system (Bio-Rad, USA).

2.11. *In vitro* trilineage differentiation

iPSCs were induced into three germ layers using STEMdiff™ differentiation kits (STEMCELL Technologies, Canada). Endodermal and mesodermal differentiation lasted 4–6 days, and ectodermal differentiation lasted 7 days. Germ layer markers AFP (Immunoway, USA), α -SMA (#19245), and β 3-tubulin (#5568) (Cell Signaling Technology, USA) were detected using immunofluorescence.

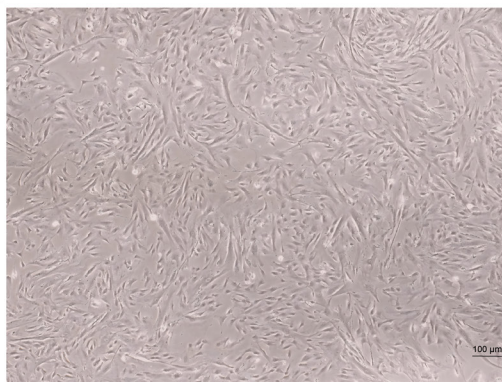
3. Results and Discussion

UDCs at passage 3 displayed a typical epithelioid morphology (Figure 1A). After episomal reprogramming, emerging iPSC colonies exhibited a compact morphology, clear boundaries, and a high nuclear-cytoplasmic ratio consistent with pluripotent stem

Table 2. Primer sequences for detection of episomal reprogramming vectors

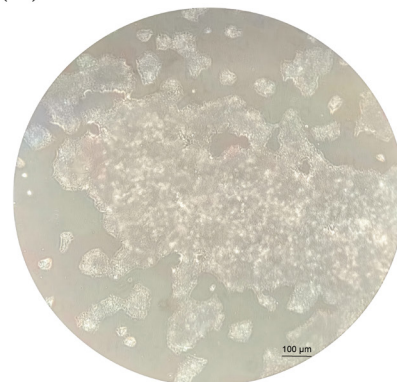
Target genes	Size (bp)	Sequence (5'-3')	
		Forward	Reverse
<i>OCT4</i>	657	AGTGAGAGGCAACCTGGAGA	AGGAACTGCTTCCTTCACGA
<i>SOX2</i>	534	CCCAGCAGACTTCACATGT	CCTCCCATTTCCTCGTTTT
<i>KLF4</i>	401	AAGGTCCCGGTCAAGAAACAG	CTTCTGCGTCACACCATTC
<i>SV40LT</i>	491	TGGGGAGAAGAATGGAAG	AGGAACTGCTTCCTTCACGA
<i>oriP</i>	544	TTCCACGAGGGTAGTGAACC	TCGGGGGTGTTAGAGACAAC
<i>EBNA-1</i>	666	ATCGTCAAAGCTGCACACAG	CCCAGGAGTCCCAGTAGTCA
microRNA302/367 cluster	322	TTTCCAAAATGTCGTAATAACCCCG	CTCCCAAAGAGTCCTGTTCTGTCCT
<i>GAPDH</i>	152	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT

(A)



UDCs

(B)



iPSCs

Figure 1. Morphological characterization of urine-derived cells (UDCs) and patient-derived induced pluripotent stem cells (iPSCs) derived from a patient with renal-predominant Fabry disease. (A) Phase-contrast image of UDCs at passage 3, showing a typical epithelial-like morphology. (B) Phase-contrast image of the established iPSCs line at passage 22, showing compact, well-defined colonies with a high nuclear-to-cytoplasmic ratio and typical human pluripotent stem cell morphology. Magnification: 4×.

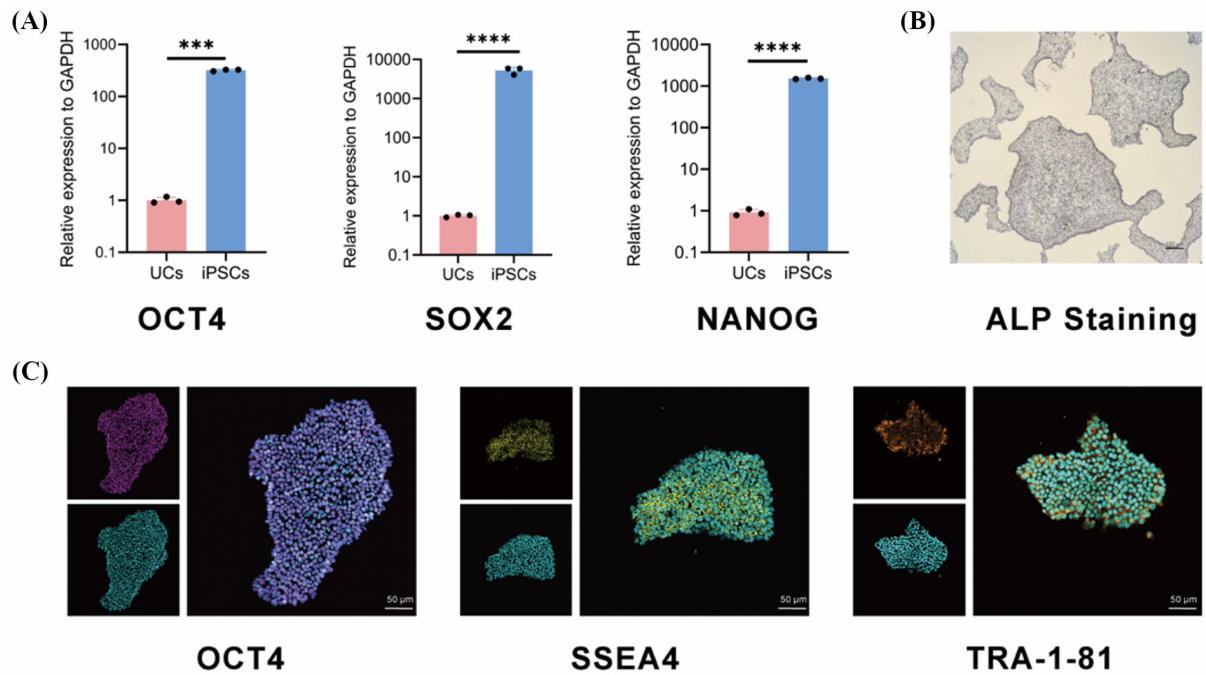


Figure 2. Pluripotency validation of patient-derived iPSCs. (A) Relative mRNA expression of pluripotency genes OCT4, SOX2, and NANOG in passage 3 UDCs and passage 22 iPSCs. Unpaired Student's *t*-test, ****p* < 0.001; ns, non-significant. (B) Alkaline phosphatase (ALP) staining of passage 22 iPSCs. 4× magnification. (C) Immunofluorescence for pluripotency markers OCT4, SSEA-4, and TRA-1-81; nuclei counterstained with DAPI. 20× magnification.

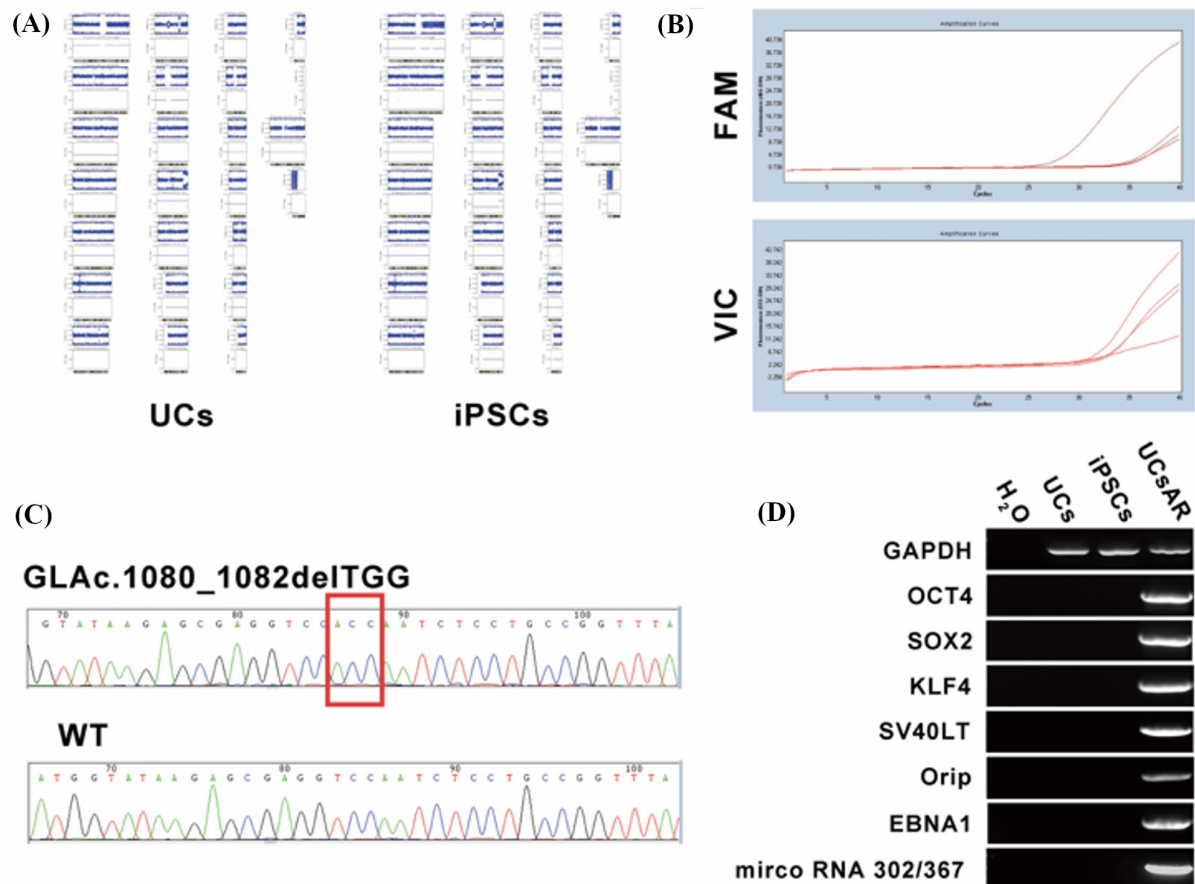


Figure 3. Genetic characterization and quality control of the established iPSC line. (A) SNP typing profiles of parental UDCs and the established iPSCs line. (B) qPCR detection of mycoplasma in iPSCs. (C) Comparison of Sanger sequencing chromatograms of the patient-derived iPSC line and WT sequences. (D) PCR analysis of exogenous episomal vector clearance.

cell characteristics (Figure 1B). Approximately 18 colonies were obtained from 1.6×10^7 UDCs. qPCR results confirmed significantly elevated expression of the endogenous pluripotency genes OCT4, SOX2, and NANOG in iPSCs compared to parental UDCs (Figure 2A). Positive ALP staining (Figure 2B) and immunoreactivity for OCT4, SSEA-4, and TRA-1-81 further verified pluripotency (Figure 2C). STR analysis confirmed full genetic matching between iPSCs and donor UDCs (Supplementary Table S1, <https://www.irdrjournal.com/action/getSupplementalData.php?ID=309>). SNP array results demonstrated genomic stability without pathogenic copy number variations or chromosomal aberrations (Figure 3A). Mycoplasma testing was negative (Figure 3B). Sanger sequencing confirmed faithful retention of the *GLA* c.1080_1082delTGG variant in iPSCs (Figure 3C). No residual integration of an episomal vector was detected

(Figure 3D). Moreover, the iPSC line exhibited a robust trilineage differentiation capacity, confirmed by specific expression of AFP, α -SMA, and β 3-tubulin (Figure 4A-C).

