# A Review on the Rare Diseases and Subsequent Orphan Drugs

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

Department of Pharmacy
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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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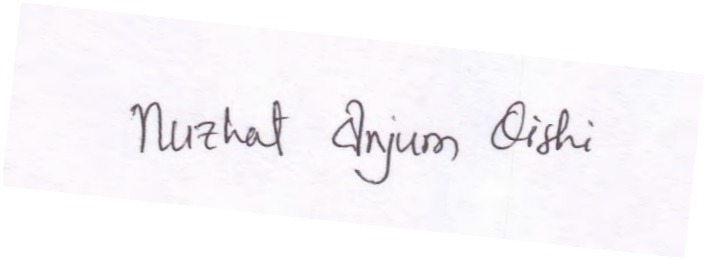

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## Approval

The thesis titled "A Review on Rare Diseases and Subsequent Orphan Drugs" submitted by Nuzhat Anjum Oishi (15146075) of Spring 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy.

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## Ethics Statement

This study does not involve any human and animal trial.

## Abstract:

Worldwide among every existing disease, approximately 5000-8000 are often referred to as rare diseases due to their uncommon, chronic, progressive, degenerative, and life-threatening characteristics. This review specifically deals with Duchenne Muscular Dystrophy, Friedreich's Ataxia, and Transthyretin Amyloid Polyneuropathy; three rare conditions each with the scope of better orphan treatment opportunities. In spite of some orphan drugs' improving results for treating each rare disease, it has been however recommended that only a precise and effective orphan treatment can provide optimal life. In response to these rare diseases, substantial cooperation among the stakeholders, funders, orphan drug developers, regulatory authorities, policymakers, governments, and immense global effort is crucial. This review paper is hence a nuanced analysis of ongoing strategies to patently identify rare diseases, three specific rare diseases profile along with their impending orphan drug candidates, and their pre-and post-development challenges.

**Keywords:** rare disease; orphan drug; Duchenne Muscular Dystrophy; Friedreich's Ataxia; Transthyretin Amyloid Polyneuropathy; treatments.

## Dedication

Dedicated to my parents, brother and my project supervisor, Dr. Md. Abul Kalam Azad.

## Acknowledgements

First of all, I would like to thank Almighty for his unlimited blessings in attempt to empower me with the strength and willingness to accomplish this project work.

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## List of Acronyms

| | |
|---|---|
| ODA | Orphan Drug Act |
| FDA | Food and Drug Administration |
| EMA | European Medicines Agency |
| DMD | Duchenne Muscular Dystrophy |
| BMD | Becker muscular dystrophy |
| FRDA | Friedreich's Ataxia |
| PIMs | Population Impact Measures |
| ATP | Adenosine tri-phosphate |
| ATTR-PN | Transthyretin Amyloid Polyneuropathy |
| AON | Antisense oligonucleotide |
| CDCs | Cardiosphere-derived Cells |
| HDAC | Histone deacetylase |
| PMO | Phosphorodiamidate morpholino oligomers |
| CAN | Cardiac autonomic neuropathy |
| HCM | Hypertrophic cardiomyopathy |
| BBB | Blood-brain barrier |
| CHF | Congestive heart failure |

# Chapter 1

# Introduction

## 1.1 Rare Disease

Worldwide the term 'Rare Disease' is used regularly to refer to any condition that might be uncommon, unique, chronic, progressive, degenerative, disabling, and often life-threatening with few or no treatment possibilities. To describe the rare disease, there is no single definition that can be commissioned and acknowledged globally. There are at least 10,000 existing diseases; among them, 5,000-8,000 are rare diseases with a prevalence rate of 0.65%-1% (Denis et al., 2010; Gong & Jin, 2012; P. Song et al., 2012). Previously, pharmaceutical companies were not interested in adopting and developing treatments for rare diseases, thus referred to as Orphan Diseases. Either inherited or acquired due to environmental factors, respective rare diseases are unique and affect a small percentage of the population (Taruscio et al., 2011). Among numerous possibilities, rare diseases can be chronic genetic disorders, rare cancer and proliferative causes, autoimmune disease, allergies, degenerative disease, or even infectious diseases (Gammie et al., 2015; Logviss et al., 2014). An initial analysis by MDO (Mondo Disease ontology) alluded that the magnitude of distinct rare diseases could be substantially higher than the generally proposed 7000 and potentially up to 10,000 (Tambuyzer et al., 2020). While individually rare diseases impact a small percentage of the population, collectively can affect a significant proportion of the general population. In a global estimation, around 350 million people are currently affected by rare diseases, of whom 30 million are in Europe and 25 million in North America (De Vrueh et al., 2013; Tambuyzer et al., 2020; Wästfelt et al., 2006). Denoting 1 out of 15 persons worldwide

might be affected by orphan diseases. Although a multitude of individuals live with rare diseases, merely 5% have an approved treatment (Ferreira, 2019; Gong et al., 2020).


*Figure 1: Resolution for rare diseases (copied from Rare Diseases International, 2021).*

## 1.2 Legislation of Rare Diseases

Most countries define rare diseases in their legislation based on the prevalence rate and threshold. In the United States of America, the Orphan Drug Act (ODA) of 1983 and the Health Promotion and Disease Prevention Amendments of 1984 described rare diseases as any disease or condition that affects less than 2,00,000 individuals in America (Ferreira, 2019; Taruscio et al., 2011). That is equivalent to a prevalence of 6.4 in 10,000 Americans (Gammie et al., 2015). The introduction of a new product for orphan diseases takes a monopoly and is achieved through certain incentives.

To incentivize orphan drug development, the US legislators embodied orphan drug policies including orphan drug designation, marketing authorization, marketing exclusivity, fast track approval, tax credits, fee waivers along with financial subsidies, and reimbursement in the national legislation (Gammie et al., 2015; P. Song et al., 2012). The United States established a landmark by enacting the ODA as the pioneer orphan drug legislation. The success of the ODA has encouraged other countries to create and implement own legislation for rare diseases, such as:

*Table 1: Country Definitions of Orphan or Rare Diseases (modified from Schouten, 2020)*

| Region : Country | Legal Framework | Definition of Rare Disease |
|---|---|---|
| Europe :<br>European Union (EU) | Article 3 of the Orphan Medicinal Product Regulation (EC) No. 141/2000<br>(December 16, 1999) | life-threatening or chronically disabling condition with a prevalence of 5 in 10,000 people (or fewer) in the 28 EU countries |
| Europe :<br>United Kingdom (UK) | The UK Rare Diseases Framework (signed up by 4 UK nations)<br>(January 2021) | |
| North America :<br>Canada | | cases fewer than 5 per 10,000 individuals (draft) |
| North America :<br>Mexico | Article 224 BIS of the General Law of Health (2012) | prevalence of less than 5 per 10,000 inhabitants |
| North America :<br>Caribbean | no orphan drug legislation or specified definition of rare diseases | |
| South America :<br>Argentina | Article 2 of the Law No. 26.689 (2011) | prevalence in the population is equal to or less than 1 in 2000 |
| South America :<br>Brazil | Article. 3 of the Ordinance No. 205<br>(December 28, 2017) | frequency less than 65 cases per 1,00,000 people |
| South America :<br>Uruguay | no orphan drug legislation or specified definition of rare diseases | |
| South America :<br>Peru | Regulation of Law No 29698 (2011) | chronic, life threatening condition with a prevalence of less than 1 per 1,00,000 people |
| Central America :<br>Panama | Law 28 (October 28, 2014) | prevalence is fewer than 1 in every 2,000 inhabitants |
| Africa : | no orphan drug legislation or specified definition of rare diseases | |

| | | |
|---|---|---|
| South Africa | | |
| Middle East :<br>UAE | Federal Law 12,<br>Decree7 (2018) | |
| Middle East :<br>Iran | | conditions with 1-5 incidence in every 10,000 people |
| Middle East :<br>Egypt | no orphan drug legislation or specified definition of rare diseases | |
| Eurasia :<br>Turkey | no orphan drug legislation or specified definition of rare diseases | |
| Eurasia :<br>Russian Federation | Article 44 of the Federal Law No. 323-FZ (November 21, 2011) | prevalence less than 10 cases per 1,00,000 people |
| Asia :<br>Japan | Japan Pharmaceutical Orphan Drug Law (October 1993) | intractable diseases (Nan-Byo) having a prevalence of less than 1 per 2,500 individuals |
| Asia :<br>Taiwan | Rare Disease Control and Orphan Drug Act (2000) | diseases of a genetic origin affecting less than 1 in 10,000 inhabitants |
| Asia :<br>South Korea | Rare Disease Management Act (2015) | prevalence threshold of fewer than 20,000 patients |
| Asia :<br>China | | prevalence rate fewer than 1 in 5,00,000 or neonatal casualty less than 1 in 10,000 |
| Asia :<br>Bangladesh | no orphan drug legislation or specified definition of rare diseases | |
| Asia :<br>Thailand | no orphan drug legislation or specified definition of rare diseases | |
| Asia :<br>Singapore | The Rare Disease Fund-RDF (July 2019) | affecting fewer than 1 in 2,000 patients |
| Asia :<br>India | National Policy for Treatment of Rare Diseases-NPTRD (July 2017) | estimates 1 case per 5000 individuals |
| Oceania :<br>New Zealand | | prevalence less than 1 in 2,000 inhabitants |
| Oceania :<br>Australia | Orphan drug program (1997) | condition affects fewer than 5 individuals in 10,000 |

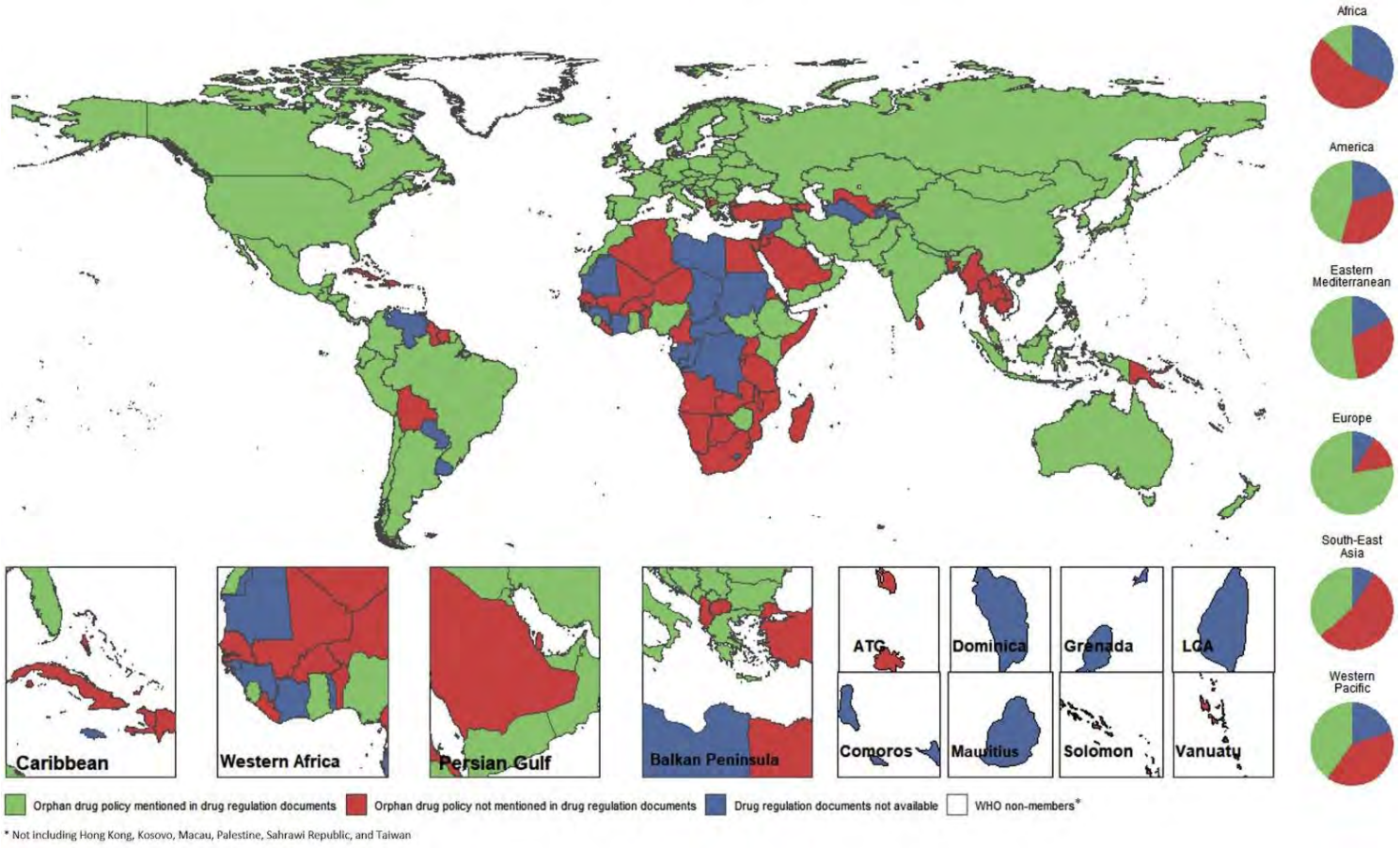


*Figure 2: Geographical distribution of orphan drug policy establishment. Green marks represent countries with orphan drug policy mentioned in drug regulation documents, red marks draw the countries with orphan drug policy not mentioned in drug regulation documents, blue marks showing countries with drug regulation documents not available and white marked countries are WHO non-members. Map not including Hong Kong, Kosovo, Macau, Palestine, Sahrawi Republic and Taiwan (adapted from Chan et al., 2020).*

## 1.3 Orphan Drug

Orphan Drugs are medicines or vaccines developed and designed to treat a rare medical condition. Orphan products include various drugs, biologics, devices, or medical food. Pharmaceutical industries rarely develop orphan medicines compared to essential medicines because companies are not social institutions and are involved professionally in producing safer drugs by conducting experimental research only when they anticipate profit. As a result, the discovery, design, and development of orphan drugs are often challenging. Challenges include difficulties in setting up

clinical studies, assessing clinical relevance, cost-effectiveness, affordability, and insufficient market opportunities (Gong et al., 2020). These criteria raise the overall drug cost and result in the drugs for rare diseases being orphaned by many leading pharmaceutical companies. Reportedly, the mean cost for orphan drugs per patient a year is 4.8 times higher than for non-orphan drugs (Ferreira, 2019). It is a dilemma for the public and private healthcare system, similarly difficult for the patients and their families. The objective criteria for orphan products are to reduce morbidity and mortality rates, notably as neonates are involved, by extending the availability and accessibility of medications despite the disease prevalence. To market an orphan product, a sponsor must apply and obtain an orphan drug designation for its product either from the Food and Drug Administration (FDA) in the USA or the European Medicines Agency (EMA) in the EU (De Vrueh et al., 2013). Receiving orphan drug designation qualifies pharmaceutical companies for incentive development, including financial grants, fee reductions, extended market exclusivity, protocol, and regulatory assistance (De Vrueh et al., 2013; Logviss et al., 2014; Sharma et al., 2010a; Tambuyzer et al., 2020; Wästfelt et al., 2006). Nowadays, precision medicines and therapeutic modalities may include small molecules, monoclonal antibodies, oligonucleotides, gene and cell therapies, protein replacement therapies, and even drug repurposing to treat and maintain a patient's optimal condition (Tambuyzer et al., 2020). Additionally, a post-approval study, including observational studies, pragmatic trials, and randomized controlled studies, is necessary to monitor the drug's safety and efficacy.

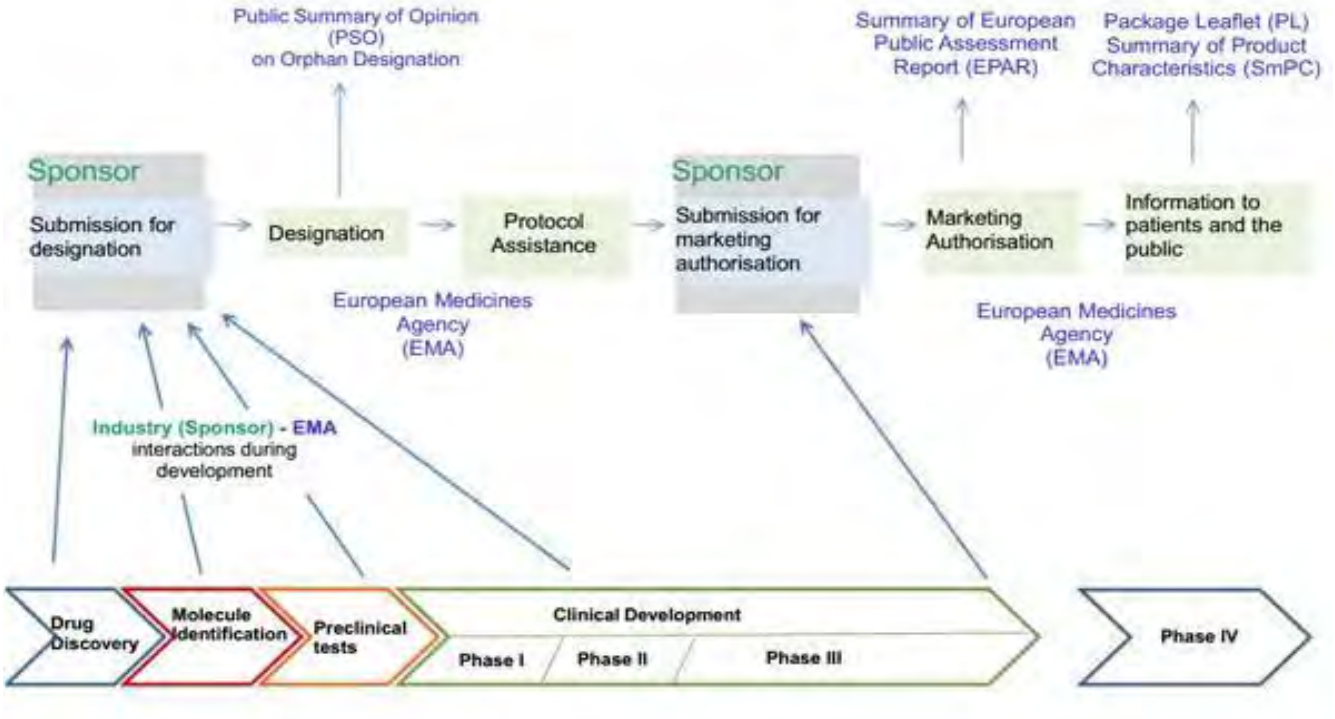


*Figure 1: Schematic drawing of Orphan Drug regulatory process in the EU. This process follows a unique stepwise pattern of developing and marketing orphan drugs. Steps are broadly divided into Drug Discovery, Preclinical, Clinical, Designation, Marketing Authorization, and Patient awareness stages. Clinical Development is divided into 3 phases, namely phases I, II, and III. Phase IV of the Clinical-stage comprises Submission for Marketing Authorization and further post-marketing stages such as Summary of European Public Assessment Report, Medication package insert within the European Medicines Agency (adapted from Saviano et al., 2015).*

## 1.4 Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) is a severe, time-limiting, rare genetic disorder characterized by progressive loss of muscles. This muscle degeneration occurs due to the alterations of a protein called dystrophin. Mutation in the dystrophin gene on an X-chromosome can occur before birth, or new mutations in the gene can happen spontaneously. Symptoms of DMD manifest in early childhood, usually by the age of 5 years, which primarily affects boys as girls are typically carriers with two X chromosomes (Ciafaloni et al., 2016). Children with DMD gradually develop weak skeletal, cardiac, and pulmonary muscles. Patients have a hard time standing up, walking, and climbing stairs. Many eventually need wheelchairs by their teenage

years (Bushby et al., 2010). The prevalence rate for DMD is less than 10 cases per 100,000 individuals (Duan et al., 2021). The diagnosis of DMD may include a creatine phosphokinase test, genetic tests, and muscle biopsy, based on the patient's symptoms and family history (Bushby et al., 2010a). Although there is no definite cure for DMD, some FDA and EMA-approved medications are on trial. FDA-approved drugs for DMD include Deflazacort (Brand name: Emflaza), Eteplirsen (Brand name: Exondys 51), Golodirsen (Brand name: Vyondys 53), Viltolarsen (Brand name: Viltepso) and Casimersen (Brand name: Amondys 45) (Duan et al., 2021). Amondys 45 is the first FDA-approved precision medicine for patients with DMD (authorized in February 2021). There are also ongoing trials on Ataluren, Prednisone, GALGT2, myostatin inhibiting drugs, SARMs, and stem cell therapy (Bushby et al., 2010; Shoji et al., 2015). DMD patients should be monitored regularly for potential heart involvement and respiratory distress. Regular physical therapy, exercises, genetic counseling, support groups, and multidisciplinary care may benefit patients (Bushby et al., 2010).

