

Duchenne and Becker Muscular Dystrophy: Implications for at-risk individuals

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Medicine in Genetic Counselling

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DECLARATION

I, Suretha Erasmus, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Genetic Counselling, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

_____ day of _____ 2009.

ABSTRACT

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are severe X-linked recessive, degenerative neuromuscular diseases. DMD/BMD are caused by deletions, duplications and point mutations in the *DMD* gene situated on the X-chromosome. Studies have shown that the risk of being a carrier for DMD/BMD has a psychosocial impact on individuals and affects their requests for DNA testing and their choices regarding reproduction. Very few articles have been published to date and this study is the first South African study to investigate the behaviours of individuals in DMD/BMD families.

The study aimed to investigate why individuals attended genetic counselling and who referred them. It also aimed to identify factors that influence at-risk individuals' decisions regarding genetic counselling, carrier testing and reproduction. The study was retrospective and data were obtained by reviewing genetic counselling files at the Division of Human Genetics, National Health Laboratory Service and the University of the Witwatersrand. The sample consisted of 79 files of families seen for genetic counselling regarding DMD/BMD from 1995 to 2008. Subjects included the maternal female relatives of affected individuals, who were all of reproductive age (15-49 years); the total number of at-risk individuals identified was 237.

Subjects were divided into three groups according to their assigned reproductive risks: low (0-9%), intermediate (10-24%) and high (>25%). The influence of reproductive risk and other identified variables on decisions to attend genetic counselling, have carrier testing and having children were analysed using chi-squared and logistic regression analysis.

Reproductive risk and relationship to the affected individuals were shown to be significant predictors of individuals' decisions. Other factors that contributed significantly to the behaviour of at-risk individuals were ethnicity, age, whether a mutation was *de novo* and whether an individual had affected children.

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LIST OF ABBREVIATIONS

%	Percentage
BMD	Becker Muscular Dystrophy
cDNA	complementary DNA
CF	Cystic Fibrosis
CI	Confidence Interval
CK	Creatine Kinase
CPD	Continuing Professional Development
CPK	Creatine Phosphokinase
CVS	Chorionic Villi Sampling
<i>DMD</i> gene	Duchenne Muscular Dystrophy gene
DMD	Duchenne Muscular Dystrophy
DNA	Deoxyribose Nucleic Acid
GC	Genetic Counselling
GP	General Practitioner
MLPA	Multiplex Ligation-Dependent Probe Amplification
NHLS	National Health Laboratory Service
NSGC	National Society of Genetic Counselors
OR	Odds Ratio
<i>P</i>	Probability
PCR	Polymerase Chain Reaction
SAIMR	South African Institute for Medical Research
SD	Standard Deviation
WITS	University of the Witwatersrand

1.0 INTRODUCTION

Duchenne Muscular Dystrophy (DMD), previously also known as Meryon's disease as it was first discovered and described by Edward Meryon, an English physician, in 1951. However, it was Duchenne de Boulogne, a French physician, who was credited with the discovery of the disease after he described it in detail in the 1960s (Emery, 2001).

Becker Muscular Dystrophy (BMD) was first described in 1934, but it was Professor P.E. Becker, a German geneticist, who first described BMD as 'a new X-chromosomal muscular dystrophy' (Emery, 2001, p.72). In 1955, he also noted that the severity of BMD differed from that of DMD but he speculated that the two diseases were possibly caused by mutations in the same allele (Emery, 2001). This was later confirmed and today it is well-known that DMD and BMD are distinct phenotypes that result from different mutations within the same gene.

1.1 Duchenne and Becker Muscular Dystrophy

DMD is a severe X-linked recessive, degenerative neuromuscular disease with an incidence of 1 in 3,500 live male births. BMD is a milder form of DMD with an incidence of 1 in every 18,000 live male births (Gatta et al., 2005). However, a South African study on 143 DMD patients showed a low prevalence (1 in 250,000) of DMD in the Black population compared to the international prevalence (Ballo, Viljoen & Beighton, 1994).

1.1.1 Clinical Presentation

DMD presents in early childhood with delayed milestones in sitting, independent standing and walking. Characteristic proximal weakness of the muscles causes toe walking, a waddling gait and difficulty in climbing. The progression of DMD is rapid and individuals are usually wheelchair bound by the age of 12 years (Emery, 2001). Cardiomyopathy occurs in all individuals with DMD after 18 years of age and scoliosis occurs in 90% of DMD affected individuals (Firth & Hurst, 2005). The common causes of death are respiratory failure and cardiomyopathy before the third decade of life (Emery, 2001).