The established iPSC line satisfies all standard criteria for a qualified human pluripotent stem cell resource. Renal injury is the most critical prognostic factor in Fabry disease, and progressive proteinuria and end-stage renal failure are the leading causes of severe clinical outcomes in affected patients (8). Fabry disease presents with prominent genotype-phenotype heterogeneity, and rare in-frame deletion variants of the *GLA* gene are closely associated with progressive renal pathological damage (9). Based on our previously unpublished data from a clinical cohort, the *GLA* c.1080_1082delTGG (p.Gly361del) variant is extremely rare in the Chinese population, with the vast majority of identified cases being sporadic. Notably, the patient in this study harbors

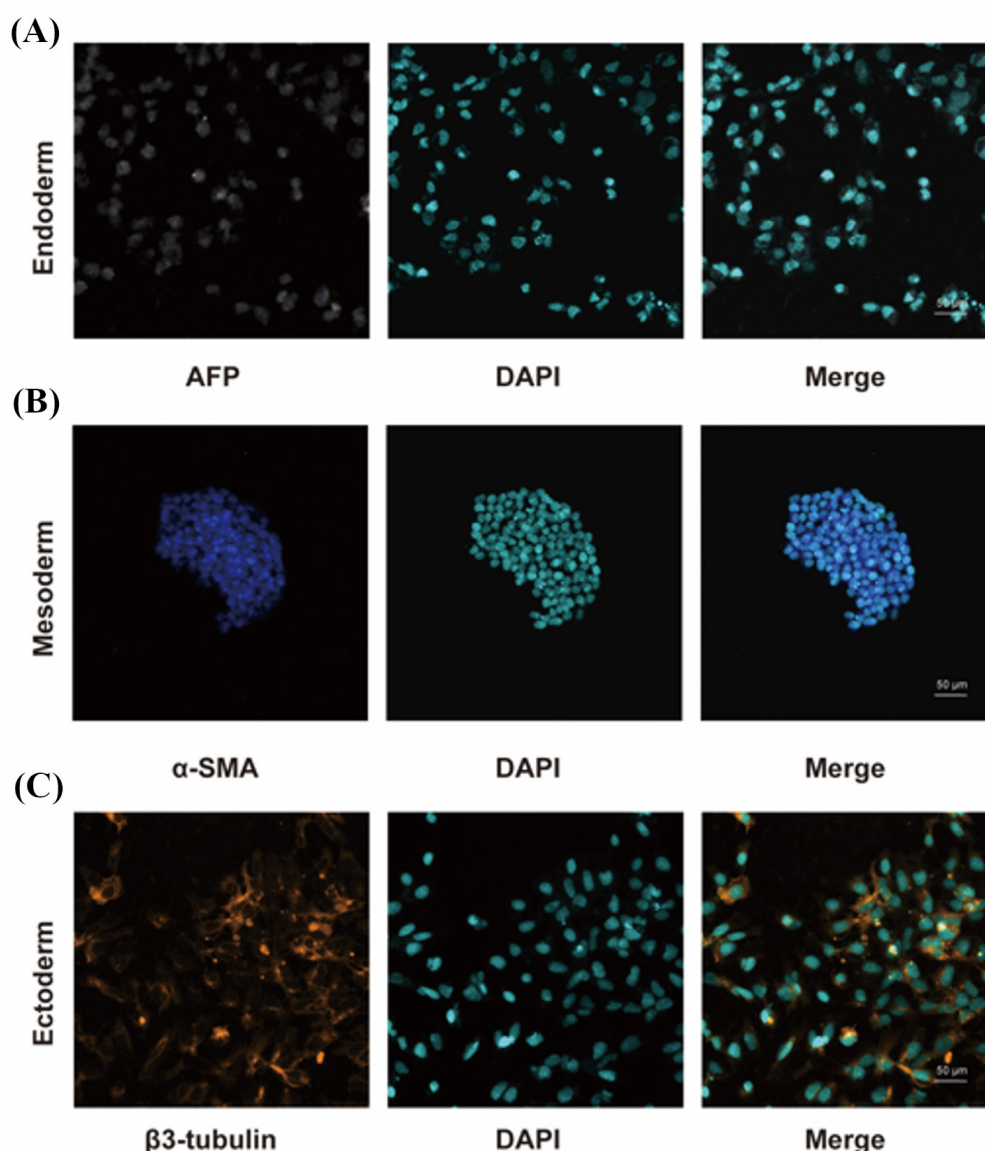


Figure 4. Immunofluorescence analysis of trilineage differentiation markers in iPSCs. (A) Endodermal marker AFP (white). (B) Mesodermal marker α -SMA. (C) Ectodermal marker β 3-tubulin. Nuclei were counterstained with DAPI.

this rare variant and manifests a typical familial renal-dominant Fabry phenotype characterized by childhood-onset proteinuria and progressive renal failure requiring transplantation. This represents an exceedingly rare clinical subtype in Chinese Fabry cohorts. More interestingly, we previously created another patient-derived iPSC line harboring an in-frame deletion in the same *GLA* codon region, it mainly exhibited neuropathic pain (10). Therefore, the unique patient-derived iPSC line that was established in this study fills the gap in cell resources for rare familial renal Fabry disease among the Chinese population. That line provides a specific and valuable cellular tool for exploring the molecular mechanism of progressive renal injury induced by the p.Gly361del variant, it elucidates the genotype-renal phenotype correlation of this rare mutation, and it facilitates subsequent mechanistic research and targeted therapeutic screening for intractable Fabry nephropathy.

This study is limited to cell line construction and basic characterization; future studies need to examine further renal lineage differentiation and perform functional phenotyping. In conclusion, this well-established iPSC line enriches the limited bank of patient-specific cell resources for Fabry disease and it provides a powerful platform for future mechanistic studies and therapeutic screening for familial renal-predominant Fabry disease.

Acknowledgements

Thanks to the patient and his family for their participation and support in this study. Thanks to the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China, for the plasmids offered in this work. We acknowledge all researchers contributing to experimental procedures and data analysis.

Funding: This research was supported by the Academic Promotion Program of Shandong First Medical University (LJ001).

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

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Received March 7, 2026; Revised June 21, 2026; Accepted June 24, 2026.

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Released online in J-STAGE as advance publication July 8, 2026.

Prenatal molecularly supported diagnosis of a fetus with urinary malformation caused by novel compound heterozygous variants in the *FAM149B1* gene

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SUMMARY: Biallelic variants in a family with sequence similarity 149 member B1 gene (*FAM149B1*, OMIM #618413) causes a range of abnormal phenotypes associated with Joubert syndrome (JS) in humans. However, the phenotypic spectrum and genetic evidence linking *FAM149B1* to ciliopathies remain limited, with no prenatal cases previously described. Here, we report the first prenatal case from a non-consanguineous Chinese family presenting with isolated right pelvicalyceal and ureteral dilation at 24 weeks of gestation. Notably, the fetus lacked cerebellar malformations or hallmark neuroimaging features such as molar tooth sign (MTS). Trio-whole-exome sequencing (Trio-WES) identified novel compound heterozygous loss-of-function variants in *FAM149B1*: paternal c.279T>A (p.Tyr93*) and maternal c.574dup (p.Ser192Phefs*7). Both variants are predicted to trigger nonsense-mediated mRNA decay (NMD), consistent with a loss-of-function mechanism, and parental segregation confirmed asymptomatic heterozygous carrier status, supporting autosomal recessive inheritance. Gene-disease validity assessment assigned a "Strong" classification to *FAM149B1*-related ciliopathy under ClinGen guidelines, enabling definitive classification of the variants as pathogenic or likely pathogenic. This case expands the phenotypic spectrum of *FAM149B1*-related disorders to include isolated urinary tract malformations in the prenatal period, where neurological manifestations may be absent or subtle. Our findings highlight the utility of prenatal exome sequencing in atypical cases and contribute to the understanding of the expanding genetic and phenotypic landscape of ciliopathies.

Keywords: *FAM149B1* gene, Joubert syndrome, urinary malformation, gene curation, prenatal diagnosis

1. Introduction

Primary cilia are microtubule-based organelles that are essential for mechanosensation, signal transduction, and cellular homeostasis (1). Defects in their assembly or function can lead to ciliopathies, a diverse group of genetic disorders characterized by dysfunctional cilia and multi-system involvement, with prominent clinical manifestations in the renal, neurological, and skeletal systems (2,3). *FAM149B1*-related disorders encompass a spectrum of genetic conditions associated with variants in the *FAM149B1* gene, which is located on chromosome 10 at the q22.2 region. The protein encoded by this gene, which contains a domain of unknown function 3719 (DUF3719) and a conserved HLR sequence motif, remains incompletely characterized. In 2019, Ranad

et al. first established the critical role of *FAM149B1* in normal ciliary biology, and identified two distinct phenotypes associated with homozygous loss-of-function variants in *FAM149B1* across four consanguineous families, presenting clinical features analogous to those of Joubert syndrome or oral-facial-digital syndrome type VI (OFD VI) (4). In 2021, the Sandy team reported another consanguineous family carrying homozygous *FAM149B1* variants and exhibiting clinical features of JS, although this family also harbored pathogenic variants in two other genes associated with neurologic disorders (5). Currently, the paucity of reported cases with *FAM149B1* variants precludes a comprehensive delineation of the associated clinical spectrum. Furthermore, absence of documented prenatal phenotypes related to *FAM149B1* variants represents a significant knowledge gap in the

prenatal diagnosis of JS-related disorders.