## 1.5 Friedreich's Ataxia

Friedreich's ataxia (FRDA) is a progressive neurodegenerative disease of a genetic origin. It is also known as autosomal recessive Spinocerebellar Ataxia (Cook & Giunti, 2017). FXN is the causative gene in this disorder and involves the repetition of GAA trinucleotide expansion mutation within the first intron of the FXN gene (Sandi et al., 2014; Yandim et al., 2013). In FRDA, the repeat expansions may adopt abnormal non-B DNA structure and induce heterochromatin-mediated gene silencing, reducing FXN gene transcription (Chutake et al., 2014; Sandi et al., 2014; Yandim et al., 2013). The reduction of frataxin is related to the length of GAA repeat, and the shorter repeat expansion length correlates with age at onset and disease severity (Cook & Giunti, 2017; Indelicato et al., 2020; Llorens et al., 2019). FRDA indications usually appear in early adolescence, and

patients might survive 20 years or more after the first onset. FRDA affects roughly 1 per 40,000 people. In Friedreich's Ataxia, several progressive neuronal degenerations causing symptoms of ataxia, dysarthria, muscle weakness, and losing sensory, coordination and balance with cardiomyopathy and diabetes can occur (Al-Mahdawi et al., 2008; F. M. Smith & Kosman, 2020). Various physical examinations, blood and urine analyses, MRI, electromyogram, electrocardiogram, genetic testing and carrier testing of at-risk relatives are performed to differentiate and diagnose FRDA (Bidichandani & Delatycki, 2017; Cook & Giunti, 2017; Indelicato et al., 2020). The symptoms and severity of each FRDA patient are different and require targeted treatments. Even though there is no specific cure yet, research is going on. Orphan-designated medications such as CTI-1601, Epicatechin, Etravirine, and gene therapy have shown promise in various clinical trials and animal-model of the disease (Bidichandani & Delatycki, 2017). Alternative treatments are mostly neurorehabilitation, including physical therapy, speech therapy, and devices like assistive cane, orthopedic braces, and wheelchairs to assist (Bidichandani & Delatycki, 2017; Schulz et al., 2009).

## 1.6 Transthyretin Amyloid Polyneuropathy

Transthyretin Amyloid Polyneuropathy (ATTR-PN) is a rare, debilitating, fatal neurodegenerative disease. In this disease, a mutated TTR gene produces misfolded transthyretin (TTR) proteins, which aggregate and deposit as amyloid (A) in the endoneurium of peripheral nerves (Adams, Ando, et al., 2021). TTR protein is synthesized and secreted mainly by hepatocytes in the liver (Hawkins et al., 2015). When TTR amyloidosis occurs, unstable TTR proteins misfold to form fibrils that deposit as amyloid. Amyloid deposits start to disrupt the function of the affected organ, particularly involving the heart or peripheral nerves. ATTR amyloidosis can either be inherited or acquired. Currently, around 50,000 people worldwide are affected by this disorder (Lovley et al.,

2021). With a short survival rate of 7 to 11 years, the onset of ATTR-PN can occur anytime in adulthood (Dohrn et al., 2021). The patient's initial symptoms are numbness and neuropathic pain in the hands and feet (Waddington-Cruz et al., 2019). ATTR-PN diagnosis includes analyzing the family history, tissue biopsy for histopathology, genetic analyses known as TTR genotyping, neurological examination, routine urine and blood analysis, ophthalmological assessment, cardiac and kidney evaluation (Adams, Ando, et al., 2021; Mazzeo et al., 2015). If transthyretin amyloid polyneuropathy is not diagnosed earlier, patients could experience severe and permanent peripheral nerve damage and premature death. Previously, the only possible treatment was liver or bone marrow transplant and supportive care. However, evolving therapeutic options such as NTLA-2001(CRISPR therapy), Patisiran (Onpattro®), Vutrisiran, Tafamidis (Vyndamax®, Vyndaqel®), Diflunsial (Dolobid®), Epigallocatechin-3-Gallate, ION-682884, Inotersen (Tegsedi®), Doxycycline (in animal models) and AG10 are currently in trial. For proper guidance, experts incorporate the patient's experience and identify the severity of the condition with regular follow-ups.

## 1.7 Objectives

- To promote national strategies and measures for rare disease and orphan drug inclusion.
- To analyze the etiology of Rarest Diseases and their treatment possibilities.
- To encourage the pharmaceutical industry to develop and market orphan drugs.

# Chapter 2

## Methodology

The establishment of this review paper is based on recent year's relevant research articles along with a few other articles from past years to integrate and justify current findings with the existing body of knowledge. In the initial stages of research, correlated articles were assembled from high-impact factor journals. Peer-reviewed journals, journal manuscripts, original research works, e-books, and books were used to enrich this review paper. The entire review was extensively searched through ResearchGate, Google Scholar, Science Direct, PubMed, SpringerLink, Mendeley, etc. This review paper incorporated various authentic journals from Orphanet Journal of Rare Diseases, Intractable and Rare Diseases Research, Degenerative Neurological and Neuromuscular Disease, Drug Discovery Today, Nature Reviews Drug Discovery, Frontiers in Molecular Neuroscience, European Journal of Neurology, American Journal of Physiology, Trends in Pharmacological Sciences, European Journal of Human Genetics, American Journal of Medical Genetics, British Medical Bulletin, International Journal of Molecular Sciences, Journal of Neurology, Neurosurgery, and Psychiatry, Molecular Therapy, Journal of Multidisciplinary Healthcare, Journal of Managed Care & Specialty Pharmacy, Journal of Pharmacy and Bioallied Sciences. Correspondingly, the Cochrane Library (Cochrane Database of Systematic Reviews) was utilized to review clinical trials of numerous ensuing and succeeding orphan drugs. Therefore, this review paper was completed with the high-quality screening of three rare diseases, few barriers to their subsequent orphan drugs, and proper citation indexing.

# Chapter 3

# Pathophysiology and possible treatments of Duchenne muscular dystrophy

## 3.1 Epidemiology

DMD is the most common form of dystrophinopathy, affecting approximately 1 in every 3,500 newborn boys (Kinali, 2009; Shoji et al., 2015). It is an X-linked recessive genetic disorder exclusively affecting males, whereas females are mostly healthy carriers (Crisafulli et al., 2020). This pediatric orphan disease usually emerges between 2 to 6 years of age (Allen et al., 2016; Mah et al., 2022; Previtali et al., 2020). In the USA and Europe, the prevalence rate of DMD is less than 10 cases per 100,000 inhabitants. In a 2006 Canadian consensus, the point prevalence of DMD was 10.3 per 100,000 males aged between 0 to 24 years (Ryder et al., 2017). In a global pooled analysis, DMD prevalence was 7.1 cases per 100,000 males, and birth prevalence was 19.8 per 100,000 live male births (Crisafulli et al., 2020; Ryder et al., 2017). The pooled global prevalence is much lower than the birth prevalence as young adults without optimal care opportunities may not survive beyond their pediatric age. Estimating the general population as a denominator, the global prevalence of DMD decreases to 2.8 cases per 100,000 individuals (Crisafulli et al., 2020). Among various studies reporting DMD prevalence, 59.1% were European, 18.2% American, 13.6% were African, and 9.1% Asian (Crisafulli et al., 2020). In another study reporting the birth prevalence rate of DMD, 72.4% were in Europe, 13.8% in America, 6.9% in both Oceania and Asia (Crisafulli et al., 2020). Female DMD carriers are usually asymptomatic and healthy. Though in less than 1 per 1,000,000 cases, females are manifesting carriers, representing 8% of the whole DMD carriers (Duan et al., 2021; T. J. Song et al., 2011). Due to progressive degeneration of muscle, the heart and diaphragm get damaged easily in DMD, leading to death often before the age of 30 (Ryder et al., 2017). A French study by Kieny reported that the improved

multidisciplinary approach toward DMD has elongated the median life expectancy up to 40.95 years (Duan et al., 2021; Kieny et al., 2013; Ryder et al., 2017). Unlike many other rare diseases, there is no study report to stratify DMD by ethnicity or ethnic groups. In a rough estimation, globally around 20,000 children are diagnosed with DMD each year. Approximately 2400 individuals live with DMD in the UK and are estimated to affect roughly 15,000 people in the US (Brabec et al., 2009; Zade et al., 2021). An additional 250,000 individuals are currently affected by dystrophinopathy in the United States. To evaluate the population impact measures (PIM) for a rare disease like DMD, a piece of epidemiological evidence is crucial in terms of the burden of disease, to identify unmet clinical needs, and to distinguish eligible target populations for drugs before marketing.

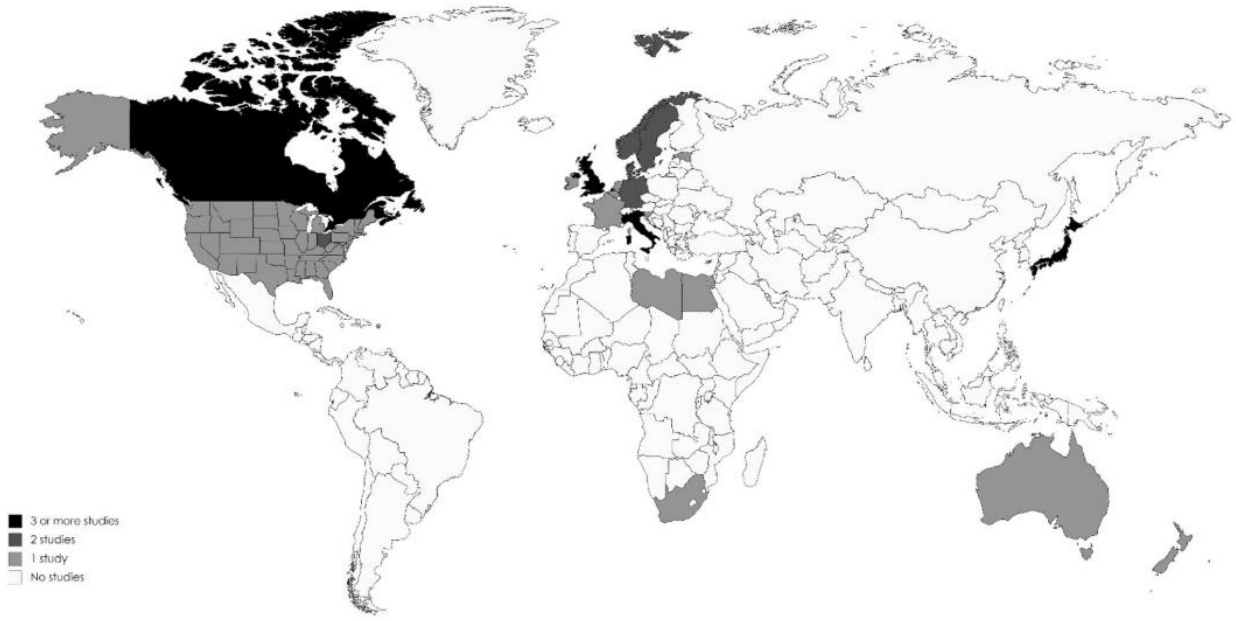


*Figure 4: Geographical distribution of the Duchenne muscular dystrophy epidemiological studies included in the systematic review. Chronologically countries with black mark pinpoint more, dark gray and gray less than that, and white pointing no conducted study on DMD (modified from Crisafulli et al., 2020).*

## 3.2 Pathogenesis

Duchenne muscular dystrophy is an X-linked mutation on the DMD gene located on Xp21 that results in dystrophin deficiency (Bushby et al., 2010a; Donkervoort et al., 2013). Dystrophin consists of three fundamental areas: the actin-binding end attaching the cellular skeleton, the central rod, and the dystroglycan-binding end connecting the dystroglycan complex within the membrane, which anchors to the extracellular matrix (Duan et al., 2021; Gao & McNally, 2015). A muscle is composed of long tubular cells called myocytes that can contract and cause flexing, and the dystrophin protein locates not too far from the membrane of myocytes. This cohesive protein links the actin-made myocyte to the mesh-like extracellular matrix outside the myocyte, and during any muscle contraction, this linkage prevents membrane damage (Bachir et al., 2017). In DMD, a genetic mutation causes this dystrophin to be extremely short, often lacking the dystroglycan-binding end, making it dysfunctional (Deconinck & Dan, 2007). Therefore, loss of connection between muscle fiber cytoskeleton and extracellular membrane occurs. During every muscle contraction, small sarcolemmal tears appear in the membrane, allowing the diffusion of various molecules into and out of the myocyte (Li et al., 2020). Among them, altered calcium channel activity increases the inflow of calcium (Mareedu et al., 2021). Calcium ions intrude through these small rips and activate calcium-dependent cellular enzymes that break down proteins, called proteases (Duan et al., 2021). By carefully regulating cellular calcium levels, generally, these proteases only break down old and damaged proteins. However, in DMD, extremely high calcium levels activate too many of these proteases, which begin to break down essential and functional proteins (Allen et al., 2016). Eventually, impaired calcium homeostasis weakens the muscle, leads to apoptotic myocyte death and, subsequently, to cell necrosis (Shoji et al., 2015). Another notable molecule that diffuses through the sarcolemma rips is creatine kinase

(CK), which leaks out of the cell and eventually into the bloodstream (Duan et al., 2021). Dystrophin deficiency causes abnormal cell membrane with increased transient local membrane disruptions, inducing this high outflow of CK (Kim et al., 2017). Measuring serum CK levels in the blood is often used as biomarker to diagnose DMD (Szigyarto & Spitali, 2018). The prime purpose of the enzyme CK is to facilitate energy storage for myocytes to use during contraction. In DMD, the progressive outflow of CK decreases energy storage capacity and weakens skeletal muscles (Moser, 1984; Shoji et al., 2015). The absence of dystrophin also produces a magnitude of free radicals and induces oxidative stress indicating oxidative damage in muscle (Duan et al., 2021). Furthermore, manipulation of these signalling pathways might alter CNS function resulting in cognitive and behavioral dysfunction (Anand et al., 2015). As muscle fiber repair mechanisms compromise, smooth muscle cells are also prone to necrosis. During the early phase of the disease, muscle repair and regeneration can occur. Subsequently, satellite cells fail to repair, and muscles no longer regenerate fast enough to keep up with the constant death of myocytes (Yanay et al., 2020). An imbalance between fiber degeneration and regeneration underlies developing fibrosis in connective tissue. Gradually, dead myocytes get replaced by fat and scar tissues (Duan et al., 2021). Since fat and scar tissue are unable to contract, muscles get disabled over time (Rugowska et al., 2021).

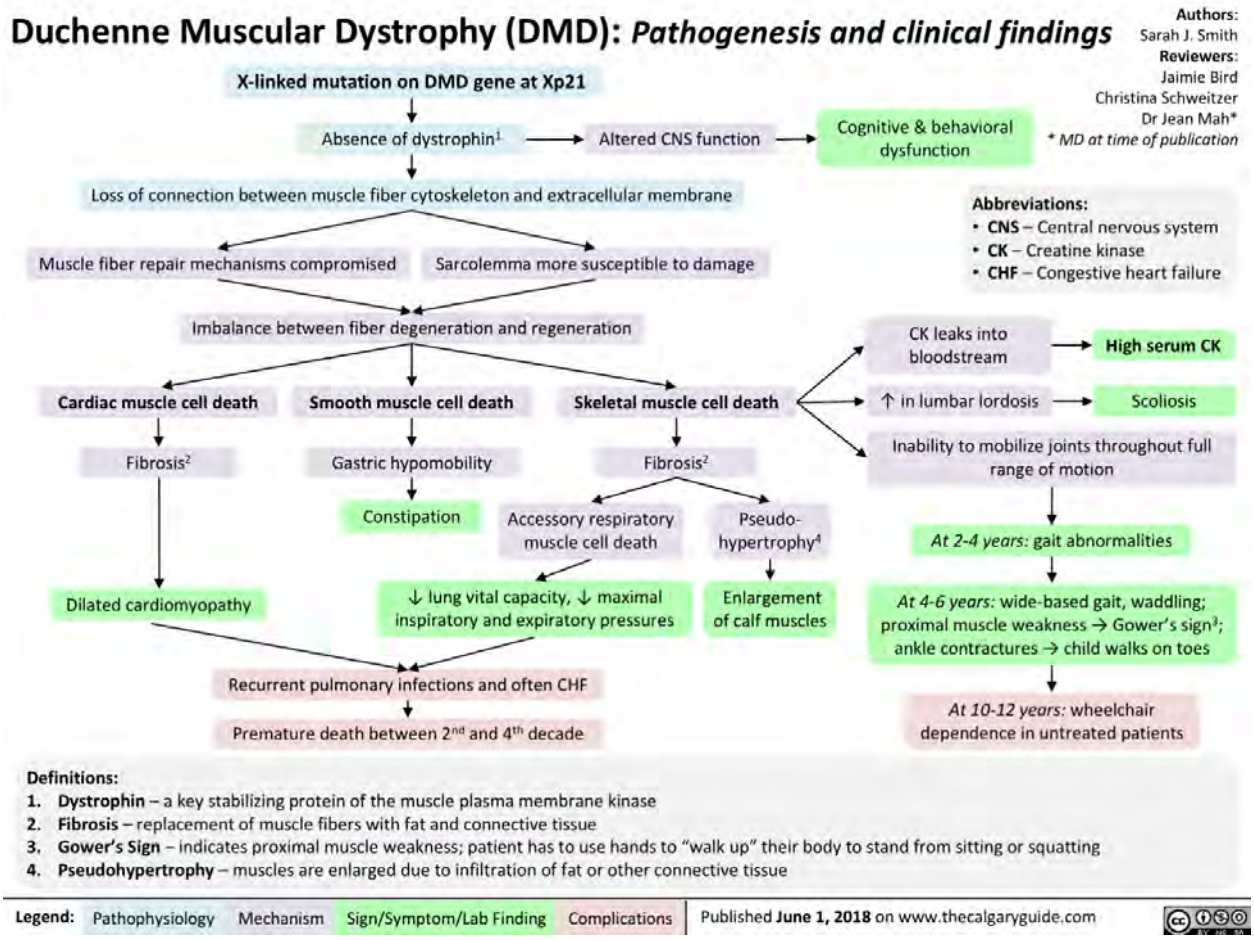


*Figure 5: Duchenne muscular dystrophy (DMD) pathogenesis and clinical findings* (copied from Smith, 2018).

