

Exploring Factors that Influence Reproductive Decision-Making in Duchenne Muscular Dystrophy Carriers

by

Caroline Kathleen Bong

BS Biological Sciences, University of Missouri, 2018

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This thesis was presented

by

Caroline Kathleen Bong

It was defended on

April 10, 2020

and approved by

John Shaffer, PhD, Assistant Professor, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh

Robin E. Grubs, MS, PhD, LCGC, Associate Professor of Human Genetics, Director, Genetic Counseling Program, Graduate School of Public Health, University of Pittsburgh

Roxanna M. Bendixen, PhD, OTR/L, FAOTA, Associate Professor, Department of Occupational Therapy, Secondary Appointment, Department of Neurology, School of Health and Rehabilitation Sciences, University of Pittsburgh

Thesis Advisor: Lauren Morgenroth, MS, CGC, Adjunct Professor, Human Genetics, Graduate School of Public Health, University of Pittsburgh

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Abstract

Background: Duchenne muscular dystrophy (DMD) is an incurable, X-linked recessive genetic disorder characterized by progressive muscle weakness and atrophy. Those affected with DMD typically experience loss of ambulation in teenage years and premature death. Females who are carriers of DMD have a 25% chance of having an affected son with each pregnancy. Little research has been explored regarding the factors that influence the reproductive choices of this population.

Methods: 141 females who are DMD carriers were anonymously surveyed to elicit reproductive decisions and other aspects of their lives. The IRB approved survey was predominantly distributed on social media sites through a specialized neuromuscular contract research organization, TRiNDS, and DMD advocacy groups. The reproductive trends and the factors that influenced their reproductive decisions were analyzed in all 141 respondents using both quantitative and qualitative analyses.

Results: Of the 92 participants who planned to have (additional) children prior to learning their carrier status and who provided clear indications of the reproductive decisions they pursued or plan to pursue, 93.5% ($n=86$) reported changed reproductive actions after learning their carrier status. Of those, 53.3% ($n = 49$) indicated they no longer plan to have (additional) children. A family history of DMD ($p<0.001$), timing when they learned of their DMD carrier status ($p=0.002$),

and the highest level of education completed ($p=0.017$) were significantly associated with preconception, prenatal, and no reproductive actions taken. The most significant factors influencing females who are DMD carriers who pursued or plan to pursue reproductive actions were having biological children ($p<0.001$), having children diagnosed with DMD ($p<0.001$), timing when they learned of their DMD carrier status ($p=0.002$), and having a family history of DMD (0.012). Understanding of their reproductive options ($p=0.014$) was also significant for these females.

Conclusions: This study demonstrated that alternative reproductive actions are commonly pursued for females who are DMD carriers after learning their carrier statuses and that certain factors were statistically significant with reproductive choices. Understanding the factors that influence reproductive decision-making has important public health implications as it may provide insight and improved psychosocial interventions for genetic counselors who work with females who are DMD carriers.

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Preface

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1.0 Introduction

Duchenne muscular dystrophy (DMD) is a rare, progressive neuromuscular genetic disorder that is inherited in an X-linked recessive pattern.¹ DMD is estimated to affect 1/3,500 to 1/5,000 live male births and is characterized by progressive muscle weakening and atrophy, which ultimately leads to loss of ambulation and life-threatening cardiopulmonary complications.^{1,2} DMD is currently an incurable disorder and the life expectancy for those affected is mid-20s to mid-30s, depending on the interventions and care routes pursued.² Females who are carriers of DMD have a 25% chance of having an affected son with DMD with each pregnancy.

Currently, there are a number of reproductive options that can be considered in both the preconception and pregnancy phase for females who are at an increased risk for having a child with an inherited genetic condition, along with family planning options that do not involve the at-risk female becoming pregnant.^{3,4} These include preimplantation genetic testing (PGT) with in vitro fertilization (IVF), utilizing a gamete donation, prenatal diagnostic testing, adoption, and not having (additional) children.³⁻⁵ Additionally, there is the reproductive option of choosing to conceive naturally without any reproductive interventions or prenatal diagnostic testing.⁵ Knowing the risk of having a child with DMD, females who are DMD carriers may consider this information when deciding which reproductive option is best for themselves, their partner, and their family.

Factors that have been identified to influence the reproductive decision-making process in females who are at an increased risk of having a child with an inherited genetic disorder consist of religious beliefs, perceptions of risk, family expectations, and the desire to have (additional) children.⁶⁻⁸ Previous studies have shown that whether or not females have previous children, along with if the children are affected, impact the decision-making process.⁷ Other factors relate to the

consequences of the genetic condition, such as the personal experiences with the condition, the perceived quality of life of those affected, and the burden of the condition on the affected child and the family.^{7,9,10}

The aim of this study is to investigate the factors that contribute to reproductive decision-making in females who are DMD carriers. An anonymous survey was developed through Qualtrics software and distributed via email, letters, in-person, and social media advertisements through Therapeutic Research in Neuromuscular Disorders Solutions (TRiNDS). DMD advocacy groups in the United States and Canada were provided with the survey link. The inclusion criteria for this study included females who were known to be DMD carriers and were 18 years of age and older. Data were collected from 141 participants and utilized to achieve the following aims:

- Evaluate the factors that influence reproductive decision-making, including factors surrounding carrier status and family history, birth and pregnancy history, and demographic features
- Analyze the collected data to assess trends of the reproductive decisions and the factors that influence the reproductive choices of females who are DMD carriers

Previous studies have evaluated reproductive decisions made by females who are DMD carriers; however, many were published prior to the current available reproductive options or utilized population-based surveillance, where the females who are carriers could not comment on the motivations of their reproductive choices.¹¹⁻¹³ The results of this study have the potential to provide an up-to-date, deeper understanding of the personal factors that influence reproductive decision-making for females who are DMD carriers. This information may offer insight and improved psychosocial interventions for genetic counselors, or other health care professionals, who work with females who are DMD carriers.

2.0 Literature Review

2.1 Duchenne Muscular Dystrophy

2.1.1 Overview

DMD is a rare, progressive neuromuscular genetic disorder that primarily affects males.¹ DMD leads to progressive proximal muscle weakness and degeneration of the skeletal and heart muscles, which ultimately causes loss of ambulation in early teens and premature death due to respiratory or dilated cardiomyopathy complications.¹ This is currently an incurable, lethal disorder with a median survival of 24 years; however, life expectancy with ventilator support and other interventions is now greater than 30 years of age.^{2,14} Furthermore, as the Food and Drug Administration has granted orphan drug designations to numerous experimental treatments, such as the corticosteroid deflazacort, the anti-fibrosis pamrevlumab, and a few exon skipping interventions, such as eteplirsen or vitolarsen, there continues to be a strong interest in developing potential therapies and cures for DMD, including gene therapy.^{15,16}

According to the National Organization for Rare Diseases (NORD), DMD is the most common childhood form of muscular dystrophies.¹⁷ Muscular dystrophies are defined as a group of clinical disorders that all have characteristics of progressive muscle weakness and degeneration due to one of the numerous identified genetic causes.¹⁸ The incidence of DMD is estimated to be 1/3,500 to 1/5,000 live male births.^{18,19} In very rare cases, females can also be affected with the classic phenotypical manifestations of DMD with an estimated incidence of 1/50,000,000 live female births.²⁰ Females diagnosed with DMD have been identified to have an X-autosome

translocation, Turner's syndrome, a structural abnormality of an X-chromosome, or a Xp21 locus deletion on the X-chromosome without the DMD pathogenic variant.²⁰

2.1.2 Molecular Genetics

DMD is caused by pathogenic variants in the *DMD* gene located on the X-chromosome.²¹ The *DMD* gene is the largest identified human gene and is responsible for coding the dystrophin protein, which is primarily found in skeletal and cardiac muscle cells.²¹ The function of this protein is to help stabilize muscles during contraction and the lack of functional dystrophin causes chronic muscle damage, muscle inflammation and wasting, and ultimately the loss of muscle function.^{19,21,22}

In 60% to 70% of cases, the type of pathogenic variants that cause DMD are large deletions within the *DMD* gene leading to disrupted reading frames and truncated protein, resulting in an absence of dystrophin protein.²³⁻²⁷ Other types of pathogenic variants, such as missense variants, nonsense variants, intronic variants, or smaller deletions or insertions affecting splice sites, have also been identified to cause DMD in a cumulative of 25% to 35% of cases.²³⁻²⁵ Lastly, large duplications account for approximately 5% to 10% of DMD cases.²³⁻²⁷ Pathogenic variants that lead to in-frameshifts and non-protein truncation allowing for the retention of partial dystrophin protein are normally associated with Becker muscular dystrophy (BMD); however there are exceptions where DMD may still be the outcome.^{21,25,28} BMD is a less severe, slower progressing proximal progressive neuromuscular genetic disorder in comparison to DMD.^{28,29}

2.1.2.1 Inheritance

DMD is a condition inherited in an X-linked recessive pattern and therefore, heterozygous females who are carriers of DMD have a 50% chance of passing the affected gene onto each of their offspring.³⁰ Male offspring who inherit the affected gene will have DMD and female offspring who inherit the affected gene will also be heterozygous carriers of DMD.³⁰ However, approximately 33% of affected individuals have de novo pathogenic variants, in which the variants arise new in the affected individual and are not inherited from a parent.^{31,32} Of these apparently de novo cases, maternal germline mosaicism occurs in an estimated 14-20% of the time.³³ One study found that the prevalence of affected males who have inherited the pathogenic variant from a carrier mother, instead of the occurrence of a de novo event, depends on type of pathogenic variant identified, with the carrier frequency ranging from 53.5% to 67.9%.³¹

2.1.3 Natural History

Average age of diagnosis is 5 years in the United States, with signs and symptoms generally appearing by the age of 2 to 5 years.³⁴ Initial symptoms include delayed milestones, as the mean age of walking is 18 months, and gait problems.^{23,34} Affected toddlers commonly have enlarged calves, trouble climbing upstairs or running, may seem clumsier than peers, and demonstrate the Gower's sign.³⁵ The Gower maneuver is frequently used by children with DMD to assist them from a sitting to standing position by starting on their hands and knees and walking their arms up their thighs until they are standing.^{35,36} This helps compensate for weakened proximal muscles in their lower extremities and pelvic girdle.³⁶

As children with DMD age, they may toe-walk or walk on the balls of their feet.²³ Affected children usually display a characteristic walk of pulling their shoulders back and pushing their

stomachs out to assist with their balance. As muscle loss progresses, children transfer to a wheelchair, which occurs at a mean age of 12 years old, especially if left untreated.¹⁹ Treatment may delay loss of ambulation, but currently no treatment is available to prevent it.^{2,37} Table 1 below describes the severity stages of DMD that correspond with the progression and motor function of affected males.

Along with loss of ambulation, 33% of affected boys will develop cardiomyopathy by the age of 14 and the majority of the rest will develop it at or after 18 years of age.³⁸ As the respiratory muscles weaken, males are at high risk to experience pneumonia, collapsed lungs, sleep hypoventilation, and/or sleep apnea.³⁹⁻⁴¹ Difficulty swallowing is reported in approximately one third of affected males, but this complication is likely underestimated.⁴² Other common complications include scoliosis; fractures; and cognitive impairments, such as intellectual disabilities (ID), autism spectrum disorder (ASD), and attention-deficit hyper activity disorder (ADHD).^{23,43} Due to the progressive cardiomyopathy and respiratory complications, death usually occurs before 30 years of age.^{2,14,19} Common characteristics of males affected with DMD based on the disease progression stage are briefly outlined in Table 1.

Table 1. Clinical Characteristics of Males Affected with DMD Patients by Stages

Stage	Clinical Characteristics
I: Diagnosis	Delayed milestones, Gower's maneuver, enlarged calves, and waddling gait
II: Early ambulatory	Toe-walking, trouble climbing upstairs or running, difficulty keeping up with peers, and clumsiness
III: Late ambulatory	Difficulty walking long distances, trouble balancing, and more frequent utilization of scooter/manual wheelchair
IV: Early non-ambulatory	Difficulty with independent walking, regular utilization of manual wheelchair, increased muscle weakness in arms and neck, breathing difficulties, and onset of cardiomyopathy
V: Late non-ambulatory	Onset of paralysis, loss of independent movement, dysphagia, retained finger usage, and retained speech

2.1.3.1 Diagnosis

DMD should be considered in boys who are displaying common DMD signs, specifically impaired muscle functioning, excessive clumsiness or frequent falls, and utilizing the Gower's sign to stand from a sitting position.²¹ When DMD is suspected in a male proband, a serum creatine phosphokinase (CK) blood test is useful in determining if the bloodstream contains an elevated level of muscle enzymes.⁴⁴ Elevated amounts of this muscle enzymes occur in males with DMD due to the lack of dystrophin protein within the muscles that result in tearing in the muscle fibers during everyday movements.^{45,46} Tears in the muscle fibers allow for excess CK to flow out from the muscles into the bloodstream.⁴⁵ The reference range for individuals without DMD is typically under 200 units/liter, while males with DMD are seen to have CK levels that are 10 to 100 times above the reference range.⁴⁴ The CK levels are highest between the ages of 2 and 5 years old in affected children and will decrease as the condition progresses.⁴⁵ Elevated CK levels in the blood is not specific to DMD and further testing should be performed.

