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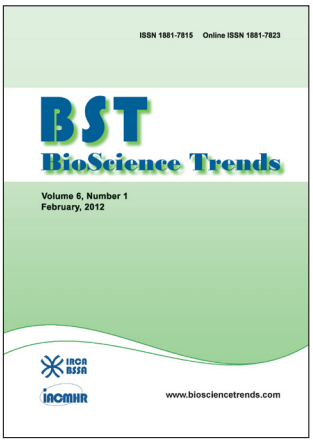

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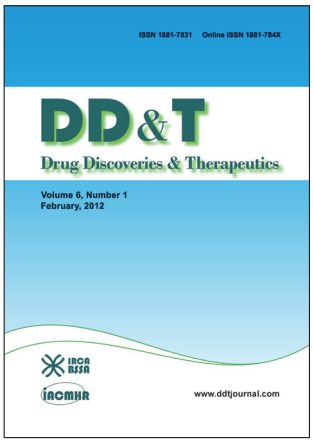

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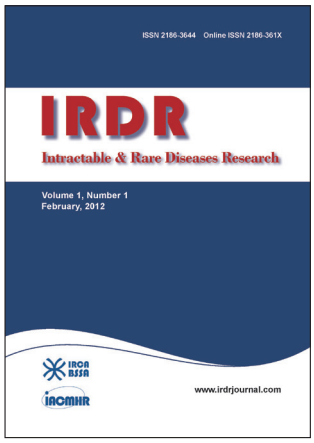

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Current diagnosis and management of rare pediatric diseases in China

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SUMMARY This review categorizes and summarizes the rare pediatric diseases that have been included in the *First List of Rare Diseases* that was jointly published by the National Health Commission and four other government departments in China in 2018. In total, 58 diseases that develop during childhood are included. These diseases involve nine organ systems, including the musculoskeletal, respiratory, immune, endocrine and metabolic, nervous, cardiovascular, hematological, urinary, and integumentary systems. Affected children often have multiorgan involvement with various presentations. Severe diseases can cause acute symptoms starting in the neonatal period that lead to increased morbidity and mortality without prompt management. Early diagnosis and treatment can significantly change the course of a disease and improve its prognosis. This work systemically reviews the status of rare pediatric diseases with a relatively high incidence in the *First List of Rare Diseases*.

Keywords rare pediatric diseases, diagnosis, management, China

1. Introduction

Most rare pediatric diseases are genetic disorders. Missed diagnosis and misdiagnosis can cause children to miss the best treatment window. In addition, both in China and the rest of the world, most children with rare diseases frequently suffer from an awkward situation due to the poorly understood pathogenesis, diagnosis, and treatment of these diseases, as well as difficulties in managing and obtaining medications for affected children. In recent years, there have been great breakthroughs in clinical research on these rare diseases. The development of numerous targeted therapeutics has provided promising results for patients. The introduction of a series of policies in China, such as the development and publication of standard treatment protocols for rare diseases, the accelerated evaluation of medications for rare diseases, the revision of drug regulations, the prioritization of rare disease treatments in the annual adjustment of the National Drug Reimbursement List, and the establishment of special funds, have significantly improved the management and accessibility of medication for rare diseases.

The *First List of Rare Diseases* was jointly published by the National Health Commission and other four government departments in China in May 2018. This list includes 121 diseases that require urgent clinical attention due to their serious disease burden and pressing social concern. All 121 of these rare diseases meet the following four conditions: *i*) low incidence and prevalence as indicated by domestic and international studies; *ii*) substantial impacts on patients and their families; *iii*) definitive diagnostic criteria; and *iv*) treatments or interventions that are affordable, or their potential management falls under national research projects even if there are no effective treatments or interventions yet (*1*). The release of the *First List of Rare Diseases* has filled a gap by defining rare diseases and ushering in a new era of using a catalogue to identify rare diseases in China. This may affect approximately 3 million patients in China (*2*). In February 2019, the Guidelines for the Diagnosis and Treatment of Rare Diseases (2019 edition) was published. It comprehensively describes the clinical management protocols for 121 rare diseases, which include disease definition, etiology, epidemiology, clinical presentations, auxiliary examinations, diagnosis and differential diagnosis, and treatment and management policies. The guidelines also propose a management process, which improves standardized diagnosis and treatment to facilitate early diagnosis and treatment of rare diseases in China (*3*). A total of 58 different rare pediatric diseases are included in the list,

and those diseases and their incidence are shown in Table 1.

2. Rare pediatric diseases of different systems

2.1. Rare pediatric diseases of the musculoskeletal system

The *First List of Rare Diseases* includes nine rare pediatric diseases that primarily involve the musculoskeletal system. They are congenital myasthenic syndrome, congenital myotonia syndrome, congenital scoliosis, hypophosphatemic rickets, osteogenesis imperfecta, Prader-Willi syndrome, progressive muscular dystrophy, severe myoclonic epilepsy in infancy, and Russell-Silver syndrome. Currently, there is no special treatment targeting the etiology of most of these rare musculoskeletal diseases. Their management usually requires multidisciplinary collaboration to treat the disease using different approaches. Management mainly includes symptomatic relief with medication and surgery, with a fundamental goal of slowing the disease progression and improving quality of life. In China, further epidemiological studies are required to better understand the prevalence of rare pediatric musculoskeletal diseases. The following is a review of several typical diseases of the musculoskeletal system, including HR and progressive muscular dystrophy, that are highly disabling and deforming.

2.1.1. Hypophosphatemic rickets

Hypophosphatemic rickets (HR) is a group of skeletal mineralization disorders characterized by hypophosphatemia due to various genetic or acquired causes. HR carries a high risk of disability and deformity. Its etiology includes gene mutations, such as the PHEX mutation, or other acquired causes that can increase the level of the phosphorus-regulating fibroblast growth factor 23 (FGF23) and result in a decreased renal phosphorus threshold and reduced intestinal absorption of calcium and phosphorus. These changes can ultimately lead to impaired bone mineralization. The disease begins in childhood and is known as rickets. Affected children often present with a square skull, a pigeon chest, beaded ribs, and bowed limbs (O- or X-shaped legs) when they start to bear weight at nearly age 1. Their symptoms can also include growth delay, multiple fractures, bone pain, and abnormal tooth development. Diagnosis of HR is based on clinical interviews, including questions about the use of anti-hepatitis B virus drugs (adefovir and tenofovir) and aminoglycosides, as well as a physical examination. Laboratory tests include blood phosphorus, calcium, parathyroid hormone, 25-hydroxyvitamin D, alkaline phosphatase, and the renal phosphorus threshold. Imaging studies can reveal skeletal deformities with a generalized reduction in bone density. Genetic tests can also be performed to identify relevant mutations. In addition, family members of an affected child should be screened to rule out the possibility of HR in order to provide prompt diagnosis and treatment (*4*).

In children with acquired HR, the cause needs to be promptly identified and any potential drug or toxic exposure needs to be halted. If the cause of HR cannot be eliminated or it is hereditary HR, standardized treatments in children can promote growth and gradually correct leg deformities as well as improve tooth mineralization. Affected children can show signs of rickets during infancy. Standardized treatment initiated in infancy can achieve satisfactory outcomes (*5*). Previously, pharmaceutical therapies often included the administration of neutral phosphate and active vitamin D. However, their efficacy was inadequate due to poor compliance as a result of prolonged use of medication and adverse reactions. The role of growth hormone in the treatment of rickets is still controversial. Therefore, a new treatment approach with better efficacy needs to be formulated. Burosumab is a recombinant fully human monoclonal antibody targeting the FGF23 antigen. It can bind to and inhibit the activity of FGF23 to increase serum phosphorus. In January 2021, the China National Medical Products Administration (NMPA) conditionally approved burosumab, under the name Crysvita, for use in children with X-linked hypophosphatemic rickets (XLH). This is the world's first approved recombinant fully human-derived monoclonal IgG1 antibody targeting FGF23. It fills the gap in the clinical treatment of XLH in China. The successful approval of this therapy has brought optimism and new hope to children with this rare disease.

In children, premature surgery should generally be avoided as their epiphyseal plates are not closed (*4*). However, when affected children have severe skeletal deformities, obvious knee varus or valgus deformities, abnormal height or body appearance, or pathological fractures that affect daily life, surgery can be considered to improve quality of life. Currently, the best treatment outcome is obtained with surgery guided by a three-dimensional reconstruction (*6*).

2.1.2. Progressive muscular dystrophy

Progressive muscular dystrophy (PMD) is a group of heterogeneous genetic defective disorders with increasing skeletal muscle weakness and atrophy as their main clinical presentation. Its incidence is estimated to be approximately 1 in 3,583 people, with approximately 70,000 patients in China at present. Currently, dozens of genes causing this disease have been identified. There are nine major types of PMD, with varying age at onset, rate of progression, range of involvement, and disease severity. Here, the diagnosis and treatment of a typical form, Duchenne/Becker muscular dystrophy, will be reviewed. Caused by the *DMD* gene, Duchenne muscular

Table 1. Rare pediatric diseases in the First List of Rare Diseases

System	Disease	Inheritance	Phenotype, MIM number	Pathogenic gene	Gene/Locus, MIM number	Incidence[#]
Musculoskeletal system	[*]Hypophosphatemic rickets, HR	XLR, AD, AR, XLD	193100, 241520, 307800, 300554	FGF23, DMP1, PHEX, CLCN5	605380, 600980, 300550, 300008	3.9/100,000
	[*]Progressive muscular dystrophy, PMD	XLR, AD, AR	310200	DMD	300377	1/3,953
	Congenital myasthenic syndrome, CMS	AR, AD	601462, 603034, 254210, 615120, 254300, 616326, 610542	CHRNA1, COLQ, CHAT, AGRN, DOK7, RAPSN, GFPT1	100690, 603033, 118490, 103320, 610285, 601592, 138292	9.2/1,000,000
	Congenital myotonia	AD	160800	CLCN1	118425	1/100,000
	Congenital scoliosis, CS	AR	618578	PAX7	167410	0.5-1/1,000
	Osteogenesis imperfecta, OI	AD	166200, 166210, 259420, 166220, 610967, 610682, 610915, 259440	COL1A1, COL1A2, IFITM5, CRTAP, P3H1, PPIB	120150, 120160, 614757, 605497, 610339, 123841	1/20,000-1/15,000
	Prader-willi syndrome, PWS	AD	176270	NDN, SNRPN	602117, 182279	1/30,000-1/10,000
	Dravet syndrome, DS	AD	607208	SCN1A	182389	1/40,000-1/20,000
	Silver-Russell syndrome, SRS	AD	180860, 618905, 616489, 618907, 618908	ICR1, IGF2, PLAG1, HMGA2	616186, 147470, 603026, 600698	1/100,000-1/30,000
Respiratory system	[*]Cystic fibrosis, CF	AR	219700	CFTR, FCGR2A, TGFB1	602421, 146790, 190180	1/25,000-1/1,800
Immune system	Primary combined immunodeficiency, CID	AR	/	IL2RG	308380	1/100,000-1/75,000
	Severe congenital neutropenia, SCN	AD, AR, XLR	202700, 613107, 610738, 612541, 615285, 616022, 617014, 618752, 300299	ELANE, GFI1, HAX1, G6PC3, VPS45, JAGN1, CSF3R, SRP54, WAS	130130, 600871, 605998, 611045, 610035, 616012, 138971, 604857, 300392	1/250,000
	X-linked agammaglobulinemia, XLA	XLR	300755	BTK	300300	1/379,000
	[*]Wiskott-Aldrich syndrome, WAS	XLR, AR	301000, 614493	WAS, WIPF1	300392, 602357	1/100,000
Endocrine and metabolic system	β-ketothiolase deficiency, BKD	AR	203750	ACAT1	607809	1/960,600
	Biotinidase deficiency, BTDD	AR	253260	BTD	609019	1/60,000
	Congenital hyperinsulinemic hypoglycemia, CHI	AD, AR	256450, 601820, 602485, 609975, 609968, 606762, 610021	ABCC8, KCNJ11, GCK, HADHSC, INSR, GLUD1, SLC16A1	600509, 600937, 138079, 601609, 147670, 138130, 600682	l/50,000-1/30,000
	Adrenal hypoplasia congenita, AHC	XLR	300200	NR0B1	300473	1/12,500
	[*]Gaucher disease, GD	AR	230800, 230900, 231000, 231005, 608013	GBA	606463	1/80,844
	Glycogen storage disease, GSD (I, II)	AR	232200, 232240, 232300	G6PC, SLC37A4, GAA	613742, 602671, 606800	1/100,000-1/20,000, 1/100,000-1/14,000
	Hepatolenticular degeneration	AR	277900	ATP7B	606882	1/30,000
	Hereditary fructose intolerance, HFI	AR	229600	ALDOB	612724	1/20,000
	Hereditary hypomagnesemia	AR, AD	602014, 154020, 248250, 611718, 248190, 613882	TRPM6, FXYD2, CLDN16, EGF, CLDN19, CNNM2	607009, 601814, 603959, 131530, 610036, 607803	1-10/40,000
	Holocarboxylase synthetase deficiency, HLCS	AR	253270	HLCS	609018	/
	Homocysteinemia	XLR	309541	CBS, HCFC1	300019	1/300,000-1/200,000
	Hyperphenylalaninemia, HPA	AR	261640, 233910, 261630, 264070, 261600	PTS, GCH1, QDPR, PCBD1, PAH	612719, 600225, 612676, 126090, 612349	1/10,397
	Inborn errors of bile acid synthesis, IEBAS	AR	607765, 235555, 613812, 214950, 616278, 617308	HSD3B7, AKR1D1, CYP7B1, AMACR, ABCD3, ACOX2	607764, 604741, 603711, 604489, 170995, 601641	1/70,000
	Laron syndrome	AR	262500	GHR	600946	/
	Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency, LCHADD	AR	609016	HADHA	600890	1/250,000
	Lysinuric protein intolerance, LPI	AR	222700	SLC7A7	603593	/
	Lysosomal acid lipase deficiency, LALD	AR	278000	LIPA	613497	1/300,000-1/40,000
	Maple syrup urine disease, MSUD	AR	248600	BCKDHA, BCKDHB, DBT	608348, 248611, 248610	1/139,000
	Medium chain acyl-CoA dehydrogenase deficiency, MCADD	AR	201450	ACADM	607008	0.66/100,000
	Methylmalonic acidemia, MMA	AR	251000, 277400	MMUT, MMACHC, PRDX1	609058, 609831, 176763	1/28,000

[#]Incidence figures are from the Guidelines for Diagnosis and Treatment of Rare Diseases (2019 Edition); [*]Diseases that were mentioned in this review.

Table 1. Rare pediatric diseases in the First List of Rare Diseases (continued)

System	Disease	Inheritance	Phenotype, MIM number	Pathogenic gene	Gene/Locus, MIM number	Incidence[#]
	Mucopolysaccharidosis, MPS	AR, XLR	607016, 309900, 252900, 252920, 252930, 252940, 253000, 253010, 253200, 601492	IDUA, IDS, SGSH, NAGLU, HGSNAT, GNS, GALNS, GLB1, ARSB, HYAL1	252800, 300823, 605270, 609701, 610453, 607664, 612222, 611458, 611542, 607071	1/100,000
	Multiple acyl-CoA dehydrogenase deficiency, ADD	AR	231680	ETFDH, ETFA, ETFB	231675, 608053, 130410	/
	N-acetylglutamate synthase deficiency, NAGSD	AR	237310	NAGS	608300	
	Neonatal diabetes mellitus, NDM	AD, AR	601410, 610374, 610582, 606176, 618856, 618857	ABCC8, KCNJ11, GCK	600509, 600937, 138079	1/500,000-1/400,000
	Ornithine transcarbamylase deficiency, OTCD	XL	311250	OTC	300461	7.1/100,000
	*Phenylketonuria, PKU	AR	261600	PAH	612349	1/11,800
	Primary carnitine deficiency, PCD	AR	212140	SLC22A5	603377	0.8-2.5 /100,000
	Progressive familial intrahepatic cholestasis, PFIC	AR	211600, 601847, 602347, 615878, 617049, 619484	ATP8B1, ABCB11, ABCB4, TJP2, NR1H4, SLC51A	602397, 603201, 171060, 607709, 603826, 612084	1/100,000-1/50,000
	Tetrahydrobiopterin deficiency, BH4D	AR	261640, 233910, 261630, 264070	PTS, GCH1, QDPR, PCBD1	612719, 600225, 612676, 126090	/
	Tyrosinemia	AR	276700, 276600, 276710	FAH, TAT, HPD	613871, 613018, 609695	1/120,000-1/100,000
	Very long chain acyl-CoA dehydrogenase deficiency, VLCADD	AR	201475	ACADVL	609575	1/100,000-1/30,000
	X-linked adrenoleukodystrophy, ALD	XLR	300100	ABCD1	300371	1/21,000-1/15,500
Nervous system	*Spinal muscular atrophy, SMA	AR, XLR	253550, 253400, 271150, 604320, 313200, 301830, 158600	SMN1, SMN2, IGHMBP2, AR, UBA1, DYNC1H1	600354, 601627, 600502, 313700, 314370, 600112	1/10,000-1/6,000
Cardiovascular system	*Cardiac ion channelopathies, CICP	AD	192500, 609621, 601144, 604772	KCNQ1, SCN5A	607542, 600163	1/2,500
	*Noonan syndrome	AD, AR	163950, 605275, 609942, 610733, 613224, 615355, 618499	PTPN11, LZTR1, KRAS, SOS1, NRAS, RIT1, MRAS	176876, 600574, 190070, 182530, 164790, 609591, 608435	1/2,500-1/1,000
Hematologic system	*Diamond-Blackfan anemia, DBA	AD	105650, 612527, 612561, 612562, 612563, 613308, 617408, 617409, 618312	RPS19, RPS17, RPL5, RPL11, RPS7, RPS10, RPL27, RPS27, RPL35	603474, 180472, 603634, 604175, 603658, 603632, 607526, 603702, 618315	1.5-5.0/100,000
	Severe congenital neutropenia, SCN	AD, AR, XLR	202700, 613107, 610738, 612541, 615285, 616022, 617014, 618752, 300299	ELANE, GFI1, HAX1, G6PC3, VPS45, JAGN1, CSF3R, SRP54, WAS	130130, 600871, 605998, 611045, 610035, 616012, 138971, 604857, 300392	1/250,000
	Fanconi anemia, FA	AR, XLR	227650, 300514, 227645, 605724, 227646, 603467, 609054	FANCA, FANCB, FANCC, BRCA2, FANCD2, FANCF, BRIP1	607139, 300515, 613899, 600185, 613984, 613897, 605882	1/160,000
	Isovaleric acidemia, IVA	AR	243500	IVD	607036	1/160,000
	*Propionic acidemia, PA	AR	606054	PCCA, PCCB	232000, 232050	0.6-0.7/100,000
Urinary system	*Alport syndrome	XLD, AR, AD	301050, 203780, 104200	COL4A5, COL4A3, COL4A4	303630, 120070, 120131	/
Integumentary system	*Hereditary epidermolysis bullosa	AR, AD	226700, 131750, 226600	LAMC2, LAMB3, LAMA3, COL7A1, MMP1	150292, 150310, 600805, 120120, 120353	/
	*Langerhans cell histiocytosis, LCH	Sporadic nonhereditary disorder	604856	BRAF V600E, MAP2K1	164757, 176872	0.5-5.4/100,000

[#]Incidence figures are from the Guidelines for Diagnosis and Treatment of Rare Diseases (2019 Edition); *Diseases that were mentioned in this review.

dystrophy (DMD) begins in childhood. China is one of the countries with the largest number of patients with this disorder, which has an incidence of about 1/3,853 (*7,8*).

The natural course of DMD usually starts with mildly delayed motor development in early childhood and progresses into motor activity decline, abnormal gait, Achilles tendon contracture, and lumbar lordosis during childhood (5 to 6 years old). Affected children can lose the ability to walk at around age 10 and can die due to cardiopulmonary failure at around age 20. Diagnosis of DMS is mainly based on serology, electromyography, muscle magnetic resonance imaging, muscle biopsy, and genetic tests. DMD can be clinically diagnosed when a child displayed obvious bilateral gastrocnemius pseudohypertrophy, combined with serology results indicating a significantly elevated blood muscle enzyme profile, and myogenic damage according to electromyography.

Although there is no cure for DMD, there have been breakthroughs in research and clinical trials on its treatment in recent years. Specifically, treatment with corticosteroids (prednisone or furazolidone) has been found to delay disease progression and extend patients' lifespans (*9*). In affected children who are older than 3, standard oral steroid treatment should be initiated before their motor functions start to decline (*7*). The development of DMD is a process of multiorgan system involvement. A series of pathological changes caused by a protein deficiency means that DMD requires a joint, multidisciplinary effort for its diagnosis and treatment. Management should target osteoporosis, muscle atrophy, and declining cardiopulmonary function. Pharmaceutical treatments, including bisphosphonates and medications for heart failure, should be given as adjuvant medications. Appropriate specialties should be consulted for any nutritional, digestive, or psychological issues. In addition, with the development of gene editing technology, gene therapy and stem cell therapy can play an important role in the relief of symptoms by increasing the expression of dystrophin. These therapies have yielded promising results, although some potential adverse effects are still under investigation (*8*).

Orthopedic surgery can be performed in children with severe skeletal deformities to maximize motor and respiratory functions. At each stage of the disease, affected children can benefit from rehabilitation, including lifelong routine physiotherapy evaluation, dietary counseling for patients on corticosteroids, pulmonary care (manual or mechanical cough assistance or noninvasive or invasive ventilation), and cardiac recovery (an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker). Standardized pharmacological treatment and rehabilitation, along with regular follow-ups to assess and treat relevant systemic symptoms, can significantly delay disease progression, prolong the patient's lifespan, and improve the patient's quality of life.

2.2. Rare pediatric diseases of the respiratory system

Cystic fibrosis (CF) is the only rare pediatric pulmonary disease included in the *First List of Rare Diseases*. CF is a multi-system disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. It is one of the most common genetic diseases in the Caucasian population. Due to the small number of CF patients reported in China, we are still in the preliminary stage of understanding this disease. More epidemiological studies are required to investigate this disease since the number of patients diagnosed with CF is rapidly increasing in China.

CFTR mutations cause abnormal production, structures, and functions of proteins, which result in chloride channel dysfunction. Decreased secretion of chloride ions and water can lead to mucus accumulation, with subsequent blockage of the lumen of the exocrine glands in the respiratory tract and pancreas. This can further cause bacterial growth and produce recurrent infections and inflammatory reactions (7). The skin can taste salty due to increased chloride in sweat. Patients with CF often have chronic bacterial infections and pathogen colonization of the respiratory tract. Once an infection occurs, it can rapidly result in diffuse bronchiectasis, which often begins in early childhood. More than 90% of pediatric patients with CF have recurrent chronic upper and lower respiratory tract infections (*10*). Gastrointestinal disorders also start in the neonatal period, with excessive viscous secretions that can cause meconium intestinal obstruction and peritonitis. Pediatric patients with CF may have salt crystals on the skin. More than 95% of male patients are infertile, which is most often a result of the absence of the vas deferens due to the incomplete development of the Wolffian tube. In general, the diagnosis of CF requires six different types of tests and studies, including laboratory tests, pulmonary function tests, imaging studies, respiratory pathogen tests, reproductive system tests, and genetic tests. Determination of the chloride ion concentration in sweat is considered to be the gold standard diagnostic test for CF (*11*). The diagnosis can usually be confirmed when typical presentations of CF are present, such as dyspnea and other pulmonary diseases, with at least one of the following abnormal results of a cystic fibrosis transmembrane conductance regulator (CFTR) test, *i*) sweat chloride ≥ 60 mmol/L or sweat chloride ≥ 40 mmol/L according to a single test; *ii*) two CFTR pathogenic mutations in the allele; and *iii*) an abnormal nasal potential difference (*12*). In addition, the presence of two CFTR pathogenic mutations on the allele or an abnormal nasal potential difference can also confirm the diagnosis of CF (*7*).

The life expectancy of children with CF does not usually exceed 10 years if appropriate interventional therapy is not provided. In clinical settings, CFTR modulators can be used as targeted therapy for CF. With

advances in research on the *CFTR* gene, clinical therapy has attempted to target *CFTR* gene defects. Ivacaftor is a typical CFTR modulator that was approved by the US Food and Drug Administration (FDA) in 2012 as the first medication to treat the underlying cause of CF (*13*). It can improve CFTR-mediated chloride secretion by restoring the function of the mutated CFTR channel and improving the function of the defective CFTR protein. In October 2019, the FDA approved Trikafta, a triple combination therapy that is effective for most CF patients. It can increase the lung capacity by 10-15% and delay the development of CF complications by correcting the most common mutations in CF. This treatment was a milestone achievement in the genetic therapy for CF because it transformed CF from a progressive disease to a chronic manageable disease (*14*). In addition, multidisciplinary comprehensive treatment is an essential approach to managing CF, which includes medications such as inhaled DNase I (α-streptokinase) to improve the clearance of airway secretions, antibiotics for at least 10-14 days to treat pulmonary infections, bronchodilators such as β-agonists and glucocorticoids to relieve asthma symptoms, and 14- or 15-membered ring macrolides to promote respiratory function and reduce the incidence of acute exacerbations. Patients with end-stage CF should receive double-lung transplantation (*15*).

At the end of the 20th century, many clinics started to use preimplantation genetic screening to facilitate the management of CF. In recent years, preimplantation genetic screening has been used to prevent the transmission of rare hereditary diseases.

2.3. Rare pediatric diseases of the immune system

Immunodeficiency diseases refer to a group of clinical syndromes in which the immune response is absent, reduced, or imbalanced due to defects in immune cells or immune molecules, which can result in reduced immune capacity against infections or immune dysfunction in the body. The *First List of Rare Diseases* includes four immune system diseases that develop during childhood: primary combined immunodeficiency disease, severe congenital neutropenia, X-linked agammaglobulinemia, and eczema-thrombocytopenia-immunodeficiency syndrome. Infection is the most common presentation and consequence of immunodeficiency diseases. It is usually serious or even fatal. Prompt prevention and aggressive control of infection are important treatment principles for immunodeficiency diseases. In addition, treatment approaches depend on whether the patient has single-organ, single-system, or multiple-system involvement. Alternative or immune reconstitution therapies can be given based on the type of immunodeficiency. General treatment can be guided by endocrine test results. Intravenous immunoglobulin infusion can be given as replacement therapy.

Eczema-thrombocytopenia-immunodeficiency syndrome (Wiskott-Aldrich syndrome, or WAS) is an X-linked recessive disease characterized by a triad of bleeding diathesis, eczema, and recurrent infections, as well as a high risk of autoimmune diseases and lymphoma. The *WAS* gene encodes the Wiskott-Aldrich Syndrome protein (WASp), which is an intracellular signaling molecule and skeletal protein specifically expressed in hematopoietic cells. It plays an important role in actin polymerization and cytoskeletal remodeling (*16,17*). Mutations in *WAS* can lead to self-activation of WASp, with subsequent morphological and functional platelet and lymphocyte abnormalities that cause different types of disease. Depending on the mutation, WAS is classified into four types in clinical settings, with the most common being the classic type. WAS almost exclusively affects males. Without a hematopoietic stem cell transplantation, the average lifespan of patients not expressing WASp is only about 15 years (*7*). Clinical diagnosis is based on a patient's medical history, physical examination, and laboratory tests (routine blood test, humoral immunity, cellular immunity, WASp expression, and genetic analysis). WASp flow cytometry and *WAS* genetic analysis are effective as confirmatory diagnostic tests (16). The classic type of WAS should be ruled out in boys with thrombocytopenia, isolated or concomitant eczema, recurrent respiratory infections, autoimmunity, and/or cancer. Further examination of a deficiency in or a reduced level of WASp expression and *WAS* gene mutations can confirm the diagnosis (*17*).

Mutations in the *WAS* gene and the degree of WASp deficiency are closely related to the clinical presentations and severity of WAS and determine the treatment options (*18*). Most affected children start to show signs of bleeding diathesis and immunodeficiency in the neonatal period, and those signs worsen as they grow. Other clinical complications, such as eczema, autoimmunity, and malignancies, can occur with different presentations and different levels of severity. Only about one quarter of patients have the classic triad of clinical presentations simultaneously. Children with typical WAS who do not receive radical therapy will eventually die from complications such as infections, bleeding, or malignancies. Thanks to breakthroughs over the past 20 years, hematopoietic stem cell transplantation therapy is currently the only curative treatment for WAS. The optimal age for transplantation is 1-2 years, with an overall survival rate greater than 90% (*16*). Some genetic therapies for WAS are currently undergoing clinical trials and have been found to cure WAS by genetic correction of autologous stem cells through viral vectors. Once its safety is improved, genetic therapy is expected to be the treatment of choice for pediatric patients with WAS who lack appropriate donors. In addition, pediatric patients with WAS also require aggressive and comprehensive treatments, such as supportive therapy with inactivated vaccines, improved nutritional, and targeted antibiotics. Affected children can receive an intravenous

immunoglobulin infusion to extend their lifespan as they wait for a hematopoietic stem cell transplantation. Immunosuppressive therapy should also be administered if there are signs of autoimmune complications.