## 3.3 Clinical Manifestations

Instead of a particular clinical feature, DMD is the overlap of distinctive pathogenetic processes involved in different stages of the disease. Initially, the ailing child struggles with basic abilities like walking, running, climbing stairs, or even getting up from the ground (Darras et al., 2018; Rugowska et al., 2021; Ryder et al., 2017). It is characterized by muscle fiber degeneration and atrophy of the pelvic area, followed by impairment of the shoulder muscles. After that, it turns into a critical phase as respiratory and heart muscles get involved. Lastly, the last stage of the disease leads to a life-threatening cardio-respiratory failure and eventually premature death (Ryder et al.,

2017). In DMD, weakness is the principal symptom as muscle fiber degeneration is the primary pathogenic process. Dystrophin deficiency is expressed in human gestation early, although patients have minimal signs until the walking age of 1 to 3 years (Ryder et al., 2017). Weakness related to DMD selectively impairs the proximal muscle before the distal limb muscle (Bushby et al., 2010). Or simply muscles of the hips, pelvic area, upper legs, and shoulders are initially impacted. Usually, it affects the lower external muscles before the upper external ones (Duan et al., 2021). Due to this muscle impairment, children face difficulty getting up from the ground and develop a unique method known as the Gowers' sign (Bushby et al., 2010a; Houang et al., 2018; Rugowska et al., 2021). It is an exclusive maneuver associated with DMD as the child must use their arms to stand up because their leg muscle is too weak (Darras et al., 2018; Rugowska et al., 2021). DMD patients are often late walkers and have short stature (Darras et al., 2018). Their growth velocity is typically slower than average. In toddlers, parents often notice enlarged calves (Zade et al., 2021). Fibrosis and infiltration of muscle tissue by fatty tissue produce this pseudohypertrophy or false enlargement of calf muscles (Houang et al., 2018). For preschoolers, the muscle weakness worsens and progresses to their trunk, legs, and arms. At school age, children develop a slightly waddling gait and lumbar lordosis (Anand et al., 2015; Darras et al., 2018). The child also faces difficulty raising their arms with a frequent falling tendency (Ryder et al., 2017). In their teen years, individuals with DMD experience progressive muscle impairment and eventually lose the ability to walk (Houang et al., 2018; Ryder et al., 2017). The loss of ambulation is followed by a gradual wheelchair-bound transition for the patient and eventually towards various orthopedic problems, such as scoliosis (Bushby et al., 2010a; Ryder et al., 2017). Heart and respiratory muscle problems also begin in the teen years. During this critical stage, patients tend to have evidence of decreased lung vital capacity and poor pulmonary function (Houang et al., 2018). The diaphragm

and other ventilation muscles weaken, decreasing maximal inspiratory and expiratory pressure by their late teen (Ryder et al., 2017). Weakened respiratory muscles make coughing difficult, leading to an increased risk of fatal respiratory infection. If not careful, simple colds can quickly progress into pneumonia for them. Along with shortness of breath, the patient may complain of having headaches, mental dullness, difficulty concentrating or staying awake, and nightmares. Recurrent pulmonary infections along with congestive heart failures (CHF) might cause premature death in DMD patients (Buddhe et al., 2018; Duan et al., 2021). In patients older than eighteen years, dystrophin deficiency weakens the muscle layer called the myocardium in the heart, and extensive myocardium scarring can develop cardiomyopathy (Darras et al., 2018). Most DMD patients in their late teens reportedly manifest symptoms of cardiomyopathy (Ryder et al., 2017). DMD also can cause conduction abnormalities in the heart (Duan et al., 2021). This subsequent impairment of the cardiac and respiratory systems is life-threatening for these patients. Patients with DMD often die in their late teens or 20s from respiratory insufficiency or cardiomyopathy, and only a few DMD patients survive beyond the third decade (Houang et al., 2018; Ryder et al., 2017). A small percentage of patients with DMD may also suffer non-progressive cognitive disability due to altered CNS function (Anand et al., 2015). According to physicians, dystrophin abnormalities in the brain may have subtle effects on cognition and behavior, including impaired intelligence and specific learning disorders (Bushby et al., 2010a). The disability to manage muscle activity-induced mechanical stresses constitute a central theme in DMD pathogenesis.

*Table 2: Signs and symptoms of DMD* (modified from Birnkrant, Bushby, Bann, Alman, et al., 2018; Birnkrant, Bushby, Bann, Apkon, et al., 2018; *Duchenne Muscular Dystrophy Fact Sheet*, 2019; Smith, 2018).

| Affected biological structure | Signs and symptoms |
|---|---|
| Skeleton and muscle | ▪ Muscle weakness<br>▪ Fatigue<br>▪ Muscle cramps<br>▪ Difficulty walking/running/jumping |

| | |
|---|---|
| | ▪ Difficulty climbing stairs<br>▪ Walking on the toes<br>▪ Gait abnormalities<br>▪ Pes cavus<br>▪ Contractures<br>▪ Pseudohypertrophy<br>▪ Scoliosis<br>▪ Hypotonia<br>▪ Myalgia or cramping |
| Lungs | ▪ Breathing difficulties<br>▪ Respiratory infections<br>▪ Sleep apnea |
| Heart | ▪ Cardiomyopathy |
| Gastrointestinal | ▪ Dysphagia<br>▪ Constipation<br>▪ Reflux<br>▪ Gastroparesis |
| Cognition | ▪ Learning disability<br>▪ Attention issues<br>▪ Behavioral issues |
| Nervous system | ▪ Developmental delay<br>▪ Motor delay |

## 3.4 Possible treatment options

### 3.4.1 Approved Treatments

#### Corticosteroids

Corticosteroids or glucocorticoids are commonly prescribed for children with DMD and are the only palliative treatment (Ryder et al., 2017). These steroids are synthetic molecules that resemble an anti-inflammatory hormone naturally produced by the body. The approved labeled indication for corticosteroids are for DMD patients older than 2 years (Bushby et al., 2010a; Duan et al., 2021). It usually increases the patient's muscle strength and functional ability by reducing tissue inflammation and immune system attacks. Types of glucocorticoids that can be prescribed for DMD are Emflaza (Deflazacort) or Prednisone (Duan et al., 2021; Hathout et al., 2016).

Deflazacort has been designated by the U.S. Food and Drug Administration (FDA) to treat DMD in 2013. Deflazacort is the first FDA-approved corticosteroid and is known as trade name Emflaza. Discovered and developed by Marathon Pharmaceuticals and later acquired by PTC Therapeutics, Emflaza has been in the market since June 2019. In Emflaza therapy, pro-corticosteroid must metabolize in the body into its active corticosteroid form. Pharmacologically it is an inactive pro-drug which is metabolized rapidly to the active drug 21-desacetyldeflazacort. When metabolized to its active form. Emflaza suppresses the immune system, thereby reducing muscle damage. It is marketed in 6mg, 18mg, 30mg, and 36mg dose tablets for oral administration and 22.75mg/ml oral suspension (Barber et al., 2013). Tracking bodyweight helps to determine the appropriate dosage as the patient grows. Considered Emflaza dosage is 0.9mg/kg/day (Bushby et al., 2010a). Another medication is Prednisone, a synthetic anti-inflammatory glucocorticoid derived from cortisone. Pharmacologically Prednisone is metabolized in the liver to its active form, prednisolone. Prednisolone prevents inflammatory mediators from being expressed. It exerts these properties by binding to glucocorticoid receptors and interrupting cytokine pathways type 1 and type 2. Prednisone is also administered orally, through tablet, or solution (concentrated or non-concentrated) (Barber et al., 2013). The preferred dosing regimen for Prednisone is 0.75mg/kg/day (Bushby et al., 2010a). Steroids may help delay the child from being wheelchair dependent (Bushby et al., 2010a). One needs to adjust the dosage as the patient grows. It is also recommended that the patient be monitored for side effects such as weight gain, growth retardation, bone demineralization, and increased fracture risk (Bushby et al., 2010a).

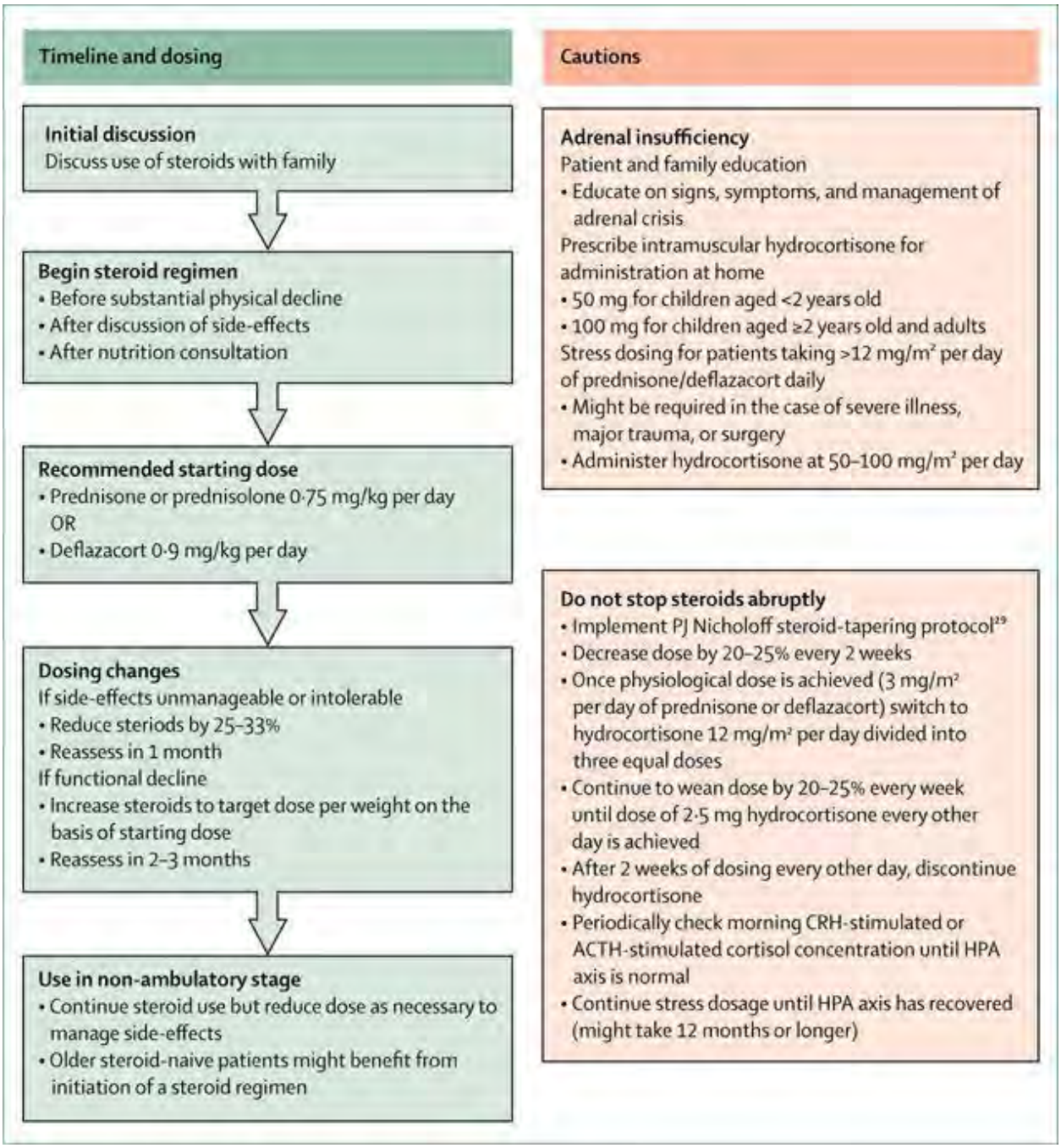


*Figure 6: Care considerations for glucocorticoid (steroid) initiation and use for patients with Duchenne muscular dystrophy ACTH=adrenocorticotropic hormone. CRH=corticotropin-releasing hormone. HPA=hypothalamic-pituitary-adrenal* (copied from Birnkrant, Bushby, Bann, Apkon, et al., 2018).

## Exon-skipping Therapy

Another potential therapy for DMD patients is exon skipping. Exon skipping is operated to restore the reading frame within a gene. In our genes, protein-coding DNA named the exons is interspersed with non-coding DNA called introns. During protein synthesis, the entire gene is first transcribed to pre-mRNA then splicing occurs and the introns are removed to produce mature mRNA (Li et al., 2020). The exons are basically concatenated. A deletion in one exon can disrupt the reading frame resulting in a premature halt in the synthesis and production of functional protein. In treating DMD, antisense oligonucleotide (AON) technology is used to mask an exon next to a mutated exon on pre-mRNA for the desired dystrophin protein (Clemens et al., 2020). It corrects the reading frame producing a shorter yet functional dystrophin protein (Servais et al., 2015). Currently, there is four approved exon skipping treatment approaches for DMD, namely Exondys 51 (Eteplirsen) for boys amenable to exon 51 skipping, Vyondys 53 (Golodirsen), and Viltepso (Viltolarsen) both amenable to exon 53 skipping, and Amondys 45 (Casimersen) amenable to exon 45 skipping (Duan et al., 2021; Servais et al., 2015). The DMD gene has 79 exons (Cazzella et al., 2012; Li et al., 2020). Among them, removal of the exon 51 of the DMD gene, along with the introns during the splicing process, occasionally can correct the reading frame of the mRNA that encodes for dystrophin (A Aartsma-Rus & Ommen, 2007; Choi & Koo, 2021; Kinali, 2009). Exondys 51 (Eteplirsen or AVI-4658), developed by Sarepta Therapeutics, is the first treatment approved by the FDA for patients with DMD (Choi & Koo, 2021; Li et al., 2020). Exondys 51 uses the phosphorodiamidate morpholino oligomer (PMO) approach (Choi & Koo, 2021; Kinali, 2009; Li et al., 2020). The recommended dose of Exondys 51 is 30 mg/kg administered once a week as a 35 to 60-minute intravenous infusion (A Aartsma-Rus & Ommen, 2007). Approximately 14% of the total Duchenne population have gene deletions that are amenable to exon 51 skipping (Duan et al., 2021; Kinali, 2009). Though FDA approved Exondys 51 to treat DMD in 2016, EMA refused

its marketing authorization in Europe. Vyondys 53 (Golodirsen, SRP-4053) is another exon-skipping therapy developed by Sarepta Therapeutics to treat DMD patients. This phosphorodiamidate morpholino oligomer drug is designed to mask exon 53 in the mRNA of the DMD gene so that protein synthesis can skip that exon and piece together the remaining exons to make a smaller yet functional dystrophin protein (Choi & Koo, 2021). The recommended dose of Vyondys 53 is 30 mg/kg administered once a week as a 35 to 60-minute intravenous infusion via an in-line 0.2-micron filter. Vyondys 53 got an accelerated FDA approval in December 2019. Roughly 8% of boys with DMD are amenable to exon 53 skipping (Duan et al., 2021). Viltepso (Viltolarsen) is also a morpholino antisense oligonucleotide to treat DMD resulting from mutations amenable to exon 53 skipping (Choi & Koo, 2021; Clemens et al., 2020). Developed by NS Pharma (parent company Nippon Shinyaku), Viltepso is specific to exon 53 and contains an artificial piece of mRNA that masks that exon before skipping. It is currently administered intravenously at an 80 mg/kg/week basis over a 60-minute infusion (Clemens et al., 2020). Viltepso was approved in Japan in March 2020, the FDA also gave conditional approval in August 2020, and the EMA has granted its orphan drug designation (Clemens et al., 2020). Lastly, Amondys 45 (Casimersen) is exon-skipping therapy for DMD patients with mutations amenable to exon 45 skipping (Choi & Koo, 2021). It is the first treatment available for around 8% of DMD patients with the exon 45 mutation (Duan et al., 2021). Amondys 45 uses phosphorodiamidate morpholino oligomer (PMO) chemistry and exon-skipping technology (Cazzella et al., 2012). The recommended dosage is 30 mg/kg of body weight of Amondys 45 administered once weekly as an intravenous infusion. It is the third exon-skipping therapy developed by Sarepta Therapeutics that the FDA gave conditional approval to in February 2021. The progression of the disease is delayed by conducting apt exon skipping therapy. The most common and unwanted side effects of exon skipping treatment are

headache, fever, common cold, cough, vomiting, nausea, diarrhea, joint pain, injection site reactions, skin rash, upper respiratory tract infection, and liver apoptosis (A Aartsma-Rus & Ommen, 2007).

## 3.4.2 Experimental Treatments

### Small Molecule Therapy

Compounds with lower molecular weights that can enter cells and affect the target (protein or other molecules) within the cell directly are small molecules. More than 90% of the therapeutics made today are of small molecules. Some ongoing experimental small molecule therapies for DMD include Givinostat, Rimeporide, Signaling Pathway Inhibition (Pamrevlumab), Signaling Pathway Upregulation (Oxandrolone). An experimental treatment to treat DMD is Givinostat (ITF2357) which works by inhibiting enzymes named histone deacetylases (HDACs) (Bettica et al., 2016). By inhibiting HDACs, Givinostat may reduce fibrosis and the death of muscle cells while enabling muscle's ability to regenerate (Bettica et al., 2016; Duan et al., 2021). Italfarmaco S.p.A. is developing Givinostat to treat DMD and BMD. Researchers published that treatment with Givinostat increased the number of muscle fibers and reduced the amount of fibrosis while attributed to the small sample size so far (Duan et al., 2021). Rimeporide (EMD 87580) is an investigational medication that inhibits sodium/proton type 1 exchanger (NHE-1) for treating DMD (Previtali et al., 2020). The muscle cells of DMD patients have an overload of calcium which contributes to further muscle damage and intracellular pH and Ca2+ influx is known to play a key role in DMD pathophysiology (Ioannou et al., 2015; Porte-Thomé et al., 2015). This plasma membrane protein, NHE-1, incidentally influences the cell's calcium level, allow cells to avoid intracellular acidification and protect them against apoptosis (Porte-Thomé et al., 2015). Thus by

blocking NHE-1 activity, Rimeporide may rectify the calcium level in the cell, reduce the sodium influx, decrease intracellular pH and induce apoptosis (Ioannou et al., 2015; Porte-Thomé et al., 2015). EspeRare Foundation has been developing Rimeporide since 2013 to treat DMD (Previtali et al., 2020). Another potential treatment is Pamrevlumab, a laboratory-developed human monoclonal antibody, which is a signal transduction inhibitor that blocks the communication of connective tissue growth factor (CTGF) (Richeldi et al., 2020). CTGF is found at abnormally high levels in the muscles of DMD patients as it promotes wound healing and fibrosis (Lipson et al., 2012; Y. Song et al., 2017). This therapy is administered into the bloodstream directly and FibroGen, Inc. developing Pamrevlumab to prevent the decline in patients' motor, lung, heart function and suppress muscle fibrosis (Lipson et al., 2012; Morales et al., 2013). Oxandrolone is an oral synthetic anabolic steroid equivalent to the male hormone testosterone (Balagopal et al., 2006; Fenichel et al., 2001). To upregulate the signaling pathway Oxandrolone mimics the action of testosterone and binds to androgen receptors in muscle cells which creates an androgen-receptor-oxandrolone complex. This androgen-oxandrolone complex stimulates transcription of several genes whose protein products may mitigate DMD symptoms (Tidball & Wehling-Henricks, 2004). Oxandrolone therapy can prevent muscle degeneration yet is not instructed for long-term DMD treatment (Balagopal et al., 2006; Tidball & Wehling-Henricks, 2004).