In situations with suggestive clinical findings and elevated serum CK levels, a diagnosis of DMD is confirmed via molecular genetic testing, typically completed on a tiered basis. Deletion/duplication genetic testing on the *DMD* gene is classically performed first, as these pathogenic variants account for 65-80% of affected males.^{21,23} If no deletions or duplications are found, sequence analysis, such as next generation sequencing, of the *DMD* gene is ordered, which detects 20-35% of males who have a pathogenic variant in the *DMD* gene.^{21,23} A recent study in 2019 performed by Andrews et al., found that molecular genetic testing for DMD has a 90.9% sensitivity rate.⁴⁷

When genetic testing does not identify a *DMD* pathogenic variant, the affected child may undergo a skeletal muscle biopsy to analyze the dystrophin protein via western blot and

immunohistochemistry.²³ Males with DMD will have undetectable molecular weight of the dystrophin protein and a dystrophin quantity between 0% and 5% on the western blot analysis.⁴⁸ As for the immunohistochemistry, affected males will be found to have either a complete absence or an almost complete absence of the dystrophin protein within their skeletal muscles.⁴⁸ However, because muscle biopsies are invasive, males affected with DMD are at an increased risk for anesthesia-related complications, and the majority of patients are now diagnosed via genetic testing, they are rarely used in the diagnostic process.^{23,49,50}

2.1.3.2 Treatment

While there is no cure for DMD at this time, there are treatment and management plans to delay the progression of the disease.⁵¹ The standard of care for males with DMD consists of long-term usage of glucocorticoids and multidisciplinary care for symptom management. Glucocorticoids, a type of steroid hormone, may delay the deterioration of muscle function and strength to prolong ambulation, with the possibility of improving respiratory and cardiac function.^{52,53} Even though glucocorticoids are standard of care, families and physicians may opt to discontinue long-term usage due to side effects that consist of “cushingoid features, obesity, abnormal glucose tolerance, growth retardation, delayed puberty, hypertension, adverse behavioral changes and increased fracture risk.”⁵⁴ A multidisciplinary care team may consist of neurologists, cardiologists, pulmonologists, physical and occupational therapists, respiratory therapists, dietitians, social workers, and additional specialized health care professionals if necessary.⁵⁵ The team commonly coordinates services such as obtaining appropriate assistive devices as mobility declines, teaching the proper stretching techniques to delay contractures, recommending specialized diet plans, prescribing heart failure medications to manage cardiomyopathy, discussing assisted ventilation as respiratory abilities decrease, and other appropriate management

strategies.⁵⁶ Along with the current standard of care recommendations, the Care Considerations established and updated in 2018 by the Center for Disease Control and Prevention encourages participation in research and clinical trials of investigational drugs that may improve patient outcomes.⁵⁶

While there are already a few FDA approved drugs shown to slow the disease progression and attempt to decrease the severity of the DMD phenotype to a BMD phenotype, clinical research is still on-going as there are numerous clinical trials for novel oral and intravenous medications that attempt to more effectively delay symptoms in these patients.⁵¹ More recently, promising animal models utilizing either adeno-associated virus gene therapies or viral vector-based gene therapies are underway.⁵⁷ These gene therapies have arisen with the hopes of essentially curing DMD by producing a protein called utrophin that mimics dystrophin and prevents the breakdown of the skeletal muscles, along with possibly regenerating the previously damaged muscles.⁵⁷ Previous gene therapies focusing on producing the dystrophin protein itself faced difficulties of strong immune responses in animal models due to immune systems viewing the newly introduced dystrophin as foreign in the body.⁵⁷ Utilizing the production of utrophin instead in gene therapies may solve the immune response problems.⁵⁷

2.2 Female Duchenne Muscular Dystrophy Carriers

2.2.1 Natural History of Manifesting Carriers

While it is extremely rare for a female to have classic DMD, carriers can exhibit some clinical symptoms of DMD with variable expressivity. These carriers are commonly referred to as

manifesting, or symptomatic, carriers and are seen in 8% to 22% of DMD carriers.^{58,59} Onset of symptoms varies greatly, from early childhood to the fourth decade of life, with an average age of 33 years.⁵⁹ Muscle weakness has been reported in 19% of female carriers, muscle pain and cramping in 5%, and left-ventricle dilation in 19%.⁶⁰ Developmental delays, enlarged calf muscles, excessive clumsiness, frequent falling, and cognitive or behavioral problems have all also been reported as signs and symptoms in manifesting carriers.⁵⁹ Approximately 8% to 10% of carriers are found to have progressive dilated cardiomyopathy and is more common in carriers displaying additional signs and symptoms.⁶¹

2.2.1.1 Molecular Genetics of Manifesting Carriers

The development of signs and symptoms in DMD carriers is most commonly attributed to the genetic mechanism called skewed X-chromosome inactivation (XCI).⁶² Due to females having two X-chromosomes, one of the X-chromosomes is (inactivated in each cell of the body to act as a dosage compensation when compared to males who only one X-chromosome.⁶² The inactivation of either the maternal or paternal X-chromosome in each cell is random and acts independently of each other, meaning females should theoretically have the maternal X-chromosome active in 50% of their cells and the paternal X-chromosome active in the other cells.⁶² Skewed XCI occurs when there is an unbalanced amount of either the maternal or paternal X-chromosome being inactivated. A common cause of this is when a female inherits a pathogenic variant on one of her X-chromosomes, leading to negative selection and predominately expressing the other X-chromosome without the variant.⁶²

In the cases of female DMD carriers, however, the skewed XCI has been reported to favor the expression of the X-chromosome with the *DMD* pathogenic variant.⁵⁹ Manifesting carriers have 49% more skewed XCI profiles favoring the chromosome with the pathogenic variant

compared to the carriers who are asymptomatic.⁵⁹ Some studies have found a direct correlation between the skewed XCI profiles and the severity of the signs and symptoms in the carriers, but this is controversial and the research is ongoing.⁵⁹

A variety of chromosome rearrangements have less commonly been attributed to cause manifesting carriers. These include monosomy X, X-autosomal translocations, compound heterozygosity for two pathogenic *DMD* variants, and X-chromosome uniparental disomy.⁶³⁻⁶⁵ Pathogenic variants in both *DMD* alleles have also been found to cause signs and symptoms in females, along with differences of sexual development in apparently affected females.^{66,67} The majority of these mechanisms typically lead to much more severe phenotypes, often mimicking the same signs and symptoms seen in affected males, than seen in manifesting carriers due to skewed XCI.⁵⁹

2.2.2 Diagnosis

Females may be evaluated for DMD carrier status for a number of reasons. They may have a known family history of DMD or they may be manifesting symptoms, with or without a known family history. There are also circumstances where a female may have no clinical findings and no known family history of DMD. In these cases, the female is typically identified after having a son who is affected or through a positive result on an expanded carrier screening, which is discussed in section 2.3.

Similar to male probands, female probands should be evaluated on their clinical findings and their blood serum CK levels.²³ Approximately 50% of female DMD carriers are found to have CK levels that are 2 to 10 times above the reference range of individuals who do not have DMD.

Like affected males, the CK levels in females who are DMD carriers have been shown to decrease with age, especially after 20 years old.⁶⁸

Even in the absence of characteristic findings for DMD or elevated serum CK concentrations in their blood, molecular genetic testing should be offered to at-risk females due to the fact that up to 50% of females who are DMD carriers will not display characteristic phenotypes or have elevated CK levels.²³ If a skeletal muscle biopsy is performed, the western blot and immunohistochemistry analysis results may demonstrate diagnostic results for female DMD carriers.⁶⁹ This is more likely to occur if the female is a manifesting carrier.⁶⁹

Diagnosing female DMD carriers is important to identify females who may benefit from appropriate screening for any potential clinical symptoms associated with DMD carrier status, along with the management and treatment of those symptoms. The identification of a positive carrier status also provides information that is necessary for the females to make informed reproductive decisions. The available reproductive decision-making options for DMD carriers are discussed in section 2.3.

2.3 Reproductive Decision-Making in Females Who Are Carriers of X-Linked Genetic Conditions

Reproductive decision-making can be defined as the choice to become a parent; however, there are several reproductive options currently available to consider throughout the decision-making process.⁷⁰ There have been numerous identified factors that influence reproductive decision-making in the context of genetic risks for certain conditions that vary depending on the

female or couple and depending on the genetic condition their children are at risk for.^{6,7,71} These factors are discussed in section 2.3.2.

Genetic disease is the leading cause of infant mortality in the United States, contributing to approximately 30-50% of the annual neonatal and infant deaths.⁷² At-risk females or couples who have an increased chance to have a child with a genetic or chromosomal condition, such as DMD, can explore the so-called “non-traditional,” or alternative, reproductive options.^{73,74} Commonly reported goals of pursuing alternative reproductive options include avoiding an affected pregnancy, identifying an affected pregnancy early enough to allow for time to consider all available options, and identifying an affected pregnancy to prepare in advance for the birth of an affected child.^{75,76}

The identification of at-risk females who are carriers of X-linked conditions has been increasing due to the availability of low-cost expanded universal carrier screening, which should ideally be offered in the preconception setting.⁷⁴ Carrier screening previously targeted a small number of highly prevalent genetic conditions in ethnic-defined populations; however, expanded universal, or pan-ethnic, carrier screening has been developed to assess hundreds of different conditions across all populations, leading to increased numbers of individuals learning of positive carrier status and possibly considering alternative reproductive options.^{74,75,77} According to one study, approximately 35% of individuals that undergo expanded universal carrier screening are found to be a carrier for at least one genetic disorder.⁷⁸ Expanded universal carrier screenings routinely offer the option for screening X-linked genetic conditions, including DMD, which would identify females who are carriers.⁷⁹

2.3.1 Reproductive Options for Females Who Are Carriers of X-Linked Genetic Conditions

Throughout the reproductive decision-making process, there are options that can be considered prior to and during pregnancy for females who are carriers for an X-linked genetic condition.^{3,4,80} There are also family planning options that do not involve the at-risk females becoming pregnant themselves.³ Figure 1 outlines the timing of the available reproductive options in regard to pregnancy status. The current reproductive technologies, tests, and options available to assist in becoming a parent are explored in further detail in the sections below.

Prior to Pregnancy	During Pregnancy	Alternatives to Pregnancy
<ul style="list-style-type: none">• Preimplantation Genetic Testing (PGT)• Gamete Donation• Natural Conception	<ul style="list-style-type: none">• Prenatal Diagnostic Testing• Prenatal Sex Determination	<ul style="list-style-type: none">• Adoption• Not Planning to Have (Additional) Children

Figure 1. Reproductive Options Prior To, During, and Without a Pregnancy

2.3.1.1 Preimplantation Genetic Testing

PGT is a preconception option that can be considered prior to pregnancy and is achieved via IVF and genetic analysis. The process of PGT consists of the eggs being fertilized within a laboratory setting to obtain embryos and one cell from each embryo being removed to screen for the at-risk genetic condition.⁸¹ Only the embryos without the condition are implanted to begin pregnancy.⁸¹ Additionally, females who are at-risk for having a son with an X-linked condition

have another option of electing to have the embryos screened for sex chromosomes and only implanting those that will become female fetuses, instead of screening for the genetic condition itself.⁸²

2.3.1.2 Gamete Donation

Gamete donation is another available reproductive option during the preconception stage of family planning for females who are X-linked carriers. Gamete donation refers to the utilization of donated eggs or sperm, which may be from anonymous or known donors, to allow for at-risk females to become parents.⁸³ In cases of females who are X-linked carriers, the process typically consists of the egg or eggs being removed from the donor and utilizing IVF to fertilize the egg in the laboratory setting. The embryo can then be implanted.

2.3.1.3 Natural Conception

Females who are X-linked carriers may decide to conceive naturally without any medical interventions prior to pregnancy. During the pregnancy stage, the female may or may not elect to pursue prenatal genetic testing to find out if the fetus has the genetic condition.⁵ If the prenatal diagnostic testing is not chosen, there is the option to test the baby after birth to determine if the genetic condition is present.⁵

2.3.1.4 Prenatal Diagnostic Testing

Prenatal diagnostic testing is the only way to diagnose or definitively rule out a genetic or chromosomal condition for a fetus during a pregnancy. There are two different procedures that can be done, depending on the timing of the pregnancy. The earliest prenatal diagnostic test is a chorionic villus sampling, or CVS. This is typically performed between 10 and 13 weeks of

pregnancy and involves the removal of chorionic villi cells from the placenta.⁸⁴ The chorionic villi cells can either be collected in a transcervical or a transabdominal procedure. Because placental cells are analyzed, there is an approximately 1% chance of confined placental mosaicism occurring, which means the DNA from the placenta is not the same as the DNA from the fetus.⁸⁵

The other prenatal diagnostic testing option is an amniocentesis that is typically performed any time after 15 weeks of pregnancy.⁸⁴ It is a transabdominal procedure that involves the removal of amniotic fluid, which contains fetal cells, from the amniotic sac.⁸⁴ A CVS and amniocentesis are both invasive procedures and hold a risk for a miscarriage, which is usually between 0.1% and 1% depending on where the procedure is being performed.^{86,87}

The information given from either of the prenatal diagnostic tests can be utilized in different ways. Some females who are X-linked carriers chose to use the information to prepare for the birth of an affected child.⁷⁵ This can allow time to coordinate a specialized delivery plan, schedule appointments with a care team to discuss the managements and treatments for after the baby is born, set up early intervention services, and so forth.⁷⁵ Other females may choose to use the information to determine if a pregnancy termination or placing the child up for adoption after birth is the right option for them.⁸⁸

2.3.1.5 Prenatal Sex Determination

Females who are X-linked carriers may choose to identify the sex of the fetus during a pregnancy in order to more accurately assess the risk of the fetus being affected. Historically, the standard for determining the fetus's sex was through a CVS procedure at 11-13 weeks.⁸⁹ If the fetus was identified as a male, further genetic testing could be performed on the chorionic villi cells to determine if the fetus carries the pathogenic variant for the X-linked condition and would be affected.⁸⁹

However, in recent years, a screening test called non-invasive prenatal testing (NIPT) has been implemented into clinical practices with a mean sensitivity of 98.9% between 7- and 20-weeks of gestation.⁹⁰ Unlike a CVS, NIPT is performed from a blood draw on the pregnant female and does not have a risk of miscarriage.⁹¹ Therefore, females who are at-risk to have a son with an X-linked condition may opt to pursue NIPT and will then decide if confirming the sex through prenatal diagnostic testing is something they are interested in if the results indicate the fetus is likely male.⁸⁹ Females who are X-linked carriers may also use these results to avoid an invasive procedure if the results indicate the fetus is likely female.⁹⁰ Because NIPT is a screening test, it is not 100% accurate in determining the fetal sex and there is the possibility of inconclusive NIPT results.^{90,91} Therefore, some females who are X-linked carriers may decide to pursue a CVS without a prior NIPT screen to avoid the possibility of inaccurate results of the fetal sex or inconclusive results.⁹¹

2.3.1.6 Additional Reproductive Options

There are also reproductive options that involve the decision of not getting pregnant or having biological children. Some females who are X-linked carriers may decide that adopting a child is the right reproductive decision for them.⁵ There are currently an estimated 3,000 adoption agencies in the United States, which are both private and public.⁹²

Additionally, some at-risk females may decide not to have any children.⁹³ While others may decide to not have any additional children, either biological or adopted.⁹⁴ Females and their partners may use various forms of birth control and contraception to assist in these decisions.

