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Investigation of Interplay of Host Microbiome among Muscular Dystrophy and Healthy Individuals

by

Taiba bibi

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Dedicated to Allah Almighty, the most beneficent and merciful, Hazrat Muhammad (S.A.W.W) who taught us the purpose of life and my father Salamat Hussain. My mother prayers have always enlightened my way throughout my life. This can't be possible without my beloved parents unwavering support, endless love and encouragement throughout my pursuit for education. It's also dedicated my work to my dearest brother Sardar Ahtisham Salamat and my sister Nida Salamat who supported me and taught me the that the best kind of knowledge is that which is learnt for its own sake. They taught me that even the toughest task can be accomplished if it is done one step at a time. I hope this achievement will fulfill the dream they envisioned for me.

CERTIFICATE OF APPROVAL

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Abstract

Muscular dystrophy is a diseases caused by mutations in genes linked with development, maintenance, functioning as well as structure, support and repair of muscles. Muscular dystrophies are a class of degenerative disorders which are hereditary in nature. These cause muscle weakness that is progressive. They are usually transmitted to the offspring, either as autosomal dominant or as recessive. They can also be transmitted as traits that are X-linked. Some cases might occur due de novo mutation. Muscular dystrophy has been associated with the microbiome of the gut, which is the group of bacteria that reside in the gastrointestinal tract of humans. Research suggests that changes in the gut microbiome may contribute to the development and progression of muscular dystrophy. Additionally, research has shown that the gut microbiome can influence immune system function, and dysbiosis or imbalanced gut microbiome may contribute to chronic inflammation, which is known to contribute to the development of muscular dystrophy. Human gut microbiome targeting would be more appropriate approach for managing the muscular dystrophy. After the ethical approval 60 questionnaire were filled by 60 healthy individual and 60 by muscular dystrophy patients. The questionnaires filled by the healthy individual and muscular dystrophy patients were statistically analyzed to study types of muscular dystrophy prevalent in Pakistan and to find the association of diet and disease with the microbiome. Stool sample of muscular dystrophy patient and healthy individual were collected and processed. In current study the difference of gut microbiota in healthy individual and muscular dystrophy was investigated through Metagenomics. The variation at class, order, and genus and species level was observed in both healthy and diseased persons. The difference in microbes in healthy and muscular dystrophy patients at class level in SFM *Alphaproteobacteria* (0.5%), *Elusimicrobia* (1%), *Spirochaetia* (1%) whereas in SF was *Actinobacteria* (7-8%), *Synergistia* and *Verrucomicrobiae*, at order level in SFM was *Aeromonadales* (1%), *Elusimicrobiales* (2%), *Spirochaetales* (1.5%) whereas in SF *Bifidoacteriales* (7%), *Erysipelotrichales* (1%) and *Peptostretococccales- Tissierellales* (2%), genus in SFM *Elusimicrobium* (2%), *Lachnospiraceae-NK4A136-group* (1%),

Megasphaera (2%), *Roseburia* (4%), *Treponema* (1%), UCG-002 (1%) and uncultured (2%).whereas in SF *[Eubacterium]-Coprostanoligenes-group* (2%), *Agathobacter* (3%), *Bifidobacterium* (12.5%), *Coprococcus* (2%), *Dialister* (3%), *Lactobacillus* (4%) and *Mitsuokella* (5%), at species level in SFM *Prevotellaceae- Bacinterium* (26%), *Treponema- Succinifaciens* (5%) in SF *Bacteroides- Xylanisolvens* (10%), *Dorea- longicatena* (4%), *Lactobacillus- ruminis* (14%), *Mitsuokella- jalaludinii* (6%), *Mitsuokella- multacida* (8%). The most abundant species in both samples were uncultured –*Alloprevotella* (SFM 26%, SF 13%), uncultured-bacterium (SFM 7%, SF 11%) and prevotella-copri (SFM 8%,SF 15%). The results indicates that the population of microbiome was different in both healthy and disease patients and diet also effects the disease and microbiome population.

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Abbreviations

2-AG	2-arachidonoylglycerol
AAV	Adeno-associated virus
ACE	Angiotensin-converting enzyme
AEA	Anandamide
AMR	Antimicrobial Resistance
BMD	Becker muscular dystrophy
CB	Cannabinoid Receptor
CGH	Comparative Genomic Hybridization
CK	Serum Creatine Kinase
CMA	Chromosomal Microarray Analyses
CMD	Congenital Muscular Dystrophy
CNBP	Cellular nucleic acid binding protein
DAPC	Dystrophin-Associated Protein Complex
DFZ	Deflazacort
DGC	Dystrophin-Glycoprotein Complex
DMD	Duchenne muscular dystrophy
DMPK	Myotonic dystrophy protein kinase
ECS	Endocannabinoid system v
EDMD	Emery-Dreifuss muscular dystrophy
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
FOS	Fructooligosaccharides
FSHD	Facioscapulohumeral Muscular Dystrophy
GIT	Gastrointestinal tract

GM	Gut Microbiota
GOS	Galactooligosaccharides
H2S	Hydrogen Sulfide
HFD	High-Fat Diet
IMF	Intra-muscular fat
LGMD	Limb-girdle muscular dystrophy
MLPA	Multiplex Ligation- Dependent Probe Amplification
MRI	Magnetic Resonance Imaging
MUFAs	Monounsaturated Fatty Acids
NGS	Next generation sequencing
OPMD	Oculopharyngeal
PCR	Polymerase Chain Reaction
PUFA	Polyunsaturated Fatty Acids
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SNP	Single Nucleotide Polymorphisms
TNF	Tumor necrosis factor
TRPC3	Transient receptor potential canonical 3

Chapter 1

Introduction

Muscular dystrophy is an illness resulting from genetic mutations associated with the processes of muscle development, upkeep, performance, and the structural support and healing of muscles [1]. This is a hereditary condition with varying degrees of severity. Within this ailment, muscle loss and reduced function can result in the inability to walk independently and, in certain instances, even lead to fatality [2].

Major kinds of muscular dystrophy includes Distal Muscular Dystrophy, Becker Muscular Dystrophy, Duchenne Muscular Dystrophy, Facioscapulohumeral Muscular Dystrophy, Congenital Muscular Dystrophy, Emery-Dreifuss Muscular Dystrophy, Limb- Girdle Muscular Dystrophy, Oculopharyngeal Muscular Dystrophy and Myotonic Dystrophy. Duchenne Muscular Dystrophy (DMD) which are caused due to dystrophin deficiency are the most prevalent type of muscular dystrophy [3].

The estimated prevalence of this disease was estimated to be 3.6 per 100,000 people worldwide, the highest prevalence was estimated in the Americans to be 5.1 per 100,000 people. The most common muscular dystrophies are DMD and BMD, with estimated prevalence rates of 4.8 per 100,000 and 1.6 per 100,000, respectively [4]. Due to the presence of two X chromosomes, men are more likely than women to have DMB and BMD, and they also do not experience as much suffering from

X-linked recessive illnesses because the unaffected chromosome typically compensates for the mutant gene [5]. Female patients with DMD rarely manifest traits resembling those found in males [6]. If women possess homozygous mutations in the dystrophin gene, they are carriers with symptoms [7].

Being a genetic condition, muscular dystrophy is inheritable and has been linked to 350 various missense, nonsense, splice site, and deletion mutations that are dispersed throughout the 65 exon coding sequence [8]. They are majorly caused due to autosomal dominant or recessive mutations [9]. Except Duchenne and Becker Muscular Dystrophy which are X-linked recessive.

Muscular dystrophy typically develops when muscular fibers cannot withstand mechanical stress. As a result, the muscle fiber's sarcolema ruptures, allowing calcium to enter the muscle cell uncontrollably. When regeneration first starts, degeneration's damage is reduced, but this is insufficient, and as time goes on, muscle cells become unable to endure damage and are eliminated by necrosis or apoptosis. Later, connective tissue and fat cells replace these muscle cells [10].

Dystrophin is a protein that is integrated into a complex of proteins located on the muscular plasma membrane's inner surface, forming what is known as the Dystrophin-Associated Protein Complex (DAPC). This complex includes not only dystrophin but also syntrophin, dystroglycans, sarcospan, and dystrobrevins. Its primary function is to play a vital role in preserving the muscle cell integrity of the outer membrane, the sarcolemma. This complex plays a vital role in numerous signaling pathways and serves as a scaffold for channel proteins. Dystroglycan functions as a receptor on the muscle cell, participating in various pathways, including MAPK signaling and the activation of the Rac1 small GTPase, and it serves as a binding site for various extracellular proteins. Conversely, Syntrophin is essential for regulating the activity of ion channel proteins. It can bind to dystrophin and dystrobrevin within the DAPC, with up to four syntrophin molecules transmitting multiple signals, including those related to ion channels and calcium pumps [11]. A vital cytolinker known as dystrophin is said to link transmembrane complexes to actin filaments, intermediate filaments, and microtubules in order to stabilize cells [12]. Mutations in dystrophin or sarcoglycans result in the

destabilization of the sarcolemma. The absence of dystrophin leads to a compromised Dystrophin-Glycoprotein Complex (DGC), and the mechanical strain from muscle contractions contributes to the deterioration of the sarcolemma and the necrosis of muscle fibers. Despite the fact that mechanical damage and sarcolemmal anomalies are crucial components of the dystrophic phenotype, neither of these mechanisms can fully explain how this disease progresses [3].

In muscular dystrophy, pathogenic cascades are aggravated by the abnormal activation of multiple signaling pathways. Research has demonstrated that loss of DGC proteins causes the activation of a number of pathogenic cascades that accelerate the course of disease [13]. Disruption of it can lead to the abnormal activation of numerous signaling pathways [14]. It is intriguing that several pathways linked to the onset of muscular dystrophy are active even before muscle cell necrosis initiates. This suggests that the absence of a functional Dystrophin-Glycoprotein Complex (DGC) disrupts normal physiological signaling in striated muscle [15]. The pattern of the DGC-lacking dystrophic disease and the muscular dystrophy brought on by transient receptor potential canonical 3 (TRPC3) overexpression and calcium influx increase were nearly identical [3]. Numerous common causes, including influx of calcium, inflammatory immune cell infiltration of muscle tissue, accumulation of profibrogenic cytokines and pro-inflammatory, activation of different enzymes that breakdown protein, the autophagous defects, and occasionally the apoptosis, are involved in cause and progression of muscular dystrophy [3].

The inflammatory response that occurs after tissue damage has a significant impact on proliferation and differentiation of muscle cells, with different regenerative stages being supported its pro-inflammatory or anti-inflammatory stages [16]. Regeneration of muscle tissue is closely regulated by a number of inflammatory signals from various immunocytes. As muscle begins to degrade, an accumulation of IL-17A+ T cells induces inflammation and speeds up tissue healing [17]. There is a possibility that an important portion of the pro-reparative function of IL- 17A+ T cells in muscle is mediated by increased accumulation of neutrophil following injury. Neutrophils are the first cells that enter injured muscle, and they are essential in removing necrotic muscle fibers, which is an essential step in activating

the repair mechanism [18].

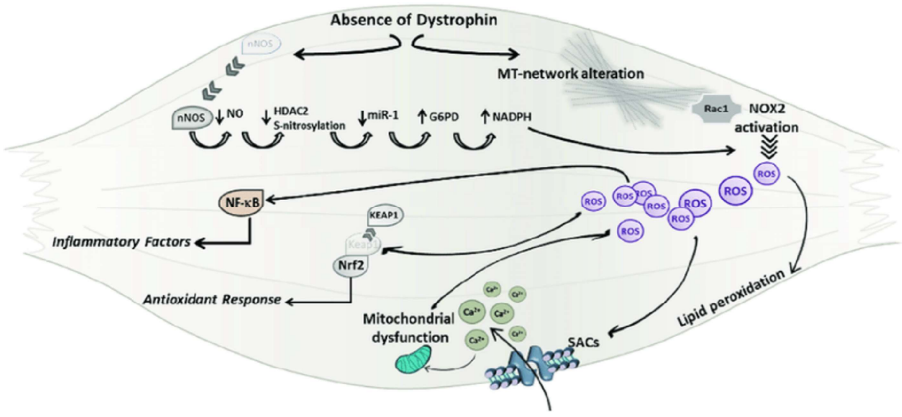


FIGURE 1.1: Mechanisms involved in muscular dystrophy [16]

As most prevalent type of Muscular Dystrophy is DMD worldwide so, most of the treatment therapies are focused on this type of muscular dystrophy. Major treatment therapies include pharmacological treatment, CRISPR-based gene editing, physical, and microdystrophin therapy, exon skipping therapy, cell-based therapy, and utrophin up regulation and stop codon read-through therapy [19].

Pharmacological treatment involves the administration of the corticosteroid prednisone, an anti-inflammatory medication known for its ability to enhance muscle strength. However, this drug is associated with various side effects. As an alternative, Deflazacort is often prescribed due to its milder side effects, including reduced risk of weight gain, immunosuppression, and osteoporosis. Another pharmacological option is the antibiotic Gentamicin, which has shown promise in improving muscle function by inhibiting the premature synthesis of a protein located in the N-terminal binding region of dystroglycan [20]. Restoring dystrophin production through exon deletion of the DMD gene and genome editing with the CRISPR/Cas9 technology leads to a functional recovery of muscle strength [21]. Novel formulations are under development, with the aim of transforming them into oligonucleotides through the utilization of viral vectors. These viral vectors, including adeno-associated viruses, are employed for gene delivery. But this method poses challenges for treatment of DMD due to very large size of its gene. Various cell-based methods are also considered for the delivery of full gene by injecting

stem cell, skeletal myoblast injection into muscle tissue. Alternative cell-based approaches are also being explored for delivering the complete gene, involving the injection of stem cells or skeletal myoblasts directly into muscle tissue. Unfortunately, the success rate is notably limited, as these cells tend to migrate away from the injection site, and their overall survival rate remains quite low [22]. Additionally, there are efforts underway to develop small molecule drugs aimed at inhibiting the generation of premature stop codons caused by nonsense mutations. This intervention helps to avert the premature termination of translation and subsequently leads to the restoration of dystrophin [23, 24]. As a substitute for dystrophin deficiency, upregulating endogenous utrophin offers tremendous promise for the therapy of DMD without posing any immunogenic risks [25].

Previous research has supported the hypothesis that the microbiota controls myocyte metabolic activity, which in turn controls muscle mass. The pro-regenerative effects of the microbiota may have been mediated by IL-17A+ T, which elevated early inflammation [26]. Through an IL-17A-dependent pathway influenced by the microbiota, IL-17A+$\gamma\delta$ T cells contributed to the initial triggering of inflammation and eventual tissue regeneration. These findings suggest potential new therapeutic targets for muscle regeneration and repair [27].

Myopathies encompass a varied range of infrequent skeletal muscle disorders, frequently associated with unfavorable prognoses. Duchenne Muscular Dystrophy (DMD) distinguishes itself as the most widespread and severe within this category 1 in 3,500 male births globally affecting approximately. [28]. Dystrophin-associated glycoprotein complex (DAPC) formation is often highlighted in Duchenne muscular dystrophy (DMD), typically arising from the deletion of one or more exons in the dystrophin gene. This complex, comprising specific proteins such as dystrophin, are essential to preserving the integrity of the structure and function of both the heart and skeletal muscles. [29]. The permanent tissue degeneration caused by the breakdown of muscle architecture is triggered by the disruption of dystrophin expression and function. This deteriorating condition is further compounded by sustained compromised autophagy, inflammation, fibrosis, and

tissue necrosis [30, 21]. Despite ongoing experimental advancements in therapies for Duchenne muscular dystrophy (DMD), a definitive cure remains elusive [32]. Consequently, the cornerstones of palliative treatment continue to be corticosteroids, more especially prednisolone (PRED) and deflazacort (DFZ). Demonstrating their efficacy in preserving functional abilities, both agents have shown positive outcomes in various models of mouse of DMD and controlled trials that are randomized. [33, 34].Yet, questions linger regarding the enduring advantages and the security of certain medical procedures. Findings from controlled trials that are randomized indicate that both deflazacort (DFZ) and prednisolone (PRED) may induce side effects such as sudden increase in weight, cognitive disorientation, feelings of despondency, issues related to growth, and the development of cataracts. [35, 36].

According to reports, the microbiome may also play a role in autoimmune disorders, just as differences in the gut microbiome may put up to the development of certain diseases [37]. Given these observations, it's reasonable to speculate that changes in the microbiome of dystrophic patients could potentially contribute to muscle damage by influencing local inflammation [38].

The microbiome present in an individual's gut is believed to be established from the surrounding environment at the time of birth [39, 40]. As a result, the microbiome can function both as a pre-established factor shaped by and interacting with the host, and as an environmental element that impacts the host's genetics to shape its phenotype. The potential to manipulate the microbiome for therapeutic objectives makes it an attractive candidate for modification. By gaining a more profound comprehension of the connections between the gut microbiome and the host genome, it may be possible to customize microbiome alterations to suit specific host genomes, thereby reducing the risk of illness [41].

1.1 Problem Statement

Host genetics and the gut microbiome can both influence metabolic phenotypes. However, whether variations in the genetic of host influences the gut microbiome

or gut microbiome impact the host phenotype is unclear. Determining whether host genetics simultaneously associates with differential microbial abundance and disease risk/progression is a central challenge and its solution has substantive potential for personalized diagnosis and/or treatments for disease.

1.2 Aim

The purpose of this study is to explore the relationship between disease (muscular dystrophy) and changes in the human microbiome.

1.3 Objectives

- To determine the prevalence of types of muscular dystrophy in local population.
- To evaluate the impact of food on disease and symptoms
- To identify the association between disease and changes of microbiome of muscular dystrophy and healthy individuals.

1.4 Scope of Study

Along with external elements lifestyle, host genetics and nutrition affect the composition of gut microbiome. Muscular dystrophy is a hereditary condition, and genome- wide studies of microbiome have the potential to uncover additional host genetic variations that influence the course of the disease via altering the microbiome's makeup and help devise better management studies.

1.5 Impact on Society

This study can provide future reference to devise better management strategies for muscular dystrophy by employing microbiome.

Chapter 2

Literature Review

2.1 Muscular Dystrophy

Muscular Dystrophies are a collection of inherited degenerative disease marked by the gradual onset of muscle weakness. They can be inherited in several ways, including autosomal dominant or recessive patterns, and they may also be linked to the X chromosome. In some instances, these conditions can arise from de novo mutations. Typically, cases with an early onset during childhood tend to exhibit a more severe phenotype. Traditionally, clinical and pathological characteristics have been the primary criteria for diagnosing muscular dystrophies. However, in recent times, the classification of most muscular dystrophies is based on molecular genetic confirmation [9].

2.2 Types of Molecular Dystrophy

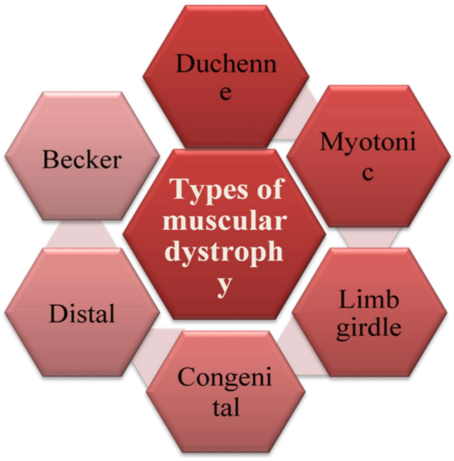


FIGURE 2.1: Types of Molecular Dystrophy [42]

2.2.1 Duchenne and Becker Muscular Dystrophies

The most prevalent and severe subtype of dystrophin - associated muscular dystrophies is Duchenne Muscular Dystrophy. It affects approximately one in 3500 – 5000 live male births. This condition is inherited in an X - linked recessive manner and is notably more severe than the milder Becker muscular dystrophy (BMD) [4].

The major cause of Duchenne and Becker muscular dystrophy is mutations in the human gene, Dystrophin, which is situated at Xp21 zone of X chromosome and encompasses about 2.4 Mb of genomic material. This gene has 79 exons encrypting mRNA of 14-Kb producing a 427-Kd membrane protein called Dystrophin [43]. DMD/BMD is clinically linked with progressive muscular weakness and atrophy that is irreversible, mainly affecting the skeletal muscles and the cardiac muscles. Diagnosis of individuals with DMD is done usually by the first 5 years of life. Prior to turning 13 years old, the patients are wheelchair dependent. By 20 years of age, the patients usually pass due to either respiratory or heart failure. Patients of BMD have a manifestation that is comparatively milder and show a later-onset of weakness of the skeletal muscle. BMD patients have the capability of independently walking before 16 years of age or later. Their life expectancy is also normal [44]. The major clinical differences that are seen between DMD and BMD are due to different mutations occuring in Dystrophin gene. In some individuals, point mutations are shown that are predominantly nonsense and/or frame-shift mutations, which are around 10–30%, 5–15% are by duplications, and intronic or 3'- and 5'-UTR alterations are about 2% [45].

Either these mutations are inherited from female carriers that are asymptomatic, which accounts for around ∼70% of cases, or they are de novo, accounting for ∼30% cases [43]. A non-functional protein is produced when a shift in the ORF, the open reading frame, results from the mutation, or a stop codon that is premature is generated by the mutation, causing the patient to develop DMD. Comparatively, production of a protein that is partially functional occurs upon the maintenance of the 10 reading frame, i.e., in-frame mutation, leading to the patients showing clinical symptoms that are milder, associated with BMD [46]. However,

the extent as well as the location of in-frame mutations has been observed to have an impact on the severity of the disease in Becker patients. The mutations causing disruption of the domain that binds actin and or the disruption of binding domain for beta-dystroglycan cause the production of Dystrophin that is non-functional, leading to the individual developing the phenotype of DMD. Furthermore, a large obliteration occuring in the domain that is in the centre (>36 exons) generally advances to a phenotype of Duchenne muscular dystrophy [47].

2.2.2 Myotonic Dystrophy

Dystrophia Myotonica is another name of Myotonic Dystrophy, is a progressive neuromuscular disorder. DM type 1 arises from an expansion of CTG triplet repeats in the DMPK gene, while the second type of DM results from an growth of CCTG tetramer repeats in the CNBP gene. Key symptoms include slow muscle relaxation (myotonia), weakness of muscles, and early cataracts. Affected people frequently also notice alterations in their cognitive functioning and cardiac arrhythmias. Genetic testing reveals that type 1 DM (DM1) has an enlarged CTG repeat in the 3' untranslated region of DM Protein Kinase (DMPK). Genetic analysis of people with DM type 2 (DM2) reveals that the cellular nucleic acid binding protein's intron (CNBP) has an enlarged CCTG repeat [29].

Upon the increase of cellular mass of CUGexp or CCUGexp RNA greater than the threshold that causes disease, the resulting titration of MBNL proteins has an impact on polyadenylation (polyA), differential splicing or the genomic expression for hundreds of 11 genes. Similarly, the CUGexp RNA expression too has an effect on cell signaling, leading to subsequent impacts on RNA processing and muscle metabolism [48].

The RNA repeats that are expanded are recognized occasionally by translational machinery in a way which leads to initiation of translation within the repeat tract, in spite of the canonical start codon absence [49]. The repeat related nonATG

(RAN) translation phenomenon causes the formation of peptides that are neurotoxic in nature. In DM biology, two major points are significant in the therapeutic context. In these, the first point is that there is retention of toxic RNAs in nuclei, forming nuclear foci. Development of foci is possibly nucleated by CUGexp/CCUGexp –MBNL interrelationship, by a mechanism dependent on signaling pathways as well as other factors. The molecular crowding in foci results in the production of an elevated concentration of binding sites of MBNL. In protein excess situations, recruitment of a MBNL fraction occurs into foci, but it can be exchanged with free MBNL that is present in the nucleoplasm, retaining activity. Both of the conditions, i.e., the protein excess and excess of RNA, might correspond more or less to the pre- symptomatic and later stages of DM1, in which the transitioning from one to the other is dependent on the CUGexp tract length. Noticeably, concentration of the latter is unfixed as extended CTG repeats growth increases in somatic cells as time progresses. There is comparatively very minor splicing dysregulation in initial stages of DM1 [50].

TABLE 2.1: Types of muscular dystrophy, cause and disease symptoms [43]

Type of Dystrophy	**Age of Onset**	**Muscles Affected**	**Protein Affected**	**Disease Symptoms**
Duchenne (DMD)	Childhood	Heart, legs, hips,	shoulders muscles	Dystrophin & Severe muscle atrophy and weakness, pneumonia, cardiomyopathy, contractures, respiratory failure, and scoliosis. Early in the 20s, death.
Becker (BMD)	Adolescence/ adulthood	Muscles in the shoulders, hips, legs, back,	heart	Dystrophin & Similar to DMD in terms of muscle weakening, but with a significantly slower rate of progression, and Heart disease.
Limb-girdle (LGMD)	Adolescence to early adulthood	Pelvic girdle	shoulder musculature	Myotilin γ- α- β- sarcoglycan & Within 20 years of the outset, walking could not be possible. cardiac issues, which can occasionally arise later
Emery-Dreifuss (EDMD)	Childhood to early teenage	Distal lower extremities	proximal upper extremities	Emerin, lamin & Early cardiomyopathy with contractures
Oculopharyngeal (OPMD)	Age 40–60 years	Eyelids, throat	Poly- Abinding protein 2	Aspiration pneumonia, dysphagia, and ptosis of the eyelids

TABLE 2.1: Types of muscular dystrophy, cause and disease symptoms [43]

Type of Dystrophy	**Age of Onset**	**Muscles Affected**	**Protein Affected**	**Disease Symptoms**
Distal muscular dystrophies	Late adolescence	Legs' anterior	posterior compartments	Dysferlin Titin & Gradually increasing, Can eventually impact the heart, lower limbs, and upper extremities. May eventually have an impact on the distal arm muscles and anterior compartment. Gradual advancement and lifelong capacity for autonomous walking.
Congenital muscular dystrophies (CMD)	At birth	Face, neck, distal lower limbs,	proximal upper limbs	Laminin α2 (merosin) FKRP LARGE Fukutin & Seizures, joint contractures, issues with speech and cognition. Most never pick up walking. Changes in white matter and anomalous structures
Facioscapulohumeral (FSHD)	Childhood to early adolescence	Shoulders, upper proximal extremities	Not identified	Anomalies of the retina, moderate hearing loss, and cardiac conduction issues

However, its severity increases in later stages, and it resembles mice that have collective loss of both MBLN1 and MBNL2 [50]. There are significant therapeutic implications to this approach, if substantially correct.

(1) Agents that act preferentially in the nucleus, antisense oligonucleotides, for instance, might show a greater impact compared to the ones that act predominantly in the cytoplasm, like siRNAs.

(2) The stabilization of expansions of CTG might prevent presentation of symptoms of DM.

(3). Foci do not consist of intractable clumps of denatured substance. Instead, These are dynamic composition that can be spread and are accessible to medications.

(4) even a moderate MBNL release, either by a decrease in the toxic RNA levels, or by CUGexp-interactions blockage, might result in the production of disproportionate 12 reversal of defects in processing of RNA, potentially improving the symptoms of the disease.

(5), The greatest affinity poly (CUG) binding protein titration of MBNL proteins, may be the primary factor in the early stages of DM1, however in the later stages, the exposure of CUGexp binding sites that are vacant may lead to a much more extensive pathological cascade. This can be addressed by reverting back to the MBNL excess criteria [51].

In the case of DM1, there is a reduction in the basal DM kinase protein expression by half because of the mutated DMPK (mutDMPK) mRNA nuclear retention. It was indicated in the initial reports that deletion of DMPK in mice leads to defects in cardiac conduction in heterozygotes as well as skeletal muscle pathology in homozygotes. Another recently done research, however, found no effect of either knockdown or knockout of DMPK on survival, development, and contraction of the muscles, function of the ventricle or cardiac conduction in rats [52]. Additionally, in monkeys, knockdown of DMPK was tolerated well [53].

2.2.3 Limb-girdle Muscular Dystrophies

Limble-Girdle Muscular Dystrophy encompasses a diverse class of Mendelian disorders characterized by progressive deterioration of the proximal limb muscles. Typically, these conditions also impact other muscle groups, including the respiratory and cardiac muscles. The presentation and clinical advancement of these conditions can exhibit considerable variation, spanning from severe forms characterized by swift onset and progression to milder variants that allow affected individuals to maintain relatively regular lives with typical levels of activity and life expectancy [54]. LGMD has evolved into a descriptive phrase that now includes the clinical images of many other diseases. The earlier definition stated that autosomal-recessive muscular dystrophies—muscular dystrophies that are milder than DMD—were inherited [55]. The development of next generation sequencing

(NGS) techniques has sped up the finding of new LGMD genes. Only 16 loci were on the list ten years ago [56].

2.2.4 Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy ranks as one of the most prevalent hereditary muscular diseases, with an incidence rate of approximately 1 in 8000 individuals [57]. The primary features used to characterize this condition include progressive muscle wasting, as well as atrophy affecting the shoulder, limb-girdle, and facial muscles in an asymmetrical manner. Additionally, individuals with this disease may also exhibit muscular atrophy in the anterior leg and weakness in the abdominal muscles [58]. The inheritance of this disease is as a trait that is autosomal dominant. Its association has been found with a repeat contraction in the D4Z4 gene locus on chromosome 4q35 as well as specific allele configuration [59].

Several features of FSHD strongly suggest the involvement of epigenetic factors. These characteristics include varied intensity and pace of progression of disease, asymmetric muscular atrophy, and gender bias in penetrance, and monozygotic twin discordance [60].