Joubert syndrome is a rare and typically autosomal recessive neurodevelopmental ciliopathy, affecting approximately 1 in every 80,000 to 100,000 infants globally each year (6). It is characterized by malformations of the posterior midbrain, cerebellar ataxia, oculomotor apraxia, dyspnea, intellectual disability, and varying degrees of developmental delay. Notably, most patients exhibit a specific malformation known as molar tooth sign, including cerebellar vermicosis hypoplasia, featuring prominent, thickened upper cerebellar peduncles, and abnormally deep interpeduncular fossa (7). In addition to these core features, most JS patients may also present with multi-organ developmental abnormalities involving the eyes, kidneys, liver, and skeleton. JS demonstrates significant genetic heterogeneity, with over 40 pathogenic genes identified to date, all encoding proteins involved in primary cilia formation or function. In the largest reported cohort, five major genes (*C5ORF42*, *CC2D2A*, *AHI1*, *CEP290* and *TMEM67*) account for 6%-9% of JS cases, three additional genes (*CSPP1*, *TMEM216* and *INPP5E*) each contribute 3%, while the remaining genes each account for JS in few families (8). However, approximately 10% of clinically suspected JS-related ciliopathies cases remain genetically unexplained (9). Notably, Joubert syndrome has also been reported as a phenotype as part of the spectrum of *FAM149B1*-associated disorders. Yet, existing literature is limited to postnatal manifestations in individuals from consanguineous backgrounds. A systematic literature search was performed in PubMed using the terms "FAM149B1", "Joubert syndrome", "ciliopathy", and "prenatal diagnosis" up to May 7th, 2026, and no prenatal cases associated with *FAM149B1* variants were identified.

This study reports, for the first time, a novel compound heterozygous loss-of-function variant in the *FAM149B1* gene in a fetus from a non-consanguineous family, representing the first prenatal case of *FAM149B1*-related disorders. This case broadens the clinical spectrum of *FAM149B1*-related disorders to include early-onset renal anomalies. Notably, the fetus did not exhibit recognizable structural abnormalities characteristic of JS, such as the MTS, highlighting the challenges and complexity of prenatal diagnosis for JS and related disorders.

2. Materials and Methods

2.1. Subjects

A 28-year-old primigravida (Figure 1A, I-2) at 23 weeks of gestation was referred to our institution for genetic counseling and potential prenatal genetics due to suspected fetal urinary system anomalies identified on ultrasound. The pregnant woman, of Chinese descent, and her husband were non-consanguineous and in good

health. The husband (Figure 1A, I-1) had a history of smoking but denied exposure to teratogens or other hazardous substances. The pregnant woman reported no medication use before or during pregnancy, and neither partner had a family history of genetic disorders. First-trimester non-invasive prenatal testing (NIPT) indicated low risk, and routine prenatal screenings were unremarkable.

2.2. Ethics approval and consent to participate

The use of human fetal tissue (fetal amniotic fluid cells) in this study was reviewed and approved by the Ethics Committee of International Peace Maternity and Child Health Hospital (GKLW-2022-14), in compliance with the provisions of the Declaration of Helsinki (as revised in 2013). All tissues were obtained with informed consent from donors, independently of any decision regarding pregnancy termination. No financial inducements or payments were made to obtain these materials. Informed consent to participate was obtained from all individuals involved in the study or their legal guardians.

2.3. Prenatal genetic diagnosis

At 24 weeks of gestation, the pregnant woman underwent amniocentesis for prenatal genetic diagnosis. Genomic DNA was extracted from amniotic fluid cells and peripheral blood samples from both parents using the Genomic DNA Extraction Kit (TIANGEN, Beijing, China). Trio-whole exome sequencing (Trio-WES) was performed as previously described (10). Sequencing libraries were constructed using the KAPA HyperExome V3 capture kit (Roche, Basel, Switzerland) and sequenced on the MGISEQ-2000 platform (MGI, Shenzhen, China) with 150-bp paired-end reads.

Raw sequencing reads were processed using SOAPnuke (v2.0) to obtain high-confidence clean reads. Clean reads were aligned to the UCSC hg19 human reference genome using the Burrows-Wheeler Aligner (BWA-MEM, v0.7.17). PCR or optical duplicates were marked and removed using Picard Tools (v2.26). Base quality score recalibration, single nucleotide variant (SNV) and small insertion/deletion (INDEL) calling, and genotyping were performed using the Genome Analysis Toolkit (GATK, v4.2) following best practices. All identified variants were annotated in VCF format and analyzed using the BGI-Varanno pipeline for variant interpretation.

In parallel, conventional chromosomal karyotype analysis and chromosomal microarray analysis (CMA) were performed on fetal samples to assess chromosomal abnormalities and genome-wide copy number variations. CMA and Trio-WES were performed as part of standard-of-care diagnostic workup in an accredited clinical laboratory, in accordance with standard medical practice. All testing was ordered by the attending clinicians for

diagnostic purposes, and informed consent was obtained from the parents prior to testing.

2.4. Sanger sequencing validation

Specific PCR primers were designed to validate

candidate pathogenic variants in *FAM149B1* (transcript NM_173348.2) identified by trio-WES, using Primer3 software (v. 0.4.0). Primers were synthesized by MAP Biotech (Shanghai, China). For amplification of exon 3 and its flanking exon-intron boundaries, the primer sequences were: forward

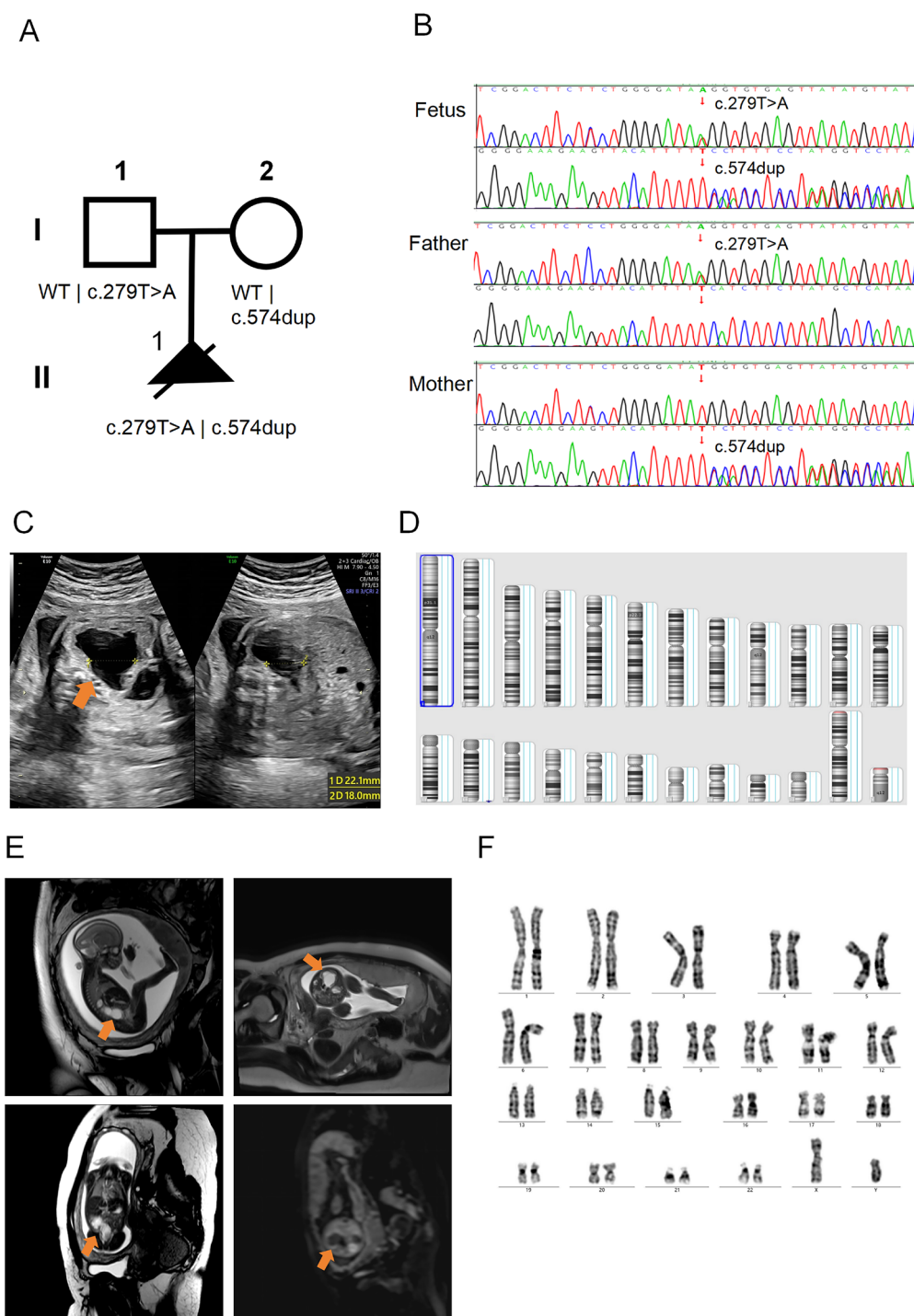


Figure 1. Pedigree, clinical, and molecular characterization of the fetus. (A) Pedigree of the family. Generations are labeled using Roman numerals (I, II). Solid symbols indicate affected individuals. (B) DNA sequencing results of the family. Sanger sequencing analysis identified two heterozygous variants associated with the phenotype of the proband in the *FAM149B1* gene, which were inherited from the parents, respectively. Red arrows indicate the variant positions. (C) Fetal ultrasound images. The arrow site marks the affected area. (D) CMA of the fetus revealed no abnormalities. (E) Fetal MRI images. The arrow site marks the affected area. (F) Chromosomal karyotype analysis of the fetus revealed no abnormalities.

5'-ATGCCACAGAAAACCTTGAGGACA-3' and reverse 5'-AAACAAAACCATACTCAAAAAGAA-3'. For exon 6 and adjacent splice regions, the primer pair was: forward 5'-ATGTAAGTGTGTTTGCATAAGACT-3' and reverse 5'-AATTCTGGATACCTGAGAAATACAT-3'.

PCR amplification and product purification were performed using the specific primers, followed by sequencing on an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using Mutation Surveyor v5.0.2 (SoftGenetics LLC) to confirm presence and zygosity of the variants in the proband and to perform segregation analysis in parental samples.