2.3.2 Factors that Influence the Reproductive Decision-Making Process

Deciding on reproductive options is a personal decision and a complex, sensitive matter.⁹⁵ Both financial and emotional resources have previously been found to impact at-risk females' capacity to not only make an informed decision that is best for them, but to also implement that decision.⁸ If a female or couple is unable to make an informed decision they may face physical, mental, and emotional challenges.⁸

Educating medical professionals to have discussions about family planning options is necessary to minimize the stress for at-risk females, along with ensuring they understand all the available options to make an informed decision.¹⁰ Genetic counseling is a process consistently found to be most helpful in aiding patients through difficult decisions.^{11,96} Genetic counselors are trained to facilitate their counselee's decision-making through discussing their counselee's goals and expectations, reviewing relevant genetic information, and giving comprehensive information on available options.⁹⁶

Factors that have been noted to influence the reproductive decision-making process in at-risk females or couples consist of religious or cultural beliefs, perceptions of risk, family expectations, and the desire to have (additional) children.^{6-8,97} Research has shown that whether or not the female has previous children, along with if the children are affected also impacts the decision-making process.⁷ Other factors revolve around the genetic condition, such as the personal experiences with the condition, a sense of responsibility to prevent a child from suffering, the perceived quality of life of those affected, and the burden of the condition on the affected child and the family as a whole.^{7,9,10}

2.3.3 Previous Studies on X-Linked Carriers Altering Reproductive Plans

There have been various studies focusing on the influence of carrier status on reproductive decision-making in females who are carriers for different X-linked conditions. Broadly, these studies have indicated that X-linked carriers were likely to change their reproductive plans, especially when the carriers were mothers of affected children.^{13,97-100} A study conducted by McConkie-Rosell et al. focused on the reproductive decisions of 28 female who are carriers of fragile X, an X-linked condition causing intellectual delays, developmental delay, and autism predominately in males.¹⁰¹ Of the 28 carriers, 19 (67%) opted to not have any more children based on their positive carrier status. When the carriers had previously not known about their carrier status, 25 (89%) would have “reduced the size of their family or not had any biological children.”^{100,101} Subsequent studies on reproductive decisions in females who are fragile X carriers and in females who are carriers for other X-linked conditions have shown similar findings.^{13,97-100} Additionally, a study of females who are carriers of fragile X with at least one affected child found that 77% of the participants decided not to have any additional biological children after learning they were carriers.⁹⁷

There have been a few previously published studies looking at the reproductive decisions made by females who are DMD carriers; however, many of the studies were published prior to the current available reproductive options, such as preimplantation genetic testing, or the studies utilized population-based surveillance where the carriers could not comment on the motivations of their reproductive choices.^{11,12} While this limits the opportunity to examine all reproductive decisions being made by females who are DMD carriers, the studies provide evidence that many females who are DMD carriers alter their reproductive plans based on their carrier statuses, similarly to carriers for other X-linked conditions.^{11,12,102,103} For instance, one study focusing on

childless females who were at-risk to be DMD carriers found that the factor most strongly correlating with the decision to pursue prenatal diagnostic testing was when the female had at least one deceased affected family member.¹¹

A subsequent study conducted by Kay et al. focused on reproductive decision-making of 14 females who are carriers for a variety of X-linked conditions. The study found that the decision to avoid an affected pregnancy was more absolute and concrete in females who were carriers and had an affected brother, compared to the decision in females who were carriers without an affected brother.¹³ The study also reported that being an female who was an X-linked carrier had led to a stronger emphasis on the importance of contraception and sterilization after completing their families to avoid an unplanned pregnancy.¹³

2.4 Psychosocial Impacts of Genetic Conditions

2.4.1 Genetic Testing

Genetic testing has been available since the 1950s when scientists discovered how to count the number of chromosomes in each cell.¹⁰⁴ Since then, genetic testing has continued to grow rapidly, evolve, and become more widely available over the years, particularly after the human genome was mapped in 2003.¹⁰⁵ According to the World Health Organization, there are over 10,000 monogenic, or genetic conditions caused by a single pathogenic gene change, identified to date, with the prevalence of approximately 10 in 10,000 worldwide.¹⁰⁶ These conditions can have serious health implications; for example, in the United States, genetic conditions account for up to 12% of hospital admissions in the adult population and a pediatric hospital in Cleveland, Ohio

found that 81% of inpatient admissions were attributed to genetic conditions.^{107,108} As the knowledge of genetic conditions and the demand for precision medicine continues to grow, so does the demand for genetic testing to identify the genetic statuses of certain conditions in individuals. Currently, there are over 75,000 genetic tests available; between 2014 to 2017, there were roughly 10 new tests entering the market every day.¹⁰⁵

Along with the rise of genetic testing, there has been a rise in the genetic conditions added to the Recommended Uniform Screening Panel for newborn screening (NBS) as another way to identify genetic conditions.¹⁰⁹ There are currently 35 conditions that are recommended for every newborn to receive screening for; however, the genetic conditions that are screened vary from state to state.¹¹⁰ The purpose of newborn screening is to identify possible genetic diagnoses as early as possible when the conditions have effective treatments or interventions that can improve prognoses.¹⁰⁹ In reference to DMD, several states are currently considering implementing newborn screening for DMD with the rise of FDA approved medications that may be more effective in managing DMD when started before symptoms arise.^{111,112} A study by Wood et al., identified that this decision would likely be supported by families, as 92.9% of expectant parents and 95.9% of parents who have a child diagnosed with an inherited muscular dystrophy, either DMD, BMD, or spinal muscular atrophy, are in favor of NBS for these conditions.¹¹³

2.4.2 Psychosocial Impacts

With the high volume of genetic testing being performed, there has been an interest in analyzing the psychosocial effects of identifying genetic condition risks on both an individual and on family dynamics.^{114,115} The ethical and clinical concerns of knowing genetic health information have led to conversations and debates not only between researchers, but also amongst the public

themselves. To date, researchers have identified numerous possible psychosocial impacts, both advantages and disadvantages, directly related to identifying statuses of varying genetic conditions.^{114,116-118}

When considering the psychosocial effects of knowing genetic risks, the broad potential beneficial impacts previously identified consist of relief from uncertainty and guilt, positive empowerment, improved family supports, and satisfaction of curiosity.^{114,115,119,120} In contrast, the reported potential adverse psychosocial impacts are mental health difficulties, including anxiety, depression, and stress; genetic stigmatization and discrimination regarding life, long-term care, and disability insurance; and disrupted family dynamics.^{114,115,119,121} There are also problematic psychosocial impacts seen specifically when an individual's genetic testing results are negative. For example, the individual may potentially experience false reassurance that the condition will not arise or may experience the impact of survivor's guilt when other family members have positive test results.^{119,122} Lastly, there can be particular detrimental psychosocial impacts when an individual receives a variant of uncertain significance. This is a result that occurs when there is currently not enough information on the identified result and the laboratories therefore cannot determine if the genetic test result is harmful or benign. This type of genetic test outcome has been seen to cause prolonged uncertainty and decisional regret for pursuing testing in the individual or family receiving this result.^{119,123}

Because there are several different categories and situations surrounding genetic information, the psychosocial impacts can vary. For example, identifying an individual's genetic status for a hereditary cancer predisposition compared to carrier testing results compared to an inherited cardiac condition results are likely to have different psychosocial effects on an individual and family.^{74,114,124,125} Furthermore, identifying genetic risks for children and symptomatic

patients, along with identifying information on incurable and ultimately lethal genetic conditions, each have their own psychosocial benefits and risks.^{114,116-118} There are also separate psychosocial analyses on individuals who are known to be at-risk for a particular genetic condition and ultimately decline genetic testing.^{119,126} For the purpose for this literature review, section 2.4.2.1 will look more closely into the psychosocial impacts of females who are carriers of a genetic condition.

2.4.2.1 Females Who Are Carriers of X-Linked Recessive Genetic Conditions

There have been some studies investigating the psychosocial impacts of females with positive carrier statuses for a range of genetic conditions, with the effects varying greatly between females who are carriers of X-linked genetic conditions compared to carriers for autosomal recessive conditions.^{13,98,127-129} For instance, previous studies found that mothers of children with X-linked conditions experienced significantly higher levels of guilt and feelings of being blamed by their partners when compared to mothers of children with autosomal recessive conditions.^{101,127} Mothers of children with X-linked carriers have also been identified to have significant levels of self-blame, feelings of responsibility, and grief.^{98,127,128} Additionally, studies have shown X-linked carriers without affected children still carry self-blame since the condition is present in the family.⁹⁸

Other detrimental psychosocial impacts reported in females who are X-linked carriers are loss of reproductive expectations, sadness, anger, depression, and overall less positive feelings in regard to themselves.^{101,128,129} The potential positive psychosocial impacts revolve around the relief in finding an explanation for the symptoms within the family, the opportunity to alert other family members of their genetic risks, and the reduction of uncertainty and anxiety for at-risk females after pursuing genetic testing.^{119,130}

As there is limited research exclusively on females who are carriers for DMD, this study will provide a first step of exploring factors on carrier status and family history, birth and pregnancy history, and demographics that potentially impact the reproductive decision-making specifically for females who are carriers of DMD. From there, the results may lead to other potential research opportunities to further assess how these factors, the reproductive decisions made, and positive carrier statuses for DMD as a whole psychosocially impact females who are DMD carriers.

3.0 Manuscript

3.1 Background

DMD is an incurable genetic disorder that is characterized by progressive proximal muscle weakness and atrophy of the skeletal and heart muscles.¹ Those affected with DMD typically experience loss of ambulation in early teenage years and premature death, most commonly due to respiratory or dilated cardiomyopathy complications.¹ The median survival is 24 years; however, life expectancy can be greater than 30 years of age depending on the interventions and care routes that are pursued.^{2,14} DMD is the most common childhood form of muscular dystrophies and it is estimated that DMD affects approximately 1 in 3,500 to 1 in 5,000 live male birth.^{17,18,19} In very rare cases, females can also be affected with DMD, with an estimated prevalence of 1/50,000,000 live female births.²⁰

DMD is caused by pathogenic variants in the *DMD* gene, which leads to chronic muscle damage, muscle inflammation and wasting, and ultimately the loss of muscle function within the skeletal and cardiac muscles.^{19,21,22} DMD is an X-linked recessive condition and females who are heterozygous carriers of DMD have a 25% chance of having an affected son with each pregnancy.³⁰

3.1.1 Reproductive Options for Females Who Are Carriers of DMD

Females who are carriers of DMD have several reproductive options.^{73,74} Preconception options include IVF with PGT for DMD, utilizing an egg donor, adoption, and no longer planning

to have (additional) children.^{5,81-83,93,94} Prenatal options include prenatal sex determination and prenatal diagnostic testing, including CVS and amniocentesis.^{84,85} Common goals of pursuing reproductive options for at-risk females include avoiding an affected pregnancy, identifying an affected pregnancy early enough to allow for time to consider all available options, and identifying an affected pregnancy to prepare in advance for the birth of an affected child.^{75,76} Additionally, females who are carriers of DMD may decide to conceive naturally without pursuing any reproductive options before or during a pregnancy.⁵

3.1.2 Factors that Influence the Reproductive Decision-Making Process

Reproductive decision-making is a personal, complex, and sensitive process.⁹⁵ Several previous studies have examined the reproductive decision-making of at-risk females for various genetic conditions. Previous factors identified that influence the reproductive decision-making process consist of religious or cultural beliefs, perceptions of risk of having an affected child, family expectations, and the desire to have (additional) children.⁶⁻⁸ Family structures, such as if the female has previous children and if the children are affected, have been noted to influence decision-making.⁷ Other factors relate to the female's personal experiences with the genetic condition, a sense of responsibility to prevent a child from suffering, the perceived quality of life of those affected, and the burden of the condition on both the affected child and the family.^{7,9,10}

3.1.3 Study Goals

This study aimed to investigate the factors that influence the reproductive decision-making specifically for females who are DMD carriers. There have been previous studies focusing on the

reproductive decisions of females who are carriers for a wide variety of genetic conditions; however, it is difficult to make the assumption that the reproductive choices of females who are carriers for one genetic condition will be influenced by the same factors that affect the reproductive choices of females who are carriers of DMD. Genetic conditions can have different genetic mechanisms, levels of severity, life expectancy, available treatment or cures, and other varying features and these factors may impact reproductive decision-making in various ways. While previous studies have evaluated the reproductive decisions made by females who are DMD carriers, many were published prior to the current available reproductive options, such as PGT, had sample sizes with less than 20 participants, or utilized population-based surveillance where the carriers could not comment on the motivations of their reproductive choices.¹¹⁻¹³

Therefore, the goal of this study is to provide an up-to-date, deeper understanding of the personal factors that influence reproductive decision-making for females who are DMD carriers. The hope is for this information to provide insight and improved psychosocial interventions for genetic counselors, or other health care professionals, who provide care for females who are DMD carriers.

3.2 Methods

The University of Pittsburgh Institutional Review Board (IRB) approved this study as Exempt under IRB: STUDY19090254 (Appendix A).

3.2.1 Study Participants

The target population for this study included females who have been diagnosed as DMD carriers and are 18 years of age and older. Males were excluded from participation, as DMD carriers are almost exclusively females due to the X-linked recessive inheritance pattern of this condition. Therefore, males with a pathogenic variant are expected to be affected with DMD and would not be classified as carriers.

3.2.2 Survey Development

The created survey for this study was adapted from Ghioffi et al. (2018) with permission (Appendix C). The survey was developed and distributed online via Qualtrics software, which meets the University Data Security standards through the University of Pittsburgh for collecting and storing data. The survey was available through an anonymous web link. It consisted of 65 questions, both multiple choice and open-ended, and utilized skip logic, so participants would only be shown relevant questions based on their pregnancy status when they were identified as carriers and their reproductive decisions. Therefore, participants were each shown between 15 and 22 multiple choice questions and three to eight open-ended questions. The survey was reviewed by two genetic counselors, a statistician, and an occupational therapy research who all have significant experience in qualitative and/or quantitative research.

The survey consisted of four sections: 1) introduction and consent, 2) diagnosis of carrier status, 3) reproductive decisions, and 4) demographic information. The introduction and consent section included the introductory script that explained the study, discussed potential risks and benefits, and provided contact information for the principal investigator and faculty chair. After

reading the introductory script, potential participants were given the option to consent and begin the survey or to decline participation and exit the survey. The introduction and consent section also included definitions for a list of reproductive terms used in the survey.

The diagnosis of carrier status section focused on background questions regarding the respondents' carrier statuses, such as how old they were when learned they were a carrier, how they were identified, their pregnancy status when they found out, and other similar questions.

There was a skip logic for the next section on reproductive decisions; certain questions were displayed differently based on whether or not the participants learned they were carriers for DMD during pregnancy. The section focused on the reproductive decisions that the participants pursued or plan to pursue. These reproductive decisions included IVF with PGT for DMD, egg donation, adoption, prenatal diagnostic testing, prenatal sex determination, no longer planning to have (additional) children, not planning to pursue any alternative options, or selecting 'other' with a free text response to elaborate. The respondents were able to select all decisions that applied to them and could further explain the decisions they made or plan to make in follow-up free text questions.