2.2.5 Congenital Muscular Dystrophies

Congenital Muscular Dystrophies, abbreviated as CMDs, represent a rare class of muscular dystrophy disorders. They are characterized by an early onset of muscular weakness, typically appearing within the first year of life. This condition is marked by severe neonatal hypotonia and distinct histological features associated with dystrophic lesions. Muscle biopsies conducted on CMD patient's exhibit significant variability, but they consistently display the dystrophic lesions' distinctive pattern. The histological variations may potentially be connected to various phases of the illness, demonstrate the seriousness of the condition, or even involve

a combination of both factors [61]. CMDs prevalence is not known well, and currently it is maintained roughly in the 1/100,000 individuals' range. This group involves not only muscles, but also other structures of the body such as the eyes, heart, and the brain. Difficulty is faced in diagnosis, and establishment of the appropriate time for performance of a muscular biopsy is not easy. However, there is considerable significance of muscular biopsy for the indication of the molecular/-genetic analysis that is correct. For any of these forms, the disease course is severe usually. The life of patients is prolonged by respiratory and nutritional support, 14 but early fatal outcome is not unexpected. Clinical diagnosis performance, as well as molecular diagnosis is of utmost importance for prognosis, genetic counselling, and anticipatory treatment as well as for prospective treatment. Currently, no treatment that is pharmacological exists for the CMDs [60].

For now, the treatment is done for improvement of the course of the disease, with prevention or treatment of pulmonary and cardiac impairment. Physiotherapy treatment is advisable for the prevention of deformity of joints, muscles retractions, and scoliosis. In case of respiratory distress, supportive treatment with respiratory support that is noninvasive is provided. Gastro-esophageal reflux correction, cardiac failure support, respiratory infection treatment, and nutritional treatment with new modes must be carried out constantly. A wider and better survival has been allowed to these patients with this treatment strategy [62].

2.2.6 Distal Muscular Dystrophies

A significant category of inheritable muscular disorders falls under the umbrella of distal muscular dystrophy. These disorders are characterized by increasing weakness and muscular atrophy, particularly affecting the hands and/or feet. Distal muscular dystrophies are classified based on several criteria, including: i) Clinical characteristics, such as onset timing: early or late and whether they primarily affect the hands or feet. ii) Evidence of involvement of the anterior or posterior compartments of the legs, as determined by Magnetic Resonance Imaging (MRI).iii) the pattern of inheritance. iv) Histopathological findings. Recent research has led to the discovery of the corresponding genes accountable for several

types of distal muscular dystrophies. Interestingly, numerous mutated genes linked to distal muscular dystrophies produce proteins involved in the Z-disk and contractile machinery, in contrast to genes found in proximal muscular dystrophies, which often encode sarcolemma proteins [63].

2.2.7 Oculopharyngeal Muscular Dystrophy

A pathological cue in many neurodegenerative diseases is protein aggregation. It is seen in a variety of disorders, including amyotrophic lateral sclerosis, Huntington's disease and Parkinson's. One distinguishing aspect of the aforementioned disorders is the formation of aggregates by the accumulating of disorderly processed and misfolded proteins that lose their physiological roles partly [64].

OPMD, that is the Oculopharyngeal muscular dystrophy, is among these proteinopathies. In this type, the mutated protein is accumulated as combined in the nuclei that are diseased. This disorder is an autosomal dominant muscular dystrophy that is rare. Its onset is later in life, usually in the late fifth decade. OPMD is associated with gradual deterioration of a particular muscle with time, eventually causing droopiness of eyelids, known as ptosis, dysphagia which is difficulty in swallowing, and proximal limb weakness. Brief repeat expansions of GCN in gene that codes for poly (A) binding protein nuclear 1 (PABPN1) leads to OPMD. A major pathological marker of the disease is nuclear aggregates formed by alanine-expanded PABPN1 in muscle fibres [65].

2.2.8 Emery-Dreifuss Muscular Dystrophy

Emery-Dreifuss Dystrophy (EDMD) is uncommon form of muscular dystrophy, and its diagnosis is critically important due to its potentially life-threatening cardiac complications. EDMD typically presents with a combination of cardiomyopathy, muscle weakness, abnormalities in cardiac conduction, and early contractures. The manifestation and severity of these symptoms can vary depending on the specific subtype of the disease. Several genes are associated with EDMD, including

LMNA, EMD, SYNE2, SYNE1, TMEM43, FHL1, SUN2, SUN1, and TTN, which encode proteins like lamin A/C, emerin, nesprin-1,nesprin-2, titin, SUN1, SUN2 FHL1, and LUMA, respectively. The clinical presentation of EDMD is distinctive, and it typically results from functional or structural defects in one or more proteins that make up the nuclear envelope, a condition known as "nuclear envelopathy." These disease mechanisms may lead to disruptions in the importation of proteins into the nucleus. The nuclear lamina, as well as the outer and inner nuclear membranes, collectively form the nuclear envelope, which serves as the structural framework of the nucleus [66].

If any of the proteins that are involved in providing the framework are affected by any deficiency or mutation, it can lead to loss of structural stability of the nucleus. For the tissues that are under stress frequently, this situation can be particularly problematic, such as the skeletal and heart muscle. The proteins involved are LMNA, emerin, nesprin-1, nesprin-2, SUN1, LUMA, and SUN2 encrypted by LMNA, EMD, SYNE1, SYNE2, SUN1, TMEM43, and SUN2 genes, respectively. Diagnosis of EDMD is tough because due to its rarity and the fact that it has phenotypic overlap with other muscular dystrophy forms, namely the LGMD and congenital muscular dystrophy. Clinical symptoms include weakness in the neck extensor, a cardiomyopathy, and classic pattern of contractures, tachyarrhythmias and bradyarrhythmias. In the case of EDMD, there are no therapies that are disease modifying available as of yet. Due to this reason, there is a need of management that comprises of treating symptoms and clinical monitoring. Substantial publications regarding the benefits of exercise, coenzyme Q10 or creatine are absent in Emery-Dreifuss muscular dystrophy. Patient monitoring should be held either in a clinic that is multidisciplinary or in a setting where coordination among different specialists as well as communication is easy, due to the complex multi-organ system complications observed in this disease [67].

Investigational therapies of EDMD were held at the laminopathy subtype (EDMD2), mainly because of the large number of patients being in this subtype, but in addition to this, it is because of the robust mouse model that is available for laminopathy. Comparatively, for emerin deficiency, a mouse model that had been developed

has a phenotype that is more subtle. Anti-thromboembolic prophylaxis, heart transplantation, respiratory management, cardiac pharmacotherapy has been an option in EDMD for quite a while. It was shown in a new research that at least in the case of cell culture, the skipping of exon 5 of LMNA is mediated by antisense oligonucleotide and this can be used successfully. Therefore, among the patients that have dominant mutations of this 17 exon, it is a promising therapeutic approach. Other strategies of molecular biology that can play a role in EDMD, like AAV-based, i.e., the adeno-associated virus, replacement of genes and techniques of gene-editing, are not yet studied formally in the case of this disease [68].

2.3 Genetic Causes

Muscular dystrophy is a medical condition wherein muscles are unable to withstand physical stress, exhibiting characteristics such as dysregulated calcium signaling, muscle disruption, and damage to the muscle plasma membrane. As muscle fibers deteriorate, they undergo a regenerative process to generate new muscle tissue. However, when degeneration surpasses the capacity for regeneration, it leads to cell necrosis or apoptosis. Then fat cells and connective tissue replaces the degenerated tissue [10].

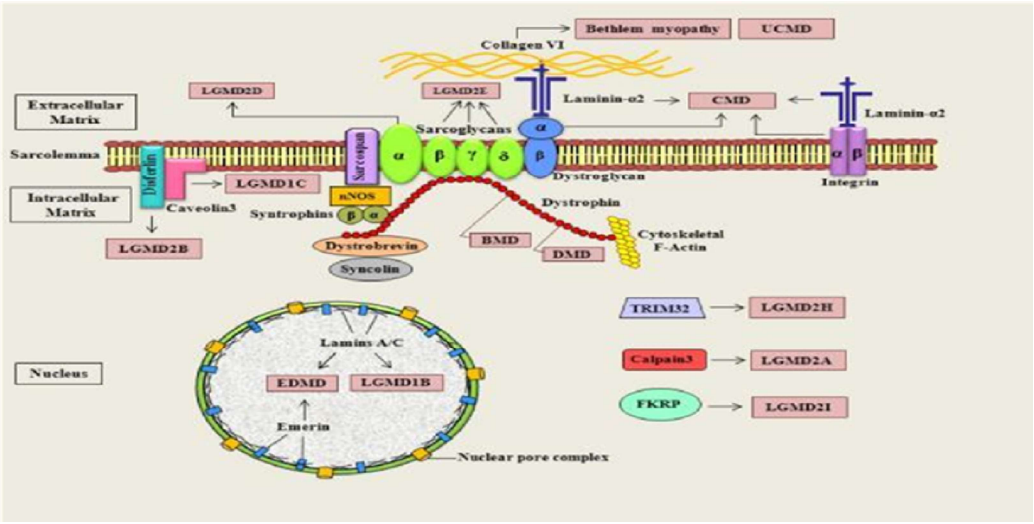


FIGURE 2.2: Genetic causes of muscular dystrophy [41]

A genetic condition, muscular dystrophy is inheritable and has been linked to 350 various missense, nonsense, splice site, and deletion mutations that are dispersed throughout the 65 exon coding sequence [8]. Mutations and variations in expressions of various genes are involved in various forms of muscular dystrophy [53].

2.4 Pathophysiology

Following major mechanisms are involved in the progression of muscular dystrophy:

2.4.1 Dystrophin Associated Protein Complex and Muscular Dystrophy

Dystrophin, a protein is found incorporated in protein complex on inner surface of muscle plasma membrane forming Dystrophin associated protein complex (DAPC).Alongside dystrophin, this complex includes syntrophin, dystroglycans, sarcospan, and dystrobrevins. The primary function of this protein complex is going to involved critical role in maintaining the stability of the sarcolemma, the outer membrane of muscle cells. Moreover, this complex is vital in various signaling pathways and serves as a scaffold for channel proteins. Dystroglycan, in particular, can be regarded as a receptor present on muscle cells that participates in a range of pathways, including those involving the MAPK signaling and Rac1 small GTPase. Moreover, it acts as a binding location for numerous extracellular proteins. In contrast, syntrophin has a vital function in controlling the activity of ion channel proteins. Up to four syntrophin molecules can bind to both dystrophin and dystrobrevin within the DAPC, facilitating the transmission of multiple signals, including those related to ion channels and calcium pumps [11]. Dystrophin is reported as a crucial cytolinker that functions to stabilize cells by connecting transmembrane complexes to actin filaments, intermediate filaments, and microtubules [12]. Sarcolemma instability is caused due to mutations in dystrophin or sarcoglycans. The functionality of DGC is compromised due to deficiency of dystrophin, and the mechanical stress brought on by muscular contraction causes

degradation of sarcolemma and necrosis of muscle fibers. Although sarcolemmal abnormalities and mechanical damage are essential factors that are involved in dystrophic phenotype, but both of them cannot explain the progression of this disease [3]. In muscular dystrophy, pathogenic cascades are aggravated by the abnormal activation of multiple signaling pathways. Research has demonstrated that loss of DGC proteins causes the activation of a number of pathogenic cascades that accelerate the course of disease [13]. And its disruption can cause abnormal activation of many signaling pathways [14]. Interestingly several pathways associated with the development of muscular dystrophy are active prior to the beginning of muscle cell necrosis, indicating that the absence of a functional DGC impairs physiological signaling in striated muscle [15]. The pattern of muscular dystrophy caused by transient receptor potential canonical 3 (TRP3) overexpression and the influx of calcium raise was almost identical to the DGC-lacking dystrophic illness [3]. It has also been discovered that aberrant myogenic signaling is present in other Muscular Dystrophy that arise from the loss of nuclear membrane proteins (e.g., lamins A/C, Emerin) or enzymes of cytoplasm (e.g., Calpin-3). These findings suggest that signaling defects are a common pathological cause in all types of Muscular Dystrophy [15]. Numerous common causes, including influx of calcium, inflammatory immune cell infiltration of muscle tissue, accumulation of pro-inflammatory and profibrogenic cytokines, activation of different proteolytic enzymes, autophagy defects, and occasionally apoptosis, are involved in cause and progression of muscular dystrophy [3].

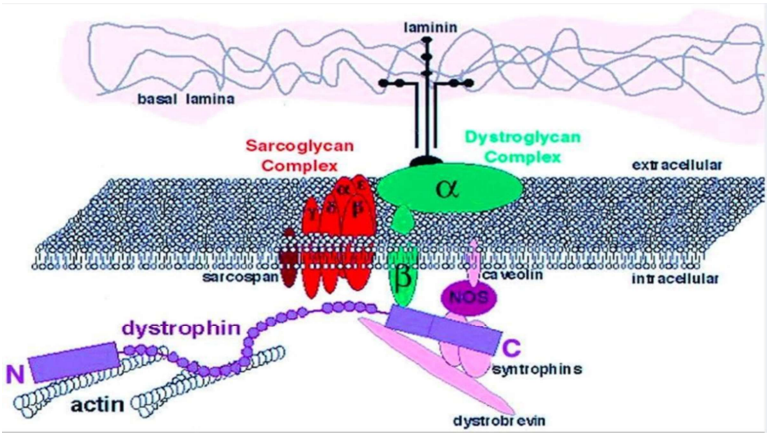


FIGURE 2.3: Dystrophin associated protein complex [69]

2.4.2 Calcium Influx and Membrane Instability

Sarcolemmal instability is one of the essential early processes in muscular dystrophy that leads to the degenerative characteristics found in the later stages of disease progression. It has been suggested that a major contributing element to the skeletal muscle degeneration in muscular dystrophy is compromised calcium (Ca2+) homeostasis [70]. The connection between destabilization of the sarcolemma and calcium homeostasis disturbance in muscles lacking dystrophin is additionally indicated by the discovery that an excess of dystrophin protein was enough to avert the rise in intracellular Ca2+ levels. A relationship between sarcolemmal destabilisation and impairment of homeostasis of calcium in muscles inadequate in dystrophin is further suggested by the finding that the dystrophin protein's overexpression was enough to stop the increase in intracellular Ca2+ levels [71]. Major features of muscular atrophy like degeneration, myofibers and regeneration and fibrosis are induced by overexpressing TRPC3, together with a rise in Ca2+ concentration [3].

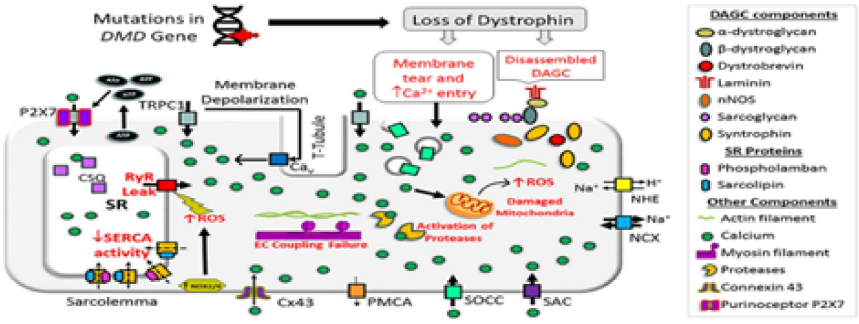


FIGURE 2.4: Mechanism of Ca2+ excess in a muscle cell lacking dystrophin [72]

One of the key ways that a high Ca2+ level contributes to muscular dystrophy is by activating calcium-dependent proteases like calpains, which ultimately induce the breakdown of proteins in muscle cells and necrosis [73]. At the age of four weeks, it results in the reduction of endogenous calpains activity and significantly reduced muscle necrosis. This implies that calpains are involved in fiber necrosis in dystrophic muscle and that their inhibition can ameliorate dystrophinopathy. [74].

2.4.3 Muscle Wasting by Immune Cell Infiltration

Studies have shown that the diaphragm and leg muscles, which experienced damage, exhibited a more gene expression associated with response of inflammation compared to the unaffected extraocular muscles [75]. Many inflammatory chemicals, such as TNF-, IL-1, and IL-6, as well as molecules that are adhere to cells, such intracellular adhesion molecule-1, are expressed more frequently in dystrophic myofibers [14]. Indeed, immune cell infiltration, which commences prior to the beginning of the primary disease and persists throughout subsequent phases, is now acknowledged as a unique in response that directly fosters inflammation and participate to the initiation and advancement of muscle lesions [76]. The main types of immune cells that enter muscle cells are neutrophils, T-cells, and macrophages [76]. Previous research has shown that systemic treatment of a monoclonal antibody dramatically reduces the number of injured fibers in mdx mice [77]. Nitric oxide produced by activated macrophages causes muscle cells to be destroyed (NO). Proinflammatory cytokines, which promote wasting of muscles by activating proteolytic systems and preventing synthesis of muscles, are likewise primarily produced by macrophages [76]. Moreover, macrophages have the ability to phagocytose damaged myofibers and subsequently present T-cells with antigens to activate them. In fact, the presence of T-lymphocytes, mast cells, eosinophils, and neutrophils in dystrophic muscle also contributes to fiber wasting [78].

2.4.4 ROS, RNS and Muscular Dystrophy

Superoxide anion, H2O2 (hydrogen peroxide), or NO can be produced as a result of excessive oxidative stress. Secondary ROS and RNS at high quantities can harm DNA, structural proteins and regulatory proteins, and lipids in membranes. There have been reports of higher lipid and protein oxidation levels in the dystrophic muscle of DMD patients [79]. NF-B is necessary for cell survival, yet data suggests that activating NF-B results in the loss of skeletal muscle. In both physiological and pathological situations, active NF-B is thought to cause

muscle atrophy through the following three mechanisms: (a) Many proteins expression in the ubiquitin-proteasome system, such as E3 ubiquitin ligase MuRF1, is increased by NF-B, which enhances the damage of skeletal muscle; (b) NF-B also elevates the numerous expression of pro-inflammatory cytokines, inflammatory cytokines, adhesion molecules for cells, and matrix-degrading enzymes, which aggravate skeletal loss of muscle mass; (c) Finally, active NF-B can prevent myofibers from regenerating after injury [80].

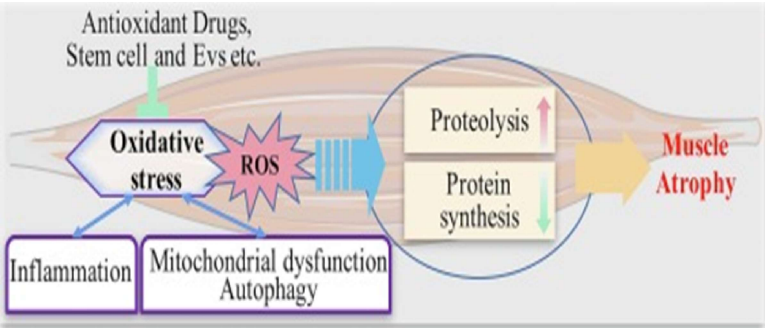


FIGURE 2.5: The function of oxidative stress in the dystrophy of skeletal muscle [81]

2.5 Diagnosis

With the rise of innovative technologies, there is an increasing utilization of genetic counseling and prenatal diagnosis. These advancements enable the detection of nearly all fetal muscular dystrophies before delivery. Amniocentesis and chorionic villus sampling are two techniques used in prenatal screening [82–84]. Furthermore, employing cell-free fetal DNA fragments discovered in the maternal plasma, relative haplotype dosage (RHDO) analysis of the plasma is utilized to sequence the fetal genome [84]. Applying preimplantation genetics involves removing a blastomere following in vitro fertilization, then collecting and analyzing DNA [85].

2.5.1 Muscle Biopsy

An additional frequently used approach for diagnosing Duchenne muscular dystrophy (DMD) involves conducting a muscle biopsy to examine the histology of

skeletal muscles. This biopsy, along with dystrophin analysis, proves valuable in confirming a DMD diagnosis, particularly in cases where genetic testing yields negative results or reveals a genetic variant of unknown significance [87]. Histological findings common to all dystrophies include irregularities in muscle fiber size, necrosis, presence of tissue macrophages, and the degree of muscle mass substitution with connective and adipose tissue. These characteristics change based on the disease's stage. [88]. Conducting an open muscle biopsy is essential to ensure a sufficient amount of muscle tissue for identifying the specific type of muscular dystrophy. Nevertheless, if the differential diagnosis is limited to Duchenne muscular dystrophy (DMD) alone, a needle biopsy may be considered appropriate [89]. After acquiring the muscle sample, the primary analysis for Duchenne muscular dystrophy (DMD) involves conducting immunocytochemistry and immunoblotting to assess the presence of the dystrophin protein [90]. It is essential to make a clear differentiation between the dystrophin protein's whole and partial absence to definitively identify Duchenne muscular dystrophy (DMD) from other dystrophinopathy phenotypes. Individuals with DMD typically exhibit no dystrophin on a western blot (or minimal presence), whereas those with Becker muscular dystrophy (BMD) may show diminished dystrophin that is smaller (80%), of normal size (15%), or larger (five percent) [91]. For individuals with Duchenne muscular dystrophy (DMD), a diagnostic criterion is the discovery of less than five percent of normal dystrophin [92, 93]. In the case of Becker muscular dystrophy (BMD), a diagnostic indicator is the identification of dystrophin levels ranging from 20 to 100% of the normal range. Following a positive muscle biopsy diagnosis of DMD, genetic testing is necessary [92, 93]

Certain healthcare providers and patients might consider bypassing the muscle biopsy test and opting for ongoing clinical exploration through genetic testing, given the biopsy's invasive and uncomfortable nature. If testing for genes is conducted and fails to identify a mutation in a patient exhibiting elevated serum CK levels and symptoms indicative of Duchenne muscular dystrophy (DMD), the subsequent diagnostic evaluation would involve a muscle biopsy. Recent clinical approaches have also been investigated for diagnosing Duchenne muscular dystrophy (DMD). Novel non-invasive techniques for DMD diagnosis have included the

use of ultrasound and magnetic resonance imaging (MRI) to measure muscle mass and track alterations over time [94, 95].

2.5.2 Serum Creatine Kinase (CK)

When Duchenne Muscular Dystrophy (DMD) is suspected, a serum creatine kinase (CK) test is often one of the first diagnostic tools employed [88]. Elevated blood CK levels, up to 10 times the normal values, can be observed in newborns with a dystrophin-related disorder.

Measuring serum creatine kinase (CK) levels in a blood sample is a simple and cost-effective test that can be conducted in the majority of hospitals. Elevated serum CK levels in Duchenne muscular dystrophy (DMD) are present from birth, and early detection at this stage facilitates prompt diagnostic intervention, contributing to an enhanced quality of life for patients throughout the course of the disease [86].

2.5.3 Multiplex Ligation-Dependent Probe Amplification (MLPA)

At present, Multiplex LLigation- dependent Probe Amplification (MLPA) stands as one of the extensively employed quantitative techniques for detecting Duchenne muscular dystrophy (DMD) [96].

MLPA is proficient in genetically screening symptomatic DMD patients, along with carrier females, utilizing either MLPA or array-MLPA. MLPA concurrently examines all 79 DMD exons within the dystrophin gene, scrutinizing the gene using a multiplex polymerase chain reaction event to check for structural changes and copy number variations. The detected copy number variations serve as indicators of deletions or duplications, signifying the presence of the disease [97].

2.5.4 Genetic Diagnostic Testing

The goal of genetic diagnostic testing is to quantitatively analyze dystrophin genes conclusively, aiming to detect the majority of mutations leading to the Duchenne muscular dystrophy (DMD) phenotype, encompassing prevalent deletions and duplications. It is advisable to prioritize testing for large deletions or duplications initially, as they are more frequent (constituting 70-80% of cases) compared to point mutations (comprising 20-30% of cases) [95]. If initial genetic diagnostic testing yields no results, the subsequent step involves a comprehensive gene sequencing qualitative approach [98. The choice of a specific standard genetic test depends on the nature of the genetic condition being investigated. Multiplex PCR involves the simultaneous amplification and analysis of fragments from the dystrophin gene. The advantages of utilizing this diagnostic tool include its widespread availability and cost-effectiveness. However, it is important to note that this method exclusively identifies deletions, making it suitable only as an initial diagnostic step [90]. Another quantitative diagnostic method will need to be used if the outcome is negative. Moreover, multiplex PCR may only detect up to ten distinct exons in a single response, not the entire dystrophin gene.Typically situated in hotspots, the targeted exons may not offer a comprehensive depiction of the mutation. Despite this, determining the endpoints of many deletions can provide valuable insights into the potential phenotype and severity of the disease, as endpoint effects impact the translational reading frame [99].

2.5.5 Next Generation Sequencing (NGS)

Next generation sequencing (NGS) in Duchenne muscular dystrophy (DMD) refers to advanced and high-throughput DNA sequencing technologies used to analyze the genetic material associated with DMD. NGS allows for the rapid and parallel sequencing of large sections of the genome, providing detailed information about the sequence and structure of the dystrophin gene, which is implicated in DMD. NGS allows for a comprehensive analysis of the entire dystrophin gene, including coding and non-coding regions, offering a more thorough examination compared

to traditional methods [100]. NGS also enables the diagnosis of the remaining uncommon mutations that may go undetected by the two aforementioned methods, through more targeted transcript analysis. A study demonstrated that NGS could effectively diagnose as many as 92% of patients with Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD) [100]. Furthermore, combining MLPA with next-generation high-throughput DNA sequencing methods can improve the accuracy of the findings [101,102].

2.5.6 Comparative Genomic Hybridization (CGH)

Using an oligonucleotides on array based, Comparative Genomic Hybridization (CGH) looks for copy number variations throughout the entire dystrophin gene [103]. It is a molecular genetics technique used to detect chromosomal imbalances or structural variations within an individual's genome. CGH enables the comparison of the DNA copy number between two different DNA samples, typically a test sample (e.g., from a patient) and a reference sample (e.g., from a healthy individual). To lower the possibility of false positives brought on by single nucleotide polymorphisms, several array probes are used. When a diagnosing physician believes that a mutation may be situated in an unusual location, CGH is especially helpful. Customized arrays that target the particular region of interest in the dystrophin gene while maintaining the requisite resolution for conclusive results can be employed with CGH. The wide-angle view that the CGH diagnostic method offers makes it possible to examine areas that are usually not examined, like the intron and the 3' and 5' regions. This capability enables CGH to identify intricately arranged mutations and precisely determine the locations of mutation breakpoints [104]. CGH is particularly esteemed for its dual capacity to identify molecular markers for Duchenne muscular dystrophy (DMD) and pinpoint specific mutations within the gene of DMD. The CGH approach is useful not only for clinical diagnosis but also for DMD research, as it enables the investigation of non-coding areas to comprehend the etiopathogenesis of mutations and the diagnostic implications of such changes. Furthermore, CGH is capable of carrying out the complex work of defining breakpoints in major rearrangements, which contributes

to the understanding and correlation of genotype versus phenotype in Duchenne muscular dystrophy [101].

2.5.7 Fluorescence in Situ Hybridization (FISH)

Large deletions and duplications can be found using fluorescence in situ hybridization (FISH), albeit this technique is not commonly used [105]. This molecular genetics method determines whether particular DNA sequences on chromosomes are present or absent. Additional genetic methods employed for diagnosing deletions and duplications in Duchenne muscular dystrophy (DMD) encompass qPCR and chromosomal microarray analyses (CMA) [106,107]. If a substantial mutation is not identified, the genetic diagnosis of Duchenne muscular dystrophy (DMD) can be conducted by screening for point mutations. Direct sequencing and denaturing high-performance liquid chromatography are commonly used to find tiny point mutations. [108,109].

2.5.8 Other Testing Technique

If one of the techniques mentioned reveals and recognizes the complete description of the mutation in dystrophin, it is recommended to forego further testing with alternative diagnostic methods. Nonetheless, in the event of a negative result in the test for duplication or deletion, to find any little deletions or point mutations, the dystrophin gene should be sequenced [98]. A thorough and comprehensive recognizes the characteristics of the mutation in dystrophin gene is essential, identifying each and every point mutation and deletion terminus. This enables the utilization of gathered information to determine how the particular mutation in the patient will impact the reading frame of the dystrophin protein gene. The primary factor in ascertaining the patient's phenotype among the various dystrophinopathy variations is the correlation with the reading frame. Additionally, this genetic comprehension might enable the patient to engage in ongoing trials for mutation-specific gene therapies [90].

2.6 Available Therapies

Pharmacological treatment includes the use of corticosteroid prednisone which is an antiinflammatory drug that enhanced muscle strength. But this drug is reported to have various side effects and an alternative drug i.e. Deflazacort is used instead as it has lesser side effects (i.e. weight gain, immunosuppression, and osteoporosis). An antibiotic Gentamicin has additionally been documented to enhance function of muscles as it inhibits premature synthesis of protein present on N-terminal of binding region of dystroglycan [21].Deletion of exons of the DMD gene, use of CRISPR/Cas9 system for genome editing are used to restore dystrophin production, which results in a functional restoration of muscle strength [110].

New formulations are being made and translated into oligonucleotides using viral vectors. Viral vectors such as adeno-associated viruses are also used for the delivery of gene. But this method poses challenges for treatment of DMD due to very large size of its gene. Various cell-based methods are also considered for the delivery of full gene by injecting stem cell, skeletal myoblast injection into muscle tissue. But success rate is quite low as these cells migrate from the site where they are injected and their survival rate is also quite low [110].