BMD presents at a mean age of 11 years with difficulty in walking and running with frequent falling (Firth & Hurst, 2005; Emery, 2001). The clinical features of BMD can be variable and wheelchair dependency can range from 12 to 70 years with varying degrees of disease progression. As with DMD, proximal muscle weakness and cardiomyopathy occur. The mean age of death in BMD affected individuals is mid-forties, with dilated cardiomyopathy being the main cause of death (Emery, 2001).

1.1.2 Pathogenesis of DMD/BMD

DMD/BMD is caused by mutations in the *DMD* gene situated at Xq21.2. The *DMD* gene produces the dystrophin protein and is the largest human gene discovered to date, with 79 exons (Gatta et al., 2005). The size of the *DMD* gene constitutes 0.1% of the total human genome and 1.5% of the X-chromosome (Muntoni, Torelli & Ferlini, 2003).

Dystrophin is mainly expressed in cardiac and skeletal muscle cells and to a lesser extent in the brain. Dystrophin interacts with membrane proteins in the sarcolemma of the muscle cells; together these proteins form the dystrophin-glycoprotein complex (Figure 1.1).

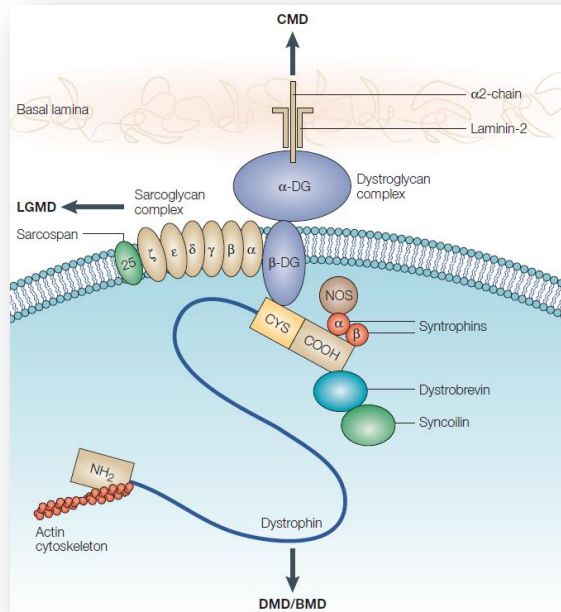


Figure 1.1: The dystrophin-glycoprotein complex (Khurana & Davies, 2003)

This complex plays an important structural role in muscle cells and also protects the muscle fibres from damage and death that may result from regular muscle contractions. The dystrophin-glycoprotein complex also plays an important role in cell signalling across membranes (Muntoni et al., 2003). Mutations in the *DMD* gene therefore affect the functionality of the dystrophin protein.

1.1.3 Genetics of Duchenne and Becker Muscular Dystrophies

1.1.3.1 Mode of Inheritance

DMD and BMD are single gene disorders that affect all ethnic groups similarly and are both inherited in an X-linked recessive manner. Females are unaffected carriers of the condition because of the normal, compensatory gene on their second X-chromosome. A female carrier has a 50% chance of passing on the X-chromosome with the *DMD* mutation to her offspring. Males who inherit the mutation present with the condition as they only have one X-chromosome. Females who inherit the mutation will be unaffected carriers. A *DMD* mutation can either be familial or as a result of a *de novo* mutation.

1.1.3.2 De novo mutations

A *de novo* *DMD* mutation is either caused by an event that occurred in the grandparental germline or the maternal germline of an affected individual. *De novo* point mutations are more often inherited from the maternal grandfather whereas deletions are more often inherited from the maternal grandmother (Helderman-van den Enden et al., 2009). Based on the assumption that males and females have equal mutation rates, it was predicted that one third of all DMD/BMD cases are due to *de novo* mutations (Morton and Lalouel (1979) cited in (Danieli & Barbujani, 1984)). It is thought that the *DMD* gene has a higher mutation rate than the average rate for other human genes because of its enormous size (Aartsma-Rus, Van Deutekom, Fokkema, Van Ommen & Den Dunnen, 2006).