**Disease-modifying Therapy**

Disease-modifying therapies (DMTs) are still investigational treatments that can reduce the activity and progression of DMD and may also treat the latent cause of different types of muscular dystrophy. DMTs offer a higher possibility to cure Duchenne using experimental treatments including CRISPR/Cas9, stop codon read-through, exon-skipping therapies and so on. The CRISPR/Cas9 is novel biomedical research of gene editing with a prospect to cure DMD entirely

(Duan et al., 2021; Tambuyzer et al., 2020). This method is based on a bacterial biologic system to protect itself from viral infections (Choi & Koo, 2021). It allows researchers to modify, delete or edit specific areas of diseased DNA to treat DMD (Choi & Koo, 2021). The CRISPR/Cas9 is short for clustered regularly interspaced short palindromic repeats (CRISPR), consisting of the Cas9 enzyme and a guide RNA (Duan et al., 2021). Cas9 enzyme cuts DNA, and the sequence inside guide RNA specifies Cas9 where to edit (Choi & Koo, 2021; Duan et al., 2021). Reportedly, the mouse model of DMD in the pre-clinical stage has shown a positive outcome (Choi & Koo, 2021). Translarna (Ataluren) is an orally bioavailable small molecule treatment that targets nonsense mutations in the DMD gene (Duan et al., 2021). Around 10-15% of DMD cases occur due to nonsense mutation (Crisafulli et al., 2020). In nonsense mutation of DMD, amino acid changes to a STOP codon resulting in premature truncation of the dystrophin protein synthesis. Translarna therapy ignores that STOP signal read-through and facilitates the muscle cell to produce a full-length functional dystrophin protein (Donkervoort et al., 2013). Translarna developed and designed by PTC Therapeutics and has conditional approval by only the EMA (Duan et al., 2021; Tambuyzer et al., 2020). An exon skipping therapy masks specific exons in a gene sequence and corrects the reading frame (A Aartsma-Rus & Ommen, 2007). Investigational yet more effective exon-skipping therapies for DMD include DS-5141 and SRP-5051. A Japanese company, Daiichi Sankyo, designed DS-5141 to treat DMD by masking exon 45 (Takaishi et al., 2017). Sarepta Therapeutics developed SRP-5051 using peptide phosphorodiamidate morpholino oligomer (PPMO) for patients amenable to exon 51 skipping (Sheikh & Yokota, 2022). DS-5141 and SRP-5051 may form short yet functional dystrophin proteins to maintain muscle fiber with occasional toxicity concerns.

**AAV-mediated Gene Therapy**

Generally, gene therapy involves correcting or silencing a faulty gene to treat a specific genetic condition (Duan et al., 2021). In AAV-mediated gene therapy for DMD, researchers are using harmless adeno-associated viruses for the target delivery of healthy genes (Tambuyzer et al., 2020). AAV is unique from other viral vectors due to its natural cell tropism and low pathogenicity (Duan et al., 2021; Rao et al., 2018). Several AAV-mediated gene therapies are under investigation to treat DMD, including GALGT2, SRP-9001, PF-06939926, SGT-001, rhLAM-111. GALGT2 (B4GALNT2) gene therapy uses adeno-associated virus (AAV) vectors to deliver the GALGT2 gene into the body (Chicoine et al., 2014). It is a surrogate gene therapy as GALGT2 enhances the production of other proteins necessary for muscle regeneration to compensate dystrophin (Chicoine et al., 2014; Manini et al., 2022; Zygmunt et al., 2019). GALGT2 gene precisely activates in skeletal and heart muscle cells (Zygmunt et al., 2019). This therapy is developed and tested in a collaboration between Sarepta Therapeutics and the Nationwide Children's Hospital. SRP-9001, PF-06939926, and SGT-001 are all micro-dystrophin gene therapy candidates (Duan et al., 2021). SRP-9001 uses a shorter yet functional DMD gene equipped into a modified virus (AAVrh74) vector and targets the muscles (Mendell et al., 2020). The Nationwide Children's Hospital and Sarepta Therapeutics designed SRP-9001. Likewise, PF-06939926 therapy uses an adeno-associated virus serotype 9 (AAV9) vector to deliver mini-dystrophin protein and Pfizer Inc. is developing this gene therapy to treat DMD (Dolsten et al., 2020; Duan, 2018; Manini et al., 2022). Additionally, the trial of SGT-001, developed by Solid Biosciences, is currently under clinical hold (Duan, 2018). The rhLAM-111 (recombinant human laminin-111) is another virus-mediated experimental gene therapy. The extracellular matrix protein, rhLAM-111, have promoted muscle healing in mdx mouse model, GRMD dog model, and adult muscle cells grown in a laboratory with the potentiality to cure DMD (Barraza-Flores et al., 2019; Goudenege et al., 2010;

Soblechero-Martín et al., 2021). Researchers are developing the treatment expecting cells infected with a virus containing rhLAM-111 protein will increase the production of alpha-7 beta-1 integrin and utrophin (Liu et al., 2008; Soblechero-Martín et al., 2021). However, a limitation in repairing dystrophin protein using viral gene therapy is the large size of the dystrophin cDNA and the small packaging capacity of adeno-associated viruses (Gao & McNally, 2015; Rao et al., 2018). Individuals previously infected with adenovirus may develop antibodies against AAV, and they need screening before undergoing AAV-mediated gene therapy.

**Other Experimental Treatments**

The draft of CAP-1002 therapy is based on treating heart conditions linked to Duchenne muscular dystrophy (DMD). CAP-1002 consists of cardiosphere-derived cells (CDCs) which are capable of developing into mature heart cells. By releasing sacks of cellular material called exosomes, CDCs modulate immune cell activity to promote heart repair (Wu et al., 2019). CAP-1002 targets multiple disease processes in DMD patients (McDonald et al., 2022). The CDCs come from the heart tissue of a healthy donor, then grown and stored in a laboratory. It is directly administered by infusion into one or more coronary arteries using standard cardiac catheterization methods. CAP-1002 is an investigational cell therapy developed by Capricor Therapeutics to treat DMD. Interim data from the ongoing trial showed a significant improvement in muscle and lung function of diseased (McDonald et al., 2022). CAP-1002 clinical trials are called HOPE, and Capricor has completed the HOPE-2, a double-blind Phase 2 clinical trial, with positive results (GlobeNewswire, 2021). According to final data from the phase 2 clinical trial, CAP-1002 improved various skeletal and cardiac functions in DMD patients (McDonald et al., 2022). For further safety and efficacy test of CAP-1002, a potential phase 3 clinical trial will soon recruit DMD patients. The FDA has granted RMAT and Orphan Drug Designation, and also a Rare

Pediatric Disease Designation for CAP-1002 treatment. Potential allergic reactions during the CAP-1002 administration might be managed with intravenous steroids and antihistamines before infusion of the therapy (Marban et al., 2020). Another investigational treatment is a dissociative steroid named Vamorolone (VBP15), which emerges as a valuable alternative to typical corticosteroid treatments. Vamorolone works similarly to corticosteroids by activating specific pathways within the cell and inhibiting others. However, Vamorolone claims to offer a more selective anti-inflammatory profile without possessing adverse effects (Conklin et al., 2018). For instance, vamorolone exhibits inhibition of the NF-kB pathway that is associated with inflammation (Conklin et al., 2018; Hoffman et al., 2018). Additionally, it binds and helps to stabilize the membranes around cells without provoking further transactivation that typical corticosteroid treatments cause (Conklin et al., 2018). Furthermore, it might also preserve heart function by inhibiting mineralocorticoid receptors in DMD patients. VISION-DMD, a Phase 2 vamorolone trial is completed, and interim data showed improvement in motor outcomes (Mah et al., 2022). Vamorolone is developed by Santhera Pharmaceuticals and ReveraGen BioPharma Inc., having orphan drug designation in the U.S. and Europe. Long-term vamorolone treatment may be mildly associated with cushingoid features, vomiting, vitamin D deficiency, and adrenal suppression (Mah et al., 2022).

### 3.4.3 Non-drug Treatments

**Physical therapy**

Physical therapy is an approach that may help slow disease progression and maintain quality of life by reducing pain to each individual's needs. In DMD patients, physiotherapy reduces pain through personalized exercise, massage, education, and advice. Soon after being diagnosed with DMD, a physiotherapist can individualize a physical therapy program to each individual's needs.

In the early developmental stage of DMD, physiotherapists identify areas of muscle weakness and manage contractions caused by the disease progression (Bushby et al., 2010). Physiotherapists work with patients, their families, and their care team to develop a treatment plan to perform at home and outside (Bushby et al., 2010). Treatment plans may include chest physiotherapy, stretching exercises, aqua-therapy, and cycling. In addition, if required, physical therapists might suggest orthosis or standing devices for boys with DMD (Bushby et al., 2010; Pedlow et al., 2019). Physiotherapists also may individualize exercises that aid strengthen throat, jaw, and tongue muscles to address any unease with daily tasks, like eating or swallowing. Despite the small sizes of trial groups, various surveys of physiotherapy clinical trials documented some improvement in patient outcomes due to physiotherapy (Gianola et al., 2013). To summarize, physical therapy may help cope with daily function, reduce the pain of muscle weakness or cramping, slow the loss of mobility, gait and posture, improve endurance, develop gross motor skills, and even maintain quality of life for DMD patients.

**Occupational Therapy**

Occupational therapy is another approach that focuses on the improvement of patients' daily lives, including assessing and treating physical, psychosocial, behavioral, cognitive, or sensory skills. Duchenne Muscular Dystrophy is a genetically inherited progressive disease and can manifest from childhood to adulthood. The progression of DMD makes it difficult for patients to carry out everyday activities at home, school, or the workplace, especially when using a wheelchair or a walker. An occupational therapist collaborates with the healthcare team to personalize and implement programs specific to the patient. The occupational therapist can determine the correct type of assistive device that can benefit each individual patient and train them accordingly, including mobility aids, cognitive aids, sensory aids, orthotic devices, or even prosthetic devices

(Akyurek et al., 2017). For DMD patients, occupational therapy also suggests ways for daily living activities. Assistive devices for personal activities of daily living include customized utensils, lightweight manual mobility devices, doorknob adapters, zippers instead of hooks and buttons, handlebars and lifts in bathrooms, corset or body jacket for better support and balance, and arm slings for muscle weakness (Akyurek et al., 2017; Stone et al., 2007). An occupational therapist also helps to operate assistive technology such as voice recording devices, power scooters, and newly developed apps to monitor movements and aid in multitasking (Akyurek et al., 2017; Bushby et al., 2010). Therefore, occupational therapy identifies ways to boost the patient's confidence and guide patients with DMD to carry out everyday tasks more efficiently.

**Speech Therapy**

Speech and language management is essential to assess and treat delayed speech and language problems for DMD patients under the guidance of a speech therapist. In patients with DMD, weakness in the facial and oral muscles results in motor speech disorder (dysarthria) and disorder of the voice (dysphonia). DMD patients have vocal asthenia due to pulmonary impairment (Fonseca et al., 2020). Initially, speech problems may include the late onset of speech, difficulties in finding words, and non-fluent aphasia. Directly consulting a speech and language pathologist for assessment and treatment is necessary on suspicion (Ball et al., 2012). For patients with DMD, speech therapists often instruct oral motor exercises, articulation therapy, expiratory and inspiratory muscle strength training, voice training, and phonetic placement techniques (Bengtsson et al., 2020). Additional compensatory strategies, voice exercises, and speech implications are incorporated by therapists when the condition deteriorates due to problems with respiratory support for speech and vocal intensity (Bushby et al., 2010b). Speech therapists often suggest using augmentative and alternative communication(AAC) resources, which involve unaided and aided

methods such as drawings, exchanging pictures, fingerspellings, and sign language (Beukelman & Light, n.d.). Speech therapists also recommend the latest communication aids, such as speech-generating devices and voice amplifiers-artificial phonation devices like electrolarynx, that speak the required message when the patient pushes a button (Heffner, 2007). Additional augmentative supports, such as intraoral devices and oral prosthetics, also supplement the natural speech of DMD patients.

# Chapter 4

# Pathophysiology and possible treatments of Friedreich's ataxia

## 4.1 Epidemiology

The progressive neurodegenerative disease, Friedreich's ataxia (FRDA), affects roughly 1 in 40,000 individuals (Delatycki & Bidichandani, 2019). It is an autosomal-recessive genetic disorder that is often fatal. FRDA is an adult-onset disease that is age-dependent, and individuals from both genders are equally affected. FRDA can occur in diverse ethnic groups worldwide with varying prevalence (Cook & Giunti, 2017). According to the available epidemiological data, FRDA is most frequent among individuals of European, North African, Middle Eastern, or Indian origin (Indo-European and Afro-Asiatic speakers). Moreover, the disease occurs mainly in Caucasian ethnicity, is rare in sub-Saharan African populations, and is very rare in the Far East (Vankan, 2013). In Indo-European descent, the carrier frequency of FRDA is 1:60, and every 1 in 20,000 people is affected (Llorens et al., 2019). A European study of Caucasian people estimates 1 in 20,000 to 1 in 50,000 individuals to have FRDA (Indelicato et al., 2020; Pandolfo, 2009; Vankan, 2013). People of North African descendants also afflict 1 per 50,000 inhabitant with a carrier frequency of 1:1000 (Llorens et al., 2019). There are approximately 2000 people with FRDA in the UK with carrier frequency between 1:60 and 1:110 (Brown et al., 2021). In other countries, the prevalence rate of FRDA is relatively low and rarely identified (Delatycki et al., 2000). FRDA may rarely exist among sub-Saharan Africans, Amerindians, and inhabitants of China, Japan, and Southeast Asia. The prevalence rate of FRDA in Japan is 1 per 1,000,000 individuals. On average, FRDA occurrence is estimated to affect about 1 per 50 000 people with an estimated carrier prevalence of about 1:110 (Delatycki et al., 2000).

*Table 3: Predicted and observed prevalence levels of FRDA based on R1b frequencies* (copied from Vankan, 2013)

| Country | R1b frequency (%) | Predicted FRDA carrier rate | Predicted FRDA prevalence | Observed FRDA prevalence |
|---|---|---|---|---|
| Northern Spain | 90 | 1 in 55 | 1 in 20 167 | 1 in 20 000 |
| Portugal | 52 | 1 in 95 | 1 in 60 167 | 1 in 105 000 |
| South West France | 80 | 1 in 62 | 1 in 25 627 | 1 in 25 000 |
| North East France | 50 | 1 in 99 | 1 in 65 340 | 1 in 65 000 |
| Italy | 45 | 1 in 110 | 1 in 80 667 | 1 in 80 000 |
| UK | 65 | 1 in 76 | 1 in 38 507 | 1 in 54 000 |
| South West Germany | 50 | 1 in 99 | 1 in 65 340 | 1 in 53 000 |
| North East Germany | 40 | 1 in 124 | 1 in 102 507 | 1 in 149 000 |
| East Austria | 25 | 1 in 198 | 1 in 261 360 | 1 in 150 000 |
| CH | 50 | 1 in 99 | 1 in 65 340 | 1 in 45 000 |
| Ireland | 85 | 1 in 58 | 1 in 22 427 | 1 in 23 000 |
| Netherlands | 54 | 1 in 92 | 1 in 56 427 | 1 in 60 000 |
| Denmark | 35 | 1 in 141 | 1 in 132 540 | 1 in 137 000 |
| Norway | 28 | 1 in 177 | 1 in 208 860 | 1 in 100 000 |
| Sweden | 20 | 1 in 248 | 1 in 410 027 | 1 in 420 000 |
| Finland | 0 | NA | NA | 1 in 750 000 |
| Czech Republic | 20 | 1 in 248 | 1 in 410 027 | 1 in 250 000 |
| Russia (European) | 16 | 1 in 336 | 1 in 636 540 | 1 in 300 000 |

## 4.2 Pathogenesis

Friedreich's ataxia (FRDA), the professed triplet-repeat disease, is the result of an over-expression of repetitive GAA trinucleotide mutation in the mitochondrial FXN gene (Yandim et al., 2013). In general, 55% of the human genome comprises repetitive DNA sequences, and the over-expression of repetitive DNA is linked to genomic instability, such as cancer and aging (Yandim et al., 2013). In FRDA, 96% of the mutation can be homozygosis consisting of abnormally expanded GAA trinucleotide repeat within intron 1 of the FXN gene and 4% may occur due to heterozygous mutation within the expanded GAA repeat on one allele and another pathogenic variant (Al-Mahdawi et al., 2008; Indelicato et al., 2020; Llorens et al., 2019). The FXN gene is located in region of chromosome 9 and provides instruction to produce a small mitochondrial protein named frataxin (Pandolfo, 2009; Sandi et al., 2014). This protein is essential for the proper functioning of mitochondria. The function of frataxin is unclear, yet it assembles cofactors called iron-sulfur (Fe-S) clusters, a key component in mitochondrial ATP production (Chutake et al., 2014; Llorens et al., 2019; F. M. Smith & Kosman, 2020). If the GAA repeat expansions produce a heterochromatin-mediated gene silencing effect, it might cause a lack of mitochondrial frataxin production and gradually decreases mitochondrial ATP production (Sandi et al., 2014). The amount of frataxin protein varies by tissue, with the highest levels in the heart, spinal cord, pancreas, and muscles used for voluntary movement (skeletal muscles) (Cook & Giunti, 2017; Delatycki & Bidichandani, 2019; Indelicato et al., 2020). During mutation in the FXN gene, abnormal trinucleotide repetition of a GAA sequence within that gene occurs (Silva et al., 2015). A healthy individual contains <36 GAA repeats in FXN alleles, whereas in patients with FRDA, the GAA sequence is repeated 100 - 1700 times within the FXN gene (Chutake et al., 2014; Silva et al., 2015; Vankan, 2013; Yandim et al., 2013). In 96% of FRDA cases, individuals have copies

600-1200 times (Llorens et al., 2019). The repeat expansion causes gene silencing, ceases frataxin synthesis and leads to FXN mRNA deficiency, subsequently reducing levels of frataxin. Due to the lack of frataxin, mitochondria are unable to incorporate iron (Fe) in the iron-sulfur cluster inside the inner mitochondrial compartment (Al-Mahdawi et al., 2008; Chutake et al., 2014; Llorens et al., 2019). As a result, mitochondrial oxidative phosphorylation decreases, reducing mitochondrial ATP production, and less energy is available for the cell to operate (Cook & Giunti, 2017). Moreover, frataxin deficiency increases the amount of free iron, surging oxidative stress (Sandi et al., 2014; F. M. Smith & Kosman, 2020). As iron accumulates inside the mitochondria and reacts with oxygen, it may create unstable oxygen radicles (Llorens et al., 2019; F. M. Smith & Kosman, 2020). Therefore, these reactive oxygen species damage DNA and protein within the cells in a process called oxidative damage (F. M. Smith & Kosman, 2020). This causes dysfunction and necrosis of mitochondria-dependent cells such as neurons, cardiomyocytes, and pancreatic beta cells (Indelicato et al., 2020). Furthermore, neuronal apoptosis and necrosis affect the posterior spinocerebellar tract (Clarke's column), brainstem, and cerebellum (Delatycki et al., 2000; Llorens et al., 2019).