2.4. Rare pediatric diseases of the endocrine and metabolic system

Hereditary metabolic diseases are caused by genetic mutations that result in disorders of enzyme or protein synthesis, defective receptors, or dysfunctional cell membranes. These can lead to the accumulation of substrates and their derivatives in the body and the development of metabolic disorders. There are about 169 rare endocrine and metabolic diseases involving various endocrine organs. The *First List of Rare Diseases* includes 32 endocrine and metabolic diseases with an age of onset in infancy or early childhood: β-ketothiolase deficiency (incidence of 1/960,600), biotinidase deficiency, primary carnitine deficiency (incidence of 2.4/100,000 in Shanghai and 3.1/100,000 in Zhejiang, China), congenital adrenal hypoplasia, Gaucher disease, glycogen accumulation disease (type I and II), hereditary fructose intolerance, hereditary hypomagnesemia, holocarboxylase synthetase deficiency, hyperhomocysteinemia (incidence of 27.5%), hyperphenylalaninemia (incidence of 1:10,397), congenital bile acid synthesis defect, Laron syndrome, long chain-3-hydroxyacyl-CoA dehydrogenase deficiency (incidence 1:250,000), lysinuric protein intolerance, lysosomal acid lipase deficiency, maple syrup urine disease (incidence of 1/139,000), medium-chain acyl-CoA dehydrogenase deficiency (incidence of 0.66/100,000), mucopolysaccharidosis, multiple acyl-CoA dehydrogenase deficiency, N-acetylglutamate synthase deficiency, neonatal diabetes mellitus, ornithine transcarbamylase deficiency, phenylketonuria (incidence of 1/11,800), progressive familial intrahepatic cholestasis, tetrahydrobiopterin deficiency, tyrosinemia, very long-chain acyl-CoA dehydrogenase deficiency, X-linked adrenoleukodystrophy, methylmalonic acidemia, congenital hyperinsulinemic hypoglycemia, and hepatolenticular degeneration. Most of these diseases still require more epidemiological studies in China (*7*). Management of these endocrine and metabolic diseases mainly includes symptomatic support, pharmacological treatments, and replacement therapies. The treatment principles are to correct metabolic disorders, alleviate symptoms, reduce or delay the occurrence of serious complications, and maintain normal or close-to-normal quality of life in patients.

2.4.1. Gaucher disease

Gaucher disease (GD) is a relatively common autosomal recessive lysosomal storage disease. GD is caused by a defective gene that results in a deficiency of β-glucocerebrosidase (GBA). Affected children are unable to hydrolyze glucocerebrosides, which leads to their accumulation in the mononuclear macrophages in the liver, spleen, bone, and the central nervous system, where they form typical storage cells, or Gaucher cells. These can ultimately cause lesions in the tissues and organs (*19*). Depending on the level of neurological involvement, GD is mainly classified into type I (non-neuropathic, adult GD), type II (acute neuropathic, infantile GD), and type III (chronic or subacute neuropathic, juvenile GD). Type I is the most common type of GD and can occur in patients in all age groups, with approximately two-thirds of patients developing the disease in childhood (*20*). Currently, the best estimate of the incidence of GD in China is from a study in Shanghai, China. That study tested the glucose brain glyoxalase activity in dried blood spots from neonates and found an incidence of 1:80,844 (*7*). A more comprehensive epidemiological survey is still required in mainland China.

The clinical characteristics of GD are progressive and involve multiple organs. All three types of disease involve hepatosplenomegaly, especially splenomegaly, as well as thrombocytopenia and anemia. In addition, pediatric patients with type I GD often have bone conditions, such as osteoporosis and long bone metaphyseal deformities, that can cause delayed growth or even disability. Pediatric patients with type II GD often have acute neurological involvement, such as medullary paralysis and seizures, that starts after birth or in infancy. Type II GD has a high mortality rate. Affected children often die before the age of 2 to 4 years. Pediatric patients with type III GD often develop the disease in childhood. Neurological involvement progresses slowly, and affected children can have a relatively long life expectancy (*7*). The diagnosis of GD requires a comprehensive examination. The main clinical sign is splenomegaly, which can be up to five times a normal-sized spleen and which is found in 87% of affected children. Children with significant splenomegaly should undergo a bone marrow examination to identify characteristic Gaucher cells *via* an enzyme activity assay. Measuring glucocerebrosidase enzyme activity is the most effective method to confirm the diagnosis of GD (*20*).

In the past, the treatment of GD was mainly symptomatic support. Since alglucerase was first used in clinical settings in 1989, enzyme replacement therapy (ERT) has gradually become the treatment of choice for GD. ERT is currently the most effective and widely used approach to managing GD. ERT can specifically reverse an enzyme deficiency, reduce glucose ceruloplasmin accumulation, correct anemia and thrombocytopenia, decrease the size of the liver and spleen, and relieve bone pain, which can significantly improve quality of life for patients (*21*). The only glucocerebrosidase enzyme currently approved in China

is imiglucerase, as it has adequate evidence-based support and obvious efficacy in the treatment of bone involvement in GD (*20*). In addition, therapy with a glucosylceramide synthase inhibitor, such as miglustat, is used in other countries, but is not a treatment option in children with GD and has not been approved in China. Other therapies include hematopoietic stem cell transplantation, which carries the risk of implantation failure and graft-related complications, and gene therapy, which requires more study before extensive clinical use.

2.4.2. Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive disorder caused by the lack of or insufficient activity of phenylalanine hydroxylase (PAH) or tetrahydrobiopterin (BH4). These conditions can result in abnormal metabolism of phenylalanine (Phe), which cannot be converted to tyrosine (Tyr). The accumulated Phe can cause a high concentration of phenylpyruvate, which can cause a series of clinical symptoms. PKU is the main type of hyperphenylalaninemia (HPA). PKU is classified differently based on different measurements (*22*). The average incidence of PKU is 1/11,800 in China (*23*).

An earlier diagnosis of PKU can result in a better treatment outcome. PKU has become one of the mandatory screening tests for newborns. It is performed by checking the level of Phe in the blood. Further analysis of a PAH deficiency or BH4 deficiency is required in newborns with confirmed PKU. Urine pterin analysis is currently an important method to diagnose a BH4 deficiency in China. In addition, the BH4 loading test, BH4-responsive PKU/HPA determination, and the blood dihydrobiopterin reductase (DHP) activity assay can also be used as auxiliary methods of diagnosing a BH4 deficiency (*24*). Affected neonates often have no clinical symptoms. The typical symptoms gradually appear 3-4 months after birth and become obvious at age 1. Clinical signs, such as yellow hair, white skin, mousy smelling urine, and delayed psychomotor development, can provide clues to diagnose PKU. Vomits and eczema are often seen in infancy (*7*).

Currently, Phe-restricted diet therapy is the main treatment approach for PKU. The principle of management is to provide the appropriate amount of Phe, with frequent adjustments based on the signs and symptoms of the disease, to facilitate a child's normal development (*25*). Although this treatment approach with a low-Phe diet can reduce the Phe concentration, it cannot stop the continued progression of neurological symptoms, which requires supplementation of BH4 and other medications. BH4 is effective in reducing the Phe concentration in patients with PKU. Sapropterin, a synthetic BH4, has a long-lasting effect in reducing the Phe concentration by 62% in patients with PKU. Pegvaliase-pqpz is the first recombinant phenylalanine ammonia enzyme (PAL) (developed by BioMarin Pharmaceuticals, USA). It can replace phenylalanine hydroxylase to convert Phe into ammonia and trans-cinnamic acid, which are eventually metabolized in the liver and excreted in the urine. Treatments with pegvaliase-pqpz can decrease the level of Phe and alleviate clinical symptoms in patients with PKU (*26*).

2.5. Rare pediatric diseases of the nervous system

Nervous system diseases are disorders of the nervous system caused by various pathological processes, such as infections, metabolic disorders, tumors, and congenital developmental abnormalities. In clinical settings, affected patients can have impaired motor, sensory, and higher nervous activity and autonomic dysfunction. Due to the highly specialized functions of the nervous system and its limited capacity for repair, the diagnosis and treatment of nervous system diseases are often difficult. Nervous system diseases account for more than one quarter of all diseases in the *First List of Rare Diseases*. Spinal muscular atrophy (SMA) is one such rare disease starting in childhood.

SMA is an autosomal recessive disorder and is the most common fatal neurogenetic disorder in infancy and childhood. SMA is caused by survival motor neuron gene 1 (*SMN1*), which encodes the survival motor neuron (SMN) protein. Mutations in *SMN1* can cause functional defects in the SMN protein (*27*). An increasing number of studies have indicated that SMA is a disorder that involves multiple organs and systems, including the cardiovascular system. About 1 in 42 people carry the *SMN1* mutation. SMA is the main genetic cause of infant mortality (*28*). Epidemiological data on SMA are required in China.

The clinical presentations of SMA in children vary significantly and mainly include muscle weakness and atrophy due to the degeneration and loss of motor neurons in the anterior horn of the spinal cord. SMA can be classified into four types based on the age of onset (prenatal or intrauterine onset), acquired motor function, and rate of disease progression. Children with type I SMA develop symptoms within 6 months of age (1 month after birth on average), with the main presentations being reduced fetal movements and severe hypotonia. Children with type II SMA can develop the disease from childhood to adolescence but usually show symptoms within the first 18 months of life, with the main presentations being progressively worsening generalized muscle weakness and hypotonia to varying degrees. Children with type IV SMA tend to develop symptoms between 30 and 60 years of age; the disease progresses slowly without affecting life expectancy (*28*). Children with different types of SMA can have various clinical symptoms. Its diagnosis is often based on electromyography. Laboratory tests, such as serum creatine kinase or genetic tests, can be performed to

corroborate the diagnosis of SMA (*7*).

The main therapeutic strategy for SMA is to increase the level of expression of the full-length SMN protein (*29*). In addition, small molecule compounds, antisense oligonucleotide gene supplementation, and stem-cell transplantation therapy can also be used in an attempt to improve nerve cells with SMA (*30*). Nusinersen (brand name: Spinraza) is a modified antisense oligonucleotide that can upregulate the level of expression of the full-length SMN protein. However, its safety and long-term tolerability still need to be investigated further because it can only be injected intrathecally, and patients require close postoperative monitoring. Nusinersen was approved by the China Food and Drug Administration (CFDA) in February 2019, making it the first medication to treat SMA in China (*27*). The clinical performance of small molecule compounds (such as novel pyridazine analogs) that regulate the splicing of exon 7 in the *SMN* gene still need to be studied further. Other approaches, such as gene supplementation therapy by introducing normal *SMN* cDNA, have achieved promising preliminary results in clinical trials. At present, SMA should be diagnosed as early as possible. Affected children can be treated with a novel medication if it is affordable. Appropriate rehabilitation exercises can be added to control clinical symptoms and slow disease progression (*31*).

2.6. Rare pediatric diseases of the cardiovascular system

The cardiovascular system is an essential system to maintain normal activity, homeostasis of the body, and metabolism. Cardiovascular diseases are currently the leading cause of death in the general population. Rare pediatric cardiovascular diseases include cardiac ion channelopathies and Noonan syndrome. Both diseases often have an early age of onset, which is usually in childhood or infancy. Like other rare pediatric diseases, rare cardiovascular diseases are often caused by genetic mutations. Attention should be paid to early prevention, timely diagnosis, and prompt treatment when managing these diseases. Pharmaceutical therapy is the most important treatment approach. At present, there are no accurate epidemiological data on this type of disease in China.

2.6.1. Cardiac ion channelopathies

Cardiac ion channelopathies (CICPs) are a large group of diseases that are caused by defective ion channels in cardiac myocytes. Arrhythmias are clearly associated with abnormal expression of the ion channel genes, which can affect protein transport and interactions directly or indirectly by interfering with the structures and functions of cardiac ion channel proteins. All of these changes can ultimately lead to the development of gene-related CICP. Mutations in multiple ion channel genes can cause various arrhythmias (*32,33*). CICP can be classified into two types, hereditary and acquired. This review will focus on the hereditary type for brevity. Hereditary CICP includes long QT syndrome (LQTS, the first CICP identified clinically), short QT syndrome (SQTS), Brugada syndrome (BRS), and catecholaminergic polymorphic ventricular tachycardia (CPVT). CPVT is a serious hereditary arrhythmia.

Clinical studies have found that patients with hereditary CICP often have sudden death or cardiac arrest as the first clinical presentation. Electrocardiographic screening can reveal abnormal QT intervals. LQTS can have torsade de pointes arrhythmia, which can cause syncope or sudden death. The terminal ventricular arrhythmia in SQTS typically presents as ventricular fibrillation (*34*). LQTS is often seen in females at a young age, whereas patients with SQTS can exhibit their first symptom as early as 1 year of age. CPVT is more common in children and adolescents without organic heart disease (*7*). The diagnosis of LQTS is currently based on the Schwartz scoring system, which includes three components (an electrocardiogram, clinical interview, and family history). The history of LQTS plays an important role in the diagnosis and determination of its subtype. Exercise and invasive electrophysiological tests have a limited role. The process of diagnosing SQTS is similar to that of diagnosing LQTS (*34*).

The treatment principles for CICP mainly include pharmaceutical therapy, lifestyle modifications, and assistive devices. Risk stratification and assessment of the disease can help clinicians make the right clinical decisions. All patients with LQTS should avoid medications that can prolong the QT interval or lower the potassium level. β-blockers remain the treatment of choice for LQTS. Clinical studies have indicated that propranolol can significantly shorten the QTc more than nadolol and metoprolol. In patients with CPVT who still experience ventricular arrhythmias or syncope despite treatment with β-blockers, verapamil may be considered, with or without a β-blocker. For pediatric patients with BRS who are not candidates for an implantable cardioverter defibrillator (ICD), treatment with quinidine should be considered (*7*). Although antiarrhythmic medications are still the preferred treatment option for CICP, non-pharmaceutical treatments have become standard care, as antiarrhythmic medications alone usually have poor outcomes or cause additional arrhythmias. ICD implantation is the only effective treatment to prevent sudden death in children with SQTS or BRS. In addition, radiofrequency catheter ablation and left stellate ganglionectomy are also important options in the clinical management of these rare pediatric diseases (*35*).

2.6.2. Noonan syndrome

Noonan syndrome (NS) is a group of autosomal dominant

disorders with similar clinical presentations caused by mutations in multiple genes. The RAS/mitogen-activated protein kinase (RAS-MAPK) signaling pathway can alter gene transcription, regulate the activity of cytoplasmic targets, and induce appropriate short- and long-term cellular responses to stimuli. Extracellular ligands (such as certain growth factors, cytokines, and hormones) can stimulate cell proliferation, differentiation, survival, and metabolism *via* the RAS-MAPK pathway. Mutations in this pathway can cause NS (*36*). Approximately 50% of patients with NS have a mutation in the *PTPN11* gene, which is a non-receptor protein tyrosine phosphatase SHP-2 that participates in a variety of intracellular signaling cascades downstream of growth factors, cytokines, and hormone receptors and which is required for normal growth and development. *PTPN11* encodes SHP-2. Mutations in *PTPN11* can lead to over-activation of SHP-2 (*37*).

Children with NS usually present with short stature (gradually occurring after 1 year of age), a characteristic facial appearance (such as wide eye spacing, ptosis, epicanthus, a low ear position, a high palatal arch, a full forehead, a low posterior hairline, a short nose, and thick lips), congenital heart defects (50−80% of patients have congenital heart disease), delayed psychomotor development, a webbed neck, an abnormal thorax (a sunken or protruding chest), and cryptorchidism (*7*). The clinical symptoms can vary significantly, with hematological disorders being the most common initial symptoms (*37*). The diagnosis of NS mainly depends on clinical presentations, although a characteristic facial appearance can change with aging, which can cause a missed diagnosis. Genetic testing is not used as the preferred diagnostic option. A clinical diagnostic scoring system can facilitate the diagnosis of NS (*38*). There is currently no targeted treatment for NS. The main goal of treatment is to maximize the child's growth so that he or she reaches normal height. The presence of congenital defects in other systems requires a multidisciplinary approach and symptomatic support to improve the patient's quality of life. Long-term growth hormone therapy can help most children reach normal adult height (*39*). However, children receiving growth hormone therapy should be monitored closely to rule out the incidence of tumors. Clinical trials are still examining use of a target therapy on the RAS/MAPK pathway in NS. Whether medications that inhibit the RAS/MAPK pathway can be safely used in children with NS needs to be studied further (*40*).

2.7. Rare pediatric diseases of the hematologic system

In the hematological system, there are six rare diseases that start in childhood and infancy: congenital pure red blood cell aplastic anemia, severe congenital neutropenia, Fanconi anemia, isovaleric acidemia, propionic acidemia, and sickle cell anemia (common in Africans and African Americans). Fanconi anemia is a multisystemic disease. If there is no cure for a hematologic disorder, then the main goal of clinical management is to ensure the growth, development, and daily activity of the affected child. The acute phase usually requires promoting anabolic metabolism while the remission phase generally involves diet therapy and medications. Typical diseases are reviewed here, including congenital pure red blood cell aplastic anemia and propionic acidemia.

2.7.1. Congenital pure red blood cell aplastic anemia

Congenital pure red blood cell aplastic anemia, an autosomal genetic disorder also known as Diamond-Blackfan anemia (DBA), is a hereditary bone marrow failure syndrome caused by mutations in the genes encoding ribosomal proteins. Mutations or deletions in the ribosomal protein genes cause haploinsufficiency, which leads to selective poor erythropoiesis in patients with DBA (*41*). In addition, impaired ribosome synthesis can affect the stability and activity of the tumor suppressor pathway involving the tumor protein p53 (TP53), which contributes to the clinical presentations of the disease, including altered erythropoiesis and increased tumor susceptibility (*7*).

Approximately 93% of affected children develop the disease within 1 year of age and can present with pallor, depression, and feeding difficulties (42). The main clinical characteristics include bone marrow failure (macrocytic anemia and significantly reduced bone marrow erythroid cells, which occur in 35% of children at birth), congenital developmental abnormalities (mainly involving the head, upper limbs, heart, and genitourinary system), and increased susceptibility to cancer and early onset of tumors. The clinical diagnosis of DBA is usually based on the following four criteria, *i*) the onset of disease is within 1 year of age; *ii*) macrocytic (or normocytic) anemia, with a normal or slightly decreased white blood cell count, and a normal or slightly increased platelet count; *iii*) significantly reduced reticulocytes; and *iv*) active myeloproliferation with low erythroid precursor cells.

The main treatments for DBA are corticosteroids and blood transfusions, which should be started promptly, as earlier treatments with glucocorticoid can achieve better outcomes. Glucocorticoids are used to maintain a stable hemoglobin level to meet the requirements of physical and cognitive development. The treatment usually increases the percentage of reticulocytes in 1-2 weeks (*43*). If there is no significant increase in reticulocytes in 4 weeks, corticosteroids should be stopped immediately. More testing should be performed to rule out bone marrow failure. Steroid treatment in children younger than 6-12 months of age can have serious adverse effects and should be replaced by blood transfusion to maintain a hemoglobin level above 80 g/L to ensure growth and development, as well as daily

activity (*7*). A blood transfusion is also a main treatment for children unresponsive to corticosteroids or for whom corticosteroids are contraindicated. When both types of treatments are ineffective, hematopoietic stem cell transplantation can be considered. In addition, gene therapy to treat defective genes to encode ribosomal protein 19 (RPS19) in patients with DBA is in clinical trials.

2.7.2. Propionic acidemia

Propionic acidemia (PA) is an autosomal recessive disorder with abnormal propionic acid catabolism caused by mutations in the gene coding for propionyl-CoA carboxylase (PCC) or methylmalonyl-CoA mutase (MUT). PA is characterized by the abnormal accumulation of the catabolic products of branched-chain amino acids (3-hydroxypropionic acid, methylcitric acid, and/or methylmalonic acid) in the plasma, urine, and other body fluids, which results in organic acidemia and a series of biochemical, neurological, and other organ system dysfunctions (*44*). The prevalence of PA is reported to be 0.6/100,000 to 0.7/100,000 in China (*7*).

PA usually starts after birth or infancy. Most neonates with PA have acute and critical disease, with a high morbidity and mortality. Early identification and treatment of these children is crucial to saving their lives and improving their outcomes (*45*). The clinical presentations of PA are not specific and can include severe and persistent metabolic acidosis, ketosis, an elevated anion gap, and hyperammonemia. In a neonate with PA, typical symptoms can begin as early as the second day of life. When there is a dramatic deterioration in the overall clinical condition of a neonate, such as vomiting, weight loss, labile body temperature, neurological involvement with hypo- or hypertonic tone, irritability, lethargy, and progression to coma or seizures, the diagnosis of PA should be ruled out. Other possible conditions, such as sepsis, should also be excluded. Blood amino acid and acylcarnitine profile tests, as well as urinary organic acid analysis, can be performed in these symptomatic children. If elevated levels of glycine, C3/C2, 3-hydroxypropionic acid, and methylcitric acid are present, the diagnosis of PA can be confirmed. In addition, genetic testing is also important in confirming the diagnosis of PA (*44*).

Children in the acute phase of PA require aggressive treatment, including removing accumulated toxic organic acids, minimizing endogenous protein catabolism, and promoting anabolism. Continuous hemofiltration has been used to rapidly remove toxins and allow the administration of a large amount of fluid without the risk of overhydration. L-carnitine can be infused during the acute phase, since it can bind to the organic acids to form water-soluble metabolites to be excreted in the urine. A long-term management plan also includes nutritional support. Adequate protein and energy intake should be ensured, but natural protein diets should be restricted. L-carnitine, betaine, and vitamin H should be supplemented. Vitamin H-containing biotin therapy can promote the catabolism of fat and carbohydrates and accelerate energy conversion. Liver transplantation may be considered in a small number of pediatric patients with PA who have frequent severe metabolic decompensation despite strict dietary control, death of siblings, or cardiomyopathy. Currently, there is no effective genetic therapy for PA (*46-48*).

2.8. Rare pediatric diseases of the urinary system

In the *First List of Rare Diseases*, the only rare pediatric-onset disease of the urinary system is Alport syndrome (AS), a hereditary basement membrane disorder. The molecular mechanism of its pathology involves three genetic mutations that alter type IV collagen, one of the major structural components of the basement membrane framework. This can result in structural and functional abnormalities of the α-3, α-4, or α-5 chains in this protein, which in turn lead to abnormal collagen structures in organs. Structural and functional impairments of type IV collagen in the glomerular, ocular, and cochlear basement membranes can result in AS (*49*).

AS can start during early childhood. It most often involves the kidneys, with glomerular hematuria being the first symptom. It can also affect the eyes and ears, causing sensorineural hearing loss and anterior lenticonus. Early diagnosis of AS is important because treatment to slow the progression of the disease depends on the stage in which treatment is initiated. The age when pediatric patients with AS transition from microalbuminuria to proteinuria is an important prognostic marker. An earlier transition indicates a worse prognosis. The diagnosis of AS relies on five components: laboratory tests (urinary analysis and routine blood and renal function tests), an ear examination (electric audiometry), an ophthalmologic examination (three lesions with diagnostic significance, anterior lenticonus, posterior polymorphous corneal dystrophy, and retinal flecks), a histopathologic biopsy (kidney, skin), and a genetic test. A genetic test is the gold diagnostic standard; it can reveal defects in the *COL4A3*, *COL4A4*, or *COL4A5* genes. Family history should be carefully reviewed since AS is a genetic disease, and affected children often have a significant family history (*49*).

Currently, there is no curative therapy for AS. Treatment aims to control urinary protein, maintain normal function, and delay the onset of renal failure. Management is mostly supportive care and renal replacement therapy. Treatment decisions should consider the patient's gender, disease stage, mutation type, and family history (especially the age of the patient's relatives at the time of end-stage renal disease).

The first line of pharmaceutical management is with angiotensin-converting enzyme inhibitors (ACEIs). Second-line treatments include angiotensin receptor blockers (ARBs) and aldosterone inhibitors. A number of studies have confirmed the safety and efficacy of ACEIs and ARBs in the treatment of chronic kidney disease in pediatric patients with AS. ACEI should be initiated in at least the 2nd stage of AS (proteinuria > 300 mg/day). It can inhibit the activation of the renin-angiotensin-aldosterone system (RAAS), adjust glomerular feedback, and decrease glomerular hyperfiltration to reduce proteinuria, delay glomerulosclerosis, and slow the progression to end-stage renal disease. During treatment, blood potassium and renal function should be monitored for possible adverse reactions. In addition to the urinary system, AS can also affect other organ systems. AS can be managed by an integrated multidisciplinary approach. Currently, studies involving animal models have identified many potential new therapies for AS, such as podocyte-targeting anti-inflammatory therapies or therapies with bone morphogenetic protein-7-like molecules, protease inhibitors, or collagen receptor antagonists, and cellular therapies targeting podocytes. Advances in basic and clinical research have advanced the treatments for children with AS (*7,49-51*).

2.9. Rare pediatric diseases of the integumentary system

Rare skin diseases can be classified into rare metabolic skin diseases, rare genetic skin diseases, and rare skin tumors. Rare genetic skin diseases are mostly caused by pathogenic genes affecting the expression of related proteins and the activities of enzymes. Epidermolysis bullosa is a rare genetic skin disease. Rare skin tumors often stem from unknown causes or fusion gene mutations. Langerhans cell histiocytosis is a rare skin tumor. It involves not only the skin mucosa but also multiple organ systems.

2.9.1. Langerhans cell histiocytosis

Langerhans cell histiocytosis (LCH) is a type of dendritic cell myeloma that is currently considered to be an inflammatory myeloid neoplasm originating from the bone marrow monocyte-macrophage system. LHC is characterized by granulomatous lesions consisting of clonal pathological histiocytes. The disease is most often seen in children with a peak age of 1-4 years (*52*), and it has an estimated annual incidence of 0.5/100,000 to 5.4/100,000 (*7*). Although the exact mechanism of LCH's pathogenesis has yet to be elucidated, activation of the MAPK pathway is present in almost all patients with LCH and is a critical driver of carcinogenesis. The oncogene *BRAF* is the gene that is most often mutated in the MAPK pathway. The *BRAF* V600E mutation has been found in 50% of patients with LCH (*53*). It is statistically correlated with an age of onset younger than 3, multisystem involvement, severe disease, permanent sequelae, resistance to first-line systemic therapy, and a high 5-year rate of recurrence. LCH is a neoplastic disease because it involves the activation and uncontrolled proliferation of pathological Langerhans cells (LC) due to the MAP kinase pathway activated by a functional *BRAF* V600E mutation (*54*).

Children with LCH can have various clinical presentations with different levels of severity, easily resulting in misdiagnosis and missed diagnosis. Affected children can have a fever, rash, central diabetes insipidus, an abnormal hematopoietic system, and bone damage, with a rash and bone damage being the most common presentations. Skin lesions most often appear in children under 2 years of age. Proptosis due to orbital bone lesions is a typical characteristic in pediatric patients (*7*). Approximately 75% of patients have bone lesions. A pathological examination is the gold standard to diagnose LCH. A typical pathological finding is the proliferation of well-differentiated histiocytes under a light microscope. In addition, clinical presentations, histology, and immunohistochemistry findings can be used to aid in diagnosis. LCH should be differentiated from other histiocytic diseases (*55*).

Determination of the management strategy in patients with LCH should comprehensively consider the clinical presentation, response to treatment, and risk of death. Partial local treatment is effective for patients with LCH and single-organ or single-system disease, whereas systemic treatment is the main therapy for patients with LCH and multisystem involvement. Patients with extensive skin lesions should receive glucocorticoids or nitrogen mustards. Patients with local bone lesions can undergo simple curettage. The first-line treatment for patients with multifocal monosystemic or multisystemic LCH is the LCH-III regimen, which was developed by the International Histiocyte Society. This treatment regimen consists of 1-2 courses of initial therapy and subsequent maintenance therapy. Daily oral steroids and weekly vincristine are given for a period of 6-12 weeks, with subsequent boluses of steroids/vincristine every 3 weeks, for a total treatment period of 12 months. In addition, 6-mercaptopurine should be included in subsequent maintenance therapy in patients with lesions involving the liver, spleen, or hematologic system (*56*). The Ras-ERK inhibitor vemurafenib can be used to treat patients who fail to respond to conventional therapies, but adverse reactions to vemurafenib should be monitored (*7*). Medications such as cytarabine and etoposide, which target myeloid tumors, and hematopoietic stem cell transplantation, have gradually become the second-line or salvage treatment options for LCH, and especially for patients with refractory LCH who have multisystem involvement but who fail to respond to first-line treatment. Patients with major organ involvement can also receive second-line treatment. However, further research should focus on drug toxicity,

the high rate of reactivation (30-50%), and sequelae. The prognosis for LCH varies widely. Children under 2 years of age without multiorgan involvement have a better prognosis than children with multisystem involvement. The latter have a higher mortality rate.

2.9.2. Congenital epidermolysis bullosa

Congenital epidermolysis bullosa (CEB) is a group of medical conditions with large blisters of the skin and mucous membranes. CEB is caused by dominant mutations in one or more genes that result in defects in the epidermal keratin or intradermal anchoring proteins with abnormal structure or function of the skin basement membrane. Patients with CEB have increased skin fragility. Minor mechanical injury can separate the epidermis from the dermis to cause blisters (*57*). CEB can be classified into three major types based on the location of blister formation: epidermolysis bullosa simplex (EBS, lesions within the epidermis), junctional epidermolysis bullosa (JEB, lesions in the center of the lamina lucida of the basement membrane zone), and dystrophic epidermolysis bullosa (DEB, lesions in the sub-lamina densa). The diagnosis of CEB requires a personal and family medical history, as well as lesion histopathology (intra- or subepidermal blisters without inflammatory cell infiltration), a skin immunofluorescence assay (negative results), and a salt-split test (fluorescent deposits on dermal sides) for further verification (*7*).