2.5. Dimensional structure analysis of the FAM149B1 mutant protein

The crystal structure of the wild-type FAM149B1 was obtained from the Uniprot database (<https://www.uniprot.org/uniprotkb/Q96BN6/entry>). Crystal structure of each mutant was then generated using Swiss model (<https://swiss.model.expasy.org/>) and PyMOL (Schrödinger Inc., New York, NY, USA).

3. Results and Discussion

3.1. Clinical and genetic findings

The pregnant woman exhibited no abnormalities in routine laboratory tests prior to pregnancy and during the early stages of gestation. The pregnancy was a spontaneous singleton intrauterine gestation. Ultrasound at 12 weeks of gestation showed that the fetal nuchal translucency measurement was within the normal range. Ultrasound at 17 weeks confirmed fetal growth appropriate for gestational age. At 23 weeks of gestation, ultrasound examination revealed the following fetal biometric measurements: biparietal diameter of 54 mm, head circumference of 194 mm, and femur length of 34 mm. Notably, right pelvicalyceal dilatation was observed, measuring 14.1 mm, accompanied by right ureteral dilation with a maximum diameter of 5.4 mm. An anechoic area measuring $10 \times 9 \times 7$ mm was also identified above the right kidney. At 26 weeks of gestation, ultrasound examination showed right double renal pelvis and 22 mm renipelvic separation of the fetus (Figure 1C). Fetal magnetic resonance imaging confirmed right duplicated renal pelvis with associated pelviectasis and right ureteral dilatation, raising suspicion of a possible ectopic ureteral orifice (Figure 1E). Additionally, a cystic lesion was identified at the upper pole of the right kidney, which appeared consistent with a benign renal cyst, no other fetal structural abnormalities were detected. CMA testing (Figure 1D) and chromosomal karyotype analysis (Figure 1F) revealed no meaningful copy number or chromosomal structural abnormalities in the fetus. To

investigate potential genetic cause and provide evidence for the prenatal diagnosis of the fetus, trio-WES was performed. After filtering out low-confidence variants with poor sequencing quality, variants with a population frequency exceeding 1% in the gnomAD database, and those predicted to be benign, the remaining variants were prioritized using "urinary system malformations" as a key phenotypic criterion. This analysis led to the identification of a novel compound heterozygous variant in the *FAM149B1* gene in the fetus: c.279T>A (p.Tyr93*) and c.574dup (p.Ser192Phefs*7), which was subsequently confirmed by Sanger sequencing (Figure 1B). The c.279T>A variant, located in exon 3, was inherited from the father, while the c.574dup variant in exon 6 was maternally inherited.

Prior to this study, only two studies had identified three pathogenic homozygous *FAM149B1* variants in consanguineous families (4,5). These frameshift and nonsense variants are severe loss-of-function mutations that lead to truncated protein products. Here, we report the first compound heterozygous variants of the *FAM149B1* gene [c.279T>A (p.Tyr93*) and c.574dup (p.Ser192Phefs*7)] in a Chinese fetal case (Table 1). A systematic literature search identified no previous reports of prenatal cases associated with *FAM149B1* variants, suggesting that this is the first such case described. Both variants are absent from population databases, including gnomAD, underscoring their rarity and novelty. Previous reports of clinical features showed that most patients of *FAM149B1* variants predominantly described with developmental delay accompanied by neurological involvement (4,5). Unlike these patients, the fetus in this study presented with isolated right pelvicalyceal and ureteral dilation, detailed ultrasound and MRI assessments revealed no cerebellar malformations or the characteristic MTS. The heterozygous carrier parents exhibited no clinical symptoms, further supporting a recessive inheritance mechanism for *FAM149B1*-related disorders. Phenotypic discrepancy further confirms that *FAM149B1*-related disorders demonstrate significant phenotypic heterogeneity and genotype-phenotype association effects, variations in mutation types and mutation sites may lead to differing degrees of protein functional abnormalities.

3.2. Pathogenicity analysis of the identified *FAM149B1* variants

Given the limited number of reported cases, we first performed gene-disease association curation, according to the framework issued by the Clinical Genome Resource (11) before pathogenicity assessment of the *FAM149B1* variants. We conducted a comprehensive literature review using PubMed and identified seven patients with three different *FAM149B1* variants (4,5). The fetal case from this study was not included due to insufficient phenotypic information. With 12 points for

Table 1. Clinical characteristics of the patients with *FAM149B1* variants

	Patients in this study (Fetus)	Reported patients (1 female, 6 male)
Ethnicity	Chinese	1 Turkish, 6 Arab
Allelic state	compound heterozygous	homozygous
Variants	1 nonsense (p.Tyr93*) and 1 frameshift (p.Ser192Phefs*7)	3 frameshift (p.Lys119Ilefs*18), 1 nonsense (p.Gln147*), 3 indel-induced frameshift (p.Gln118Hisfs*20)
Developmental delay/Intellectual disability	—	7/7
Molar tooth sign	—	5/6
Ocular motor apraxia/strabismus	—	7/7
Ptosis	—	5/7
Ataxia	—	3/7
Duane Syndrome	—	3/7
Nystagmus	—	3/7
Progressive ophthalmoplegia	—	3/7
Mesoaxial polydactyly	—	5/7
Mild skeletal dysplasia	—	3/7
Macrocephaly	—	3/7
Renal involvement	+	0/7
Cardiac defects	—	1/7
Lateralization defects	—	1/7
Liver involvement	—	0/7

Table 2. Gene-disease association curation for *FAM149B1* and autosomal recessive JS-related ciliopathy

	Evidence Type	Case Information Type	Guidelines			Scores	
			Default	Range	Max	Points	Tally
			(per variant)	(per variant)		(Max)	
Genetic Evidence	Case-Level Data						
	Variant Evidence	Two variants in trans and at least one de novo or a predicted/proven null variant	2	0–3	12	21 (12)	12
		Two variants (not predicted/proven null) with some evidence of gene impact in trans	1	0–1.5			
	Total Genetic Evidence Points (Maximum 12)						
Experimental Evidence	Evidence Category	Evidence Type	Guidelines			Scores	
			Default	Range	Max	Points	Tally
	Functional Alteration	Patient cells	1	0–2	2	1	1
		Non-patient cells	0.5	0–1		0	
	Total Experimental Evidence Points (Maximum 6)						

genetic evidence and 1 point for experimental evidence, the gene-disease relationship between *FAM149B1* and JS related ciliopathy is classified as "Strong" (Table 2). Thus, all evidence criteria outlined in the ACMG/AMP guidelines can be applied (12,13). Additionally, in contrast to the three previously reported variants, which are all clustered within the DUF3719 domain, these two variants described in this study are located outside this domain (Figure 2A). The variants p.Tyr93* and p.Ser192Phefs*7 occur in the first third of the *FAM149B1* protein sequence, leading to premature termination of translation and severe structural truncation (Figure 2B). These alterations are predicted to result in marked protein instability and are highly likely to trigger nonsense-mediated mRNA decay, consistent with a

loss-of-function pathogenic mechanism of *FAM149B1* disorders. Therefore, the p.Tyr93* nonsense variant was classified as likely pathogenic (PVS1+ PM2_supporting), while the p.Ser192Phefs*7 frameshift variant was classified as pathogenic (PVS1+ PM2_supporting+ PM3).

JS has strong clinical heterogeneity, affected individuals may show involvement of other organs to varying degrees classified into multiple clinical subgroups: JS with ocular involvement (JS-O), JS with renal involvement (JS-R), JS with oculorenal involvement (JS-OR), JS with hepatic involvement (JS-H), JS with orofaciocigital involvement (OFDVI syndrome), *etc* (8,9). The renal involvement features in JS patients include loss of corticomedullary differentiation and small

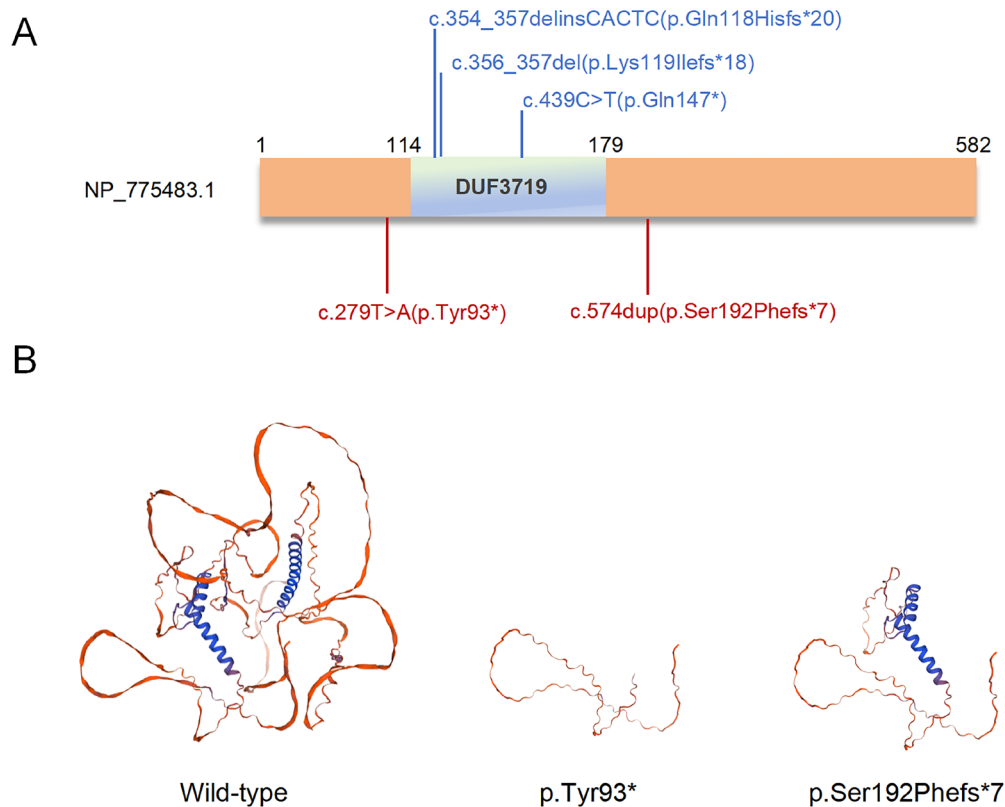


Figure 2. Distribution and structural analysis of the mutants. (A) The diagram illustrates the schematic structure of the FAM149B1 protein domain. Variants recorded in previous studies (blue) are annotated above the protein schematic, while the heterozygous frameshift variants identified in this cohort (red) are mapped below and aligned with the corresponding structural context. DUF3719 resemble domain that belongs to DUF families that may be functionally important. (B) Crystal structure analysis indicates that, compared to the wild-type FAM149B1 protein, the p.Tyr93* and p.Ser192Phefs* mutants are predicted to cause severe disruption of the protein structure.

cyst formation, most commonly in those with pathogenic variants in *CEP290*, *TMEM67*, *AH11*, *NPHP1*, *RPGRIP1L* and *TMEM237* (14,15). The absence of the neurological phenotype observed in this fetus as well as its differences from previous cases may be multifactorial. First, this may be due to the limited number of reported patients, resulting in an incomplete understanding and characterization of its phenotypic spectrum. Secondly, the clinical data for this fetal case are primarily derived from assessments during the second trimester, the fetus was still at an early stage of development, and certain extrarenal or neurological features commonly associated with *FAM149B1*-related disorders might not yet have been apparent, the limitations of the evaluation methods for mild neurologic involvement restrict the comprehensive characterization of the phenotype (16). Finally, the majority of previously reported patients are from Saudi Arabia, phenotypic heterogeneity related to different ethnic backgrounds also cannot be ruled out. Different residual protein activity and genetic background may contribute to phenotypic heterogeneity.