## 4.3 Clinical Manifestations

FRDA is a rare early-onset degenerative disorder affecting various organ systems due to dysregulation in frataxin (FXN) protein and impaired mitochondrial function, characterized by motor, cognitive and autonomic dysfunction (F. M. Smith & Kosman, 2020). The FXN mutation leads to a decrease in the production of frataxin protein and can cause progressive sensory-motor loss, neurodegeneration, muscle atrophy, fatigue, brain iron accumulation, and eventual death by hypertrophic cardiomyopathy (Sandi et al., 2014; F. M. Smith & Kosman, 2020). FRDA can affect multiple organs and produce a range of symptoms (F. M. Smith & Kosman, 2020). In particular,

the nervous system gets damaged, and the loss of neurons causes ataxia (Al-Mahdawi et al., 2008; Cook & Giunti, 2017; Llorens et al., 2019). At first, the patient may exhibit musculoskeletal abnormalities, such as walking difficulty associated with muscle weakness and loss of proprioception (Llorens et al., 2019). It affects the legs and gradually moves up to the torso. Due to muscle denervation patients are unable to move in a coordinated way (Bidichandani & Delatycki, 2017). Loss of coordination and muscle strength leads to motor disability as the disease progresses. Pes cavus and hammer toes are also two frequently seen deformities in FRDA patients (Cook & Giunti, 2017; Indelicato et al., 2020). Eventually, individuals with limb and gait ataxia get wheelchair-bound within 15 years and then develop a bedridden state (Delatycki & Bidichandani, 2019; Llorens et al., 2019). As the muscle weakness progresses most individuals develop increased spasticity (Bidichandani & Delatycki, 2017; Cook & Giunti, 2017). A feeling of exhaustion is also common. In later stages, patients often become severely impaired with various health complications including cardiomyopathy, hearing loss, blindness (Cook & Giunti, 2017). Individuals with FRDA show indications usually in childhood or puberty. One of the most common symptoms of FRDA is dysarthria and dysphagia. Dysarthria affects the muscular control of speech in the patient, thus altering voice quality, speech clarity, and intelligibility (Llorens et al., 2019). Gradually the coordination of mouth and pharynx muscles reduces, and swallowing difficulty known as dysphagia occurs (Cook & Giunti, 2017). Acute aspiration of gastric contents into the lower respiratory tract is also a common occurrence in critically ill patients. The severity and progression of FRDA might impact the respiratory and vocal tract motor systems. The disorder also affects other organs like the heart and endocrine pancreas (Llorens et al., 2019). In patients with FRDA, pancreatic β-cell dysfunction and insulin resistance causes insulin deficiency and may lead to diabetes mellitus. Approximately 10% of FRDA patients develop diabetes, and 20%

develop glucose intolerance (Bidichandani & Delatycki, 2017; Cook & Giunti, 2017). The diabetic symptoms in FRDA generally include extreme thirst, frequent urination, weight loss, fatigue, and blurry vision (Cook & Giunti, 2017). Frataxin deficiency commonly result in another severe condition called cardiac neuropathy. Cardiac autonomic neuropathy (CAN) is frequently associated with diabetes mellitus, which leads to cardiomyopathy (Llorens et al., 2019; F. M. Smith & Kosman, 2020). Roughly 75% of people with FRDA develop various heart abnormalities. Around 59% FRDA patients die due to cardiomyopathy and sever hypertrophic cardiomyopathy (HCM) is the most common causality of death in FRDA patients with a mean age of death at 36.5 years (Cook & Giunti, 2017; Delatycki & Bidichandani, 2019; Llorens et al., 2019; F. M. Smith & Kosman, 2020). Other cause reason of mortality can be stroke, ischemic heart disease and pneumonia (Cook & Giunti, 2017). Primary symptoms of declining heart function include extreme fatigue, shortness of breath, chest pain, lightheadedness, leg swelling, difficulty breathing while lying flat, and heart palpitations (F. M. Smith & Kosman, 2020). Individuals with higher GAA repeats in the FXN gene manifest FRDA later in life but more severely than those with fewer repeats (Cook & Giunti, 2017; Indelicato et al., 2020; Llorens et al., 2019). Clinical features usually manifest between ages 10 to 15 (Bidichandani & Delatycki, 2017; Delatycki & Bidichandani, 2019). People with longer expansion tend to have more sever phenotype and late-onset of ≥25 years (Cook & Giunti, 2017; Indelicato et al., 2020; Llorens et al., 2019). In very rare cases, disease onset can be as early or late as <5 years or >75 years respectively. FRDA patients mostly get wheelchair-dependent within 10 to 20 years after the first appearance of symptoms (Cook & Giunti, 2017). The mental capabilities of people with FRDA usually remain intact but a long-term condition can cause several psychological and adaptive issues (Lynch et al., 2021).

## 4.4 Possible treatment options

For Friedreich's ataxia (FRDA) no cure exists and no disease-modifying therapies have been approved yet. However, many symptoms and following complications can be treated to help individuals maintain a quality of life. A multi-specialty team approach is essential to treat patients with FRDA. There are also several ongoing investigational therapies to prevent and treat the underlying cause. Currently, management of Friedreich's ataxia follows a symptom-management approach, delivered by an interdisciplinary team (Cook & Giunti, 2017).

### 4.4.1 Experimental Treatments

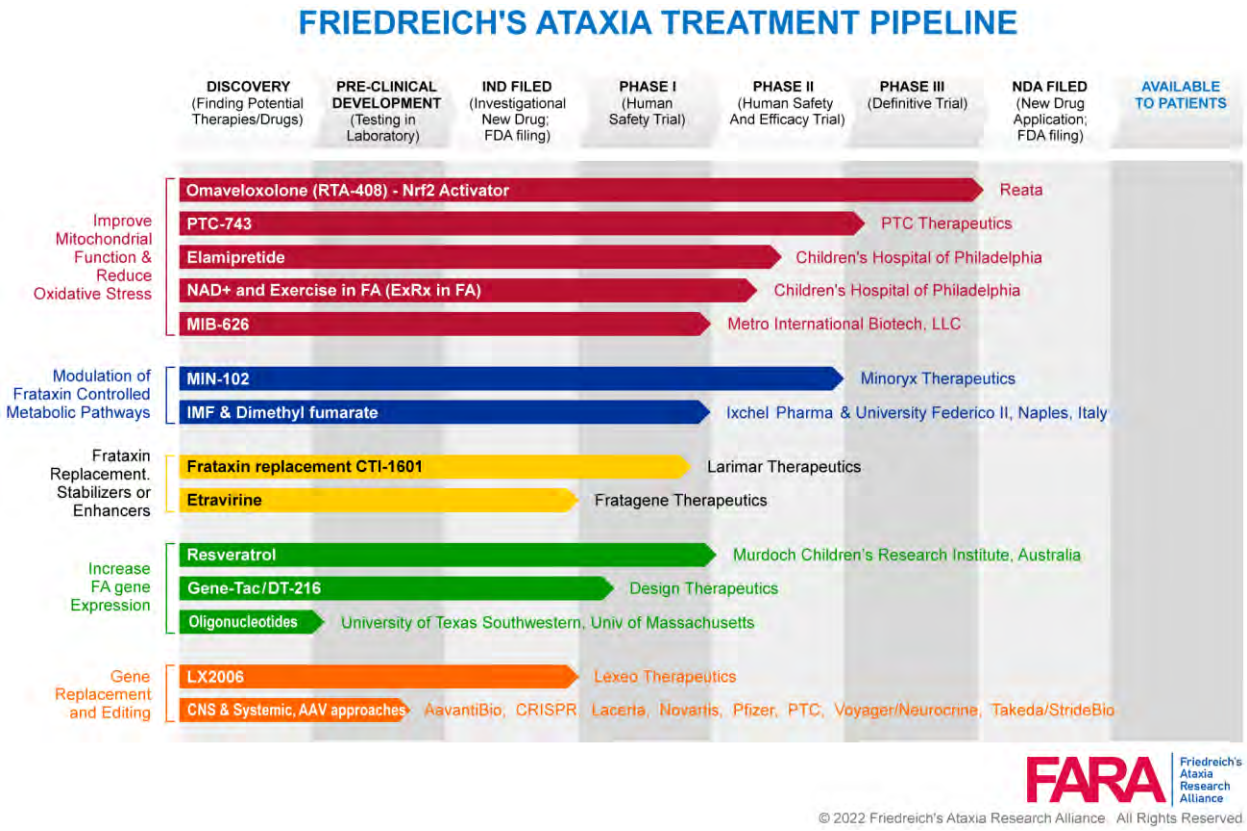


*Figure 7: Therapeutic pipeline for FRDA, including the clinical development stage for each approach as well as the organizations performing research, as of April 2019. Figure provided by J. Farmer of the Friedreich's Ataxia Research Alliance* (adapted from Gottesfeld, 2019).

**CTI-1601**

CTI-1601 is an investigational protein replacement therapy developed by Larimar Therapeutics to treat individuals with FRDA. Friedreich's ataxia (FRDA) is a hereditary neuromuscular disorder caused by a genetic defect that inhibits the FXN gene expression. FXN encodes for the frataxin protein, which is an essential mitochondrial function. Inadequate amounts of the frataxin protein affect mitochondria and the energy production system, and the lack of frataxin eventually leads to symptoms that include loss of coordination, progressive muscle weakness, and diminishing eyesight. CTI-1601 is a 24.9kDa fusion protein and consists of the transactivator of transcription (TAT) protein transduction domain, in which the mechanism of action relies on the cell-penetrating ability of the TAT peptide (Lynch et al., 2021; Mess et al., 2020). CTI-1601 utilizes platform technology by using a carrier protein to deliver the frataxin protein to the mitochondria of the patient (Tai et al., 2018). Mitochondria then process this carrier-bound frataxin to become mature frataxin, which plays an active role in mitochondrial metabolism. This therapy may restore protein levels, helping mitochondria to carry out their essential function within cells. Higher mature frataxin protein levels may help to minimize patients' symptoms and prevent FRDA progression. CTI-1601 therapy is administered via subcutaneous injection into mice models and increased its lifespan up to 53% (Vyas et al., 2012). For fast track development, the FDA has allotted CTI-1601 an orphan drug and rare pediatric disease designation (Tai et al., 2018).

**Epicatechin**

Epicatechin is a well-studied polyphenol (flavan-3-ol) to treat FRDA. The (+)-epicatechin and (-)-epicatechin are two stereoisomers of flavonol catechin, which naturally originate in fruits, red wine, dark chocolate, green tea, guarana berry, and other plants (Qureshi et al., 2021; Rodríguez et al., 2020; Schwarz et al., 2018). FRDA is a genetic disorder caused by mutations in the gene

encoding for frataxin protein. This protein is essential for the normal functioning of mitochondria, the energy factories of the cells. The lack of frataxin leads to a progressive impairment of muscle coordination, loss of muscle strength, sensation, and impaired speech, vision, and hearing. Epicatechin therapy may exhibit health-promoting biological activity, improving mitochondrial function and plasma levels of frataxin protein, and boost metabolism in patients with FRDA (Schwarz et al., 2018; Zhang et al., 2019). A study reported that epicatechin treatment assert antioxidative properties, reduces oxidative stress and helps restore mitochondrial biogenesis in senile mice (Gavrilova, 2017; Rodríguez et al., 2020). It may reduce the risk of cardiovascular and neurodegenerative diseases and improve the structure of skeletal muscles and muscle strength in animal model (Rodríguez et al., 2020; Schwarz et al., 2018). Epicatechin is administered orally as capsules (Gavrilova, 2017; Qureshi et al., 2021). Recent research suggested that (+)-epicatechin can be more potent than (-)-epicatechin (Gavrilova, 2017). Cardero Therapeutics and the Mayo Clinic have identified Epicatechin as a potential treatment for FRDA and published the phase 2 trial outcome in September 2020 (Gavrilova, 2017; Gottesfeld, 2019; Zesiewicz et al., 2020).

**Etravirine**

Etravirine is another investigational treatment option for FRDA. It is mainly an anti-viral medication approved by the FDA to treat human immunodeficiency virus (HIV) infections (Gottesfeld, 2019; Zesiewicz et al., 2020). A screening study showed that the therapy may benefit FRDA treatment as etravirine increases frataxin protein in cells (Rufini et al., 2022). Etravirine also confers protection against oxidative stress by elevating mitochondrial aconitase activity in cells derived from FRDA patients (Gottesfeld, 2019; Rufini et al., 2022). Researchers believe that etravirine treatment improves the production of frataxin in the cells by increasing the translation of existing frataxin mRNA or preventing frataxin degradation and does not increase the frataxin

gene expression, avoiding any potential issues of the FXN gene over-activation (Delatycki & Bidichandani, 2019; Gottesfeld, 2019; Zesiewicz et al., 2020). Fratagene Therapeutics (Rome, Italy) currently holds a U.S. patent on the use of etravirine as a therapeutic option for FRDA (Gottesfeld, 2019). Patients receive different dosages of etravirine orally (Rufini et al., 2022). A Phase 2 trial launched in March 2020; however, no experimental data is available for etravirine activity in animal models (Rufini et al., 2022). The IRCCS Eugenio Medea Scientific Institute will conduct further trial in collaboration with the University of Rome Tor Vergata in Italy.

**Gene Therapy**

Another experimental therapeutic approach to treat FRDA is gene therapy. Gene therapy involves an intracellular delivery of genomic material and induces a therapeutic effect into existing cells to prevent or cure a disease. As FRDA originates from a single gene mutation, there is a possibility to treat this disorder by gene therapy. Gene therapy for FRDA is the approach of introducing a new gene to the cell to compensate for a mutated FXN gene, and newly transferred genetic material alters how a protein or group of proteins produce inside the cell. There are two kinds of approaches to gene therapy, in-vivo and ex-vivo, and in-vivo gene therapy seems to be the best strategy as the treatment can access CNS and post-mitotic FRDA cells easier (Ocana-Santero et al., 2021). The gene is inserted into a cell by using a genetically engineered (vector) delivery system. The therapy uses either viral vectors that are benign or non-viral vectors of physical and chemical systems (Evans-Galea et al., 2014). Viral vector-based delivery utilizes adeno-associated virus, retroviral and lentiviral vectors, or herpes simplex virus type 1, and nonviral-based delivery includes liposomes, nanoparticles, polymers, ultrasound, or laser-based magnetic energy (Evans-Galea et al., 2014; Ocana-Santero et al., 2021; Zesiewicz et al., 2020). Strong preclinical proof suggests that a vector can correct the mutated FXN gene or treat any damaged system in the body, including

neurological and cardiac symptoms, inspiring pharmaceutical companies to develop programs in FRDA gene therapy (Gottesfeld, 2019; Zhang et al., 2019). Voyager Therapeutics is currently investigating VY-FXN01 and is in the preclinical stage. A project supported by GENEFA is also investigating methods to modify vectors and improve genetic delivery across the blood-brain barrier (BBB) to treat FRDA. Researchers published in Nature Medicine that using a viral vector prevented and corrected cardiac damage in mice by restoring heart function and reversing heart enlargement (Zesiewicz et al., 2020). At the same time, Pfizer focuses on synthetic lipid nanoparticles (LNP) system, a codon-optimizing FXN mRNA, to treat FRDA (Gottesfeld, 2019; Zhang et al., 2019). International collaborations between GENEFA, AAV Life, and Voyager Therapeutics have created Third Rock Ventures, which also fund and develop gene therapy for FRDA (Evans-Galea et al., 2014). Gene therapy is administered via intravenous (IV) injection, and the dosage depends on the patient weight (Ocana-Santero et al., 2021). Individuals previously exposed to the delivery viruses are unsuitable candidates for gene therapy (Tai et al., 2018).

### 4.4.2 Non-drug Treatments

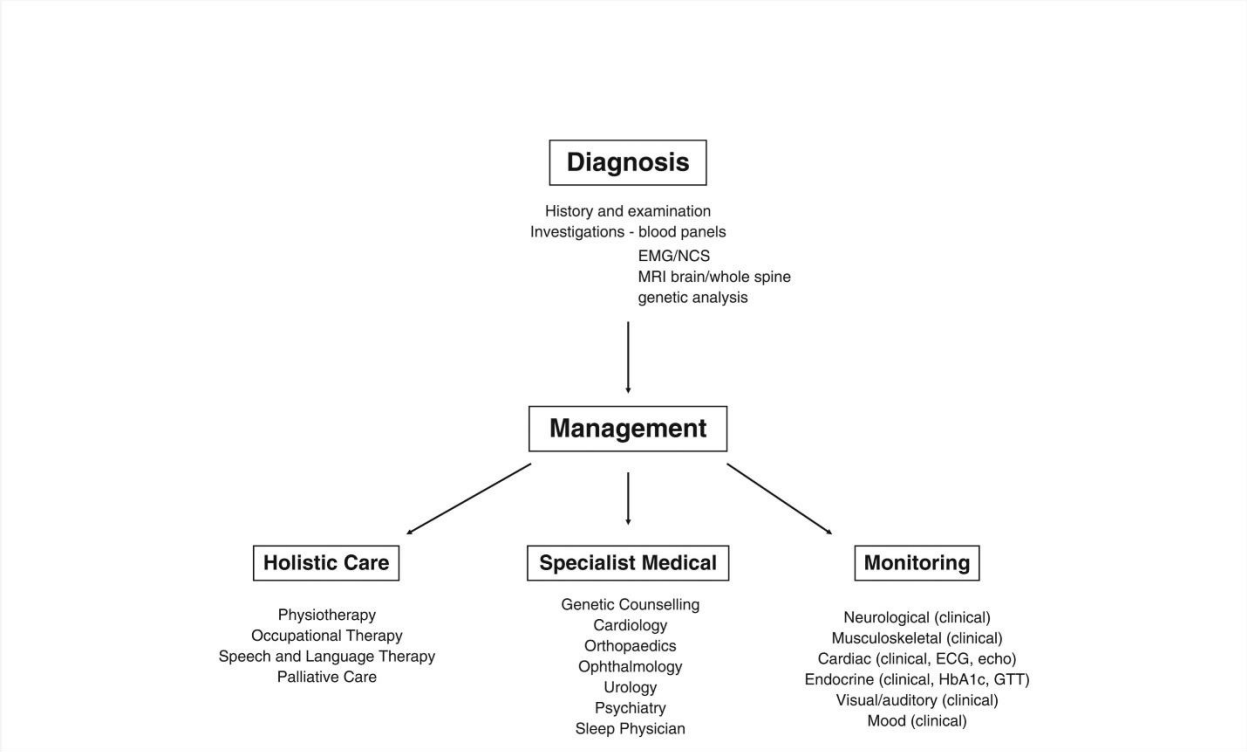




*Figure 8: Flow chart for the diagnosis and management of Friedreich's ataxia* (adapted from Cook & Giunti, 2017).