Children with CEB can have signs of skin lesions starting at birth. The severity of lesions depends on the type of disease. The clinical characteristics of CEB include increased skin fragility to mechanical forces and blister formations after trauma. Histopathology reveals intra- or subepidermal blisters without inflammatory cell infiltration. Children with CEB and critical lesions can die during infancy. There is currently no cure for CEB. Multidisciplinary comprehensive treatments are the main approach to treating CEB, with the major therapies being symptomatic support, injury prevention, infection control, and promotion of wound healing. The skin wound needs to be adequately assessed, including the area of the lesion and its morphology (intact blisters versus erosions, chronic versus acute, and exudative versus non-exudative), in order to implement a wound care plan (*58*). Specific skin management includes wound care, pain management, and treatment of pruritus. Non-adhesive foam, modified absorbent pads, lipido-colloid dressings, and contact covers can be used to protect non-exudative wounds. Exudative wounds can be managed with aqueous fibers, calcium alginate, and topical antimicrobials (*e.g.*, antibiotics, silver-containing dressings, and medical-grade honey). Sedatives, such as phenobarbital or chloral hydrate, can be given to children with irritability due to pain. The most commonly used medications for pruritus are antihistamines. Gabapentin, an antiepileptic medication, has also been found to reduce pruritus in children with chronic kidney disease or a burn. All of these can be used for analgesia in children. Genetic and cell-based studies on molecular therapies have yielded promising results and have provided a new approach to managing CEB. However, their reliability, practicality, and safety need to be studied further. If secondary squamous cell carcinoma is suspected, a skin biopsy should be performed. If confirmed, surgical excision is required (7,57-59).

3. Conclusion

Due to the extremely low incidence of rare pediatric diseases, there is generally no precedent for their treatment. As medicine has advanced, however, clinical treatments and drugs for some rare pediatric diseases have become available. For many rare diseases, early treatment can significantly improve its prognosis and the patient's quality of life. Dietary treatment in infancy can help children with PKU to develop normally, while significant delays in treatment can lead to severe mental and physical disabilities. Neonatal screening will improve viability and effectively prevent death and disability. Determining the novel molecular mechanisms that cause hereditary rare diseases can also help to treat common diseases. Research on rare pediatric diseases is an important aspect of rare diseases and is expected to develop into a systematic and substantive discipline. At present, some rare diseases have been covered by medical insurance in some regions or in the Chinese population, but there is no policy directly targeting rare pediatric diseases. As diagnosis and treatment of rare diseases in China advances further, diagnosis and treatment of rare pediatric diseases should also advance further.

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Review

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Off-label medication use in rare pediatric diseases in the United States

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SUMMARY Many pediatric patients with rare diseases use drugs off-label due to limited data in pediatric patients. Off-label treatment remains an important public health issue for neonates, infants, children, and adolescents, especially for pediatric patients with rare diseases. For patients with rare diseases, the majority of medications have no or limited information in labelling for pediatric use. Children present unique considerations in clinical trials due to ethical and clinical concerns, which have limited and even discouraged testing of drugs in the pediatric population. Numerous legislative measures have been enacted to address barriers in pediatric drug testing. This research reviewed off-label medication use in rare pediatric diseases, evaluated recent medication uses in pediatric clinical practice, discussed key regulations for rare pediatric diseases, and summarized recent drug approvals for rare pediatric diseases. This study demonstrates the ongoing medical need for newly approved medications to treat pediatric rare diseases and revealed the positive impact of regulations from the Orphan Drug Act of 1983 to the Research to Accelerate Cures and Equity (RACE) for Children Act on drug development and off-label medication practice in rare pediatric disease management. This article provides informative historical background and current considerations of off-label use of medications in neonates, infants, children, and adolescents with rare diseases.

Keywords rare diseases, off-label drug use, pediatrics, infants, children.

1. Introduction

While many drugs have been approved by the U.S. Food and Drug Administration (FDA) for use in adults, the lack of studies in children often leads to off-label usage of drugs in children. A drug will only have FDA approved labelling for use in children if the FDA has determined the drug's safety and effectiveness for a particular condition in children. There are delays in conducting clinical trials with children due to a lack of financial incentives for sponsors to conduct drug trials with children. Also, many diseases are less common in children than in adults, so it requires more time to recruit child participants. In addition, there are often concerns regarding ethics, harm, and consent, making it difficult to obtain institutional review board approval to conduct clinical trials with children. The risk of improper dosing or usage of drugs in children may cause harm. In addition, the resulting hospitalizations and administration of off-label treatments are major health care cost drivers and concerns.

There have already been several governmental legislations that have been set up to address off-label use in children. For instance, the Pediatric Research Equity Act (PREA) of 2003 requires pharmaceutical companies to study effects of new drugs on children if the drugs have the potential to be prescribed to children (*1*). Under the PREA policy, when sponsors submit a new drug application to the FDA for approval in adults, they also have to provide information on safety and efficacy of the drug in children. Studies under PREA are mandatory and those under the Best Pharmaceuticals for Children Act (BPCA) are voluntary. BPCA gives pharmaceutical companies six additional months of patent use for drugs already on the market if the companies conduct clinical trials for children (*2*). These regulations not only support the need for pediatric safety, efficacy, and dosing information, but also enhance transparency of the drug approval process.

Unfortunately, these laws have very limited application to orphan therapies for rare diseases. The majority of rare diseases start in childhood and more than half of patients with rare diseases are children. Increasing rates of off-label prescribing patterns in

children were observed from 2006 to 2015, particularly for unapproved conditions (*3*). With this consideration in mind, the objective of this article is to review current considerations and historical background of off-label use of medication in pediatric patients with rare diseases.

2. Off-label use for rare pediatric diseases

There are over 7,000 known rare diseases, with many new ones being discovered (*4*). According to the Orphan Drug Act of 1983, each rare disease affects fewer than 200,000 people in the United States (*5*). Although the number of patients affected for each rare condition is small, approximately 25-30 million are affected by those rare diseases in the United States (*4*). Approximately 80% of rare diseases are genetic in origin, and approximately half of affected individuals are children (*6*).

While significant advances in the development and approval of rare disease therapies have been made, most rare conditions still have no treatments. Only 5-7% of rare diseases have an FDA approved drug (*7*). For example in 2017, FDA approved *cerliponase alfa* (Brineura™) as the first treatment for neuronal ceroid lipofuscinosis type 2 (CLN2) disease (*8*). CLN2 disease, also known as late infantile neuronal ceroid lipofuscinosis (NCL), is a form of Batten's Disease which is a rare, autosomal recessive, pediatric neurodegenerative disease that results from pathogenic variants in the gene encoding lysosomal enzyme tripeptidyl peptidase 1 (TPP1) (*9*). Before *cerliponase alfa*, there were no approved pharmacological treatments for CLN2 other than drugs for symptom management.

There are many challenges in developing new drugs for rare diseases. Besides ethical issues related to enrolling children in clinical trials, rare pediatric diseases are frequently underdiagnosed because of the heterogeneity in disease presentation and limited clinical expertise outside of a few specialized centers. Additionally, many rare diseases have poorly characterized natural histories. Phenotypic diversity within a disorder adds to the complexity in describing its natural history. Furthermore, because the number of patients for each rare disease is small, the study design is often restricted in clinical development programs. Clinical endpoints, such as potential biomarkers, are often not well-defined and may not have regulatory precedence.

Since most rare conditions have no FDA approved treatments, physicians treating patients with rare diseases strongly rely on off-label drug use. Unfortunately, rare diseases are not often investigated within peer-reviewed journal articles and the results from failed clinical trials are rarely published. Due to a lack of communication of benefits for off-label use and limited diffusion of off-label information, many physicians who treat individuals with rare diseases are unaware of potential benefits of off-label use of therapies for their patients. Even if they are aware, information that is available generally is not specific to a particular rare disease, leaving substantial uncertainty on how to care for these patients using the off-label drug. Table 1 summarizes current needs and challenges of using off-label medications in rare pediatric diseases.

Another important barrier for individuals with rare diseases is obtaining insurance coverage for off-label therapies. In general, insurers and pharmacy benefit managers (PBM) will not reimburse off-label use of drugs or medical devices. Due to the rejection of coverage of off-label therapies for rare diseases, patients are required to mostly pay out of pocket or provide additional paperwork which delays patient access. These patients are forced to pay thousands of dollars just to

Table 1. Current needs and challenges of using off-label medication in rare pediatric diseases

Needs and Challenges	Rationale
Current needs of using off-label medication	Lack of studies in children often leads to off-label usage of drugs in children. There are delays in conducting clinical trials with children due to • Lack of financial incentives for sponsors to conduct drug trials with children • Many diseases are less common in children than in adults, so it requires more time to recruit child participants • Concerns regarding ethics, harm, and consent, making it difficult to obtain institutional review board approval to conduct clinical trials with children Unique challenges in conducting clinical trials in rare pediatric diseases: • Frequently underdiagnosed because of the heterogeneity in dis-ease presentation and limited clinical expertise outside of a few specialized centers • Natural histories are poorly characterized and phenotypic diversity within a disorder adds to the complexity • Study design is often restricted due to a small number of patients for each disorder • Clinical endpoints, *e.g.* biomarkers, are often not well-defined and there may be no regulatory precedence
Challenges of using off-label medication	There is substantial uncertainty for physicians to care for their patients with rare diseases using the off-label drug due to • Rare diseases are not often investigated within peer-reviewed journal articles • Results from failed clinical trials are rarely published • Lack of communication of benefits for off-label use and limited diffusion of off-label information • Even the information that is available generally is not specific to a particular rare disease

access therapies prescribed off-label (*10,11*). This results in an equality issue where only the wealthy can afford off-label treatment, while patients with lower incomes cannot.

3. Medication uses in recent pediatric clinical practice

Pediatric patients with rare diseases represent a population which has a high likelihood for off-label drug use. As an example, several biologics are approved for the indication of juvenile idiopathic arthritis (JIA). However, many children with JIA continue to have active disease despite treatment and are treated with other off-label biologics, including anakinra, ustekinumab and golimumab (*12*). Infliximab and golimumab, which are not approved for JIA, are considered potential treatment options, particularly in JIA patients with rheumatoid factor positive where previous therapy was ineffective or not tolerated (*13*). Table 2 shows the off-label biologic agents for treatment of JIA. A retrospective study showed ~5% of JIA patients were prescribed off-label biologic agents as their first-course treatment and more than 20% of patients were prescribed off-label biologics as their second-course therapy (*14*). The proportion of children with an off-label biologic disease-modifying antirheumatic drug (DMARDs) prescription after JIA diagnosis increased over time (Figure 1). Near 15% of patients were treated with off-label biologic DMARDs on average over the ten-year period from 2009 to 2018, and off-label use of biologics was increasing from 0.0% in 2009 to 17.2% in 2018, peaking at 28.3% in 2015.

An important reason for use of off-label drugs is to improve access to new treatments or to address the medical needs and preferences of patients. In general, off-label use of medicines is not supported by the same level of evidence as drugs FDA approved for pediatric use. This may result in increased uncertainty on efficacy as well as the risk for toxicity and other adverse events. Recently, more studies have been conducted to describe off-label use of new treatments, such as biologics in children with rare diseases (*15-17*).

4. Regulations for rare pediatric diseases

Since the number of patients is small for each rare disease, there is a lack of financial incentives for sponsors to conduct drug trials for these conditions. Legislation has promoted orphan drug development.

Table 2. Biologic agents for treatment of juvenile idiopathic arthritis

Mechanism of action	Generic Name	Route	FDA Approval Date for JIA	FDA First Approval Date
TNF inhibitor	Adalimumab	Injection	2/22/2008	12/31/2002
	Certolizumab Pegol*	Injection	NA	4/22/2008
	Etanercept	Injection	5/27/1999	11/2/1998
	Infliximab*	Infusion	NA	8/24/1998
	Golimumab*	Injection/Infusion	NA	4/24/2009
Binds to CD80/CD86 and inhibits T-cell costimula-tory signal	Abatacept	Injection/Infusion	10/28/2008	12/23/2005
IL-1 inhibitor	Canakinumab	Injection	5/10/2013	6/17/2009
	Rilonacept	Injection	NA	2/27/2008
	Anakinra*	Injection	NA	11/14/2001
IL-6 inhibitor	Tocilizumab	Injection/Infusion	4/30/2013	1/8/2010
Binds CD20 on B-cell	Rituximab*	Infusion	NA	11/26/1997
IL-12/IL-23 inhibitor	Ustekinumab*	Injection	NA	9/25/2009

CD, cluster of differentiation; TNF, tumor necrosis factor; IL, interleukin; JIA, juvenile idiopathic arthritis. *Off-label uses of biologic agents to treat juvenile idiopathic arthritis.

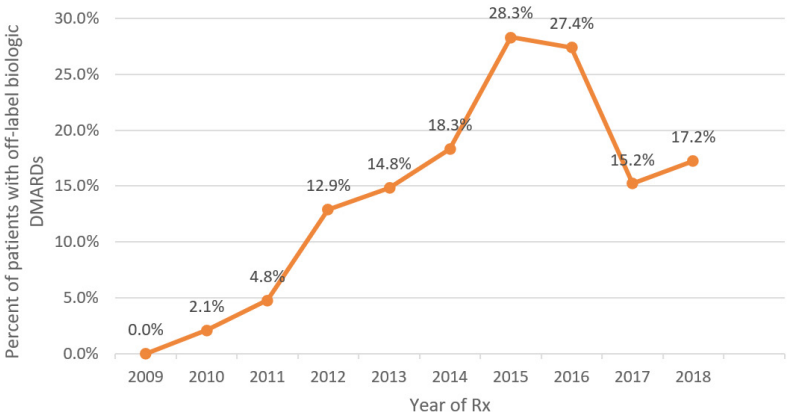

Figure 1. Annual proportions of patients receiving off-label biologic disease-modifying antirheumatic drug (DMARDs) after juvenile idiopathic arthritis diagnosis by calendar year, 2009-2018 (*33,34*).

The Orphan Drug Act of 1983 made substantial progress in promoting development of products for diagnosis and treatment of rare diseases (*5*). The policy created economic incentives to promote development of new treatments for rare diseases, including: *i*) 7 years of market exclusivity for approved orphan products; *ii*) 25% tax credit for clinical study costs and user fee waivers; *iii*) eligibility to apply for FDA Orphan Grants program to support clinical research.

To motivate sponsors to conduct drug trials for life-threatening rare pediatric diseases, the Creating Hope Act was established in 2012. As part of the act, the rare pediatric disease priority review voucher program (PRV) was created (*18*). The act changed the way that pharmaceutical companies look at rare pediatric diseases dramatically. In the prior 20 years, there were very few drugs developed for treating the pediatric population that were FDA approved for children. But after the PRV was approved, many pharmaceutical companies started to develop drugs for children with cancer and other life-threatening illnesses because of the incentives. These incentives include: *i*) vouchers awarded when a new drug is approved for a rare pediatric disease (*e.g.*, Duchenne muscular dystrophy); *ii*) future product gets a 6-month priority review timeline (instead of usual 10-month standard review). Since 2012, 28 rare pediatric disease vouchers have been awarded by the FDA (*19*).

A large portion of rare diseases are rare cancers. Altogether, there are about 6,000 types of cancer identified (*20*). Nineteen of these cancers are common diseases (*e.g.*, lung, colorectal, breast, pancreas, prostate) and the remaining cancers are rare diseases under US Public Law (*21,22*). Rare cancers account for about 20% of all cancers diagnosed (*21*) and all forms of pediatric cancer are rare by definition. DeSantis *et al.* reported that more than two-thirds (71%) of cancers occurring in children and adolescents are rare cancers compared with less than 20% of cancers diagnosed in patients aged 65 years and older. The 5-year relative survival for rare cancers is poorer than that for common cancers among both males (55% *vs.* 75%) and females (60% *vs.* 74%). While survival rates have improved for certain pediatric cancers, malignant neoplasms were the third leading cause of death, representing 9% of overall deaths among children and adolescents (*23*).

In August 2017, the Research to Accelerate Cures and Equity (RACE) for Children Act was signed into law as part of the 2017 FDA Reauthorization Act (*24*). The purpose of the RACE for Children Act was to promote development of new cancer treatments for children. The RACE Act gives the FDA authority to require any new cancer drug to be studied in pediatric cancers if the molecular target of the cancer drug is relevant. Cancer drugs with orphan designations are no longer exempt from PREA requirements. RACE for Children Act amended PREA to require pediatric investigation of certain targeted cancer drugs based on molecular mechanisms of action rather than the clinical indication for original Novel Drug Applications (NDAs) and Biologics License Applications (BLAs) submitted on or after August 18, 2020, unless a deferral or waiver is granted. Table 3 highlights important regulations and core content for rare pediatric diseases including rare cancers in the last 10 years (2011-2021).

Table 3. Important regulations and core content for rare pediatric diseases including rare cancers in the last 10 years (2011-2021)

Public Law	Subsequent Law	Program/Regulation for Rare Pediatric Disease
21st Century Cures Act ● Enacted on December 13, 2016 ● To help accelerate medical product development and bring new innovations and advances to patients who need them faster and more efficiently ● Major driver of 21st Century Cures Act was patients with rare diseases ● Renewed the Creating Hope Act of 2012 ○ The rare pediatric disease priority review voucher pro-gram (PRV) was created	**Advancing Hope Act of 2016** ● Strengthens and extends the PRV through September 30, 2020	**Rare Pediatric Disease Priority Review Vouchers Program** ● Intended to create a market incentive for the development of drugs for rare pediatric diseases through the establishment of a priority review voucher ● Voucher awarded when a new drug approved for a rare pediatric disease (*e.g.*, Duchenne muscular dystrophy) ● Future product gets a 6-month priority review time clock (instead of usual 10-month standard review) ● Vouchers can be sold
Food and Drug Administration Reauthorization Act (FDARA) ● Signed into law on August 18, 2017 ● Revises and extends the user-fee programs for drugs, medical devices, generic drugs, and biosimilar biological products ● Updates the 2003 Pediatric Research Equity Act (PREA)	● **Research to Accelerate Cures & Equity Act (RACE)** ○ Enacted on August 18, 2017 ○ Incorporated as Title V of the FDARA ○ Aim to promote research into, and development of, new treatments for children with cancer	**Research to Accelerate Cures & Equity Act (RACE)** ● Provides the FDA the authority to require any new cancer drug to be studied in pediatric cancers for which the molecular target of the cancer drug is relevant ● Amended PREA to require pediatric investigation of certain targeted cancer drugs based on molecular mechanisms of action rather than the clinical indication for original BLAs/NDAs submitted on or after August 18, 2020, unless a deferral or waiver is granted ● Cancer drugs with orphan designations no longer exempt from PREA requirements

Table 4. Number of novel drug approvals (NDA) by the Center for Drug Evaluation and Research (CDER) from 2017 to August 2021

Number (%)	2017	2018	2019	2020	2021 (as of August)
All NDAs approved	46	59	48	53	36
NDAs for rare diseases (adults and children)	18 (39%)	34 (58%)	21 (44%)	31 (58%)	17 (47%)
NDAs for rare pediatric diseases	6 (33%)	9 (26%)	7 (33%)	15 (48%)	8 (47%)

5. Recent drug approvals for rare pediatric diseases

Thanks to the regulations set up specifically for rare pediatric diseases, recent orphan drug development has increased the availability of treatments. The study by Kimmel *et al.* showed that among 402 orphan indications approved by the FDA between 2010 and 2018 (*25*), 136 (33.8%) were for pediatric orphan indications. There is an increasing trend in the number of FDA-approved pediatric orphan indications. For instance, there were eight pediatric orphan indications in 2010, 18 in 2014, 12 in 2015, 27 in 2017, and 29 in 2018. Most of the pediatric orphan indications used existing drugs and many targeted the same disease. However, there is still a substantial unmet need for treatments in most pediatric rare diseases.

On the FDA website, we evaluated novel drug approvals by the Center for Drug Evaluation and Research (CDER) for rare pediatric diseases from 2017 to August 2021 (Table 4). For instance, in 2019, 21 of the 48 novel drugs approved by CDER (44%) were for treatments of rare diseases (*26*). Among these 21 orphan indications, seven (33%) were for rare pediatric diseases. In 2020, 31 of CDER's 53 novel drug approvals (58%) were approved to treat rare diseases (*27*). The percentage of orphan indications for rare pediatric diseases was much higher in 2020 (15 out of 31, 48%). Notable examples of novel approvals of 2020 to treat pediatric rare diseases include Evrysdi (risdiplam) to treat patients two months of age and older with spinal muscular atrophy (SMA), a rare and often fatal genetic disease affecting muscle strength and movement (*28*) and Lampit (nifurtimox), to treat Chagas disease (a rare parasitic disease which, if left untreated, can cause congestive heart failure) in children less than 18 years of age (*29*). For 2021, as of August, the percentage of orphan indications for rare pediatric diseases remained high (47%).

Table 5 lists drug name and indication for recent novel drug approvals for rare pediatric diseases from 2017 to August 2021. Duchenne muscular dystrophy is the most studied rare condition with NDA each year from 2017 to 2021, except for 2018. The number of NDA for rare cancers remains small, ranging from 1 to 3 each year from 2017 to 2021.

6. Discussion

Off-label treatment remains an important public health issue for neonates, infants, children, and adolescents, especially for pediatric patients with rare diseases. Many pediatric patients with rare diseases use drugs off-label due to limited data for pediatric patients. Lack of studies in children often leads to off-label usage of drugs. There are delays in conducting clinical trials with children due to lack of financial incentives for sponsors to conduct drug trials with children, and difficulties to obtain institutional review board approval owing to ethical and clinical concerns.

There are over 7,000 known rare diseases and ~50% of people affected by rare diseases are children. While significant advances in the development and approval of rare disease therapies have been made, most rare conditions still have no FDA approved treatments. Unique challenges in conducting clinical trials in rare pediatric diseases, *e.g.* poorly characterized natural history of the disease, and a small number of patients for each disorder contribute to the delays in conducting clinical trials with children.

We support FDA efforts in promoting orphan drug development for rare pediatric diseases. For instance, recent developments in legislation, in particular, the rare pediatric disease priority review voucher program has been successful. As shown in the Kimmel study, it is encouraging that there is an increasing trend in the number of FDA-approved pediatric orphan indications over time. We found that the percentage of orphan indications for rare pediatric diseases increased to near 50% in 2020 and 2021 among the novel drug approvals by CDER from 2017 to August 2021. In addition, the RACE for Children Act was set up to promote the development of new treatments for rare cancers in children, which are a large portion of rare diseases. Although the number of new drug approvals for rare cancers remains small from 2017 to 2021, it may be too early to see the impact from the RACE for Children Act. There should be a significant increase in new drug approvals for rare cancers in children in the near future.

In addition, several efforts from FDA to address unique challenges in conducting clinical trials in rare diseases, including regulatory innovations and flexibilities significantly contributed to several orphan drug approvals. These include adaptive trial design, external control arm based on real-world data, *etc.* The FDA describes the adaptive trial design as "*a clinical trial design that allows for prospectively planned modifications to one or more aspects of the design based on accumulating data from subjects in the trial*"

Table 5. Recent novel drug approvals for rare pediatric diseases, 2017-August 2021

Year	2017	2018	2019	2020	2021
1	Hemlibra for hemophilia A who have developed antibodies called Factor VIII (FVIII) inhibitors	Crysvita for x-linked hypophosphatemia (XLH)	Adakveo for vasoocclusive crisis	Artesunate for severe malaria	Evkeeza for homozygous familial hypercholesterolemia
2	Mepsevii for mucopolysaccharidosis type VII (MPS VII)	Epidiolex for Lennox-Gastaut syndrome and Dravet syndrome	Egaten for fascioliasis	Danyelza for refractory or relapsed neuroblastoma	Amondys 45 for Duchenne muscular dystrophy
3	Benznidazole for Chagas disease	Elzonris for blastic plasmacytoid dendritic cell neoplasm (BPDCN)	Oxbrytax for sickle cell disease	Dojolvi for fatty acid oxidation disorders	Rylaze for acute lymphoblastic leukemia and lymphoblastic lymphoma
4	Brineura for a specific form of Batten disease	Asparlas for acute lymphoblastic leukemia (ALL)	Rozlytrek for metastatic solid tumors	Ebanga for Zaire Ebola virus infection	Fexinidazole for human African trypanosomiasis
5	Bavencio for metastatic Merkel cell carcinoma	Vitrakvi solid tumors with a biomarker called a neurotrophic receptor tyrosine kinase (NTRK) gene fusion	Trikafta for cystic fibrosis	Evrysdi for spinal muscular atrophy	Rezurock for chronic graft-versus-host disease after failure of at least two prior lines of systemic therapy
6	Emflaza for Duchenne muscular dystrophy	Takhzyro for types I and II hereditary angioedema	Vyondys 53 for Duchenne muscular dystrophy	Imcivree for obesity associated with pro-opiomelanocortin deficiency	Bylvay for pruritus
7		Diacomit for Dravet syndrome	Ga-68-DOTATOC for somatostatin receptor positive neuroendocrine tumors (NETs)	Inmazeb for Zaire Ebola virus infection	Nexviazyme for late-onset Pompe disease
8		Moxidectin for onchocerciasis due to Onchocerca volvulus		Koselugo for neurofibromatosis type 1	Skytrofa for short stature due to inadequate secretion of endogenous growth hormone
9		Symdeko for cystic fibrosis with a certain type of genetic mutation		Lampit for Chagas disease	
10				Orladeyo for hereditary angioedema	
11				Oxlumo for hyperoxaluria type 1	
12				Retevmo for metastatic medullary thyroid cancer	
13				Viltepso for Duchenne muscular dystrophy	
14				Zokinvy for Hutchinson-Gilford Progeria Syndrome	
15				Tazverik for epithelioid sarcoma	

(*30*). One potential advantage of adaptive trial design is statistical efficiency. An adaptive design may provide the same statistical power with smaller expected sample size or shorter expected duration, which works well for rare diseases since the number of patients for each rare disease is small.

The FDA website's page titled "*Rare Diseases: Natural History Studies for Drug Development Guidance for Industry*" states that natural history study data may be used as an external control for clinical investigations (*31*). Natural history study data were used to support several orphan drug approvals. For instance, in 2015, the FDA approved asfotase alfa (Strensiq™), for the treatment of patients with perinatal/infantile- and juvenile-onset hypophosphatasia (HPP) (*32*). The approval of asfotase alfa was based on two multicenter, single-arm, phase 2 interventional studies in 68 treated patients, compared with 48 patients with similar age and HPP characteristics from a retrospective natural history study. In addition, the FDA has set up the Orphan Products Natural History

Grant Program (*19*), which has supported a number of natural history studies to address several challenges during the clinical development programs of drugs and biological products for rare diseases.

Although substantial efforts and significant advances in the development and approval of rare disease therapies have been made, there is still a substantial unmet need for new treatments in most pediatric rare diseases. Currently less than 10% of rare diseases have an FDA-approved drug. Even though some rare conditions have FDA approved drugs, some patients continue to use off-label medications due to active disease, disease progression, patient preference or others. For instance, in the article by Yu *et al.* to evaluate biologics for children with JIA, many children continue to be treated with off-label biologics.

There is substantial uncertainty for physicians to care for their patients with rare diseases using off-label drugs. To address this issue, there are several areas potentially for the scientific community to assist. For instance, peer-reviewed journal articles could promote and encourage submission and publication of articles investigating off-label medication use in rare pediatric diseases in a format such as a case report, review article or others. In addition, it would be helpful for clinical and scientific societies to promote and encourage information sharing on off-label medication use, and to create a forum to allow discussion of potential benefits or challenges for rare pediatric diseases through webinars, workshops or others.

In conclusion, the present study demonstrates the ongoing medical need for newly approved medications to treat pediatric rare diseases and revealed the positive impact of regulations from the Orphan Drug Act of 1983 to the RACE for Children Act on drug development and off-label medication practice in rare pediatric disease management. This article provides informative historical background and current considerations of the off-label use of medication in neonates, infants, children, and adolescents with rare diseases.

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Review

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Effect of nutritional intervention on nutritional status among children with disorders of amino acid and nitrogen metabolism (AANMDs): A scoping review

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SUMMARY Disorders of amino acid and nitrogen metabolism (AANMDs) occur due to an enzyme deficiency in a normal biochemical pathway. Nutritional intervention is recognized as the mainstay of treatment for children diagnosed with AANMD. Hence, this scoping review aimed to identify the nutritional interventions available in managing AANMD disorders and their effects on nutritional status. A systematic search using PRISMA Extension for Scoping Reviews (PRISMA-ScR) method was conducted across 4 databases: PubMed, ScienceDirect (Elsevier), EBSCOhost and Cochrane Central Register of Controlled Trials (CENTRAL). Inclusion criteria for the study to be selected are: subjects aged less than 18-year-old, article published in English, utilized an experimental design and published within the past 20 years. A total of 22 articles were included in this review. The majority of the subjects are boys (55.6%) and employed a randomized controlled trial (RCT) study design (45.4%). Nutritional interventions were categorized into 4 categories which are: "protein substitute" (n = 5), "protein substitute with modified composition" (n = 6), "nutrient supplementation (n=8)", and "distribution and dosage of protein substitute (n = 3)". The most frequently assessed outcomes were biochemical parameters that gauge the effectiveness of metabolic control (68.2%). Overall, "protein substitute enriched with inhibitive amino acids", "long-chain polyunsaturated fatty acids supplementation", and "evenly distributed protein substitute" demonstrated beneficial effects towards the nutritional status, especially in terms of biochemical parameters. In summary, nutritional intervention plays a significant role in improving the nutritional status of AANMD patients. Further investigations of nutritional intervention among AANMD children using a meta-analysis approach are necessary for better comprehension of their impact in management of AANMD disorders.