DUF3719 is a conserved domain found in eukaryotes, comprising approximately 70 amino acids. It features a conserved HLR sequence motif and two fully conserved residues—tryptophan(W) and histidine(H)

(4). XBX-4, the homologous protein of FAM149B1 protein in nematodes, contains the DUF3719 domain and participates in maintaining ciliary homeostasis through the highly conserved DYF-18/CCRK and DYF-5/MAK kinase cascade (17). The non-DUF3719 region of the FAM149B1 protein exhibits high conformational flexibility, may participate in regulating intracellular transport in cilia by interaction with BROMI-CFAP20, and this interaction is enhanced in the presence of CCRK (18). Truncating variants that disrupt the structural integrity of proteins may impair these interactions and interfere with normal ciliary assembly and function. Fibroblasts from *FAM149B1*-deficient patients exhibit abnormally elongated cilia, abnormal accumulation of IFT machinery components at the ciliary tips, and significant dysregulation of SHH signaling (4). However, whether different *FAM149B1* variants exert differential effects on primary cilia development requires further in-depth research.

3.3. Pregnancy outcome

After clarifying the pathogenicity and clinical significance of the two *FAM149B1* variants, the couple received comprehensive genetic counseling. Considering

severity of the *FAM149B1*-related disorder, they opted to terminate the pregnancy at 28 weeks at other medical institutions. Meanwhile, the family was informed about reproductive options for future pregnancies, including prenatal diagnosis following natural conception and preimplantation genetic testing.

Given the novelty of the fetal phenotype, detailed clinical documentation would have been invaluable for phenotypic correlation. However, the pregnancy was terminated at an external institution, and no autopsy or photographic records of the fetal dysmorphic features were available despite efforts to obtain them. Consequently, the phenotypic characterization is limited to prenatal ultrasound and MRI findings. Owing to absence of relevant experiments covering transcript-level expression analysis, protein function investigation and ciliary functional assessment, we are currently unable to fully clarify the biological effects of the identified variants in depth from mechanistic and biological aspects. Our finding highlights the challenge of diagnosing JS spectrum disorders prenatally when typical neuroimaging features are absent, further suggest the potential association between *FAM149B1* variants and renal abnormalities. More evidence is needed to elucidate the association between variation and fetal phenotype in the future.

In conclusion, we report the first prenatal case carrying pathogenic *FAM149B1* variants with renal manifestations, further expanding the genotypic and phenotypic spectrum associated with *FAM149B1*. It underscores the importance of genetic evaluation following the detection of abnormalities on prenatal imaging and diagnostic value of exome sequencing in fetuses with atypical or isolated structural anomalies.

Acknowledgements

We are deeply grateful to the patients and their family for participating in this study.

Funding: The study was supported by the National Key Research and Development Program of China (2022YFC2705202), the Shanghai Municipal Health Commission (2024ZZ2005), the Program of Innovative Research Team of High-Level Local Universities in Shanghai (SHSMU-ZDCX20212200), the project of Shanghai Key Laboratory of Embryo Original Diseases (Shelab202301 and Shelab202404), and the SJTU Transmed Awards Research (20240202).

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

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Mol Biol Cell. 2022; 33:ar79.

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Received March 20, 2026; Revised June 3, 2026; Accepted
June 24, 2026.

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Released online in J-STAGE as advance publication July 8,
2026.

Traditional Chinese medicine research on rare diseases: Publication trends and future perspectives

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SUMMARY: Traditional Chinese medicine (TCM) has increasingly attracted the attention of researchers as a potential complementary or supportive approach for selected rare diseases, but the characteristics and evidentiary strength of this literature remain unclear. This Correspondence summarizes publication patterns identified for the 121 conditions in the First Chinese Rare Disease List using Chinese and English sources searched from database inception through June 30, 2021. Fifty-five rare diseases were identified in 3,030 TCM-related publications, though the ten most frequently studied conditions accounted for more than 80% of the literature. Generalized myasthenia gravis, idiopathic pulmonary fibrosis, and multiple sclerosis together accounted for nearly half of all publications. Clinical studies and case reports or experience summaries predominated, whereas experimental studies represented only 9.03%. Oral herbal formulas were the most frequently reported intervention, and more than 95% of publications originated from mainland China. These patterns indicate growing interest but a fragmented, geographically concentrated, and methodologically limited evidence base; publication volume should not be interpreted as evidence of efficacy or safety. Future research should prioritize mechanistic studies, standardized and innovative clinical designs, rigorous safety and quality-control procedures, real-world evidence, patient-centered outcomes, and international collaboration to clarify the potential roles and limitations of TCM in rare disease management.

Keywords: traditional Chinese medicine, rare diseases, publication trends, clinical evidence, research methodology

1. Introduction

Rare diseases, though individually rare, collectively affect millions worldwide. In China, the official Rare Disease List has expanded from 121 conditions in 2018 to 207 in 2023, reflecting growing recognition in policy (1-3). For most of these diseases, however, effective treatments remain scarce and prohibitively expensive. Rare diseases are clinically heterogeneous and often associated with substantial unmet needs, although standard care varies widely across diseases (4-6).

In this context, traditional Chinese medicine (TCM)-related approaches have attracted attention in research on selected rare diseases, mainly as potential complementary or supportive strategies. Rooted in a holistic framework, TCM emphasizes syndrome differentiation. This diagnostic process identifies patterns

of physiological imbalance and guides the use of multi-component herbal formulas tailored to each patient (7,8). Rather than targeting a single molecular pathway, TCM aims to restore overall physiological equilibrium. This individualized framework may partly explain why TCM-related approaches have attracted interest in research on selected rare diseases characterized by insidious onset, progressive courses, and multisystem involvement (9).

Conditions such as systemic sclerosis (SSc), generalized myasthenia gravis (GMG), and multiple sclerosis (MS) exemplify this complexity. All are chronic, autoimmune-mediated disorders with heterogeneous presentations and no definitive cure. Previous reports by our group suggested potential clinical relevance and patient acceptance of TCM-related approaches in SSc; however, these observations should not be interpreted as definitive evidence of efficacy (10,11). TCM-related

interventions have been reported in GMG and MS, often alongside conventional treatments, but the strength and quality of this evidence remain uncertain (12-18).

Despite these promising signals, the literature on TCM for rare diseases remains poorly characterized. Which diseases have attracted sustained interest in research? What types of studies and therapeutic interventions dominate? This article seeks to examine these critical questions, offering a concise overview of observations regarding TCM research on rare diseases, based on the literature, and highlighting directions for future investigation.

2. Key observations from publications

Data source and scope: We based our observations on TCM-related publications involving the First Chinese Rare Disease List (CRDL) of 121 conditions (19). Publications were searched in Chinese and English sources, including CNKI, Baidu Academic, DUXIU, PubMed, Bing Academic, and Microsoft Bing, from database inception to June 30, 2021. TCM-related research was broadly defined as publications involving Chinese herbal medicine, compound prescriptions, acupuncture, moxibustion, tuina, rehabilitation-related TCM approaches, integrated Chinese and Western medicine, syndrome differentiation, or related interventions. The analysis examined publication patterns and disease distribution; it was not designed as a systematic review or an assessment of efficacy, safety, or evidence quality. The detailed search strategy is provided in Supplementary File (<https://www.irdrjournal.com/action/getSupplementalData.php?ID=313>).

Concentration on a subset of diseases: Among the 55 rare diseases with TCM publications, just ten accounted for over 80% of all papers. Three conditions stand out: GMG, idiopathic pulmonary fibrosis (IPF), and MS, which together accounted for nearly half of the literature. GMG alone accounted for more than one-fifth of all publications (Table 1).

Emphasis on chronic rare disease management: What unites these highly researched diseases? Nearly all share a common profile: they are chronic, multisystem disorders with adult onset, are poorly understood, and have multifactorial origins. GMG and IPF exemplify this pattern. Both involve complex immune dysregulation rather than a single genetic defect, and both require long-term, multi-system management. This clinical complexity may explain why these diseases have attracted attention in research on TCM-related studies.

The concentration of publications on GMG, IPF, MS, and SSc may reflect clinical familiarity, disease prevalence, research trends, availability of TCM interventions, chronic disease course, and publication bias. Therefore, this distribution should be interpreted as attention in research rather than evidence that TCM is more effective in treating these diseases.

Predominance of clinical studies: Clinical studies (37.25%) and case reports or experience summaries (31.25%) accounted for the largest share of 3,030 TCM publications on rare diseases. These were followed by literature reviews and theoretical studies, while experimental studies were the least frequent, accounting for only 9.03% of the total. This distribution demonstrates that TCM research on rare diseases remains primarily oriented toward clinical observation and empirical experience rather than mechanistic investigation (Figure 1). The evidence level appears limited. Most publications were clinical studies, case reports, or experience summaries, whereas experimental studies accounted for only 9.03%. We did not perform a formal risk-of-bias assessment, and the broad category of "clinical studies" may include heterogeneous designs. Therefore, these publications should be viewed as preliminary signals for future research rather than evidence sufficient to establish efficacy or safety.