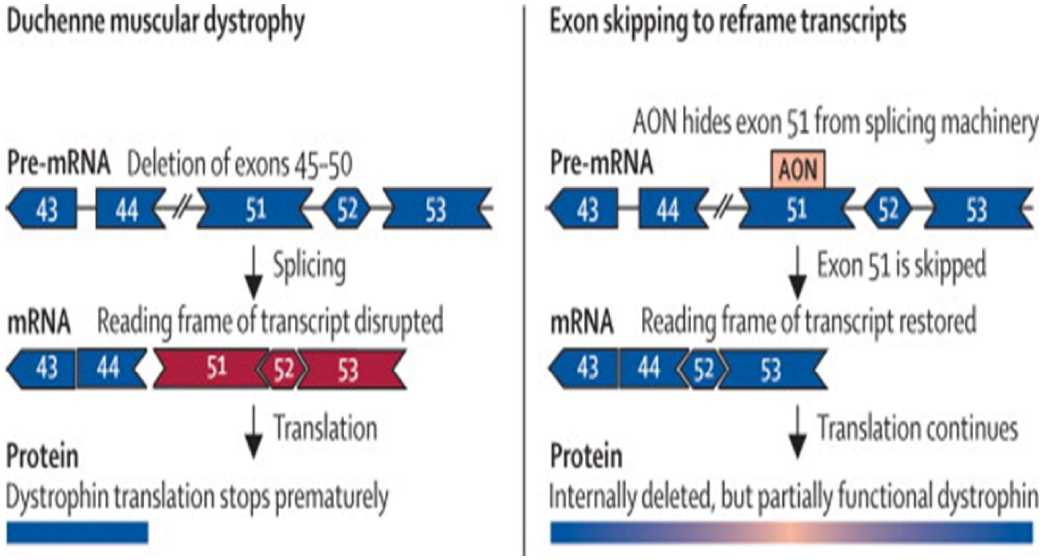


FIGURE 2.6: Exon skipping to treat DMD transcripts using antisense [111]

Furthermore, small molecule medications are being created to prevent production of premature stop codons by nonsense mutations which further prevents premature termination of translation, which restores dystrophin [112,113]. Upregulating endogenous utrophin holds significant potential for the treatment of DMD without raising any immunogenic issues asit can act as an alternative for dystrophin deficiency [114].

2.6.1 Pharmacological Treatment

Two corticosteroids, deflazacort (Emflaza) and prednisone, can stop the progression of some kinds of muscular dystrophy and strengthen muscles. But long-term use of these medications can thin the bone and cause weight gain, which raises the risk of fracture. The first medication approved by the Food and Drug Administration (FDA) for the treatment of some people with Duchenne muscular dystrophy (DMD) is eteplirsen (Exondys 51), a more recent drug. Additionally, the FDA-approved medication golodirsen is used to treat some DMD individuals who have a particular genetic mutation. Heart medications such as ACE inhibitors (angiotensin-converting enzyme Inhibitors) or beta blockers are administered if muscular dystrophy damages the heart [21].

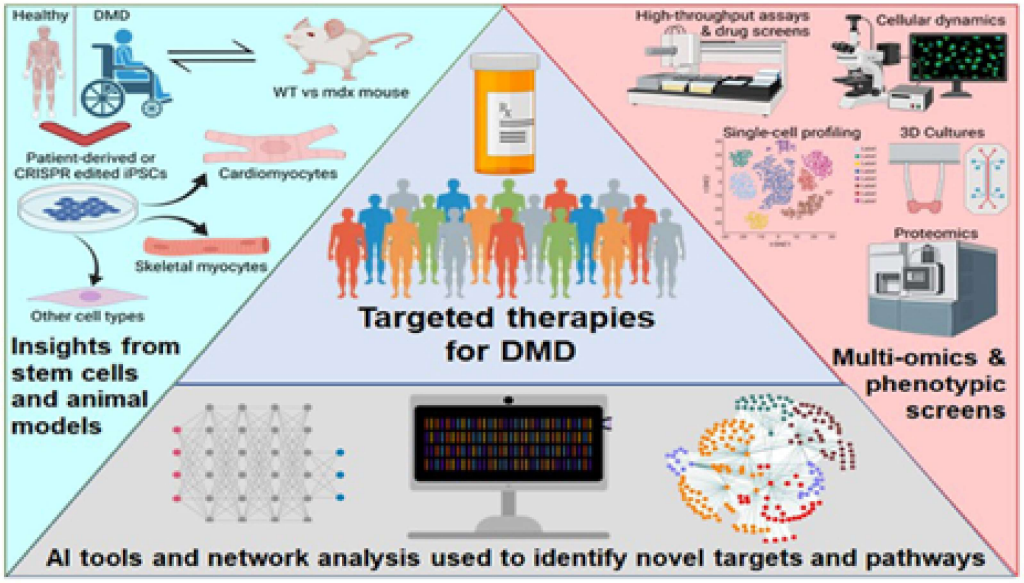


FIGURE 2.7: Schematic of the approaches to broaden the therapeutics available to DMD patients [115]

2.6.2 Gene Delivery via Viral Vectors

Adeno-associated virus (AAV) is one of the viral vector systems currently being explored as a vital treatment for muscular dystrophy. In the case of micro-dystrophins, which are partially functional proteins, nearly half of the typical dystrophin amino acid sequence has been omitted.

Interestingly, the "Becker-like" dystrophins naturally present in individuals with Becker muscular dystrophy (BMD) closely resemble the biochemically altered dystrophin introduced through gene therapy. Notably, the dystrophins generated by AAV are noticeably smaller in size than those observed in patients with Becker muscular dystrophy [116]. A major issue that requires more research is reactions to the recovered dystrophin or AAV viral proteins [117].

2.6.3 Stop Codon Read Through Therapy

A mutated stop signal, distinct from the usual termination signal found at the end of a gene, can be detected through readthrough therapy. This treatment aims to encourage the cell to bypass the stop codon, allowing for the correct interpretation of genetic instructions and ultimately the production of a functional dystrophin protein. To achieve a therapeutic impact, readthrough medications often require multiple administrations.

Ataluren, a small-molecule drug designed for stop codon readthrough, has exhibited variable enhancements in 6-minute walk times [118]. Based on research findings, it has been observed that 30% of boys with DMD, who possessed a stop codon, exhibited residual dystrophin in their muscles even without the administration of the drug ataluren. However, as of now, there is no evidence indicating a drug-induced increase in dystrophin levels within the muscles of these patients [119]. Any therapy based on genome editing therapy must first undergo comprehensive characterization to ensure that it only modifies possible off-target locations when necessary [120].

2.6.4 Exon Skipping Therapy

In the pursuit of developing treatments for Duchenne muscular dystrophy (DMD), the focus has been on exon skipping as a therapeutic strategy to restore dystrophin when faced with an out-of-frame dystrophin mutation. Exon skipping, or the transition of out-of-frame to in-frame deletions, could be accomplished through exon deletions during genome editing or by using oligonucleotides or U7 snRNPs that express an antisense sequence. The process of exon skipping entails the removal of an additional DMD gene exon adjacent to a patient's deletion mutation, effectively changing an out-of-frame DMD mutation into an in-frame Becker muscular dystrophy (BMD) mutation.

This can be accomplished using exon deletions during genome editing, antisense-expressing oligonucleotides, or U7 snRNPs. In the case of modified U7 snRNP genes, they have been delivered using AAV vectors. Unlike the typical antisense region that hybridizes with histone RNA, this modification targets a dystrophin exon. Here, the intended target is premRNA rather than mRNA, resembling the exon skipping process achieved with oligonucleotides.

Clinical trials are currently exploring AAV-mediated RNA editing as a potential treatment for exon 2 duplications. Another approach for achieving exon skipping involves the use of oligonucleotide medications that bind to pre-mRNA before splicing. These oligonucleotide techniques have experimented with various drug chemistries, yielding varying levels of success [121].

Clinical investigations have shown that exon skipping seems to halt the development of the disease, and regulatory approval is being considered. Unfortunately, therapies based on AON-exon skipping temporarily restore dystrophin expression, necessitating scheduled injections throughout the patient's lifetime [122]. Reservations about in vivo genetic modification may be allayed by advancements in cell therapy, especially those that affect cell viability, regeneration, and distribution [123].

2.7 Association of Microbiome with Muscular Dystrophy

The microbiome, a collection of microorganisms residing in the human gastrointestinal tract, has been associated with muscular dystrophy. Muscles gradually deteriorate and weaken as a result of the muscular dystrophy group of genetic diseases. According to research, changes in the gut microbiota could potentially contribute to the initiation and advancement of muscular dystrophy. Studies in muscular dystrophy animal models indicate that changes in the gut flora can aggravate muscle inflammation and injury. Furthermore, scientific investigations have revealed that the gut microbiome has the potential to influence the functioning of the immune system. When there is dysbiosis or an imbalance in the gut microbiome, it may contribute to chronic inflammation, a known factor in the development of muscular dystrophy [62].

2.7.1 Impact of Microbiome on Immune Cells

Muscle cell proliferation and differentiation are significantly impacted by the inflammatory response that follows tissue damage, with various regenerative stages being assisted by its pro-inflammatory or anti-inflammatory stage [16].

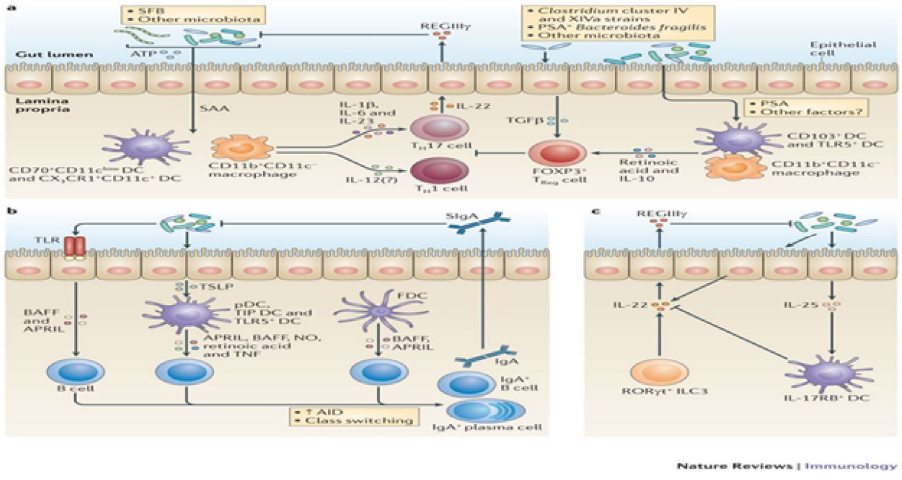


FIGURE 2.8: Immune modulation and gut microbiome in inflammatory diseases [124]

Several inflammatory signals from different immunocytes are closely regulated in how quickly muscle tissue regenerates. A buildup of IL-17A+ T cells causes inflammation as muscle starts to deteriorate and expedites tissue recovery [17]. It's possible that enhanced neutrophil accumulation after injury mediates a significant percentage of the pro-reparative effect of IL-17A+ T cells in muscle. The first cells to enter injured muscle are neutrophils, which are crucial for eliminating dead muscle fibers, which is a necessary step in triggering the repair pathway [18].

TABLE 2.2: Possible bacterial metabolites effects on mass of skeletal muscles health [125,126,127]

MODELS OF HEALTHY OR STANDARD DIETS		
MODELS OF GM MODULATION, DIET, DAMAGE TYPE REFERENCES	EFFECTS OF GM ON MUSCLE MASS, PHENOTYPE, AND/OR FUNCTION	OTHER RELATED EFFECTS
Male GF and PF C57BL/6J male mice (6-8 wk old)[12]	Effects of GM depletion:	
Standard chow diet (R36 Lactamin, Stockholm, Sweden): 3.5% cellulose (%weight), 22.9% protein (%energy), 67.1% carbohydrate, and 9.6% fat	↓ Muscle weight, ↓ locomotion and grip strength	↑ Serum corticosterone
	↑ FoxO3/pAMPK degradation pathway with:	Alteration of metabolism, notably related to amino acids glycine and alanine, bile acids and choline in liver and serum
	↑ Atrogin-1 and MuRF1 atrophic markers	
	↑ Transcription of genes inducing BCAA catabolism, ↓ oxidative capacity, ↑ amino acids such as glycine and alanine, ↓ transcription of genes involved in NMJ function and troponin	
	At least partly normalized in GF mice transplanted with GM from PF mice, or treated with SCFAs acetate, propionate, and butyrate	
GF or SPF C57Bl/6J male mice (2 mo or 6-7 mo old, respectively) treated or not with metronidazole for 4 wk[65]	Effect of the antibiotic metronidazole:	
	In SPF mice	
Standard chow diet	↓ Weight of hind limb muscles	↑ Fecal proteobacteria
	↓ Myofiber surface area in the tibialis anterior	
	↑ In the gastrocnemius of factors involved in:	
	Protein breakdown, that is, *FoxO3, Hdac4, myogenin, MuRF1, atrogin-1*	
	Circadian clock and metabolism, that is, *Cry2, Ror-β, E4BP4, Per2, FOX01, PPARγ,* and *adiponectin*	
	In GF mice	
	↓ Weight of hind limb muscles	↓ Body weight
	↓ *FOX01* and *Pdk4,* ↑ clock gene *Bmal1,* ↓ *Per2*	
Male C57BL/6 mice treated or not at 14 wk by treatment with a broad-spectrum antibiotics cocktail (ampicillin, streptomycin, colistin, and vancomycin)	Effects of the broad-spectrum antibiotics cocktail:	
	↓ Endurance	↓ Transporters *FFAR3* (*Gpr41*) and sodium/glucose cotransporter 1 *Sglt1* in ileum
	↓ Extensor digitorum longus (EDL) muscle fatigue index in an ex vivo contractile test	
For 21 d	↓ Muscle glycogen levels	
Or for 10 d followed by a 11 d natural recolonization (NAT group)[66]		
69.2% of cereals, 20.2% of vegetal proteins, 6.0% of animal proteins, and 4.6% of mineral and vitamin cocktail (SAFE A03)	Normalized following natural reseeding (NAT group)	
Male Institute of Cancer Research (ICR) mice supplemented or not for 6 wk with *L. plantarum* TWK10[60]	Effect of *L. plantarum* TWK10 (one or both doses):	
	↑ Relative muscle weight (%)	↓ Body weight and epididymal fat pad

2.7.2 Role of Microbiome Metabolites in Disease Progression

Research has demonstrated that the gut microbiome has an impact on the generation of short-chain fatty acids (SCFAs), which metabolites are known to support muscle growth and recovery. When there is an imbalance in the gut microbiome, it can result in reduced SCFA production. This reduction in SCFAs may potentially play a role in the muscle wasting observed in individuals affected by muscular dystrophy [61].

2.8 Factors Affecting Gut Microbial Composition and Function

2.8.1 Diets

Nonetheless, a prior study proposed that genetics of the host may play a secondary role in influencing makeup of the microbiome, possibly due to challenges in standardizing conditions across individuals. In contrast, a recent study has demonstrated that environmental factors exet a more significant influence than host genetics in determining the gut microbiome composition in hosts [128]. Within environmental factors, dietary elements, which exert a strong influence on microbiota composition, can swiftly and consistently modify the structure of gut microbial communities [129]. Furthermore, dietary interventions have the potential to enhance pig performance and pork quality by influencing the diversity and makeup of the microbiome [130,131]. Significantly, the quantity of dietary fat has been consistently identified as a factor influencing the composition of gut microbiota. An extensive meta-analysis of sequencing-based research including people and animals, for example, revealed that a high-fat diet (HFD) affects microbial diversity and consistently alters the structure of gut microbial communities. This includes

an observed increase in the Firmicutes/Bacteroidetes ratio, which showed a significant correlation with the fat content [132]. This could explain the observed phenomenon of pigs developing obesity following a diet heavy in fat consumption[133]. Additionally, Kong et al. proposed that consuming a high-fat diet (HFD) has been shown to reduce the prevalence of opportunistic pathogens like Bacteroides, Alistipes, and Anaerotruncus while simultaneously increasing the levels of several beneficial bacteria like Lactobacillus, Prevotella, Alloprevotella, and Clostridium sensu stricto. [134]. Hence, in the formulation of pig feed, it is crucial to add fat judiciously to preserve the equilibrium of gut microbiota. Furthermore, aside from the quantity of dietary fat, the use of distinct types of dietary fat can variably alter both the makeup and roles of the gut microbiota [135,136]. The antibacterial capabilities of SCFAs and MUFAs are now recognized. Consuming a diet rich in these lipids, for instance, can lower the risk of gastrointestinal infections by preventing the growth of common causative organisms like Salmonella and E. coli [137, 138]. Furthermore, in the diet of mammals, there exist three primary types of fats categorized by saturation: saturated fatty acids (SFA), and polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). These fats have the potential to influence the composition of pig gut microbiota. As an example, pigs that were given palm oil exhibited notable alterations in the structure of the bacterial community, causing Proteobacteria to become more abundant and Firmicutes to become less abundant. [136]. Furthermore, Prevotella was more prevalent in pigs fed oleic acid [139]. Additionally, a diet high in flaxseed oil may improve the gut immunity and health of pigs during intrauterine growth retardation; this is because the gut microbiota and mucosal fatty acid profile of the animals have changed. When taken as a whole, dietary fat types and gut microbiota structure are tightly associated, providing new awareness into the appropriate use of fat in diet [140].

Moreover, recently, dietary fiber has garnered growing interest among researchers due to its crucial role in influencing microbiome of gut. Dietary cellulose, constituting a feed component resistant to efficient digestion by digestive enzymes that are monogastric, stands as the gut microbiota's main source of fuel. This suggests that consuming an appropriate quantity of dietary fiber could potentially increase the

quantity of a certain microbiome [141]. In addition to encouraging the growth of certain taxa of bacteria that break down fiber, including SCFA and the microbiota, increasing fiber to a diet also helps complete pigs. Furthermore, they discovered that increasing the amount of fiber in the diet raises the pig gut microbiota's α- and β-diversity indices [130]. Crucially, in the gastrointestinal tract, dietary fiber decreases the amount of pathogenic bacteria while simultaneously raising the percentage of good bacteria. [142,143,144]. This could contribute to enhancing both gastrointestinal health and the excellence of meat in pigs [145,146]. However, various studies have explored how dietary fiber from different sources can yield distinct impacts on the variety and composition of microbes. For instance, a previous investigation indicated that xylan promotes the growth of Bifidobacterium, while glucan has an adverse impact on it in the ileum and cecum of pigs [147]. Additionally, compared to wheat bran, alfalfa diets increased the proportion of Bacteroidetes and Firmicutes in weaned pigs [148]. Although the aforementioned studies show that there is a significant relationship between gut microbiota and diet fiber, more research is necessary to determine the precise mechanism by which dietary fiber influences the gut microbiota of pigs [147].

2.8.2 Host Genetics

The gut microbiota's composition is significantly influenced by host genetics [41]. Increasing evidence indicates the heritability of certain bacterial taxa in both humans and pigs [41, 149, 150, 151, 152, 153]. Previous studies in pigs have suggested that there are discernible differences in the difference in the intestinal microbiota of lean and obese pigs [133, 154, 155]. Using high-throughput pyrosequencing, it has been established that the core microbiomes of lean Yorkshire pigs and fat Rongchang pigs differ [154]. Furthermore, the first four genera of Landrace pigs are slender, whereas those of Jinhua pigs are obese [133]. A recent investigation has proposed that Shaziling pigs with obesity exhibit increased α-diversity and elevated levels of beneficial bacteria, such as notably, over an extended period of time, the gut microbial compositions of varied purebred pigs were randomly placed together and showed increased similarity while maintaining breed-specific

proportions. [156]. This suggests that differences in gut microbiota are causally influenced by host genetics. Akkermansia, and SMB53, spread across 17 areas of the pig genome [157]. Reverter et al. recently pinpointed single nucleotide polymorphisms (SNPs) exhibiting a pleiotropic effect linked to the quantity of butyrate-producing bacteria (Faecalibacterium, Butyrococcus, and Coprococcus). Furthermore, using heritability estimations, a study conducted on two herds of pigs reared in identical farm settings and fed the same diet discovered 37 potential genes linked to the gut microbiota in addition to tens of heritable bacterial taxa. Remarkably, 13 of these 37 potential genes have been linked to metabolism, including levels of insulin and blood glucose as well as obesity [153]. These findings support the importance of host genetics in maintaining control over the microbiota's makeup. To gain a deeper comprehension of the intricate relationships between gut microbiota taxa and host genotype as well as to find markers of genetic and potential genes that can be incorporated into pig breeding programs to alter the composition of microbes and ultimately improve host performance, more research in this area is microbiota of pigs [158].

2.8.3 Antibiotic

Antibiotics are used in intensive swine husbandry systems frequently incorporated into pig feed or water to combat infections affecting the respiratory system and gastrointestinal tract, while also fostering the growth and development of pigs. Moreover, the selection of specific antibiotics is often linked to particular diseases, age groups, or farm management practices. For example, in many animal species, penicillins were frequently used for the prevention and treatment of septicemia, respiratory infections, and urinary tract infections. Actinobacillus pleuropneumonia and Pasteurella multocida-induced respiratory illnesses were commonly treated with tetracyclines. Colistin found its primary application in addressing gastrointestinal issues in piglets and weaners, whereas tylosin was predominantly utilized for fatteners and sows [159]. Antimicrobial resistance (AMR), which is present in the food chain and can spread to consumers, is facilitated by the widespread use

of antibiotics in food animals [160]. Therefore, in order to support effective antibiotic usage, a thorough understanding of the existing use pattern of antibiotics in cattle is important. This might slow the spread of AMR in animal husbandry. Furthermore, there has been a renewed focus on the correlation between antibiotic exposure and host physiology, recognizing the pivotal function of microbiome of gut in the modern understanding of host physiology. A study indicated that the administration of antibiotics impacts the growth performance of pigs, myofiber composition, and lipid metabolism. Changes in the gut microbiota may be linked to these consequences [161]. Nevertheless, the prolonged utilization of subtherapeutic doses of antibiotics has the potential to elevate the levels of pathogenic microorganisms, disrupting normal intestinal function. This can result in adverse effects on the proportionate bacterial population, hence causing illnesses [162,163,164].

Specifically, intestinal infections may arise and postweaning diarrhea may ensue as a result of extended antibiotic use. This is attributed to a decrease in bacterial diversity and the provocation of gut inflammation [165]. Research conducted on piglets revealed that supplementing subtherapeutic antibiotics from postnatal day 7 to 42 resulted in noticeable changes to the small and large intestines' microbial makeup. These included a decrease in the bacteria Clostridium, Bacillus, and Sharpea in the stomach, duodenum, and jejunum digesta as well as a little decrease in Prevotella in the colon. These modifications had an impact on the metabolic phenotype [166,167]. This has been shown that some antibiotics, such as penicillin, tylosin, sulfamethazine, and chlortetracycline, significantly change the makeup of the gut microbiome in developing pigs and upset the equilibrium [168,169,170]. Nonetheless, swine agriculture is home to an increasing number of antibiotic-resistant bacteria [171]. The imbalance in the microbiota can impact the host's utilization and uptake of nutrients, as well as influence lifelong metabolic phenotype and diseases, including obese person [172,173,174,175]. Owing to growing safety concerns associated with antibiotics, several countries have imposed restrictions on their use in animal feed. Therefore, comprehending the long-term effects and the precise mode of action of antibiotics becomes crucial for developing effective alternatives in pig farming [176].

2.8.4 Prebiotics and Probiotics

Prebiotics are indigestible compounds that specifically promote the development and function of good bacteria and other microorganisms in the digestive system. They essentially serve as food for these beneficial microbes, promoting their proliferation. The fermentation of prebiotics by these microbes produces short-chain fatty acids, which contribute to a healthier intestinal environment. Common examples of prebiotics include inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS) [177]. On the other hand, probiotics are live microorganisms (usually bacteria or yeast) that, when administered in adequate amounts, confer health benefits to the host. Probiotics work by enhancing the balance of the intestinal microbiota, preventing the colonization of harmful bacteria and supporting the immune system. Lactic acid bacteria (e.g., Lactobacillus, Bifidobacterium) and certain yeast strains are often used as probiotics in animal feed [178].

Probiotics and prebiotics contribute to the balance and diversity of the gut microbiota. A diverse and well-balanced microbiota is associated with several health benefits, including improved nutrient absorption, enhanced resistance to infections, and the prevention of inflammatory conditions [179].

Both prebiotics and probiotics contribute to the overall health of the gastrointestinal tract in animals. This has several implications for production performance, as a healthy gut microbiota is linked to improved nutrient absorption, better resistance to pathogens, and enhanced overall well-being. Consequently, animals supplemented with prebiotics and probiotics often exhibit improved growth rates, feed efficiency, and disease resistance [180] .It important to note that the effectiveness of prebiotics and probiotics can vary depending on factors such as the species of the animal, the specific strains used, and the overall management practices. Additionally, ongoing research is continually refining our understanding of the interactions between these additives and the host's physiology. Furthermore, prebiotics have the capacity to regulate lipid metabolism, leading to an increased intramuscular fat (IMF) level in finishing pigs [181].

2.9 Gap Analysis

There is scarcity of human studies specifically focusing on the gut microbiome individual with muscular dystrophy. Most existing research has been conducted in animal models. Closing the gap with comprehensive human studies is essential for understanding the revalance of gut microbiome to muscular dystrophy and human health.

2.10 Research Questions

1. What type of gut microbiome are present associated with muscular dystrophy?
2. What is the functional implications of gut microbiome in muscular dystrophy in normal patients?

Chapter 3

Material and Methods

3.1 Chemicals

PBS buffer(sigma), MRS media (Biolab), agarose (Bioworld), glycerol (Merck), crystal violet (Sigma), kovac's reagent (AnalaR), hydrogen peroxide (paradise pharma) Alphanapthol (Sigma), MR - VP broth (Alpha biosciences), ethanol (AnalaR), Safranin solution (Sigma), Gram Iodine (Scharlau).

3.2 Apparatus

Petri plate, beaker, conical flask, wire loop, spirit lap, micropipette, cotton swab, measuring cylinder, spatula, microscopic slides, dropper, falcon tube, eppendorf and reagent bottles.

3.3 Equipment

Laminar flow hood, incubator, weighing balance, autoclave, vortex, microwave, water bath and pH meter.

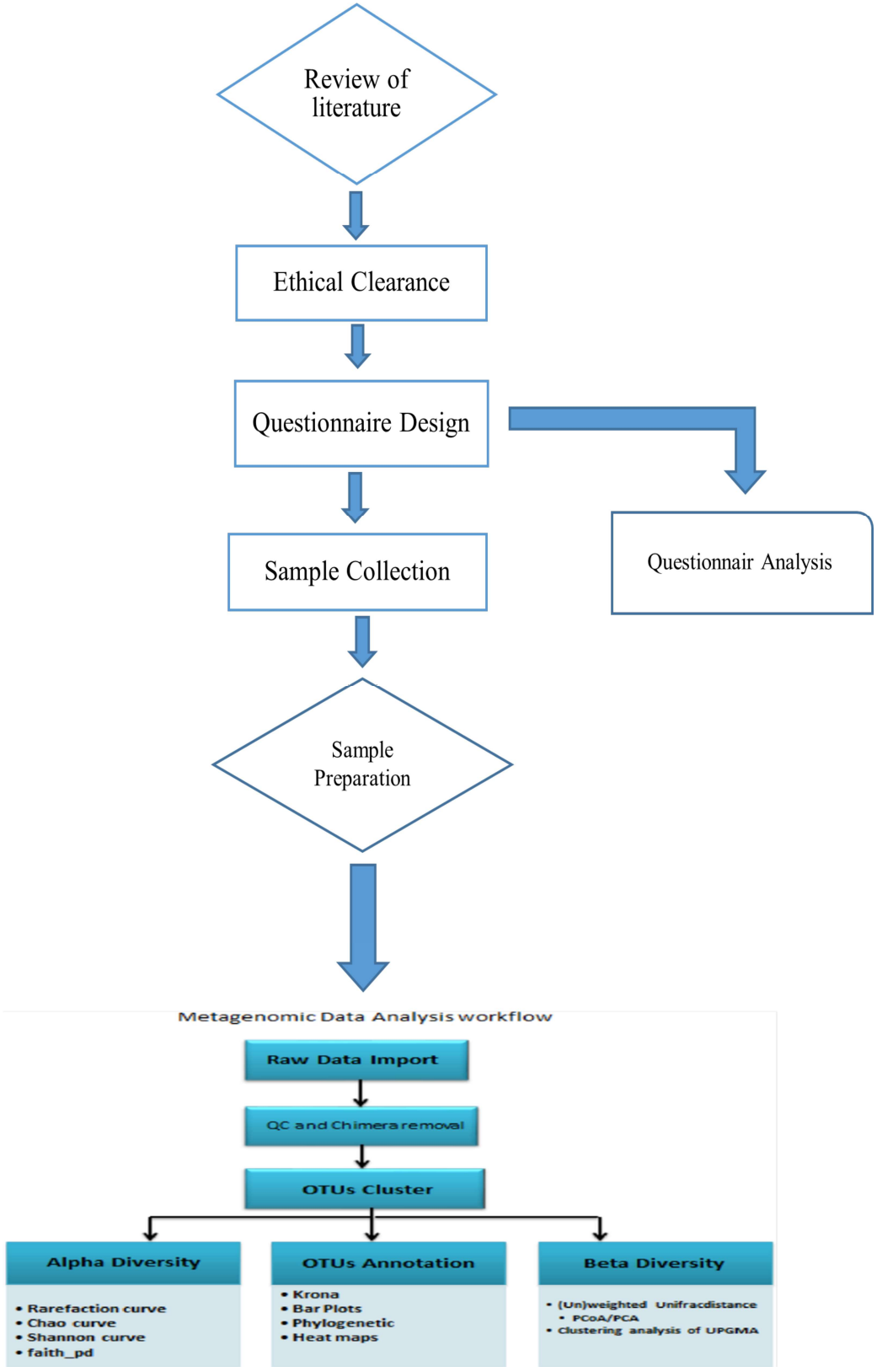


FIGURE 3.1: Flow Chart shows Major Steps of Methodology

3.3.1 Ethical Clearance

Ethical clearance was received from ethical committee of "Department of bioinformatics and Biosciences" (Appendix 1).