Keywords nutrition therapy, amino acid metabolism, inborn errors, nutritional status

1. Introduction

The prevalence of amino acid and nitrogen metabolism disorders (AANMDs) at birth is 26.31 per 100,000 live births worldwide (*1*). AANMDs are a group of rare, heterogeneous genetic diseases that arise due to the deficiency of an enzyme, its co-factors, or a transporter that results in the disruption of an amino acid in a metabolic pathway (*2*). When the body is unable to break down a particular amino acid, toxic metabolites begin to accumulate in the blood, urine, body tissues as well as the brain (*3*). When these toxic molecules accumulate in the brain, they harm neurons leading to neuronal damage, neuronal death as well as impaired synaptic plasticity and excitability. This often manifests as learning, behavioural, and emotional difficulties. This accumulation also inhibits energy production within neuron cells resulting in cell swelling and acute encephalopathy (*4*). Other common neurological symptoms of AANMDs include seizures, progressive mental retardation, psychomotor retardation, ataxia, as well as changes in episodic consciousness (*5*). These clinical signs and symptoms

are often precipitated by fasting, catabolism, pyrexia, intercurrent illness, injury, and the overconsumption of intact proteins (*6*).

Nutritional therapy has remained the cornerstone of treating AANMDs since it was first used to successfully treat phenylketonuria (PKU; MIM ID #261600); the "poster child" of metabolic diseases; in 1951 (*7*). The primary goal of dietetic management is to reduce toxic metabolite production and accumulation as well as maintain blood amino acid indices within a non-neurotoxic range (*8*). Furthermore, it is prudent to maintain good nutrition in order to support normal physiological protein synthesis and prevent catabolism (*9*). Dietetic interventions primarily include restricting the intake of naturally-occurring proteins (*10*), introducing protein substitutes that are free of precursor amino acids, and the intake of adequate amounts of low protein food to meet energy requirements (*11*). Medical nutrition therapy (MNT) has been found to favourably affect the nutritional indices of AANMD patients. In this study, patients were thought the importance of metabolic control, provided with low protein recipes and low protein products as well as regularly followed-up at metabolic clinics (*12*).

A literature review revealed that most existing studies only summarise the types of nutritional interventions available for AANMD patients. However, none have thoroughly investigated the effects that these nutritional interventions have on nutritional indices (*11*). Furthermore, instead of consolidating all nutritional interventions into one article, only a handful of studies have examined the nutritional impact of supplementing PKU patients with specific nutrients (*13-15*). Therefore, a scoping review was performed to identify and map the available data on the impact of nutritional intervention on the nutritional indices of AANMD patients (*16*). A scoping review was employed to identify the types of nutritional interventions available as well as investigate their impact on the nutritional indices of children and adolescent with AANMDs.

2. Materials and Methods

2.1. Study design

PRISMA-ScR (PRISMA extension for Scoping Reviews) was used to draft the protocol that was used in this scoping review (*17*). For the purposes of this scoping review, nutritional interventions were defined as the prescription of natural food, medical food or formula, and dietary supplements (*8*). Studies were selected for inclusion only if the participants were below the age of 18 and had been diagnosed with AANMD. One justification for only including patients from this age group was the high prevalence of failure to thrive (FTT) among children with AANMD. Apart from that, studies that had been published between 2001 to 2021 in full text and in English were included in this scoping review. Studies that had been published 20 years ago instead of 5-10 years ago were included in this scoping review in view of the scarcity of research in this field.

Additionally, only studies that tracked and reported changes in nutritional indices; such as anthropometry measurements, biochemical parameters, clinical outcomes, or dietary intake; were included in this scoping review. Lastly, the design of these studies had to be experimental. This included randomised controlled trials (RCT), pre- and post-studies as well as quasi-experimental studies. Only studies with the types of the study designs outlined in the published literature were selected for inclusion (*18*). This was because an experimental study design is commonly used to evaluate research questions on therapeutic agents. This is like our scoping review which aims to assess the effects of nutritional interventions. Therefore, only studies that met all the above-mentioned criteria were included in this scoping review regardless of the length of the intervention or the sample size.

2.2. Search strategy

This scoping review utilised the bibliographic research methodology for the literature search. The relevant studies were searched on electronic bibliographic databases, such as PubMed®/MEDLINE (National Library of Medicine), ScienceDirect® (Elsevier), EBSCOhost, and Cochrane Central Register of Controlled Trials (CENTRAL). A literature search was also performed on an additional resource; the Journal of Inherited Metabolic Disorders (JIMD), to ensure that the search for AANMD-related studies would be extensive. Furthermore, the reference lists of the included studies as well as AANMD-related systematic reviews and clinical guidelines were also reviewed and screened. The first author drafted search strategies which was then cross-checked by the second author to ensure that important keywords had not been omitted from the search. The completed search results were then exported into Mendeley (version 1.19.8), a citation management software. The keywords used in the search were divided into two categories: *i*) synonyms for "nutritional intervention", such as "nutritional management"; and *ii*) the names of all AANMDs. A list of AANMDs was retrieved from Nutrition Support Protocols: The Ross Metabolic Formula System (2005) (19). The Boolean operator "AND" was used to combine keywords from both categories while "OR" was used to combine phrases within each category. Table 1 presents a list of all the keywords that were used during the search.

2.3. Study selection

The studies that were exported to Mendeley (version

Table 1. Key search term in the scoping review

Nutritional Treatment	Disorders of Amino Acid and Nitrogen Metabolism (AANMDs)
Nutritional Intervention	Phenylketonuria
Nutritional Approach	Tyrosinemia
Nutritional Strategies	Maple Syrup Urine Disease
Nutritional Management	Isovaleric Acidaemia OR 3-Methylcrotonylglycinuria OR 3-Methylglutaconic Aciduria OR 3-Hydroxy-3-Methylglutaric Aciduria
Nutritional Education	Homocystinuria
Dietary Treatment	Glutaric Aciduria OR 2-Ketoadipic Aciduria
Dietary Intervention	Propionic Acidaemia OR Methylmalonic Acidaemia
Dietary Approach	Urea Cycle Defects OR Urea Cycle Disorders
Dietary Strategies	Nonketotic Hyperglycinemia
Dietary Management	
Dietary Education	

1.19.8) were first screened for duplication after which duplicate studies were removed from the folder. The title of each study was then read, one-by-one, to determine its relevance to the research questions after which irrelevant studies were removed from the folder. The abstract of each study was then read to determine if it answered the research questions. Finally, the full text of each study was read to confirm its eligibility as some of the population characteristics were not reported in the abstract. The studies selected by the first author were then checked by the second author to ensure that every study met the eligibility criteria of this scoping review.

2.4. Data charting

Data from the eligible studies was charted in a standardised table that presented six important components: *i*) study characteristics (authors, year of publication, country, study design, and type of AANMD diagnosed), *ii*) intervention features, *iii*) intervention length, *iv*) study outcome, *v*) study results, and *vi*) summary. The outcome of each study was further divided into four main categories: *i*) anthropometry measurements, *ii*) biochemical parameters, *iii*) clinical parameters, and *iv*) dietary intake. In the end, three types of tables were presented in this scoping review as multiple study designs were included. This made it difficult to coherently present all the data in a single table. The charted table was reviewed by the second author to ensure that it was comprehensible.

3. Results

3.1. Study selection and characteristics

A total of 1,755 studies were identified from the electronic databases. After removing duplicate studies, 1,679 study titles remained to be screened. At this stage, 65 studies were retrieved and assessed for eligibility by reading the abstracts of each study. Of the remaining 35 full-text studies, 13 studies were excluded as the participants were above the age of 18 (n = 5), six were excluded as they did not meet the study design criterion (n = 7), and one was excluded because it did not report nutritional indices as the outcome (n = 1). Therefore, a total of 22 studies were selected for inclusion in this scoping review (Supplemental Tables S1-3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=84*). The selection process is outlined in Figure 1.

In terms of study characteristics, the selected studies comprised of 652 participants (range: 7 to 109 participants). 55.6% of the participants were male and 44.4% were female. However, three of the selected studies did not provide gender characteristics (*20-22*). Most of the studies recruited participants that had been diagnosed with PKU (n = 17, 73.7%) (*22-37*). This was followed by glutaric aciduria type 1 (GAT1; MIM ID #231670) (n = 2, 10.5%) (*38,39*). Only single studies investigated patients with maple syrup urine disease (MSUD; MIM ID #248600) (*21*), methylmalonic aciduria (MMA; MIM ID #251000) or propionic aciduria (PA; MIM ID #606054) (*40*), and urea cycle disorders (CPS1D, OTCD, ASSD, ASLD, ARG1D, and NAGSD with MIM IDs #237300, #311250, #215700, #207900, #207800, #237310; respectively) (*20*). Eight (36.4%) of the studies were published in the United Kingdom (UK) (*23-25,32,34,35,37,41*), followed by Germany (n = 5) (*22,26,27,36,39*), the United States of America (US) (n = 4) (*20,21,38,40*), Italy (n = 3) (*28-30*), and Egypt (n = 1) (*33*). Only one study was performed in multiple countries; namely France and the UK (*31*). In terms of study design, five studies (22.7%) used a pre-post design without control arms (*20,21,23,33,40*) while seven studies (31.8%) used a non-randomised prospective interventional design with one or more control arms (*26,34,36-39,41*). Four out of six studies had only one control group while the other two studies had more than one treatment group. The remaining ten studies (45.4%) used a randomised controlled trial (RCT) design (*22,24,25,27-32,35*). Of the ten studies, four were a double-blind RCT (*22,27-29*) and four were a crossover RCT (*24,25,32,35*).

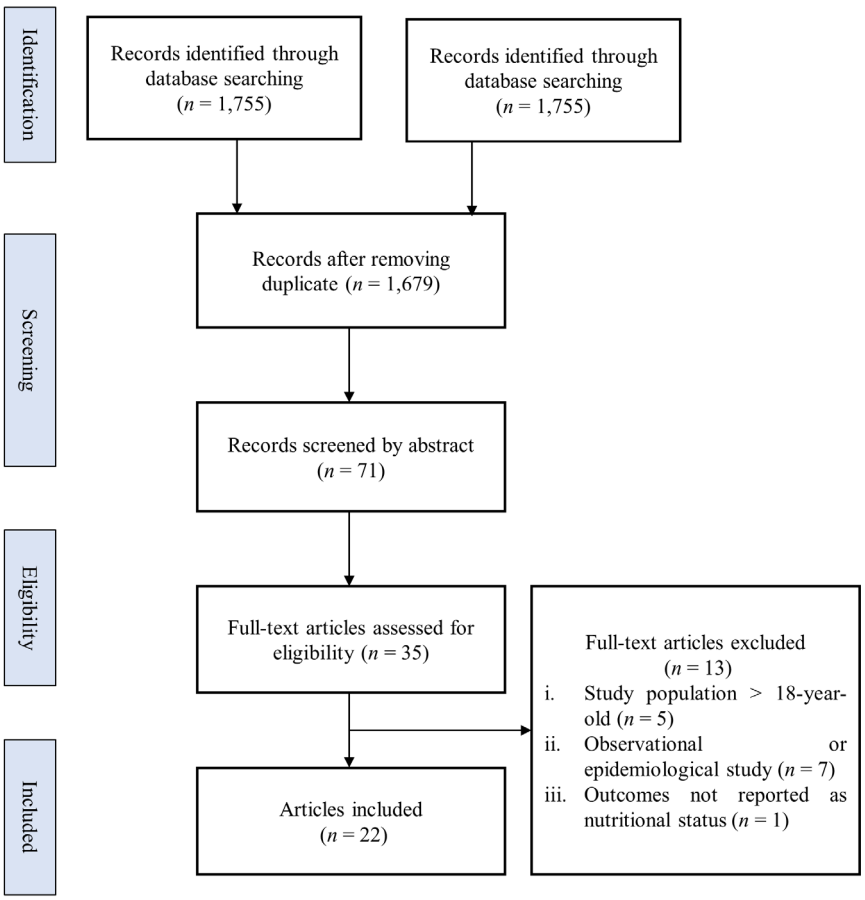

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram depicting the literature search and study selection process.

3.2. Nutritional interventions and outcomes

For the purposes of this scoping review, nutritional interventions were classified into four main categories: *i*) "protein substitute", *ii*) "protein substitute with modified composition", *iii*) "nutrient supplementation", and *iv*) "distribution and dosage of protein substitute". Table 2 depicts a summary of each category of nutritional intervention and outcome measurements.

3.2.1. Medical formula/protein substitute

In this scoping review, the terms "medical formula" and "protein substitute" are interchangeable. Four protein substitutes that had been customised for different types of AANMDs were identified. Two out of five studies revealed an improvement in anthropometric parameters; such as weight and height; as well as biochemical parameters; such as of plasma amino acid concentrations, protein indices, and vitamin indices; in UCD and MMA or PA patients that had been supplemented with medical formulas that were free of essential amino acid and free of methionine and valine, respectively (*20,40*). Apart from that, two studies that utilised a formula enriched with a group of amino acids that compete with lysine and BCAA uptake into the brain demonstrated a significant increment in the uptake of inhibitive amino acid substrate among GAT1 and MSUD patients, indicating the neuroprotective properties of the protein substitute in question. Metabolic control was also found to be significantly improved (*21*). On the other hand, two studies found that GAT1 patients supplemented with a lysine-free, tryptophan-reduced, and arginine-fortified formula had significantly higher total arginine intake than the control group (*38,39*).

3.2.2. Protein substitute with modified nutritional composition

Six studies employed the use of protein substitutes that had modified nutritional compositions as part of their nutritional interventions. The term "modified nutritional composition" was defined as a protein substitute with a different amino acid profile and macronutrient content other than a conventional protein substitute". Of the six studies, five studied the effect that casein glycomacropeptide (CGMP)-based protein substitutes with conventional amino-acid-based protein substitutes had on the weight, metabolic control,

Table 2. Summary of nutritional strategies and outcomes measures

Nutritional strategies	Descriptions
Protein substitute/Medical formula (*n* = 5)	- Formula free of non-essential amino-acid (*20*) - Formula enriched with selenium, zinc, alpha-linolenic acid, and a group of amino acids that compete with BCAA for uptake into the brain (*21*) - Formula free of methionine and valine (*40*) - Medical formula with low lysine (0 mg) and fortified with arginine (90 mg), reduced tryptophan (5mg) (*38,39*)
Protein substitute with modified nutritional composition (*n* = 6)	- Low CHO protein substitute with a CHO/Protein-equivalent ratio 0.5:1 (*35*) - Casein glycomacropeptide (CGMP) based protein substitute (*32-34,37,41*)
Distribution and dosage of protein substitute (*n* = 3)	- Prolonged-release phenylalanine free protein substitute (*30*) - Administration of protein substitute evenly (shorter time gap) throughout the day (*25*) - Higher dosage of protein substitute compared with an alternative dose (*24*)
Nutrient supplementation (*n* = 8)	- Infant protein substitute supplemented with prebiotics (*42*) - LC-PUFA-supplementation (*22,26,28,36*) - EFA-supplemented phenylalanine free formula (*27,29,31*)
Outcomes	**Parameters**
Anthropometric measurements (*n* = 7)	- Body weight (*20,40*) - Body length/height (*19,38,40*) - Body mass index (*20,34,35,37,38,40,41*) - Head circumference (*19,38,40*)
Biochemical parameters (*n* = 15)	- Markers that gauge the effectiveness of metabolic control (*etc*: Phe, Leu, Gly) (*21,23-26,30,32-35,37-41*) - Indices of protein status (*20,21,30,33,40*) - Micronutrients' status (*21,33,40,41*) - Liver and kidney profile (*33*)
Clinical outcomes (*n* = 7)	- Neurological functions (*22,29*) - Cognitive function (*22,26,29,39*) - Rates of cerebral uptake for amino acid substrates (*21,38*) - Gastrointestinal tolerance (*23*)
Dietary Intake (*n* = 10)	- Total energy and macronutrient intake (*21,24,27,31,34,35,37*) - Specific amino acid intake (*25,38,39*) - Essential fatty acid intake (*27,31*)

BCAA: Branched chain amino acid; CHO: Carbohydrate; LC-PUFA: Long-chain polyunsaturated fatty acids; EFA: Essential fatty acids; Phe: Phenylalanine; Leu: Leucine; Gly: Glycine.

and total macronutrient intake of PKU patients (*32-34,37,41*). With the exception of Zaki *et al.* (2016) (*33*), the other three studies concluded that CGMP-based protein substitutes led to a significantly higher blood phenylalanine level (*32,34,41*). Furthermore, it was also found to stabilise phenylalanine concentrations with less fluctuations (*32*). One of the three studies concluded that CGMP-based protein substitutes significantly improved whole blood and plasma selenium levels in comparison to amino-acid-based protein substitutes (*41*). However, some studies did not observe any changes in anthropometric parameters (*34,37,41*). Another study compared the effect of a protein substitute with a lower carbohydrate/protein (CHO/PRO) ratio of 0.5:1 and a traditional protein substitute with a CHO/PRO ratio of 1:1 and found no significant differences in plasma phenylalanine concentrations and weight changes, indicating the feasibility of this protein substitute (*35*).

3.2.3. Nutrient supplementation

Of the 22 studies, the most common nutritional strategy was supplementation with a specific nutrient. Long-chain polyunsaturated fatty acid (LC-PUFA) was the most common nutrient supplement for PKU patients (*n* = 7). It was either added in the phenylalanine-free protein substitute (*27,29,31*) or given as a sole supplement (*22,26,28,36*). LC-PUFA was administered in the form of precursor essential fatty acids (EFA); particularly linoleic acid (LA) and α-linolenic acid (ALA) or their eicosanoids derivatives; such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA). These seven studies demonstrated favourable outcomes in terms of plasma LCPUFA. In terms of physiological outcomes, three studies used visual evoked potentials (VEPs) as an indicator of neurological (visual) function (*22,29*) while four studies assessed motor development and mental

or cognitive performance (*22,26,29,39*). One study found a significant improvement in visual function; as evidenced by a shortening in the VEPs (*36*); while another study reported a significant improvement in the motor development index (*26*). Additionally, one pilot intervention study that examined the tolerability and efficacy of a protein substitute supplemented with prebiotics revealed a significant reduction in median stool pH among PKU patients (*42*).

3.2.4. Distribution and dosage of protein substitute intake

Two studies investigated the effects of protein substitute distribution on blood phenylalanine control. A direct and traditional method is to manipulate the timing of the protein substitute by increasing the frequency of administration throughout a more extended period in a day (*25*). The other method is to prolong the release or duration of action of a conventional protein substitute to mimic the effect of frequent protein substitute administrations by adding a chemical substance known as sodium alginate (*30*). Both studies showed a significant improvement in blood phenylalanine control; as evidenced by lower phenylalanine values and smaller fluctuations in 24-hour plasma phenylalanine. Apart from achieving better metabolic control, better protein indices were also reported. In terms of dosage, one study found that participants on the higher doses of protein substitutes had a significant decrease in median plasma phenylalanine concentrations (*24*).

4. Discussion

By adopting the review protocol described in PRISMA-ScR (PRISMA extension for Scoping Reviews), this scoping review successfully addressed and detailed the evolution of nutritional interventions for AANMDs by reviewing literature published over the past 20 years. Unlike existing studies that only address the historical evolution, dosage, and distribution of protein substitutes among PKU patients (*43,44*), this scoping review contributes to existing knowledge by exploring multiple nutritional interventions and their impact on the nutritional indices of different types of AANMDs instead of a single disorder.

This scoping review demonstrated that most nutritional interventions incorporate a medical formula or protein substitute of different properties and characteristics as a treatment plan. Apart from that, it also found that most of the selected studies involved participants that had been diagnosed with PKU. One possible explanation for these two findings is the successful nutritional intervention of PKU; the paradigm AANMD; using a low-protein or phenylalanine-free formula back in the 1960s (*45*). Since the successful treatment of PKU, medical formula has emerged as a cornerstone in AANMD management. The primary treatment mechanism of medical formulas lies in their nutritional compositions which omit substrates that are associated with neurotoxicity, hence preventing neurological damage and metabolic crisis (*46*). As seen in the studies included in this scoping review, the medical formulas of the early 2000s supplied patients with complete and balanced macronutrients as well as additional vitamins and minerals to meet the recommended nutrient intake and improve growth and overall protein indices (*20,40*). As one of the medical ethical principles of healthcare research is non-maleficence, it is not feasible to conduct a randomised controlled trial (RCT) to assess the efficacy of medical formulas by excluding medical formula from the normal diet of some participants as it will endanger their health (*47*). As such, a pre-test post-test interventional study is the best study design with which to investigate the effect of supplementing AANMD patients with medical formulas.

Over the past two decades, significant advances in the clinical neurological research of metabolic diseases have facilitated the creation of improved medical formulas. Prior to a study by Strauss *et al.* (2010) (*21*), the principle of competitive inhibition in the dietary treatment of amino acid disorders was largely unknown. Thanks to this neuroimaging study, it is now well known that large neutral amino acids; such as phenylalanine, tryptophan, leucine, methionine, isoleucine, tyrosine, histidine, valine, and threonine; as well as cationic amino acids; such as lysine and arginine; are transported across the blood-brain barrier (BBB) *via* the common transporters; L1-neutral amino acid transporter (LAT1) and cationic transporter (y^+ system); respectively (*48,49*). This principle has been played a critical role in the formulation of a new medical formula as the theory posits that a high concentration of a single acid or a group of amino acids will inhibit the uptake of another offensive amino acid as they share a common transporter thereby reducing exposure to cerebral toxins. Unlike the traditional formula which only omits the offending amino acids, the improved medical formula is enriched with amino acids that compete with leucine or lysine for brain uptake thereby increasing the potential of improving metabolic control.

Dietary supplementation is another treatment modality for AANMD patients. Studies have established that patients diagnosed with PKU, MSUD, and other AANMDs have significantly lower concentrations of plasma, erythrocyte docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) primarily due to a vegan-like diet which excludes DHA-rich food; such as fish and other whole animal food (*50-52*). Given the pivotal role that DHA plays in visual and neurological development, PKU patients are supplemented with long-chain polyunsaturated fatty acids (LCPUFA) to improve plasma fatty acid indices. The role of LCPUFA supplementation in improving plasma DHA indices

has sparked considerable research into supplementing medical formulas with either precursor essential fatty acids or their eicosanoids derivatives; such as DHA and/or AA; to address fatty acid deficiencies among children with AANMDs. While it has been assumed that the conversion of ALA to EPA to DHA is limited in humans (*53,54*), the studies presented thus far demonstrate that both types of supplementation lead to significant improvements in plasma DHA indices as well as total essential fatty acid intake. This suggests that ALA sources; such as safflower or canola oil; can be used as an alternative but it can be incorporated into protein substitutes to improve palatability. Nevertheless, there is mixed evidence on the ability of LCPUFA supplementation to improve neurological function among PKU patients. However, the different outcomes might be due differences in the testing instruments that were used to assess cognitive and mental development.

It has been hypothesised that patients of metabolic diseases are at higher risk of an imbalance in the microbiome due to the nature of their disease requires them to adhere to a strict dietary regime. To reduce the risk of accumulating toxic compounds, such dietary regimes always lack elements or nutrients that the patient is unable to metabolise (*55*). For instance, a breastfeeding-restricted infant will lack the oligosaccharides normally present in breast milk. This renewed interest in introducing a phenylalanine-free infant formula that contains a specific mixture of prebiotic oligosaccharides for PKU children. The results of a small-scale pilot study showed a significant reduction in stool pH while maintaining *bifidobacteria* levels, which reduces the risk of infection (*23*). However, due to small sample size, the findings of this study cannot be extrapolated to the whole PKU population. Therefore, a larger sample size should be gathered prior to conducting future studies on prebiotics in infant protein substitute to fully evaluate the health benefits.

There has been an increase in the number of studies investigating alternative sources of protein substitutes in recent years. Due to its unique physiological properties, casein glycomacropeptide (CGMP), a whey protein derived from cheese production, has attracted considerable interest as a potential substitute for phenylalanine-free amino acid-based formulas. CGMP had been proven to promote satiety (*56*), decrease the rate of amino acid absorption, thereby reducing fluctuations in plasma phenylalanine (*57,58*); and improve taste acceptability and palatability of the medical food and formula, thereby improving dietary compliance (*59*). However, although this whey protein derivative demonstrated some physiological benefits in adult participants, it contains residual phenylalanine (equivalent of ~1.8 mg/g cGMP protein) which might adversely affect metabolic control in PKU patients (*60*). Based on the included studies, children and adolescents who were given CGMP-AA, a mixture of CGMP and essential and conditional amino acids, had significantly higher levels of plasma phenylalanine in comparison to those given a phenylalanine-free formula. However, these findings differ from a meta-analysis (*61*) and few clinical trials which only found a negligible difference in phenylalanine concentrations between the two groups (*58,62,63*). Participant age could explain the disparity of the findings as the only participants included in this scoping review were children and adolescents while the other studies included adults. As studies have shown that phenylalanine concentrations in PKU patients increase with age, it may mask the significant increase in phenylalanine concentrations among adult participants prescribed with CGMP (*64*). Furthermore, children are more susceptible to fever and recurrent infections which may further increase phenylalanine fluctuations (*61*). Nevertheless, the introduction of CGMP as a replacement for traditional phenylalanine-free protein substitutes has successfully improved acceptability in terms of taste, mouthfeel, texture, and smell among PKU children (*34*). It has been suggested that adjusting the amount of dietary phenylalanine from natural food among PKU patients receiving CGMP-AA protein substitute can significantly lower phenylalanine concentrations (*32*). Furthermore, studies have acknowledged the critical role that large neutral amino acid (LNAA) play in decreasing blood phenylalanine concentrations by inhibiting and competing with phenylalanine in the gastrointestinal tract and across the blood-brain-barrier (*65*). Studies involving adult participants reported some benefits of supplementing PKU patients with LNAA among which was decreased phenylalanine concentrations (*66*), increased tyrosine concentrations, which is always lower than reference value among PKU patients (*67*); and improving cognitive performance (*68*). Nevertheless, there is no direct comparison between the effects of increased LNAA in CGMP-AA protein substitute on metabolic control and a CGMP-AA formula to meet the minimum safe amino acid intake levels. Hence, we recommend that future studies attempt to identify an optimum amino acid profile of CGMP-AA protein substitute for safe metabolic control and taste acceptability among PKU children given the physiological benefits of CGMP-AA protein substitute.

The rapid absorption kinetics of the free AA in protein substitutes is known to affect the plasma phenylalanine concentrations of PKU patients over time (*69*). This has led to new technology in the dietary approach of PKU treatment, more specifically a "prolonged release" amino acid formula capable of mimicking the physiological protein absorption kinetics of healthy children (*65*). Although benefits were observed in terms of metabolic control and protein indices (*30*), the general lack of research on this type of protein substitute warrants more studies in this emerging field in order for PKU children to reap the most nutritional benefits. Furthermore, more

large-scale and multicentre studies of PKU patients need to be conducted to determine the optimum dosage of protein substitute required to support normal growth and control phenylalanine indices.

The key strength of this scoping review is the inclusion of nutritional indices; such as anthropometry, biochemical, clinical, and diet; as it helps paint a clearer picture of the clinical effects of a particular nutritional treatment which leaves ample room for discussion. However, this scoping review is not without its limitations. Firstly, although this scoping review was only limited to interventional or experimental study designs, most of the studies did not utilise RCT; the gold standard of measuring the efficacy of a new intervention or treatment (*70*). Some studies utilised a quasi-experimental design, where the intervention and control groups were not selected at random; while some studies did not even have a control group. Therefore, it was impossible to conclusively deduce if changes in nutritional indices and metabolic control were due entirely to the intervention in question (*18*). Secondly, the sources of information used to search for relevant studies were limited to online electronic databases and hand-searching reference lists of past systematic and integrative studies. Furthermore, the exclusion of grey literature, such as unpublished thesis, technical reports, conferences, and proceedings may have resulted in fewer studies being included in this scoping review. As such, the efficacy of each nutritional intervention could not be determined due to the small number of studies on each intervention. Lastly, a statistical analysis was not carried out to pool the effect of each nutritional intervention and its study outcome.

5. Conclusion

In conclusion, the prescription of protein substitutes remains at the core of dietary treatment for AANMDs. Major advances in nutrition and dietetics research have facilitated the development of protein substitutes with modified nutritional properties and components; such as CGMP-based protein substitutes, prolonged-released protein substitutes, and protein substitute fortified with specific nutrients or certain amino acids; that have a neuroprotective effect. This scoping review showed that formula supplemented with LC-PUFA has a positive impact on plasma DHA and EPA indices as well as dietary fatty acid intake. Similarly, fortifying protein substitutes with specific amino acids holds great promise according to the neurological concept proposed in this scoping review. At the same time, there is a great potential of administering prolonged release protein substitutes in improving metabolic control. Nevertheless, the safety and efficacy of CGMP-AA protein substitute in the treatment of PKU children remain unclear. In the future, efforts should be increased to study the effects of prebiotic supplementation and a protein substitute with a lower carbohydrate (CHO) content as only a handful of studies have been conducted. Furthermore, due to the negligible number of studies on non-PKU-related AANMDs, we urge that more research on novel nutritional strategies, be it new protein substitute formulations, nutrient supplementation, or innovations in nutritional education; such as using mobile technology; be carried to cater to the nutritional needs of patients with other AANMDs.

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Mini-Review

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A review of Alström syndrome: a rare monogenic ciliopathy

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SUMMARY Alström syndrome is a rare monogenic ciliopathy caused by a mutation to the *Alström syndrome 1* (*ALMS1*) gene. Alström syndrome has an autosomal recessive nature of inheritance. Approximately 1,200 cases of Alström syndrome have been identified worldwide. Complications of the disease are likely caused by dysfunctional cilia with complications arising early in life. The known complications of Alström syndrome have been reported to impact multiple major organ systems, including the endocrine system, cardiac system, renal system, sensory system, and hepatic system. The symptoms of Alström syndrome have great variability in presentation and intensity but often lead to organ damage. This has resulted in a shortened lifespan for individuals affected by Alström syndrome. Individuals with the disease rare exceed the age of 50. Currently, there are no specific treatments for Alström syndrome that can cure the disease, prevent the complications, or reverse the complications. Current management involves management of symptoms with the goal of improving quality of life and lifespan. This review aims to summarize the current knowledge on the epidemiology, diagnosis, pathophysiology, complications, management, and prognosis of Alström syndrome. In addition to that, this review also aims to raise awareness and encourage research on Alström syndrome as the condition has a huge impact on affected individuals.