Diversity of therapeutic approaches: Oral herbal formulas predominated overall (59%), but their application varied by disease. GMG, a neuromuscular disorder, was mainly treated with medication, likely

Table 1. Rare diseases with the highest numbers of TCM-related publications

ID*	Top 10 rare diseases in TCM-related publications**	Number (n)	Proportion (%)
032	Generalized myasthenia gravis	665	21.95%
055	Idiopathic pulmonary fibrosis	498	16.44%
076	Multiple sclerosis	314	10.36%
102	Retinitis pigmentosa	192	6.34%
004	Amyotrophic lateral sclerosis	182	6.01%
037	Hepatolenticular degeneration	164	5.41%
077	Multiple system atrophy	157	5.18%
112	Systemic sclerosis	153	5.05%
081	Neuromyelitis optica	116	3.83%
088	Paroxysmal nocturnal hemoglobinuria	86	2.84%

*Disease ID in the First Chinese Rare Disease List (CRDL) of 121 conditions. **Publications were searched in Chinese and English sources, including CNKI, Baidu Academic, DUXIU, PubMed, Bing Academic, and Microsoft Bing, from database inception to June 30, 2021. *Note:* The number of publications and each proportion on top 10 rare diseases were counted. The results of the analysis are shown in the table. The table shows publication counts and proportions of TCM-related publications identified for 121 CRDL-listed rare diseases. Publication volume should not be interpreted as evidence strength, disease burden, or clinical importance.

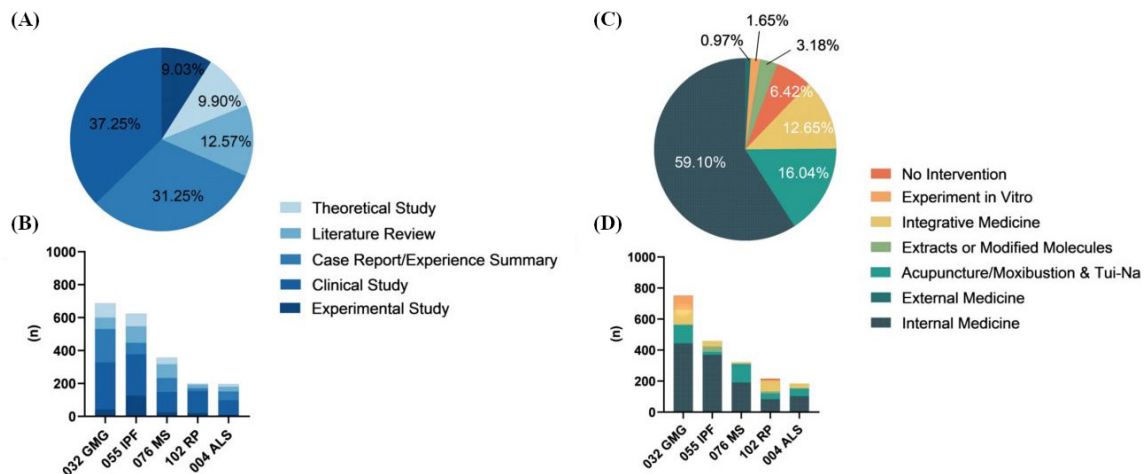


Figure 1. Distribution of study types among TCM-related publications on rare diseases. (A) Types of studies in publications on all diseases; (B) Types of studies in publications on the top 5 rare diseases; (C) Types of interventions in publications on all diseases; (D) Types of interventions in publications on the top 5 rare diseases. *Note:* Publications were categorized by study and intervention types. Publications on all diseases were compiled as different percentages in pie charts and stacked bar charts for the top 5 rare diseases. These categories describe publication types and intervention categories only; they do not indicate evidence quality, efficacy, safety, or clinical recommendation strength. *Abbreviations:* GMG, generalized myasthenia gravis; IPF, idiopathic pulmonary fibrosis; MS, multiple sclerosis; RP, retinitis pigmentosa; ALS, amyotrophic lateral sclerosis.

targeting systemic immune regulation. In contrast, IPF was treated with herbal extracts or modified molecules, perhaps reflecting a focus on a more targeted pulmonary intervention. Acupuncture and integrative therapies were also often used, underscoring the multimodal nature of TCM (Figure 1).

3. Implications and future directions

The patterns observed in the publications highlight several implications for the role of TCM in rare disease management.

3.1. The concentration of research on chronic rare diseases is not incidental

Concentrated research on chronic rare diseases points to a fundamental alignment between the holistic, syndrome-based approach of TCM and the clinical realities of diseases for which conventional targeted therapies are often limited. Conditions such as GMG, IPF, and SSc exemplify this pattern. Care for these diseases is mainly symptomatic and supportive care, an area where TCM-related approaches have been explored. The predominance of such conditions in the TCM publications therefore sends an important message. Future research may clarify whether TCM-related approaches have potential roles in treating the complex, multisystem manifestations of selected rare diseases.

This is not to suggest that TCM has no role in hereditary rare diseases. In conditions such as hemophilia, where monogenic defects are well-defined, TCM has been used adjunctively to manage bleeding complications and associated musculoskeletal symptoms.

However, the literature on such applications remains sparse, averaging fewer than three publications annually since 1958. This contrast reinforces the conclusion that TCM's primary contribution lies in complex, non-Mendelian conditions.

3.2. Attention in research has steadily increased over time

After a single publication in 1958, the field remained dormant for decades. A turning point came in around 2011, when annual publications began to increase steadily, coinciding with growing international awareness following the first Rare Disease Day in 2008. By 2019, annual output had peaked at 240 publications.

However, this increasing trend has revealed significant gaps that must be addressed. Experimental research is still scarce, accounting for less than 10% of publications. Mechanistic studies need to be conducted to move beyond empirical observation. Clinical evidence is predominated by case reports and uncontrolled studies, highlighting the need for well-designed trials that incorporate TCM syndrome differentiation. Given the inherently small patient populations in rare diseases, conventional large-scale randomized trials are often unfeasible. Therefore, clinical trial designs require not only standardization, including rigorous controls and consistent outcome measures, but also methodological innovation. Moreover, the wealth of clinical experience calls for systematic synthesis, an area where data mining techniques could prove valuable in distilling the expertise of renowned TCM practitioners.

Beyond traditional clinical trials, real-world data offer a complementary pathway. Large-scale patient registries,

electronic health records, and prospective observational studies can discern the effectiveness of TCM in routine practice, where treatments are individualized and long-term outcomes matter most. Such evidence, while not replacing randomized trials, could inform clinical decisions and generate hypotheses for further investigation.

In summary, TCM rare disease research is predominantly case-based, with few experimental studies. The future integration of N-of-1 randomized controlled trials (RCTs), Bayesian trials, and comprehensive patient registries will be crucial to transforming individualized pattern differentiation into high-quality evidence. This shift from mere observation to rigorous validation will enable the field to overcome the limitations of small samples and to clarify the potential role and limitations of TCM-related approaches in rare disease research.

3.3. The international dimension warrants attention

Currently, over 95% of TCM publications on rare diseases originate from mainland China, with fewer than 3% published in international journals. This geographic concentration, compounded by regulatory differences and challenges in herbal quality control, limits global attention.

3.4. To bridge these gaps, we propose several priorities for future research:

Mechanistic studies need to be conducted to elucidate the molecular pathways modulated by TCM formulas, moving beyond empirical observation towards scientific validation. For GMG and SSc, this might involve immune profiling and biomarker discovery; for IPF, it may focus on fibrotic pathways.

Standardized clinical trial designs are essential to enhancing scientific validity. This includes rigorous controls, consistent outcome measures, and incorporation of TCM syndrome differentiation. Pragmatic trials that reflect real-world clinical practice could bridge the gap between explanatory efficacy studies and clinical applicability. TCM may be discussed as a potential complementary or supportive approach for further study but not as an established alternative to evidence-based therapy.

Safety monitoring and quality control should be a central component of future studies. Patients with rare diseases often receive prolonged and complex standard therapies, and TCM interventions may involve multi-component herbal formulas or combined modalities. Future research should therefore include adverse event monitoring, quality control of herbal products, dose and formulation reporting, and evaluation of potential herb–drug interactions with standard therapies.

Real-world evidence should be systematically collected and compiled. Patient registries, electronic

health records, and prospective observational studies can complement clinical trials by capturing long-term outcomes and treatment patterns in routine practice.

A *TCM-specific evidence framework* is needed to transcend conventional RCT limitations. An integrated evidence framework may help organize heterogeneous evidence from case reports, observational studies, mechanistic research, and trials, but it should complement rather than replace rigorous clinical evaluation. For rare diseases with limited numbers of patients, approaches such as rare disease registries, N-of-1 trials, Bayesian designs, and real-world evidentiary studies may provide additional evidence-generating strategies.

International collaboration is needed to harmonize research methodologies, facilitate regulatory recognition, and expand the global acceptance of TCM in rare disease management. Joint initiatives, including multinational patient registries and collaborative clinical trials, could accelerate progress.

Patient-centered research should be prioritized. Understanding patient preferences, treatment adherence, and quality of life outcomes will be essential to demonstrating the value of TCM beyond symptom control. The high rate of TCM acceptance among patients with SSc in our study suggests that patient perspectives may be a powerful driver of integration.

3.5. Limitations

Several limitations should be acknowledged. First, this Correspondence is based on published literature and may be affected by publication bias, language bias, and database coverage limitations. Second, the analysis was based on the first CRDL of 121 diseases and publications available up to June 30, 2021, so it does not represent the expanded list of 207 diseases or subsequent literature. Third, we did not assess the risk of bias, evidence quality, or the efficacy, safety, or clinical appropriateness of individual TCM interventions. Finally, concentrated publications on selected diseases may reflect research trends, clinical familiarity, disease prevalence, or availability of interventions rather than stronger clinical evidence.

In conclusion, TCM-related publications suggest growing interest in research on selected rare diseases, but the current evidence base remains fragmented and preliminary. Future studies should use rigorous designs, standardized interventions, safety monitoring, and clinically meaningful outcomes.

Acknowledgements

The authors wish to thank all of the study participants for their contributions. The authors would also like to thank Prof. Li Ding-Guo and Secretary General Chen Zhi-Qiang (of the Shanghai Rare Disease Prevention and Control Foundation) for their assistance in writing

this manuscript. Prof. Liu Ming-Yuan contributed to the internal discussions.