3.3.2 Questionnaire Design

Based on the literature reviewed questionnaire was designed (Appendix 2).

3.3.2.1 Inclusion Criteria

All participants with below mentioned characteristics were included,

- Individuals with good general health status
- No infection or recurrent infection since last three months
- Not used any antibiotics, antifungals, recreational and other drugs during last three months

3.3.2.2 Exclusion Criteria

Participants were excluded if there were was any evidence of below mentioned disorders,

- Diabetes complications
- Metabolic disorders (blood pressure)
- Individuals with any aliment (cancer, genetic disorder and other disease)

3.3.2.3 Statistical Analysis

For presenting percentage the types of muscular dystrophy and for diagnosis level graph were drawn using excel version Based on the inclusion and exclusion criteria SF filled were analyzed to find the possible association of diet, type of muscular dystrophy and symptoms using SPSS and Regression model was applied.

3.4 Sample Selection

Based on the above mentioned exclusion and inclusion criteria questionnaire were filled from 60 healthy people and 60 muscular dystrophy with informed consent.

Exclusion and inclusion criteria was followed for the collection of stool samples. They were also analyzed for their integrity and appearance visually to check if they were fresh or not.

3.5 Sample Collection

The muscular dystrophy patient were approached with the collaboration of Muscular Dystrophy Organization of Pakistan. Informed consent was obtained from the individuals who agreed to donate the samples.

As part of their consent, donors agreed to the unspecified research on their stool samples that may be helpful for the scientific community and taken before the breakfast. The collected samples were anonymized and assigned and identity prior to analyses. Samples were collected in sterile plastic containers kept in ice and brought to the lab within 24 hours of collection for further processing. Stool samples did not contain urine or water.

3.6 Metagenomics

Two samples, one of muscular dystrophy patient and other for healthy individuals was sent for Metagenomics analysis. For this purpose 3-4ml PBS was added in eppendrof tubes and 1.5 grams stool sample was added into it and homogenized using vortex.

3.6.1 16S Ribosomal RNA (rRNA)

The 16S ribosomal RNA (rRNA) sequence consists of nine hypervariable regions that are alternated with conserved regions. These nine hypervariable regions (V1-V9) are located inside the bacterial 16S gene and range in length from roughly 30 to 100 base pairs. These regions play a role in shaping the tiny ribosomal subunit's secondary structure. To achieve taxonomic classification, sequencing specific hypervariable regions alone is adequate, eliminating the need to sequence the entire gene [182].

3.6.2 DNA Extraction

300 μl of samples are collected. These samples are homogenized in 500 μl of lysis solution. The homogenized samples are then incubated for 20-30 minutes at 60^{o}C. This step helps in breaking down cell membranes and releasing DNA. After the initial incubation, 20 μl of 20% SDS (Sodium Dodecyl Sulphate) is added. SDS helps to break down cell membranes and denature proteins. Additionally, 25 μl of proteinase K is added. An enzyme called proteinase K breaks down proteins, including nucleases that could damage DNA. This step is crucial for protein removal and DNA protection. The samples are incubated at 56^{o}C overnight. This extended incubation period allows for thorough digestion of proteins and complete lysis of cells, releasing DNA into the solution. 500 μl of PCI solution (phenol: chloroform: isoamyl alcohol) is added to the samples. The mixture is then centrifuged at 13000 rpm for 10 minutes [183]. The purpose of this step is to separate

the DNA-containing aqueous phase from proteins, cell debris, and other contaminants, which remain in the organic phase. The aqueous phase obtained from the PCI treatment is transferred to a new tube. After that, 500 μl of chloroform and isoamyl alcohol (C:I, 24:1) are added, and it is centrifuged once more for ten minutes at 13000 rpm. This step further purifies the DNA by removing any remaining contaminants. The aqueous layer obtained from the C:I treatment is transferred to a new tube. To precipitate the DNA, add 500 μl of cold isopropanol and 55 μl of sodium acetate. The samples are incubated at -20^{o}C for 60 minutes. This step allows the DNA to precipitate out of the solution. The samples are centrifuged for 10 minutes at 13,000 rpm after incubation. The supernatant (liquid above the pellet) is discarded, leaving behind the DNA pellet. After treating the DNA pellet with 500 μl of 70% ethanol, it is centrifuged for 10 minutes at 7500 rpm.This step is crucial for washing away any remaining impurities. To get rid of any leftover ethanol, the DNA pellet is air-dried following the ethanol wash. The DNA pellet is then re-suspended in TE buffer (Tris-EDTA), providing a stable environment for the DNA. The re-suspended DNA is stored at 4^{o}C until further use [184].

3.6.3 Agarose Gel Electrophoresis

One gram of agarose was dissolved in one hundred milliliters of 1X TAE buffer (Tris Acetic acid EDTA) for the purpose of gel electrophoresis. TAE buffer provides the necessary ions for the electrophoresis process. The solution is heated to create a clear agarose gel solution.7 μl of Fluorescent dye Ethidium Bromide bonds to DNA and enables visibility when exposed to UV light., is added to the gel solution. The gel solution is poured into a gel casting tray with inserted combs. Combs create wells for loading DNA samples.After solidification, the gel caster (containing the solidified gel with wells) is transferred to a gel tank filled with 1X TAE buffer. Combs are carefully removed from the solidified gel in the gel caster. 7 μl of extracted DNA is mixed with 3 μl of 6X bromophenol blue dye (loading dye), which helps in tracking the progress of the electrophoresis.The DNA-loading dye mixture is loaded into the wells of the gel. The gel is subjected to electrophoresis under specific parameters: 500 mA of current, 75 volts for 60 minutes. These conditions

facilitate the migration of DNA fragments through the gel matrix based on their size. After electrophoresis, the gel is visualized under a UV Trans-Illuminator. The UV light activates the Ethidium Bromide, causing the DNA bands to fluoresce [185]. A Bio Doc Analyzer is used for capturing an image of the gel. The gel picture is used to compare the migration pattern of the sample DNA bands with a 1-kilobase (KB) DNA ladder. The ladder serves as a molecular weight marker, allowing estimation of the sizes of the sample DNA fragments [186].

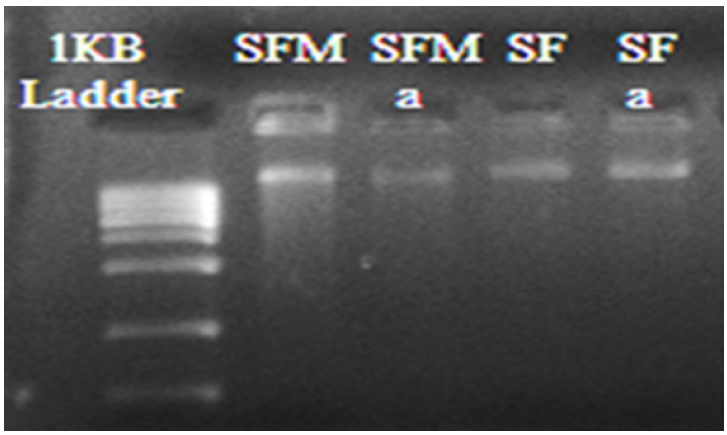


FIGURE 3.2: Gel electrophoresis image

The initial well of the gel received a 1-kilobase (KB) ladder, while the subsequent wells were filled with DNA samples characterized by their robust integrity and sizes exceeding 20 kilobases. Both the samples were extracted in duplicate, Sample with best QC proceeded further for sequencing.

3.7 DNA Quantification

The quantity of DNA was assessed using the Thermo Scientific Multi Skan Go Instrument, where the quality was indicated by the 260/280 ratio, and the concentration was expressed in ng/μl.

TABLE 3.1: Sample Concentration

Sr. No	**Sample ID**	**Nucleic Acid 260/280**	**Nucleic Acid Conc. in (ng/μl)**
1	SFM	1.82	790

TABLE 3.1: Sample Concentration

Sr. No	**Sample ID**	**Nucleic Acid 260/280**	**Nucleic Acid Conc. in (ng/μl)**
2	SFMa	1.76	550
3	SF	1.81	610
4	SFa	1.74	575

Chapter 4

Results

4.1 Sample Collection

Sample collection were carried out during the month of august. After the ethical clearance stool samples was collected from the city of Islamabad.

Questionnaire were filled from 60 healthy and 60 muscular dystrophy patient with informed consent. One stool sample from healthy individual and one sample from muscular dystrophy patient was taken for Metagenomic analysis.

4.2 Questionnaire Analysis

4.2.1 Prevalence/Types of Muscular Dystrophy

A total of 60 muscular dystrophy patients participated in this study.

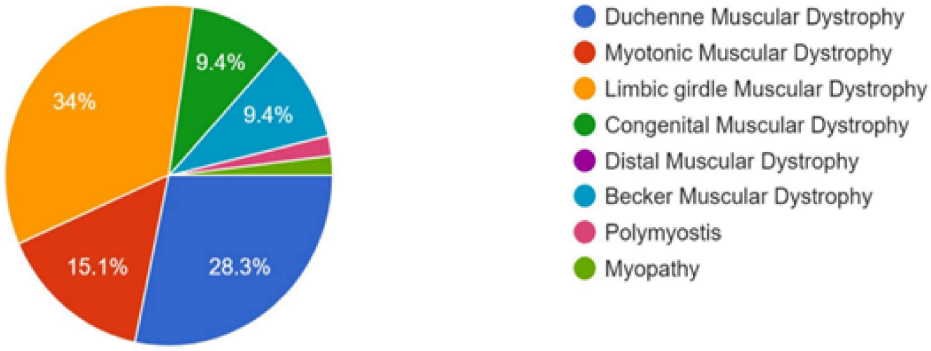


FIGURE 4.1: A pie chart showing prevalence of different types of Muscular Dystrophies

According to the data that was collected it can be concluded that limbic girdle is the most prevalent kind of Muscular Dystrophy among the individuals that participated in this study. The prevalence of Limbic Girdle muscular dystrophy is 34%, Duchenne muscular dystrophy is 28.3%, Myotonic muscular dystrophy is 15%, and prevalence of Congenital and Becker muscular dystrophy is 9.4%. The differences of prevalence can be seen as compared to those mentioned in literature review due multiple factors such as ethnicity, origin, diet and lifestyle [18].

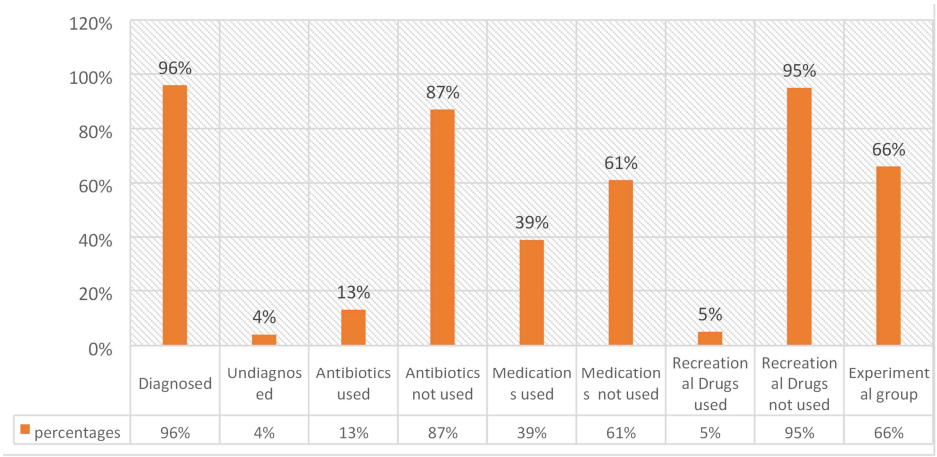


FIGURE 4.2: A Bar Graph showing Questionnaire Analysis of Experimental Group

The prevalence of diagnosed muscular dystrophy patients was 96% and that of undiagnosed patients were 4% in total sample size of 60 individuals. Among the diseased individuals who participated in the study 87% had not used antibiotics

and 13% had used antibiotics ,39% had not used medications and 61% had used medications, 5% has used and 95% had not used the recreational drugs during the previous three months. The individuals shortlisted as experimental group for the collection of stool samples were those who had not used any antibiotics, medications, recreational drugs and were diagnosed with the disease and their prevalence was found to be 66% in total sample size of 60 individuals.

4.2.2 Association of Diet and Muscular Dystrophy

TABLE 4.1: ANOVA Test

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	56.84829	28.42415	523.0814	2.12E-37
Residual	58	3.151709	0.05434		
Total	60	60			

TABLE 4.2: Regression model show the association of diet and muscular dystrophy

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
Diease Patients	0.240385	0.036142	6.651112	1.13E-08	0.168039	0.312731	0.168039	0.312731
Normal Subjects	0.393162	0.062831	6.257435	5.11E-08	0.267392	0.518933	0.267392	0.518933

The regression analysis reveals a strong overall fit for the model, as indicated by a multiple R of 0.9734 and an R-square value of 0.9475, suggesting that approximately 94.75% of the variability in the dependent variable can be explained by the independent variables. The ANOVA results further support the significance of the model, with a highly significant F-statistic of 523.08 ($p < 0.001$). The coefficients for the predictors, Disease Patients, and Normal Subjects, also show significant relationships with the dependent variable. The coefficient for Disease Patients is 0.2404 ($p < 0.001$), indicating a positive relationship, while the coefficient for

Normal Subjects is 0.3932 ($p < 0.001$), suggesting a stronger positive association. However, without intercept values, the interpretation of these coefficients is limited.

Upon analyzing the residuals, it's evident that the predicted values closely match the observed values, with small variations indicated by the residuals. However, some residuals have notably larger values, particularly in observations 1, 4, 16, 18, 36, and 50, suggesting potential outliers or anomalies in the data. Further investigation into these specific cases may be warranted to ensure the robustness of the model.

The regression analysis suggests that both Disease Patients and Normal Subjects have a significant impact on the dependent variable.

4.2.3 Association of Diet, Symptoms and Type of Muscular Dystrophy

TABLE 4.3: ANOVA Test

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	56.57146	28.28573	478.5052	2.34544E-36
Residual	58	3.428536	0.059113		
Total	60	60			

TABLE 4.4: Regression model show the association of diet symptoms and types of muscular dystrophy

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
symptoms	0.347179	0.023354	14.86621	1.92E-21	0.300431620	0.393926	0.300432	0.393926
Types of Disease	0.078539	0.014036	5.59562	6.26E-07	0.050443606	0.106635	0.050444	0.106635

The coefficient for Symptoms was found to be 0.347 ($t = 14.866$, $p < 0.001$), suggesting that, while controlling for Types of Disease, a one-unit increase in

Symptoms is associated with a 0.347 unit increase in diet. The 95% confidence interval for this coefficient ranges from 0.300 to 0.394.

In this case, a higher t-value (14.866) indicates a more significant relationship. The p-value (< 0.001) suggests that the relationship between Symptoms and diet is statistically significant. In other words, it's unlikely to be due to random chance. The interpretation "while controlling for Types of Disease" means that the analysis considered the potential influence of Types of Disease, and the reported effect is specifically related to Symptoms and diet after adjusting for Types of Disease. A one-unit increase in Symptoms is associated with a 0.347 unit increase in diet." This implies that as the level of Symptoms increases by one unit, we can expect diet to increase by 0.347 units, assuming all other factors (Types of Disease) are held constant. The interpretation indicates a statistically significant positive association between Symptoms and diet, after accounting for the influence of Types of Disease, and provides a range of values for the estimated effect with a high level of confidence.

The coefficient for Types of Disease was 0.079 ($t = 5.596$, $p < 0.001$), indicating that, holding Symptoms constant, a one-unit increase in Types of Disease is associated with a 0.079 unit increase in diet. The 95% confidence interval for this coefficient ranges from 0.050 to 0.107.

This represents the estimated change in the dependent variable (diet) for a one-unit increase in the independent variable (Types of Disease), while holding the variable 'Symptoms' constant. The coefficient estimate's distance from zero standard deviations is expressed as the t-value. In this case, a higher t-value (5.596) indicates a statistically significant relationship.

The p-value (< 0.001) suggests that the relationship between Types of Disease and diet is highly significant, meaning it's unlikely to be due to random chance. A one-unit increase in Types of Disease is associated with a 0.079 unit increase in diet." This implies that, with Symptoms held constant, an increase of one unit in Types of Disease is associated with an increase of 0.079 units in diet. The interpretation indicates a statistically significant positive association between Types of Disease

and diet, after adjusting for the influence of Symptoms, and provides a range of values for the estimated effect with a high level of confidence.

The present study employed multiple regression analysis to examine the relationship between the dependent variable, diet, and two independent variables, namely Symptoms and Types of Disease. The results of the analysis are summarized below. The interpretation indicates that the overall model is highly statistically significant, meaning that the combined influence of the independent variables explains a significant amount of the variation in diet. The high R^2 value further emphasizes the model's ability to account for a substantial portion of the variability in diet based on the chosen predictors.

4.3 Metagenomics

To characterize the microbiome with and without muscular dystrophy, 16S rRNA gene sequencing was used to investigate 2 fecal samples from healthy and muscular dystrophy people. SFM represents the control samples and SF was the muscular dystrophy sample.

4.4 Taxonomic Bar Plots

4.4.1 Class Bar Plots

The most prevalent class in SFM was, *Bacteroidia* (47%), *Clostridia* (37%) followed by *Negativicutes* (4%), *Coriobacteriia* (0.5%), whaereas in SF the *Bacteroidia* (32%), *clostridia* (33%), *Negativicutes* (6%) *Coriobacteria* (2%).The difference in variation at class level in SFM was *Alphaproteobacteria* (0.5%), *Elusimicrobia* (1%), *Spirochaetia* (1%) whereas in SF was *Actinobacteria* (7-8%), *Synergistia* and *Verrucomicrobiae* (figure 4.3).

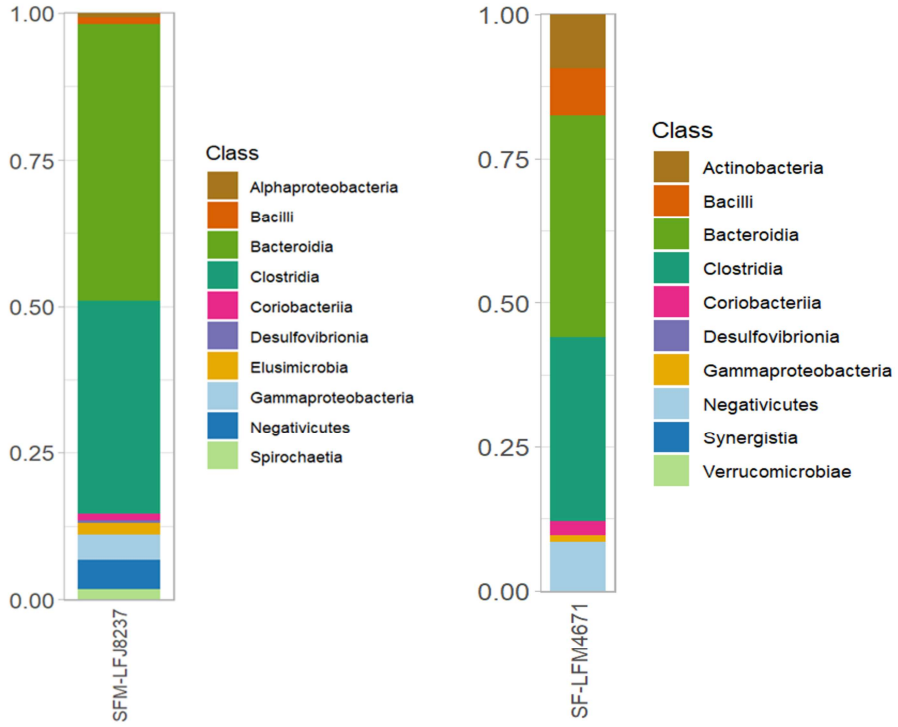


FIGURE 4.3: Taxonomic Class Taxa Bar Plot showing Classes of bacteria in SFM and SF and their abundance

4.4.2 Order Taxa Bar Plot

In order level the abundant phyla in SFM was, *Bacteroidales* (53%), *Oscillospirales* (20%), *Lachnospirales* (15%) where as in SF was *Bacteriodales* (40%), *Oscillospirales* (6-7%), *Lachnospirales* (12%) while *Burkholderdales* was least abundant 1.5% in SFM and 0.5% in SF.

The difference in variation at order level in SFM was *Aeromonadales* (1%), *Elusimicrobiales* (2%), *Spirochaetales* (1.5%) whereas in SF *Bifidoacteriales* (7%), *Erysipelotrichales* (1%) and *Peptostretococccales- Tissierellales* (2%) (figure 4.4).

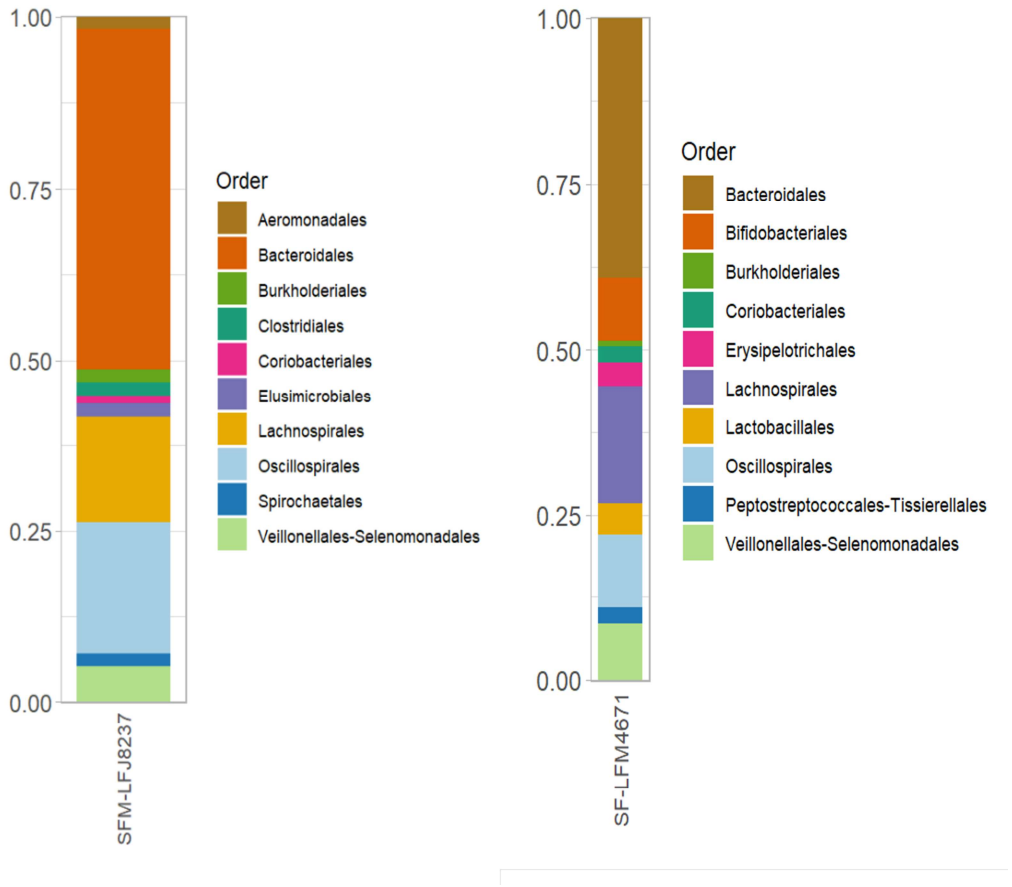


FIGURE 4.4: Taxonomic Order Taxa Bar Plot showing the orders of bacteria in SFM and SF and their abundance

4.4.3 Genus Bar Plots

In figure 4.5 the difference in variation at genus level was also observed. The healthy and disease person. Metagenomic analysis revealed only three genus were common between these two samples *Allopreveteolla.* (SFM 6%, SF 2%), *Faecalibacterium* (SFM 10%, SF 3%) and *Preveotella* (SFM 48%, SF 58%). The genus that were different in SFM *Elusimicrobium* (2%), *Lachnospiraceae-NK4A136-group* (1%), *Megasphaera* (2%), *Roseburia* (4%), *Treponema* (1%), UCG-002 (1%) and uncultured (2%).whereas in SF *[Eubacterium]-Coprostanoligenes-group* (2%), *Agathobacter* (3%), *Bifidobacterium* (12.5%), *Coprococcus* (2%), *Dialister* (3%), *Lactobacillus* (4%) and *Mitsuokella* (5%) in figure 4.5.

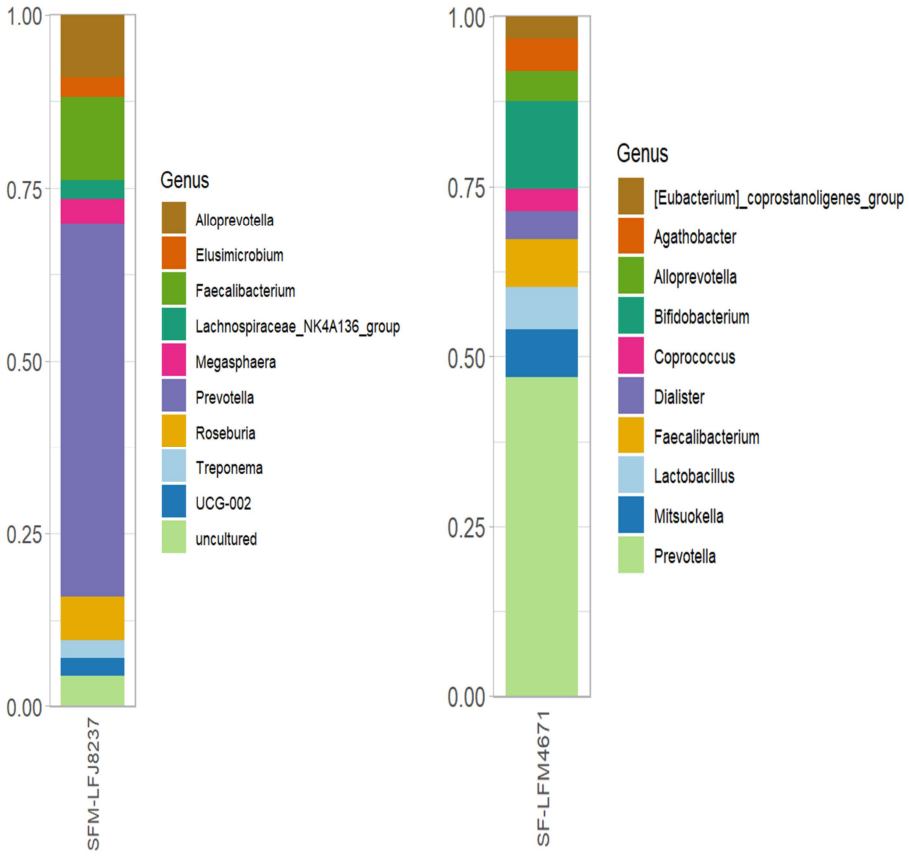


FIGURE 4.5: Taxonomic Order Taxa Bar Plot showing the Genus of bacteria in SFM and SF and their abundance

4.4.4 Species Taxa Bar Plot

The difference in variation at species level was also observed. The healthy and disease person Metagenomic analysis revealed only three species were common between these two samples uncultured –*Alloprevotella* (SFM 26%, SF 13%), uncultured-Bacterium (SFM 7%, SF 11%) and *Prevotella- copri* (SFM 8%, SF 15%). The species that were different between SFM and SF was *Prevotellaceae- Bacterium* (26%), *Treponema- Succinifaciens* (5%) in SFM, whereas in SF was *Bacteroides-Xylanisolvens* (10%), *Dorea- Longicatena* (4%), *Lactobacillus- Ruminis* (14%), *Mitsuokella-jalaludinii* (6%), *Mitsuokella- multacida* (8%). The meatagenome of healthy person also reveal the presence of *Trichuris- trichiura* (whipworm) a parasite that reside in the large intestine, appendix, and cecum of host (Figure 4.6).