1.1.3.3 Mutation types

Mutations responsible for DMD are mostly nonsense or frame-shift mutations that result in barely detectable, truncated protein products, whereas mutations that cause BMD are

mostly in-frame and result in the production of less but partly functional dystrophin (Firth & Hurst, 2005; Muntoni et al., 2003). This ‘reading-frame model’ first presented by Monaco, Bertelson, Liechti-Gallati, Moser and Kunkel (1988) still holds true and is 90% accurate in determining whether an individual has the DMD or BMD phenotype (Muntoni et al., 2003; Monaco et al., 1988).

Large deletions in the *DMD* gene are the major cause of DMD/BMD and account for 65% of DMD cases and 85% of BMD cases. Other *DMD* gene mutations include point mutations and duplications (Table 1.1) (Darras, Korf & Urion, 2008; Gatta et al., 2005).

Table 1.1: DMD/BMD causing mutations.

	DMD	BMD
Large deletions	~65%	~85%
Duplications	~5-10%	~5-10%
Point mutations	~25-30%	~5-10%

Most of the mutations occur in two defined hotspot regions, which include exons 2-19 and 44-55 (Muntoni et al., 2003). Modern day molecular techniques are sensitive and advanced, and can detect all the different types of mutations.

1.1.4 Diagnostic testing for confirmation of DMD/BMD in affected individuals

In South Africa, DMD/BMD is first diagnosed by the clinical history and physical examination of an affected individual. The serum creatine phosphokinase concentration is evaluated and confirmation of the diagnosis is made with molecular genetic testing. If no deletion or duplication is detected, the diagnosis can be confirmed by sequencing of the

DMD gene or by muscle biopsy to measure the amount of dystrophin in the muscle fibres. The diagnostic tests are elaborated below.

1.1.4.1 Serum Creatine Phosphokinase concentration

The first step in the diagnosis of DMD/BMD after clinical evaluation is the determination of serum creatine kinase (CK) levels, also known as creatine phosphokinase (CPK). CK is an enzyme that assists with the release of energy from creatine in the body and is mainly present in cells with high energy requirements, primarily the brain, thyroid, skeletal and heart muscles (Martin, 2007). DMD/BMD individuals have increased serum CK levels as a result of leakage from damaged muscle fibers (Ozawa, Hagiwara & Yoshida, 1999) and are elevated to more than ten times the normal value in DMD individuals and five times in BMD individuals (Darras et al., 2008).

1.1.4.2 Genetic testing

Multiplex polymerase chain reaction (PCR) and Southern Blotting techniques have been the two commonly used molecular techniques to screen for *DMD* mutations. Multiplex PCR is a method that simultaneously amplifies the exons 2-20 and 42-53 which includes the two hotspot regions. Almost all the DMD/BMD deletions are situated in these hotspot regions. The multiplex PCR method can detect 98% of ‘hotspot’ deletions in affected males (Gatta et al., 2005; van Essen et al., 1997). However, multiplex PCR does not detect duplications and southern blotting is therefore used as a complimentary method. Southern blotting is a technique used to detect deletions and duplications by using cDNA probes to detect the site of mutations along the entire *DMD* gene (Prior & Bridgeman, 2005).

Two of the newer techniques used are the Multiplex Ligation-Dependent Probe Amplification (MLPA) and gene sequencing techniques. The MLPA method is a more rapid and efficient technique and can be used to screen for deletions and duplications in all 79 exons of the *DMD* gene (Gatta et al., 2005). Gene sequencing detects small mutations generally not detected with the other available techniques. These mutations include: deletions, duplications, single-base changes, and splicing mutations (Darras et al., 2008).

In South Africa in 1987, the first screening service for carrier detection and prenatal diagnosis was started at the Department of Human Genetics, University of Cape Town (Ballo, Hitzeroth & Beighton, 1991). Up to 2007, deletion screening to confirm the diagnosis of DMD/BMD was routinely offered. The multiplex PCR method was used to test for common deletions in the following exons 2-4,6,9,11-13,16,17,19,25,32,34,41-52,60 and 66. In 2008, a newer and more effective MLPA test was offered to screen for deletions and duplications in the *DMD* gene. It is not yet possible to detect point mutations at the Division of Human Genetics, NHLS/WITS or any other laboratory in South Africa and samples with suspected point mutations or other small mutations can be sent overseas for sequencing analysis of the full *DMD* gene. However, this option is only available to patients who can afford this testing.