## Physical Therapy

Physical therapy aims to minimize pain, deformity, and disability while achieving a higher quality of life (Maring & Croarkin, 2007). In physiotherapy, every disorder is different, and only a qualified physiotherapist can identify and recommend proper relief (Lynch et al., 2021; Maring & Croarkin, 2007). Before starting a treatment plan, the physiotherapist conducts a preliminary evaluation, including complaints assessment, functional abilities, balance, body alignment, gait, coordination, muscle flexibility, and cardiovascular response to activity (Corben et al., 2014). Physiotherapists prescribe exercises that may help FRDA patients maintain the use of their limbs

for longer (Cook & Giunti, 2017; Corben et al., 2014). A trained physiotherapist may include various exercise approaches to treat FRDA, such as aerobic exercises, strengthening exercises, stretching exercises, gait training, balance exercises, and cardiovascular conditioning (Cook & Giunti, 2017; Lynch et al., 2021). Low-intensity strengthening exercises may improve hip and shoulder posture, facilitate limb function, reduce scoliosis pain and maintain trunk control (Lynch et al., 2021). Basic strength routines with light weights and sufficient rest periods are suggested. Passive stretching can help wheelchair-dependent patients to prevent contractures and spasticity (Cook & Giunti, 2017; Corben et al., 2014). A physiotherapist may instruct hamstring stretch and plantar fascia stretch to treat FRDA patients (Corben et al., 2014). Implementing gait training is for patients with proprioception to improve mobility (Cook & Giunti, 2017; Milne et al., 2017). Coordination activities can be incorporated while executing daily tasks (Milne et al., 2017). Physiotherapists often recommend using devices like reverse-brake-system walker or rollators to practice stepping, power scooter, and mirrors for visual feedback (Harris-Love et al., 2004; Maring & Croarkin, 2007). Balance exercises are also instructed therapies to maintain or improve balance while sitting, standing, and moving (Corben et al., 2014; Milne et al., 2017). Physiotherapists even suggest stationary bicycles or water workouts for moderate cardiovascular exercises (Maring & Croarkin, 2007; Tai et al., 2018). As it is, physiotherapies can manage the symptoms of FRDA with a positive influence on patients by improving overall fitness (Maring & Croarkin, 2007).

**Occupational Therapy**

Occupational therapy is a personalized treatment that establishes an appropriate plan to address patient-specific needs (Corben et al., 2014). Occupational therapy can assist people of all ages with physical, sensory, or cognitive difficulties. FRDA is a progressive disease with an impaired nervous system and muscle control. In FRDA, an occupational therapist can help identify aspects

of daily life by guiding patients or caregivers on home adaptations and suggesting techniques to make tasks manageable (Cook & Giunti, 2017). The most common manifestation of FRDA is walking difficulty as patients gradually experience a loss of mobility (Corben et al., 2014). Therefore, occupational therapists may recommend and guide FRDA patients on using assistive prescribed devices such as standing frames, cane, walker, scooters or wheelchair to help mobility (Akyurek et al., 2017; Cook & Giunti, 2017). In FRDA, occupational therapy also offers individualized techniques and strategies to cope with mental fatigue (Corben et al., 2014). Physiotherapy combined with occupational therapy may improve speech (dysarthria) and swallowing (dysphagia) difficulties in FRDA patients (Cook & Giunti, 2017; Corben et al., 2014; Tai et al., 2018). As the disorder progresses, occupational therapists suggest potential approaches like electronic devices with buttons, voice-activated software, nonslip mats, weighted cutlery, lidded cups, etcetera to adjust in daily life (Cook & Giunti, 2017). Occupational therapy in FRDA is very precise and exclusive for each patient.

**Surgical Procedures**

Physicians may suggest surgical procedures to control FRDA symptoms in severe scoliosis, foot deformities, swallowing difficulties, or heart conditions (Corben et al., 2014). Due to loss of muscle strength, people with FRDA may develop a sideways curvature of the spine known as scoliosis. Over 90% of FRDA patients with early-onset develop intermediate to severe scoliosis. Compared to other curve correction treatments, scoliosis surgery has a higher success rate to maintain spinal formation (Corben et al., 2014). Surgery also can help correct gait, and foot deformities such as pes cavus, that cause pain or affect mobility (Corben et al., 2014; Evans-Galea et al., 2014). Surgical procedures for foot deformities may include tendon lengthening surgery, tendon transfer surgery and deformity correction, stabilizing with plaster or internal fixation, joint

fusion surgery. The surgical procedure gastrostomy may relieve issues related to swallowing difficulties or dysphagia (Bidichandani & Delatycki, 2017; Corben et al., 2014). In FRDA patients with cardiomyopathy, the heart muscle becomes enlarged, thickened, and weakened (Cook & Giunti, 2017). Heart surgery may become essential in the critical stage of FRDA associated with progressive cardiomyopathy (Lynch et al., 2021). Heart surgery for FRDA patients includes septal myomectomy, implanted devices (pacemaker), heart transplant (Bidichandani & Delatycki, 2017; Corben et al., 2014). In FRDA treatment, surgical procedure is usually the last resort (Corben et al., 2014). As FRDA is a multisystem disease, each surgical procedure require different anesthesia techniques to prevent lethal situations.

# Chapter 5

# Pathophysiology and possible treatments of Transthyretin Amyloid Polyneuropathy

## 5.1 Epidemiology

ATTR-PN is a rare neurodegenerative disease caused by mutations in the TTR gene. The global prevalence of ATTR-PN was approximately 5000 to 10,000 individuals (Waddington-Cruz et al., 2019). However, a recent publication estimated the incidence of ATTR-PN is 38,000 people worldwide (Waddington-Cruz et al., 2019). The average age of onset for ATTR-PN is 61.5 years and men are more frequently affected (Dohrn et al., 2021). Transthyretin amyloidosis is considered very rare in most places throughout the world. ATTR-PN endemic countries include Portugal, Sweden, and East Asia (Japan, China, Taiwan, and South Korea) (Kapoor et al., 2019; Mazzeo et al., 2015; Waddington-Cruz et al., 2019). In endemic regions, ATTR-PN may affect every 1 in 1000 individuals (Adams, Ando, et al., 2021). In a few areas of northern Portugal, ATTR-PN prevalence is 1 per 1,108 people (Pinto et al., 2018). Among Americans of European descent, ATTR-PN is less frequent. The U.S. and most regions of Europe have 1 in 100,000 ATTR-PN patients. In individuals of Sicilian origin, the prevalence of ATTR-PN is 8.8 per 1,000,000 (Mazzeo et al., 2015). Approximately 5000 ATTR-PN cases are in Brazil (Pinto et al., 2018). In ATTR-PN studies, 70% population showed the presence of four common genetic mutations in TTR gene, namely Val30Met (47.6%), Ser77Tyr (10%), Ala97Ser (6.5%) and Phe64Leu mutation (4.4%) (Waddington-Cruz et al., 2019). Following is the global distribution of ATTR-PN cases with common genetic mutations:

*Table 4: Global Distribution of Reviewed ATTR-PN Cases*(adapted from Waddington-Cruz et al., 2019)

| Country | Country Total | Ala97Ser | Phe64Leu | Ser77Tyr | Val30Met | Other |
|---|---|---|---|---|---|---|
| France | 17.9% | 0% | 0% | 34% | 48.5% | 17.5% |
| Japan | 17% | 0% | 0% | 0% | 82.6% | 17.4% |
| China | 13.1% | 2.8% | 0% | 16.9% | 32.4% | 47.9% |
| Italy | 10.7% | 0% | 41.4% | 0% | 36.2% | 22.4% |
| Taiwan | 6.5% | 94.3% | 0% | 2.9% | 0% | 2.9% |
| Germany | 4.8% | 0% | 0% | 0% | 50% | 50% |
| Spain | 3.5% | 0% | 0% | 0% | 42.1% | 57.9% |
| Portugal | 3.3% | 0% | 0% | 0% | 88.9% | 11.1% |
| Greece | 3.1% | 0% | 0% | 0% | 100% | 0% |
| Sweden | 2.6% | 0% | 0% | 0% | 35.7% | 64.3% |
| Ireland | 2.2% | 0% | 0% | 0% | 0% | 100% |
| Argentina | 2% | 0% | 0% | 0% | 72.7% | 27.3% |
| Turkey | 2% | 0% | 0% | 0% | 36.4% | 63.6% |
| Brazil | 1.5% | 0% | 0% | 0% | 100% | 0% |
| United States | 1.1% | 0% | 0% | 0% | 16.7% | 83.3% |
| Australia | 0.6% | 0% | 0% | 0% | 0% | 100% |
| Belgium | 0.6% | 0% | 0% | 0% | 100% | 0% |
| Finland | 0.6% | 0% | 0% | 0% | 0% | 100% |
| Poland | 0.6% | 0% | 0% | 0% | 0% | 100% |
| Romania | 0.6 | 0 | 0 | 0 | 0 | 100 |
| South Korea | 0.4 | 0 | 0 | 0 | 0 | 100 |
| Russia | 0.4 | 0 | 0 | 0 | 0 | 100 |
| Slovenia | 0.4 | 0 | 0 | 0 | 0 | 100 |
| Switzerland | 0.4 | 0 | 0 | 0 | 50 | 50 |
| Czech Republic | 0.2 | 0 | 0 | 0 | 0 | 100 |
| Denmark | 0.2 | 0 | 0 | 0 | 100 | 0 |
| Holland | 0.2 | 0 | 0 | 0 | 100 | 0 |
| India | 0.2 | 0 | 0 | 0 | 0 | 100 |
| Malaysia | 0.2 | 0 | 0 | 0 | 0 | 100 |
| Norway | 0.2 | 0 | 0 | 0 | 100 | 0 |
| United Kingdom | 0.2 | 0 | 0 | 0 | 0 | 100 |

## 5.2 Pathogenesis

ATTR amyloidosis is caused by the misfolding of a protein called transthyretin which aggregates and deposits as amyloids in various tissues and organs (Lovley et al., 2021). ATTR-PN is a rare genetic condition caused by mutations in the TTR gene. Mutations in the apolipoprotein A-1 (APOA1) or gelsolin (GSN) genes can also be associated with the abnormal buildup of TTR protein (Kapoor et al., 2019). TTR gene on chromosome 18 mainly encodes for transthyretin protein (Luigetti et al., 2020). Transthyretin is primarily produced in the liver, and its role is to transport vitamin A and thyroxin hormones (Carroll et al., 2022). To carry Vitamin A or thyroxin, four individual transthyretin proteins must be attached or bound to each other to form a tetramer protein complex. In ATTR-PN, those four units are unstable and prone to breaking up. The defective proteins form abnormal clumps known as amyloid fibrils build up in different tissues and accumulation of amyloids can cause various tissue and organ damage (Rintell et al., 2021). Approximately 140 pathogenic mutations in the TTR gene are identified and each one is associated with different patterns of organ involvement, age of onset, and disease progression (Dohrn et al., 2021). Val30Met pathogenic mutation in the TTR gene is most prevalent (Mazzeo et al., 2015). In the Val30Met mutation, amino acid valine (Val) is substituted by another amino acid named methionine (Met) at the 30th position of the protein sequence. Other common mutations such as Thr60Ala, Glu89Gln, Ser50Arg, Phe64Leu, Ala97Ser, Ser77Tyr, and Ser77Phe follow the same naming pattern (Mazzeo et al., 2015). In most cases, ATTR-PN occurs due to inheriting a mutated TTR gene, which gives rise to misfolded transthyretin protein. It is called hereditary transthyretin amyloidosis or hATTR or ATTRv and is inherited in an autosomal dominant manner (Dohrn et al., 2021). In other cases, proteins may alter due to age, and are referred to as wild-type transthyretin amyloidosis (Adams, 2013; Bezerra et al., 2020). The defective proteins deposit as

amyloid in different tissues, particularly those of the nerves and heart (Lovley et al., 2021). This deposition of amyloid ultimately leads to the typical symptoms of ATTR-PN.

## 5.3 Clinical Manifestations

ATTR-PN is a multisystem disorder, manifesting a variety of signs and symptoms. The ATTR-PN onset varies as symptoms may manifest anytime between the ages of 30 to 70 (Lovley et al., 2021). Transthyretin amyloidosis is caused by misfolded transthyretin protein (Dohrn et al., 2021). The TTR gene encodes for the transthyretin protein, which is essential to transport vitamin A and thyroxine to the circulatory system (Kapoor et al., 2019). However, a mutated form of transthyretin is unable to execute, and forms clumps or amyloids in the nervous system, heart, kidneys, spleen, and eyes (Adams, Ando, et al., 2021; Luigetti et al., 2020). Nerve impairment and heart problems are characteristic of ATTR-PN disorder (Waddington-Cruz et al., 2019). Individuals with early-onset ATTR-PN, usually have a family history of the disorder and experience a rapid progression of symptoms (Nativi-Nicolau et al., 2021). Late-onset ATTR-PN progresses more slowly and often involves the heart (Adams, Ando, et al., 2021; Nativi-Nicolau et al., 2021). Initially, it affects the peripheral nerves residing outside the brain and spinal cord, which are responsible for controlling sensation and movement in the upper and lower limbs (Mazzeo et al., 2015; Waddington-Cruz et al., 2019). In ATTR-PN, amyloid deposits accumulate and damage these nerves leading to a condition known as peripheral neuropathy. The most typical presentation is sensory-motor polyneuropathy, which commonly manifests as abnormal sensations in the hands and feet, such as numbness, tingling, or burning (Luigetti et al., 2020; Mazzeo et al., 2015). Carpal tunnel syndrome (CTS) is one of the most common and early signs of peripheral neuropathy (Adams, Ando, et al., 2021; Mazzeo et al., 2015). Loss of sensation, paresthesia, impaired coordination, losing thermal sensitivity, and muscle weakness are symptoms characteristic of peripheral neuropathy in ATTR-

PN disorder (Ando et al., 2013). Psychogenic pain or allodynia is also familiar to patients with early-onset, and even paralysis may occur if motor nerves are affected (Ando et al., 2013). People with ATTR-PN may also experience typical symptoms of autonomic neuropathy, including nausea, vomiting, diarrhea, constipation, gastrointestinal issues, urinary incontinence, abnormal sweating and erectile problems (Kapoor et al., 2019; Luigetti et al., 2020). On the contrary, individuals with late-onset ATTR-PN tend to cause fewer symptoms related to autonomic impairment (Mazzeo et al., 2015). Postural hypotension symptoms may also occur in ATTR-PN patients. Accumulation of amyloid deposits in the heart causes cardiomyopathy, which leads to heart failure and is the primary cause of death in ATTR-PN patients (Dohrn et al., 2021; Waddington-Cruz et al., 2019). Clinical symptoms may include fatigue, shortness of breath, heart palpitations and abnormal heart rhythm (arrhythmia), swelling in the legs, angina (chest pain), lightheadedness, dizziness or fainting, sleeping disturbances, blood in urine (Adams, Ando, et al., 2021). Other organs can also be damaged by amyloid fibrils causing vision problems, dry eyes, headaches, seizures, kidney failure, and splenomegaly. Drastic and unintended weight gain (megarexia) or weight loss (anorexia) is also complications related to amyloid neuropathy (Adams, Ando, et al., 2021; Kapoor et al., 2019).

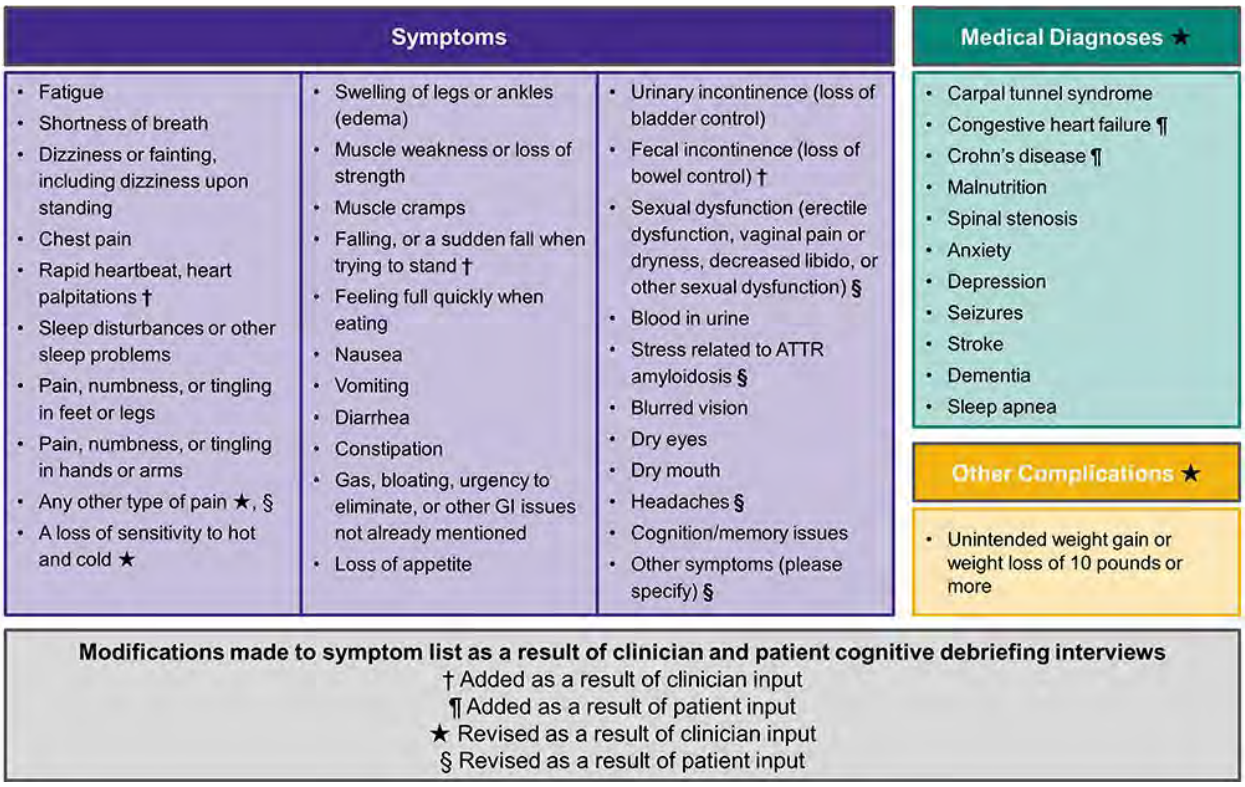


*Figure 9: Modifications to ATTR-PSS (ATTR Patient Symptom Survey) symptom list. Conditions listed in "medical diagnoses" and "other" categories were originally included as part the symptom list. At the suggestion of clinicians, these conditions were removed from the symptom list and added as 2 new items to the ATTR-PSS (as represented by the * symbol). Revised symptoms (represented by the * and § symbols) were modified for language/clarity; the updated language is reflected in the figure* (copied from Rizio et al., 2020).

The underlying genetic cause of the disease may affect its onset, as well as its course. For patients with early-onset V30M (Val30Met) mutation, neuropathic symptoms usually appear within 30 to 50 years. In V30M mutation, heart conditions are uncommon with early-onset, and among patients with late-onset cardiomyopathy is more common (Ando et al., 2005; Kapoor et al., 2019). The Ser50Arg and Ala97Ser mutations are also associated with neuropathic symptoms (Luigetti et al., 2020). In contrast, patients with V122l (Val122lle) or L111M (Leu111Met) mutations typically experience heart problems and have mild peripheral nerve involvement (Luigetti et al., 2020). Individuals in their 50s with T49A (Thr49Ala) mutation may develop symptoms of moderate neuropathy and severe cachexia followed by cardiomyopathy (Mazzeo et al., 2015).