The last section of demographic information included questions about the participants reproductive history, number of children diagnosed with DMD, religious affiliation, ethnicity, and other relevant demographic questions.

3.2.3 Survey Recruitment and Distribution

The recruiting process was completed through a number of methods. Females who are DMD carriers were contacted via email, letters, or in-person to ask their permission to receive an email with the survey link or to take a flyer with the survey link. Therapeutic Research in

Neuromuscular Disorders Solutions (TRiNDS) and DMD advocacy groups located in the United States and Canada posted an IRB approved advertisement on their social media sites. The DMD advocacy groups included CureDuchenne, DuchenneXchange, Jesse's Journey, Jett Foundation, Little Hercules Foundation, and Parent Project Muscular Dystrophy. Within the introductory script of the survey, there was also a sentence asking potential participants to forward the survey link to other females who may match the inclusion criteria and who may be willing to complete the survey. The advertisement provided in the emails, the social media postings, or printed as flyers contained study information and how interested potential participants could access the survey (Appendix D). The survey remained open for two months from December 2019 to February 2020.

Participants were consented to participate in the study by reviewing the introductory script and selecting "Yes, I consent to participate [Begin Survey]" before beginning the survey (Appendix B). Within the introductory script, participants were informed that survey participation was voluntary and could be discontinued at any point. The introductory script also described that participants could decline to answer any questions that they did not wish to answer. No compensations or incentives were offered to participants for their participation.

3.2.4 Data Analysis

The data from the anonymous survey responses were recorded in Microsoft Excel and descriptive statistics were calculated. The data were analyzed via Fisher's exact test within Stata/SE statistical software for comparison between various factors and the reproductive decisions pursued. Pearson's chi-squared test within Stata/SE statistical software was used in place of Fisher's exact test when all of the expected counts in the two-way contingency tables were greater than 5. P-values less than 0.05 were considered statistically significant.

While qualitative analysis was not the main methodical approach for the study, thematic analyses on the free text responses were performed in Microsoft Excel by the lead researcher and distributed to the other study investigators for review. Since participants could skip questions, data analysis was performed for every item regardless of the number of survey respondents for each one.

3.3 Results

The survey was available for access for approximately four weeks and opened by 176 individuals. Within one day of the DMD advocacy groups being provided with the IRB approved advertisement with the survey link, 61 individuals opened the survey and within one week, 132 individuals opened the survey. Thirty-four participants exited the survey prior to finishing it for unknown reasons, and one participant declined consent. Of those who exited the survey, 29 (85.3%) did not answer any of the questions, while 5 (14.7%) answered between one and 11 questions without finishing the survey. One hundred forty-one participants consented and finished the survey, providing either partial or full responses for the multiple-choice questions. Of these participants, 121 completed every multiple-choice question. For the purpose of this study, data from all 141 participants who finished the survey were analyzed regardless if whether the survey partially or fully completed; therefore, some questions have response rates less than n=141. The median completion time of the survey was five minutes and 48 seconds. Based on the study recruitment methods, it is unknown how many females who are DMD carriers and 18 years of age and older were made aware of the survey. Therefore, a response rate cannot be calculated. Figure

2 illustrates the participant inclusion process, along with the breakdown of the participants included in the various analyses conducted.

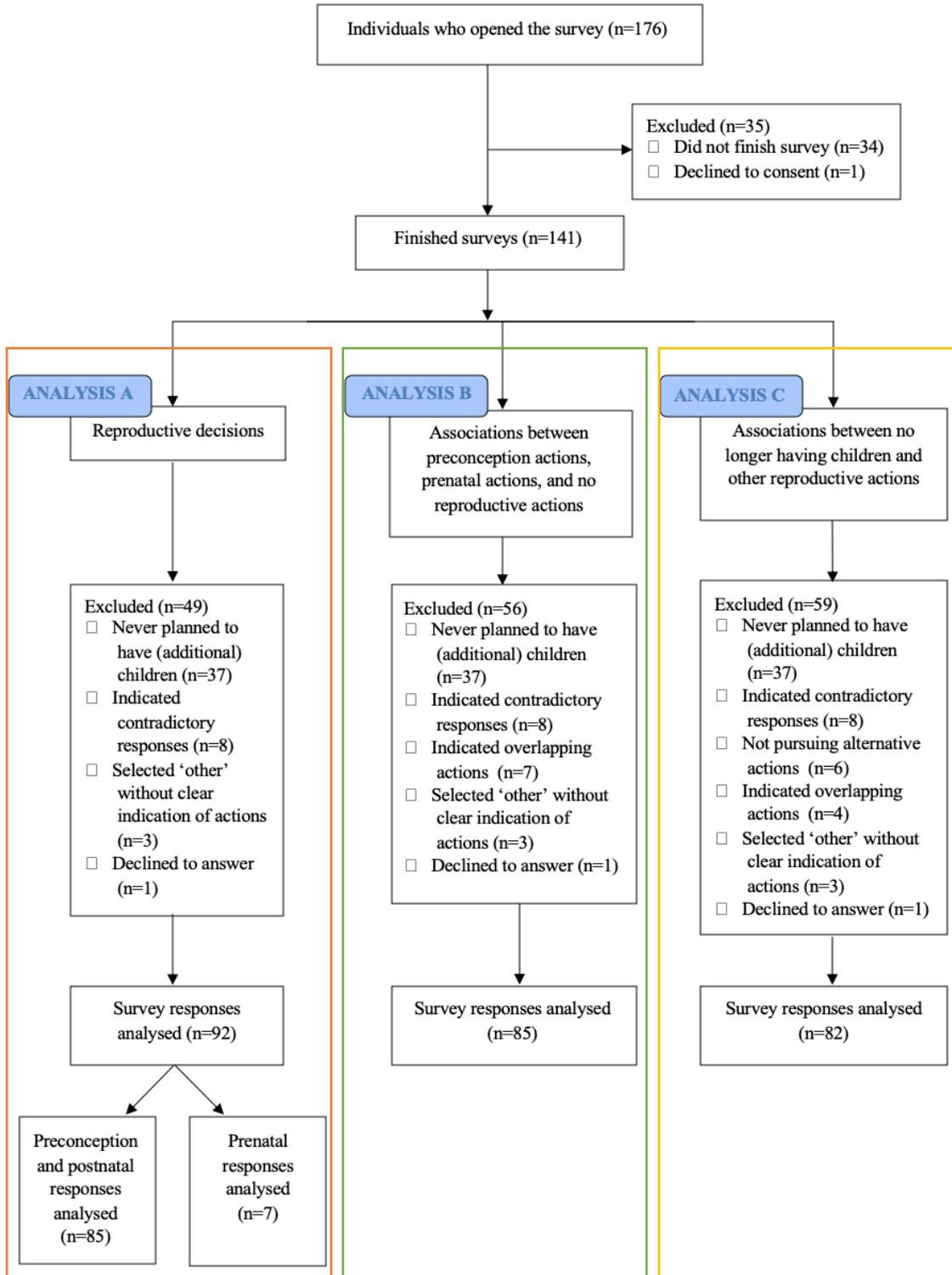


Figure 2. Participant Inclusion and Exclusion Criteria for Analyzes Performed

3.3.1 Demographic Information

The respondents were predominately Caucasian (92.1%) and just under two-thirds (64.4%) of the 141 participants indicated a religious affiliation. Excluding out the 28 (20.3%) participants who declined to state their annual household income, the responses consisted of relatively equal amounts below \$75,000 (52.7%) and exceeding \$75,000 (47.3%). Likewise, the respondents were nearly equal for highest completed education level, with 51.8% reported completing some college, high school, or less and 48.2% reported completing a bachelor's degree or higher (Table 2).

Table 2. Participant Demographic Information (N=141)

Characteristic	<i>n</i>	Responses, n	Percentage
Ethnicity	139		
Caucasian		128	92.1%
Hispanic		4	2.9%
Asian or Pacific Islander		3	2.2%
Multiracial		3	2.2%
African American		1	0.7%
Religious Affiliation	141		
None		45	31.9%
Catholic		31	22.0%
Protestant		27	19.1%
Christian		17	12.1%
Hindu		3	2.1%
Others		13	9.2%
Decline to state		5	3.5%
Identified Gender	141		
Female		140	99.3%
Male		1	0.7%
Highest level of education	141		
High school or less		22	15.6%
Some college		51	36.2%
Bachelor's degree or higher		68	48.2%

Table 2 Continued			
Annual household income	138		
<\$34,999		26	18.8%
\$35,000 - \$49,999		11	8.0%
\$50,000 - \$74,999		21	15.2%
\$75,000 - \$99,999		16	11.6%
>\$100,000		36	26.1%
Decline to state		28	20.3%

The majority of respondents (91.5%) learned of their positive DMD carrier status while not pregnant and 8.5% of participants learned of their carrier status during a pregnancy. Of the responding females, 58.5% learned of their carrier status at or after the age of 30 and 41.5% learned of their carrier status under the age of 30. The majority of the respondents, 69.5%, did not have family history of DMD in any of their male relatives (Table 3).

Table 3. Carrier Status and Family History (N=141)

Characteristic	<i>n</i>	Responses, n	Percentage
Age at learning carrier status	135		
0-9		2	1.5%
10-19		18	13.3%
20-29		36	26.7%
30-39		60	44.4%
40 and older		19	14.1%
Pregnancy status at time of learning carrier status	141		
Pregnant		12	8.5%
Preconception/Postnatally		129	91.5%
Family History of DMD	141		
Yes		43	30.5%
No		98	69.5%

Additional information was collected on the respondent's birth and pregnancy histories. There was a wide range in the number of children the respondents had; however, the majority of the responding females who are carriers had one son diagnosed with DMD (60.7%). Of the respondents with one or more children with DMD, 57.5% did not have any biological children before their first son was diagnosed and the majority did not have any additional children after their son was diagnosed with DMD (73.6%) (Table 4).

Table 4. Birth and Pregnancy History (N=141)

Characteristic	<i>n</i>	Responses, n	Percentage
Number of Children	141		
None		20	14.2%
1		28	19.9%
2		54	38.3%
3		31	22.0%
4 or more		8	5.7%
Children diagnosed with DMD	140		
None		34	24.3%
1		85	60.7%
2		21	15.0%
3 or more		0	0%
Age when oldest son with DMD was born	104		
18-20		7	6.7%
21-25		20	19.2%
26-30		35	33.7%
31-35		25	24.0%
36-40		17	16.3%
Biological children before oldest son with DMD was born	106		
None		61	57.5%
1		29	27.4%
2		14	13.2%
3 or more		2	1.9%

Table 4 Continued

Pregnancies after oldest son with DMD was diagnosed	105		
Yes		36	34.3%
No		69	65.7%
<hr/>			
Births after oldest son with DMD was diagnosed	106		
Yes		28	26.4%
No		78	73.6%

3.3.2 Reproductive Decisions

The analysis exploring the reproductive decisions (Analysis A in Figure 2) evaluated the reproductive actions selected or indicated by the respondents. An exclusion criterion used for this analysis included “indicated contradictory responses,” which was defined as selecting both reproductive actions and have not or do not plan to pursue any additional actions. Of the 92 respondents, 86 participants (93.5%) indicated that after learning their positive carrier status, alternative reproductive decisions were taken or plan to be taken. Six of the 92 (6.5%) indicated they did not pursue or do not plan to pursue any alternative options. The most prevalent reproductive action was no longer planning to have (additional) children with 49 of the 92 respondents (53.2%) indicating it was the only action they pursued or plan to pursue and 53 (57.6%) indicating it was at least one of the actions they pursued or plan to pursue. This was followed by IVF with PGT for DMD with 20 respondents (21.7%) indicating it was at least one of the actions they pursued or plan to pursue. 17 of the 92 respondents (18.5%) indicated prenatal diagnostic testing was at least one of the actions they took or plan to take.

In section 3.3.2.1. and 3.3.2.2., the specific reproductive actions the 141 respondents selected are described in more detail based on whether they learned of their carrier status during a pregnancy or while not pregnant.

3.3.2.1 Reproductive Decisions While Pregnant

Twelve of the 141 (8.5%) females who are DMD carriers were pregnant at the time of learning their positive carrier status. Two of the 12 (16.7%) chose to pursue prenatal diagnostic testing, either a CVS or amniocentesis, and nine of the 12 (75.0%) decided against pursuing a diagnostic test. One (8.3%) participant reported planning a diagnostic test, but miscarried prior to the procedure. For respondents who underwent prenatal diagnostic testing, both fetuses were found to be male and unaffected with DMD. Both pregnancies were continued. Five of the ten (50.0%) respondents who did not pursue prenatal diagnostic testing made the decision after finding out the fetus was female.

The respondents were asked of the reproductive options they pursued or planned to pursue following the pregnancy where they learned of their positive carrier status. After excluding the respondents who never planned to have additional children prior to learning their DMD carrier status, those who selected contradictory responses, and those who selected “other” without providing clear indications of their decisions when asked to elaborate, 7 of the 92 participants (7.6%) were pregnant at the time of learning their positive carrier status and were included in the analysis. Six (85.7%) reported they are no longer planning to have additional children and one (14.3%) reported planning to pursue prenatal sex determination and/or prenatal diagnostic testing in future pregnancies. The respondent planning to pursue prenatal reproductive actions indicated the desire to have IVF with PGD for future pregnancies, but is unable due to the expense. No respondents indicated they were planning to pursue the options of adoption or egg donation.

3.3.2.2 Reproductive Decisions While Not Pregnant

After excluding the respondents who never planned to have additional children prior to learning their DMD carrier status, those who selected contradictory responses, and those who selected “other” without providing clear indications of their decisions when asked to elaborate, 85 of the 92 participants (92.4%) were not pregnant at the time of learning their positive carrier status. As the respondents were asked to select all the reproductive actions they pursued or plan to pursue, 117 total reproductive actions were reported.

The most prevalent reproductive action was no longer planning to have (additional) children, with 47 of the 85 respondents (55.3%) indicating it was at least one of the actions they pursued or plan to pursue. Twenty of the 85 respondents (23.5%) indicated IVF with PGT for DMD was at least one of the actions they pursued or plan to pursue, while 16 of the 85 respondents (18.8%) indicated prenatal diagnostic testing was at least one of the actions they took or plan to take. A total of ten respondents (11.8%) pursued or plan to pursue prenatal sex determination as at least one of their reproductive actions and ten respondents (11.8%) pursued or plan to pursue adoption as at least one of their reproductive actions. Finally, six (7.1%) did not or do not plan to pursue any alternative options.