1.1.4.3 Muscle biopsy

Muscle biopsy is not the preferred method for testing because it is an invasive test. A muscle biopsy followed by Western Blotting or Immunohistochemical staining of dystrophin however, is an accurate technique to confirm the clinical diagnosis of DMD/BMD. Dystrophin levels are nearly depleted in muscle biopsies from DMD affected

individuals whereas in BMD, affected individuals can have dystrophin levels between 20 and 50% in their muscle tissue (Darras et al., 2008). In the situation where genetic testing failed to detect a gene mutation, a muscle biopsy can be performed to confirm a diagnosis of DMD/BMD.

1.2 Genetic Counselling

Genetic counselling is an essential service for individuals affected with, or at-risk of being carriers of DMD/BMD. Genetic counselling is a holistic service that offers medical information, psychosocial support and continued management to individuals and families. Genetic counselling is defined by the National Society of Genetic Counselors (NSGC) as: *“Genetic Counseling is the process of helping people understand and adapt to the medical, psychological, and familial implications of the genetic contributions to disease. This process integrates:*

- *Interpretation of family and medical histories to assess the chance of disease occurrence or recurrence.*
- *Education about inheritance, testing, management, prevention, resources and research.*
- *Counseling to promote informed choices and adaptation to the risk or condition”*

(Resta et al., 2006).

Although the primary aim of genetic counselling is to inform individuals about their risks, helping them understand their risks and assisting them in making informed decisions, the genetic counselling consultation involves both counselling and testing services (Smerecnik, Mesters, Verweij, de Vries & de Vries, 2009).

DMD and BMD are incurable, disabling conditions and lead to premature demise. The female relatives, including the mothers, sisters, nieces, aunts, cousins and daughters (of BMD individuals) therefore request genetic counselling and carrier testing to get an indication of the risk that their offspring will be affected with the conditions (van Essen et al., 1997).

1.2.1 The family pedigree

Pedigree construction is an inexpensive, reliable and non-invasive method of obtaining family information (Wolpert & Speer, 2005). A pedigree is usually constructed as a three generation family tree with information on different diseases or genetic conditions in a family, the age of disease onset, death, age and causes of the deaths. This information should be obtained for first, second and third degree relatives but generally the information gets less accurate with an increase in the distance of relatedness (Wattendorf & Hadley, 2005). The family pedigree is useful in that it gives an indication of biological relationships in the family. It is important to keep in mind that families and the information around individuals in a family constantly change, and it is therefore important to update the family pedigree regularly (Wolpert & Speer, 2005).

Constructing a pedigree is useful in genetic counselling because it serves as a tool to assist in the diagnosis of a condition, the inheritance pattern and in calculating risks of individuals to either carry a mutation or develop a condition. With pedigree construction, an added benefit is the psychosocial information that is often revealed, which can be used in establishing a counsellor-client relationship (Veach, LeRoy & Bartels, 2003). In genetic counselling the information obtained with a family pedigree can be used to provide

information about reproductive risks and can also function in the early diagnosis and management of certain conditions.

The pedigree can also be used as a tool when informing individuals about their risk. The pedigree provides the necessary framework to help individuals understand their risks and the risks to their offspring (Smerecnik et al., 2009).

1.2.2 Risk perception

Risk plays an important role in genetic counselling; it is essential to present risk information accurately, thus allowing individuals to make informed decisions based on their perception of their risks (Smerecnik et al., 2009; Parsons & Clarke, 1993). In genetic counselling, individuals are given a mathematical number for their risk but it has been shown that individuals have difficulty in understanding and perceiving their risk when presented in a numerical way (Sivell et al., 2008). However, Sivell, et al. (2008) and Smerecnik, et al. (2009) performed systematic reviews and found that individuals' accuracy of their risk perception improved with genetic counselling.

It is not only the perception of risk that influences decisions; the burden of the disease has also been shown to have an influence on the individual's decisions (Smerecnik et al., 2009). Other factors that contribute to complicated decision-making are an individual's coping mechanisms, worry and anxiety about the implications of their risk (Smerecnik et al., 2009), previous experiences, environmental factors, profession, diet and other genetic or family history factors (Sivell et al., 2008). All these factors combined and considered will impact on the individual's ability to make a decision appropriate to them.