*Figure 10: Substantial overlap and mixed phenotype within both neurologic and cardiac features. In hereditary transthyretin amyloidosis point mutations in the transthyretin gene can lead to a predominately neurologic or cardiac phenotype, however, there is often substantial overlap and a mixed phenotype with both neurologic and cardiac features is common (replicated from Benson et al., 2020).*

## 5.4 Possible treatment options

### 5.4.1 Approved Treatments

#### Patisiran

Onpattro (Patisiran) is the first approved medication for ATTR-PN that targets both wild-type and mutant TTR mRNA (Adams, Polydefkis, et al., 2021; Coelho, Adams, et al., 2020). In August 2018, the FDA and EMA both approved Onpattro, manufactured by Alnylam Pharmaceuticals, to treat adults with ATTR-PN (Kristen et al., 2019; Tambuyzer et al., 2020). Onpattro uses a technology that researchers call RNA interference (RNAi), and molecules named small interfering RNAs, or siRNAs, which regulate gene expression or the number of protein cells (Sekijima et al., 2018). By harnessing this pathway, the therapy selectively targets mutated genes and shuts the production of faulty proteins down (Kristen et al., 2019; Tambuyzer et al., 2020). ATTR-PN is an inherited disorder caused by mutations in the TTR gene and mutated TTR gene produces misfolded transthyretin protein in the liver (Coelho, Adams, et al., 2020). Abnormal transthyretin in the liver leads to the deposition of amyloid fibrils within nerve cells, the heart, and the gastrointestinal tract

(Adams et al., 2018; Coelho, Adams, et al., 2020). Onpattro medication or a double-stranded small interfering ribonucleic acid (siRNA) encapsulates inside the lipid nanoparticles, which delivers the siRNA to liver cells (Russo et al., 2020). After arriving in hepatocytes, Onpattro selectively targets and cleavages TTR mRNA, reducing the expression of the faulty TTR gene and lessening the production of transthyretin (Adams et al., 2018; Russo et al., 2020). Onpattro is administered via intravenous (IV) infusion once every 3 weeks, and for patients with less than 100 kg weight, the recommended dose is 0.3 mg/kg of actual body weight (Coelho, Adams, et al., 2020; Russo et al., 2020; Sekijima et al., 2018). As is the case, transthyretin protein mainly transports vitamin A and thyroxine in the blood circulation and cerebrospinal fluid. Onpattro therapy designedly reduces transthyretin and transthyretin-dependent vitamin A accordingly(Kristen et al., 2019). As vitamin A levels in the blood decrease, patients using Onpattro treatment need to take a daily vitamin A supplement (Adams, Ando, et al., 2021; Russo et al., 2020).

**Inotersen**

Tegsedi (Inotersen or ISIS 420915) is an approved antisense oligonucleotide (ASO) therapy to treat adult patients with ATTR-PN (Brannagan et al., 2020; Gales, 2019). Tegsedi is designed to obstruct the production of misfolded transthyretin and prevent the buildup of amyloid deposits (Tambuyzer et al., 2020). ATTR-PN is a hereditary progressive condition caused by a mutation in the transthyretin (TTR) gene, which contains the information necessary for the production of the transthyretin protein (Gales, 2019). The mutation results in the production and accumulation of abnormal transthyretin, especially in the heart, kidneys, nervous system, and eyes (Doğan & Kürtüncü, 2019; Gales, 2019). Abnormal protein deposits damage these organs over time and lead to the symptoms of ATTR-PN (Brannagan et al., 2020). Tegsedi is a chemically modified RNA molecule that produces a dose-dependent reduction of TTR mRNA (Tambuyzer et al., 2020).

Through antisense therapy, Tegsedi binds to TTR mRNA and stops it from being read by ribosomes, halting the overall synthesis and production of transthyretin (Coelho, Adams, et al., 2020; Gales, 2019). It is a once-weekly injection administered 284-300 mg dosage regimen subcutaneously, inhibiting the hepatic production of both ATTRv and ATTRwt (Gales, 2019; Russo et al., 2020). Since 2018, EC, FDA, and Health Canada have authorized to market of Tegsedi for adults with stage 1 and 2 ATTR-PN (Coelho, Yarlas, et al., 2020; Gales, 2019). Tegsedi also has marketing approval from the Brazilian Health Regulatory Agency. The global marketing license of the Tegsedi is held by Ionis Pharmaceuticals and its subsidiary Akcea Therapeutics, and PTC Therapeutics has licensed commercialization rights for the treatment in Latin America (Gales, 2019). According to FDA, Tegsedi causes low platelet counts and is not recommended for patients with existing thrombocytopenia (Merrill D. Benson et al., 2018; Gales, 2019). Additionally, Tegsedi can causes glomerulonephritis and may not be prescribed for patients with renal dysfunction (Gales, 2019). Close monitoring of platelet counts and renal function is recommended in patients taking Tegsedi (Brannagan et al., 2020; Doğan & Kürtüncü, 2019; Russo et al., 2020). Furthermore, Tegsedi is associated with an increased risk of vitamin A deficiency, and patients require 3000 IU of vitamin A per day (Doğan & Kürtüncü, 2019).

**Physical Therapy**

The objective of physical therapy is to improve muscle strength and reduce pain (Jesson et al., 2020). Physiotherapy with physical rehabilitation is applicable in a wide range of neurological, neuromuscular, cardiovascular, and respiratory conditions (Dohrn et al., 2021). The patient might be advised and trained in several exercises to help with nerve function (Filipova et al., 2020). In ATTR-PN, the nervous systems is affected and physiotherapy can reduce some of the pain and restricted movement associated with peripheral neuropathy (Jesson et al., 2020). ATTR-PN

physiotherapy largely depend on the extent of symptom progression and organ damage (Adams, 2013). A qualified physiotherapist may include various exercise approaches to treat ATTR-PN, namely aerobic exercises, flexibility exercises, strength training, balance exercises (Jesson et al., 2020). Prescribed aerobic exercises include brisk walking, swimming, low-impact aerobics sessions, stationary cycling, or a combination of all, increasing muscular activity, heart rate, and breathing. Flexibility exercises (stretching) help joints to reduce the risk of injury and with the proper guidance, stretching can be performed at home or clinic. Stretching targets osteoarticular flexing of calf muscles, hamstring muscles, and plantar fascia (Dohrn et al., 2021). Strength training involves lifting weights to regain lost muscle strength and targets muscles on the core, hip, knee, and ankle (Jesson et al., 2020). Strength training carries a risk of injury and must be performed under physiotherapist supervision. Balance exercises may restore balance and relieve the feeling of stiffness in older patients (Jesson et al., 2020). The physiotherapist also prescribes braces to protect injured nerves or muscles. After evaluating the patient's physical fitness, a physiotherapist must determine the duration and type of exercises for the ATTR-PN patient (Jesson et al., 2020).

**Occupational Therapy**

Occupational therapy can help patients with neuropathies to improve their quality of life. This therapy is a personalized method of treatment to improve a patient's necessary motor skills to perform everyday functions, as occupational risk can occur due to any physical, psychological, social, or environmental causes (Cavero-Carbonell et al., 2022; Lovley et al., 2021). Transthyretin amyloid polyneuropathy is an ascending peripheral neuropathy caused by mutations in the transthyretin (TTR) gene and is the most serious hereditary polyneuropathy of adult-onset. In ATTR-PN, amyloid deposits accumulate in the nervous system by affecting the upper and lower

limbs followed by severe and even complete paralysis (Cavero-Carbonell et al., 2022). Occupational therapy can help to improve limb function by guiding patients to avoid dangerous movements or postures. The severity of the disease escalates depending on the type of the mutation and the stage of disease progression (Cavero-Carbonell et al., 2022). Depending on the severity of ATTR-PN, occupational therapy is often prescribed with physiotherapy. The therapist can teach self-care activities that may impose a risk of falling or tripping. Occupational therapists also instruct how to change positions to avoid the sudden risk of blood pressure drop or fainting. Moreover, occupational therapy is combined with recreation therapy to prevent patients from being isolated and help them socialize, reduce stress and anxiety (Moisset et al., 2020; Sekijima et al., 2018). An occupational therapist aims to ease the patient's hardship by focusing on overall health and ensuring appropriate interventions to satisfy their specific needs on time (Cavero-Carbonell et al., 2022).

**Liver Transplant**

Liver transplantation or orthotopic liver transplantation is a medical procedure of surgically replacing a diseased liver with a complete or partial healthy liver from a donor (Adams, 2013). The liver is essential for blood filtration, bile production and excretion, enzyme activation, metabolism of fats, proteins, and carbohydrates. It is also crucial for the removal of degraded metabolic products. Transthyretin is a liver-produced protein encoded by the TTR gene and once released into the circulation its role is to transport vitamin A and thyroxin hormone (Carroll et al., 2022). To function properly, four individual transthyretin must attach and form a four-unit complex. In ATTR-PN, the four-unit protein complex is unstable, and dissociated TTR protein tends to misfold and aggregate to form fibrils, which deposits as amyloid (Luigetti et al., 2020; Pinto et al., 2018). The build-up of the amyloid starts to disrupt the function of various organs,

leading to progressive multisystem dysfunction (Luigetti et al., 2020). Therefore, performing a liver transplant can slow the progression of ATTR-PN disorder by suppressing the primary source of mutant TTR and preventing the formation of defective TTR protein (Adams, 2013). Based on the Coutinho staging system of 0 to 3, patients in stage 1 of the ATTR-PN are best suited for a liver transplant (Ando et al., 2013). Individuals at early stage 2 may also be eligible. But liver transplant surgery at the last stage of ATTR-PN might not be beneficial as transplantation cannot treat formerly accumulated amyloids in the body (Ando et al., 2013). In addition, liver transplantation is not practical to operate for inhibiting wild-type TTR synthesis (Gales, 2019). After determining eligibility, physicians perform other tests to ensure vital parameters (Ando et al., 2013; Hawkins et al., 2015). After complete or partial transplantation, the patient is kept under observation for a few days and discharged within few weeks after the operation (Chaudhry et al., 2018). A partially transplanted liver normally grows to full size within a year. The expected outcome of a liver transplant depends on the type of mutation in the TTR gene, patient age, the severity of symptoms, and the presence of amyloids in organs (Ando et al., 2013; Hawkins et al., 2015; Kapoor et al., 2019). Liver transplantation is associated with high-risk complications, including blood clots, bile duct complications, rejection or failure of the donated liver, kidney damage, seizures, strokes, and even glaucoma (Adams, 2013; Kapoor et al., 2019). Moreover, anti-rejection immunosuppressant exhibits potential side effects (Hawkins et al., 2015). Thus, liver transplant surgery is only recommended in patients with early-stage and considered as last-resort measure for ATTR-PN treatment.

## 5.4.2 Experimental Treatments

**Eplontersen**

Eplontersen, formerly known as AKCEA-TTR-LRx or ION-682884, is a second-generation drug targeted at TTR and is an antisense inhibitor of transthyretin production (Luigetti et al., 2020; Russo et al., 2020). In ATTR-PN, mutations in the TTR gene lead to the abnormal folding and accumulation of a protein called transthyretin (TTR) in various tissue-organ of the body, particularly in the nervous system and the heart. Eplontersen therapy is co-developed by Ionis Pharmaceuticals and AstraZeneca to treat all forms of transthyretin amyloidosis (ATTR). This second-generation RNA-targeted therapy developed on Ionis' ligand conjugated antisense raises ASO potency at least 10-fold in mice (Carroll et al., 2022; Russo et al., 2020). It is designed to reduce the production of the TTR protein and prevent the buildup of toxic amyloid clumps by terminating the translation of the TTR mRNA into faulty transthyretin protein. Eplontersen is administered subcutaneously via a single subcutaneous injection (Luigetti et al., 2020). It is currently in Phase 3 trials and the FDA has granted Eplontersen an orphan drug designation with incentives (Carroll et al., 2022).

**Dolobid**

Dolobid (Diflunisal) is a salicylic acid derivative frequently defined as a nonsteroidal anti-inflammatory drug (NSAID) (Dohrn et al., 2021; Hawkins et al., 2015). Although derived from salicylic acid, its chemical structure is different from aspirin and also acts as a non-selective TTR stabilizer (Luigetti et al., 2020; Pinto et al., 2018). It is currently used off-label for the treatment of ATTR-PN as dolobid inhibits the progression of neuropathy and preserves the quality of life in people with ATTR-PN (Dohrn et al., 2021; Hawkins et al., 2015; Pinto et al., 2018). ATTR-PN is a progressive disease characterized by the accumulation of amyloids in different organs and tissues; however, diflunisal inhibits amyloid fibril formation and suppresses amyloid fibrils by binding to the T4 binding sites of TTR protein (Adams, 2013; Carroll et al., 2022; Kapoor et al.,

2019). The purpose of diflunisal is also to reduce pain, inflammation, and fever. Dolobid works as prostaglandin synthase inhibitors by blocking the activity of enzymes that synthesize prostaglandins. Dolobid tablets are administered orally for mild to moderate pain (Hawkins et al., 2015). Common side effects of long term diflunisal use may include nausea, vomiting, dizziness, drowsiness, headache, rash, gastrointestinal pain, congestive heart failure (CHF), and renal function impairment (Carroll et al., 2022; Hawkins et al., 2015; Luigetti et al., 2020).

**Doxycycline and Tauroursodeoxycholic acid**

The combination therapy of doxycycline and tauroursodeoxycholic acid (TUDCA) is another investigational treatment option for ATTR-PN patients that only works on non-fibrillar TTR deposits (Kapoor et al., 2019). Doxycycline is an antibiotic and tauroursodeoxycholic acid TUDCA is a bile acid (Ando et al., 2013; Carroll et al., 2022). In ATTR-PN, a mutation in the TTR gene produces misfolded transthyretin and is characterized by the deposits of transthyretin (TTR) amyloid in multiple organs, particularly in peripheral nerves and the heart. Doxycycline and TUDCA are both amyloid matrix solvents that work by disrupting or dissolving the deposited amyloid fibrils (Kapoor et al., 2019; Sekijima et al., 2018). Along with the removal of TTR deposits, this drug combination therapy activity leads to tissue repair (Adams, 2013). This oral fixed-dose drug combination may also overcome physical limitations by lowering the inflammatory stress with an acceptable toxicity profile (Ando et al., 2013). This oral combination treatment was last tested for ATTR-PN patients in a Phase 2 trial and reported mild skin redness (Hawkins et al., 2015).

**SOM0226**

SOM0226, also known as Tolcapone and CRX-1008, is an investigational repositioned compound to treat ATTR-PN (Reig et al., 2015). It is a repositioned compound with a newly identified activity as a potent kinetic TTR stabilizer and TTR fibril disruptor (Bezerra et al., 2020; Carroll et al., 2022). In ATTR-PN, the amyloid fibrils are formed by native TTR tetramer dissociation into unstable monomers that unfold and aggregate in various organs. TTR serves by transporting thyroxine hormone and vitamin A, and SOM0226 imitates the thyroxine-transthyretin binding process in the circulation to stabilize the TTR tetramer (Bezerra et al., 2020; Russo et al., 2020). SOM0226 (Tolcapone) was first approved to treat Parkinson's disease due to its activity as a catechol-O-methyltransferase (COMT) inhibitor (Bezerra et al., 2020; Russo et al., 2020). SOM Biotech entered into a global licensing agreement with Corino Therapeutics in May 2017 for further development as CRX-1008 to treat ATTR-PN. SOM0226 can effectively cross the blood-brain barrier (BBB), and currently, it is in a Phase 2 trials study to treat ATTR-PN (Bezerra et al., 2020; Carroll et al., 2022; Russo et al., 2020). Repositioning existing medicines can speed a treatment development and lower its cost as safety is established, facilitating SOM0226 usage in ATTR-PN prevalent countries like Brazil and Portugal (Reig et al., 2015).

**Vutrisiran**

Vutrisiran (previously ALN-TTRsc02) is a second generation investigational siRNA conjugate to treat ATTR-PN (Bezerra et al., 2020; Carroll et al., 2022). Alnylam Pharmaceuticals is developing this GalNAc-siRNA conjugated drug for amyloid polyneuropathy (Coelho, Adams, et al., 2020; Russo et al., 2020). The buildup and accumulation of abnormal deposits of TTR proteins in different organs and tissues characterize ATTR-PN. Subcutaneously administered vutrisiran targets faulty TTR mRNA, which is the intermediate messenger molecule between the TTR gene and transthyretin protein (Carroll et al., 2022; Coelho, Adams, et al., 2020). By binding to mRNA,

Vutrisiran triggers a natural process called RNA interference that promotes the destruction of TTR mRNA and RNAi may also prevent the production of the faulty transthyretin protein that causes ATTR-PN (Russo et al., 2020). Although Vutrisiran do not efficiently cross the blood–brain barrier (BBB), promising phase 3 result was revealed in April 2021 (Carroll et al., 2022). Vutrisiran has an orphan drug designation from the FDA and European Union EU.

**Vyndaqel**

Vyndaqel (Tafamidis) is a novel treatment developed by Pfizer to treat patients with neurodegenerative ATTR-PN disorder (Adams, 2013). A functional TTR protein is a tetrameric complex of four identical transthyretin and mutations in the TTR gene destabilize the soluble tetrameric complex, causing the accumulation of monomeric TTR proteins in various organs. Vyndaqel the orally applicable benzoxazole medication acts as a kinetic transthyretin stabilizer (Dohrn et al., 2021; Hawkins et al., 2015; Luigetti et al., 2020). It binds to the unstable tetrameric transthyretin complex and prevents it from breaking down into monomeric TTR molecules (Carroll et al., 2022; Dohrn et al., 2021; Kapoor et al., 2019). It blocks abnormal signal transduction and slows the progress of peripheral neuropathy (Adams, 2013). Tafamidis is recently approved as an oral treatment for ATTR-PN patients in Europe and the United States (Carroll et al., 2022; Dohrn et al., 2021). Currently, 40 other countries also approved Vyndaqel for ATTR-PN patients including Japan, European countries, Brazil, Mexico, Argentina, Russia, and South Korea (Dohrn et al., 2021; Hawkins et al., 2015; Kapoor et al., 2019; Luigetti et al., 2020). The most common adverse effects of Vyndaqel therapy include urinary tract and vaginal infections, stomach aches, and diarrhea (Pinto et al., 2018).

# Chapter 6

# Pre and post development challenges

## 6.1 Diversity and inclusion

From the point of view of epidemiology, a disease might be classified into common, rare, or ultra-rare categories, which can impact individuals regardless of race, ethnicity, income, and nationality. Approximately 85% of rare diseases are considered ultra-rare, as prevalence is 1 per 1,000,000 individuals (Berger et al., 2021). While having lower disease prevalence, rare diseases are scattered among diverse nationalities. With wide biological diversity, these disparate rare diseases affect 6-8% global population, creating an immense burden (Nguengang Wakap et al., 2020). This often initiates an enormous challenge in the development of precision medicine. The rare disease community is often small and negligible, heterogeneous, and widely dispersed, which restricts obtaining inclusive data (Nguengang Wakap et al., 2020). According to an enumeration, 80% of rare diseases are not race-specific and emerge due to individual genetic mutations (Giugliani et al., 2019; Molster et al., 2016). The inclusion of diverse samples in genomic research has become paramount. Rare diseases are also easily misdiagnosed due to the complex biology, limited knowledge of the natural history of the disease and their similarity with symptoms of various known diseases (Vandeborne et al., 2019). A limited number of countries specialize in orphan treatments and have study sites, leading to difficulties in patient recruitment and acquiring large amounts of high-quality patient data (Lanar et al., 2020). Patient cohorts from certain income levels and the commoners, who face disparities in access to care, are largely under-represented in research and clinical trials. For accurate and impactful analysis in orphan drug development diverse and inclusive research workforce to investigate rare diseases is required.