3.3.3 Data Associations with Reproductive Decisions

Data analyses were performed to compare associations and trends between various factors and the reproductive decisions participants indicated they pursued or plan to pursue. Twelve factors were analyzed including factors on carrier status and family history, birth and pregnancy history, and demographics of the females who are DMD carriers. The factors related to carrier status and family history consist of: the age at the time of learning positive DMD carrier status,

pregnancy status at the time of learning positive DMD carrier status, if genetic counseling was received, how well they feel they understand their reproductive options, and if they have a family history of DMD. Birth and pregnancy history factors consist of: if they have biological children, if they have children diagnosed with DMD, the age when their oldest child with DMD was born, and if they had biological children before their oldest child with DMD was born. The analyzed demographic factors consist of: religious affiliation, highest level of education completed, and annual household income.

3.3.3.1 Preconception, Prenatal, and No Actions Taken

Reproductive decisions of the participants were first categorized into preconception actions taken, prenatal actions taken, and no actions taken. Preconception actions taken consisted of options of IVF with PGT for DMD, egg donation, adoption, and/or no longer planning to have (additional) children. Prenatal actions taken included prenatal sex determination and/or prenatal diagnostic testing, such as CVS or amniocentesis. No actions taken consisted of did not or do not plan to pursue any alternative options.

The analysis exploring possible associations between preconception actions, prenatal actions, and no reproductive actions (Analysis B in Figure 2) included the exclusion criterion “indicated contradictory responses,” which was defined as selecting both reproductive actions and have not or do not plan to pursue any additional actions. The exclusion criterion of “indicated overlapping actions” was defined as respondents who indicated actions within multiple categories, i.e. both preconception actions and prenatal taken, for this specific analysis. Overall, 85 participants provided interpretable answers on the reproductive options they previously pursued or plan to pursue. Table 5 depicts the results for the demographic features and the reproductive preconception actions, prenatal actions, and no actions taken.

Of females who learned of their carrier status under the age of 30, 66.7% (28/42), 23.8% (10/42), and 9.5% (4/42) indicated preconception actions, prenatal actions, and no actions taken, respectively. This is compared to the females who learned of their carrier status at or over the age of 30, where 91.9% (34/37) reported preconception actions, 5.4% (2/37) reported prenatal action, and 2.7% (1/37) reported no actions taken. This suggests the age at which female DMD carriers learn their positive carrier statuses influences their reproductive decisions ($p=0.022$).

Likewise, there was a significant difference between the reproductive actions of the participants with a family history of DMD, meaning they have family members affected with DMD, not including if they have a child who is diagnosed, versus the participants without a known family history ($p<0.001$). Of the respondents with a family history, 57.6% (19/33), 24.2% (8/33), and 18.2% (6/33) indicated preconception actions, prenatal actions, and no actions taken, respectively versus the respondents with no family history where 92.3% (48/52) indicated preconception actions, 7.7% (4/52) indicated prenatal actions, and 0% (0/52) indicated no actions taken.

Lastly, it was determined that the reproductive actions pursued were significantly different based on the highest level of education ($p=0.017$). Of the respondents who completed some college, high school, or less, 69.0% (29/42) indicated preconception actions, 16.7% (7/42) indicated prenatal actions, and 14.3% (6/42) indicated no actions taken versus the respondents who completed a bachelor's degree or high and indicated 88.4% (38/43), 11.6% (5/43), and 0% (0/43) for preconception actions, prenatal actions, and no actions taken, respectively.

No significant difference was identified between the pregnancy status at the time of learning DMD carrier status, if genetic counseling was received, how well they feel they understand their reproductive options, if they have biological children, if they have children

diagnosed with DMD, the age when their oldest child with was DMD was born, if they had biological children before their oldest child with DMD was born, their religious affiliation, or their annual household income.

Table 5. Associations with Preconception, Prenatal, and No Reproductive Actions

Age at Time of Learning DMD Carrier Status (N=79)			
	<30	30+	Total
Preconception action taken	28	34	62
Prenatal action taken	10	2	12
No action taken	4	1	5
Total	42	37	p=0.022
Pregnancy Status at Time of Learning DMD Carrier Status (N=85)			
	Pregnant	Preconception/ Postnatal	Total
Preconception action taken	6	61	67
Prenatal action taken	1	11	12
No action taken	0	6	6
Total	7	78	p=1.000
Received Genetic Counseling (GC) (N=83)			
	GC Services	No GC Services	Total
Preconception action taken	53	12	65
Prenatal action taken	11	1	12
No action taken	5	1	6
Total	69	14	p=0.866
Understanding of Reproductive Options (N=85)			
	Extremely or very well	Moderately, slightly, or not well at all	Total
Preconception action taken	43	24	67
Prenatal action taken	11	1	12
No action taken	4	2	6
Total	58	27	p=0.166
Family History of DMD (N=85)			
	Family hx	No Family hx	Total
Preconception action taken	19	48	66
Prenatal action taken	8	4	12
No action taken	6	0	6
Total	33	52	p<0.001
Biological Children (N=85)			
	Yes	No	Total
Preconception action taken	57	10	67
Prenatal action taken	9	3	12
No action taken	3	3	6
Total	69	16	p=0.065

Table 5 Continued

Children Diagnosed with DMD (N=84)			
	None	1+	Total
Preconception action taken	15	51	66
Prenatal action taken	6	6	12
No action taken	3	3	6
Total	24	60	p=0.065
Age When Oldest Son with DMD was Born (N=61)			
	<26	26+	Total
Preconception action taken	15	37	52
Prenatal action taken	1	5	6
No action taken	0	3	3
Total	16	45	p=0.702
Biological Children Before Oldest Son with DMD was Born (N=61)			
	Yes	No	Total
Preconception action taken	19	33	52
Prenatal action taken	3	3	6
No action taken	2	1	3
Total	24	37	p=0.550
Religious Affiliation (N=83)			
	Religious affiliations	No religious affiliations	Total
Preconception action taken	43	23	66
Prenatal action taken	6	6	12
No action taken	2	3	5
Total	51	32	p=0.352
Level of Education (N=85)			
	Bachelor's degree or higher	Some college, high school degree, or less	Total
Preconception action taken	38	29	67
Prenatal action taken	5	7	12
No action taken	0	6	6
Total	43	42	p=0.017
Annual Household Income (N=66)			
	\$75,000+	<\$75,000	Total
Preconception action taken	25	27	52
Prenatal action taken	6	5	11
No action taken	1	2	3
Total	32	34	p= 0.902

Bolded values are statistically significant.

3.3.3.2 No Longer Having (Additional) Children and Other Actions Taken

The reproductive decisions of the participants were then categorized into two reproductive options: no longer planning to have (additional) children and all other reproductive actions taken.

The category of all other reproductive actions taken consisted of the options of IVF with PGT for DMD, egg donation, adoption, prenatal sex determination, and/or prenatal diagnostic testing, such as CVS or amniocentesis.

The analysis (Analysis C in Figure 2) defined the exclusion criterion “indicated contradictory responses” as selecting both reproductive actions and have not or do not plan to pursue any additional actions. Specifically for this analysis, the exclusion criterion of “indicated overlapping actions” was defined as respondents who indicated no longer planning to have (additional) children, along with another reproductive action. Overall, 82 participants provided interpretable answers on the reproductive decisions they previously pursued or plan to pursue. Table 6 depicts the measures of associations between the factors and the reproductive actions of no longer planning to have (additional) children and other actions taken.

Similar to the data analysis between preconception actions, prenatal actions, and no actions taken, the action of no longer having (additional) children and other reproductive actions taken was significantly different between the participants’ ages at the time of learning their positive DMD carrier statuses was significant between. Of the participants who learned of their carrier status under the age of 30, 34.9% (15/43) indicated no longer having (additional) children versus 88.2% (30/34) of the participants who learned of their carrier status at or after the age of 30 ($p < 0.001$).

Additionally, whether or not the respondents have a family history of DMD, not including if they have a child who is diagnosed, was found to be significantly different between the action of no longer having (additional) children and other reproductive actions taken ($p = 0.012$). Of the respondents with a family history, 35.7% (10/28) reported no longer having (additional) children and 64.3% (18/28) reported other preconception or prenatal actions taken. This is compared to

64.8% (35/54) of respondents without a family history who indicated no longer have (additional) children and the 35.2% (19/54) who indicated other preconception or prenatal actions taken.

It was determined that the reproductive actions pursued was significantly different between how well the participants feel they understand their reproductive options ($p=0.014$). Of the participants who felt they understand their reproductive options extremely well or very well, 45.5% (25/55) reported no longer having (additional) children and 54.5% (30/55) reported other reproductive actions taken. In comparison to the participants who felt they understood their reproductive actions moderately well, slightly well, or not well at all, 74.1% (20/27) indicated no longer having (additional children) and 25.9% (7/27) indicated other preconception or prenatal action taken.

Significant differences were determined between the reproductive actions of the females with biological children and the females who do not have biological children. Of the females with biological children, 64.7% (44/68) reported no longer having additional children in contrast to the 7.1% (1/14) of females who do not have biological children and are no longer planning to have children ($p<0.001$).

Finally, the reproductive action of no longer having (additional) children and other reproductive actions were significantly different between whether or not the participants have children diagnosed with DMD ($p<0.001$). Of the participants who have one or more children diagnosed with DMD, 67.2% (39/58) and 32.8% (19/58) indicated no longer having additional children and taking other preconception or prenatal actions, respectively. In comparison, 21.7% (5/23) of participants without a child diagnosed with DMD were no longer planning to have (additional) children and the 78.3% (18/23) who reported other reproductive actions taken.

No significant difference was identified between the pregnancy status at the time of learning DMD carrier status, if genetic counseling was received, the age when their oldest child with DMD was born, if they had biological children before their oldest child with DMD was born, their religious affiliation, their highest level of education of completed, or their annual household income.

Table 6. Associations with No Longer Having (Additional) Children and Other Reproductive Actions

Age at Time of Learning DMD Carrier Status (N=77)			
	<30	30+	Total
No longer planning to have additional children	15	30	45
Other action(s) taken	28	4	32
Total	43	34	p<0.001*
Pregnancy Status at Time of Learning DMD Carrier Status (N=82)			
	Pregnant	Preconception/ Postnatal	Total
No longer planning to have additional children	6	39	45
Other action(s) taken	1	36	37
Total	7	75	p=0.121
Received Genetic Counseling (GC) (N=80)			
	GC Services	No GC Services	Total
No longer planning to have additional children	39	5	44
Other action(s) taken	28	8	36
Total	67	14	p=0.232
Understanding of Reproductive Options (N=82)			
	Extremely or very well	Moderately, slightly, or not well at all	Total
No longer planning to have additional children	25	20	45
Other action(s) taken	30	7	37
Total	55	27	p=0.014*
Family History of DMD (N=82)			
	Family hx	No Family hx	Total
No longer planning to have additional children	10	35	45
Other action(s) taken	18	19	37
Total	28	54	p=0.003*
Biological Children (N=82)			
	Yes	No	Total
No longer planning to have additional children	44	1	45

Table 6 Continued

Other action(s) taken	24	13	37
Total	68	14	p<0.001
Children Diagnosed with DMD (N=81)			
	None	1+	Total
No longer planning to have additional children	5	39	44
Other action(s) taken	18	19	27
Total	23	58	p<0.001
Age When Oldest Son with DMD was Born (N=59)			
	<26	26+	Total
No longer planning to have additional children	26	14	40
Other action(s) taken	14	5	19
Total	40	19	p=0.565
Biological Children Before Oldest Son with DMD was Born (N=59)			
	Yes	No	Total
No longer planning to have additional children	15	25	40
Other action(s) taken	7	12	19
Total	22	37	p=0.961*
Religious Affiliation (N=81)			
	Religious affiliations	No religious affiliations	Total
No longer planning to have additional children	29	15	44
Other action(s) taken	20	17	37
Total	49	32	p=0.277*
Level of Education (N=82)			
	Bachelor's degree or higher	Some college, high school degree, or less	Total
No longer planning to have additional children	22	23	45
Other action(s) taken	22	15	33
Total	44	38	p=0.339*
Annual Household Income (N=65)			
	\$75,000+	<\$75,000	Total
No longer planning to have additional children	15	19	34
Other action(s) taken	15	16	31
Total	30	35	p= 0.730*

* indicates that Chi-squared analysis was performed instead of Fischer's exact test. Bolded values are statistically significant.

3.3.4 Qualitative Analysis of Decision-Making

For the participants who learned of their positive carrier status for DMD when they were not pregnant, they were asked about the reproductive options they pursued or planned to pursue, followed by a choice to elaborate on their decisions via two free text questions, “What factors influenced your decision?” and “Everyone makes different decisions about their reproductive choices. Think back to when you made that decision. Why did you choose the options you did?” The participants who learned of their positive carrier status during a pregnancy were asked about the reproductive options they plan to pursue in the future, followed by the opportunity to answer the same two free text questions. Two or more themes were indicated for the majority of the participant responses.

Responses were analyzed based on if the females indicated they were no longer plan to have (additional) children, if they pursued or plan to pursue alternative preconception and/or reproductive actions outside of no longer having (additional) children, and if females did not or do not plan to pursue alternative actions. This was based on the varying themes indicated and the varying prevalence of these themes for the different specified reproductive choices.

3.3.4.1 No Longer Having (Additional) Children

For the 37 respondents who indicated they are no longer planning to have (additional) children as at least one of their reproductive actions, there was a leading theme (19/37) of being unwilling to take the risk of having (another) child who is affected:

I think my son's chances and emotional well-being are better served with him being the only one affected. Two boys with Duchenne would be difficult, he is better supported this way.

Nine of the 37 respondents expressed the severity of DMD was a reason why they chose to no longer have (additional) children. Respondents indicated their knowledge of DMD and the disease trajectory impacted their decision, with several of the respondents describing DMD as a “devastating” or “awful” condition.

Additionally, 9/37 felt personal responsibility to avoid passing on the pathogenic variant. Differing from being unwilling to take the risk, this theme arose when the females used first person language of them not wanting to pass on the variant or not wanting to put a child through the condition:

I couldn't stand the thought of passing on DMD to a son or carrier status to a daughter, so I decided not to have children.

When discussing the choice of no longer having (additional) children, other reasons included the cost of alternative options (7/37), to best support their child(ren) who are diagnosed (6/37), and the choice was the best decision for the family as a whole (4/37). Table 7 depicts all the themes and the number of females who indicated those themes during their decision to no longer have (additional) children.

Table 7. Thematic Analysis of Factors that Influence No Longer Having (Additional) Children

Theme (N=37)	Responses, n
Unwilling to take the risk of having child who is affected	19
Severity of DMD	9
Responsibility to avoid passing on variant	9
Cost of alternative options	7
Support affected children	6
Best decision for family	4
Prevent potential children from suffering	3
Maternal psychosocial or physical concerns	3
Avoid an affected pregnancy	2
Parental relationships	2

Table 7 Continued

Death of an affected child or family member	1
External pressure to avoid passing on variant	1
*Responses will not sum to N=37 as multiple themes could be cited by each respondent.	