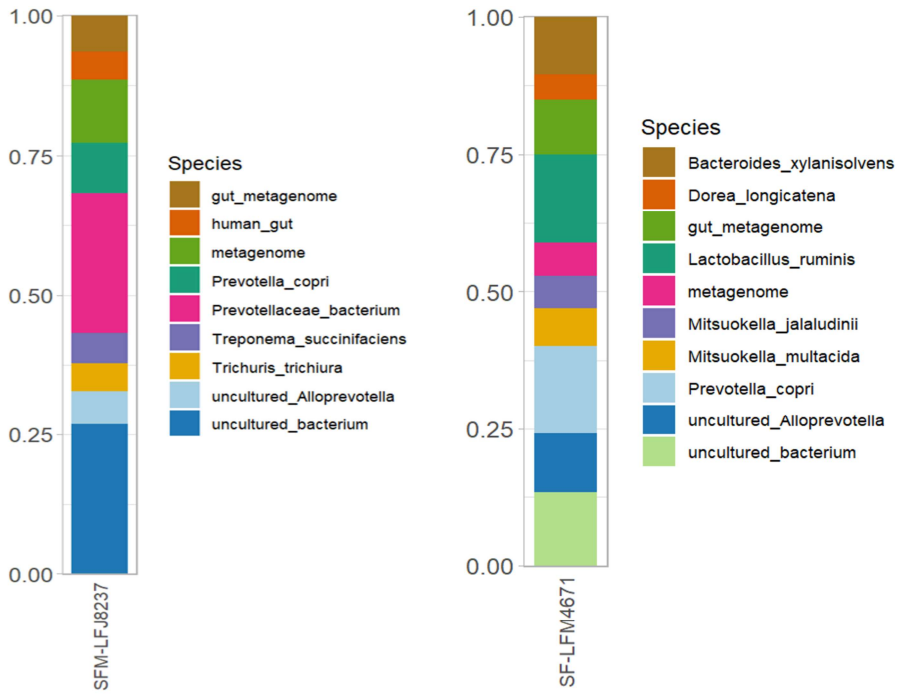


FIGURE 4.6: Taxonomic Order Taxa Bar Plot showing the species of bacteria in SFM and SF and their abundance

4.5 Heat Map

4.5.1 Order (heat map)

Heat map is the graphical representation of the data for better visualization and understanding of the events with in the dataset. Red color indicates high expression in that region whereas blue color referred to low expression. As shown in the order level heatmap SFM (control sample) the expression of *Lachnospirates*, *Oscillospirales*, *Veillonellales- Selenomonadles*, *Erysipelotrichales* and *Coriobacteriales* were low whereas the highly expressed orders for *Peptostreptococcales-Tissierellales*, *bacteroidales* and *Lactobacillales* was observed. Whereas the expression order for *Lachnospirates*, *Oscillospirales*, *Veillonellales- Selenomonadles*, *Erysipelotrichales* and *coriobacteriales* were highly expressed in SF and orders for *Peptostreptococcales- Tissierellales*, *Bacteroidales* and *Lactobacillales* was observed low (figure 4.7).

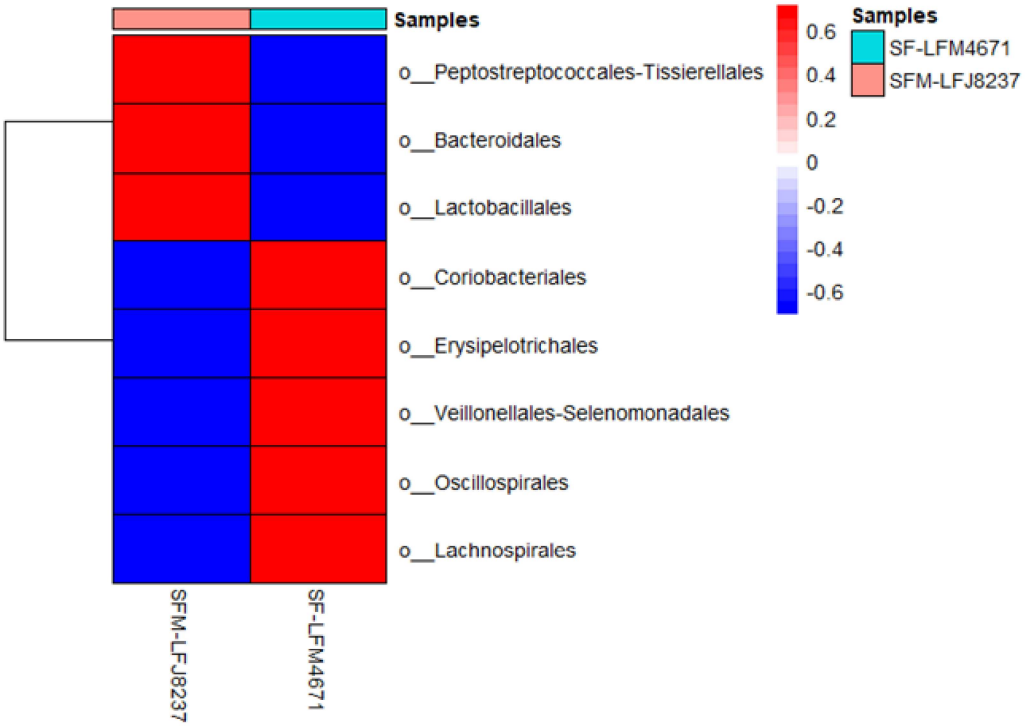


FIGURE 4.7: Heat map of order of Samples SFM (controlled) and SF (diseased)

4.5.2 Species (heat map)

As shown in the figure 4.8 in the heartmap the expression of species in SFM for *s-Lactobacillus-ruminins*, *s-Coprococuss-comes*, *s-Eubacterium-rectale*, *s-uncultured-Alloprevotella* and s-uncultured-organism are highly expressed and these species are low expressed in SF.

Whereas the species in SFM for *s - Bacteroides*, s- *Mitosukella - jalaludinni*, *s - Mitosukella-multacida*, s-uncultured −*Wautersiella*, *s - Prevotella-Copri*, *s-metagenome*, *s-Roseburia - inlinivorans*, *s - Butyrvibrion-crossotus* and *s-Prevotella - stercocrea* were low expreesed and these species are highly expressed in SF.

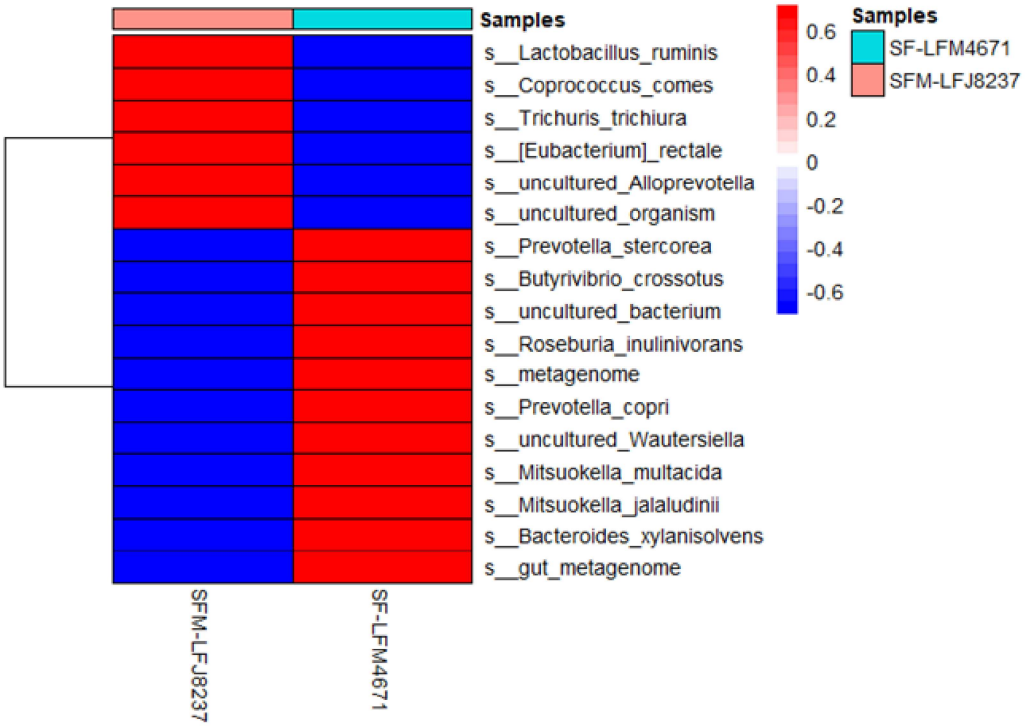


FIGURE 4.8: Species heat map of samples SFM (controlled) and SF (diseased)

4.6 Krona Plots

TABLE 4.5: Detailed comparison of SFM and SF gut microbiome based on krona plot

Species name	**SFM (%)**	**SF (%)**
s-Prevotella-bacterium	3	
s-uncultured-Alloprevotella	2	3
g-Muribaculaceae	1	
g-Faecalibacterium	8	5
Uncultured-bacterium	1	
Eubacterium-coprostanoligenes-group	2	
g-Lachnospiraceae-NK4136-group	2	
Blautia	1	2
s-Butyrivibrio-crossotus	1	
g-Clostridium-senso-stricto-1	2	
g-Megasphaera	2	
s-Bacteoides-xylanisolvens	1	3
g-Mitsuokella	1	

TABLE 4.5: Detailed comparison of SFM and SF gut microbiome based on krona plot

Species name	**SFM (%)**	**SF (%)**
c-Bacili	1	
g-Sutterella	1	
s-uncultured bacterium	2	
s-treponema-sucinifaciens	2	
p-Actinobacteriota	1	
Prevotellaceae-bacterium	5	
s-Prevotella-copri	3	4
s-uncultured bacterium	1	
s-Prevotella sterocorea	1	
g-prevotella	36	34
g-Bifidobacterium		9
g-Collinsella		2
Agathobacter		3
Roseburia		2
Gut-metagenome		2
Oscillospiraceae		2
Romboustia		2
s-Mitosuokella-multacida		2
s-Mitosuokella jalaludinii		2
s-lactobacillius-ruminis		4

4.7 Alpha Diversity

Shannon index is a measure of diversity within a colony of microbes. Both the evenness (distribution of abundances) and richness (number of distinct species) of the species present are considered by the index. If the Shannon index is close to 0, it suggests low diversity.

This could mean that one or a few species dominate the community, and there is limited variability in species abundances. A Shannon index value of SFM 9.241996

and in SF 9.142553714 suggests that the gut microbiome in the analyzed stool sample is diverse. There are likely many different species present, and the abundances of these species are relatively evenly distributed.

Higher diversity in the gut microbiome is often associated with a healthier state. A diverse microbial community is thought to contribute to various functions, including metabolic processes, immune system regulation, and overall gut health.

The diversity of the gut microbiome can be influenced by factors such as diet, lifestyle, and other environmental exposures. Certain diets, high in fiber and a variety of nutrients, are often associated with increased microbial diversity.

A Simpson diversity index value of SFM was 0.999802and in SF 0.999778094 in a metagenomic analysis of a stool sample indicates a very low level of diversity within the microbial community. The Simpson index quantifies the probability that two individuals randomly selected from the community belong to the same species.

A Simpson index value close to 1 suggests low diversity, meaning that one or a few species dominate the microbial community, and there is limited variability in species abundances.

In this context, a Simpson index aof 0.999778094 means that there is a very high probability (almost 100%) that two randomly selected individuals from the microbial community will belong to the same species. This indicates a highly uneven distribution of species, with one or a few species dominating the community.

An inverse Simpson index value of 4506.421184 in stool Metagenomics indicates a very high level of diversity within the microbial community. The inverse Simpson index is the reciprocal of the Simpson index, and both indices are commonly used to quantify diversity in ecological and metagenomic studies.

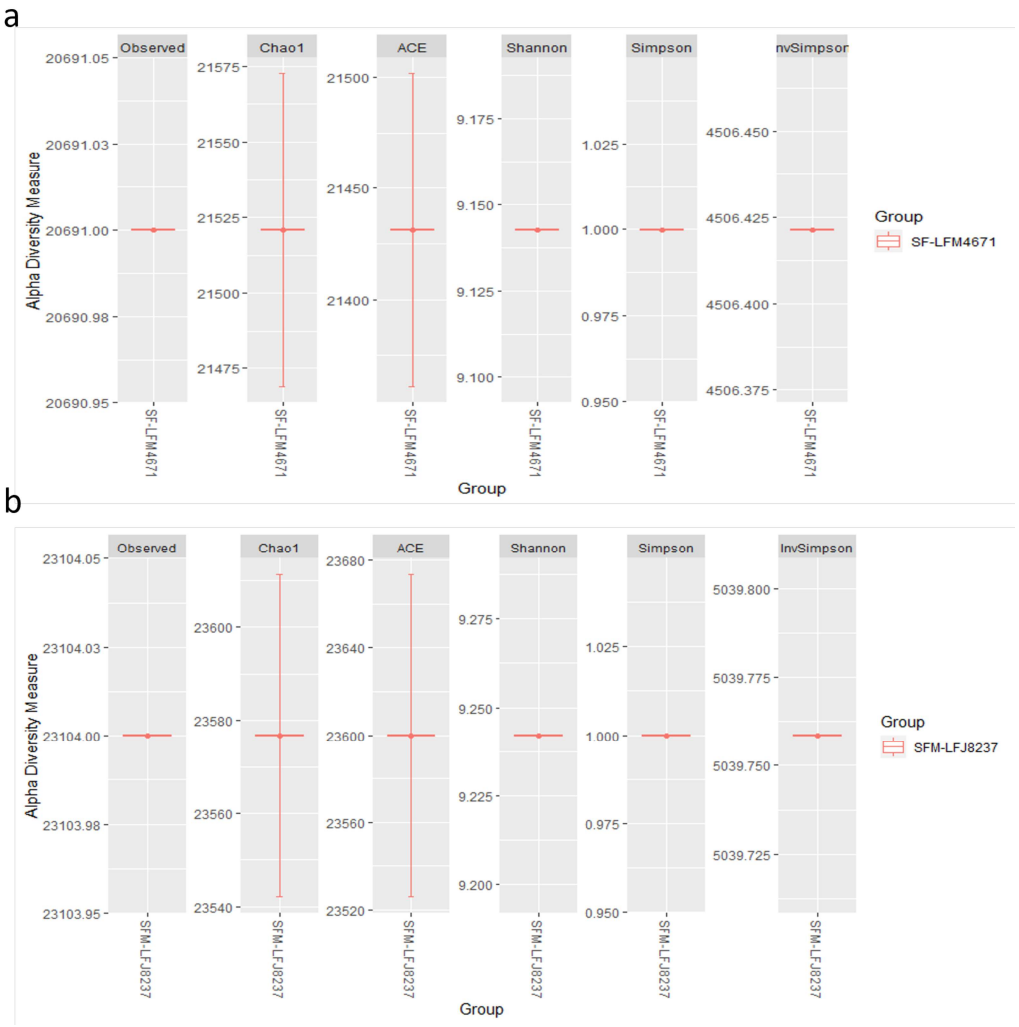


FIGURE 4.9: Shows the alpha diversity in SF (a) and SFM (b)

A high inverse Simpson index suggests a diverse microbial community with many different species, and the abundances of those species are relatively evenly distributed. In other words, there is a rich variety of microbial taxa in the stool sample.

The high value indicates that no single species dominates the community, and there is a more equitable distribution of species abundances. This is often associated with a healthy and balanced microbial community.

Chapter 5

Discussion

The intricate interplay between the human health and gut microbiome is an ever-evolving and captivating realm of study. The preservation of mass of skeletal muscles is aided by the gut bacteria. For instance, in germ-free mice mass of skeletal muscle exhibited a decrease in mass compared to skeletal muscle in pathogen-free mice. However, the loss of muscle mass in mice without gut microbes could be partially restored by transplanting pathogen-free mice's fecal microbiome into germ-free mice [26,187]. Likewise, piglets that are germ-free display reduced body weight and lean mass compared to their counterparts with normal intestinal microbiota [187]. There are noticeable differences in fiber type composition among muscles, both within individual animals and across different animals. [188]. Crucially, improving the ratio of slow-twitch or oxidative muscle fibers, which possess enhanced oxidative metabolism capabilities, is associated with superior meat quality compared to those predominantly composed of fast-twitch or glycolytic fibers [189]. Accumulating information suggests that the microbiome of gut significantly influences the development of muscle fibers. Transplanting fecal microbiota has shed light on the connection between muscle fiber properties and gut microbiota. Germ-free mice were analyzed subsequent to the introduction of a microbial gut population obtained from obese Rongchang pigs exhibited an elevated presence of slow-contracting fibers, a decrease in fiber size, and a lower ratio of fast IIB fibers [154]. Moreover, it was observed that germ-free piglets, upon colonization with

microbiota sourced from normal piglets, exhibited a consistent and partial restoration of slow-twitch muscle fibers. These findings emphasize the involvement the effects of gut bacteria on the properties of muscle fibers and provide an avenue for investigating the impact of gut microbiota on meat quality. The process relating to the function of skeletal muscle and the microbiota have gradually become more elucidated, and later on, there has been a focus on studying the contributions of bacterial metabolites to the change in the kinds of muscle fibers [187].

In recent decades, significant insights have been gained regarding the relationship between the gut microbiota and parameters including contractile and metabolic qualities, as well as skeletal muscle mass [190, 191, 26].. Despite this progress, the exact biochemical pathways that connect gut microbes to the operation of skeletal muscle are still mostly unknown. SCFAs and KBs are believed to confer diverse favorable effects to host metabolism energy through a proposed mechanism involving intracellular signaling. Peroxisome proliferator-activated receptor-gamma (PPARc) and/or G-protein couple receptor41, GPR43, and G-protein couple receptor 109A activation are required for this signaling pathway to function [192, 193].

Furthermore, current finding highlights the endocannabinoid system is a crucial regulator that mediates communication between the host and the gut flora [194, 195].

A complicated lipid cell-signaling system that is essential to both human health and disease is the endocannabinoid system (ECS). Despite the fact that the molecules in number linked to the ECS is constantly increasing, the lipid mediator's 2-arachidonoylglycerol (2-AG) and anandamide (AEA) are still regarded as important participants. Mostly, these compounds activate two G-coupled receptors that are dispersed differently throughout the body: cannabinoid receptor type 1 (CB1) and type 2 (CB2) [196]. Even though endocannabinoids, also known as plant cannabinoids, have great potential as complementary and/or alternative treatments, their possible application in skeletal muscle problems has not yet been thoroughly investigated [198]. Recent studies have shown that: (i) 2-AG stimulates proliferation in both human and murine skeletal muscle cell precursors

(myoblasts), but inhibits differentiation into mature muscle cells (myotubes); (ii) 2. In vivo, AG inhibits the development of skeletal muscle.; (iii) DMD-affected skeletal muscles in humans and mice exhibit a hyperactive endocannabinoid system (ECS); and (iv) In dystrophic animals, pharmacological suppression of the endocannabinoid CB1 receptor avoids locomotor impairment, lowers muscle inflammation, and stimulates the development of both satellite and myoblast cells into mature myotubes [196].

Numerous metabolic, neurological, and inflammatory illnesses are intimately connected with the growth and/or advancement of gut microbial dysbiosis [197, 198]. Accordingly, an increasing amount of research indicates that therapeutic interventions—like the intestinal bacteria transplantation or the use of probiotic- or prebiotic products—hold promising potential to address a variety of disorders by reversing disruptions in the gut microbiota. (i.e., dysbiosis) [199]. Patients with Duchenne muscular dystrophy (DMD) commonly experience nutritional, metabolic, and gastrointestinal challenges [200,201]. It is predicted that over half of children with Duchenne muscular dystrophy are overweight or obese due to chronic persistent inflammation, prolonged sedentary behavior, and prolonged usage of anti-inflammatory steroid medicines. On the other hand, underweight problems in adults and adolescents with DMD are frequently linked to poor swallowing function, insufficient dietary fiber, and decreased intestinal motility [202, 203].The commonly used experimental model of DMD, mdx mice, and exhibits comparable pathology characteristics [204].

The research showed that dystrophic mice had unique microbial alterations, including an increased *Prevotellaceae* family abundance. The *Helicobacteriaceae*, *Clostridiales_vadinBB60* families, *Peptococcaceae* and *Saccharimonadaceae*, on the other hand, had reduced relative abundances. Previous research reveals that members of the *Prevotellaceae* family, which includes about 40 different species, thrive in inflammatory microenvironments that are frequently found in diseases like inflammatory bowel disease, arthritis, and colorectal cancer. This research helps to explain this transition [205,206]. On the other hand, it is demonstrated a negative

correlation between *Saccharimonadaceae* abundance and the emergence of malfunctioning metabolism [207]. Notably, deflazacort (DFZ), a standard treatment for DMD, improved various pathological features in mdx mice and concurrently restored levels of *Prevotellaceae*, *Saccharimonadaceae*, and *Clostridiales_vadinBB60*. This suggests the potential involvement of these altered in these intestine bacterial taxa in the observed disease features. DFZ also induced changes in the relative mdx mice did not change the abundance of two families, which were raised and decreased, respectively: *Desulfovibrionaceae* and *Erysipelotrichaceae* [208].

The gut microbiota (GM) plays a crucial role in metabolizing organic substrates, derived from either undigested food in the intestinal tract or natural secretions such as mucopolysaccharides and cell debris. In the host tissues, GM has a role in the creation and control of gasses like nitric oxide and hydrogen sulfide (H2S), as well as neurotransmitters including histamine, serotonin, γ-amino butyric acid (GABA), and catecholamines. The production of these molecules, influenced by the intestinal bacteria, not only affects microbiome interactions through a "quorum" sensing system but also impacts gastro intestinal defense and is associated with distinct neurophysiological, metabolic, and intestinal characteristics. Numerous signaling pathways, including neuroendocrine circuitry like vagal nerves and enteric neurons, as well as the regulation of inflammatory profiles and systemic metabolites, may be involved in these effects [209,210].

Various byproducts produced by the gut microbiota (GM), including phenolic products, short chain fatty acids (SCFAs) bile acids from intestinal bacteria, and conjugated linoleic acid, have demonstrated the potential to enhance muscle glucose homeostasis, energy expenditure, protein synthesis, and physical performance. These effects are attained by direct targeting of skeletal muscle, interorgan cross talks, and/or intestinal permeability control. Numerous research have provided copious evidence on the distinct advantages of the primary GM SCFA metabolites—acetate, and butyrate—on insulin responses, blood glucose, and skeletal muscle function. Notably, recent studies showed that giving germ-free (GF) mice a combination of these SCFAs partially corrected the skeletal muscle damage brought on by GM deficiency, especially by increasing muscle strength [26].

The modification of intestinal permeability resulting from dysbiosis has been linked to persistent metabolic and inflammatory conditions, which may have an impact on the pathophysiology of muscles [191, 211, 212]. This idea was supported by the use of animal models with intestinal physical injury or infection to show that a particular strain of Escherichia coli can stop muscular atrophy. By using the NLRC4 inflammasome pathway, white adipose tissue's synthesis of insulin-like growth factor-1 (IGF-1) mediates this protective effect. The phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway in skeletal muscle is subsequently activated as a result [213].

Numerous studies highlight an affirmative correlation in relation to eubiosis and fitness. Notably, a ramp maximal activity test was used to assess peak oxygen uptake in healthy adults between the ages of 18 and 35. The results showed that, regardless of diet, there was an association between increasing gut microbiota (GM) diversity and peak oxygen absorption. Elevated fecal butyrate production and large concentrations of important butyrate-producing taxa, such as *Erysipelotrichaceae*, *Roseburia*, *Clostridiales* and *Lachnospiraceae*, were also linked to this increased GM diversity [214].

Genetic myopathies, including Steinert disease (myotonic dystrophy type 1 or MD1),limb-girdle muscular dystrophy (LGMD), Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), are associated changes in inflammation and metabolism. Patients with DMD and LGMD exhibit a drastic decrease in absorption of protein and an increase in catabolism of protein, a phenomenon potentially preserved in BMD patients. Insulin resistance in MD1 leads to the dysregulation of protein metabolism. These pathologies also feature alterations in lipid and muscle metabolisms. Additionally, systemic inflammation is a characteristic feature of these diseases, with elevated levels of TNFα observed in muscle biopsies of DMD patients compared to healthy subjects. Interestingly, higher TNFα levels are correlated with better muscle function, underscoring the intricate evolution of inflammation processes throughout the course of the disease [215].

Certain bacteria, such as *Akkermansia muciniphila* (*A. muciniphila*) and *Prevotella copri* (*P. copri*), have been linked to varying metabolic outcomes. P. copri

has shown associations with both positive and negative impacts on glucose tolerance and insulin sensitivity. On the other hand, while in genetically obese and diabetic mice, as well as in obese mice generated by diet, *A. muciniphila* levels are typically thought to be inversely correlated with metabolic diseases. However, other studies have found that *A. muciniphila* abundance is enhanced in mice fed a high-fat, high-sucrose diet. These results demonstrate the intricate connections between particular bacterial species and the consequences of metabolic health [216].

Prevotella copri is the most prevalent species of the many species that inhabit the human stomach. An estimated 40% of the general human population is thought to be affected, with some individuals having relative abundances higher than 50% [217].

Due to its correlation with low-fat, high-fiber diets, *Prevotella copri* is more common in non-Western populations [218; 219]. It is frequently associated with favorable health outcomes, such as decreased visceral fat and enhanced glucose metabolism [220, 221]. Nevertheless, there are contradictory studies that link *P. copri* to harmful diseases like persistent gut inflammation, hypertension, and insulin resistance [222]. The role of *P. copri* in health outcomes appears to be complex, and further research is needed to clarify its diverse associations [223].

Recent studies have revealed that the prevalent *Prevotella copri* ancestor is composed of at least four distinct species-level lineages, each exhibiting less than 95% average nucleotide identity with one another [217]. Additionally, there is significant variability in the utilization of polysaccharides among *P. copri* isolates, suggesting distinct metabolic patterns within this lineage [224]. The contradictory findings on P. copri and human health could be attributed to the differences in strain-specific metabolic variables, food preferences, and conventional taxonomic classifications and species boundaries. This emphasizes how important it is to fully characterize the diversity of microbes in the human microbiome in order to have a more detailed understanding of its functions [225].

Apart from *Prevotella copri*, the human gut is home to multiple other *Prevotella* species, including *P. rectalis*, *P. histicola*, and *P. stercorea*. Few of these species have just been identified through genomes retrieved from human gut metagenomes [226]. Based on the metagenomes of healthy human guts, *P.sterocrea* is probably the second most common and prevalent species in the guts of healthy humans, behind *P. copri* [227, 228]. The range of other *Prevotella* species, however, is far less well understood. To the best of our knowledge, *P. rectalis* and *P.ihumii*, for example, were recently isolated from people who did not appear to be ill and have not been linked to any medical disorders [229]. Although unclassified *Prevotella* are routinely identified by DNA-based investigations of the gut microbiome, these analyses are not always accurate in identifying the precise species. This limitation may arise from the uncharacterized diversity within the *Prevotella* lineage, making it challenging to identify specific species accurately [230, 231].

Given the significance of *Prevotella copri* in the intestinal microbiome of human and its roles in human health, as well as possible link to harmful circumstances, the hypothesis was formed that uncharacterized *Prevotella* species could also be essential for the functions human gut. It was reasoned that these uncharacterized species might possess comparable qualities with *P. copri*. Therefore, an evaluation of the entire variety and dispersal of *Prevotella* in the gut of human might be improved by evaluating their functions in health. From metagenomes of human gut *Prevotella* species are recovered were examined for their genomes, and the results showed that *P. copri* and *P. stercorea* are the primary relatives of the most common and reasonably numerous species in this context. The unique gene repertoires of these animals most likely represent adaptations to specific metabolic environments. Interestingly, *P. stercorea* and similar species have carbohydrate esterases but lack numerous genes involved in xylan metabolism, indicating they collaborate with *P. copri* to metabolize dietary fiber [230, 231].

Mitsuokella, a bacterial genus within the family *Alcaligenaceae*, encompasses various species, such as *Mitsuokella dentalis* and *Mitsuokella multiacidus*. These bacteria are commonly encountered in the human gastrointestinal tract, oral cavity, and diverse environmental sources. *Mitsuokella* species are recognized for their

capacity to generate acetic acid and succinic acid through fermentation [232].They have been identified in dental plaque and fecal samples, constituting a component of the normal microbial flora in these settings. While certain *Mitsuokella* species have been linked to human infections, they are generally regarded as commensal bacteria without established pathogenicity. Similar to many bacterial genera, the comprehension of *Mitsuokella* and its species undergoes continuous refinement through ongoing research in microbiology and microbial ecology [233].

In a determinative bacteriology study conducted in 2004, it was observed that certain bacterial genera, including *Selenomonas* (producing acetate and propionate), *Mitsuokella* (producing acetate), *Megasphaera* (producing lactate), and *Lactobacillus* (producing lactate), exhibited the capability to generate short-chain fatty acids (SCFAs), comprising lactate and propionate [234].

Bacteroides xylanisolvens is a species of bacteria within the *Bacteroides genus*, which is commonly found in the human gut microbiome. While specific information about *Bacteroides xylanisolvens* may be limited, here are some general functions and characteristics associated with *Bacteroides* species, which could provide insights into the potential roles of *Bacteroides xylanisolvens* [235]. *Bacteroides* species, including *Bacteroides xylanisolvens*, are known for their ability to break down complex carbohydrates that are resistant to digestion by human enzymes. This includes the fermentation of dietary fibers and other complex polysaccharides. Bacteroides species often form complex interactions within the microbial community of the gut, participating in cross-feeding and competition with other bacteria [236].

Dorea is a genus of bacteria that belongs to the phylum *Firmicutes.* It is commonly found in the human gut microbiome. The gut microbiome is a complex community of microorganisms, including bacteria, fungi, and viruses, that inhabit the gastrointestinal tract. The composition and diversity of the gut microbiome can vary among individuals. Bacteria in the genus Dorea are known for their ability to metabolize complex carbohydrates through fermentation, *producing* short-chain fatty acids (SCFAs) as byproducts [237]. SCFAs can have various effects on the host, including influencing immune function and providing a source of energy for

colonocytes. *Dorea* species may engage in metabolic interactions with the host and other microbes in the gut. These interactions can influence nutrient utilization and energy metabolism [233].