Even with the different contributing factors influencing decision-making, it is still crucial to give accurate risks as this is the primary foundation of informed decision-making. Bayes' theorem is commonly used in genetic counselling to calculate accurate risks. The theorem calculates the prior probability that an individual carries the disease causing mutation and then adapts that probability with new information that becomes available, e.g. test results or the birth of healthy children. In essence, the theorem continually uses information that becomes available and then combines the probabilities that an event will arise or not (Turnpenny, Ellard & Emery, 2007). This is useful because carrier risk is not definitive and can change as more genetic information on the family becomes available (Parsons & Clarke, 1993).

1.2.3 Carrier testing for at-risk individuals

Testing individuals at-risk of carrying a *DMD* mutation is considered important because it can assist in refining risks, informed decision making and clarify prenatal options. Before testing an at-risk individual, testing of the *DMD*/BMD affected individual needs to be done. It is not always possible to detect the disease causing mutation in an affected individual. It is also not always possible to test an affected individual for the family mutation because of reticence, geographical distance, or death of the affected individual.

1.2.3.1 Testing options

- ***In families with a known mutation***

The multiplex-PCR or MLPA methods can be used for carrier detection where the family mutation in the *DMD* gene is known. For point mutations, *DMD* gene sequencing can be performed on the specific gene section for detection of the mutation.

- ***In families without a known mutation***

The most commonly used method when the family mutation is unknown, is linked marker analysis for carrier detection. *DMD* intragenic polymorphic markers are used to detect the presence of the high risk X-chromosome. This method is limited however, by the number of informative markers in the *DMD* gene and in families with a single affected male.

- ***In families with no proband***

In families with no available proband, the preferred method of defining risk is linked marker analysis to try and track the high-risk X chromosome in the family. Healthy unaffected brothers of the proband can be useful as they will always have the low-risk X chromosome.

1.2.4 Prenatal Diagnosis

Prenatal diagnosis is offered to women with a family history of DMD/BMD and to women with an increased risk of germline mosaicism as a result of a *de novo* event. A *de novo* event is assumed where there is no family history of DMD/BMD and the affected individual is the first one in the family. The first step in prenatal diagnosis is to determine the gender of the fetus by invasive testing or ultrasound examination. This would be followed with molecular testing for the disease causing mutation, in a male fetus. Female fetuses are not tested for the *DMD* mutation because they are not at risk of being affected with the condition but rather at 50% risk of being unaffected carriers.

The two invasive techniques routinely used for prenatal diagnosis are chorionic villi sampling (CVS), a technique used to obtain chorionic villi (placental tissue) between 10 and 13 weeks gestation, and amniocentesis, where amniocytes present in the amniotic fluid are extracted, cultured and analysed. Amniocentesis is a second trimester test, ideally performed between 16-22 weeks of pregnancy. Both procedures have a risk for miscarriage. Linkage analysis or molecular testing can now be performed on the chorionic villi sample or the cultured fetal cells from the amniotic fluid to confirm the presence or absence of the high risk X-chromosome or *DMD* mutation in the fetus.

DMD/BMD are fatal conditions with no successful curative therapeutic interventions to date (Scime & Rudnicki, 2008). The options for parents once a positive result is obtained in a fetus, are either to terminate the pregnancy or to keep the affected baby. CVS offers an early result and a termination of pregnancy at an early stage in pregnancy can be less traumatic physically. A termination of pregnancy in the second trimester can be more traumatic because of experienced fetal movement and a longer time period for maternal bonding with the baby.

1.3 Psychosocial aspects of being a carrier of a genetic disease

A qualitative study by Williams and Schutte (1997) of 34 at-risk individuals showed that after genetic testing, non-carriers experienced relief from the burden of fear and also expressed freedom to continue with their reproductive planning. However, carriers experienced feelings of sadness, as well as the loss of hope to have unaffected children and grandchildren. Interestingly, it was found that both carriers and non-carriers had difficulty informing family members of their genetic status. Carriers that already had affected

children did not experience any reassurance after receiving confirmation of their carrier status (Williams & Schutte, 1997).