Keywords genetic disorder, rare disease, cilia, reduced lifespan

1. Introduction

Alström syndrome is a rare condition that was first revealed in the literature in 1959 by Carl-Henry Alström from Sweden (*1*). The syndrome is an autosomal recessive genetic disorder. The disorder is caused by a mutation to the *Alström syndrome 1* (*ALMS1*) gene and affects many systems in the body (*2,3*). The symptoms of Alström syndrome usually first arise in infancy and further develop during childhood and later in life. Common complications of Alström syndrome include endocrine complications, cardiac complications, renal complications, hepatic complications, complications with vision and complications with hearing (*4*). The symptoms and complications of Alström syndrome vary greatly in severity. Currently there are no specific treatments for Alström syndrome with the management currently involving management of symptoms with the goal of improving quality of life and lifespan (*2*).

This review aims to summarize the current knowledge of Alström syndrome. Furthermore, this paper also aims to promote further research and develop awareness of Alström syndrome.

2. Epidemiology

Alström syndrome is a rare condition, and while current incidence is unknown, estimates have created a range from 1 in 500,000 to 1 in 1,000,000 (*5*). The rare nature of the disease has potentially resulted in many cases of Alström syndrome being undiagnosed. Worldwide, approximately 1200 cases of Alström syndrome have been identified (*6*). The condition affects both sexes equally (*7*).

3. Pathophysiology

Alström syndrome is caused by a mutation in the *ALMS*1 gene. It is an autosomal recessive condition meaning that both copies of the *ALMS1* gene need to be mutated for the syndrome to occur (*2*). The *ALMS1* gene is located on the short arm of chromosome 2 (*8,9*).

The pathophysiology of the symptoms of Alström syndrome has potential links to the ALMS1 protein. The ALMS1 protein is a protein that is found in primary cilia within the centrosomes and the basal bodies. The protein is comprised of 4,169 amino acids (*10*). ALMS1

protein has a significant impact on the function of cilia, with a paper published in 2007 suggesting that the absence of the protein results in an impairment in the formation of cilia (*11*). In addition to that, the ALMS1 protein has also been shown to be related to energy metabolism homeostasis, cell differentiation, ciliary signalling pathways, cell cycle control and intracellular trafficking (*9*). These facts aid in the classification of Alström syndrome as a ciliopathy, a disorder that results in abnormal cilia function or formation (*12,13*). While more research needs to be conducted on the pathophysiology of Alström syndrome, there appears to be a significant relationship between the *ALMS1* gene, ALMS1 protein, cilia function and the disorder.

4. Diagnosis

Due to the wide range of symptoms and great variability in presentations, the diagnosis of Alström syndrome is often challenging. Furthermore, certain symptoms have a delayed presentation until later in life, meaning that the diagnosis during early life is often missed (*14*). The characteristic features of Alström syndrome often become more evident as a child grows, meaning that the diagnosis and clinical suspicion of Alström syndrome become more evident in late childhood. A study published in 2007 established a set of criteria that aid in the diagnosis of Alström syndrome (*2*). The molecular diagnosis of Alström syndrome is established when a patient is found to have two *ALMS*1 mutations, with one mutation coming from each parent. This screening is done by molecular genetic testing (*14*).

5. Complications

The main complications of Alström syndrome are summarized in Table 1.

5.1. Endocrine complications

Alström syndrome has an impact on endocrine and metabolic function resulting in endocrine complications. The main endocrine complications are related to growth, pubertal development, obesity, and diabetes mellitus (*15*).

A common complication of Alström syndrome is obesity and early onset diabetes mellitus. *ALMS1* mutations allow glucose transport through the actin cytoskeleton, which helps in insulin-mediated Glucose transporter type 4 (GLUT4) transport. Among the various metabolic derangements in cases with Alström syndrome, a study shows that 76% had obesity and 37% had type 2 diabetes mellitus. These patients had very high fasting and mixed-meal test (MMT) insulin resistance indices, higher MMT glucose, insulin, and C-peptide values, higher haemoglobin A1c (HbA1c), and higher prevalence of type 2 diabetes mellitus (*16*).

Table 1. Summary of the major complications of Alström syndrome

Items	Complications
Endocrine	Obesity and early onset diabetes mellitus Short Stature Hypogonadism and testicular fibrosis (in males) Gynecomastia (in males) Issues with menstruation (in females) Poor breast development (in females)
Cardiovascular	Dilated cardiomyopathy Early onset coronary artery disease Early onset Hypertension
Sensory	Progressive vision loss Progressive sensorineural hearing loss
Renal/Urinary	End-stage renal disease Renal cysts Recurrent urinary tract infections
Hepatic	Non-alcoholic fatty liver disease Cirrhosis
Respiratory	Recurrent respiratory tract infections

It has been found that extreme insulin receptor and β-cell failure are the two main factors responsible for the glucose metabolism alterations in Alström syndrome (*17*). A cross-sectional cohort study showed that patients may develop early child obesity, but the body mass index, waist circumference, and body fat decreased with age as insulin resistance increased. This insulin resistance is also associated with increased levels of triglycerides in patients with Alström syndrome (*15*).

Growth complications are also a major endocrine issue of Alström syndrome. Almost 98% of adults with Alström syndrome are in the 5th percentile or less for height. The growth complications are likely to arise during puberty as studies conducted have shown that pre-pubertal heights of children with Alström syndrome are not dissimilar to their peers (*18*). No study has specifically investigated growth hormone (GH) deficiency in a large population. Small sample studies have shown that Alström syndrome patients may be functionally GH deficient. Tested Alström syndrome patients had an inadequate GH reserve to growth hormone release hormone-arginine (GHRH-arg) leading to a low growth velocity despite advanced bone ages and normal insulin like growth factor 1 concentration. Growth hormone deficiency accounts for the short stature in some patients with Alström syndrome, while the advanced bone age and normal early growth may be due to hyperinsulinism (*19,20*). A specific defect in the signal transduction of insulin action accounts for the existence of insulin resistance in the presence of growth hormone deficiency (*21*).

Pubertal development is another endocrine complication of Alström syndrome. In males, hypogonadotropic hypogonadism and testicular fibrosis

have been reported to halt or delay puberty. This has resulted in patients with Alström syndrome developing gynecomastia (*18*). In females, insulin resistance has resulted in patients with low concentrations of plasma gonadotropin, which have resulted in symptoms such as hirsutism, irregular menses, amenorrhea, or precocious puberty. Breast development is often poor in patients with Alström syndrome (*22*). Limited studies have been done on male or female fertility in patients with Alström disease, although there have been reports of females with the condition that have given uncomplicated birth to healthy infants (*23*).

5.2. Cardiac complications

Cardiac complications are common in Alström syndrome. The severity and symptoms of cardiac complications have great variability in patients with Alström syndrome (*24*). In Alström syndrome, heart failure due to a form of dilated cardiomyopathy is a frequent finding, occurring in approximately 60% of cases and representing the most common cause of death (*25,26*). The mechanism of cardiomyopathy is not currently known (*27*).

Dilated cardiomyopathy presents in infancy in 50% of cases and, if treated successfully, apparent atypical recovery of cardiac function within 3 years can occur with restitution of near normal cardiac function into adult life (*26*). Importantly, infantile congestive heart failure can recur in adolescence or adulthood with a poor prognosis for affected patients (*26,28*).

There are suggestions that hemodynamic changes associated with large artery stiffening have been shown to lead to maladaptive changes of myocardial hypertrophy and can contribute to development of left ventricular failure in patients with Alström syndrome. However, in a study published in 2007, it did not find clear associations between left ventricular structure and function and parameters of large artery function, suggesting that primary cardiac pathology is likely to play an important role in the pathogenesis of cardiomyopathy in Alström syndrome (*26*). The primary cardiac pathology concept is supported by another paper published in 2017, which states that although some patients had a history of significant cardiac risk factors for many years, they were unable to find a relationship between metabolic derangements and cardiac abnormalities (*27*). Furthermore, another study, which was published in 1996 found that clinically and histologically, the cardiomyopathy associated with Alström syndrome in their five patients was indistinguishable from other sporadic and familial forms of isolated cardiomyopathy (*25*).

Histopathology of the heart in such cases has not revealed any specific findings other than a variable degree of cardiac dilatation and fibrosis (*26,29*). Most cases of myocardial fibrosis are gradual and non-reversible; so, the infantile onset of dilated cardiomyopathy and the high rate of resolution are difficult to explain. As an example a paper published in 2012 speculates that the myocardial fibrosis and the infantile dilated cardiac myopathy may follow different pathogenic processes (*28*).

Comorbidities associated with Alström syndrome could also lead to early onset coronary artery disease. A 2012 study suggests that patients with Alström syndrome should be assessed for classical coronary risk factors and investigated specifically to exclude coronary artery disease (*30*).

Echocardiography is critical in establishing the diagnosis, guiding therapy, and determining an overall prognosis (*31*). Cardiac magnetic resonance imaging not only provides pathological insights, but gives a chance to detect early functional changes, track the natural history and progression of the disease, and assess the impact of therapeutic interventions, as well as guide referral for transplantation in patients with Alström syndrome (*32*). Early diagnosis allows greater opportunities to introduce therapies for cardiac complications of Alström syndrome. This is particularly true of cardiomyopathy, which presents acutely in childhood in 45% of cases and is potentially fatal if unrecognized and recurs (*33*).

5.3. Sensory complications

Progressive loss of vision is a common finding in cases of Alström syndrome. Like many other features of Alström syndrome, disease severity and age of onset can differ considerably. The process of visual impairment begins within the first decade of life, usually between birth and 15 months of age (*22*). The impairment begins with the loss of visual acuity, photophobia, and horizontal nystagmus (*2,22,34*). This is followed by loss of residual light perception, which eventually progresses to complete loss of light awareness within the second decade of life (*22*). Photopic electroretinogram findings in the beginning of the disease show evidence of cone dystrophy. Follow-up photopic electroretinogram testing reveals how rapidly the visual impairment progresses to typical cone-rod dystrophy (*35*).

Auditory impairment is also a classic complication of Alström syndrome. The onset and severity of hearing loss can vary significantly between individuals. The hearing loss is progressive, and up to 70% of patients develop some degree of high frequency sensorineural hearing loss within the first decade of life (*36*). Many patients with Alström syndrome suffer from chronic otitis media, which contributes to progressive hearing loss (*4,36*). Histopathology findings of a 2015 study have shown atrophy of the stria vascularis and degeneration of the organ of Corti (*37*). This may play a role in the pathophysiology of auditory impairment.

5.4. Renal complications

Renal complications are common in patients with Alström syndrome. The renal complications have high variability and often slowly progress. Onset of symptoms and complication are usually late in childhood or during adulthood. Significant renal complications include end-stage renal disease, which can occur during late childhood in some patients (*38*). Renal cysts are another renal feature of Alström syndrome (*39*). Interstitial fibrosis and hyalinization of tubules are visible on histopathological investigation (*40*).

5.5. Hepatic complications

Complications related to the liver are common in most patients with Alström syndrome although there is great variability in symptoms and severity. Elevated liver enzymes are often present since childhood and patients with Alström syndrome often have a high liver fat concentration (*15*). Portal hypertension and associated symptoms are also a common hepatic complication of Alström syndrome. Non-alcoholic fatty liver disease and cirrhosis are also more likely in patients with Alström syndrome when compared to the general population (*41*). Chronic active hepatitis, steatohepatitis, hepatic fibrosis, and cirrhosis are common findings on liver biopsy in patients with Alström syndrome (*42*).

5.6. Other complications

Upper and lower respiratory tract infections are often a complication of Alström syndrome. The symptoms very in severity and can result in pulmonary fibrosis, pulmonary hypertension, and impaired pulmonary function (*2,14*).

Urinary symptoms are also a complication of Alström syndrome with patients presenting with lower urinary tract symptoms and abdominal pain prior to urination. Recurrent urinary tract infections and urinary strictures can also arise as a complication (*2,43*).

Hypertension is also a common and significant complication of Alström syndrome. Studies have shown that approximately 40% of patients with Alström syndrome have hypertension. Furthermore, there have been cases of patients with hypertension at an age of 2 (*2,14*).

6. Management

Currently there are no specific treatments for Alström syndrome that can cure the disease, prevent the complications, or reverse the complications. A multidisciplinary approach is currently preferred to detect, predict, and treat the complications of Alström syndrome. Regular monitoring *via* blood tests of levels of various markers including: liver enzymes, blood glucose and gonadotropins are suggested. Cardiac monitoring with the use of echocardiography is also suggested. Management of sensory deficits is essential, and this is especially significant in young children. A study published in 2007 has outlined the assessments and interventions that should be considered in patients with Alström syndrome (*2*) (Table 2).

Table 2. Common assessments/investigations in patients with Alström syndrome

Items	Nvestigations/Assessments
Endocrine	Fasting blood glucose, HbA1c Height, weight, body mass index Gonadal function test (in males)
Cardiovascular	Electrocardiography Echocardiography Blood pressure
Sensory	Visual acuity Electroretinogram Audiometric assessment
Renal/Urinary	Urinalysis Renal function test
Hepatic	Liver function test/liver enzymes

7. Prognosis

Due to the wide range of symptoms, complications and severities of complications, the prognosis for patients with Alström syndrome can greatly vary. That being said, the disease often results in severe complications such as organ failure. As a result, patients with Alström syndrome generally have a reduced lifespan. Patients rarely exceed the age of 50 (4).

8. Conclusion

Alström syndrome is a rare genetic disorder caused by mutations to the *ALMS1* gene. Complications of the disease are likely caused by dysfunctional cilia with complications arising early in life. The symptoms of Alström syndrome have great variability in presentation and intensity and can affect a great range of organ systems. Complications include: endocrine complications, sensory complications, renal complications, hepatic complications, and cardiac complications. Due to the wide range of complications and the high rates of organ damage and failure, lifespan is greatly reduced in individuals with Alström syndrome. There are no specific treatments for Alström syndrome with the current treatment only aiming to manage the complications of the condition. Alström syndrome has a huge impact on affected individuals, and more research should be conducted on this subject.

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Brief Report

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Effects of a personalized home-based training program among patients suffering from Marfan syndrome: a pilot randomized and controlled study

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SUMMARY Marfan syndrome (MFS) is an autosomal hereditary pathology affecting 1:5000 peoples. Alteration of the fibrillin 1 gene (FBN1) results in haplo-insufficiency of the FBN1 protein mainly altering the vascular system. International recommendations have gradually allowed MFS patients to perform training programs because of its potential benefits. However, to date, there are no data on the effect of a long training period in these patients. The aim of the present study is to investigate the effect of a 3-month personalized home-based training on quality of life (QoL) of patients suffering from MFS. At least 50 MFS patients were included in the study. They were randomly placed into 4 groups: control group; endurance; resistance and endurance + resistance training groups. The training program lasted 3 months and is performed at patients' home. There were 2 training sessions per week telemonitored by a specialist of physical activity and cardiology. Pre and post-training evaluations were performed at the Bichat-Paris Hospital, France. They consisted of assessing psychometrics based on self-administered questionnaires (FiRST, GPAQ, ISP-25, MOS SF-36) and physiological parameters such as the peak oxygen consumption, aorta diameter, cardiac ventricle function and skeletal muscle power at rest and during exercise. Our preliminary results showed an improvement of 50% in QoL, cardiorespiratory fitness and skeletal muscle power in a patient who completed the combined training program. This experimental approach might be a new alternative way for MFS patients' care that may improve their QoL, cardiorespiratory fitness and skeletal muscle power.

Keywords Marfan, telerehabilitation, personalized training

1. Introduction

Marfan syndrome (MFS) is an autosomal dominant genetic pathology affecting the cardiovascular system, the respiratory system, skeletal muscles, bones and eyes. This syndrome is the result of a mutation in the gene encoding fibrillin type I protein (FBN1), which forms a microfibrillar network interacting with elastin fibers to form the standard extracellular matrix.

In MFS patients, the most serious risk is a progression to aortic dissection, which is associated with a high rate of mortality (*i.e.* 1% per hour during the first 48 hours). Several studies have highlighted anomalies in the left ventricle (LV) size, function, myocardial deformation and myocardial fibrosis (*1,2*). It has also been shown that the cause of LV dysfunction was also due to mutation of the FBN1 gene, related to the Transforming Growth Factor (TGF) pathway (*3*). Patients also have a slender morphotype with low muscle mass (*4*). In addition, they suffer from a decrease maximal quadriceps strength associated with a decrease in muscular mass (*5*). Musculoskeletal pains reduce endurance capacity as well (*6*).

All the aforementioned disorders are associated with pain and disability, which affect professional activity, leisure, and family life. Furthermore, aortic dilation and the associated risk of aortic dissection are a source of anxiety for these patients (Peters *et al.* 2001) and depression is often found in patients MFS (*7*). In addition, quality of life (QoL) assessed by the SF-36 questionnaire is very impaired in these patients (*8*), particularly in patients with the largest aortic dilations.

Therefore, it is essential to find a new global approach that should be proposed to reduce the cardiovascular impact but also the psychological impact (*7*) of MFS.

Physical activity (PA) may play a vital role in secondary aortic dilation and dissection prevention in patients with MFS in association with routine clinical management based on prescription of treatment with beta-blockers and annual monitoring of aortic diameter (*9*). As reported by the new 2020 guidelines on sports cardiology and exercise in patients with cardiovascular disease of the European Society of Cardiology (ESC) (*10*) the prescription of regular physical activity is highly beneficial for patients with cardiovascular diseases and reduced their mortality. In patients with aortopathy, regular PA has a positive effect on hypertension and concomitant future risk of dissection. Moreover, regular PA appeared to be well tolerated in MFS patients and induced beneficial physical effects (*11*). However, to our knowledge, this is the only existing small prospective cohort study conducted in these patients and there are no randomized controlled trials or any prospective data regarding risks of more intensive PA practice in MFS patients. Finally, home-based training may represent a new alternative way for MFS patients' care. Indeed, it has been demonstrated that heart rate monitoring and the Internet were effective tools for management of patients after coronary artery disease by improving their cardiopulmonary fitness after a 12-week home-based program (*12*).

In this context, the present study aimed at determining the impact of a 3-month personalized home-based training on the QoL of MFS patients.

2. Materials and Methods

This study was designed as an interventional, monocentral, prospective and randomized controlled trial at the Marfan National Reference Center in the Hospital of Bichat, Paris, France. They were 3 phases during the study: Phase 1) baseline evaluations at the hospital; Phase 2) post 3-month home-based personalized training evaluations at the hospital; Phase 3) Phone call evaluations 3 months after the end of training. All patients involved in the study provided written informed consent before entering the study. The study complies with the Declaration of Helsinki. The institutional ethics committee approved the study protocol (#2020-A01751-38), the French Society of Cardiology promotes the study and informed consent will be obtained from the subjects.

2.1. Population

Patients with MFS aged between 18 and 65 years were included in the study. The patients had to receive an optimal and stable dose of ACE inhibitors and Beta-blockers. They also had to be able to realize physical exercise and to possess social security insurance. The contradictions for randomization in the study were: pregnant patients, patients with cardiovascular diseases not due to MFS, an aortic dissection, an aortic diameter > 45 mm, an aortic regurgitation, a non-controlled resting hypertension (diastolic blood pressure > 90 mmHg and systolic blood pressure > 140 mmHg), who were non reachable by phone, who participated in another research project, who refused or who were not able to sign the informed consent form. Participants eligible for research were randomized into 4 experimental groups: Group 1 (control: no training), Group 2 (aerobic training), Group 3 (muscle strengthening training) and Group 4 (endurance + resistance training). The R program, with envelopes opened when the patient was randomized generated a randomization list.

2.2. Evaluations during visits 1 and 2

Cardiovascular capacity at rest was assessed before and after the training program. Patients underwent the following evaluations: *i*) Electrocardiogram (ECG) (200S-Cardioline). *ii*) Resting blood pressure assessment using an automatic tensiometer (dynamap®). *iii*) A standard echocardiography and a tissue Doppler imaging were performed to assess the ejection fraction (EF) of the left ventricle (LV), its diastolic diameter (LVDD), the systolic interventricular septum thickness (IVSTs), the global systolic and diastolic function of the LV (*i.e.* S, E and A waves of the lateral and septal walls) and the right ventricle (RV) (*i.e.* S wave, TAPSE and E/A peak), a full aorta analysis (*i.e.* ring, Valsalva sinus, tubular aorta, arch, descending thoracic aorta, and abdominal aorta) and a measure of the vena cava diameters during inspiration and expiration (Vivid 9 Dimension® ultrasound apparatus-GE Healthcare). A 2D strain echocardiography was performed to assess the global systolic longitudinal strain (GLS) of the LV and the RV.

2.3. Assessment of oxygen consumption (VO_2) before and after the training program

Patients performed a VO_2 test on an ergometric bike. This exercise intensity was increased by 10 watts every minute until patients reach a peak of VO_2 or until voluntary cessation. The ventilatory threshold 1 (VT1), the oxygen absorption efficiency slope (OUES), the ratio ventilation (VE) on carbon dioxide production (VCO_2) (VE/VCO_2) as a prognostic value was measured and a spirometry performed to evaluate the following parameters: forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC and peak expiratory flow 75% (PEF75).

2.4. Assessment of heart rate and blood pressure

Heart rate (HR) was monitored during the test. Two HR references were obtained, one at the VT1 (HRVT1) and

one at the peak of (HRpeak VO_2). HRVT1 + 10-20% was used for targeting the intensity of training sessions and HRPeak VO_2 was the HR limit to be reached during training sessions. The systolic blood pressure (SBP) and the diastolic blood pressure (DBP) were also carried out during the test. If the SBP was ≤ 160 mmHg for at least 20 minutes, then the patient was allowed to continue the research. If this SBP was > 160 mmHg during exercise, then the patient was excluded from research (*11,13-15*). In parallel, the rating of perceived exertion (RPE) was evaluated during the test (*16*).

2.5. Muscular capacity evaluations

Patients performed one countermovement jump (cm), one squat jump (cm), repeated jumps (nb/min) and an evaluation of the one-repetition maximum (1RM) based on 3 consecutive squats. The muscular capacity evaluations consisted of the evaluation of the muscle power and the maximal force contraction using a linear encoder (Gymaware®).

After these muscular capacity evaluations, patients performed different types of muscle-building movements under supervision (*i.e.* step, dynamic squat, isometric squat, crunch, abdominal and low back sheathing, isometric contraction of the quadriceps (chair position) and concentric contractions of the biceps (dumbbells of 1-5 kg) (Supplemental Figure S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=82*).

2.6. Body composition

The body composition of MFS patients was measured using a bioimpedance scale (Tanita Body composition Analyzer BC-420MA). The bodyweight and the percent of fat mass (BF%), of lean mass (LM%) and of water were assessed. The body mass index (BMI) was calculated using the following formula: BMI = kg/m^2, where kg is a patient's weight in kilograms, and m^2 is the patient's height in meters squared.

2.7. Psychometric questionnaires

The assessment of QoL, level of physical activity, pain and pre-workout self-esteem were given to all patients included in the study. QoL was assessed by the MOS SF36 (Medical Outcome Study Short Form - 36) (*17*) questionnaire. The GPAQ (Global Physical Activity Questionnaire) (*18*) evaluated daily physical activity in sixteen questions. The FiRST (Fibromyalgia Rapid Screening Tool) scale assessed pain of patients (*19*) and the ISP-25 (Inventaire du Soi Physique-25) questionnaire assessed self-esteem of patients (*20*).

2.8. Personalized home-based training program

The training program followed the last ESC guidelines recommendations for physical activity (*10*). All patients included in training groups performed a 3-month personalized home-based training program with 2 sessions per week (Figure 1). The prescribed intensity of exercise was based on recommendations made by the European and American learned societies (*9,21*). These sessions were performed according to the level of patients evaluated during the initial cardiopulmonary and muscle capacity tests.

2.8.1. Endurance training (Group 2)

Endurance training consisted of a training circuit (TC) to lead patients to reach a target HR intensity evaluated during visit 1 baseline evaluations (Table 1). The intervals of recovery times, efforts, and number of series were adapted according to aerobic capacity of the

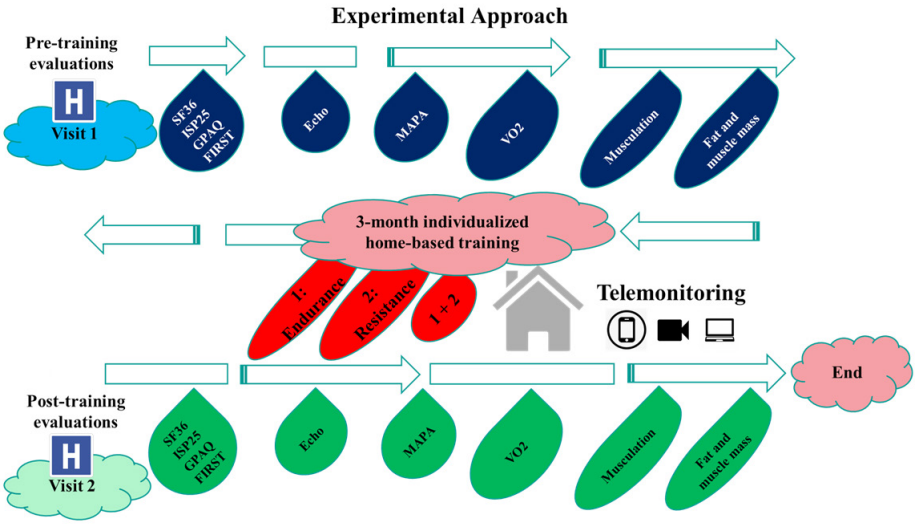

Figure 1. Illustration of the experimental approach.

Table 1. Description of the endurance training circuit

Time × number se-ries	Total time	Exercise time	Recovery time	Number of workshops	RPE scale
15"-15" × 1	5' à 10'	15"	15"	3-6	3/10
15"-10" × 1	5' à 10'	15"	10"	3-6	4/10
15"-5" × 1	5' à 10'	15"	5"	3-6	5/10
20"-10' × 1	5' à 10'	20"	10"	3-6	5/10
20"- 5" × 1	5' à 10'	20"	5"	3-6	6/10
30"-15" × 1	5' à 10'	30"	15"	3-6	6/10
30"-10" × 1	5' à 10'	30"	10"	3-6	7/10
45"-10" × 1	5' à 10'	45"	10"	3-6	8/10
50"-10" × 1	5' à 10'	50"	10"	3-6	8/10

RPE: rating of perceived exertion.

Table 2. Description of the resistance training circuit

Time × number se-ries	Total time	Exercise time	Recovery time	Number of workshops	RPE scale
20"-30" × 1	5' à 10'	20"/8r	< 30" or more	3-6	3/10
20"-30" × 1	5' à 10'	20"/8r	< 30" or more	3-6	4/10
20"-1' × 1	5' à 10'	20"/8r	< 1' or more	3-6	5/10
20"-3' × 1	5' à 10'	20"/8r	< 3' or more	3-6	5/10
30"-3' × 1	5' à 10'	30"/8r	< 3' or more	3-6	7/10
30"-3' × 1	5' à 10'	50"/12r	< 3' or more	3-6	7/10

RPE: rating of perceived exertion.

patients. Globally, effort times were 15 to 50 seconds interspersed with recovery phases of 15 to 50 seconds and the series duration was 3 to 12 minutes. The training session time was 60 minutes. Exercises were performed at bodyweight or with low additional loads (20% of the 1RM maximum).

2.8.2. Resistance training (Group 3)

The resistance training consisted of a TC composed of a sum of series each comprising two times: 1) "effort" and 2) "recovery" (Table 2). Each series included a succession of dynamic or static exercises, chosen individually according to the initial assessment, and a rest time. The number of repetitions during the effort was between 6 and 12. The recovery times were higher than the effort time from 30 seconds to 3 minutes. The total duration of the workout was 60 minutes. All muscles were solicited in both specific and exclusive work (*i.e.* lower limb type, abdominal belt, or upper limb) or combined (*i.e.* grouping together several muscle groups). Initially, patients carried out work of coordination of gestures specific to muscular reinforcement. Then, they performed muscle-building sessions to body weight. Finally, the various exercises were performed with a higher load that can range from 30% to 50% of the 1RM depending on the capabilities of the patients.

2.8.3. Combination of endurance and resistance training (Group 4)

This training program consisted of 2 sessions alternating 1 endurance TC session and 1 resistance TC session per week. The session of endurance was performed at a moderate-intensity corresponding to the target HR of VT1 and lasted 60 minutes. The session of resistance was performed at the HR of VT1, lasted 60 minutes and integrated muscular loads corresponding to 30-50% of the 1RM.

2.9. Telemonitoring

The investigators followed the training program weekly based on phone interviews and vidioconferences. Patients had a cardiofrequencemeter and a tensiometer before beginning the training at home. During each session of exercise, HR, DBP, SBP and the RPE scale were monitored. Patients were informed at visit 1 of the criteria for alarm at rest and for stopping exercise. These criteria were: at rest: a SBP of > 140 mmHg and a DBP of > 90 mmHg; on exertion: the SBP must remain ≤ 160 mmHg) (*12,14-16*). Patients also had a training booklet made by the investigators at their disposal to understand the exercises to be done.