3.3.4.2 Alternative Preconception or Prenatal Actions

The free text responses from 29 females who are DMD carriers and pursued or plan to pursue alternative reproductive actions outside of no longer having (additional) children as at least one of their reproductive actions were qualitatively analyzed. There were three main themes approximately evenly discussed between the respondents. The first major theme (8/29) was the feeling of responsibility to avoid passing on the pathogenic DMD variant:

Watching my brother die a terrible death. I did not want that for my children. Not passing this disease “down the line.”

Of the free text responses from ten participants who indicated IVF with PGT for DMD was their only reproductive action they pursued or plan to pursue, five indicated their feelings of responsibility.

Several respondents (6/29) who pursued or plan to pursue alternative preconception or prenatal options indicated the desire to have (additional) children who are unaffected with DMD. Likewise, 6/29 of these respondents also discussed their choices based on the cost of these alternative options. This was the main theme of the females (6/29) who selected prenatal sex determination and/or prenatal diagnostic testing as at least one of the reproductive decisions they pursued or plan to pursue:

We had desperately wanted another baby before my son was diagnosed and the DMD diagnosis did not change that desire. We couldn't afford IVF so decided to accept the risk and have a CVS done in order to establish DMD.

Other respondents referenced their unwillingness to take the risk of having (another) child who is affected (5/29), their desire to prevent potential children from suffering (5/29), the severity of DMD (5/29), their desire to avoid a pregnancy that is affected (4/29), and the death of a family member who was affected with DMD (4/29). Table 8 depicts all the themes and the number of females who indicated those themes during their decision to pursue alternative preconception or prenatal reproductive actions.

Table 8. Thematic Analysis of Factors that Influence Preconception and Prenatal Actions

Theme (N=29)	Responses, n
Responsibility to avoid passing on variant	8
Desire to have children who are unaffected	6
Cost of alternative option	6
Prevent potential children from suffering	5
Unwilling to take the risk of having child who is affected	5
Severity of DMD	5
Avoid an affected pregnancy	4
Death of an affected child or family member	4
Maternal psychosocial or physical concerns	2
Best decision for family	1
Religious beliefs	1
Support a child who is affected	1
Gain information during pregnancy	1
*Responses will not sum to N=30 as multiple themes could be cited by each respondent.	

3.3.4.3 No Alternative Actions Pursued

Free text responses from two of the respondents who did not or do not plan to pursue alternative reproductive options discussed three different themes. One participant indicated religious belief as the deciding factor in the decision, while the other participant indicated both the cost of other alternative options and the desire to have (additional) children:

I was not financially able to pursue any of the available options. I wanted more children and my boys were doing well, so I knew I would love another baby regardless.

3.4 Discussion

The aim of this study was to explore the reproductive choices and the factors that influence reproductive decision-making in females who are DMD carriers. Associations between the participants' responses regarding these factors and their reproductive choices have provided insights into aspects that have impacted their decisions, which are discussed in the sections below.

Additionally, the response time for this survey also provided insight into the level of interest in this topic for females who are DMD carriers. This study had a relatively short recruitment period of four weeks; however, the number of survey responses were much higher than expected, with an original goal of 20 respondents. This is an important occurrence, as it revealed that this is a relevant and meaningful topic to females who are DMD carriers. The rapid response rates likely indicate that these females want to share their experiences with reproductive decision-making and hear the experiences of females who faced similar situations.

Another key insight this study revealed was the worrisome gap in the participants' knowledge and understanding of reproductive options. Less than one quarter (22.7%) of the participants were previously aware of all seven reproductive options discussed in the survey; however, 63.8% stated they understand the available reproductive options 'extremely well' or 'very well.' This discrepancy demonstrates that there may be a failure in healthcare services to these females in regard to properly informing them of their reproductive choices. These findings

indicate that there is a crucial necessity for continued education on all the available reproductive options for these females undergoing reproductive decision-making.

3.4.1 Reproductive Decisions

This study showed that the majority of the females who are DMD carriers pursued or plan to pursue alternative reproductive actions after learning their positive DMD carrier. After excluding the respondents who never planned to have additional children prior to learning their DMD carrier status, those who selected contradictory responses, and finally those who selected “other” without providing clear indications of their decisions when asked to elaborate, 93.5% of the remaining participants reported alternate reproductive actions. While there is limited research on the reproductive decisions of females who are DMD carriers, a study performed by Ghioffi et al. investigated the reproductive actions of at-risk couples who are carriers for the same autosomal recessive genetic condition. Of the couples who were carriers for severe or profound conditions, where DMD would be classified, 76% reported alternative reproductive actions.⁷⁵ The decreased number of individuals altering their reproductive action compared to the results from our study may be due to a variety of factors. These include items that studies have previously identified in influencing reproductive decisions, such as the difference in the mode of inheritance, the difference between the genetic conditions themselves, and the influence of both members of the couple being a carrier instead of only the female.^{7,127,128,131}

Overall, the most prevalent reproductive decisions within this study population was no longer having (additional) children, followed by IVF with PGT for DMD, and then prenatal diagnostic testing. This trend was consistent with finding in the Ghioffi et al. study regarding the

reproductive decisions-making of individuals who are at risk to have a child with a genetic condition.⁷⁵

3.4.2 Reproductive Decisions While Pregnant

The reproductive options that females have who are identified as X-linked carriers during a pregnancy are limited compared to the reproductive options available for females who are identified when they are not pregnant. Furthermore, several of these options are dependent on the gestational age of the pregnancy. There has been minimal research examining how learning their positive carrier statuses during a pregnancy has impacted the future reproductive choices of females who are X-linked carriers. For this reason, participants were asked what their pregnancy status was when they were identified as carriers. The pregnancy status was ultimately found to not be statistically significant factor related to future reproductive plans.

Of the ten participants who were identified as DMD carriers during a pregnancy and did not decide to pursue prenatal diagnostic testing, it was unsurprising that all five participants whose fetuses were determined to be female declined the procedures. This is a common trend seen in females who are carriers for an X-linked recessive condition, as the risk of having a miscarriage is notably higher than the risk of a female fetus being fully affected.⁸⁹

The other five participants who chose to decline prenatal diagnostic testing were all for different reasons, all of which are frequently reported explanations of why the decision to not pursue prenatal diagnostic testing is made and are consistent with other study findings.^{75,94,132} These included a miscarriage prior to testing being performed, fear of a procedure related miscarriage, religious beliefs, desire to continue the pregnancy regardless of a diagnosis, and identification of the maternal carrier status in the third trimester.

3.4.3 Reproductive Decisions While Not Pregnant

According to the American College of Obstetricians and Gynecologist, the ideal time to perform carrier screening, if the patient desires, is during the preconception setting since it allows for the widest range of available reproductive options.¹³³ Eighty-five respondents learned of their positive carrier statuses while not pregnant and reported interpretable reproductive decisions they made or plan to make. These only included the participants who were still planning to have additional children prior to knowing their carrier statuses. Similar to the analysis of all 92 responses, the majority of this subgroup (92.9%) reported altering their reproductive actions after learning their positive carrier status. This study's finding was slightly higher than, but overall consistent with, a study performed by Johansen et al. on the reproductive actions of at-risk couples who are carriers for autosomal recessive or X-linked recessive genetic conditions. After learning of their positive carrier statuses for profound or severe conditions during the preconception stage, 84% of participants reported alternative actions.¹³²

However, the actual reproductive actions of this study participants were different compared to the participants in the study conducted by Johansen et al. Half of the participants in this study population, 55.3%, reported no longer planning to have additional children as at least one of the actions they pursued or plan to pursue and 23.5% reported IVF with PGT as at least one of the reproductive choices they took or plan to take. Conversely, only 3.4% participants in the study by Johansen et al. who are carriers for profound or severe conditions reported no longer planning to have additional children as at least one of the actions planned, while 64% reported IVF with PGT as at least one of the reproductive choices they pursued or plan to pursue.¹³² Johansen et al. evaluated reproductive decisions made by participants who were carriers for a wide range of genetic conditions while the present study focused on reproductive decisions made by females who

are carriers for DMD and the varying risks faced by participants may explain the difference in their reproductive choices. Furthermore, difference between the two studies may be due to 99.2% of the participants in the Johansen et al. study being from the United States, where IVF is available and where the rate of IVF procedures has continued to increase over the past decade.^{132,134} As this study did not inquire on geographical location, further research should be performed to determine whether location within both the United States and the world impacts the reproductive decisions to pursue IVF with PGT for females who are DMD carriers. Lastly, the differing results between the two studies may also be due to differences in income between this study and the study by Johansen et al., as IVF with PGT is typically thousands of dollars and not covered by most insurances.^{135,136} While previous studies support that household income is associated with pursuing IVF for infertility, this cannot be concluded for those who are carriers for a genetic condition as the study by Johansen et al. did not evaluate for income and this study did not identify household income to be statistically significant between preconception actions, prenatal actions, or taking no reproductive actions.¹³⁷

3.4.4 Associations with Preconception, Prenatal, and No Actions Taken

The factors related to carrier status and family history, birth and pregnancy history, and demographics of the study population were all analyzed for associations between preconception actions taken, prenatal actions taken, and no reproductive actions taken after the females learned of their carrier status.

For carrier status and family history factors, it was most surprising that approximately one fifth of females with a family history of DMD pursued or plan to pursue no reproductive actions, compared to 0% of the females without a family history who pursued or plan to pursue no

alternative actions. The trend identified in this study is contradictory to a previous qualitative study performed via participant interviews by Kay et al., which found that females who are carriers for various X-linked conditions, 64.3% of the participants were females who are carriers for DMD, were more concrete in their decisions to avoid an affected pregnancy and to avoid having a child who is affected when they had an affected brother compared to those without an affected brother.¹³ Furthermore, it was unexpected that those with a family history were more likely to rely on prenatal diagnostic testing than those without a family history. A prior qualitative study by Kelly et al. on females who were fragile X carriers found that the participants experiences with the condition impacted their reproductive decisions by dissuading them to rely on prenatal diagnostic testing, with over two thirds deciding to pursue preconception actions instead.⁹⁴ Along with previous literature findings, this result was also surprising based on the trajectory, symptoms, and lifespan DMD. It seemed more likely that those with a family history of DMD, who saw the effects of the condition and possibly lost family members to it, would be more likely to pursue preconception reproductive actions to try to prevent an affected pregnancy from occurring.

At 91.9%, the majority of females who learned of their carrier status at or after the age of 30 years took or plan to take preconception actions and were less likely to pursue prenatal actions or no actions at all compared to females who learned of their carrier status under the age of 30. This latter group of participants were much more likely to pursue both prenatal actions at 23.8% and take no reproductive actions at 9.5%. The risk of a pregnancy being affected with a chromosomal condition, such as Down syndrome or trisomy 18, and other pregnancy complications both increase with maternal age, particularly at or after the age of 35, which is classified as advanced maternal age.¹³⁸⁻¹⁴⁰ Therefore, additional investigations looked at older ages and no statistical significances were found in females who learned of their carrier status after 35

years of age (data not shown). Interestingly, the younger the age cut-off the more significant this factor was found. The statistical significance between when females learned of their carrier status and their reproductive actions decreased as the age cutoff increased. Therefore, this does not support that the increasing risks for external pregnancy complications, unrelated to DMD, may also be playing a role between this factor and reproductive choices. Without further exploration into the different motivations between these age groups, it is difficult to say what is driving this significant difference in reproductive actions.

It was unexpected that none of the factors regarding birth and pregnancy history were found to be statistically significant in influencing the reproductive decision-making, as this contradicts previous findings in the literature. Prior studies have shown that whether or not at-risk individuals for having children with a genetic condition have prior children, along with if the children are affected, impact the reproductive decision-making processes.^{7,12} However, if the females within this study population had biological children and if they had children diagnosed with DMD were both near significant.

For the demographic factors, the highest-level education completed was found to be significant on the reproductive decision-making for females who are DMD carriers. Notably, none of the females who completed a bachelor's degree or higher reported that they did not or do not plan to pursue any alternative actions. In comparison, 14.3% females who completed some college, high school, or less did not or do not plan to take any reproductive alternative actions. A possible explanation for this difference is that the females who are DMD carriers with higher education may have better access, understanding, and/or awareness of the preconception and prenatal reproductive options available compared to the other females who are DMD carriers.

3.4.5 Associations with No Longer Having (Additional) Children and Other Actions Taken

As 62.4% (n=53) of the 85 respondents included within the analysis of associations with preconception actions, prenatal actions, and no actions taken indicated at least one of their planned reproductive actions included no longer planning to have (additional) children and 57.6% (n=49) reported that no longer having (additional) children was their only planned reproductive action, further associations on the same 12 factors were analyzed between the reproductive option of no longer planning to have (additional) children and all other preconception or prenatal actions taken.

The finding that the majority of the participants in this study are no longer planning to have (additional) children is consistent with preceding studies on the reproductive decisions of females who are carriers for other X-linked recessive conditions.^{13,98-100} For example, a study conducted by McConkie-Rosell et al. focused on the reproductive decisions of female carriers of fragile X and found that 19 out of the 28 females (67%) opted to not have any more children based on their positive carrier status.¹⁰¹

Similar to the prior reproductive options category of preconception actions, prenatal actions, and no reproductive actions, both the age at the time of learning DMD carrier status and a family history of DMD were significant factors. Additionally, how well the females feel they understood their reproductive decisions was found to be significant when comparing the reproductive action of no longer planning to have (additional) children and other actions taken.

For the age at the time of learning DMD carrier status, it is noteworthy that of the females who learned of their carrier status at or over the age of 30 years old, almost 90% are no longer planning to have (additional) children. In contrast, approximately two thirds of the females who learned of their positive carrier status under the age of 30 took or plan to take other reproductive actions. An explanation for this significant finding may be the difference in the percent of

respondents who reported already having biological children between the two groups of respondents. Of the females who learned of their carrier status prior to age 30, 67.4% did not report having any biological children at the time of the survey versus 100% of the females who learned of the carrier status at or after the age of 30 reported having biological children. Furthermore, as described in section 3.4.4, the younger the age cut-off the more significant the factors were on influencing reproductive decisions. Therefore, this once again does not indicate that the increasing risks for external pregnancy complications may be impacting the significance between the age groups.