Funding: This work was supported by the Shanghai Health Commission's Program for Young Personnel in Healthcare [2022YQ058]; the Shanghai Municipal Education Commission's Key Innovation Team from High-level Local Universities in Shanghai (Phase II); and Shanghai University of Traditional Chinese Medicine's Program for Students Engaged in Scientific and Technological Innovation [202210268298].

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

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Received May 10, 2026; Revised July 22, 2026; Accepted August 14, 2026.

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Released online in J-STAGE as advance publication August 18, 2026.

Fulminant myocarditis and sudden death associated with thymoma-related autoimmunity

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SUMMARY: Fulminant myocarditis (FM) is a severe, rapidly progressive and life-threatening condition of myocarditis associated with infectious or autoimmune etiologies. Here, we present three patients with an invasive thymoma who had FM, which eventually led to sudden death. The most frequent clinical presentations at onset include palpitations, orthopnea, and acute worsening of myasthenia gravis (MG). Highly elevated myocardial biomarkers and positive autoantibodies against cardiac muscle, skeletal muscle, and neuromuscular junctions were detected in serum. The electrocardiograph (ECG) findings progressed to life-threatening ventricular arrhythmias. Although high-dose methylprednisolone or intravenous immunoglobulin was administered along with advanced respiratory support, profound hemodynamic collapse rapidly occurred, and these patients eventually died within days. This case series underscores the importance of rapid recognition of FM associated with thymoma and early aggressive supportive and immunomodulatory interventions, including consideration of mechanical circulatory support when clinically indicated.

Keywords: invasive thymoma, fulminant myocarditis, sudden cardiac death, autoantibody

1. Introduction

Fulminant Myocarditis (FM) is a form or a syndrome of severe myocarditis that presents with sudden-onset acute heart failure, cardiogenic shock, or life-threatening arrhythmia (1). Infections, autoimmune diseases, and drug toxicity are the main triggers for the development of FM (2,3), among which, viral infection is the most common etiology (4). Thymoma-associated immunologic paraneoplastic disorders (PNDs) involve multiple systems, including neuromuscular, central nervous system (CNS), hematologic, endocrine, cutaneous, gastrointestinal, renal, cardiac (5), and other systems (6). The frequency of myocarditis associated with thymoma is less than 1% (7). Pathologically speaking, WHO subtypes B2 and AB are the most prevalent forms associated with thymoma (7).

Here, we briefly present 3 cases of FM associated with invasive thymoma and multiple autoantibodies that were treated at a single centre (Huashan Hospital). Written informed consent for publication of this report

and the accompanying clinical images was obtained from their legal representatives. We aim to emphasize the need for early diagnosis and aggressive interventions to improve the clinical outcome for this devastating, rare complication.

2. Shared clinical pattern of invasive thymoma-associated fulminant myocarditis

In these patients, the age at diagnosis was 30 to 43 years with invasive thymoma, the pathology of which was WHO B2-B3 and Masaoka staging III-IV. Ocular onset, or generalized onset of myasthenia gravis (MG), the most prevalent form of thymoma-associated autoimmunity, was the initial clinical presentation. All three patients rapidly developed myasthenic crisis (MC), a life-threatening condition with respiratory failure requiring ventilation. Highly elevated titers of anti-Acetylcholine receptor antibody (AChR-Ab), anti-Titin antibody, anti-skeletal muscle antibody, and anti-cardiac muscle antibody were identified. These were

potential culprit antibodies for multisystem involvement, including neuromuscular junction impairment, myositis and myocarditis.

The clinical characteristics and serological antibodies of three cases are presented in Table 1. After admission, progressively decreased blood pressure and malignant arrhythmia occurred (Figure 1). The bedside echocardiography identified motion abnormality in the multi-segmental left ventricular wall. Serum testing identified elevated cardiac biomarkers, such as troponin T (TnT), pro-Brain natriuretic peptide (BNP), and heart-type Fatty Acid-Binding protein (H-FABP). Initially, the electrocardiograph (ECG) revealed sinus tachycardia and ST-segment elevations, which progressed to life-threatening ventricular arrhythmias. Profound hemodynamic collapse rapidly occurred, followed by a gradual loss of consciousness. Despite timely ventilatory support and immunotherapies, resuscitation failed for these patients. The time from cardiac presentations to sudden death was 4 to 5 days.

These cases shared the same clinical patterns, including invasive or advanced thymoma, thymoma-associated myasthenia gravis, myasthenic crisis, high titers of anti-striational or anti-cardiac antibodies, rapid cardiac deterioration, malignant arrhythmia, and sudden death.

3. Clinical implications for early recognition and aggressive intervention

Thymomas are associated with a variety of autoimmune disorders. Pathophysiological mechanisms are complex

and include decreased expression of autoimmune regulator (AIRE), MHC Class II, and T regulatory cells (Tregs) (6). The above mechanisms cause production of organ-specific autoantibodies, including those against the myocardium. Pathogenic antibodies detected in FM associated with thymoma are anti-heart antibodies (AHA), which are present in up to 60% of patients with inflammatory cardiomyopathy and their relatives (8). These autoantibodies probably target cardiac autoantigens, particularly cardiac α -myosin heavy chain (also known as myosin 6) and β -myosin heavy chain (also known as myosin 7) isoforms (9). Diagnostic value of AHA as compared with myocardial biopsy was highlighted in a previous study (10). The role of AHA has been repeatedly shown in development of inflammatory and autoimmune diseases of the myocardium and pericardium, as well as in progression of genetically determined cardiomyopathy (11).

Due to the high mortality rate of FM, early identification and intervention are of critical importance. Once a healthy individual or a non-cardiac disease patient shows rapid heart failure, it is very important to consider the possibility of FM. When a suspected patient is admitted, routine tests including physical examinations, blood chemistry, ECG, and emergency echocardiography should be conducted (12). In certain patients with a history of thymoma and MG, clinical presentation of arrhythmia warrants immediate cardiac evaluations and early intervention. More specifically, immunotherapies using large doses of glucocorticoids and intravenous immunoglobulin (IVIG) are necessary. Plasma or blood

Table 1. The clinical characteristics and serological antibodies of three cases

Characteristics	Case 1	Case 2	Case 3
Sex	Male	Female	Male
Age	30	43	40
Thymoma Pathology (WHO)	B2	B2	B3
Thymoma Pathology (Masaoka)	IIIB	III	IV
Resection status for thymoma	One resection	No resection, only biopsy	Two resections
Antibody profiles:			
AChR-Ab (nmol/L)	36.2	42.96	11.51
anti-Titin antibody	+++	+++	+++
anti-skeletal muscle antibody	1:320	1:320	1:1000
anti-cardiac muscle antibody	1:320	1:320	1:1000
Cardiac manifestations	blood pressure rapidly dropped, motion abnormality in the multi-segmental left ventricular wall, ventricular arrhythmia, cardiac biomarkers elevated	heart failure and malignant arrhythmia, cardiac biomarkers elevated	difficulty in breathing, cardiac biomarkers elevated
Myasthenia Gravis (MG) or other autoimmunity	Ocular onset of MG	Generalized onset of MG	Generalized onset of MG
Interventions and Therapies	non-invasive mechanical ventilation, plasmapheresis, IVIG and IVMP	endotracheal intubation, plasmapheresis and IVMP	non-invasive mechanical ventilation, plasmapheresis, IVIG and IVMP
Time from cardiac presentation to sudden death	5 days	4 days	5 days

Abbreviations: AChR, acetylcholine receptor; IVIG, intravenous immunoglobulin; IVMP, intravenous methylprednisolone.

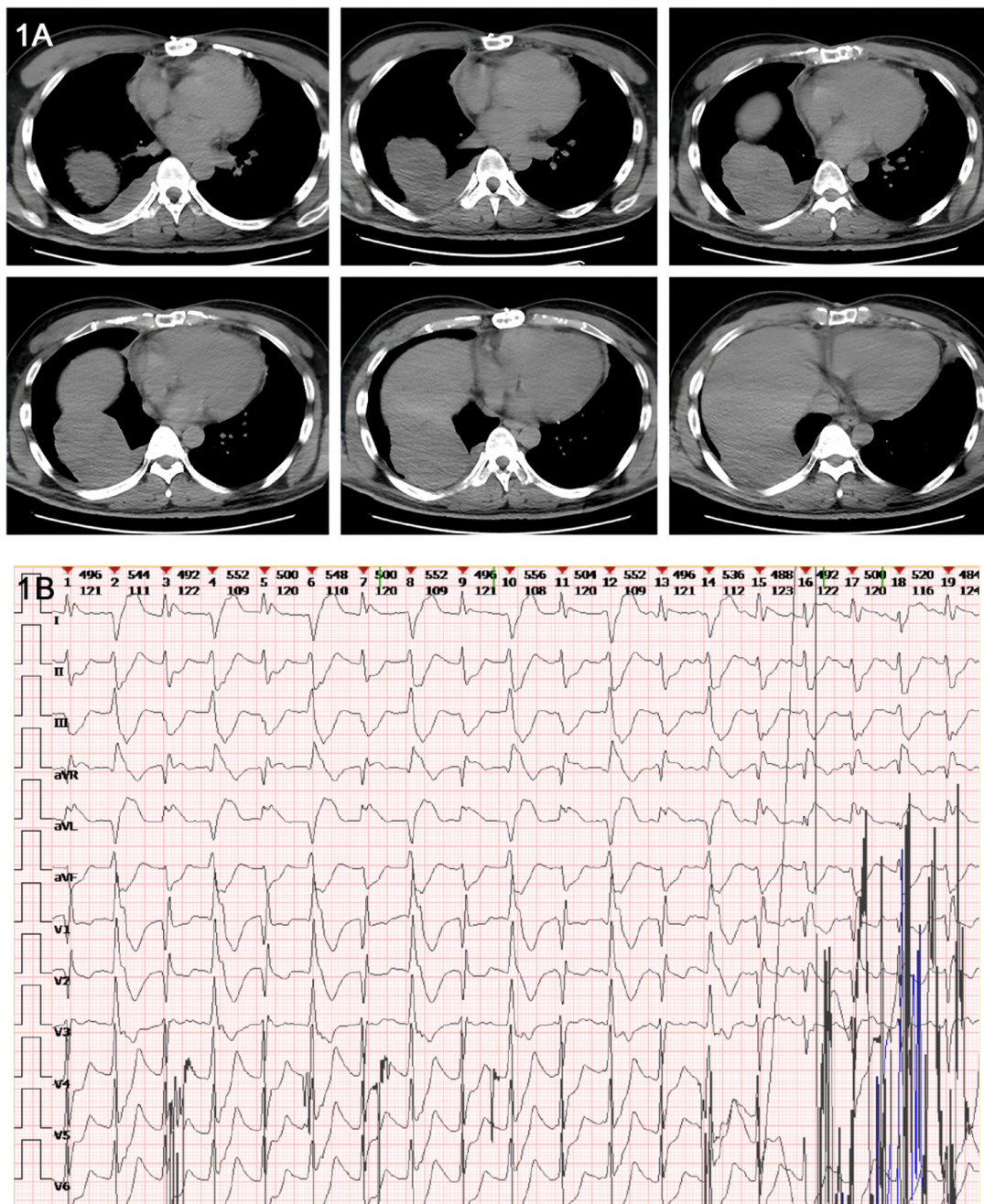


Figure 1. Representative radiological and electrocardiographic findings in Case 1. (A) Chest CT scan showing a large thoracic mass and pleural effusion; **(B)** ECG showing ventricular arrhythmia and ST-segment changes.

purification and life-support techniques, including intra-aortic balloon pump (IABP), extracorporeal membrane oxygenation (ECMO), assisted mechanical ventilation, and temporary pacemaker implantation if necessary, should be employed if needed (2). Despite timely initiation of immunotherapies, all our cases failed treatment. This failure may be due to an excessively high tumour burden, which involves the pericardium. More aggressive intervention, such as ECMO, should be initiated early for these cases.