Depending on variations of these parameters as training progresses, the investigators adjusted the exercise time and the recovery time in order to vary the intensity of the training. Moreover, a mismatch between the target training HR (*i.e.* HRVT1) and the HR communicated by the patient caused an adaptation of the work intensity, if necessary.

2.10. Statistical analyses

The ANOVA between the trained patients and the control patients was performed. Then the data of the 3 trained

subgroups was compared by ANOVA. Qualitative data were reported as a percentage, and quantitative data as means and standard deviation. All results were expressed as mean ± SD. The Software used was SPSS 20. Differences were considered significant at P values ≤ 0.05.

3. Results and Discussion

Regular physical activity is highly beneficial for patients with cardiovascular disease. Indeed, physically active patients have a decrease in cardiovascular mortality and in the risk of increasing the severity of their pathology (*22*). Moreover, regular, moderate to intense exercises have been reported to significantly lower blood pressure and therefore may have a significant impact on the appearance of cardiovascular diseases and aorthopathy (*23-25*). Patients with MFS might also benefit from regular physical activity but, to date, there are no randomized studies evaluating the longitudinal effect of exercise on survival or risk of aortic dissection in patients with aorthopathy including MFS patients (*26*).

Only two studies performed on a mouse model of MFS demonstrated that a five-month training consisting of moderate to intense dynamic exercises induced either an improvement of the aortic wall elasticity (*27*) or no significant reduction of the aortic diameter (*28*) compared to sedentary control animals. Only one study performed in patients with MFS demonstrated the feasibility of training in such patients and no adverse events were found after the three-week training program during their one-year follow-up (*11*). Hence, there still is no evidence of contraindications for an exercise prescription in patients with MFS. Moreover, there is a need to investigate the precise effects of training in such patients has been recently mentioned (*10,26*). In this context, the preliminary results of our study that is underway are very encouraging. At this time, 8 patients (means: age: 30 ± 2 years; height: 178 ± 2 cm; weight: 66 ± 6 kg) completed the 3-month combined training protocol. There is a tendency towards an increase of the score for their QoL between pre- and post-training (74 ± 15 *vs.* 91 ± 6 ≈ + 25%). The evolution of their physiological parameters post-training also showed encouraging results. The VO2peak increased from 24 ± 4 to 27 ± 5 mL/min/kg ($p = 0.05$), the slope of VE/VCO_2 tended to decrease from 34 ± 19 to 24 ± 2, the pulsed wave speed decreased from 5.3 ± 0.4 to 5.1 ± 0.4 ms ($p < 0.001$), the Aix decreased from 24 ± 12 to 16 ± 13 ($p < 0.001$), the FEVG tended to increase from 60 ± 11 to 64 ± 12 %, and the 1RM increased from 64 ± 29 to 96 ± 25 kg ($p < 0.05$). Interestingly, despite the moderate intensity of training, the aortic diameter did not change between pre and post training (39.0 ± 52 *vs.* 39.3 ± 4.6 mm). This potentially demonstrates the safety of the training program and its feasibility for Marfan patients. We think that the other results from the whole population study will follow the previously described results and permit us to propose a new healthcare for MFS patients in the future based on adapted physical activity and telemonitoring.

Telemonitoring is a unique feature of the training, facilitating control and compliance of the performed physical exercises by patients at home. In a review comparing studies performing telemonitoring or a traditional rehabilitation program in patients with cardiovascular diseases, authors reported that telemonitoring was as relevant as traditional training to induce benefits with no adverse effects in such patients (*29*). Again, based on our preliminary results, it is believed that patients with MFS might also benefit from an adapted home-based training program.

In conclusion, this personalized home-based training program appears to improve the QoL of MFS patients. It is anticipated that this specific training program associating mild to moderate regular endurance and resistance exercises should be prescribed because of its known positive effects on global health. It is also believed that this study will prove that telerehabilitation is a safe intervention and that it can help to prevent thoracic aortic dissection by implementing it into standard preventive care for patients with MFS.

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Brief Report

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Alteration of skeletal and cardiac muscles function in *DBA/2J mdx* mice background: a focus on high intensity interval training

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SUMMARY Duchenne muscular dystrophy (DMD) is a recessive hereditary myopathy due to deficiency of functional dystrophin. Current therapeutic interventions need more investigation to slow down the progression of skeletal and cardiac muscle weakness. In humans, there is a lack of an adapted training program. In animals, the murine *Mdx* model with a *DBA/2J* background (D2-*mdx*) was recently suggested to present pathological features closer to that of humans. In this study, we characterized skeletal and cardiac muscle functions in males and females D2-*mdx* mice compared to control groups. We also evaluated the impact of high intensity interval training (HIIT) in these muscles in females and males. HIIT was performed 5 times per week during a month on a motorized treadmill. Specific maximal isometric force production and weakness were measured in the *tibialis anterior* muscle (TA). Sedentary male and female D2-*mdx* mice produced lower absolute and specific maximal force compared to control mice. Dystrophic mice showed a decline of force generation during repetitive stimulation compared to controls. This reduction was greater for male D2-*mdx* mice than females. Furthermore, trained D2-*mdx* males showed an improvement in force generation after the fifth lengthening contraction compared to sedentary D2-*mdx* males. Moreover, echocardiography measures revealed a decrease in left ventricular end-diastolic volume, left ventricular ejection volume and left ventricular end-diastolic diameter in sedentary male and female D2-*mdx* mice. Overall, our results showed a serious muscle function alteration in female and male D2-*mdx* mice compared to controls. HIIT may delay force loss especially in male D2-*mdx* mice.

Keywords cardiac function, muscle function, force production, HIIT, cardiomyopathy

1. Introduction

Duchenne muscular dystrophy (DMD) is a chronic and degenerative disease characterized by a progressive weakness of skeletal, respiratory and cardiac muscles due to deficiency of functional dystrophin (*1,2*). DMD has an incidence rate affecting closer to 1/5000 male births but also females, being asymptomatic carriers of mutations (*3,4*). Cardiomyopathy is the main cause of death of DMD patients, due to myocardial tissue lesions associated with systolic dysfunction (*5*). Male DMD patients exhibit cardiomyopathy, and myocardial fibrosis but females mostly develop later dilated cardiomyopathy (*6,7*). The development of cardiomyopathy is often due to relative physical inactivity and exercise intolerance inherent to their disability and progressive muscle wasting (*8*). DMD also alters skeletal muscles making them more susceptible to damage caused by high-force contractions like eccentric contractions both *in situ* and *in vitro* (*9,10*). Consequently the disease contributes to a muscle mass loss and leads to progressive loss of locomotion in DMD patients (*11,12*). These events may be related to several mechanisms such as, failure in neuromuscular transmission, reduced muscle excitability, impaired calcium release and uptake in the sarcoplasmic reticulum, and/or contractile impairment (*13,14*).

Today, there is no treatment for DMD. In healthy subjects, exercise induces beneficial effects, but its effects on DMD patients are still not well known. A new form of training, namely high intensity interval training (HIIT) has gained in popularity in clinics. Despite its potential beneficial effects in humans (*15,16*), no data are available on the effect on cardiac and skeletal muscles function in DMD patients and

mdx mice. Studies focusing on the effect of exercise in *mdx* mice reported contrasting results (*17-19*). These discrepancies may be attributed to the type of exercise, the age of animals and their genotype. Concerning this last point, it was recently demonstrated that *C57BL/10 mdx* mice, the commonly used *mdx* mouse model for DMD, exhibited a less severe disease progression in skeletal and cardiac muscles than in a *DBA/2J* mdx (D2-*mdx*) mice background when both were compared to their control peers (*20*). *D2-mdx* congenic mice are generated by backcrossing *C57BL/10-mdx* mice to *DBA/2J* inbred mice for several generations to generate D2-*mdx* mice (*20*). These mice showed a lower hind limb muscle weight, fewer myofibers, increased fibrosis and fat accumulation, and muscle weakness compared to *C57BL/10-mdx* mice (*20*). Thereafter, data on the pathophysiological profile of this mouse model in different genders are still missing. Authors suggested that D2-*mdx* mice might represent a more robust animal model of DMD that may be used to determine new strategies of treatment.

The goal of the present study was to explore gender differences and functional properties of skeletal and cardiac muscles in 9-month old D2-*mdx* mice compared to their age-matched *DBA/2J* control peers and to determine the effects of a 4-week HIIT on skeletal and cardiac muscle function in female/male, sedentary/trained and control/D2-*mdx* mice.

2. Materials and Methods

2.1. Animals

All experimental protocols were performed in accordance with the national and European legislations and were approved by the institutional Ethics Committee Charles Darwin (project 01362.02). D2-*mdx* mice used in this study have been described previously and were generously provided by D. Coley (The Jackson Laboratory, Bar Harbor, ME, USA) (*20*). *C57BL/10-mdx* mice were backcrossed to *DBA/2J* inbred mice (Stock No. 000671) for several generations using a marker-assisted, speed congenic approach to generate the D2.B10-congenic strain (also referred to as *DBA/2J-mdx* or D2-*mdx*) as Stock No. 013141 (The Jackson Laboratory jax#01314). The *DBA/2J* background was used for control mice.

The experimental protocols followed the European directives on animal rights (86/609/CEE) and were approved by the institutional Ethics Committee "Charles Darwin" (project 01362.02). Animals were housed under standard conditions (20-22 °C, 12 h-12 h light-dark cycle), in normal cages with *ad libitum* access to water and food. 9-month old D2-*mdx female* (n = 12) and male (n = 12) mice were used in this study. They were compared to control *DBA/2J* female (n = 10) and male (n = 10) mice. Mice were weighed before starting the training protocol and then were divided into sedentary (SED) or HIIT groups after familiarization period and the identification of the most tolerant mice to running (Table 1).

After 4 weeks of training, animals were sacrificed and heart, *gastrocnemius* (Gastro) and *tibialis anterior* (TA) muscles were immediately weighed and isolated for further analyses (Table 1).

2.2. Running performance

All mice were tested before and after the training period. They were familiarized during 2 days with a motorized treadmill (LE8710MTS-Bioseb) at a running speed of 5 cm/s during 30 minutes before beginning the HIIT protocol. After this period, control *DBA/2J* (5 male and 5 female) and D2-*mdx* (8 male and 8 female) mice groups performed a maximal running speed (MRS) test every week. The remaining mice were divided into sedentary groups. The MRS test was determined with a running test in which the speed was gradually increased. It consisted of running 10 minutes at 10 cm/s followed by speed increments of 5 cm/s every minute until mice could no longer maintain the treadmill pace, then speed was recorded (*21*). Trials end at exhaustion as defined by the mouse touching the shock grid more than three times.

2.3. High intensity interval training (HIIT) protocol

After a 5-minute warm-up period at 10 cm/s, the HIIT protocol consisted of 10 repetitions of 30 seconds of high-intensity at 40 cm/s (80-90% of MRS) running followed by 1 minute of low intensity 15 cm/s (30-40% of MRS) running, 5 days/week over 1 month. The intensity of training was adapted every week after the MRS test. During the HIIT period, sedentary animals remained in their cages in the treadmill room with water and diet *ad libitum*.

2.4. Force-generating capacity and fragility of skeletal muscle

Force-generating capacity and fragility were evaluated by measuring the *in situ* TA muscle contraction in response to nerve stimulation, as previously described (*17*). The absolute maximal force that was generated during isometric contractions in response to electrical stimulation (frequency of 75 to 150 Hz, train of stimulation of 500 milliseconds) was measured. Absolute maximal force was determined at L0 (length at which maximal tension was obtained during the tetanus). It was normalized to the muscle mass as an estimate of specific maximal force, an index of muscle weakness.

Fragility (*i.e.* susceptibility to contraction-induced injury in D2-*mdx* mice) was estimated from the force decrease resulting from lengthening contraction-

Table 1. Body and muscle weights of female and male sedentary or trained *DBA/2J*, control and D2-*mdx* mice

Items	Sedentary mice				HIIT mice			
	Male CTRL SED ($n = 5$)	Female CTRL SED ($n = 5$)	Male D2-*mdx* SED ($n = 4$)	Female D2-*mdx* SED ($n = 4$)	Male CTRLHIIT ($n = 5$)	Female CTRL HIIT ($n = 5$)	Male D2-*mdx* HIIT ($n = 8$)	Female D2-*mdx* HIIT ($n = 8$)
Body weight (g)	29.3 ± 0.6	29.6 ± 0.4	25.6 ± 0.3**	20.1 ± 0.7^{***bbb}	27.8 ± 1.2	29.5 ± 0.3	25.0 ± 0.7**	20.2 ± 0.3^{***bbb}
Gastro (mg)	123 ± 6.9	115 ± 1.9	77.0 ± 1.6***	63.9 ± 1.5***	127 ± 8.2	130.2 ± 4.0	68.7 ± 1.2***	63.4 ± 1.8***
TA (mg)	33.2 ± 1.6	29.0 ± 1.8	31.0 ± 1.3	29.7 ± 1.4	32.8 ± 1.8	32.7 ± 2.0	22.2 ± 1.6$^{*** §§}$	25.4 ± 1.4***
Heart (mg)	169 ± 0.0	133 ± 0.0^{bb}	146 ± 0.0**	96 ± 0.0^{**bb}	160 ± 0.0	133 ± 0.0	148 ± 0.0**	104 ± 0.0^{**bbb}
Heart/Body weight (mg/g)	5.8 ± 0.2	4.7 ± 0.2^{bb}	5.7 ± 0.2	4.8 ± 0.2	5.8 ± 0.2	4.6 ± 0.1^{bb}	5.9 ± 0.2	5.1 ± 0.1^{bb}

CTRL: Control *DBA/2J* mice; D2-*mdx*: dystrophic mice; HIIT: high intensity interval training group; Gastro: *gastrocnemius muscle*; TA: *tibialis anterior muscle*. Data are expressed as means ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$ D2-*mdx vs.* Control; $^{§§}p < 0.01$ HIIT *vs.* sedentary; $^{bb}p < 0.01$; $^{bbb}p < 0.001$ male *vs.* female. $n = 5$ animals per group and sex in control mice; $n = 4$ animals in sedentary male and female D2-*mdx* groups; $n = 8$ animals in HIIT male and female D2-*mdx* groups.

induced injury. Nine lengthening contractions (eccentric contractions) of the TA muscles were performed in D2-*mdx* and control mice, each separated by a 60-second rest period. Maximal isometric force was measured 1 minute after each lengthening contraction and expressed as a percentage of the initial maximal force. After contractile measurements, the animals were sacrificed with cervical dislocation.

2.5. Echocardiography

Echocardiography was performed before and after the training period, on anesthetized mice under isoflurane (induction with 2% and maintained with 0.5%) as previously described (*18*). Non-invasive measurements of left ventricular (LV) dimensions were done using echocardiography-Doppler (Vivid 7 Dimension/Vivid7 PRO; GE Medical System Co, Vélizy, France) with an ultrasound probe at a 9-14 MHz frequency range. The following measurements were performed: diastolic (IVSd) and systolic intreventricular septal (IVSs) and posterior wall thicknesses (LVPWd and LVPWs), Left ventricle end-diastolic (LVEDD) and end-systolic diameters (LVESD) and heart rate. LV shortening fraction (LVSF) was calculated using the formula: (LVEDD-LVESD)/LVEDD × 100. LV ejection volume (LVEV), LV end-diastolic (EDV), and end-systolic (ESV) volumes were calculated using a half-ellipsoid model of the LV. From these volumes, LV ejection fraction (LVEF) was calculated using the formula: (EDV -ESV)/EDV × 100. The LV thickness/LV radius ratio (h/r) was also assessed in all animals.

2.6. Statistical analysis

All data are presented as the mean ± SEM. A two-way ANOVA followed by the Tukey post-hoc procedure was used to determine the effects of training and the animal genotype. We also evaluated the effects of training and the animal's gender with a two-way ANOVA test. The significance level was set at $p < 0.05$. Data were analyzed using the statistical package GraphPad Prism version 6.02 for Windows (La Jolla, California).

3. Results and Discussion

In this study, we investigated the function of skeletal and cardiac muscles in males and females with a *DBA/2J mdx* (D2-*mdx*) background. Then, we evaluated the impact of HIIT on these tissues in dystrophic mice.

3.1. Animals and muscles characteristics

Sedentary male and female D2-*mdx* mice showed a significant decrease in body weight (BW) compared with sedentary control *DBA/2J* mice. The decline of BW was especially marked in sedentary D2-*mdx*

female mice compared to sedentary control females (Table 1; $p < 0.0001$). The decrease in muscle mass of Gastro was observed also in both sedentary D2-*mdx* gender compared to control groups ($p < 0.0001$) (Table 1). No sex or genotype difference in TA muscle mass was observed in sedentary groups. However, mass loss was also observed in the heart of sedentary D2-*mdx* mice compared with sedentary control groups ($p < 0.0001$). A significant decrease was especially shown in control and D2-*mdx* females compared to males in both genotypes (Table 1).

Trained D2-*mdx* groups remain lighter compared to trained control *DBA/2J* mice and showed a larger decrease of BW in trained female D2-*mdx* compared to trained male D2-*mdx* (Table 1; $p < 0.001$). In addition, HIIT did not improve Gastro and TA mass in trained females and males of both genotypes. Trained D2-*mdx* males showed a significant reduction in TA mass compared to sedentary D2-*mdx* males (Table 1; $p < 0.05$). Heart mass was also lower in trained D2-*mdx* groups especially in females compared to control trained mice (Table 1; $p < 0.001$).

Overall, the decrease of BW in sedentary and trained D2-*mdx* mice might be linked to the mass loss of cardiac and skeletal muscles (*i.e.* Gastro). This could be explained by the fact that dystrophin absence in tissue impaired regeneration and muscle growth inducing loss of mass and muscle function (*22,23*).

3.2. Functional impairment and fragility in the tibialis anterior muscle

A decrease in absolute maximal isometric force (Po) generated by TA muscle was observed in the male and female D2-*mdx* group compared to control *DBA/2J* mice (Figure 1A; $p < 0.001$). The TA specific maximal isometric force (sPo), was also significantly lower in D2-*mdx* mice compared to control groups, without gender difference (Figure 1B; $p < 0.001$).

During repetitive eccentric contractions, a large force decrease was demonstrated in sedentary D2-*mdx* mice from the third to tenth lengthening contraction compared to the control *DBA/2J* group (Figure 2A; $p < 0.001$). This decrease was particularly pronounced in male D2-*mdx* mice compared to female D2-*mdx* (Figure 2A; $p < 0.001$). In this context, several studies have confirmed that loss of muscle mass is always associated with decrease of force generation in *mdx* mice (*17,18*). This last point reinforces the fact that D2-*mdx* mice are characterized by a loss of BW associated with decline of the Po and sPo as found in DMD patients. However, D2-*mdx* females showed a better resistance to muscle damage caused by lengthening contraction compared to D2-*mdx* males in sedentary groups. Although no sex difference was reported in skeletal muscle mass, the male D2-*mdx* generated a lower force during contractions. These findings are in agreement with the

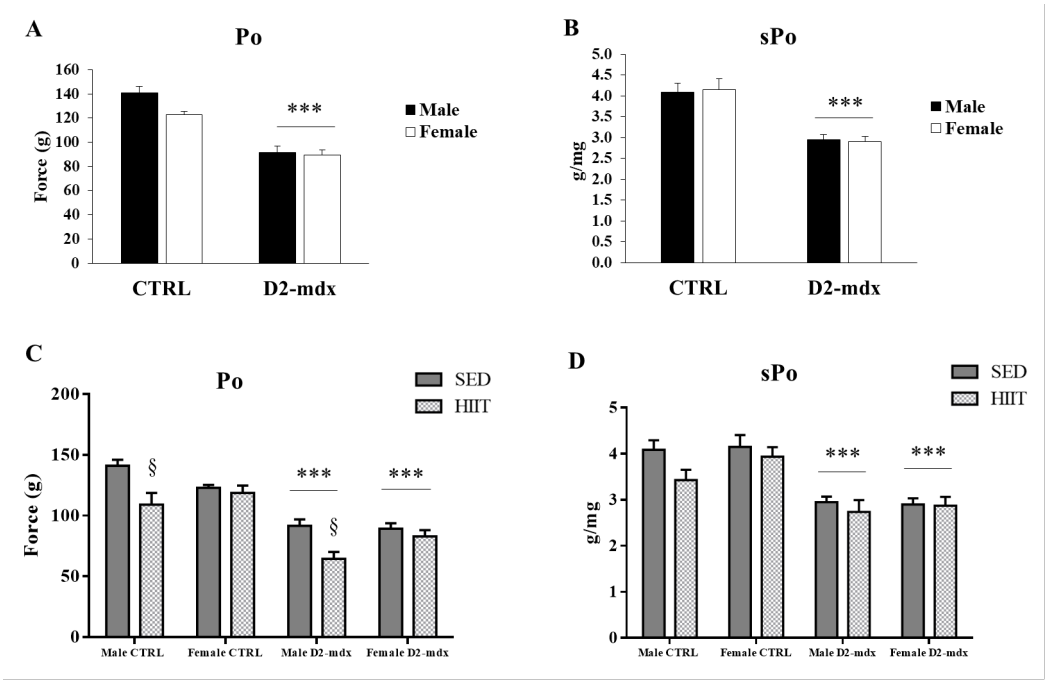

Figure 1. Absolute (Po) and specific (sPo) maximal force generation of *tibialis anterior* muscle in *DBA/2J* male and female control and D2-*mdx* mice (A) Absolute (Po) maximal force in male and female groups of sedentary control and D2-*mdx* mice. **(B)** Specific (sPo) maximal force in male and female groups of sedentary control and D2-*mdx* mice. **(C)** Absolute (Po) maximal force in sedentary or trained control and D2-*mdx mice*. **(D)** Specific (sPo) maximal force in sedentary or trained control and D2-*mdx mice*. Data are expressed as means ± SEM. CTRL: control *DBA/2J* mice; D2-*mdx*: *mdx* mice; SED: sedentary mice; HIIT: high intensity interval training group. $^{***}p < 0.001$ D2-*mdx vs.* control; $^{\S}p < 0.05$ HIIT vs. sedentary $n = 5$ animals per group and sex in control mice; $n = 4$ animals in sedentary male and female D2-*mdx* groups; $n = 8$ animals in HIIT male and female D2-*mdx* groups.

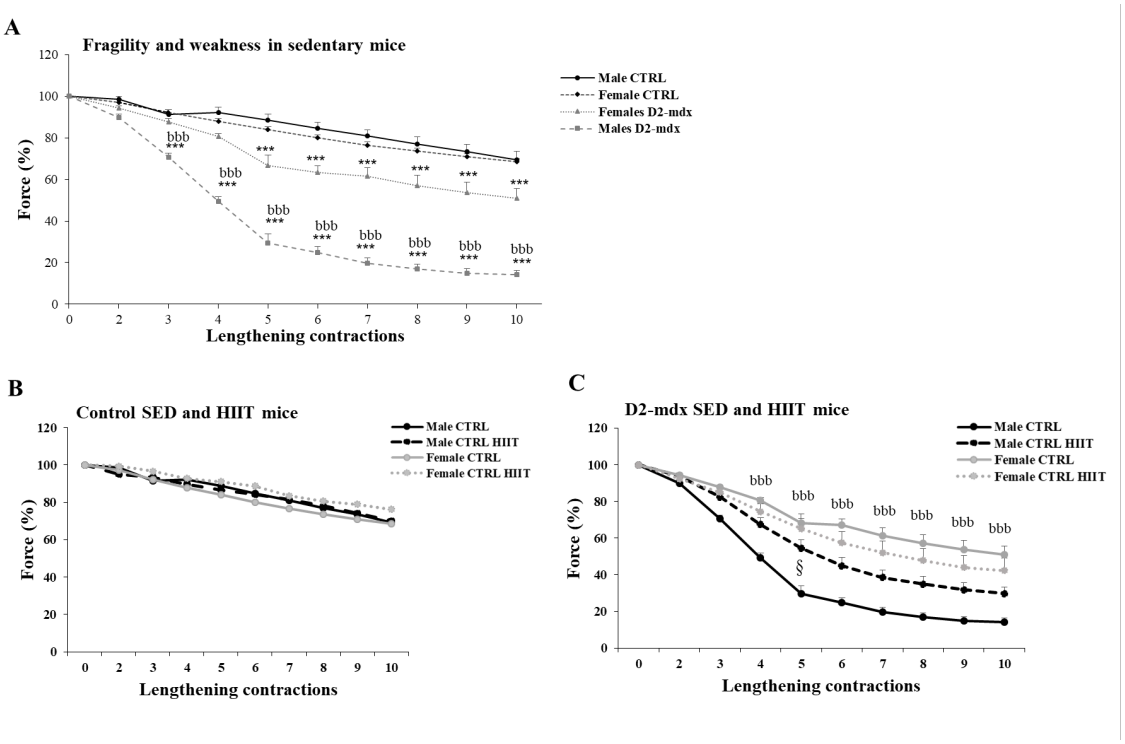

Figure 2. Fragility and weakness in tibialis anterior muscle in *DBA/2J* male and female control and D2-*mdx* mice (A) Force after lengthening contractions in sedentary male or female control and D2-*mdx* mice **(B)** Force after lengthening contractions in trained or sedentary male and female control group **(C)** Force after lengthening contractions in trained or sedentary male and female D2-*mdx* group. Data are expressed as means ± SEM; CTRL: control *DBA/2J mice*; HIIT: high intensity interval training group; D2-*mdx*: mdx mice, $^{***}p < 0.001$ D2-*mdx vs.* Control; $^{bbb}p < 0.001$ male *vs.* female; $^{\S}p < 0.05$ HIIT *vs.* sedentary; $n = 5$ animals per group and sex in control mice; $n = 4$ animals in sedentary male and female D2-*mdx* groups; $n = 8$ animals in HIIT male and female D2-*mdx* groups.

previous study of Van Putten *et al.* 2019, indicating that D2-*mdx* females outperformed compared to D2-*mdx* males. To sum up, *DBA/2J* mice are characterized by a loss of BW associated with a deficit in force generation with a greater impact on males.

The sedentary D2-*mdx* group, trained D2-*mdx* male and female mice, still generated a lower Po compared to control *DBA/2J* mice (Figure 1C; $p < 0.001$). Trained males in the D2-*mdx* and control groups showed a decline of Po compared to their sedentary peers (Figure 1 C; $p < 0.05$). Furthermore, TA specific maximal isometric force (sPo) was significantly lower in trained D2-*mdx* male and female mice compared to control groups (Figure 1D; $p < 0.001$). Contrary to Po, no sex effect was detected in sPo of trained groups (Figures 1D).

The fragility test showed no difference in control male and female mice of sedentary and trained groups. Until the tenth lengthening contraction, all control mice were able to maintain 70%, on average, of their force production (Figure 2B). In the D2-*mdx* group, females were still stronger in force generation than males independent of training status (Figure 2C, $p < 0.001$). Furthermore, trained D2-*mdx* males generated greater force especially after the fifth lengthening contraction compared with sedentary D2-*mdx* male mice (Figure 2C; $p < 0.05$).

These results demonstrated a delayed force production loss of the TA in male D2-*mdx* mice during repetitive eccentric contractions. However, our protocol training did not significantly increase the TA Po and sPo or muscle mass in D2-*mdx* or control mice. This is in contrast with other studies using a custom program of HIIT. In these studies, 24 month-old male and female control (C57BL/6J) mice underwent a 10-minute HIIT starting with 3 minutes of warm up at 8 m/min followed by intervals of 1 minute sprint at 13 m/min interspersed with a 1 minute period of relative rest at 8m/min. They performed 3 sessions/week for 2 or 4 months. This training induced an increase in muscle mass, an enlargement of fibers and an improvement of grip strength in trained mice compared to a sedentary group (*24,25*). In addition, the HIIT program (*i.e.* treadmill inclination 25°: 10 intervals of 4 minutes at 85-90% of VO2max interspersed with 2 minutes active rest at 5 m/min, 5 days/week for 2 months) improved metabolic dysfunction induced by High Fat Diet (HFD) and decreased the body weight and percentage of fat mass in 10-week old mice with a diet-induced obesity phenotype (*26*). In contrast, our HIIT program, was more intense (*i.e.* 10 repetitions of 30 seconds sprint interspersed with 1 minute of low intensity running; 5 days/week) and shorter in total duration (*i.e.* 1 month). These methodological differences and the age of animals might explain these differences and support the development of a standard training program to fully determine the impact of training in DMD.