## 6.2 Safety concerns

Developing drugs to treat rare diseases represents unique challenges as verifying drug safety and efficacy comes foremost. The drug discovery process ends when one lead compound is found for a drug candidate. The clinical development process then starts, initially determining its safety profile in phase 1 (Muglia & DiGiovanna, 1998). In the development of precision medicine consistent drug safety, quality and efficacy are a must, accompanying dedicated equipment, skilled staff, cGMP quality, and adequate patient documentation. To ensure a safety profile each development step also requires regular planning or update (Sharma et al., 2010b). However, the safety assessment for rare medicines can be weakened by limited disease information and difficulty determining endpoints, placebo, and biomarkers. The paucity of data from small numbers of patients further challenges clinical trial design, which assesses safety, efficacy, or risk-benefit ratio (Simoens, 2011). Due to human error during the clinical development of precision medicine, safety data, including documented adverse events, laboratory data, and historical data, are often mislaid. Although precision medicine requires prospective clinical trials, inadequate safety profiling and lack of postmarketing pharmacovigilance are a matter of concern. In the postmarketing phase of an orphan medication, all available sources need to be assessed and monitored to ensure a safety profile, which is also challenging (Mazzucato et al., 2014). Active surveillance in pharmacovigilance addresses efforts to develop safe and efficacious drugs for rare diseases, allowing precise data for postmarketing risk management (Simoens, 2011).

## 6.3 Ethical issues

In addition to small potential markets, clinical development for rare diseases poses unique scientific and ethical challenges (Hartman et al., 2020). Many rare diseases have no treatment

opportunities, which often raise moral challenges. If potential orphan medications exist to treat particular rare diseases timely, there will be a good prognosis for individuals to lead a decent life. Patient rights and equity of resources are two crucial ethical issues perceived by participants. Research in rare diseases is often performed in high-income countries and biases individuals from certain races and income levels (Kontoghiorghe, 2014). More ethical problems are faced by patients as systemic inequalities result in widespread disparities, making a timely diagnosis and adequate treatment considerably harder. This results in serious health consequences and raises ethical issues. Ethical questions associated with clinical testing of orphan medications, population screening, and epidemiology testing on rare diseases are also a matter of concern. Additionally, over 40% of progressive and life-threatening rare diseases affect children and reminds the critical need to address special ethical considerations for children participating in rare disease clinical trials (Lee et al., 2020). Bias toward children and different contributions to the pediatric and adult form of RDs is also ethically questioned (Mazzucato et al., 2014; Ollendorf et al., 2018). Expensive medical procedures are also an ethical issue. High expenditure for drug development is mainly related to marketing costs, and therefore, precision medicine development and production is a matter of dilemma for pharmaceutical industries (Ollendorf et al., 2018). To make a profit from available orphan treatments, manufacturers increment higher prices per patient. Ethicists question whether to let a patient suffer due to inability to pay or facing a high financial burden. Ethical aspects of rare diseases are relevant to health care professionals, members of clinical ethics committees, researchers and bio-scientists, policymakers, and patients, who must address these obstacles (Simoens, 2011).

## 6.4 Financial Considerations

Living with a rare disease can be physically, mentally, and even financially challenging (Molster et al., 2016). Due to a small patient pool and the higher cost of launching on the market, orphan medications can appear less promising for the pharmaceutical companies' investment (Kontoghiorghe, 2014). Companies set extraordinarily high prices for orphan drugs to recoup R&D costs, including unsuccessful attempts at drug development (Ollendorf et al., 2018). Financial barrier thus burdens direct healthcare costs such as Rx medications and their administration, healthcare equipment, medical tests, specialist visits, hospitalizations, and health transport. Despite a considerable financial contribution towards development costs from Government organizations, over-priced orphan drug conditions have no solution (Ollendorf et al., 2018). The unit cost for orphan drugs is 25x more expensive than commonly prescribed non-orphan drugs. Nowadays, 88% of orphan drugs cost more than $10,000, such as Zolgensma, the most costly drug yet approved to treat rare spinal muscular atrophy (Kerpel-Fronius et al., 2020). Another causality for this economic burden is the length of clinical development for orphan drugs. From initial discovery to the marketplace, any novel orphan medication pathway requires more than 10 to 15 years, with clinical trials alone taking 12.3 years on average (Annemieke Aartsma-Rus et al., 2021). Additionally, many are unaware of the financial burden from lifestyle changes, including home or auto modifications, medical foods, and leave paid employment or forced retirement. Living with any rare health condition can also burden indirect and non-medical costs, such as professional caregivers, non-health transport, social services, and inability to work or early retirement (Kontoghiorghe, 2014). In current health insurance systems, orphan drugs must compete for funds with drugs prescribed for common diseases. Expenditure for rare diseases exceeds the amount an insurer is willing to pay, leading to patients facing a high financial burden. Reportedly 55% of direct medical expenses for rare diseases in the US and 18% in the UK are not

covered by insurance (Jayasundara et al., 2019). Another hidden financial consideration of rare diseases is travel, and patients incur various travel cost burdens while visiting dedicated centers. Pharmaceutical companies also encounter financial obstacles while justifying the investment of bringing a rare disease therapy to market. In 2019, the economic burden of rare diseases reached nearly $1 trillion in the U.S. (EveryLife Foundation For Rare Diseases, 2019). The financial burden associated with rare diseases is an economic question and requires business models that can make it in manufacturers' financial interest to behave more ethically.

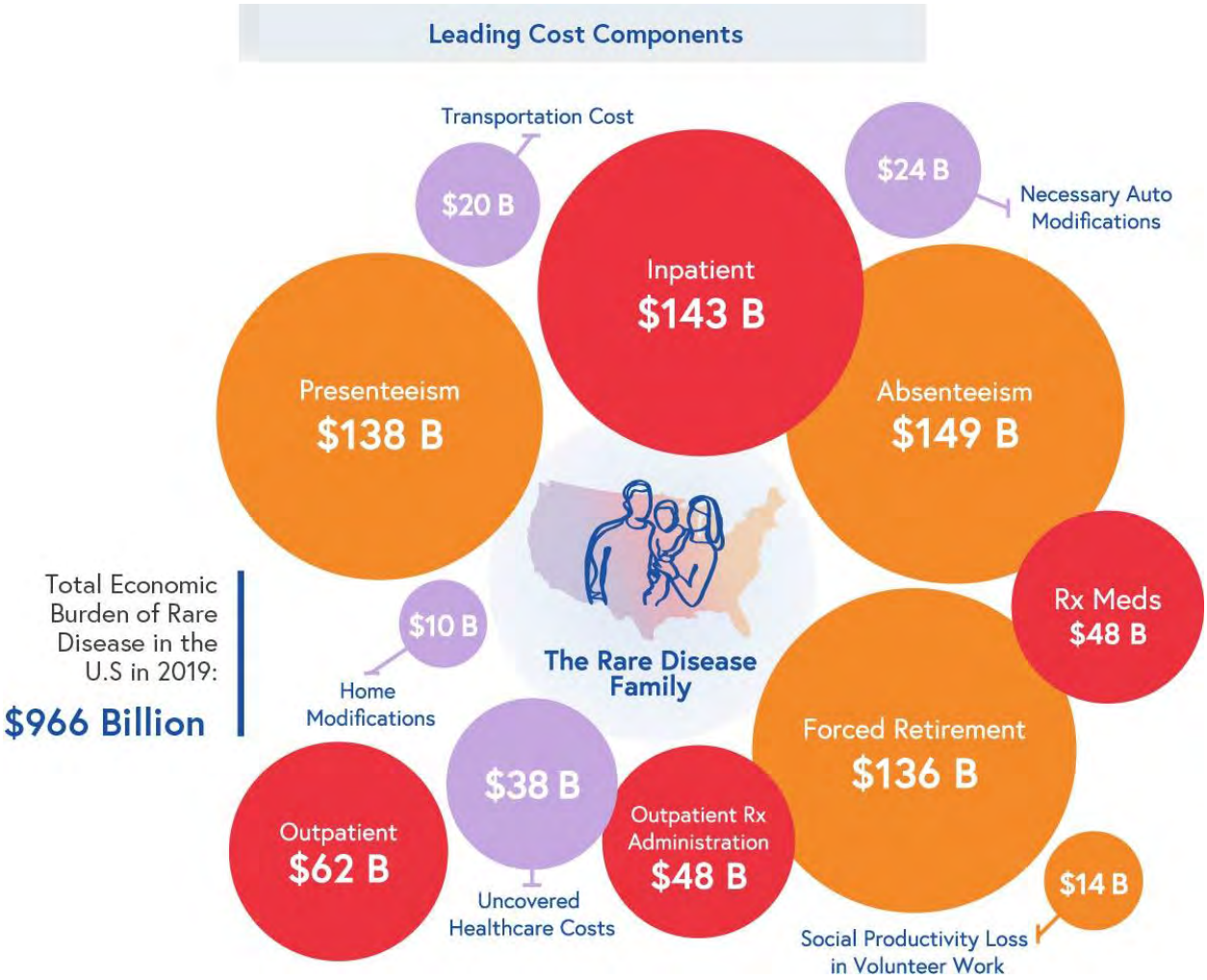


*Figure 11: Statistical analysis of total economic burden and leading cost components of Rare Disease in the U.S in 2019 (adapted from EveryLife Foundation For Rare Diseases, 2019).*

## 6.5 Reimbursement Barriers

Reimbursement is a zero-sum game in which high-cost medicines are funded out of a pre-determined drug budget. Advanced treatments, such as orphan medication may bring long-term, durable, and potentially curative benefits to patients, along with challenges for reimbursement agencies linked to pricing and reimbursement (Simoens, 2011). The financial burden associated with any prescription drug involves the ratio of un-reimbursed expenditures to household income. Orphan medical care not reimbursed by insurance was estimated to be $166 million, which costs 0.57x more than non-orphan medication. Most systems annually employ a siloed healthcare budgeting process known as zero-based budgeting that minimizes increases in pharmaceutical spending. Therefore increased annual spending on a new drug is often funded by reduced spending on other medicine or silos (medical devices, healthcare services, social services) (Tambuyzer, 2010). The orphan drug growth usually assisted with a range of government incentives that accelerate the pace of scientific breakthroughs, R&D, and new product launches. Orphan drugs transact a bulk of pharmaceutical spending with an estimated 18% of total drug spending by 2024. However, as orphan drugs become a large part of pharmaceutical spending, bureaucratic attitudes often sour, and incentives might be in jeopardy. The system is often more concerned with managing drug budget targets than managing total costs for the approach. As a result, pharmaceutical companies are increasingly cannibalizing their innovations. This unstable reimbursement situation usually results in reduced reimbursement and scaling back the programs that have made these therapies possible. Reimbursement barriers also discourage or prevent health care practitioners from helping patients achieve any lifestyle modifications. Orphan drugs that are often high priced should reimburse from public funds for better general accessibility (Ollendorf et al., 2018).

# Chapter 7

# Conclusion and Future Approaches

## 7.1 Conclusion

Worldwide various global health crises and financial tragedies are being witnessed, and one of the emphasized solutions is to provide an effective and secure precision medication in the shortest possible timeframe to as many people as possible. So far, a limited number of orphan drug candidates are approved after positive clinical trials and are available for distribution, which is not enough. To address and treat unmet medical needs precise drug candidates must be developed. However, the journey is challenging as R&D sectors and policymakers have to pursue various precautionary measures for each rare disease. Rare disease researchers often face difficulty in meeting standard clinical trial requirements. The technological development and legislation of orphan acts, such as the Orphan Drug Act of 1983 and the 21st Century Cures Act with tempting ODD incentives, facilitate and motivate the quest for orphan medication. Nowadays, several pharmaceutical companies and research institutes are only dedicated to rare disease investigations and developing orphan drug candidates. Approximately 80% of rare diseases occur due to genetic mutations that can be hereditary or acquired. Current market trends and industry predictions suggest addressing unmet medical needs considering unique genotypic and phenotypic information. A better understanding of human genetics and genomics can facilitate the discovery of innovative therapy or the refinement of existing ones. Therefore, the commercialization of orphan drugs is likely to increase. However, economic evaluations of orphan drugs are difficult as clinical data are limited and have no relevant comparators. It is a matter of concern as manufacturers are free to set drug's introductory pricing, and thus, companies might create a

monopoly market. An innovative study design that appreciates evolving regulatory guidance and incorporates patients and families, researchers, regulatory authorities, key opinion leaders, patient advocacy organizations, and technology companies into the drug development process is crucial. Moreover, it requires a pharmaceutical paradigm that can make it in manufacturers' financial interest to behave more ethically because lifesaving orphan drugs are costly but offer societal benefits. Furthermore, many diseases that were rare are not considered orphans anymore, such as diabetes, which was defined as a rare condition by the ancient Egyptians, has proper diagnoses and medications now. Despite the myriad challenges, the ongoing pipeline of orphan drugs indicates pharma's enthusiasm for rare diseases has not weakened. It is possible because orphan medications came a long way in terms of legislation, technological advancement, and patient recruitment. There is still a gap in terms of information, and scientists have been trying relentlessly to unfold the mystery about rare diseases and precision medicines as much as they can. To conclude, it must be admitted that globalization, cooperation, and coordination are necessary to solve these drug development endeavors of rare medical mysteries.

## 7.2 Future Approaches

### 7.2.1 Web-based Recruitment

Participant recruitment is an essential practice in developing precision medicine (Lanar et al., 2020). Nowadays, technological advancement has given an edge to expand knowledge beyond the borderline. Globalizing trials via an online platform can rapidly lead all currently un-diagnosable diseases into a globally coordinated diagnostic and research pipeline. This opportunity is crucial in orphan drug development as patients typically wait for 6 to 8 years before being diagnosed. In the context of rare diseases, online recruitment, hence, can provide a remarkable opportunity for

connecting patient groups with rare conditions and lower diagnosis rates. In this method, patients can easily be pre-screened, determine their eligibility for the study and access the study information, and communicate with patient advocacy groups representing each rare disease category via their own social media networks (Applequist et al., 2020). Web-based applications can also establish a direct link to the patients, provide access to defined study populations and relevant patient data on rare diseases. If reliable web-based recruitment forms, it will offer a broader range of orphan drug development possibilities based on data from patient interviews (Mazzucato et al., 2014). Digital solutions like E-recruitment can bring together patients, researchers, and industry as leaving no one behind is the future of rare diseases. Therefore, a globally accessible online platform with reliable information, consultation, and a chance to enlist is a must in this technology-based era.

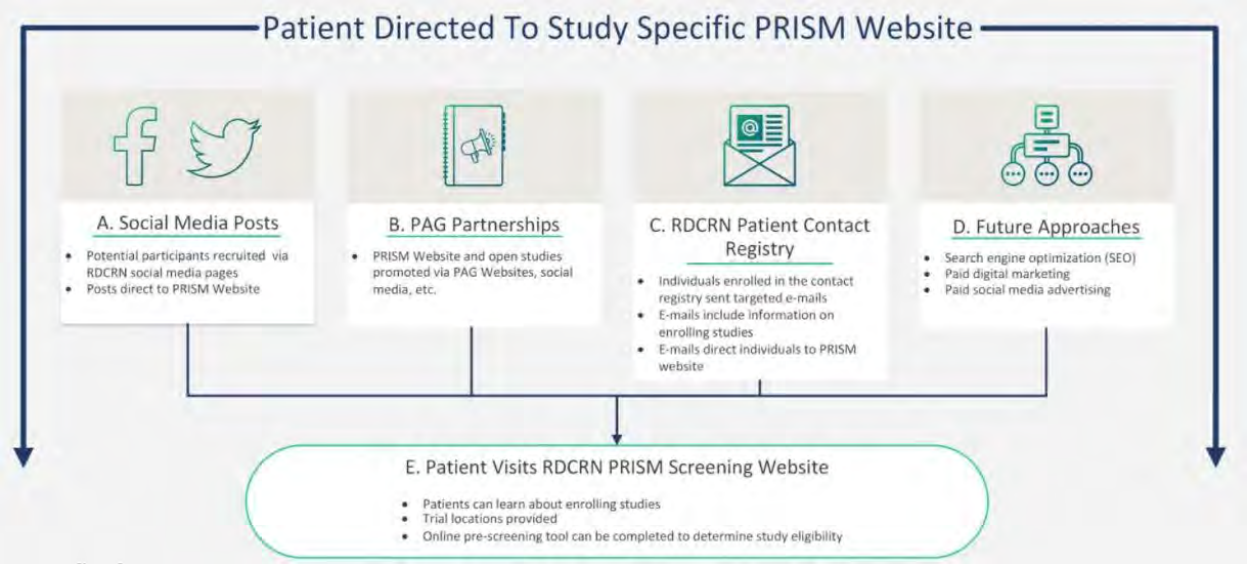


*Figure 12: Comprehensive approach to identification and recruitment of potential subjects to PRISM Website. A, Potential study participants engage with social media posts shared by the RDCRN explaining an enrolling study. B, Potential study participants engage with social media posts and other communications shared by Patient Advocacy Groups (PAGs). C, Individuals subscribed to the RDCRN Patient Contact Registry receive targeted e-mails containing an overview of enrolling studies. A, B, and C each lead individuals directly to the study-specific PRISM Website. D, Approaches that can be incorporated in addition to A, B, and C. E, Patients learn more about the enrolling study via the Website and answer questions to determine their potential eligibility for that study* (replicated from Applequist et al., 2020).

### 7.2.2 Artificial Intelligence

The future promises great advancements and personalized treatments with the introduction of novel treatments and approaches to care. For rare diseases, such a novel approach is Artificial Intelligence attributing to the necessity of orphan drug discovery and development (Paul et al., 2021). Artificial intelligence (AI) refers to the simulation of human intelligence in machines that are programmed to think like humans and mimic their actions. The term AI can also be applied to any machine that exhibits traits associated with a human mind, such as problem-solving (FRANKENFIELD, 2021). With this assumption, the 1970s saw a proliferation of AI research, specifically in Biomedicine. Despite the lack of general interest, in the beginning, AI found its way into healthcare and began to be applied to several biomedical problems (Brasil et al., 2019). The novel AI computer systems can be used in various medical science applications, including diagnosing patients, managing medical records, end-to-end drug discovery and development, digital consultation, virtual nurses, health monitoring, and precision medicine (Paul et al., 2021). After analyzing all variants against the most comprehensive data sets, AI provides 90% accurate detection of disease-causing variants, especially in infants with rare diseases (Schaefer et al., 2020). Additionally, AI can assist in structure-based drug discovery by predicting the 3D protein structure, and the design is followed by the chemical environment of the target protein site, helping to predict the effect of a compound on the target with safety considerations before further development. In the clinical trial process, AI can save time and resources by providing accurate information (De La Vega et al., 2021). AI-driven approaches in genetics and genomics look for precise mutations and links to disease from the information in DNA. Fabric GEM is an AI-driven tool for faster diagnosis of rare genetic conditions, established AI algorithms to enable clinical labs, hospital systems, and country-sequencing programs to gain actionable genomic insights (De

La Vega et al., 2021). In addition, AI transforms genomic data into actionable insights. With the help of AI, body scans may spot cancer or rare diseases early and predict the health issues people might face based on their genetics (Brasil et al., 2019; Schaefer et al., 2020). Moreover, precision medicine is also one of the most valued fields for AI as it requires considerable data analysis from several disruptive innovations. AI in rare diseases can provide real-time data without any human error, save time and resources, assist research, and may reduce physician stress (Schaefer et al., 2020). Like many other approaches, artificial intelligence is not free from associated risks, followed by an analysis of the ethical challenges of AI in healthcare. However, the application of Artificial Intelligence is growing rapidly in the rare medical field, especially in hospitals and research centers because computers can process and respond to data quickly and accurately to provide better treatment outcomes. Therefore, in case of rare conditions, it is crucial to minimize ethical risks of AI implementation and consider how to integrate AI into clinical practices for better feedback, guidance, and support for staying healthy.

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