Mothers of children with an X-linked condition, when they are confirmed as carriers, experience more guilt and blame, and feel more stigmatised because of their carrier status compared to parents of children with autosomal recessive diseases (James, Hadley, Holtzman & Winkelstein, 2006). Guilt experienced by the mothers of affected children can easily turn into depression with feelings of hopelessness and helplessness. Fathers of children with X-linked diseases are more likely to blame their partners than those with children with autosomal recessive diseases (James et al., 2006).

A study by Marteau, van Duijn, and Ellis (1992) on the effect of genetic screening on a group of individuals at risk of being carriers for a recessive disease, found that carriers are less optimistic about their future health than non-carriers. The authors suggested that confirmation of carrier status might have a subtle influence on an individual's self-perception (Marteau et al., 1992).

1.4 Behaviour of at-risk individuals

At-risk individuals base their decisions both on the cognitive and emotional considerations and their decisions may not always seem logical to medical professionals (Decruyenaere, Evers-Kiebooms, Denayer & Welkenhuysen, 1998).

1.4.1 Decision to undergo carrier testing

Prior to genetic testing, at-risk individuals experience fear; fear to have an affected child, fear that carrier children can pass the gene on to the next generation and fear that the results will prove their belief that they are responsible for transferring the gene to their offspring (Williams & Schutte, 1997).

To make a decision is difficult and naturally a number of factors play a role in this process. The individuals' perceived risk plays a major role in their desire to take up carrier testing (Decruyenaere et al., 1998). A systematic review, focussing on published quantitative surveys and qualitative research, on cystic fibrosis (CF) carrier testing identified factors that would affect decisions. CF is a debilitating recessive genetic condition marked by the build-up of thick, sticky mucus in the lungs and digestive tract which is responsible for chronic lung disease, pancreatic insufficiency and a shortened life-span. Three factors that strongly influenced decisions to undergo CF carrier testing were the perceived benefits of testing, the perceived barriers to carrier testing and having/desiring children (Chen & Goodson, 2007). A review on retrospective, descriptive studies showed that the benefits most recognized by individuals are knowing one's carrier status and preventing affected children (Decruyenaere et al., 1998). Individuals who perceived carrier testing as beneficial are more likely to undertake testing. A perceived barrier to testing is the thought about attached stigma. Individuals who do not perceive stigma as negative are more likely to be tested. Individuals with a strong desire to have children, are twice as likely to have carrier testing than individuals with weaker intentions to have children (Chen & Goodson, 2007). Interestingly, at-risk individuals with unaffected children are less likely to have carrier testing possibly because of their perception that they are at a lower risk (Chen & Goodson, 2007).

Additional factors that play a role in the decision to undergo carrier testing are knowledge about the condition, socioeconomic background and ethnic group. People with more knowledge, higher socioeconomic status or White are more likely to be tested (Chen & Goodson, 2007). Factors that play a role in individuals' decision to decline carrier testing, are the reluctance to terminate a pregnancy and a perceived low risk of being a carrier (Decruyenaere et al., 1998). Individuals that considered their options and ultimately decided to have carrier testing, have a low tolerance for ambiguous test results and the cost of the test (Chen & Goodson, 2007).

Although the decision to have carrier testing is dependent on a number of factors, the decision is a highly subjective one and will differ between individuals. Some will see it as a benefit whereas others may see it as unnecessary. A questionnaire based study on at-risk carriers for haemophilia, an X-linked bleeding disorder, showed that only 49% of the individuals had carrier testing although more than 95% of the individuals in the study thought that carrier testing is useful (Varekamp et al., 1990). A reason for the lower percentage of individuals that had testing compared to those who thought it helpful, is because the more distant at-risk individuals in a family (cousins and nieces) were less informed about the availability of carrier testing (Varekamp et al., 1990).

1.4.1.1 In DMD/BMD families

In a study by Eggers, Pavanello, Passos-Bueno and Zatz (1999) where information was obtained retrospectively from files and telephonic interviews with patients, the decisions of 263 at-risk individuals were recorded after they received genetic counselling. In the study it was shown that more DNA tests were requested by individuals from higher socio-

educational levels and/or if they had one or more individuals in the family that had died as a result of DMD (Eggers et al., 1999). The experience of having lost an affected DMD individual greatly influenced the behaviour of at-risk individuals regarding genetic testing (Eggers et al., 1999). They also showed that an individual's decision to proceed with testing depends on beliefs about termination of pregnancy (Eggers et al., 1999). A major reason for carrier testing in DMD/BMD is to assist in the reproductive decisions of at-risk individuals.