Lactobacillus ruminis is a species of bacteria that is commonly found in the gastrointestinal tract, particularly in the rumen of ruminant animals such as cows and sheep. *Lactobacillus ruminis* is recognized as an indigenous species naturally present in the human gastrointestinal tract (GIT) [238]. Lactobacillus species, including *L. ruminis*, are often associated with promoting gut homeostasis. They contribute to the balance of the gut microbiota and help prevent the overgrowth of harmful bacteria. Previous research has emphasized the potential immune modulatory properties of Lactobacillus ruminis [239, 240], indicating its potential role in influencing the immune system. Moreover, there is supporting evidence suggesting that Lactobacillus ruminis may contribute to the suppression of antibiotic-resistant pathogens [241].

Chapter 6

Conclusion and Recommendations

Muscular dystrophies are a class of degenerative disorders which are hereditary in nature. Muscular dystrophy is responsible for muscle weakness that is progressive. Skeletal muscle growth and development, which makes up around 50% of a mammal's body mass, indirectly affects the amount and quality of pork by affecting things like, meat color, juiciness, tenderness, and pH and drip loss. In general, a number of variables, including as exercise, genotype, hormones, nutrition, breed, muscle location environmental temperature, affect how muscles grow and develop. Interestingly, new studies suggest that the growth, development, and function of muscles can be impacted by preserving a stable and diversified gut flora. Therefore, there is a perception that the gut microbiota could be a viable biological target for increasing the amount and quality of muscle. The first objective was to determine the prevalence of type of muscular dystrophy. Questionnaire were filled by the 60 healthy and 60 muscular dystrophy subjects with informed consent. Limbic Girdle Muscular Dystrophy was the most prevalent kind of muscular dystrophy among the individuals that participated in this study. The prevalence of Limbic Girdle Muscular Dystrophy was 34%, followed by Duchenne Muscular Dystrophy 28.3%, Myotonic Muscular Dystrophy 15%, and Becker Muscular Dystrophy is 9.4%. The differences of prevalence can be seen as compared to those mentioned in literature review due to multiple factors like diet and lifestyle. The prevalence of diagnosed

muscular dystrophy patients was 96% and that of undiagnosed patients were 4% in total sample size of 60 individuals. Among the diseased individuals who participated in the study 87% had not used antibiotics and 13% had used antibiotics ,39% had not used medications and 61% had used medications, 5% has used and 95% had not used the recreational drugs during the previous three months. The individuals shortlisted as experimental group for the collection of stool samples were those who had not used any antibiotics, medications, recreational drugs and were diagnosed with the disease and their prevalence was found to be 66% in total sample size of 60 individuals.

Second objective meant to evaluate the impact of diet on disease and symptoms indicates that data of muscular dystrophy patient also showed the association. The coefficient for Symptoms was found to be 0.347 ($t = 14.866$, $p < 0.001$), suggesting that, while controlling for Types of Disease, a one-unit increase in Symptoms is associated with a 0.347 unit increase in diet. The 95% confidence interval for this coefficient ranges from 0.300 to 0.394. In this case, a higher t-value (14.866) indicates a more significant relationship. The p-value (< 0.001) suggests that the relationship between Symptoms and diet is statistically significant. In other words, it's unlikely to be due to random chance. The interpretation indicates a statistically significant positive association between Symptoms and diet, after accounting for the influence of Types of Disease, and provides a range of values for the estimated effect with a high level of confidence. Both Symptoms and Types of Disease appeared as significant predictors of diet, contributing to the strength of the regression model. These findings offer valuable insights into the factors influencing diet, paving the way for a more understanding of the relationships among the variables under investigation. To evaluate the difference between muscular dystrophy and healthy people Metagenomics was performed. To ensure the presences of bacterial strains gene sequence analysis was done. In taxonomic bar plots the most prevalent class SFM was , *Bacteroidia* (47%), *Clostridia* (37%) followed by *Negativicutes* (4%), *Coriobacteriia* (0.5%), whereas in SF the *Bacteroidia* (32%), *clostridia* (33%), *Negativicutes* (6%) *Coriobacteria* (2%). At order level the most abundant was in control sample was *Bacteroidales* (53%) and in muscular dystrophy patient was *bacteriodales* (40%). The difference in variation at order level in SFM was

Aeromonadales (1%), *Elusimicrobiales* (2%), *Spirochaetales* (1.5%) whereas in SF *Bifidoacteriales* (7%), *Erysipelotrichales* (1%) and *Peptostretococccales- Tissierellales* (2%) . At genus level the genus preveotella is 48% in control sample and 58% in muscular dystrophy patient. The genus that were different in SFM *Elusimicrobium* (2%), *Lachnospiraceae-NK4A136-group* (1%), *Megasphaera* (2%), *Roseburia* (4%), *Treponema* (1%), UCG-002 (1%) and uncultured (2%).whereas in SF *[Eubacterium]-Coprostanoligenes-group* (2%), *Agathobacter* (3%), *Bifidobacterium* (12.5%), *Coprococcus* (2%), *Dialister* (3%), *Lactobacillus* (4%) and *Mitsuokella* (5%) In the species bar plot the specie uncultured –*Alloprevotella* was 26%, and *Prevotella-copri* (8%) in control sample and uncultured –*Alloprevotella* (13%) and *prevotella-copri* (15%) in muscular dystrophy patient. The species that were different between SFM and SF was *Prevotellaceae-Bacterium* (26%), *Treponema-Succinifaciens* (5%) in SFM, whereas in SF was *Bacteroides-Xylanisolvens* (10%), *Dorea-Longicatena* (4%), *Lactobacillus-Ruminis* (14%), *Mitsuokella- jalaludinii* (6%), *Mitsuokella-multacida* (8%). The results indicates that the population of microbiome was different in both healthy and disease patients and diet also effects the disease and microbiome population.

The current study revealed that population of microbiome varied among the muscular dystrophy and healthy subjects. It can be concluded that addressing the microbiome in genetic disorders can help to manage the severity of disease and can have potential for therapeutic purpose.Further research involving both healthy and disease participants will advance our understanding of the microbiota-skeletal muscle axis by elucidating its underlying mechanisms at the levels of metabolism, immunity, inflammation, hormones, and neurotransmission, as well as providing insight into its pathophysiological consequences.

It will additionally delineate the regulatory impact of specific bacteria on the processes of muscle protein synthesis and degradation.

In light of the significant impact that the gut microbiota (GM) has on endocrine, metabolic, and inflammatory processes, more computer modeling and experimental studies are desperately needed. The objective is to find novel tailored instruments that target the gut microbiota in particular with the aim of maintaining

the health of skeletal muscle in the setting of hereditary illnesses, as previously stated.

Studies on microbiota-based interventions and the incorporation of in silico or mathematical modeling, considering interactions of bacteria, are expected to enhance personalized guidance regarding supplements, nutrients and/or physical activity to address weakness of muscles.

Bibliography

[1] Michele, D.E. and K.P. Campbell, Dystrophin-Glycoprotein Complex: Post-translational Processing and Dystroglycan Function * 210. Journal of Biological Chemistry, 2003. 278(18): p. 15457-15460.

[2] Heydemann, A., K.R. Doherty, and E.M. McNally, Genetic modifiers of muscular dystrophy: Implications for therapy. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2007. 1772(2): p. 216-228.

[3] Millay, D.P., Goonasekera, S. A.Sargent, M. A.Maillet, M.Aronow, B. J.Molkentin, J. D, Calcium influx is sufficient to induce muscular dystrophy through a TRPC-dependent mechanism. Proc Natl Acad Sci U S A, 2009. 106(45): p. 19023-8

[4] Salari, N., Fatahi, B.Valipour, E.Kazeminia, M.Fatahian, R.Kiaei, A.Shohaimi, S.Mohammadi, M., Global prevalence of Duchenne and Becker muscular dystrophy: a systematic review and meta-analysis. J Orthop Surg Res, 2022. 17(1): p. 96.

[5] Nozoe, K. T,Akamine, R. T,Mazzotti, D. R,Polesel, D. N,Grossklauss, L. F,Tufik, S,Andersen, M. L..Moreira, G. A..,Phenotypic contrasts of Duchenne Muscular Dystrophy in women: Two case reports. Sleep Sci, 2016. 9(3): p. 129-133.

[6] Fujii, K.,Minami, N.,Hayashi, Y.,Nishino, I.,Nonaka, I.,Tanabe, Y.,Takanashi, J.,Kohno, Y., Homozygous female Becker muscular dystrophy. Am J Med Genet A, 2009. 149a(5): p. 1052-5.

[7] Li, S.,Li, Q.,Chen, X.,Liu, W.,Sun, X., Duchenne muscular dystrophy in a female patient with a karyotype of 46,X,i(X)(q10). Tohoku J Exp Med, 2010. 222(2): p. 149-53.

[8] Durbeej, M., Laminin-α2 Chain-Deficient Congenital Muscular Dystrophy: Pathophysiology and Development of Treatment. Curr Top Membr, 2015. 76: p. 31-60.

[9] Narayanaswami, P.,Weiss, M.,Selcen, D.,David, W.,Raynor, E.,Carter, G.,Wicklund, M.,Barohn, R. J.,Ensrud, E.,Griggs, R. C.,Gronseth, G.,Amato, A. A.,Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology, 2014. 83(16): p. 1453-63.

[10] Wallace, G.Q. and E.M. McNally, Mechanisms of muscle degeneration, regeneration, and repair in the muscular dystrophies. Annu Rev Physiol, 2009. 71: p. 37-57.

[11] Constantin, B., Dystrophin complex functions as a scaffold for signalling proteins. Biochimica et Biophysica Acta (BBA) - Biomembranes, 2014. 1838(2): p. 635-642.

[12] Prins, K. W.,Humston, J. L.,Mehta, A.,Tate, V.,Ralston, E.,Ervasti, J. M., Dystrophin is a microtubule-associated protein. J Cell Biol, 2009. 186(3): p. 363-9.

[13] Chakkalakal, J. V.,Thompson, J.,Parks, R. J.,Jasmin, B. J., Molecular, cellular, and pharmacological therapies for Duchenne/Becker muscular dystrophies. Faseb j, 2005. 19(8): p. 880-91.

[14] Delfń, D. A.,Xu, Y.,Peterson, J. M.,Guttridge, D. C.,Rafael-Fortney, J. A.,Janssen, P. M., Improvement of cardiac contractile function by peptide-based inhibition of NF-κB in the utrophin/dystrophin-deficient murine model of muscular dystrophy. J Transl Med, 2011. 9: p. 68.

[15] Bhatnagar, S. and A. Kumar, Therapeutic targeting of signaling pathways in muscular dystrophy. J Mol Med (Berl), 2010. 88(2): p. 155-66.

[16] Tidball, J.G., Regulation of muscle growth and regeneration by the immune system. Nat Rev Immunol, 2017. 17(3): p. 165-178.

[17] Cascabulho, C. M.,Beghini, D. G.,Meuser-Batista, M.,Penido, C.,Henriques-Pons, A., Chemotaxis and Immunoregulatory Function of Cardiac $\gamma\delta$ T Cells in Dystrophin-Deficient Mice. J Immunol, 2016. 197(9): p. 3531-3544.

[18] Nishimura, D.,Sakai, H.,Sato, T.,Sato, F.,Nishimura, S.,Toyama-Sorimachi, N.,Bartsch, J. W.,Sehara-Fujisawa, A., Roles of ADAM8 in elimination of injured muscle fibers prior to skeletal muscle regeneration. Mech Dev, 2015. 135: p. 58-67.

[19] Wilton-Clark, H. and T. Yokota, Biological and genetic therapies for the treatment of Duchenne muscular dystrophy. Expert Opin Biol Ther, 2023. 23(1): p. 49-59.

[20] Yao, S.,Chen, Z.,Yu, Y.,Zhang, N.,Jiang, H.,Zhang, G.,Zhang, Z.,Zhang, B., Current Pharmacological Strategies for Duchenne Muscular Dystrophy. Front Cell Dev Biol, 2021. 9: p. 689533.

[21] Lovering, R.M., N.C. Porter, and R.J. Bloch, The muscular dystrophies: from genes to therapies. Phys Ther, 2005. 85(12): p. 1372-88.

[22] Escobar-Huertas, J. F.,Vaca-González, Juan Jairo,Guevara, Johana María,Ramirez-Martinez, Angelica M.,Trabelsi, Olfa.,Garzón-Alvarado, D. A., Duchenne and Becker muscular dystrophy: Cellular mechanisms, image analysis, and computational models: A review. Cytoskeleton, 2024. n/a(n/a).

[23] Welch, E. M.,Barton, E. R.,Zhuo, J.,Tomizawa, Y.,Friesen, W. J.,Trifillis, P.,Paushkin, S.,Patel, M.,Trotta, C. R.,Hwang, S.,Wilde, R. G.,Karp, G.,Takasugi, J.,Chen, G.,Jones, S.,Ren, H.,Moon, Y. C.,Corson, D.,Turpoff, A. A.,Campbell, J. A.,Conn, M. M.,Khan, A.,Almstead, N. G.,Hedrick, J.,Mollin, A.,Risher, N.,Weetall, M.,Yeh, S.,Branstrom, A. A.,Colacino, J. M.,Babiak, J.,Ju, W. D.,Hirawat, S.,Northcutt, V. J.,Miller, L. L.,Spatrick,

P.,He, F.,Kawana, M.,Feng, H.,Jacobson, A.,Peltz, S. W.,Sweeney, H. L., PTC124 targets genetic disorders caused by nonsense mutations. Nature, 2007. 447(7140): p. 87-91.

[24] Gonzalez-Hilarion, S.,Beghyn, T.,Jia, J.,Debreuck, N.,Berte, G.,Mamchaoui, K.,Mouly, V.,Gruenert, D. C.,Déprez, B.,Lejeune, F., Rescue of nonsense mutations by amlexanox in human cells. Orphanet J Rare Dis, 2012. 7: p. 58.

[25] Loro, E.,Sengupta, K.,Bogdanovich, S.,Whig, K.,Schultz, D. C.,Huryn, D. M.,Khurana, T. S., High-throughput identification of post-transcriptional utrophin up-regulators for Duchenne muscle dystrophy (DMD) therapy. Sci Rep, 2020. 10(1): p. 2132.

[26] Lahiri, S.,Kim, H.,Garcia-Perez, I.,Reza, M. M.,Martin, K. A.,Kundu, P.,Cox, L. M.,Selkrig, J.,Posma, J. M.,Zhang, H.,Padmanabhan, P.,Moret, C.,Gulyás, B.,Blaser, M. J.,Auwerx, J.,Holmes, E.,Nicholson, J.,Wahli, W.,Pettersson, S., The gut microbiota influences skeletal muscle mass and function in mice. Sci Transl Med, 2019. 11(502).

[27] Mann, A. O.,Hanna, B. S.,Muñoz-Rojas, A. R.,Sandrock, I.,Prinz, I.,Benoist, C.,Mathis, D., IL-17A-producing $\gamma\delta$T cells promote muscle regeneration in a microbiota-dependent manner. J Exp Med, 2022. 219(5).

[28] Crisafulli, S.,Sultana, J.,Fontana, A.,Salvo, F.,Messina, S.,Trifirò, G., Global epidemiology of Duchenne muscular dystrophy: an updated systematic review and meta-analysis. Orphanet J Rare Dis, 2020. 15(1): p. 141.

[29] Cirak, S.,Feng, L.,Anthony, K.,Arechavala-Gomeza, V.,Torelli, S.,Sewry, C.,Morgan, J. E.,Muntoni, F., Restoration of the dystrophin-associated glycoprotein complex after exon skipping therapy in Duchenne muscular dystrophy. Mol Ther, 2012. 20(2): p. 462-7.

[30] Sandri, M.,Coletto, L.,Grumati, P.,Bonaldo, P., Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. J Cell Sci, 2013. 126(Pt 23): p. 5325-33.

[31] De Palma, C.,Perrotta, C.,Pellegrino, P.,Clementi, E.,Cervia, D., Skeletal muscle homeostasis in duchenne muscular dystrophy: modulating autophagy as a promising therapeutic strategy. Front Aging Neurosci, 2014. 6: p. 188.

[32] Sheikh, O. and T. Yokota, Advances in Genetic Characterization and Genotype-Phenotype Correlation of Duchenne and Becker Muscular Dystrophy in the Personalized Medicine Era. J Pers Med, 2020. 10(3).

[33] Bushby, K.,Finkel, R.,Birnkrant, D. J.,Case, L. E.,Clemens, P. R.,Cripe, L.,Kaul, A.,Kinnett, K.,McDonald, C.,Pandya, S.,Poysky, J.,Shapiro, F.,Tomezsko, J.,Constantin, C., Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol, 2010. 9(1): p. 77-93.

[34] Griggs, R. C.Miller, J. P.Greenberg, C. R.Fehlings, D. L.Pestronk, A.Mendell, J. R.Moxley, R. T.,King, W.,Kissel, J. T.,Cwik, V.,Vanasse, M.,Florence, J. M.,Pandya, S.,Dubow, J. S.,Meyer, J. M., Efficacy and safety of deflazacort vs prednisone and placebo for Duchenne muscular dystrophy. Neurology, 2016. 87(20): p. 2123-2131.

[35] Matthews, E.,Brassington, R.,Kuntzer, T.,Jichi, F.,Manzur, A. Y., Corticosteroids for the treatment of Duchenne muscular dystrophy. Cochrane Database Syst Rev, 2016. 2016(5): p. Cd003725.

[36] Biggar, W.D., A. Skalsky, and C.M. McDonald, Comparing Deflazacort and Prednisone in Duchenne Muscular Dystrophy. J Neuromuscul Dis, 2022. 9(4): p. 463-476.

[37] De Luca, F. and Y. Shoenfeld, The microbiome in autoimmune diseases. Clin Exp Immunol, 2019. 195(1): p. 74-85.

[38] Tulangekar, A. and T.E. Sztal Inflammation in Duchenne Muscular Dystrophy–Exploring the Role of Neutrophils in Muscle Damage and Regeneration. Biomedicines, 2021. 9, DOI: 10.3390/biomedicines9101366.

[39] Costello, E. K.,Stagaman, K.,Dethlefsen, L.,Bohannan, B. J.,Relman, D. A., The application of ecological theory toward an understanding of the human microbiome. Science, 2012. 336(6086): p. 1255-62.

[40] Walter, J. and R. Ley, The Human Gut Microbiome: Ecology and Recent Evolutionary Changes. Annual review of microbiology, 2011. 65: p. 411-29.

[41] Goodrich, J. K.,Davenport, E. R.,Beaumont, M.,Jackson, M. A.,Knight, R.,Ober, C.,Spector, T. D.,Bell, J. T..Clark, A. G.,Ley, R. E., Human genetics shape the gut microbiome. Cell, 2014. 159(4): p. 789-99.

[42] Mah, J. K.,Korngut, L.,Fiest, K. M.,Dykeman, J.,Day, L. J.,Pringsheim, T.,Jette, N., A Systematic Review and Meta-analysis on the Epidemiology of the Muscular Dystrophies. Can J Neurol Sci, 2016. 43(1): p. 163-77.

[43] Zhang, Z.,Pinto, A. M.,Wan, L.,Wang, W.,Berg, M. G.,Oliva, I.,Singh, L. N.,Dengler, C.,Wei, Z..Dreyfuss, G., Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. Proc Natl Acad Sci U S A, 2013. 110(48): p. 19348-53.

[44] Emery, A.E., Population frequencies of inherited neuromuscular diseases–a world survey. Neuromuscul Disord, 1991. 1(1): p. 19-29.

[45] Guo, H., J.B. Callaway, and J.P. Ting, Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med, 2015. 21(7): p. 677-87.

[46] Chen, X. and G.Y. Yan, Novel human lncRNA-disease association inference based on lncRNA expression profiles. Bioinformatics, 2013. 29(20): p. 2617-24.

[47] Aartsma-Rus, A.,Van Deutekom, J. C.,Fokkema, I. F.,Van Ommen, G. J.,Den Dunnen, J. T., Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve, 2006. 34(2): p. 135-44.

[48] Kuyumcu-Martinez, N.M., G.S. Wang, and T.A. Cooper, Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. Mol Cell, 2007. 28(1): p. 68-78.

[49] Zu, T.,Cleary, J. D.,Liu, Y.,Bañez-Coronel, M.,Bubenik, J. L.,Ayhan, F.,Ashizawa, T.,Xia, G.,Clark, H. B.,Yachnis, A. T.,Swanson, M. S.,Ranum, L. P. W., RAN Translation Regulated by Muscleblind Proteins in Myotonic Dystrophy Type 2. Neuron, 2017. 95(6): p. 1292-1305.e5.

[50] Sznajder Ł, J.,Michalak, M.,Taylor, K.,Cywoniuk, P.,Kabza, M.,Wojtkowiak-Szlachcic, A.,Matłoka, M.,Konieczny, P.,Sobczak, K., Mechanistic determinants of MBNL activity. Nucleic Acids Res, 2016. 44(21): p. 10326-10342.

[51] Querido, E.,Gallardo, F.,Beaudoin, M.,Ménard, C.,Chartrand, P., Stochastic and reversible aggregation of mRNA with expanded CUG-triplet repeats. J Cell Sci, 2011. 124(Pt 10): p. 1703-14.

[52] Carrell, S. T.,Carrell, E. M.,Auerbach, D.,Pandey, S. K.,Bennett, C. F.,Dirksen, R. T.,Thornton, C. A., Dmpk gene deletion or antisense knockdown does not compromise cardiac or skeletal muscle function in mice. Hum Mol Genet, 2016. 25(19): p. 4328-4338.

[53] Pandey, S. N.,Kesari, A.Yokota, T.Pandey, G. S., Muscular Dystrophy: Disease Mechanisms and Therapies. Biomed Res Int, 2015. 2015: p. 456348.

[54] Nigro, V., S. Aurino, and G. Piluso, Limb girdle muscular dystrophies: update on genetic diagnosis and therapeutic approaches. Curr Opin Neurol, 2011. 24(5): p. 429-36.

[55] Nigro, V., Molecular bases of autosomal recessive limb-girdle muscular dystrophies. Acta Myol, 2003. 22(2): p. 35-42.

[56] Nigro, V. and G. Piluso, Next generation sequencing (NGS) strategies for the genetic testing of myopathies. Acta Myol, 2012. 31(3): p. 196-200.

[57] Deenen, J. C.,Arnts, H.,van der Maarel, S. M.,Padberg, G. W.,Verschuuren, J. J.,Bakker, E.,Weinreich, S. S.,Verbeek, A. L.,van Engelen, B. G., Population-based incidence and prevalence of facioscapulohumeral dystrophy. Neurology, 2014. 83(12): p. 1056-9.

[58] Sacconi, S., L. Salviati, and C. Desnuelle, Facioscapulohumeral muscular dystrophy. Biochim Biophys Acta, 2015. 1852(4): p. 607-14.

[59] Orrell, R.W., Facioscapulohumeral dystrophy and scapuloperoneal syndromes. Handb Clin Neurol, 2011. 101: p. 167-80.

[60] Cabianca, D. S.,Casa, V.,Bodega, B.,Xynos, A.,Ginelli, E.,Tanaka, Y.,Gabellini, D., A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell, 2012. 149(4): p. 819-31.

[61] Bönnemann, C. G.,Wang, C. H.,Quijano-Roy, S.,Deconinck, N.,Bertini, E.,Ferreiro, A.,Muntoni, F.,Sewry, C.,Béroud, C.,Mathews, K. D.,Moore, S. A.,Bellini, J.,Rutkowski, A.,North, K. N., Diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord, 2014. 24(4): p. 289-311.

[62] Wheeler, T. M.,Leger, A. J.,Pandey, S. K.,MacLeod, A. R.,Nakamori, M.,Cheng, S. H.,Wentworth, B. M.,Bennett, C. F.,Thornton, C. A., Targeting nuclear RNA for in vivo correction of myotonic dystrophy. Nature, 2012. 488(7409): p. 111-5.

[63] Evilä, A.,Vihola, A.,Sarparanta, J.,Raheem, O.,Palmio, J.,Sandell, S.,Eymard, B.,Illa, I.,Rojas-Garcia, R.,Hankiewicz, K.,Negrão, L.,Löppönen, T.,Nokelainen, P.,Kärppä, M.,Penttilä, S.,Screen, M.,Suominen, T.,Richard, I.,Hackman, P.,Udd, B., Atypical phenotypes in titinopathies explained by second titin mutations. Ann Neurol, 2014. 75(2): p. 230-40.

[64] Boland, B.,Yu, W. H.,Corti, O.,Mollereau, B.,Henriques, A.,Bezard, E.,Pastores, G. M.,Rubinsztein, D. C.,Nixon, R. A.,Duchen, M. R.,Mallucci, G. R.,Kroemer, G.,Levine, B.,Eskelinen, E. L.,Mochel, F.,Spedding, M.,Louis, C.,Martin, O. R.,Millan, M. J., Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nat Rev Drug Discov, 2018. 17(9): p. 660-688.

[65] Galluzzi, L.,Kepp, O.,Chan, F. K.,Kroemer, G., Necroptosis: Mechanisms and Relevance to Disease. Annu Rev Pathol, 2017. 12: p. 103-130.

[66] Astejada, M. N.,Goto, K.,Nagano, A.,Ura, S.,Noguchi, S.,Nonaka, I.,Nishino, I.,Hayashi, Y. K., Emerinopathy and laminopathy clinical, pathological and

molecular features of muscular dystrophy with nuclear envelopathy in Japan. Acta Myol, 2007. 26(3): p. 159-64.

[67] Fanin, M. and C. Angelini, Protein and genetic diagnosis of limb girdle muscular dystrophy type 2A: The yield and the pitfalls. Muscle Nerve, 2015. 52(2): p. 163-73.

[68] Dowling, J. J.,D. Gonorazky H,Cohn, R. D.,Campbell, C., Treating pediatric neuromuscular disorders: The future is now. Am J Med Genet A, 2018. 176(4): p. 804-841.

[69] Sweeney, H.L. and E.R. Barton, The dystrophin-associated glycoprotein complex: what parts can you do without? Proc Natl Acad Sci U S A, 2000. 97(25): p. 13464-6.

[70] Culligan, K. and K. Ohlendieck, Diversity of the Brain Dystrophin-Glycoprotein Complex. J Biomed Biotechnol, 2002. 2(1): p. 31-36.

[71] Denetclaw, W. F., Jr.,Hopf, F. W.,Cox, G. A.,Chamberlain, J. S.,Steinhardt, R. A., Myotubes from transgenic mdx mice expressing full-length dystrophin show normal calcium regulation. Mol Biol Cell, 1994. 5(10): p. 1159-67.

[72] Avila-Medina, J.,Mayoral-Gonzalez, I.,Dominguez-Rodriguez, A.,Gallardo-Castillo, I.,Ribas, J.,Ordoñez, A.,Rosado, J. A.,Smani, T., The Complex Role of Store Operated Calcium Entry Pathways and Related Proteins in the Function of Cardiac, Skeletal and Vascular Smooth Muscle Cells. Front Physiol, 2018. 9: p. 257.

[73] Goll, D. E.,Neti, G.,Mares, S. W.,Thompson, V. F., Myofibrillar protein turnover: the proteasome and the calpains. J Anim Sci, 2008. 86(14 Suppl): p. E19-35.

[74] Schroder, E. A.,Wang, L.,Wen, Y.,Callahan, L. A. P.,Supinski, G. S., Skeletal muscle-specific calpastatin overexpression mitigates muscle weakness in aging and extends life span. J Appl Physiol (1985), 2021. 131(2): p. 630-642.

[75] Porter, J. D.,Khanna, S.,Kaminski, H. J.,Rao, J. S.,Merriam, A. P.,Richmonds, C. R.,Leahy, P.,Li, J.,Guo, W.,Andrade, F. H., A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. Hum Mol Genet, 2002. 11(3): p. 263-72.

[76] Evans, N. P.,Misyak, S. A.,Robertson, J. L.,Bassaganya-Riera, J.,Grange, R. W., Immune-mediated mechanisms potentially regulate the disease time-course of duchenne muscular dystrophy and provide targets for therapeutic intervention. Pm r, 2009. 1(8): p. 755-68.

[77] Wehling, M., M.J. Spencer, and J.G. Tidball, A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice. J Cell Biol, 2001. 155(1): p. 123-31.

[78] Hodgetts, S.,Radley, H.,Davies, M.,Grounds, M. D., Reduced necrosis of dystrophic muscle by depletion of host neutrophils, or blocking TNFalpha function with Etanercept in mdx mice. Neuromuscul Disord, 2006. 16(9-10): p. 591-602.

[79] Haycock, J. W.,MacNeil, S.,Jones, P.,Harris, J. B.,Mantle, D., Oxidative damage to muscle protein in Duchenne muscular dystrophy. Neuroreport, 1996. 8(1): p. 357-61.

[80] Li, H., S. Malhotra, and A. Kumar, Nuclear factor-kappa B signaling in skeletal muscle atrophy. J Mol Med (Berl), 2008. 86(10): p. 1113-26.

[81] Changchien, C. Y.,Lin, Y. H.,Cheng, Y. C.,Chang, H. H.,Peng, Y. S.,Chen, Y., Indoxyl sulfate induces myotube atrophy by ROS-ERK and JNK-MAFbx cascades. Chem Biol Interact, 2019. 304: p. 43-51.