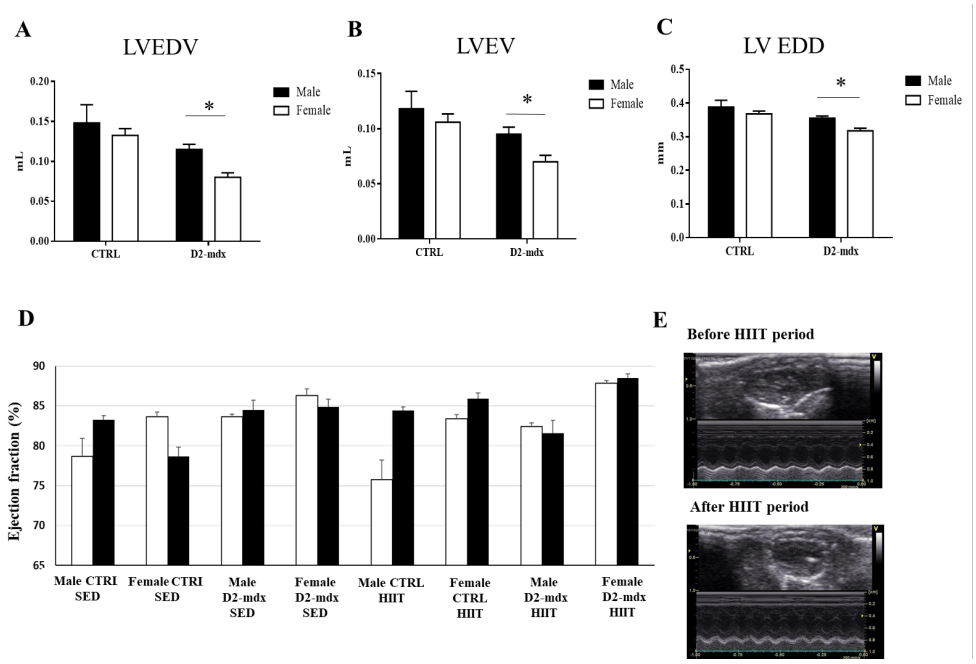

Figure 3. Impact of HIIT on cardiac function in control and D2-*mdx*, male and female *DBA/2J* mice. (A) Left ventricular end-diastolic volume (LVEDV) in male and female of control and D2-*mdx* sedentary groups **(B)** Left ventricular ejection volume (LVEV) in male and female of control and D2-*mdx* sedentary groups **(C)** Left ventricular end-diastolic diameter (LVEDD) in male and female of control and D2-*mdx* sedentary groups. **(D)** Ejection fraction in myocardium in male and female control and D2-*mdx* groups before and after HIIT period. **(E)** Electrocardiography of heart before and after HIIT session. Data are expressed as means ± SEM. $^{*}p < 0.05$; D2-*mdx vs.* Control; HIIT: high intensity interval training group; SED: sedentary group. $n = 5$ animals per group and sex in SED mice; $n = 4$ animals in SED male and female D2-*mdx* groups; $n = 5$ animals per group for control male and female HIIT mice; $n = 8$ animals per group for D2-*mdx* male and female HIIT mice. White bars = pre training; black bars = post training.

3.3. Functional alterations in cardiac muscle of male and female D2-*mdx* mice

Echocardiography evaluations showed a significant alteration of the LV structure in sedentary male and female D2-*mdx* mice compared to the *DBA/2J* control group. A significant decrease of LVEDV, LVEV and LV EDD was observed in D2-*mdx* mice compared to control group (Figure 3A, B, C; $p < 0.05$). No significant sex difference was observed in either genotype. This finding validated the installation of pathology in this mouse model (*21*) closer to the one observed in humans.

No significant difference was found between pre and post training for all echocardiographic variables between trained controls and *D2-mdx* groups *vs.* sedentary mice. Figure 3D represents two representative electrocardiographs of the myocardium before (white bars) and after (black bars) the HIIT period and a measure of the ejection fraction, which is a global parameter of cardiac function. This is contrary to other studies reporting severe muscle and heart damage (*27,28*) in trained *mdx* mice (C57Bl/10ScSn$^{mdx/mdx}$) compared to their sedentary peers. Theses discrepancies highlight the importance of the impact of the genetic background onto the phenotype of mice used for studies in the DMD field. Future studies are needed to explore the adaptive potential of *DBA/2J mdx* mice to other HIIT protocols and determine new potential training programs for DMD patients.

4. Conclusion and Perspectives

The present study underlines functional impairments in skeletal and cardiac muscles of male and female D2-*mdx* mice with an original evaluation of the impact of HIIT in these tissues. These results might be related to metabolic disorders and to a higher susceptibility to weakness in dystrophic muscle as reported previously (*29,30*).

This study also demonstrated the potential feasibility, safety and beneficial (*i.e.* delayed TA generation force loss and no impact on cardiac function) effect of HIIT for DMD care management in the future.

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

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Brief Report

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Serum neurofilament light chain is not a useful biomarker of central nervous system involvement in women with Fabry disease

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SUMMARY Neurofilament Light Chain (NfL) serum concentration is a new noninvasive marker of neurodegenerative disorders. Fabry disease (FD) leads to accumulation of glycosphingolipids in tissues leading to progressive damage of critical body systems and organs, including peripheral and central nervous system. There are no established serum markers of neurodegeneration in FD. Our cross-sectional single-center study was designed to prove the concept that serum NfL levels could reflect the severity of cognitive impairment and indirectly, the level of central nervous system involvement in women at earlier stages of FD. Twelve women with a diagnosis of FD confirmed by genetic tests and 12 matched healthy subjects were included. Serum concentrations of NfL were measured in all subjects together with neuropsychological tests that included Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment Scale (MoCA). Quality of life was assessed with the Short Form Survey (SF-36). FD patients and healthy subjects did not differ with respect to serum NfL concentration, results of neuropsychological tests and quality of life. There was a significant positive correlation between NfL and globotriaosylosphingosine (lyso-Gb3) concentration in women with FD ($R = 0,69$, $p = 0.01$). There was also a correlation between NfL concentration and MoCA score but not MMSE score. Receiver operating characteristic (ROC) analysis showed that the best predictor for Mild Cognitive Impairment in both groups was eGFR. Serum NfL concentration does not appear to predict the degree of nervous system involvement in women with FD.

Keywords biomarker, neurodegeneration, quality of life

1. Introduction

Fabry disease (FD), is an ultra-rare lysosomal storage disease inherited as an X-linked disorder. FD is caused by a deficiency of the lysosomal enzyme alpha-galactosidase A (α-Gal A; E.C. 3.2.1.22). GLA gene, located on X chromosome at Xq22, encodes a 429 amino acid precursor that is processed to a 398 amino acid glycoprotein functioning as a homodimer. The mutation of GLA leads to a deficiency or absence of the enzyme α-galactosidase A (α-Gal A), which catalyzes the hydrolysis of globotriaosylceramide.

Alpha-Gal A deficiency leads to progressive accumulation of glycolipids, and globotriaosylosphingosine (lyso-Gb3) in different cells of the body, leading to damage and dysfunction of affected organs. The most affected cells and tissues in FD include glomerular podocytes, cardiomyocytes, endothelial cells, vascular muscles and peripheral and central nerves. It all leads to dysfunction and failure of vital organs including the heart, kidneys and nervous system. The severity of the disease depends on the gender, age and the type of mutation. Males with classic phenotype have the highest risk of complications and early symptoms, while younger women mostly become affected by FD later in life (*1*).

Neurofilaments are the main component of the neuronal cytoskeleton. Light (NfL), intermediate (NfM) and heavy (NfH) chains have been distinguished on the basis of their molecular mass. The serum concentration of NfL and their importance as a marker of central nervous system diseases have been demonstrated in several recent studies (*2,3*). Neuronal damage and physiological changes of the central nervous system (CNS) cause release of neurofilaments. This translates into elevated levels of NfL in the cerebrospinal fluid and ultimately in the blood, where its concentration reflects the rate at which NfL is released from the neurons (*3*). Several studies have shown a strong positive correlation between NfL in the blood and in the cerebrospinal fluid (*4*). Serum NfL concentration positively correlated with severity of various diseases of the central nervous system including multiple sclerosis, amyotrophic lateral sclerosis, frontotemporal

dementia, Alzheimer's disease, traumatic brain injuries and degeneration of the nervous system associated with HIV infection (*2*).

The main physiologic factor influencing the NfL concentration is the age of the patients. With aging NfL concentration in healthy subjects increased by 2.2% annually. After the age of 60, a further significant increase in NfL concentration is observed (*5*). These changes could be attributed both to aging itself and aggregation of comorbidities. It has been well proven that patients with FD are characterized with much faster brain aging compared to the healthy population (*6*). Patients with FD are at increased risk of developing cognitive dysfunction and most of them also have symptoms of depression (*7*). Patients diagnosed with severe depression have more cognitive impairment compared to the general population (*8*). It also was shown that in major depressive disorder higher levels of NfL were observed (*9*). However, it has not been confirmed in FD that the cognitive impairment is due to depressive symptoms but its risk factors include male gender, patients with classic disease phenotype, lower intelligence quotient (IQ) and a history of stroke (*10,11*).

Many clinical tests have been developed to assess cognitive functions, each of which assesses specific domains of cognitive functioning, but in a different aspect. Screening tests play a key role in allowing each clinician to perform an initial assessment of cognitive impairment. The best validated tests used for screening for cognitive impairment include the Mini Mental State Examination (MMSE) and the Montreal Cognitive Assessment Scale (MoCA) (*12,13*). MMSE and MoCA were used and well-validated in recent studies assessing cognitive impairment in patients with FD (*11,14*).

The aim of the study was to assess whether the serum concentration of NfL could be a marker of the early central nervous system involvement and cognitive impairment in women with FD.

2. Materials and Methods

The study was approved by the Local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All patients gave informed written consent to participate in the study.

Twenty-four subjects were enrolled, including 12 women with confirmed FD and 12 matched healthy controls. The study was conducted between March and October 2020. The characteristics of the study population is provided in Table 1. Diagnosis of FD was based on the blood concentration of α-Gal A, lyso-Gb3 and on genetic tests. The tests were performed using the Dry Blood Spot method (DBS). Individual results are presented in Table 2. Only one woman from the study group has been qualified for enzyme replacement therapy.

The patients with FD included in our study came from three different families. The degree of kindship and

Table 1. Clinical characteristics of the study population

No.	Age	eGFR	Education	MMSE	MoCA	SF-36	Diabetes	Stroke
Fabry disease group								
1	42	88.8	12	28	28	62	No	No
2	21	129.5	12	26	29	28	No	No
3	64	45.8	8	24	24	87	Yes	Yes
4	42	91.6	17	30	29	62	No	No
5	45	113.1	17	30	29	23	No	No
6	71	32.4	12	30	24	74	No	Yes
7	51	63.5	17	25	25	19	No	No
8	22	127.9	12	30	29	28	No	No
9	42	111.5	17	28	27	27	No	No
10	47	94.7	17	28	25	35	No	No
11	20	133.7	12	30	30	15	No	No
12	40	110.7	17	30	28	39	No	No
Control group								
1	42	116.2	17	30	30	18	No	No
2	68	95.3	12	25	23	63	No	No
3	36	116.3	13	26	26	52	No	No
4	30	108.7	20	30	30	26	No	No
5	37	109.7	20	30	30	23	No	No
6	53	94.4	17	30	26	22	No	No
7	48	129.7	17	30	30	44	No	No
8	54	68.7	12	26	24	67	Yes	No
9	41	42	12	29	28	74	No	No
10	76	34.4	12	24	19	84	Yes	No
11	73	46	12	29	24	100	No	No
12	54	68.7	12	26	24	67	Yes	No

eGFR: estimated Glomerular Filtration Rate; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment Scale; SF-36:The Short Form 36 Health Survey.

Table 2. Type of genetic variant, globotriaosylosphingosine and α-galactosidase A concentration in blood in women with Fabry disease

Patients No	Mutation	Lyso-Gb3 [ng/mL]	α-Gal A [μmol/L/h]
1	c.680G>A(p.Arg227Gln)	4.6	4.7
2	c.680G>A(p.Arg227Gln)	4.4	1.4
3	c.439g>A (p.Gly147Arg)	6.8	1.7
4	c.439g>A (p.Gly147Arg)	8	1.9
5	c.439g>A (p.Gly147Arg)	10.2	3.3
6	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	22.7	0.4
7	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	12.8	1.2
8	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	4.6	3
9	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	5.2	1.6
10	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	11	2.4
11	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	7.1	1.8
12	c.138C>G (p.His46Gln) c.153G>T (p.Met51Ile) c.167G>T (p.Cys56Phe)	23.9	0.6

Lyso-Gb3: globotriaosylosphingosine; α-Gal A - α-galactosidase A.

family trees of the patients are presented in Figure 1.

The control group consisted of 12 healthy women, matched to women with FD for age, education level and kidney function. The exclusion criteria were the diagnosis of any disease of the central nervous system other than associated with FD, a disability that would hinder any of the study procedures such as hearing or vision loss, chronic kidney disease with eGFR < 30 mL/min, dementia, acute functional psychiatric disorder and uncontrolled hypertension (systolic BP > 130 mmHg or diastolic BP > 80 mmHg).

Every patient completed a quality-of-life questionnaire The Short Form 36 Health Survey (SF-36) and two screening tests assessing cognitive function, MoCA and MMSE.

The MoCA and MMSE are commonly used as the screening neuropsychological scales. In MMSE, the most commonly used cutoff point for the diagnosis of dementia is a score of 24 points or less. The maximum score for the MoCA test is 30 points; a result of 26 or more points is defined as normal. A score from 19 to 25 is considered as mild cognitive impairment (MCI) (*13*).

During the same visit blood was collected in a fasting state after an overnight rest from all participants to determine serum concentration of NfL, creatinine, urea, calcium, phosphate, parathyroid hormone (PTH) and blood hemoglobin. The concentration of NfL was

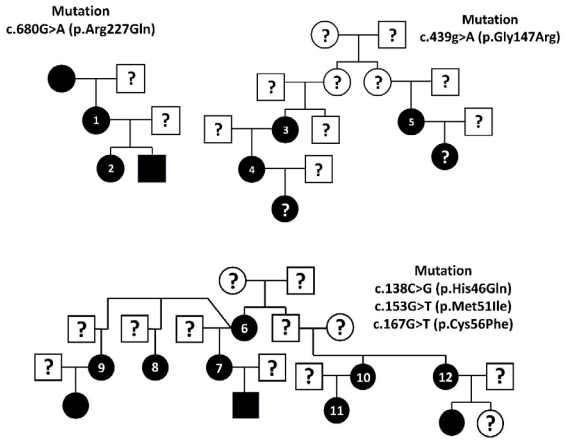


Figure 1. Family trees of three families with Fabry disease included in our study.

assessed with a Neurofilament Light Polypeptide (NEFL) ELISA Kit (antibodies-online GmbH, Aachen, Germany). Other parameters were measured using routine automated laboratory methods in a local laboratory.

The mean value and standard deviation were calculated for each normally distributed variable. For non-normally distributed variables median value with the interquartile range (IQR) was calculated. Analysis of the normality of the distribution was performed with the Shapiro-Wilk test, and on the basis of its results, the parametric *t*-test or the non-parametric Mann-Whitney *U* test was used. Receiver operating characteristic (ROC) curves were drawn to assess the value of serum concentration of NfL, eGFR, lyso-Gb3 and α-Gal A indicating presence of mild cognitive impairment in the MoCA test. Statistica 13.1 software was used to perform the statistical analysis.

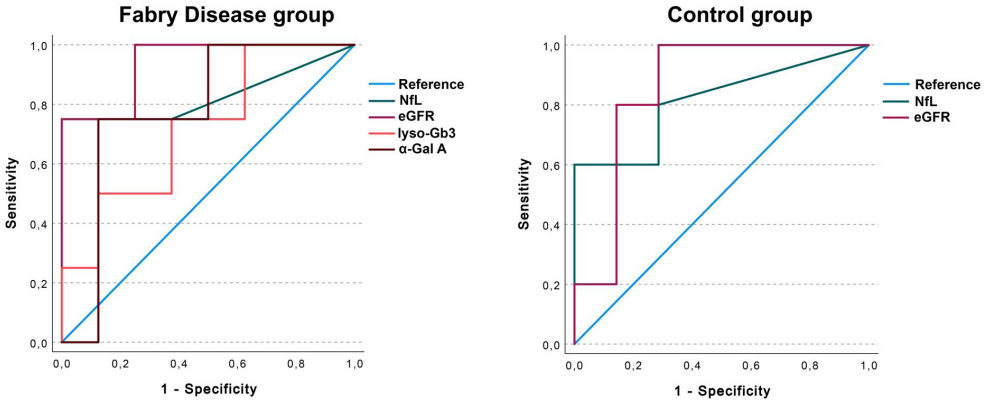
3. Results and Discussion

Serum NfL concentration did not significantly differ between groups (0,053 ± 0,1 *vs.* 0,048 ± 0,09 ng/mL; $p = 0.9$). Women with FD also had similar blood hemoglobin, serum phosphate and PTH. Serum calcium concentration was higher in the FD group than in healthy women (2.38 ± 0.08 *vs.* 2.28 ± 0.11 mmoL/L, respectively; $p = 0.03$). MMSE, MoCA and SF-36 scores were also similar in each group.

In women with FD there was a significant positive correlation between age and serum PTH concentration ($R = 0.62$, $p = 0.03$). The same correlation was not seen in the control group.

In the FD group there was also a significant positive correlation between NfL and lyso-Gb3 concentration ($R = 0.69$, $p = 0.01$).

In the control group a significant negative correlation was found between serum NfL and hemoglobin ($R = -0.8$, $p = 0.001$) MoCA score ($R = -0.6$, $p = 0.04$), and a positive correlation between NfL and serum PTH



Variable	AUC	*a*	*b*	95% CI of AUC
NfL	0,734	0,169	0,166	0,403-1,066
eGFR	0,938	0,074	0,000	0,792-1,083
lyso-Gb3	0,781	0,143	0,050	0,500-1,062
A-Gal A	0,719	0,159	0,170	0,406-1,031

Variable	AUC	*a*	*b*	95% CI of AUC
NfL	0,814	0,139	0,023	0,543-1,086
eGFR	0,857	0,117	0,002	0,628-1,086

NfL – Neurofilament Light Chain
eGFR – estimated Glomerular Filtration Rate
lyso-Gb3 - globotriaosylosphingosine
α-Gal A - α-galactosidase A

Figure 2. Predictors of mild cognitive impairment and receiver operating characteristic curves.

concentration (R = 0.8, p = 0.0009). These correlations were not present in women with FD.

In the control group there was a strong negative correlation between age and MoCA score (R = -0.83, p = 0.0009) and a positive correlation between age and SF-36 score (R = 0.6, p = 0.04). In the FD group only a significant correlation between age and MoCA score was observed (R = -0.85, p = 0.0004).

ROC analysis showed that the best predictor for MCI in both groups was eGFR. Area under the curve (AUC) for women with FD was 0.938 (95% CI: 0.792 - 1.083) and in the control group 0.857 (95% CI: 0.628 - 1.086). Detailed information on ROC analysis results is provided in Figure 2.

Our cross-sectional single-center study was designed to prove the concept that serum NfL levels could reflect the severity of cognitive impairment and indirectly, the level of CNS involvement in women at earlier stages of FD without previous clinical symptoms of nervous system involvement. The hypothesis behind the study was that damage to the neurons in FD patients will result in an increase in concentration of NfL in cerebrospinal fluid and its penetration into the peripheral circulation, which can be assessed by measuring their serum levels. Loeffler T, *et al.* in a mouse model showed that NfL concentration can be a valuable biomarker not only in typical neurodegenerative diseases but also in other diseases that have an additional neuronal component, like lysosomal storage diseases, *e.g.*, Gaucher disease (*15*). Erante D, *et al.* confirmed that NfL is a good biomarker of neurodegeneration in Niemann-Pick disease (*16*). Ru Y, *et al.* described NfL as a biomarker of neurodegeneration in neuronal ceroid lipofuscinosis type 2 (CLN2 disease), another lysosomal storage disease. These authors showed in a canine model a significant correlation between serum NfL concentration and disease progression. Differently, in the human part of their study they were unable to show correlations between NfL concentration and CLN2 severity or age of the patients. However they showed, that NfL concentration decreased by 50% after enzyme replacement therapy was initiated (*17*).

The above cited studies assessed the utility of NfL as a biomarker for the nervous system involvement in some lysosomal storage diseases. To our knowledge, there have been no similar studies in patients with FD. In FD women usually have a milder disease course than men, which is due to a random inactivation of the X chromosome. However, severe involvement of such target organs as the heart or kidneys is quite common (*18*). Despite residual enzyme activity, women with FD develop characteristic symptoms with age, including central nervous system symptoms. However, the clinical manifestation is more varied and the symptoms appear about 10 years later compared to men. The median interval between the onset of early FD symptoms and correct diagnosis is even more delayed in women, with a delay of 19 years on average (*19*). The identification of the marker of early nervous system involvement in women with FD could be particularly clinically relevant since women with this disease develop symptoms much later than men but the nervous system is most frequently involved. The symptoms of FD significantly interfere with patients daily functioning, which contributes to a significantly reduced lifespan. Studies show that the life expectancy of men with Fabry disease is 15 to even 20

years shorter, and that of women is 6 to 10 years shorter compared to the average life expectancy in the population (*20*). In our study, we were not able to confirm that NfL concentration is a clinically useful biomarker for an assessment of the degree of nervous system involvement in women with FD. It may be due to the young age of the patients included in the study and the fact that most of them had no or moderate typical symptoms of the disease from other organs than the nervous system. In most of our study subjects genetic tests were performed due to diagnosis of FD in their relatives.

The α-Gal activities and the lyso-Gb3 concentration are the serum markers commonly used in the diagnosis and monitoring of FD. Measurement of α-Gal activity in plasma or leukocytes, which is the reference method for laboratory confirmation of the diagnosis in men, is often inconclusive in female patients whose enzymatic activity can range from low to normal values. In our study 25% of women with FD had normal α-Gal activity and the lyso-Gb3 concentration was above normal in all cases. The basis underlying variability of the phenotype in women is still poorly understood, but the role of X chromosome inactivation appears to be most important (*21*). In FD, lyso-Gb3 levels are always elevated in men, but only between 40 and 60% in women. Lyso-Gb3 levels in women increase with age and are within the normal range in childhood. However, when symptomatic FD is suspected in adult women, both measurement of α-Gal A and plasma lyso-Gb3 activity improves the diagnostic value (*18*).

In our study the concentration of lyso-Gb3 did not correlate with the results of the tests validated for diagnosis of cognitive dysfunction and with the results of the SF-36. Despite no difference in NfL concentration between study and control group, a positive significant correlation between the concentration of lyso-Gb3 and NfL in the populations of women with FD was seen. An accumulation of lyso-Gb3 deposits as a result of α-Gal A deficiency causes damage to nerve cells with subsequent pro-inflammatory activity (*22,23*). It is possible that due to early diagnosis of the disease and lack of previous neurologic symptoms in our study group, the risk of neurodegeneration was low and thus no difference in serum NfL concentration between the two groups was observed.

Studies conducted so far have shown that patients with FD have significant cognitive deficits (*24*). Most of the studies attributed the changes of cognitive functioning in the course of FD to the accumulation of glycosphingolipids in the cerebral circulation (*25*). In our study, however, no relationship was found between lyso-Gb3 concentration and cognitive impairment. Many psychological tests have been developed for screening for cognitive impairment in clinical practice. They differ in their sensitivity and specificity. The most commonly used screening test in the diagnosis of cognitive impairment is MMSE. Körver S, *et al.* showed that MMSE did not allow screening for MCI in patients with FD and it may lose its predictive value when the cognitive impairment is milder, less prevalent and occurs predominantly in the executive domain, as appears to be the case in FD (*14*). Our results are consistent with the finding that MMSE cannot accurately distinguish patients with subtle cognitive impairment from patients without clinically detectable cognitive impairment. Therefore MoCA should be preferred to MMSE in populations at risk for MCI or with early-stage dementia. In a study conducted by Körver S, *et al.* MoCA questionnaire classified 21% of patients with FD as having MCI, compared with 11% in the reference group (*14*). These data are consistent with our results. In our study more patients were identified as having MCI with the MoCA test compared to MMSE.

In our study, the mean score in MMSE and MoCA test was similar in FD women and control subjects. Löhle M, *et al.* also did not find any significant difference in the tests accessing cognitive functions between the patients with FD and healthy subjects. Their study group was larger, included both women and men in different stages of FD and with both classic and late type *(11)*. In contrast our study included only women without any signs of central nervous system involvement.

Our study results indicate that neither depression nor disease severity, time since FD symptoms, and enzyme activity predicted a cognitive dysfunction. The analysis found no association between cognitive impairment and test scores.

Kidney disease is one of the major complications of FD and is associated with a continuous accumulation of glycosphingolipids throughout the nephron. This leads to progressive loss of GFR and eventually to renal failure. The kidney and brain share similar hemodynamic abilities, such as vasoregulation of the microcirculation. Studies have shown that lower eGFR was associated with increasing severity of chronic white matter hyperintensities (CWMH), and patients with more stable eGFR had fewer strokes than those with rapidly progressive kidney disease (*26*). Our study also suggests the importance of eGFR levels as a predictor of MCI in this patient group. Therefore, further research into the relationship between eGFR levels and MCI in FD patients is warranted (*9*).

In our study, we did not show an association between elevated serum calcium levels in women with FD and serum PTH. In one previous study the authors tried to elucidate the pathomechanism of calcium-phosphate disturbances in patients with FD using a mouse model GlatmTg (CAG-A4GALT). The study results showed a relationship between hypercalcemia and hypercalciuria and secondary hyperthyroidism (*27*). This may suggest that patients with FD are at increased risk of accelerated bone resorption and osteomalacia (*28*).

Our study has limitations, because we have assessed the concentration of NfL only in the peripheral

circulation. However, previous studies showed that the concentration of NfL in the blood strongly correlates with its concentration in the cerebrospinal fluid (*29,30*). Another limitation is a small study group, which is a result of the ultra-low incidence of the disease in the population and of the selection of only the female patients without any previous signs of central nervous system involvement typical for FD.

4. Conclusions

The results of our study did not confirm the relation between the degree of nervous system involvement in women with FD and serum NfL levels. Therefore serum NfL cannot be considered as a useful marker of cognitive impairment in this disease. Our study also showed that MoCA is the preferred test to detect mild cognitive impairment in FD.

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

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Current status of specific pediatric chronic diseases in Japan: National measures, disease types, treatment availability, copayment assistance, and research

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SUMMARY In Japan, specific pediatric chronic diseases include 788 diseases in 16 groups of diseases, 278 categories, and 845 subcategories as of November 1, 2021. The national specific pediatric chronic diseases measures – also called the Medical Aid Program for Chronic Pediatric Diseases of Specified Categories (MAPChD) – was established in Japan in 1974 and enshrined in law in 2005. Patients with specific pediatric chronic diseases can receive government support and welfare in several ways: *i)* MAPChD requires diagnosis and medical treatment at designated hospitals and by designated physicians so that patients receive optimal treatment; *ii)* the copayment rate for medical expenses is reduced to 20% and a maximum is set depending on household income; and *iii)* the analysis of specific pediatric chronic diseases from different perspectives is continuously being promoted. In addition to these three aspects, various measures are being implemented to provide ongoing support for patients with specific pediatric chronic diseases as they grow up.

Keywords specific pediatric chronic diseases, Japan, Medical Aid Program for Chronic Pediatric Diseases of Specified Categories (MAPChD)

1. Introduction

In Japan, specific pediatric chronic diseases must met the following conditions as specified by the Ministry of Health, Labor, and Welfare: *i)* the disease must be chronic, *ii)* the disease must be life-threatening, *iii)* the disease must have symptoms and treatment that reduce the patient's quality of life over a prolonged period, *iv)* the disease must cause a prolonged burden and involve expensive care, and *v)* affect children under 18 years of age (including those under 20 years of age if they continue to require treatment after reaching 18 years of age) (*1*).

The national specific pediatric chronic diseases measures – also called the Medical Aid Program for Chronic Pediatric Diseases of Specified Categories (MAPChD) – was established in Japan in 1974 and enshrined in law in 2005 under the Child Welfare Act (*2*). Initially, the program covered nine groups of diseases including inborn errors of metabolism, hemophilia, pediatric cancer, chronic nephritis and nephrotic syndrome, pediatric asthma, diabetes, collagen diseases, chronic heart diseases, and endocrine diseases. The number of covered diseases has increased (*3*). As of November 1, 2021, the MAPChD covered 788 diseases in 16 groups of diseases, 278 categories, and 845 subcategories (*4*).

MAPChD provides support for patients and families in various ways, including designated hospitals, designated physicians, and specified copayments. Financial assistance for medical expenses can significantly reduce the financial burden on patients and their families. As medical technology makes significant advances, research is being focused on various aspects including the patient's living conditions, information and communication technology (ICT) applications, and improvement of the patient's quality of life.

Here, the current status of specific pediatric chronic diseases in Japan is described in terms of national measures, disease types, treatment availability, copayment assistance, and research.

2. Development of national measures for specific pediatric chronic diseases in Japan and classification of those diseases

2.1. Development of the national measures

In Japan, the medical benefits program for pediatric diseases began in 1968 that for inborn errors of

metabolism, then for hemophilia that began in 1969. The project to research the treatment for pediatric cancer was started in 1971, for chronic nephritis and nephrotic syndrome and for pediatric asthma in 1972 (Table 1).

By integrating above programs and projects, the national specific pediatric chronic diseases measures (also called MAPChD) were established in Japan in 1974, which covers nine groups of diseases including inborn errors of metabolism, hemophilia, pediatric cancer, chronic nephritis and nephrotic syndrome, pediatric asthma, diabetes, collagen diseases, chronic heart diseases, and endocrine diseases (*5*). The number of diseases covered has increased. As of November 1, 2021, the MAPChD covered 788 diseases in 16 groups of diseases, 278 categories, and 845 subcategories (*4*).

2.2. Classification of specific pediatric chronic diseases

Specific pediatric chronic diseases in Japan currently include 788 diseases in 16 groups of diseases. Details on the groups of diseases, categories, and subcategories are shown in Table 2.