In regard to whether or not the females had a known family history of DMD, not including if they have a child who is diagnosed, it was again surprising that the females without a family history of DMD were much more likely to be no longer planning to have (additional) children, with nearly two thirds making this decision. In comparison, approximately one third of the females who have a family history of DMD indicated they were no longer planning to have (additional) children. These findings are once more incongruous to the previous findings by Kay et al. and Kelly et al.^{13,94}

How well the females feel they understood their reproductive decisions was likewise found to be significant when comparing the reproductive action of no longer planning to have (additional) children and other actions taken. The females who felt they only understood their reproductive moderately well, slightly well, or not well at all were less inclined to pursue other reproductive actions, with only one fourth indicating this decision. In contrast, 54.5% of females who feel they understand their reproductive options extremely well or very well reported taking other reproductive actions. It is possible that they females who did not feel they understood all of their

reproductive options extremely or very well were less comfortable pursuing alternative reproductive decisions outside of no longer planning to have additional children.

Unlike the previous reproductive categories of preconception actions taken, prenatal actions taken, and no actions taken, two of the four factors included in the birth and history factors were found to be significant and were more consistent with prior literature findings.^{7,12} These consisted of if the females who are DMD carrier have biological children and if they have children diagnosed with DMD.

The majority of the females who were no longer planning to have additional children already had previous biological children, compared to the females who are DMD carriers and do not have biological children. Of the latter group, only 7.1% of the females indicated they are no longer planning to have children after learning their carrier status, compared to almost two thirds of females who have biological children and are no longer planning to have additional children. A likely explanation for this deviation is the females who already have biological children may have had their hope of being a mother fulfilled, while the females without children have not and are therefore more willing to pursue other reproductive actions.

The finding that having children who are affected with DMD is statistically significant in affecting the reproductive actions of these females is consistent with other published literature. While there are limited studies on the reproductive decisions of females who are DMD carriers, a prior population-based surveillance study conducted by Nabukera et al. identified 59.8% of mothers of males diagnosed with DMD or BMD did not have any additional children.¹² The results from this study population actually identified elevated rates with 67.2% of the females with children diagnosed with DMD indicating they are no longer planning to have additional children. One of the possible explanations for the prior study's decreased finding is their population also

included females who were mothers of children diagnosed with BMD, a less severe muscular dystrophy and may have had a less impact on subsequent reproductive decisions. Contrarily, the majority females in this study without any of their children being diagnosed with DMD indicated they pursued or plan to pursue other reproductive actions with only 21.7% reporting they are no longer planning to have (additional) children.

None of the factors were found to be statistically significant on reproductive decision-making within the final group of demographic factors. Additionally, when qualitative analysis was performed, which is further discussed in section 3.4.6 below, only 3.0% of participants indicated religious beliefs were a factor in their reproductive actions. These results were surprising as previous studies have identified that religious beliefs impacted reproductive decision-making for at risk females. For example, a qualitative study on females who are carriers for fragile X syndrome by Raspberry et al. noted that religious beliefs and faith were mentioned by 28.7% of the females when discussing the factors that to influence the participants' reproductive decision-making processes.^{6-8,97} A possible explanation for this deviation is that Fragile X syndrome is not a fatal condition, unlike DMD, and there are various supportive therapies or medications to help alleviate some of the clinical manifestations of Fragile X syndrome.¹⁴¹ Therefore, those at-risk for having an affected child with Fragile X syndrome may be more willing to trust in their religion compared to those at-risk for having a child with DMD.

3.4.6 Qualitative Analysis of Decision-Making

The qualitative analysis revealed that being unwilling to take the risk of having (another) child who is affected was a prevalent theme followed by the feeling of responsibility to avoid passing on the pathogenic variant. Over half of the 64 respondents' free text answers referenced

one or both of these themes as reasons that impacted their reproductive decision-making. This finding supports previous literature findings that perceptions of risk and a sense of responsibility to prevent passing on the condition are factors that influence reproductive choices of individuals who are carriers of genetic diseases.^{6,7,97}

Similarly, the other recurrent reasons for reproductive choices included the severity of DMD, preventing potential future children from suffering, the desire for (additional) children who are not affected with DMD, making the decision that would allow the females to best support their children who are affected, and making the decision that is best for the family as whole. These factors have also been previously reported as factors that impact the reproductive decision-makings of carriers.^{6,7,9,10,97}

Just under a fifth of the study participants cited that the cost of alternative options, particularly IVF with PGT for DMD, was a major factor in their reproductive plans. This is rarely mentioned in other studies, which is unexpected, as briefly discussed in section 3.4.3, IVF with PGT typically costs between \$11,000 and \$40,000 per IVF cycle and is rarely covered by insurance companies, especially within the United States.^{135,136} Therefore, it is understandable that 61.5% of the study participants who indicated cost was a factor during their reproductive planning further explained that IVF with PGT for DMD was a reproductive action they would like to pursue; however, it is not a financially feasible option.

3.4.7 Study Limitations

In this study, there are several limitations. A primary limitation is the possibility of sampling bias as the study was advertised through DMD advocacy groups and a contract research organization involved in clinical research for DMD, Therapeutic Research is Neuromuscular

Disorders Solutions (TRiNDS). Therefore, the survey respondents may exclude females who are DMD carriers and are less involved within the DMD community, such as females without children who are affected. Females who are DMD carriers who are involved in the advocacy groups and aware of clinical research for DMD may be more inclined to share their experiences and participate in studies related to DMD, such as this survey. Females involved in the advocacy groups or clinical research trials may have also been provided with more education on DMD and subsequently their reproductive risks and options compared to other females who are DMD carriers. Likewise, as the majority of the recruitment took place online and through social media, the study sample may exclude the females who are DMD carriers with limited access to a computer, including those of lower socioeconomic status.

Another study limitation is the possibility of recall bias, as the survey responses are dependent on the participants' accurate and complete memories. The survey also asked which reproductive options the participants have pursued or are planning to pursue. It is not guaranteed that the decisions the females are planning to pursue will correlate with their future actions. Furthermore, the survey was anonymous, thus the data analysis was on self-reported responses and could not be verified via participants' medical records.

Lastly, a limitation of this survey was the study sample demographics, as the majority of the participants were Caucasian and learned of their positive DMD carrier while they were not pregnant. Geographical location was also a demographic factor that was not asked about within the survey. Because of this, the knowledge of the areas of where the survey responses are from and the possible impact on reproductive decisions are limited.

3.4.8 Future Directions

The results of this study offer preliminary evidence into the factors that are associated with the reproductive decision-making process for females who are DMD carriers. As research on the reproductive decisions of females who are DMD carriers is limited, the results warrant further qualitative investigation of these factors through structured interviews or focus groups to gain a deeper understanding into how these factors motivated reproductive choices. Future research should also explore the psychosocial effects of being a carrier for DMD and how these factors impact not only their reproductive decisions, but other aspects of the females' lives and their families.

Additionally, there were limited participants in this study who learned of their positive DMD carrier status during pregnancy and a reasonable direction would involve further research on this subgroup.

3.5 Conclusion

This study offers data regarding the factors that influence the reproductive decision-making of females who are DMD carriers, demonstrating that alternative reproductive actions are commonly pursued after learning their carrier statuses and that certain factors are statistically significant with the reproductive choices that are made. Twelve factors were analyzed independently within two different reproductive decision categories. The first category compared the reproductive options of preconception action taken, prenatal action taken, and no action taken; the second category compared no longer planning to have (additional) children and other

reproductive actions taken. The age at the time of learning their positive DMD status and a family history of DMD were statistically significant factors on the reproductive decisions made in both categories. The highest level of education completed was a significant factor on the reproductive decisions between preconception actions, prenatal actions, versus no actions taken. How well the females feel they understand their reproductive options, if they have biological children, and if they have children diagnosed with DMD were all significant factors on the reproductive choices between no longer planning to have additional children and other reproductive actions taken. The pregnancy status at the time of learning positive DMD carrier status, if genetic counseling was received, the age at when their oldest child with was DMD was born, if they had biological children before their oldest child with DMD was born, their religious affiliation, their highest completed level of education, and their annual household income were not statistically significant factors for the reproductive decisions made in either category. Thematic analysis of the females' free text responses identified being unwilling to take the risk of having (another) child who is affected and the feeling of responsibility to avoid passing on the pathogenic variant were prevalent themes indicated. These results support the hypothesis that the age at the time of learning their positive DMD status, how well females who are DMD carriers feel they understand their reproductive options, and a feeling of responsibility to avoid passing on the pathogenic *DMD* variant influence reproductive decision-making of females who are DMD carriers; however, additional research is needed to gain a deeper understanding into how these factors motivate reproductive choices. Being aware of the factors that influence reproductive decision-making for females who are DMD carriers may provide insight and improved psychosocial interventions for genetic counselors, or other health care professionals, who care for females who are DMD carriers and are undergoing their own reproductive decision-making processes.

4.0 Research Significance to Genetic Counseling and Public Health

The goal of this study was to gain a more comprehensive understanding of the factors that influence the reproductive decision-making in females who are DMD carriers. In this process, more was learned about their motivations and reproductive choices as well as their understanding of the available options. The results of this study may lead to more informed clinical practice of healthcare providers, including genetic counselors, obstetricians and gynecologists, and family physicians, who provide genetic counseling for females who are DMD carriers. These implications are important within both the fields of public health and genetic counseling.

Public health is based on three core functions: assessment, policy development, and assurance.¹⁴² This study can be specifically contextualized within two of the essential services under the assurance core function: “inform, educate, and empower people about health issues” and “assure a competent public health and personal health care workforce.”¹⁴³

For the first essential public health service, “inform, educate, and empower people about health issues,” the results of this study revealed gaps in the participants’ knowledge and understanding of the reproductive options available to them. Less than one quarter of the participants (22.7%, n=141) were previously aware of all seven reproductive options discussed in the survey. However, when the participants were asked how well they understand the available reproductive options, 63.8% (n=141) stated ‘extremely well’ or ‘very well.’ This discrepancy demonstrates the necessity for continued education on all the available options.

Making sure females are aware of all their reproductive options may be achieved via implementing the second essential public service, “assure a competent public health and personal health care workforce” serve females who are carriers for DMD. While DMD is considered a rare

condition, affecting 1 in 3,500-5,000 males, there are multiple other X-linked recessive conditions that affect females.¹⁸ Educating healthcare professionals on both the available reproductive choices and how to effectively explain those options to patients who are DMD carriers will not only benefit those females, but may also benefit a wide diversity of patients who are carriers of other X-linked recessive genetic diseases. Because the identification of at-risk females who are carriers for various X-linked conditions continues to rise due to the increasing popularity, affordability, and access to expanded carrier screening, this is a pertinent healthcare matter.⁷⁴

It is essential that these females are provided with information regarding all of the available reproductive options in order for them to make both an informed and autonomous decision that is right for them and their family. Studies have found education on both genetics and reproductive options have positive psychosocial impacts for females who are carriers as anxiety may be lessened with a better understanding of these matters.^{79,100} Therefore, this study also has implications for genetic counselors, who have the significant role of educating and counseling patients who are DMD carriers about their reproductive options and the genetics of DMD.

Furthermore, according to the Genetic Counseling Practice-Based Competences as established by the Accreditation Council for Genetic Counseling genetic counselors, “promote client-centered, informed, non-coercive and value-based decision-making.”¹⁴⁴ One of the main goals of this study was to provide more insight into the factors that may influence the reproductive decisions of females who are DMD carriers in order to help genetic counselors serve as effective decision facilitators for future patients. These results may assist genetic counselors during patient conversations about factors that have impacted reproductive choices of other females who are DMD carriers. This may help patients explore which factors are most important to them in order to facilitate a decision that aligns with their own reproductive goals. Additionally, discussing the

previous trends and factors in other females who are DMD carriers creates the possibility of decreasing isolation and stigma for patients who are possibly making sensitive, personal, and difficult decisions.

Appendix A Institutional Review Board Approval

University of Pittsburgh Institutional Review Board

Human Research Protection Office
3500 Fifth Avenue, Suite 106
Pittsburgh, PA 15213
Tel (412) 383-1480
www.hrpo.pitt.edu

APPROVAL OF SUBMISSION (Exempt)

Date:	November 14, 2019
IRB:	STUDY19090254
PI:	Caroline Bong
Title:	Exploring Factors Influencing Reproductive Decision-Making in Duchenne Muscular Dystrophy (DMD) Carriers

The Institutional Review Board reviewed and approved the above referenced study. The study may begin as outlined in the University of Pittsburgh approved application and documents.

Approval Documentation

Review type:	Initial Study
Approval Date:	11/14/2019
Exempt Category:	(2)(i) Tests, surveys, interviews, or observation (non-identifiable)

Approved Documents:	<ul style="list-style-type: none">• Listening to Women: Exploring Factors Influencing Reproductive Decision-Making in DMD Carriers Survey, Category: Data Collection;• HRP-721 - WORKSHEET - Exemption_Tests Surveys Public Behavior_Version_0.01.docx, Category: IRB Protocol;• Introductory Script_Version_0.01.pdf, Category: Recruitment Materials;
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As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others. The HRPO Reportable Events policy, Chapter 17, is available at <http://www.hrpo.pitt.edu>.

Clinical research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS).

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, [Carolyn Ivanusid](#).

Please take a moment to complete our [satisfaction Survey](#) as we appreciate your feedback.

Appendix B Introductory Script to Survey

Informed Consent

Thank you for considering participating in this survey. This research project is being conducted by Caroline Bong in fulfillment of a master's degree in Genetic Counseling at the University of Pittsburgh.

We hope to learn about patient experiences and decisions after learning of positive Duchenne muscular dystrophy (DMD) carrier screening results. Specifically, what options do women or couples pursue after learning that they are carriers of DMD and what factors are associated with decision-making. If you decide to participate, you will be asked to complete an online survey containing approximately 20 questions about your demographic background and preferences regarding reproductive options. The survey is expected to take about 10-15 minutes to complete.

Your participation is voluntary, and you may discontinue at any point. Please note that you can decline to answer any questions you do not wish to answer. The answers from your survey will not be connected to your name or any other identifying information. Survey responses are anonymous, and all data collected will be de-identified.

There are no risks to you for your participation in this study, except for the potential emotional distress that may be caused by answering questions about your pregnancy history, your feelings regarding reproductive options, and your future family planning. Should you experience any adverse reactions to taking the survey, you may notify the principal investigator, who will put you into contact with a licensed genetic counselor. It is possible that you will not benefit directly by participating in this study; however, the study may improve the genetic counseling of future patients who are DMD carriers.

This study is approved by the University of Pittsburgh's Institutional Review Board. Any questions or concerns may be addressed to me, Caroline Bong (ckb24@pitt.edu) or my faculty chair, Lauren Morgenroth, MS, CGC (lmorgenroth@trinds). If you have any questions about your rights as a research subject or wish to talk to someone other the research team, please call the University of Pittsburgh Human Subjects Protection Advocate toll-free at 866-212-2668.