[82] Nevin, J.,Nevin, N. C.,Dornan, J. C.,Sim, D.,Armstrong, M. J., Early amniocentesis: experience of 222 consecutive patients, 1987-1988. Prenat Diagn, 1990. 10(2): p. 79-83.

[83] Ta, M. H.,Tran, T. H.,Do, N. H.,Pham le, A. T.,Bui, T. H.,Ta, V. T.,Tran, V. K., Rapid method for targeted prenatal diagnosis of Duchenne muscular dystrophy in Vietnam. Taiwan J Obstet Gynecol, 2013. 52(4): p. 534-9.

[84] Parks, M.,Court, S.,Cleary, S.,Clokie, S.,Hewitt, J.,Williams, D.,Cole, T.,MacDonald, F.,Griffiths, M.,Allen, S., Non-invasive prenatal diagnosis of Duchenne and Becker muscular dystrophies by relative haplotype dosage. Prenat Diagn, 2016. 36(4): p. 312-20.

[85] Lee, S. H.,Kwak, I. P.,Cha, K. E.,Park, S. E.,Kim, N. K.,Cha, K. Y., Preimplantation diagnosis of non-deletion Duchenne muscular dystrophy (DMD) by linkage polymerase chain reaction analysis. Mol Hum Reprod, 1998. 4(4): p. 345-9.

[86] Mendell, J. R.,Shilling, C.,Leslie, N. D.,Flanigan, K. M.,al-Dahhak, R.,Gastier-Foster, J.,Kneile, K.,Dunn, D. M.,Duval, B.,Aoyagi, A.,Hamil, C.,Mahmoud, M.,Roush, K.,Bird, L.,Rankin, C.,Lilly, H.,Street, N.,Chandrasekar, R.,Weiss, R. B., Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol, 2012. 71(3): p. 304-13.

[87] Tuffery-Giraud, S.,Saquet, C.,Chambert, S.,Echenne, B.,Marie Cuisset, J.,Rivier, F.,Cossée, M.,Philippe, C.,Monnier, N.,Bieth, E.,Recan, D.,Antoinette Voelckel, M.,Perelman, S.,Lambert, J. C.,Malcolm, S.,Claustres, M., The role of muscle biopsy in analysis of the dystrophin gene in Duchenne muscular dystrophy: experience of a national referral centre. Neuromuscul Disord, 2004. 14(10): p. 650-8.

[88] Emery, A.E., The muscular dystrophies. Lancet, 2002. 359(9307): p. 687-95.

[89] Muntoni, F., Is a muscle biopsy in Duchenne dystrophy really necessary? Neurology, 2001. 57(4): p. 574-5.

[90] Bushby, K.,Finkel, R.,Birnkrant, D. J.,Case, L. E.,Clemens, P. R.,Cripe, L.,Kaul, A.,Kinnett, K.,McDonald, C.,Pandya, S.,Poysky, J.,Shapiro, F.,Tomezsko, J.,Constantin, C., Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol, 2010. 9(1): p. 77-93.

[91] Voit, T.,Stuettgen, P.,Cremer, M.,Goebel, H. H., Dystrophin as a diagnostic marker in Duchenne and Becker muscular dystrophy. Correlation of immunofluorescence and western blot. Neuropediatrics, 1991. 22(3): p. 152-62.

[92] Saad, F.A., G. Siciliano, and C. Angelini, Advances in Dystrophinopathy Diagnosis and Therapy. Biomolecules, 2023. 13(9).

[93] Hoffman, E. P.,Fischbeck, K. H.,Brown, R. H.,Johnson, M.,Medori, R.,Loike, J. D.,Harris, J. B.,Waterston, R.,Brooke, M.,Specht, L., Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. N Engl J Med, 1988. 318(21): p. 1363-8.

[94] Shklyar, I.,Geisbush, T. R.,Mijialovic, A. S.,Pasternak, A.,Darras, B. T.,Wu, J. S.,Rutkove, S. B.,Zaidman, C. M., Quantitative muscle ultrasound in Duchenne muscular dystrophy: a comparison of techniques. Muscle Nerve, 2015. 51(2): p. 207-13.

[95] Takeshima, Y.,Yagi, M.,Okizuka, Y.,Awano, H.,Zhang, Z.,Yamauchi, Y.,Nishio, H.,Matsuo, M., Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. J Hum Genet, 2010. 55(6): p. 379-88.

[96] Hegde, M. R.,Chin, E. L.,Mulle, J. G.,Okou, D. T.,Warren, S. T.,Zwick, M. E., Microarray-based mutation detection in the dystrophin gene. Hum Mutat, 2008. 29(9): p. 1091-9.

[97] Gatta, V.,Scarciolla, O.,Gaspari, A. R.,Palka, C.,De Angelis, M. V.,Di Muzio, A.,Guanciali-Franchi, P.,Calabrese, G.,Uncini, A.,Stuppia, L., Identification of deletions and duplications of the DMD gene in affected males and carrier females by multiple ligation probe amplification (MLPA). Hum Genet, 2005. 117(1): p. 92-8.

[98] Falzarano, M. S.,Scotton, C.,Passarelli, C.,Ferlini, A., Duchenne Muscular Dystrophy: From Diagnosis to Therapy. Molecules, 2015. 20(10): p. 18168-84.

[99] den Dunnen, J.T. and A.H. Beggs, Multiplex PCR for identifying DMD gene deletions. Curr Protoc Hum Genet, 2006. Chapter 9: p. Unit 9.3.

[100] Okubo, M.,Minami, N.,Goto, K.,Goto, Y.,Noguchi, S.,Mitsuhashi, S.,Nishino, I., Genetic diagnosis of Duchenne/Becker muscular dystrophy using next-generation sequencing: validation analysis of DMD mutations. J Hum Genet, 2016. 61(6): p. 483-9.

[101] Wang, Y.,Yang, Y.,Liu, J.,Chen, X. C.,Liu, X.,Wang, C. Z.,He, X. Y., Whole dystrophin gene analysis by next-generation sequencing: a comprehensive genetic diagnosis of Duchenne and Becker muscular dystrophy. Mol Genet Genomics, 2014. 289(5): p. 1013-21.

[102] Wang, D.,Gao, M.,Zhang, K.,Jin, R.,Lv, Y.,Liu, Y.,Ma, J.,Wan, Y.,Gai, Z.,Liu, Y., Molecular Genetics Analysis of 70 Chinese Families With Muscular Dystrophy Using Multiplex Ligation-Dependent Probe Amplification and Next-Generation Sequencing. Front Pharmacol, 2019. 10: p. 814.

[103] Hegde, M. R.,Chin, E. L.,Mulle, J. G.,Okou, D. T.,Warren, S. T.,Zwick, M. E., Microarray-based mutation detection in the dystrophin gene. Hum Mutat, 2008. 29(9): p. 1091-9.

[104] Bovolenta, M.,Neri, M.,Fini, S.,Fabris, M.,Trabanelli, C.,Venturoli, A.,Martoni, E.,Bassi, E.,Spitali, P.,Brioschi, S.,Falzarano, M. S.,Rimessi, P.,Ciccone, R.,Ashton, E.,McCauley, J.,Yau, S.,Abbs, S.,Muntoni, F.,Merlini, L.,Gualandi, F.,Ferlini, A., A novel custom high density-comparative genomic hybridization array detects common rearrangements as well as deep intronic mutations in dystrophinopathies. BMC Genomics, 2008. 9: p. 572.

[105] Xiao, Y., X. Jiang, and R. Wang, Screening for DMD/BMD deletion carriers by fluorescence in situ hybridization. Genet Test, 2003. 7(3): p. 195-201.

[106] Pastore, L.,Caporaso, M. G.,Frisso, G.,Orsini, A.,Santoro, L.,Sacchetti, L.,Salvatore, F., A quantitative polymerase chain reaction (PCR) assay completely discriminates between Duchenne and Becker muscular dystrophy deletion carriers and normal females. Mol Cell Probes, 1996. 10(2): p. 129-37.

[107] Jang, W.,Kim, Y.,Han, E.,Park, J.,Chae, H.,Kwon, A.,Choi, H.,Kim, J.,Son, J. O.,Lee, S. J.,Hong, B. Y.,Jang, D. H.,Han, J. Y.,Lee, J. H.,Kim, S. Y.,Lee, I. G.,Sung, I. K.,Moon, Y.,Kim, M.,Park, J. H., Chromosomal Microarray Analysis as a First-Tier Clinical Diagnostic Test in Patients With Developmental Delay/Intellectual Disability, Autism Spectrum Disorders, and Multiple Congenital Anomalies: A Prospective Multicenter Study in Korea. Ann Lab Med, 2019. 39(3): p. 299-310.

[108] Bennett, R. R.,den Dunnen, J.,O'Brien, K. F.,Darras, B. T.,Kunkel, L. M., Detection of mutations in the dystrophin gene via automated DHPLC screening and direct sequencing. BMC Genet, 2001. 2: p. 17.

[109] Torella, A.,Trimarco, A.,Blanco Fdel, V.,Cuomo, A.,Aurino, S.,Piluso, G.,Minetti, C.,Politano, L.,Nigro, V., One hundred twenty-one dystrophin point mutations detected from stored DNA samples by combinatorial denaturing high-performance liquid chromatography. J Mol Diagn, 2010. 12(1): p. 65-73.

[110] Robinson-Hamm, J.N. and C.A. Gersbach, Gene therapies that restore dystrophin expression for the treatment of Duchenne muscular dystrophy. Hum Genet, 2016. 135(9): p. 1029-40.

[111] Monaco, A. P.,Bertelson, C. J.,Liechti-Gallati, S.,Moser, H.,Kunkel, L. M., An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics, 1988. 2(1): p. 90-5.

[112] Welch, E. M.,Barton, E. R.,Zhuo, J.,Tomizawa, Y.,Friesen, W. J.,Trifillis, P.,Paushkin, S.,Patel, M.,Trotta, C. R.,Hwang, S.,Wilde, R. G.,Karp, G.,Takasugi, J.,Chen, G.,Jones, S.,Ren, H.,Moon, Y. C.,Corson, D.,Turpoff, A. A.,Campbell, J. A.,Conn, M. M.,Khan, A.,Almstead, N. G.,Hedrick, J.,Mollin, A.,Risher, N.,Weetall, M.,Yeh, S.,Branstrom, A. A.,Colacino, J. M.,Babiak, J.,Ju, W. D.,Hirawat, S.,Northcutt, V. J.,Miller, L. L.,Spatrick, P.,He, F.,Kawana, M.,Feng, H.,Jacobson, A.,Peltz, S. W.,Sweeney, H. L., PTC124 targets genetic disorders caused by nonsense mutations. Nature, 2007. 447(7140): p. 87-91.

[113] Gonzalez-Hilarion, S.,Beghyn, T.,Jia, J.,Debreuck, N.,Berte, G.,Mamchaoui, K.,Mouly, V.,Gruenert, D. C.,Déprez, B.,Lejeune, F., Rescue of nonsense mutations by amlexanox in human cells. Orphanet J Rare Dis, 2012. 7: p. 58.

[114] Loro, E.,Sengupta, K.,Bogdanovich, S.,Whig, K.,Schultz, D. C.,Huryn, D. M.,Khurana, T. S., High-throughput identification of post-transcriptional utrophin up-regulators for Duchenne muscle dystrophy (DMD) therapy. Sci Rep, 2020. 10(1): p. 2132.

[115] Sun, C.,Shen, L.,Zhang, Z.,Xie, X., Therapeutic Strategies for Duchenne Muscular Dystrophy: An Update. Genes (Basel), 2020. 11(8).

[116] Odom, G.L., P. Gregorevic, and J.S. Chamberlain, Viral-mediated gene therapy for the muscular dystrophies: successes, limitations and recent advances. Biochim Biophys Acta, 2007. 1772(2): p. 243-62.

[117] McCullagh, K.J. and R.C. Perlingeiro, Coaxing stem cells for skeletal muscle repair. Adv Drug Deliv Rev, 2015. 84: p. 198-207.

[118] Campbell, C.,Barohn, R. J.,Bertini, E.,Chabrol, B.,Comi, G. P.,Darras, B. T.,Finkel, R. S.,Flanigan, K. M.,Goemans, N.,Iannaccone, S. T.,Jones, K. J.,Kirschner, J.,Mah, J. K.,Mathews, K. D.,McDonald, C. M.,Mercuri, E.,Nevo, Y.,Péréon, Y.,Renfroe, J. B.,Ryan, M. M.,Sampson, J. B.,Schara, U.,Sejersen, T.,Selby, K.,Tulinius, M.,Vílchez, J. J.,Voit, T.,Wei, L. J.,Wong, B. L.,Elfring, G.,Souza, M.,McIntosh, J.,Trifillis, P.,Peltz, S. W.,Muntoni, F., Meta-analyses of ataluren randomized controlled trials in nonsense mutation Duchenne muscular dystrophy. J Comp Eff Res, 2020. 9(14): p. 973-984.

[119] Hufton, M. and H. Roper, Variations in Duchenne muscular dystrophy course in a multi-ethnic UK population: potential influence of socio-economic factors. Dev Med Child Neurol, 2017. 59(8): p. 837-842.

[120] Bolukbasi, M.F., A. Gupta, and S.A. Wolfe, Creating and evaluating accurate CRISPR-Cas9 scalpels for genomic surgery. Nat Methods, 2016. 13(1): p. 41-50.

[121] Takeda, S., P.R. Clemens, and E.P. Hoffman, Exon-Skipping in Duchenne Muscular Dystrophy. J Neuromuscul Dis, 2021. 8(s2): p. S343-s358.

[122] Voit, T.,Topaloglu, H.,Straub, V.,Muntoni, F.,Deconinck, N.,Campion, G.,De Kimpe, S. J.,Eagle, M.,Guglieri, M.,Hood, S.,Liefaard, L.,Lourbakos, A.,Morgan, A.,Nakielny, J.,Quarcoo, N.,Ricotti, V.,Rolfe, K.,Servais, L.,Wardell, C.,Wilson, R.,Wright, P.,Kraus, J. E., Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. Lancet Neurol, 2014. 13(10): p. 987-96

[123] McCullagh, K.J. and R.C. Perlingeiro, Coaxing stem cells for skeletal muscle repair. Adv Drug Deliv Rev, 2015. 84: p. 198-207.

[124] Bakhtiar, Syeda M.,LeBlanc, Jean Guy,Salvucci, Emiliano,Ali, Amjad,Martin, Rebeca,Langella, Philippe,Chatel, Jean-Marc,Miyoshi, Anderson,Bermúdez-Humarán, Luis G.,Azevedo, Vasco, Implications of the human microbiome in inflammatory bowel diseases. FEMS Microbiology Letters, 2013. 342(1): p. 10-17.

[125] Kotlyarov, S., Role of Short-Chain Fatty Acids Produced by Gut Microbiota in Innate Lung Immunity and Pathogenesis of the Heterogeneous Course of Chronic Obstructive Pulmonary Disease. Int J Mol Sci, 2022. 23(9).

[126] Di Ciaula, A.,Garruti, G.,Lunardi Baccetto, R.,Molina-Molina, E.,Bonfrate, L.,Wang, D. Q.,Portincasa, P., Bile Acid Physiology. Ann Hepatol, 2017. 16(Suppl. 1: s3-105.): p. s4-s14.

[127] Bousselamti, A.,El Hasbaoui, B.,Echahdi, H.,Krouile, Y., Psychomotor regression due to vitamin B12 deficiency. Pan Afr Med J, 2018. 30: p. 152.

[128] Rothschild, D.,Weissbrod, O.,Barkan, E.,Kurilshikov, A.,Korem, T.,Zeevi, D.,Costea, P. I.,Godneva, A.,Kalka, I. N.,Bar, N.,Shilo, S.,Lador, D.,Vila, A. V.,Zmora, N.,Pevsner-Fischer, M.,Israeli, D.,Kosower, N.,Malka, G.,Wolf, B. C.,Avnit-Sagi, T.,Lotan-Pompan, M.,Weinberger, A.,Halpern, Z.,Carmi, S.,Fu, J.,Wijmenga, C.,Zhernakova, A.,Elinav, E.,Segal, E., Environment

dominates over host genetics in shaping human gut microbiota. Nature, 2018. 555(7695): p. 210-215.

[129] David, L. A.,Maurice, C. F.,Carmody, R. N.,Gootenberg, D. B.,Button, J. E.,Wolfe, B. E.,Ling, A. V.,Devlin, A. S.,Varma, Y.,Fischbach, M. A.,Biddinger, S. B.,Dutton, R. J.,Turnbaugh, P. J., Diet rapidly and reproducibly alters the human gut microbiome. Nature, 2014. 505(7484): p. 559-63.

[130] Pu, G.,Li, P.,Du, T.,Niu, Q.,Fan, L.,Wang, H.,Liu, H.,Li, K.,Niu, P.,Wu, C.,Zhou, W.,Huang, R., Adding Appropriate Fiber in Diet Increases Diversity and Metabolic Capacity of Distal Gut Microbiota Without Altering Fiber Digestibility and Growth Rate of Finishing Pig. Front Microbiol, 2020. 11: p. 533.

[131] Zhao, J.,Liu, P.,Wu, Y.,Guo, P.,Liu, L.,Ma, N.,Levesque, C.,Chen, Y.,Zhao, J.,Zhang, J.,Ma, X., Dietary Fiber Increases Butyrate-Producing Bacteria and Improves the Growth Performance of Weaned Piglets. J Agric Food Chem, 2018. 66(30): p. 7995-8004.

[132] Bisanz, J. E.,Upadhyay, V.,Turnbaugh, J. A.,Ly, K.,Turnbaugh, P. J., Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host Microbe, 2019. 26(2): p. 265-272.e4.

[133] Yang, H.,Xiang, Y.,Robinson, K.,Wang, J.,Zhang, G.,Zhao, J.,Xiao, Y., Gut Microbiota Is a Major Contributor to Adiposity in Pigs. Front Microbiol, 2018. 9: p. 3045.

[134] Kong, C.,Gao, R.,Yan, X.,Huang, L.,Qin, H., Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. Nutrition, 2019. 60: p. 175-184.

[135] Huang, E. Y.,Leone, V. A.,Devkota, S.,Wang, Y.,Brady, M. J.,Chang, E. B., Composition of dietary fat source shapes gut microbiota architecture and alters host inflammatory mediators in mouse adipose tissue. JPEN J Parenter Enteral Nutr, 2013. 37(6): p. 746-54.

[136] Yang, F.,Zhang, S.,Tian, M.,Chen, J.,Chen, F.,Guan, W., Different Sources of High Fat Diet Induces Marked Changes in Gut Microbiota of Nursery Pigs. Front Microbiol, 2020. 11: p. 859.

[137] Gunness, P.,Williams, B. A.,Gerrits, W. J.,Bird, A. R.,Kravchuk, O.,Gidley, M. J., Circulating triglycerides and bile acids are reduced by a soluble wheat arabinoxylan via modulation of bile concentration and lipid digestion rates in a pig model. Mol Nutr Food Res, 2016. 60(3): p. 642-51.

[138] Yang, J. T.,Chen, Y. J.,Huang, C. W.,Wang, Y. C.,Mersmann, H. J.,Wang, P. H.,Ding, S. T., Docosahexaenoic Acid Suppresses Expression of Adipogenic Tetranectin through Sterol Regulatory Element-Binding Protein and Forkhead Box O Protein in Pigs. Nutrients, 2021. 13(7).

[139] Han, Q.,Huang, X.,Yan, F.,Yin, J.,Xiao, Y., The Role of Gut Microbiota in the Skeletal Muscle Development and Fat Deposition in Pigs. Antibiotics (Basel), 2022. 11(6).

[140] Che, L.,Zhou, Q.,Liu, Y.,Hu, L.,Peng, X.,Wu, C.,Zhang, R.,Tang, J.,Wu, F.,Fang, Z.,Lin, Y.,Xu, S.,Feng, B.,Li, J.,Jiang, P.,Wu, D.,Chen, D., Flaxseed oil supplementation improves intestinal function and immunity, associated with altered intestinal microbiome and fatty acid profile in pigs with intrauterine growth retardation. Food Funct, 2019. 10(12): p. 8149-8160.

[141] Sappok, M. A.,Peréz Gutiérrez, O.,Smidt, H.,Pellikaan, W. F.,Verstegen, M. W.,Bosch, G.,Hendriks, W. H., Adaptation of faecal microbiota in sows after diet changes and consequences for in vitro fermentation capacity. Animal, 2015. 9(9): p. 1453-64.

[142] Jiang, X.,Lu, N.,Xue, Y.,Liu, S.,Lei, H.,Tu, W.,Lu, Y.,Xia, D., Crude fiber modulates the fecal microbiome and steroid hormones in pregnant Meishan sows. Gen Comp Endocrinol, 2019. 277: p. 141-147.

[143] Wu, J.,Xiong, Y.,Zhong, M.,Li, Y.,Wan, H.,Wu, D.,Liu, Q., Effects of purified fibre-mixture supplementation of gestation diet on gut microbiota, immunity and reproductive performance of sows. J Anim Physiol Anim Nutr (Berl), 2020. 104(4): p. 1144-1154.

[144] Xu, C.,Cheng, C.,Zhang, X.,Peng, J., Inclusion of Soluble Fiber in the Gestation Diet Changes the Gut Microbiota, Affects Plasma Propionate and Odd-Chain Fatty Acids Levels, and Improves Insulin Sensitivity in Sows. Int J Mol Sci, 2020. 21(2).

[145] Lindberg, J.E., Fiber effects in nutrition and gut health in pigs. J Anim Sci Biotechnol, 2014. 5(1): p. 15.

[146] Han, P.,Li, P.,Zhou, W.,Fan, L.,Wang, B.,Liu, H.,Gao, C.,Du, T.,Pu, G.,Wu, C.,Zhang, Z.,Niu, P.,Huang, R.,Li, H., Effects of various levels of dietary fiber on carcass traits, meat quality and myosin heavy chain I, IIa, IIx and IIb expression in muscles in Erhualian and Large White pigs. Meat Sci, 2020. 169: p. 108160.

[147] Wu, X.,Chen, D.,Yu, B.,Luo, Y.,Zheng, P.,Mao, X.,Yu, J.,He, J., Effect of different dietary non-starch fiber fractions on growth performance, nutrient digestibility, and intestinal development in weaned pigs. Nutrition, 2018. 51-52: p. 20-28.

[148] Mu, C.,Zhang, L.,He, X.,Smidt, H.,Zhu, W., Dietary fibres modulate the composition and activity of butyrate-producing bacteria in the large intestine of suckling piglets. Antonie Van Leeuwenhoek, 2017. 110(5): p. 687-696.

[149] Kurilshikov, A.,Wijmenga, C.,Fu, J.,Zhernakova, A., Host Genetics and Gut Microbiome: Challenges and Perspectives. Trends Immunol, 2017. 38(9): p. 633-647.

[150] Turpin, W.,Espin-Garcia, O.,Xu, W.,Silverberg, M. S.,Kevans, D.,Smith, M. I.,Guttman, D. S.,Griffiths, A.,Panaccione, R.,Otley, A.,Xu, L.,Shestopaloff, K.,Moreno-Hagelsieb, G.,Paterson, A. D.,Croitoru, K., Association of host genome with intestinal microbial composition in a large healthy cohort. Nat Genet, 2016. 48(11): p. 1413-1417.

[151] Goodrich, J. K.,Davenport, E. R.,Beaumont, M.,Jackson, M. A.,Knight, R.,Ober, C.,Spector, T. D.,Bell, J. T.,Clark, A. G.,Ley, R. E., Genetic Determinants of the Gut Microbiome in UK Twins. Cell Host Microbe, 2016. 19(5): p. 731-43.

[152] Goodrich, J. K.,Davenport, E. R.,Clark, A. G.,Ley, R. E., The Relationship Between the Human Genome and Microbiome Comes into View. Annu Rev Genet, 2017. 51: p. 413-433.

[153] Chen, C.,Huang, X.,Fang, S.,Yang, H.,He, M.,Zhao, Y.,Huang, L., Contribution of Host Genetics to the Variation of Microbial Composition of Cecum Lumen and Feces in Pigs. Front Microbiol, 2018. 9: p. 2626.

[154] Yan, Honglin,Diao, Hui,Xiao, Yi,Li, Wenxia,Yu, Bing,He, Jun,Yu, Jie,Zheng, Ping,Mao, Xiangbing,Luo, Yuheng,Zeng, Benhua,Wei, Hong,Chen, Daiwen, Gut microbiota can transfer fiber characteristics and lipid metabolic profiles of skeletal muscle from pigs to germ-free mice. Scientific Reports, 2016. 6(1): p. 31786.

[155] Xiao, Yingping,Kong, Fanli,Xiang, Yun,Zhou, Weidong,Wang, Junjun,ang, Hua,Zhang, Guolong,Zhao, Jiangchao, Comparative biogeography of the gut microbiome between Jinhua and Landrace pigs. Scientific Reports, 2018. 8(1): p. 5985.

[156] Ma, J.,Duan, Y.,Li, R.,Liang, X.,Li, T.,Huang, X.,Yin, Y.,Yin, J., Gut microbial profiles and the role in lipid metabolism in Shaziling pigs. Anim Nutr, 2022. 9: p. 345-356.

[157] Pajarillo,E.A.,Chae, J. P.,Balolong, M. P.,Kim, H. B.,Seo, K. S.,Kang, D. K., Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. J Microbiol, 2014. 52(8): p. 646-51.

[158] Crespo-Piazuelo, D.,Migura-Garcia, L.,Estellé, J.,Criado-Mesas, L.,Revilla, M.,Castelló, A.,Muñoz, M.,García-Casco, J. M.,Fernández, A. I.,Ballester, M.,Folch, J. M., Association between the pig genome and its gut microbiota composition. Sci Rep, 2019. 9(1): p. 8791.

[159] Lekagul, A., V. Tangcharoensathien, and S. Yeung, Patterns of antibiotic use in global pig production: A systematic review. Vet Anim Sci, 2019. 7: p. 100058.

[160] Ferri, M.,Ranucci, E.,Romagnoli, P.,Giaccone, V., Antimicrobial resistance: A global emerging threat to public health systems. Crit Rev Food Sci Nutr, 2017. 57(13): p. 2857-2876.

[161] Yan, H.,Yu, B.,Degroote, J.,Spranghers, T.,Van Noten, N.,Majdeddin, M.,Van Poucke, M.,Peelman, L.,De Vrieze, J.,Boon, N.,Gielen, I.,Smet, S.,Chen, D., Antibiotic affects the gut microbiota composition and expression of genes related to lipid metabolism and myofiber types in skeletal muscle of piglets. BMC Vet Res, 2020. 16(1): p. 392.

[162] Hedman, H.D., K.A. Vasco, and L. Zhang, A Review of Antimicrobial Resistance in Poultry Farming within Low-Resource Settings. Animals (Basel), 2020. 10(8).

[163] Fouhse, J.M., R.T. Zijlstra, and B.P. Willing, The role of gut microbiota in the health and disease of pigs. Animal Frontiers, 2016. 6(3): p. 30-36.

[164] Schokker, D.,Zhang, J.,Zhang, L. L.,Vastenhouw, S. A.,Heilig, H. G.,Smidt, H.,Rebel, J. M.,Smits, M. A., Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One, 2014. 9(6): p. e100040.

[165] Gresse, R.,Chaucheyras-Durand, F.,Fleury, M. A.,Van de Wiele, T.,Forano, E.,Blanquet-Diot, S., Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol, 2017. 25(10): p. 851-873.

[166] Mu, C.,Yang, Y.,Su, Y.,Zoetendal, E. G.,Zhu, W., Differences in Microbiota Membership along the Gastrointestinal Tract of Piglets and Their Differential Alterations Following an Early-Life Antibiotic Intervention. Front Microbiol, 2017. 8: p. 797.

[167] Mu, C.,Yang, Y.,Yu, K.,Yu, M.,Zhang, C.,Su, Y.,Zhu, W., Alteration of metabolomic markers of amino-acid metabolism in piglets with in-feed antibiotics. Amino Acids, 2017. 49(4): p. 771-781.

[168] Kim, H. B.,Borewicz, K.,White, B. A.,Singer, R. S.,Sreevatsan, S.,Tu, Z. J.,Isaacson, R. E., Microbial shifts in the swine distal gut in response to the

treatment with antimicrobial growth promoter, tylosin. Proc Natl Acad Sci U S A, 2012. 109(38): p. 15485-90.

[169] Looft, T.,Allen, H. K.,Cantarel, B. L.,Levine, U. Y.,Bayles, D. O.,Alt, D. P.,Henrissat, B.,Stanton, T. B., Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations. Isme j, 2014. 8(8): p. 1566-76.

[170] Allen, H. K.,Looft, T.,Bayles, D. O.,Humphrey, S.,Levine, U. Y.,Alt, D.,Stanton, T. B., Antibiotics in feed induce prophages in swine fecal microbiomes. mBio, 2011. 2(6).

[171] Monger, X. C.,Gilbert, A. A.,Saucier, L.,Vincent, A. T., Antibiotic Resistance: From Pig to Meat. Antibiotics (Basel), 2021. 10(10).

[172] Sprockett, D., T. Fukami, and D.A. Relman, Role of priority effects in the early-life assembly of the gut microbiota. Nat Rev Gastroenterol Hepatol, 2018. 15(4): p. 197-205.