The 16 groups of diseases include: *i)* malignant neoplasms, *ii)* chronic kidney diseases, *iii)* chronic respiratory diseases, *iv)* chronic heart diseases, *v)* endocrine diseases, *vi)* connective tissue diseases, *vii)* diabetes mellitus, *viii)* inborn errors of metabolism, *ix)* hematologic diseases, *x)* immune diseases, *xi)* neuromuscular diseases, *xii)* chronic digestive disorders, *xiii)* a syndrome involving chromosomal or genetic alterations, *xiv)* skin diseases, *xv)* skeletal dysplasia, and

Table 1. Development of national measures to combat specific pediatric chronic diseases in Japan

Year	Program/Projects	Details
1968	Medical Benefits Program	A medical benefits program for inborn errors of metabolism is started.
1969	Medical Benefits Program	A medical benefits program for hemophilia is started.
1971	Project to Research Treatment	A project to research the treatment of childhood cancer is started.
1972	Project to Research Treatment	A project to research the treatment of chronic nephritis and nephrotic syndrome and a project to research the treatment of pediatric asthma are started.
1974	Program for Research into the Treatment of specific pediatric chronic diseases	The Program is created to cover 9 groups of diseases by integrating the disease-specific projects listed above and by adding diabetes, collagen diseases, chronic heart diseases, and endocrine diseases.
2005	Medical Aid Program for Chronic Pediatric Diseases of Specified Categories (MAPChD) is enshrined in law	As of April 1, 2005, the Program was moved under the Child Welfare Act and its content was modified. In addition to its expansion to 11 groups of diseases, the criteria for specified chronic pediatric diseases (symptoms of the diseases) and a medical copayment based on household income were included.
2021	List Update of MAPChD	26 diseases are added to the list; the MAPChD covers 788 diseases (without comprehensive diseases*) in 16 groups of diseases.

*A comprehensive disease is a single name for a concept pertaining to a large number of disease groups.

Table 2. The number of specific pediatric chronic diseases in Japan (category and subcategory)

No.	Disease group	Category	Subcategory
I	Malignant neoplasm	6	91
II	Chronic kidney disease	21	51
III	Chronic respiratory disease	12	14
IV	Chronic heart disease	67	99
V	Endocrine disease	41	92
VI	Connective tissue disease	5	24
VII	Diabetes mellitus	1	7
VIII	Inborn error of metabolism	14	138
IX	Hematologic disease	26	52
X	Immune disease	11	56
XI	Neuromuscular disease	41	100
XII	Chronic digestive disease	16	44
XIII	Syndrome involving chromosomal or genetic alterations	1	35
XIV	Skin disease	11	16
XV	Skeletal dysplasia	2	17
XVI	Vascular disease	3	9
Total		278	845

Data source: *Reference 4*.

Table 3. Criteria and maximum copayment limit based on the household tax and annual income for patients with specific pediatric chronic diseases

Classification	Criteria based on the household's residence tax and annual income	Maximum copayment (Japanese yen)		
		Regular	Severe*	Mechanical support such as a ventilator
Household on welfare		0	0	0
Low income I	Household exempt from residence tax (Annual income of less than 800,000 Japanese yen)	1,250	1,250	500
Low income II	Household exempt from residence tax (Annual income less than 2,000,000 Japanese yen)	2,500	2,500	
General income I	Residence tax of less than 71,000 Japanese yen (Annual income of less than 4,300,000 Japanese yen)	5,000	2,500	
General income II	Residence tax of less than 251,000 Japanese yen (Annual income of less than 8,500,000 Japanese yen)	10,000	5,000	
High income	Residence tax of more than 251,000 Japanese yen (Annual income of more than 8,500,000 Japanese yen)	15,000	1,0000	
Meals during hospitalization		50% of expenses		

*"Severe" refers to: A patient with a severe condition who requires expensive medical care for a prolonged period; this is a patient whose monthly medical expenses exceed 50,000 Japanese yen more than 6 times in one year.

xvi) vascular diseases.

3. Designated physicians and designated hospitals for specific pediatric chronic diseases in Japan

In order to promote diagnosis and treatment accessibility for patients with specific pediatric chronic diseases, measures to designate physicians and hospitals were implemented in Japan. These measures play an important role in early diagnosis, early treatment, and reduction of the medical burden.

The designated physicians need to have at least five years of experience in the diagnosis or treatment of the disease and be certified by a relevant medical society or complete training required by government. The designated hospitals are medical facilities that have sufficient capacity to respond to the specific pediatric chronic diseases and that have designated physicians on staff and appropriate equipment available. Enacted on January 1, 2015, the Partial Amendment of the Child Welfare Act stipulates that when a patient with a specific pediatric chronic disease applies for assistance with medical expenses, the application form (medical certificate) must be completed by a designated physician.

Information on designated physicians and designated hospitals for specific pediatric chronic diseases are published by local governments. For instance, 3,304 designated physicians and 1,474 designated hospitals in Tokyo are listed with the Bureau Social Welfare and Public Health of the Tokyo Metropolitan Government as of August 31, 2021 (*6*).

4. Assistance with medical expenses related to specific pediatric chronic diseases in Japan

When a patient is diagnosed with a specific pediatric chronic disease by a designated physician, the patient can apply for the assistance with medical expenses from the MAPChD in order to reduce the financial burden on patients and their families. In Japan, the copayment rate for specific pediatric chronic diseases has been reduced from 30% to 20%, and a maximum is set depending on household income (7).

MAPChD has six classes that determine the maximum copayment based on the household's residence tax and annual income (Table 3): *i)* a household on welfare that is exempt from medical expenses according to the Ministry of Health, Labor and Welfare; *ii)* low income I, which is a household with annual income of less than 800,000 Japanese yen that is exempt from residence tax; *iii)*. low income II, which is a household with an annual income of less than 2,000,000 Japanese yen that is exempt from residence tax; *iv)* general income I, which is a household with an annual income of less than 4,300,000 Japanese yen that is subject to residence tax of less than 71,000 Japanese yen; *v)* general income II, which is a household with an annual income of less than 8,500,000 Japanese yen that is subject to residence tax of less than 251,000 Japanese yen; and *vi)* high income, which is a household with an annual income of more than 8,500,000 Japanese yen that is subject to residence tax of more than 251,000 Japanese yen.

The copayment is free for a household on welfare. The maximum limit of copayment is 1,250 – 15,000 Japanese yen for general patients, depending on the household's residence tax and annual income.

5. Research on specific pediatric chronic diseases in Japan

As medical technology makes significant advances, research is being focused on various aspects including

Table 4. The major research programs promoted by the Information Center for Specific Pediatric Chronic Diseases in Japan in 2020

Research direction	Research content
Online resources	① Creating websites for the general public with content explaining specific pediatric chronic diseases to patients and their families. ② Studying visits to the Center's portal and its dissemination of information. ③ Studying content for children (videos)
Collation of patient data	① Studying the coding of specific pediatric chronic diseases based on ICD-10. ② Guidelines for the use of registry data by academia and the private sector, how consent should be obtained, and guidelines on the handling of past registry data. ③ Studying registration in the database for children with s specific pediatric chronic diseases (2015-2018). ④ Design and development of a registry database and improvement of its accuracy.
Financial burden of patient treatment	Research on the medico-economic value of pediatric treatment
Patient quality of life	① Support for children with specific pediatric chronic diseases using the International Classification of Functioning in Life (ICF). ② Organizing disability welfare policies and systems from the patients' perspective: An attempt to provide information using ICT.
National measures	① A study to create and conduct a training program (e-learning) for specialists in specific pediatric chronic diseases. ② A study on measures for specific pediatric chronic diseases in coordination with the Japanese Society of Pediatrics and its subcommittees and related academic societies. ③ A study on specific pediatric chronic diseases that may qualify as designated intractable diseases.
Follow-on support	① A study on support for the independence of patients with specific pediatric chronic diseases; ascertaining the status of social participation after adulthood. ② A study on support for the independence of patients with specific pediatric chronic diseases: Revision of the guidelines on transition assistance for patients with specific pediatric chronic diseases.

Data source: *Reference 8*.

the patient's living conditions, ICT applications, and improvement of the patient's quality of life.

For instance, research groups at the Information Center for Specific Pediatric Chronic Diseases in Japan are conducting research on aspects such as provision of information, collation of patient data, assessment of financial burdens, and follow-on support. The research conducted in 2020 is summarized in Table 4, and research was conducted in the following main areas: *i)* online resources; *ii)* collation of patient data; *iii)* the financial burden of patient treatment; *iv)* patient quality of life; *v)* national measures; and vi) follow-on support.

6. Perspectives for the future

In Japan, patients with specific pediatric chronic diseases can receive government support and welfare from designated physicians and designated hospitals and assistance with medical expenses. In addition, specific pediatric chronic diseases are being researched. That said, more attention needs to be paid to follow-on support for patients with specific pediatric chronic diseases, such as disease progression with age or the development of sequelae (*9*). Some patients have difficulty entering the workforce, which leads to an increased psychological and financial burden on the patient and the patient's family (*10*). National measures to combat specific pediatric chronic diseases and the continued enhancement of those measures are expected to help more patients to benefit from treatment availability, copayment assistance, research, and follow-on support.

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Updated genetic studies of Marfan syndrome in China

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SUMMARY Marfan syndrome (MFS) is an autosomal dominant connective tissue disease that affects multiple systems such as the ocular, skeletal, and cardiovascular systems. This disease is relatively rare and has no effective treatment except for symptomatic treatment. As a result, early detection, early intervention, and preventing the occurrence of adverse cardiovascular outcomes are crucial to the diagnosis and treatment of MFS. The rapid development of gene sequencing technology has facilitated the detection of MFS at the genetic level, allowing a more accurate and efficient diagnosis of the disease. Therefore, research on MFS-related genes has become a topic of interest. This article reviews the recent progress of genetic research on MFS in China.

Keywords Marfan syndrome (MFS), gene sequencing, *FBN1*, *TGFBR2*, base editor 3 (BE3)

Marfan syndrome (MFS) is an autosomal dominant connective tissue disease with a prevalence of 2-3/10,000 (*1*). According to an epidemiological survey in Taiwan, the total prevalence of MFS in the Chinese population is 10.2/100,000 (*2*), which is slightly different from the worldwide figure. MFS affects multiple organs and systems, including the eyes, cardiovascular system, musculoskeletal system, and possibly even the lungs, skin, and central nervous system. Cardiovascular diseases, including aortic dilation and dissection, are the leading cause of death in patients with MFS (*3*). Due to multi-organ involvement and the absence of an effective treatment except for symptomatic treatment, early detection and prevention of disease progression are crucial. The most common cause of MFS is a mutation in the fibrinogen 1 (*FBN1*) gene, which occurs in more than 90% of patients with MFS (*4*). Therefore, detection of mutations in the *FBN1* gene in patients with MFS, the relationship between gene mutation sites and phenotypes, and whether there are other gene mutations related to MFS have become hot topics in MSF research in China.

Since 1991, when the mutation of *FBN1* gene was identified as a pathogenic factor of MFS (*5*), approximately 2,900 mutated loci have been identified in the *FBN1* gene. Clinically, patients with *FBN1* mutations exhibit a range of phenotypes from mild to severe. A single mutation cannot fully explain the heterogeneity of clinical phenotypes in patients with MFS. In 2019, the Beijing Anzhen Hospital of Capital Medical University evaluated the genotype and phenotype in 180 patients by sequencing their DNA. The patients were divided into two groups according to clinical manifestations: an aortic dissection group and an aortic aneurysm group. Results indicated that frameshift mutations and nonsense mutations in *FBN1* were significantly more prevalent in patients with aortic dissection, while missense mutations in the *FBN1* gene were more prevalent in patients with aortic aneurysm (*6*). That said, a strong genotype-phenotype association between *FBN1* variants and MFS had not been reported. To resolve that situation, researchers at the Beijing Anzhen Hospital collected medical records and genetic tests from 131 patients with MFS in 2020 (*7*), and they found 82 low-frequency harmful loci in the *FBN1* gene, including 38 genes that had not been reported in the HGMD database. They also found that eight patients had two mutations (more than one SNP or INDEL locus) in the *FBN1* gene, and patients with two mutations in the *FBN1* gene exhibited a more significant MFS phenotype compared to other family members. They also found that patients with MFS had mutations in other genes, such as *PKD1*, *PKD2*, *FLNA*, *NKX2-5*, and *ACVRL1*. The frequency of mutations in the *PKD1* gene was much higher than that in other genes, and mutations in the *PKD1* gene were detected in a total of 27 people. This analysis also indicated that there are many genes associated with heart disease, such as *TTN, NEFH*, *PLEC*, *CASQ2*, and *SYNE1*. In the study, patients were divided into four groups depending on their aortic disease phenotype: 1): Aortic widening group; 2): Aortic aneurysm group; 3): Aortic dissection group; 4):

No aortic disease group. A statistical analysis with a *t*-test revealed a significant age difference between patients without an aortic disease phenotype and those with aortic aneurysm or aortic dissection ($P < 0.05$). Patients with aortic aneurysm or aortic dissection were mainly between 20 and 40 years of age, while patients without aortic disease were younger on average and the age distribution varied. The researchers ultimately created genotype-phenotype correlation maps and they combined sequencing results with various databases to screen and sequence affected genes. Genes were classified depending on the strength of the association between gene mutations and MFS. The top 10 candidate genes associated with a disease phenotype or the disease were *FBN1*, *MED12*, *TGFBR2*, *SMARD*, *FBN2*, *TP53*, *CDH1*, *FN1*, *COL4A3*, and *COL4a2*. These findings provide new ideas for further scientific research.

Although the *FBN1* gene is mutated in more than 90% of patients with MFS, *FBN1* mutations are not detected in 10% of patients clinically diagnosed with MFS, suggesting that atypical types of mutation or other genes may cause MFS. To look for atypical mutations or other mutations that cause MFS, the First Hospital Affiliated with Nanjing Medical University recruited 19 volunteers from three Han Chinese families between 2012 and 2016 to conduct a family-based study on 19 individuals using full exome sequencing (WES) (*8*). After DNA samples were collected, whole exon sequencing was performed, and quality control, mapping and variant calling were performed. Based on the literature regarding OMIM and MFS and the American Society of Medical Genetics (ACMG) standards and guidelines, genes were divided into eight previously reported MFS-related genes, 125 MFS-related genes from gene cards, and previously unknown genes. In the end, Sanger sequencing was performed after all remaining mutations were manually detected using an integrated genome viewer (IGV 2.3.80). A novel loss-of-function indel of *FBN1* (c. 5027 _5028insTGTCCTCC, p.D1677Vfs*8), a second novel loss-of-function indel (c.5856delG, p.S.1953Lfs*27), and a nonsense mutant (c. 8034C>A, p.Y2678 *) were found in those families. Moreover, all mutation sites were located in the highly conserved amino acid region (calcium-bounding epidermal growth factor (EGF) domain) across different species. These different types of loss-of-function (LOF) variations in *FBN1* are located in the cbEGF region and cross-species conserved domains and had not been previously reported.

Patients with type 2 MFS carry a mutation in the transforming growth factor β receptor 2 (*TGFBR2*) gene. In 2018, the Hypertension Diagnosis and Treatment Center of Fuwai Hospital, Chinese Academy of Medical Sciences conducted a study on two families with type 2 MFS, and for the first time researchers found that patients with MFS2 carried pathogenic mutations located in the *TGFBR2* gene transmembrane domain (*9*). They sequenced the *FBN1* gene and the *TGFBR2* gene. Their protein structures were predicted and a genotype-phenotype analysis was performed on the screened carriers of the *TGFBR2* gene transmembrane domain missense mutation. Results indicated that all carriers (100%, 8/8) of the *TGFBR2* gene transmembrane domain missense mutation met the main diagnostic criteria for cardiovascular involvement, with ascending aortic dilation, aortic root dilation, aortic dissection, or some other serious clinical phenotype of cardiovascular disease. Of the carriers, 75% (6/8) had abnormal skeletal involvement, and only 12.5% (1/8) of those met the main diagnostic criteria. None of the *TGFBR2* mutation carriers had ocular involvement. Results also indicated that carriers of two new *TGFBR2* gene missense mutations (p.137K(c.110T > A) and p. G43D (c.128G > A)) in families with type 2 MFS were more likely to develop aortic dilation or aortic dissection.

The recently developed base editor (BE) system is a novel technique involving the creation of a BE by fusing the protein dCas9 to deaminase. In contrast to traditional genome technologies based on CRISRP/Cas9, a BE edits specific loci through C-to-T or G-to-A transformations without requiring the formation of double-strand breaks (DSBs). The BE system provides a safer genome editing tool with low off-target effect (*10,11*). The Third Hospital Affiliated with Guangzhou Medical University conducted an experiment using BE3 to correct mutated MFS-related genes (*12*). They modeled the T7498C mutation of *FBN1* using CRISPR/Cas9 in combination with ssODN in HEK293T cells and then designed a specific single guide RNA (sgRNA). Mutant cells in this model were transfected with correctional sgRNA and BE3 expression plasmids. Three days later, the cells were collected, and genomic DNA was extracted and used as template to amplify the target sequence. PCR was performed, and the results were compared to those for the wild type to verify allele correction. Correction occurred in 10 of the 20 models, and ideal C-to-T correction at T7498C was performed in eight, demonstrating the high efficiency of the BE technique in correcting *FBN1*T7498C. The researchers then assembled immature oocytes and single sperm donated by patients with MFS and a heterozygous *FBN1*T7498C mutation after *in vitro* maturation intracytoplasmic sperm injection (ICSI) and partially injected BE3 mRNA and corrected sgRNA 16-18 hours later. Control samples were injected with BE3 mRNA and scrambled sgRNA and cultured. A total of 7 experimental embryos and 7 control embryos were obtained. Genome-wide amplification was performed, and genotyping was performed using Sanger sequencing. Results indicated that the rate of allele correction in the tested embryos was close to 100%. The researchers then performed deep sequencing and a comprehensive analysis of the test samples, the results of which revealed the high efficiency and precision of gene correction. Finally, the researchers tested non-target mutations

by deep sequencing and whole genome sequencing to demonstrate the safety of the aforementioned procedures, providing a theory and direction for further research. Studies and the significance of their findings are summarized in Table 1.

FBN1 gene research is also a hot topic in Europe, but European studies have reported slightly different results.

In a French study (*13*), the investigators took the mRNA of the *FBN1* gene from adventitial fibroblasts from multiple sites in 5 patients with thoracic aortic aneurysm or dissection. RT-PCR was then used to study the differences in levels of expression of *FBN1* subtypes between normal people and patients with MFS and differences in levels of expression of the three subtypes *FBN1_001* (ENST00000316623.5, NM_000138), *FBN1_004* (ENST00000559133.1), and *FBN1_009* (ENST00000561429.1) in relation to clinical phenotypes. This was the first time that *FBN1* alternative splicing was identified as a potential mechanism of clinical variability in MFS. In a Dutch study (*14*), 14 individuals from 2 families underwent next-generation sequencing (NGS) gene panel diagnostics. An *FBN1* mutation at site c.1453C>T, p.(Arg485Cys) was found to be a pathogenic mutation that leads to autosomal

Table 1. Research in China and several European countries

Lead author	Results	Significance
Shijun Xu (2019)	Frameshifts and nonsense mutations of the *FBN1* gene significantly more prevalent in patients with aortic dissection, while missense mutations of the *FBN1* gene were more frequent in patients with aortic aneurysm.	These results have laid the foundation for the study of the genotype-phenotype association between *FBN1* variation and MFS and may have guiding significance for the treatment of patients with MFS.
Yuduo Wu (2020)	1). 82 low-frequency harmful loci were identified in the *FBN1* gene, including 38 novel loci. 2). Patients with two mutations in the *FBN1* gene exhibited a more significant MFS phenotype. 3). Patients with MFS also have mutations in other genes, such as *PKD1* and *PKD2*; mutations in the *PKD1* gene were the most prevalent. 4). Many heart disease-related genes such as *TTN* and *NEFH* were noted. Patients without an aortic disease phenotype and patients with aortic aneurysm or aortic dissection differed significantly in age.	Many new MFS mutation sites and double mutation sites were identified, further confirming the pathogenicity of the *FBN1* gene in patients with MFS. A mutation in the *FBN1* gene is the main factor leading to aortic dissection or aneurysm in patients, and a double mutation site is the key factor aggravating the phenotype of patients
Zhening Pu (2018)	1). Two new sites of *FBN1* gene dysfunction (c.5027_5028insTGTCCTCC, p.D1677Vfs*8; c.5856delG, p.S1953Lfs*27) and a nonsense mutant (c.8034C>A, p.Y2678*) were identified. 2). All mutation sites were located in the highly conserved amino acid region (calcium binding epidermal growth factor (EGF) domain) in different species.	For the first time, different types of loss-of-function (LOF) variants of *FBN1* were identified in the cbEGF region and in the cross-species conserved domain.
Lin Zhang (2018)	The carriers of two new *TGFBR2* gene missense mutations p.137K(c.110T>A) and p.G43D(c.128G>A) in families with type 2 Marfan syndrome were more likely to develop aortic dilation or aortic dissection.	For the first time, results revealed that patients with MFS2 carried pathogenic mutations located in the transmembrane domain of the *TGFBR2* gene.
Yanting Zeng (2018)	In HEK293T cells, CRISPR/Cas9 combined with ssODN was used to establish the T7498C model of an *FBN1* mutation, and then the BE3 system was used for gene correction. Correction occurred in 10 of the 20 models, and ideal C-to-T correction at 7,498 was performed in eight. The safety of these procedures was verified with deep sequencing and whole genome sequencing to detect non-target mutations.	This study proved the safety of gene editing in patients with MFS and provided a theory and direction for further research
Louise Benarroch (France) (2019)	RT-PCR was used to study the differences in levels of expression of FBN1 subtypes between normal people and patients with MFS. Three subtypes were identified: *FBN1_001*, *FBN1_004*, and *FBN1_009*. The main isoform was *FBN1_001*, and it was significantly reduced in skin and adventitial fibroblasts of patients with MFS	This was the first time that *FBN1* alternative splicing was identified as a potential mechanism of clinical variability in MFS.
Eline Overwater (Netherlands) (2018)	An *FBN1* mutation caused by site c.1453C>T, p.(Arg485Cys) was found to be a pathogenic mutation that leads to autosomal dominant MFS, which is characterized by high clinical variability and apparently isolated early onset familial abdominal aortic aneurysms.	This study confirmed the high degree of clinical variability associated with *FBN1* variation and it provided new insights into the genetic pattern of *FBN1* variation c.1453C>T, p.(Arg485Cys)
Sinem Yalcintepe (Turkey) (2020)	Three mutated loci were identified: NM_000138.4(*FBN1*):c.229G>A(p.Gly77Arg), NM_000138.4(*FBN1*):c.165-2A>G(*novel*), NM_000138.4(*FBN1*):c.399delC (p.Cys134ValfsTer8) (*novel*).	The two novel pathogenic mutations have been added to the genotype-phenotype spectrum of clinical features of MFS. This case report emphasized the role of molecular analysis in the diagnosis of MFS.
Fatemeh Bitarafan (Iran) (2020)	5 mutations in *FBN1* and *2* mutations in *TGFBR2* were identified in 7 patients with MFS. *Novel* mutation sites were NM_000138.4(*FBN1*):c.3833G>A(p.Cys1278Tyr), NM_000138.4(*FBN1*):c.6288C>A(p.Cys2096*), and NM_003242.6(*TGFBR2*):c.1085A>G (p.His362Arg).	Early and accurate molecular diagnosis leads to better management and improved life expectancy, so the results of this study should greatly improve genetic counselling for families with MFS in Iran.

dominant MFS, which is characterized by high clinical variability and apparently isolated early onset familial abdominal aortic aneurysms. In a case report from Turkey (*15*), three patients with a prediagnosis MFS underwent gene sequencing, and three mutated loci were identified: NM_000138.4(*FBN1*):c.229G>A(p.Gly77Arg),NM_000138.4(*FBN1*):c.165-2A>G(*novel*), NM_000138.4(*FBN1*):c.399delC (p.Cys134ValfsTer8) (*novel*). The two latter mutations, which were not detected in the parents' genes, have been termed new pathogenic mutations and were added to the genotype-phenotype spectrum of clinical features of MFS. This case report emphasized the role of molecular analysis in the diagnosis of MFS. In Iran (*16*), 7 patients with MFS were screened for 14 genes (including *FBN1* and *TGFBR2*), and 5 mutations in *FBN1* and 2 mutations in *TGFBR2* were found. The novel mutation sites were NM_000138.4(*FBN1*):c.3833G>A(p.Cys1278Tyr), NM_000138.4(*FBN1*):c.6288C>A(p.Cys2096*), and NM_003242.6(*TGFBR2*):c.1085A>G (p.His362Arg). In addition, *FBN1* gene testing in patients with MFS has been reported in Italy, Spain, and elsewhere.

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Letter

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GPR30: A new potential therapeutic target in human testicular germ cell tumors

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SUMMARY The G protein-coupled estrogen receptor (GPR30) is suggested to exert a role in non-nuclear estrogen signalling and is over-expressed in a variety of hormone dependent tumors. It is well known that estrogens and xenoestrogens are involved in testicular germ cell tumorigenesis. Different studies show that down regulation of estrogen receptor β (ERβ) associates with GPR30 over-expression both in human testicular carcinoma *in situ* (CIS) and seminomas and that the mitogenic role exerted by 17β-oestradiol induces the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) through GPR30. In conclusion, the exposure to oestrogens or oestrogen-mimics, in some as of yet undefined manner, diminishes the ERβ-mediated growth restraint in CIS and in human testicular seminoma, indicating that GPR30 could be considered a potential therapeutic target to design specific inhibitors.

Keywords GPR30, testicular cancer, estrogen receptor, seminomas

The estrogen receptor β (ERβ) subtype is the principal mediator of oestrogen action in promoting germ cell survival and development. After activation, these receptors, in association with various coactivators as RING-finger protein 4 (RNF4) and repressors as POZ-AT HOOK Zinc Finger -Containing Protein 1 (PATZ1), act as nuclear transcription factors for targeted genes. It has been well documented in literature that ERβ is instead down regulated in seminomas and embryonal cell carcinomas (*1-7*).

In the last few years, G protein-coupled receptor 30 (GPR30) was demonstrated to be capable of mediating estrogen actions in a wide variety of cell types including germ cells and Sertoli cells; in fact, it has been shown that GPR30 may mediate actions important for Sertoli cell function and maintenance of normal testis development and homeostasis. GPR30 has been recently found to bind 17β-estradiol (E2) with high affinity and to mediate estrogenic signals controlling the proliferative effects of E2 in ER-negative SKBr3 breast cancer cell lines since GPR30 depletion, by using antisense oligonucleotides or RNA interference (RNAi) strategies, abrogated E2-stimulated growth in these cells (*8,9*). GPR30 activates numerous cell signaling pathways including calcium mobilization, adenylyl cyclase, MAP kinase and phosphatidyl inositol 3- kinase, in large part *via* the transactivation of epidermal growth factor receptors (EGFRs) (*10*). These observations led to the hypothesis that GPR30 activation may represent an alternative pathway for estrogen-mediated activity in high grade and advanced stage in various epithelial tumors that are more often ER negative.

Recent published studies correlate the GPR30, and ERβ expression in testicular human carcinoma *in situ* (CIS) and seminomas (*8*) (Table 1). First, the down-regulation of ERβ, observed in seminomas, was in accordance with our previously published data, and from animal models and human cell culture studies suggesting that ERβ may control cell proliferation during germ cells cancer progression (*8,9*). These considerations induce to hypothesize that exposure to estrogens, in some as of yet undefined manner, diminishes the ERβ-mediated growth restraint in spermatagonia, which favors unscheduled cell proliferation. The affected spermatogonia or their descendants may then be able to escape normal cell cycle regulation and be at a higher risk of undergoing malignant transformation (*9*).

Recently, we have shown that ERβ interacts with High Mobility Group A1 (HMGA1) and PATZ1 in normal germ cells, while down regulation of ERβ is concomitant with transcriptional coregulators HMGA1 and PATZ1 over-expression and cytoplasmic localization both in human testicular seminomas and in TCam-2 seminoma cell line (*5,6*). We also observed

Table 1. Immunohistochemical markers in TGCTs subtypes

Items	HMGA1	HMGA2	PATZ1	GPR30	ERβ	RNF4
Seminoma	+©	-	+©	+	-	-
Embryonal carcinoma	+©	+	+©	+	-	-
Teratoma	-	-	+©	+/-	-	-
Yolk sac	-	+	+©	+	-	-

Notes: +, expressed; +© cytoplasmic localization; -, not expressed; +/- variable expression.

that 17β-oestradiol induces an HMGA1 increased cytoplasmic expression correlates with an ERβ down-regulation in TCam2 cell line (*5*). In addition, our group has published that GPR30 is over-expressed in human testicular seminomas, that are more often ERα/β negative (*9*) (Table 1).

The relationship between estrogen signaling and its multiple regulatory interactions with growth factor and other kinase signaling pathways involves complex patterns of genomic and non genomic cross-talk. Estrogen, as well as many of the classic ER antagonists and estrogen receptor modulators (SERMs, including fulvestrant and tamoxifen) activate signaling pathways *via* GPR30 (*9,10*). In addition, in our recent published study we have shown by using the TCam2 seminoma cell line that 17β-estradiol induces Extracellular signal-Regulated Kinase 1 and 2 (ERK1/2) activation and c-fos increased expression in absence of ERβ and in presence of GPR30 (9). Studies that evaluate GPR30 expression in relation to the classical steroid receptors (ERα/β) and response to chemotherapy are needed to elucidate the value of GPR30 as a prognostic indicator (*8,9*). Since many G protein-coupled receptors, including GPR30, induce EGFR phosphorylation, the inter-receptor cross-talk demonstrated by this paradigm represents a novel opportunity for therapeutic intervention (*8,9*). Therefore, the expression or function of GPR30 with selective agonists and/or antagonists could be an effective treatment strategy, in conjunction with standard chemotherapy. In fact, we have demonstrated that G15, a new selective GPR30 antagonist, inhibits estrogen-induced proliferation in TCam2 seminoma cell line (*3,9,10*).

In conclusion, the design of specific GPR30 inhibitors could represent a useful molecular target to block neoplastic germ cells with a high proliferative rate suggesting its potential therapeutic role for the treatment of CIS and seminomas.

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

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IRDR
Intractable & Rare Diseases Research

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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, 2010).

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