In conclusion, FM is a rare and fatal complication associated with invasive thymoma. These cases highlight that patients with invasive or recurrent thymoma who

develop worsening myasthenic symptoms and new cardiac manifestations, such as dyspnea, palpitations, hypotension, arrhythmia, or elevated cardiac biomarkers, may rapidly progress to fatal fulminant myocarditis and therefore require immediate cardiac evaluation and aggressive early intervention.

Funding: This study is supported by financial grants from the National Natural Science Foundation of China (82571592 and 82471426), and the National Clinical Collaboration Project for Major and Difficult Diseases with Integrated Traditional Chinese and Western Medicine (No.ZDYN-2024-A-076).

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

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Received March 6, 2026; Revised June 28, 2026; Accepted July 7, 2026.

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Released online in J-STAGE as advance publication July 15, 2026.

Skeletal involvement in Joubert syndrome: From low bone mineral density to severe secondary osteoporosis

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SUMMARY: Joubert syndrome (JS) is a rare ciliopathy characterized by neurological and multisystem manifestations; however, skeletal involvement remains poorly recognized. We report two genetically confirmed pediatric patients with JS illustrating different degrees of bone impairment. The first patient, a 2.5-year-old boy with a TMEM67 mutation, presented with severe hypotonia, profound immobility, multiple fractures, and markedly reduced bone mineral density (DXA Z-score -5.875), fulfilling the criteria for secondary osteoporosis and requiring bisphosphonate therapy. The second patient, a 5-year-old girl with an OFD1 mosaic mutation, showed delayed motor development and reduced bone mineral density (DXA Z-score -2.2) without fractures and was managed conservatively. These cases demonstrate the broad spectrum of skeletal manifestations in JS, ranging from low bone mineral density to severe secondary osteoporosis. Reduced mobility and chronic hypotonia may contribute to impaired bone mineralization. Increased awareness and early bone health surveillance, including fracture assessment and densitometric evaluation, may facilitate timely diagnosis and improve management of bone complications in patients with Joubert syndrome.

Keywords: Joubert syndrome, secondary osteoporosis, low bone mineral density, hypotonia, pediatric osteoporosis, fractures

1. Introduction

Skeletal involvement in Joubert syndrome (JS) remains underrecognized, despite well-described neurological and multi-organ manifestations of this ciliopathy (1-3). Importantly, bone mineralization abnormalities in JS may range from asymptomatic low bone mineral density (BMD) to severe secondary osteoporosis with fractures. Here, we present two pediatric patients illustrating this phenotypic spectrum and highlight the clinical implications for bone health monitoring.

JS (OMIM 213300) is a rare neurodevelopmental disorder characterized by cerebellar and brainstem malformations, including the molar tooth sign on neuroimaging (2,4). Hypotonia and delayed motor development are common and may contribute to reduced mechanical loading, a known risk factor for impaired bone mineralization (5). However, skeletal complications remain insufficiently described in clinical practice (3,4).

In pediatric populations, it is important to distinguish between low BMD and osteoporosis. According to International Society for Clinical Densitometry (ISCD) criteria, pediatric osteoporosis requires both a clinically significant fracture history and low BMD (Z-score ≤ -2.0), whereas reduced BMD alone does not establish

the diagnosis (6,7).

We present two genetically confirmed patients with JS and contrasting skeletal phenotypes (Table 1).

The first patient (Figure 1A), a 2.5-year-old boy with a TMEM67 mutation, presented with severe hypotonia, inability to sit independently, and markedly reduced mobility. He developed multiple fractures during infancy, accompanied by progressive skeletal deformities (Figure 1B), including rib and humeral abnormalities. DXA revealed profoundly decreased BMD (Z-score -5.875). Based on the combination of fracture history and densitometric findings, secondary osteoporosis was diagnosed, and bisphosphonate therapy was initiated (7-9).

In contrast, the second patient, a 5-year-old girl with an OFD1 mosaic mutation, demonstrated milder neurological involvement and achieved independent ambulation, albeit delayed. Despite the presence of dysmorphic skeletal features and mild spinal abnormalities, she had no history of fractures. DXA revealed reduced BMD (Z-score -2.2), but she did not meet criteria for osteoporosis and was managed conservatively with monitoring (10,11).

These cases illustrate the broad phenotypic spectrum of skeletal involvement in JS, ranging from severe

Table 1. Comparative clinical characteristics of the two patients

Parameter	Patient 1	Patient 2
Age	2.5 years	5 years
Genotype	TMEM67	OFD1 (mosaic)
Neurological severity	Severe hypotonia	Moderate hypotonia
Mobility	Non-ambulatory	Independent walking (delayed)
Fracture history	Multiple fractures	None
Skeletal deformities	Severe (thorax, humeri, spine)	Mild (genu valgum, spine curvature)
DXA (Z-score)	-5.875	-2.2
Diagnosis	Secondary osteoporosis	Low BMD
Treatment	Bisphosphonates	Observation

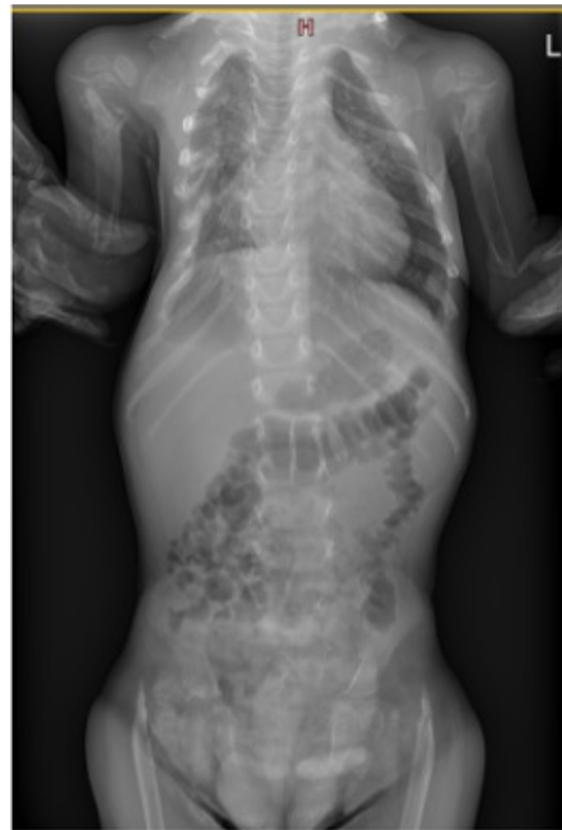
(A)**(B)**

Figure 1. Skeletal manifestations in Joubert syndrome. (A) Clinical appearance of patient 1 showing severe hypotonia and skeletal deformities; **(B)** Radiographic findings demonstrating reduced bone mineralization and skeletal abnormalities associated with severe secondary osteoporosis. *Note:* Written informed consent for publication of this report and any accompanying images was obtained from the patients' legal guardians.

osteoporosis with fractures to subclinical reductions in BMD without overt complications.

2. Clinical implications

These observations highlight several important clinical considerations. Skeletal involvement in Joubert syndrome may be underestimated because fractures are not present in all patients with impaired bone mineralization. Chronic hypotonia and reduced mobility are likely major contributors to skeletal fragility, as mechanical loading plays an essential role in bone mass acquisition during childhood. In addition, nutritional deficiencies, delayed

motor development, and limited physical activity may further compromise bone health. The marked difference between our two patients highlights the clinical heterogeneity of skeletal manifestations in JS.

First, skeletal complications in JS may remain clinically silent and therefore underdiagnosed, particularly in patients without a history of fractures (3). Second, severe hypotonia and limited mobility appear to contribute significantly to bone fragility and increase fracture risk (10,11). Third, reduced BMD may be present even in relatively mobile patients and may not necessarily fulfill the criteria for osteoporosis.

Given these findings, routine bone health

assessment should be considered in selected patients with JS, particularly those with significant hypotonia, delayed motor development, or reduced mobility. Such evaluation may include fracture history, biochemical assessment, and DXA measurement when feasible (7-10). Increased awareness among pediatricians and neurologists may allow earlier diagnosis and timely intervention aimed at preventing fractures and improving quality of life.

The potential role of ciliopathy-related mechanisms in bone metabolism remains unclear but may contribute to the observed variability in skeletal involvement (4). This hypothesis warrants further investigation.

3. Conclusion

Skeletal manifestations in Joubert syndrome represent a clinically relevant but underrecognized spectrum, ranging from asymptomatic low BMD to severe secondary osteoporosis with fractures. Increased awareness and appropriate monitoring may improve early detection and management of bone complications in this population.

Funding: None.

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

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Received February 26, 2026; Revised June 18, 2026; Accepted June 23, 2026.

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Released online in J-STAGE as advance publication July 31, 2026.



Intractable & Rare Diseases Research

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

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

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

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

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

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

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6. Manuscript Preparation

Manuscripts are suggested to be prepared in accordance with

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

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

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

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

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

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

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

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Manuscripts should be submitted to *Intractable & Rare Diseases Research* online at <https://www.irdrjournal.com>. Receipt of your manuscripts submitted online will be acknowledged by an e-mail from Editorial Office containing a reference number, which should be used

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(As of August 2026)

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