[173] Yu, M.,Zhang, C.,Yang, Y.,Mu, C.,Su, Y.,Yu, K.,Zhu, W., Long-term effects of early antibiotic intervention on blood parameters, apparent nutrient digestibility, and fecal microbial fermentation profile in pigs with different dietary protein levels. J Anim Sci Biotechnol, 2017. 8: p. 60.

[174] Schokker, D.,Zhang, J.,Vastenhouw, S. A.,Heilig, H. G.,Smidt, H.,Rebel, J. M.,Smits, M. A., Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs. PLoS One, 2015. 10(2): p. e0116523.

[175] Stark, C. M.,Susi, A.,Emerick, J.,Nylund, C. M., Antibiotic and acid-suppression medications during early childhood are associated with obesity. Gut, 2019. 68(1): p. 62-69.

[176] Schulfer, A. F.,Schluter, J.,Zhang, Y.,Brown, Q.,Pathmasiri, W.,McRitchie, S.,Sumner, S.,Li, H.,Xavier, J. B.,Blaser, M. J., The impact of early-life sub-therapeutic antibiotic treatment (STAT) on excessive weight is robust despite transfer of intestinal microbes. Isme j, 2019. 13(5): p. 1280-1292.

[177] Mu, C. and W. Zhu, Antibiotic effects on gut microbiota, metabolism, and beyond. Appl Microbiol Biotechnol, 2019. 103(23-24): p. 9277-9285.

[178] Li, J.,Yang, K.,Ju, T.,Ho, T.,McKay, C. A.,Gao, Y.,Forget, S. K.,Gartner, S. R.,Field, C. J.,Chan, C. B.,Willing, B. P., Early life antibiotic exposure affects pancreatic islet development and metabolic regulation. Sci Rep, 2017. 7: p. 41778.

[179] Roberfroid, M., Prebiotics: the concept revisited. J Nutr, 2007. 137(3 Suppl 2): p. 830s-7s.

[180] Grela, E. R.,Świątkiewicz, M.,Florek, M.,Bakowski, M.,Skiba, G., Effect of Inulin Source and a Probiotic Supplement in Pig Diets on Carcass Traits, Meat Quality and Fatty Acid Composition in Finishing Pigs. Animals (Basel), 2021. 11(8).

[181] Brahe, L.K., A. Astrup, and L.H. Larsen, Can We Prevent Obesity-Related Metabolic Diseases by Dietary Modulation of the Gut Microbiota? Adv Nutr, 2016. 7(1): p. 90-101.

[182] Johnson, Jethro S.,Spakowicz, Daniel J.,Hong Bo-Young,Petersen, Lauren M.,Demkowicz Patrick,Chen Lei,Leopold, Shana R.,Hanson, Blake M.,Agresta, Hanako O..Gerstein Mark,Sodergren Erica,Weinstock, George M., Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature Communications, 2019. 10(1): p. 5029.

[183] Köchl, S., H. Niederstätter, and W. Parson, DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. Methods Mol Biol, 2005. 297: p. 13-30.

[184] Waye, J. S.,Presley, L. A.,Budowle, B.,Shutler, G. G.,Fourney, R. M., A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts. Biotechniques, 1989. 7(8): p. 852-5.

[185] Sambrook, J. and D.W. Russell, Purification of nucleic acids by extraction with phenol:chloroform. CSH Protoc, 2006. 2006(1).

[186] Ghatak, S., R.B. Muthukumaran, and S.K. Nachimuthu, A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech, 2013. 24(4): p. 224-31.

[187] Qi R.,Sun J.,Qiu X.,Zhang Y.,Wang J.,Wang Q.,Huang J.,Ge L.,Liu Z., The intestinal microbiota contributes to the growth and physiological state of muscle tissue in piglets. Sci Rep, 2021. 11(1): p. 11237.

[188] Kallabis, S.,Abraham, L.,Müller, S.,Dzialas, V.,Türk, C.,Wiederstein, J. L.,Bock, T.,Nolte, H.,Nogara, L.,Blaauw, B.,Braun, T.,Krüger, M., High-throughput proteomics fiber typing (ProFiT) for comprehensive characterization of single skeletal muscle fibers. Skelet Muscle, 2020. 10(1): p. 7.

[189] Wen, W.,Chen, X.,Huang, Z.,Chen, D.,Yu, B.,He, J.,Luo, Y.,Yan, H.,Chen, H.,Zheng, P.,Yu, J., Dietary lycopene supplementation improves meat quality, antioxidant capacity and skeletal muscle fiber type transformation in finishing pigs. Anim Nutr, 2022. 8(1): p. 256-264.

[190] Bäckhed, F.,Manchester, J. K.,Semenkovich, C. F.,Gordon, J. I., Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A, 2007. 104(3): p. 979-84.

[191] Grosicki, G.J., R.A. Fielding, and M.S. Lustgarten, Gut Microbiota Contribute to Age-Related Changes in Skeletal Muscle Size, Composition, and Function: Biological Basis for a Gut-Muscle Axis. Calcif Tissue Int, 2018. 102(4): p. 433-442.

[192] Schwab, M.,Reynders, V.,Ulrich, S.,Zahn, N.,Stein, J.,Schröder, O., PPARgamma is a key target of butyrate-induced caspase-3 activation in the colorectal cancer cell line Caco-2. Apoptosis, 2006. 11(10): p. 1801-11.

[193] Layden, B. T.,Angueira, A. R.,Brodsky, M.,Durai, V.,Lowe, W. L., Jr., Short chain fatty acids and their receptors: new metabolic targets. Transl Res, 2013. 161(3): p. 131-40.

[194] Cani, P. D.,Plovier, H.,Van Hul, M.,Geurts, L.,Delzenne, N. M.,Druart, C.,Everard, A., Endocannabinoids–at the crossroads between the gut microbiota and host metabolism. Nat Rev Endocrinol, 2016. 12(3): p. 133-43.

[195] De Vos, W. M.,Tilg, H.,Van Hul, M.,Cani, P. D., Gut microbiome and health: mechanistic insights. Gut, 2022. 71(5): p. 1020-1032.

[196] Lannotti, F.A., V. Di Marzo, and S. Petrosino, Endocannabinoids and endocannabinoid-related mediators: Targets, metabolism and role in neurological disorders. Prog Lipid Res, 2016. 62: p. 107-28.

[197] Di Marzo, V., New approaches and challenges to targeting the endocannabinoid system. Nat Rev Drug Discov, 2018. 17(9): p. 623-639.

[198] Lannotti, F. A.,Pagano, E.,Guardiola, O.,Adinolfi, S.,Saccone, V.,Consalvi, S.,Piscitelli, F.,Gazzerro, E.,Busetto, G.,Carrella, D.,Capasso, R.,Puri, P. L.,Minchiotti, G.,Di Marzo, V., Genetic and pharmacological regulation of the endocannabinoid CB1 receptor in Duchenne muscular dystrophy. Nat Commun, 2018. 9(1): p. 3950.

[199] Gentile, F.,Doneddu, P. E.,Riva, N.,Nobile-Orazio, E.,Quattrini, A., Diet, Microbiota and Brain Health: Unraveling the Network Intersecting Metabolism and Neurodegeneration. Int J Mol Sci, 2020. 21(20).

[200] Morais, L.H., H.L.t. Schreiber, and S.K. Mazmanian, The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol, 2021. 19(4): p. 241-255.

[201] Kho, Z.Y. and S.K. Lal, The Human Gut Microbiome - A Potential Controller of Wellness and Disease. Front Microbiol, 2018. 9: p. 1835.

[202] Pane, M.,Vasta, I.,Messina, S.,Sorleti, D.,Aloysius, A.,Sciarra, F.,Mangiola, F.,Kinali, M.,Ricci, E.,Mercuri, E., Feeding problems and weight gain in Duchenne muscular dystrophy. Eur J Paediatr Neurol, 2006. 10(5-6): p. 231-6.

[203] Brumbaugh, D.,Watne, L.,Gottrand, F.,Gulyas, A.,Kaul, A.,Larson, J.,Tomezsko, J., Nutritional and Gastrointestinal Management of the Patient With Duchenne Muscular Dystrophy. Pediatrics, 2018. 142(Suppl 2): p. S53-s61.

[204] Mulè, F., A. Amato, and R. Serio, Gastric emptying, small intestinal transit and fecal output in dystrophic (mdx) mice. J Physiol Sci, 2010. 60(1): p. 75-9.

[205] Kleessen, B.,Kroesen, A. J.,Buhr, H. J.,Blaut, M., Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. Scand J Gastroenterol, 2002. 37(9): p. 1034-41.

[206] Hofer, U., Pro-inflammatory Prevotella? Nature Reviews Microbiology, 2014. 12(1): p. 5-5.

[207] Ortega-Hernández, A.,Martínez-Martínez, E.,Gómez-Gordo, R.,López-Andrés, N.,Fernández-Celis, A.,Gutiérrrez-Miranda, B.,Nieto, M. L.,Alarcón, T.,Alba, C.,Gómez-Garre, D.,Cachofeiro, V., The Interaction between Mitochondrial Oxidative Stress and Gut Microbiota in the Cardiometabolic Consequences in Diet-Induced Obese Rats. Antioxidants (Basel), 2020. 9(7).

[208] Liu, T. W.,Park, Y. M.,Holscher, H. D.,Padilla, J.,Scroggins, R. J.,Welly, R.,Britton, S. L.,Koch, L. G.,Vieira-Potter, V. J.,Swanson, K. S., Physical Activity Differentially Affects the Cecal Microbiota of Ovariectomized Female Rats Selectively Bred for High and Low Aerobic Capacity. PLoS One, 2015. 10(8): p. e0136150.

[209] Rastelli, M., P.D. Cani, and C. Knauf, The Gut Microbiome Influences Host Endocrine Functions. Endocr Rev, 2019. 40(5): p. 1271-1284.

[210] Fung, T.C., C.A. Olson, and E.Y. Hsiao, Interactions between the microbiota, immune and nervous systems in health and disease. Nature Neuroscience, 2017. 20(2): p. 145-155.

[211] Ticinesi, A.,Nouvenne, A.,Cerundolo, N.,Catania, P.,Prati, B.,Tana, C.,Meschi, T., Gut Microbiota, Muscle Mass and Function in Aging: A Focus on Physical Frailty and Sarcopenia. Nutrients, 2019. 11(7).

[212] Ponziani, F.R. and A. Gasbarrini, Sarcopenia in Patients with Advanced Liver Disease. Curr Protein Pept Sci, 2018. 19(7): p. 681-691.

[213] Schieber, A. M.,Lee, Y. M.,Chang, M. W.,Leblanc, M.,Collins, B.,Downes, M.,Evans, R. M.,Ayres, J. S., Disease tolerance mediated by microbiome E. coli involves inflammasome and IGF-1 signaling. Science, 2015. 350(6260): p. 558-63.

[214] Estaki, M.,Pither, J.,Baumeister, P.,Little, J. P.,Gill, S. K.,Ghosh, S.,Ahmadi-Vand, Z.,Marsden, K. R.,Gibson, D. L. , Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome, 2016. 4(1): p. 42.

[215] Cruz-Guzmán Odel, R., M. Rodríguez-Cruz, and R.E. Escobar Cedillo, Systemic Inflammation in Duchenne Muscular Dystrophy: Association with Muscle Function and Nutritional Status. Biomed Res Int, 2015. 2015: p. 891972.

[216] Cani, P.D., Human gut microbiome: hopes, threats and promises. Gut, 2018. 67(9): p. 1716-1725.

[217] Tett, A.,Huang, K. D.,Asnicar, F.,Fehlner-Peach, H.,Pasolli, E.,Karcher, N.,Armanini, F.,Manghi, P.,Bonham, K.,Zolfo, M.,De Filippis, F.,Magnabosco, C.,Bonneau, R.,Lusingu, J.,Amuasi, J.,Reinhard, K.,Rattei, T.,Boulund, F.,Engstrand, L.,Zink, A.,Collado, M. C.,Littman, D. R.,Eibach, D.,Ercolini, D.,Rota-Stabelli, O.,Huttenhower, C.,Maixner, F.,Segata, N., The Prevotella copri Complex Comprises Four Distinct Clades Underrepresented in Westernized Populations. Cell Host Microbe, 2019. 26(5): p. 666-679.e7.

[218] Wu, G. D.,Chen, J.,Hoffmann, C.,Bittinger, K.,Chen, Y. Y.,Keilbaugh, S. A.,Bewtra, M.,Knights, D.,Walters, W. A.,Knight, R.,Sinha, R.,Gilroy, E.,Gupta, K.,Baldassano, R.,Nessel, L.,Li, H.,Bushman, F. D.,Lewis, J. D., Linking long-term dietary patterns with gut microbial enterotypes. Science, 2011. 334(6052): p. 105-8.

[219] David, L. A.,Maurice, C. F.,Carmody, R. N..Gootenberg, D. B.,Button, J. E.,Wolfe, B. E.,Ling, A. V.,Devlin, A. S.,Varma, Y.,Fischbach, M.

A.,Biddinger, S. B.,Dutton, R. J.,Turnbaugh, P. J., Diet rapidly and reproducibly alters the human gut microbiome. Nature, 2014. 505(7484): p. 559-63.

[220] Kovatcheva-Datchary, P.,Nilsson, A.,Akrami, R.,Lee, Y. S.,De Vadder, F.,Arora, T.,Hallen, A.,Martens, E.,Björck, I.,Bäckhed, F., Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. Cell Metab, 2015. 22(6): p. 971-82.

[221] Asnicar, F.,Berry, S. E.,Valdes, A. M.,Nguyen, L. H.,Piccinno, G.,Drew, D. A.,Leeming, E.,Gibson, R.,Le Roy, C.,Khatib, H. A.,Francis, L.,Mazidi, M.,Mompeo, O.,Valles-Colomer, M.,Tett, A.,Beghini, F.,Dubois, L.,Bazzani, D.,Thomas, A. M.,Mirzayi, C.,Khleborodova, A.,Oh, S.,Hine, R.,Bonnett, C.,Capdevila, J.,Danzanvilliers, S.,Giordano, F.,Geistlinger, L.,Waldron, L.,Davies, R.,Hadjigeorgiou, G.,Wolf, J.,Ordovás, J. M.,Gardner, C.,Franks, P. W.,Chan, A. T.,Huttenhower, C.,Spector, T. D.,Segata, N., Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nat Med, 2021. 27(2): p. 321-332.

[222] Dillon, S. M.,Lee, E. J.,Kotter, C. V.,Austin, G. L.,Gianella, S.,Siewe, B.,Smith, D. M.,Landay, A. L.,McManus, M. C.,Robertson, C. E.,Frank, D. N.,McCarter, M. D.,Wilson, C. C., Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. Mucosal Immunol, 2016. 9(1): p. 24-37.

[223] Pedersen, H. K.,Gudmundsdottir, V.,Nielsen, H. B.,Hyotylainen, T.,Nielsen, T.,Jensen, B. A.,Forslund, K.,Hildebrand, F.,Prifti, E.,Falony, G.,Le Chatelier, E.,Levenez, F.,Doré, J.,Mattila, I.,Plichta, D. R.,Pöhö, P.,Hellgren, L. I.,Arumugam, M.,Sunagawa, S.,Vieira-Silva, S.,Jørgensen, T.,Holm, J. B.,Trošt, K.,Kristiansen, K.,Brix, S.,Raes, J.,Wang, J.,Hansen, T.,Bork, P.,Brunak, S.,Oresic, M.,Ehrlich, S. D.,Pedersen, O., Human gut microbes impact host serum metabolome and insulin sensitivity. Nature, 2016. 535(7612): p. 376-81.

[224] Fehlner-Peach, H.,Magnabosco, C.,Raghavan, V.,Scher, J. U.,Tett, A.,Cox, L. M.,Gottsegen, C.,Watters, A.,Wiltshire-Gordon, J. D.,Segata, N.,Bonneau,

R.,Littman, D. R.., Distinct Polysaccharide Utilization Profiles of Human Intestinal Prevotella copri Isolates. Cell Host Microbe, 2019. 26(5): p. 680-690.e5.

[225] De Filippis, F.,Pasolli, E.,Tett, A.,Tarallo, S.,Naccarati, A.,De Angelis, M.,Neviani, E.,Cocolin, L.,Gobbetti, M.,Segata, N.,Ercolini, D., Distinct Genetic and Functional Traits of Human Intestinal Prevotella copri Strains Are Associated with Different Habitual Diets. Cell Host Microbe, 2019. 25(3): p. 444-453.e3.

[226] Hayashi, H.,Shibata, K.,Sakamoto, M.,Tomita, S.,Benno, Y., Prevotella copri sp. nov. and Prevotella stercorea sp. nov., isolated from human faeces. Int J Syst Evol Microbiol, 2007. 57(Pt 5): p. 941-946.

[227] Almeida, A.,Nayfach, S.,Boland, M.,Strozzi, F.,Beracochea, M.,Shi, Z. J.,Pollard, K. S.,Sakharova, E.,Parks, D. H.,Hugenholtz, P.,Segata, N.,Kyrpides, N. C.,Finn, R. D., A unified catalog of 204,938 reference genomes from the human gut microbiome. Nat Biotechnol, 2021. 39(1): p. 105-114.

[228] Pasolli, E.,Asnicar, F.,Manara, S.,Zolfo, M.,Karcher, N.,Armanini, F.,Beghini, F.,Manghi, P.,Tett, A.,Ghensi, P.,Collado, M. C.,Rice, B. L.,DuLong, C.,Morgan, X. C.,Golden, C. D.,Quince, C.,Huttenhower, C.,Segata, N., Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. Cell, 2019. 176(3): p. 649-662.e20.

[229] Anani,H.,Guilhot, E.,Andrieu, C.,Fontanini, A.,Raoult, D.,Fournier, P. E., Prevotella ihumii sp. nov., a new bacterium isolated from a stool specimen of a healthy woman. New Microbes New Infect, 2019. 32: p. 100607.

[230] Petersen, L. M.,Bautista, E. J.,Nguyen, H.,Hanson, B. M.,Chen, L.,Lek, S. H.,Sodergren, E.,Weinstock, G. M., Community characteristics of the gut microbiomes of competitive cyclists. Microbiome, 2017. 5(1): p. 98.

[231] Chen, R. Y.,Mostafa, I.,Hibberd, M. C.,Das, S.,Mahfuz, M.,Naila, N. N.,Islam, M. M.,Huq, S.,Alam, M. A.,Zaman, M. U.,Raman, A. S.,Webber,

D.,Zhou, C.,Sundaresan, V.,Ahsan, K.,Meier, M. F.,Barratt, M. J.,Ahmed, T.,Gordon, J. I., A Microbiota-Directed Food Intervention for Undernourished Children. N Engl J Med, 2021. 384(16): p. 1517-1528.

[232] Alberdi, A.,Andersen, S. B.,Limborg, M. T.,Dunn, R. R.,Gilbert, M. T. P., Disentangling host-microbiota complexity through hologenomics. Nat Rev Genet, 2022. 23(5): p. 281-297.

[233] Sommer, F. and F. Bäckhed, The gut microbiota–masters of host development and physiology. Nat Rev Microbiol, 2013. 11(4): p. 227-38.

[234] Ramayo-Caldas, Y.,Mach, N.,Lepage, P.,Levenez, F.,Denis, C.,Lemonnier, G.,Leplat, J. J.,Billon, Y.,Berri, M.,Doré, J.,Rogel-Gaillard, C.,Estellé, J., Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. Isme j, 2016. 10(12): p. 2973-2977.

[235] Chassard, C.,Goumy, V.,Leclerc, M.,Del'homme, C.,Bernalier-Donadille, A., Characterization of the xylan-degrading microbial community from human faeces. FEMS Microbiol Ecol, 2007. 61(1): p. 121-31.

[236] Cooper, S.W., D.G. Pfeiffer, and F.P. Tally, Evaluation of xylan fermentation for the identification of Bacteroides ovatus and Bacteroides thetaiotaomicron. J Clin Microbiol, 1985. 22(1): p. 125-6.

[237] Chen, J., A. Wang, and Q. Wang, Dysbiosis of the gut microbiome is a risk factor for osteoarthritis in older female adults: a case control study. BMC Bioinformatics, 2021. 22(1): p. 299.

[238] Tannock, G. W.,Munro, K.,Harmsen, H. J.,Welling, G. W.,Smart, J.,Gopal, P. K., Analysis of the fecal microflora of human subjects consuming a probiotic product containing Lactobacillus rhamnosus DR20. Appl Environ Microbiol, 2000. 66(6): p. 2578-88.

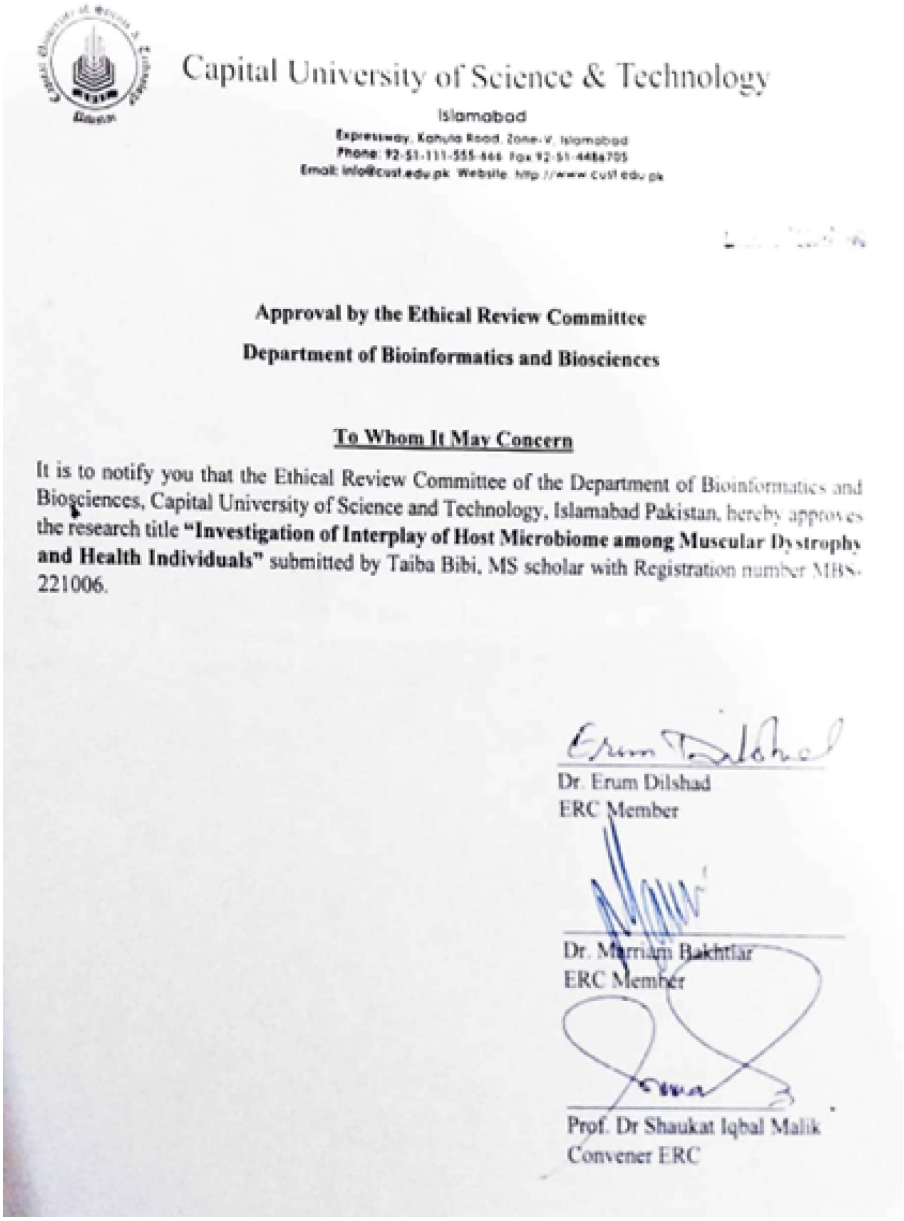
[239] Neville, B. A.,Forde, B. M.,Claesson, M. J.,Darby, T.,Coghlan, A.,Nally, K.,Ross, R. P.,O'Toole, P. W., Characterization of pro-inflammatory flagellin

proteins produced by Lactobacillus ruminis and related motile Lactobacilli. PLoS One, 2012. 7(7): p. e40592.

[240] Taweechotipatr, M.,Iyer, C.,Spinler, J. K.,Versalovic, J.,Tumwasorn, S., Lactobacillus saerimneri and Lactobacillus ruminis: novel human-derived probiotic strains with immunomodulatory activities. FEMS Microbiol Lett, 2009. 293(1): p. 65-72.

[241] Yun, J. H.,Yim, D. S.,Kang, J. Y.,Kang, B. Y.,Shin, E. A.,Chung, M. J.,Kim, S. D.,Baek, D. H.,Kim, K.,Ha, N. J., Identification of Lactobacillus ruminus SPM0211 isolated from healthy Koreans and its antimicrobial activity against some pathogens. Arch Pharm Res, 2005. 28(6): p. 660-6.

Appendix A



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Approval by the Ethical Review Committee

Department of Bioinformatics and Biosciences

To Whom It May Concern

It is to notify you that the Ethical Review Committee of the Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad Pakistan, hereby approves the research title **"Investigation of Interplay of Host Microbiome among Muscular Dystrophy and Health Individuals"** submitted by Taiba Bibi, MS scholar with Registration number MBS-221006.

Dr. Erum Dilshad
ERC Member

Dr. Marriam Bakhtiar
ERC Member

Prof. Dr Shaukat Iqbal Malik
Convener ERC

Appendix B

Capital University of Science and Technology,
Department of Biosciences and Bioinformatics

Disease Assessment Questionnaire

In order to assess the association of microbiome and muscular dystrophy disease, we need to review your medical and family history, focusing on the history of Muscular Dystrophy.

The first part of the questionnaire focuses on your personal and medical history, the second part on your family history. If you are uncertain about any information, please write in your best approximation or write unknown. You may decline to answer any or all of the questions at this time or at any later time.

- Please take some time before you fill this form to gather as much of your family history information as possible.
- We will not use the information you provide us to contact your family members. Names of family members are used only as a reference and to reduce our errors.
- Your personal and family history will be kept confidential.
- You may be contacted in the future about queries and further information.
- You are free to leave at any time.
- After completing the questionnaire, you may wish to make a copy of it for your record.
- If you have any questions, please contact our office at (which number do add?) or email at taibasalamat88@gmail.co and aqdasiftikhar123@gmail.com.

Please circle one:

I **do** / **do not** agree to share my personal and family history information for research purposes and for deductions to be made and results to be published on the information that I provided.

Name:_____ Signature:_____ Date:

Personal History

Name:_____,_____,
First name Middle name Family name

What is your date of birth?____/____/_____

What is your occupation? _____

Marital Status:

Single/widowed:

Married:

Divorced:

Age:

Please choose which ethnic/racial background best describes you and your biological mother and father.

Self:

Maternal side:

Paternal side:

What is your religious affiliation? _____

General Medical History

How is your health in general?

Do you have any other underlying health conditions? Y / N / U
If yes, what type(s) and at what age(s) were you diagnosed?

Do you smoke or use tobacco products: Currently? Y N **Previously?** Y / N

If yes, what do you use and how much?

Do you drink alcohol beverages? Y / N **If yes, how often?**

1-3/week_____4-6/week_____>6/week_____other:

Do you use other recreational drugs? Y / N

If yes, what do you use and how often?

Do you have diabetes? Y / N

Do you have any metabolic disorder? Y / N

Have you used any antibiotics in previous 3 months? Y / N

If yes, list please

Name: _____ **Reason**:

Name: _____ **Reason**:

Name: _____ **Reason**:

If you currently take medication daily or on a regular basis, please list medication and reason for taking it

Medication Reason

Medication Reason

Diet

What do you have in?

Breakfast:
Brunch:

Lunch:
Supper:
Dinner:
Do you have a special diet? Y / N

If yes please list

Screening History

Have you ever been diagnosed with muscular dystrophy? Y / N

Type:

Age Diagnosed:

Treatment used:

If you currently take medication daily or on a regular basis, please list medication and reason for taking it (for treatment of Muscular Dystrophy).

Medication Reason

Medication Reason

Medication Reason

Duration of Treatment being used?

Please check the following boxes if you have the following symptoms

Symptom	**Y/N**
Difficulty in walking	
Waddling gait	
Frequent falls and clumsiness	

Difficulty in getting up from sitting position	
Difficulty in climbing stairs	
Walking on toes	
Frequent falls	
Difficulty rising from the floor	
contractures	
Scoliosis (curved spine)	
Breathing and swallowing problems	
Shoulder deterioration	

Mild facial weakness	
Lordosis (inward curve of lumbar spine)	
Droopy eyes	
Poor vision	
Cataracts	
Double vision	
Problems with upper gaze	
Muscle weakness in face	
Difficulty in opening and close eyes	
Trouble smiling or puckering lips	
Difficulty in chewing	
Weight loss	
Cardiac irregularities	

Family Members/ Relatives

If you have any family members or Relatives who had or are suffering from muscular Dystrophy please list

Name	Sex	Age	Relationship to you	Deceased	Muscular Dystrophy Type	Age at Diagnosis
	M / F			Y / N / U		
	M / F			Y / N / U		
	M / F			Y / N / U		
	M / F			Y / N / U		
	M / F			Y / N / U		
	M / F			Y / N / U		
	M / F			Y / N / U		

	M / F			Y / N / U		
	M / F			Y / N / U		

Reviewed by:_____ **Date:**