If you know of any women who are DMD carriers and older than the age of 18 years old that you think you would be willing to complete this online survey, please forward this survey link to them.

- Yes, I consent to participate [Begin Survey]
- No, I decline to participate [Exit Survey]

Appendix C Survey Content



Thank you for participating.

S1.

Thank you for considering participating in this survey. This research project is being conducted by Caroline Bong in fulfillment of a master's degree in Genetic Counseling at the University of Pittsburgh.

We hope to learn about patient experiences and decisions after learning of positive Duchenne muscular dystrophy (DMD) carrier screening results. Specifically, what options do women or couples pursue after learning that they are carriers of DMD and what factors are associated with decision-making. If you decide to participate, you will be asked to complete an online survey containing approximately 20 questions about your demographic background and preferences regarding reproductive options. The survey is expected to take about 10-15 minutes to complete.

Your participation is voluntary, and you may discontinue at any point. Please note that you can decline to answer any questions you do not wish to answer. The answers from your survey will not be connected to your name or any other identifying information. Survey responses are anonymous, and all data collected will be de-identified.

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about your pregnancy history, your feelings regarding reproductive options, and your future family planning. Should you experience any adverse reactions to taking the survey, you may notify the principal investigator, who will put you into contact with a licensed genetic counselor. It is possible that you will not benefit directly by participating in this study; however, the study may improve the genetic counseling of future patients who are DMD carriers.

This study is approved by the University of Pittsburgh's Institutional Review Board. Any questions or concerns may be addressed to me, Caroline Bong (ckb24@pitt.edu) or my faculty chair, Lauren Morgenroth, MS, CGC (lmorgenroth@trinds). If you have any questions about your rights as a research subject or wish to talk to someone other the research team, please call the University of Pittsburgh Human Subjects Protection Advocate toll-free at 866-212-2668.

If you know of any women who are DMD carriers and older than the age of 18 years old that you think you would be willing to complete this online survey, please forward this survey link to them.

- Yes, I consent to participate [Begin Survey]
- No, I decline to participate [Exit Survey]

S2.

Thank you for participating in our survey. We appreciate hearing about your experience.

The following is a list of terms that are used in the survey; it may be helpful for you to review these terms prior to completing the survey:

Amniocentesis: A test done during pregnancy where a thin needle is inserted

through a pregnant woman's belly area into her uterus (womb) to collect a small amount of amniotic fluid from the womb. This is a way to diagnose a baby with Duchenne muscular dystrophy before birth.

Chorionic villus sampling (CVS): A test done during pregnancy where a thin flexible tube is entered through a pregnant woman's cervix (birth canal) to collect a small amount of the placenta (afterbirth). The test may also be done with thin needle inserted through a pregnant woman's belly area into her uterus (womb) to collect a small amount of the placenta. This is a way to diagnose a baby with Duchenne muscular dystrophy before birth.

Egg donation: A woman donating an egg to different woman who cannot or does not want to use her own eggs to have a child. Egg donation can be used to prevent passing on a genetic condition to a child.

In vitro fertilization (IVF): When an egg is collected from a woman's ovaries and fertilized by sperm within a lab. The fertilized eggs are put back into a uterus (womb) to begin a pregnancy.

Sex determination: A test performed during a pregnancy to determine if the baby is male or female.

Preimplantation genetic testing (PGT): A test done before the fertilized eggs are transferred to a uterus (womb) during the IVF process. The test identifies genetic conditions within the fertilized egg. This is a way to prevent passing on a genetic condition to a child.

Please click the next button to get started.

Let's start with background questions.

1. Let's get started with some background questions. How old were you when you learned that you carry a difference or mutation in the Duchenne muscular dystrophy gene?

2. How did you learn you were a carrier for Duchenne muscular dystrophy gene?

- An OB/GYN, or other childbirth provider, or prenatal genetic counselor recommended routine carrier screening
- My son was diagnosed with Duchenne muscular dystrophy after he was born and carrier testing was done after
- A family member was diagnosed with Duchenne muscular dystrophy and carrier testing was done after
- Other (please specify):

3. Did you seek genetic counseling to discuss your carrier status?

- Yes, with a genetic counselor
- Yes, with a healthcare provider other than a genetic counselor
- No, but I may consider it in the future
- No, I don't plan to pursue genetic counseling

3.A. What are the reasons you decided not to pursue genetic counseling?

4. Prior to reading the descriptions in question S2 , which reproductive

options were you previously aware of? Please choose all that apply.

- In vitro fertilization (IVF) with preimplantation genetic testing (PGT)
- Egg donation
- Adoption
- Prenatal diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis
- Prenatal sex determination
- Deciding to not have (additional) children
- Deciding to not pursue any alternative reproductive options
- Other (please specify):

5. How well do you feel you understand the reproductive choices that are available?

- Extremely well
- Very well
- Moderately well
- Slightly well
- Not well at all

6.

Were you pregnant when you learned you were a carrier for Duchenne muscular dystrophy?

- Yes
- No

Preconception or postnatal

7.

Which of the following best describes your situation at the time you learned you are a carrier for Duchenne muscular dystrophy?

- I had no biological children and had no specific plans to conceive
- I had no biological children and was thinking about trying to conceive in the next year
- I had no biological children and was actively trying to conceive
- I had biological daughters or biological sons without Duchenne muscular dystrophy
- I had a biological son who was not yet diagnosed with Duchenne muscular dystrophy
- I had a biological son who was already diagnosed with Duchenne muscular dystrophy
- Other (please specify):

8. After learning you are a carrier for Duchenne muscular dystrophy, what option(s) did you pursue or are you planning to pursue? Choose all that apply to you.

- In vitro fertilization (IVF) with preimplantation genetic testing (PGT) for Duchenne muscular dystrophy
- Egg donation
- Adoption
- Prenatal diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis
- Prenatal sex determination
- No longer planning to have additional children

- Never planned to have additional children
- Not planning to pursue any alternative options
- Other (please specify):

8.A. What factors influenced your decision?

8.B. Everyone makes different decisions about their reproductive choices. Think back to when you made that decision. Why did you choose the options you did?

Now we are going to ask you some question about yourself

9. How many biological children have you given birth to?

- None
- 1
- 2
- 3
- 4 or more

10. How many children do you have diagnosed with Duchenne muscular dystrophy?

- None

- 1
- 2
- 3 or more

10.A. How old were you when your oldest son with Duchenne muscular dystrophy was born?

10.B. Before having your son who was diagnosed with Duchenne muscular dystrophy, how many biological children did you have?

- None
- 1
- 2
- 3 or more

10.C. After your son was diagnosed with Duchenne muscular dystrophy, did you have additional pregnancies?

- Yes
- No

10.D. After your son was diagnosed with Duchenne muscular dystrophy, did you give birth to any additional biological children?

- Yes
- No

11. Have any other males in your family been diagnosed with Duchenne muscular dystrophy?

- Yes
- No

11.A. How are/were they related to you?

12. What is your religious affiliation?

- Rather not say
- Protestant
- Catholic
- Mormon
- Jewish
- Buddhist
- Hindu
- Muslim
- No religious affiliation
- Other:

13. What is the race you identify with?

- White
- African American
- Hispanic

- Asian or Pacific Islander
- Native American
- Multiracial

14. What gender do you identify as?

- Female
- Male
- Other

15. What is the highest level of education you completed?

- High school or less
- Some college
- Bachelor's degree or higher

16. What is your current household income in U.S. dollars? This is the income from all adults who live in your home.

- Rather not say
- Less than \$20,000
- \$20,000 - \$34,999
- \$35,000 - \$49,999
- \$50,000 - \$74,999
- \$75,000 - \$99,999
- Over \$100,000

Prenatal

7. Which of the following best describes your situation at the time you were pregnant and learned you are a carrier for Duchenne muscular dystrophy?

- I had no biological children
- I had biological daughters or biological sons without Duchenne muscular dystrophy
- I had a biological son who was not yet diagnosed with Duchenne muscular dystrophy
- I had a biological son was already diagnosed with Duchenne muscular dystrophy
- Other (please specify):

8.

After learning you are a carrier for Duchenne muscular dystrophy, did you pursue prenatal diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis for the condition?

- Yes
- No

8.A. Everyone makes different decisions about getting testing done before the baby is born during pregnancy. Think back to when you made that decision. How did you decide not to pursue prenatal diagnostic testing?

8.A. Everyone makes different decisions about getting testing done before the

baby is born during pregnancy. Think back to when you made that decision. How did you decide to pursue prenatal diagnostic testing?

8.B. What did the final result of the prenatal diagnostic testing show?

- The pregnancy was found to be a female
- The pregnancy was found to be a male and not affected with Duchenne muscular dystrophy
- The pregnancy was found to be a male and affected with Duchenne muscular dystrophy

8.C. The outcome after learning the final test results was that:

- The pregnancy was continued
- The pregnancy was miscarried
- The pregnancy was terminated

8.D. We are interested to hear your experiences with deciding whether or not to get a prenatal test, and how things went after the test. What was the experience like for you?

9. What option(s) do you plan to pursue in the future? Choose all the apply to you.

- In vitro fertilization (IVF) with preimplantation genetic testing (PGT) for Duchenne muscular dystrophy

- Egg donation
- Adoption
- Prenatal diagnostic testing such as chorionic villus sampling (CVS) or amniocentesis
- Prenatal sex determination
- No longer planning to have children
- Never planned to have additional children
- Not planning to pursue any alternative options
- Other (please specify):

9.A. What factors influenced your decision?

9.B. Everyone makes different decisions about their reproductive choices. Think back to when you made that decision. Why did you choose the options you did?

Now we are going to ask you some questions about yourself

10. Now we are going to ask you some questions about yourself. How many biological children have you given birth to?

- None
- 1
- 2

- 3
- 4 or more

11. How many children do you have diagnosed with Duchenne muscular dystrophy?

- None
- 1
- 2
- 3 or more

11.A. How old were you when your oldest son with Duchenne muscular dystrophy was born?

11.B. Before having your son who was diagnosed with Duchenne muscular dystrophy, how many biological children did you have?

- 0
- 1
- 2
- 3 or more

11.C. After your son was diagnosed with Duchenne muscular dystrophy, did you have additional pregnancies?

- Yes

No

11.D. After your son was diagnosed with Duchenne muscular dystrophy, did you give birth to any additional biological children?

Yes

No

12. Have any other males in your family been diagnosed with Duchenne muscular dystrophy?

Yes

No

12.A. How are/were they related to you?

13. What is your religious affiliation?

Rather not say

Protestant

Catholic

Mormon

Jewish

Buddhist

Hindu

Muslim

No religious affiliation

Other:

14. What is the race you identify with?

White

African American

Hispanic

Asian or Pacific Islander

Native American

Multiracial

15. What gender do you identify as?

Female

Male

Other

16. What is the highest level of education you completed?

High school or less

Some college

Bachelor's degree or higher

17. What is your current household income in U.S. dollars? This is the income from all adults who live in your home.

- Rather not say
- Less than \$20,000
- \$20,000 - \$34,999
- \$35,000 - \$49,999
- \$50,000 - \$74,999
- \$75,000 - \$99,999
- Over \$100,000

Decline

7. After learning you are a carrier for Duchenne muscular dystrophy, what option(s) did you pursue or are you planning to pursue now? Choose all the apply to you.

- In vitro fertilization (IVF) with preimplantation genetic testing (PGT) for Duchenne muscular dystrophy
- Egg donation
- Adoption
- Prenatal diagnostic testing, such as chorionic villus sampling (CVS) or amniocentesis
- Prenatal sex determination
- No longer planning to have additional children
- Never planned to have additional children
- Not planning to pursue any alternative options
- Other (please specify):

7.A. What factors influenced your decision?

7.B. Everyone makes different decisions about their reproductive choices. Think back to when you made that decision. Why did you choose the options you did?

Now we are going to ask you some questions about yourself

8. Now we are going to ask you some questions about yourself. How many biological children have you given birth to?

- None
- 1
- 2
- 3
- 4 or more

9. How many children do you have diagnosed with Duchenne muscular dystrophy?

- None
- 1
- 2
- 3 or more

9.A. How old were you when your oldest son with Duchenne muscular

dystrophy was born?

9.B. Before having your son who was diagnosed with Duchenne muscular dystrophy, how many biological children did you have?

- 0
- 1
- 2
- 3 or more

9.C. After your son was diagnosed with Duchenne muscular dystrophy, did you have additional pregnancies?

- Yes
- No

9.D. After your son was diagnosed with Duchenne muscular dystrophy, did you give birth to any additional biological children?

- Yes
- No

10. Have any other males in your family been diagnosed with Duchenne muscular dystrophy?

- Yes
- No

10.A. How are/were they related to you?

11. What is your religious affiliation?

- Rather not say
- Protestant
- Catholic
- Mormon
- Jewish
- Buddhist
- Hindu
- Muslim
- No religious affiliation
- Other:

12. What is the race you identify with?

- White
- African American
- Hispanic
- Asian or Pacific Islander
- Native American
- Multiracial

13. What gender do you identify as?

- Female
- Male
- Other

14. What is the highest level of education you completed?

- High school or less
- Some college
- Bachelor's degree or higher

15. What is your current household income in U.S. dollars? This is the income from all adults who live in your home.

- Rather not say
- Less than \$20,000
- \$20,000 - \$34,999
- \$35,000 - \$49,999
- \$50,000 - \$74,999
- \$75,000 - \$99,999
- Over \$100,000

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Appendix D Social Media Advertisement for Survey Participation

Women Who Are Duchenne Muscular Dystrophy Carriers

We want to hear from you!

Participants will answer a short research survey that takes 10-15 minutes about reproductive decision making

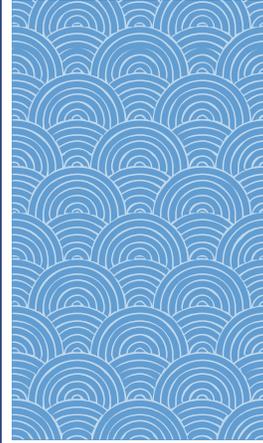
Who will qualify:
Women who are known carriers of Duchenne muscular dystrophy and are 18 years or older

Survey Link:

https://pitt.co1.qualtrics.com/jfe/form/SV_ebXswV4OI4IWPKR

This study has received IRB approval from the University of Pittsburgh

If you have any questions regarding the study, please contact
Caroline Bong: ckb24@pitt.edu or Lauren Morgenroth, MS,
CGC: lmorgenroth@trinds.com



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