1.4.2 Reproductive decisions of at-risk individuals

At-risk individuals and their partners facing reproductive decisions often experience high levels of anxiety and need to consider a number of options that would be acceptable and available to them. These reproductive decisions include the choice to have children, the timing of when to start a family, or once pregnant, the decision to continue with prenatal diagnosis and possible termination of a pregnancy, or choosing the non-biological route of adoption or oocyte donation. The anxiety couples experience when making decisions is exacerbated by the risk to have an affected child, anticipation of a painful prenatal diagnostic procedure and the associated miscarriage risk, the difficulty to know what to do if the at-risk individual is pregnant with an affected child, and lastly the knowledge that choosing any one of the options will have psychological consequences (Kadir et al., 2000).

Decisions about reproductive choices are usually dynamic (Sawyer et al., 2006) and change either with new information that becomes available or with the coping strategies of the at-risk individuals and their partners. A few factors may influence at-risk individuals'

decisions and include amongst others, religion, family pressure and previous experience with an affected individual (Kadir et al., 2000).

Studies on families with haemophilia, showed that individuals with a family history of the condition, chose to have children at a later stage in their life (Tedgård, Ljung & McNeil, 1999). A large proportion of at-risk haemophilia carriers and CF carriers chose not to have any children/have more children mainly because they did not want to pass the defective gene on to their children but also because of a previous experience with the condition and not wanting to terminate an affected child (Henneman et al., 2001; Kadir et al., 2000).

Interestingly, the mean number of children born to at-risk haemophilia carriers did not differ from the number of children born to individuals not at risk of carrying the mutation (Tedgård et al., 1999). However, this study and others showed that the majority of at-risk individuals that had an affected child chose not to have further pregnancies (Kelly, 2009; Tedgård et al., 1999). It has been shown that approximately 75% of individuals with affected children who chose to have more children elected not to have prenatal diagnosis (Kelly, 2009).

In families with haemophilia, the decision of at-risk individuals to continue with prenatal diagnosis depends strongly on their beliefs about termination of pregnancy after a positive result and also on the presence of a family history. Individuals in favour of termination of an affected pregnancy are more likely to opt for prenatal diagnostic testing (Tedgård et al., 1999). Women that do not approve of termination tend to decide against prenatal diagnosis. These women are also more likely not to have further children after the birth of an affected child (Tedgård et al., 1999). It is important that individuals realise that prenatal diagnosis does not necessarily have to be followed by termination of the pregnancy, it can

simply function as preparation for the couple for the outcome or necessary medical intervention after the birth of an affected child.

Most studies on reproductive behaviour are retrospective, and the decisions made by individuals in a counselling session or immediately after receiving information about carrier status are not definitive. A study that looked at the decisions individuals made about future reproduction compared with their actual behaviour, found that more than half of the individuals who planned not to have further children continued to have more children. These behaviours were strongly linked with the individual's experience with an affected child or their sense of disease burden. The authors also found that once an affected child was born, parents also changed their minds regarding prenatal diagnosis and termination of pregnancy (Sawyer et al., 2006).

1.4.2.1 In DMD/BMD families

Eggers et al. (1999) categorised the genetic risk of having a DMD affected son as very low (0-4%), low (5-9%), intermediate (10-24%) and high (>25%). They found that women with a high risk did not differ in their reproductive and testing decisions from those women with lower genetic risks ($\leq 24\%$) (Eggers et al., 1999). In their study on at-risk individuals, they also found that sisters of affected DMD individuals did not reproduce less and did not request DNA tests more frequently than their at-risk cousins, aunts and nieces. They also showed that 75% of the sisters had an intermediate to high genetic risk (Eggers et al., 1999).

However, Hutton and Thompson (1976) showed, with their questionnaire based study that included 122 at-risk females, that the risk of being a carrier discouraged ~81% of women from having children. They also showed that the number of women who chose to have children increased with a decrease in their risk to have affected children (Hutton & Thompson, 1976). A record based study on potential DMD carriers in Wales from 1971-1986 showed that many women at high risk of being carriers chose not to have children or deliberately delayed having children (Norman, Rogers, Sibert & Harper, 1989). A prospective study on 574 at-risk individuals showed that mothers of affected boys more often chose not to have further children (Zatz, 1983).

1.4.3 Uptake of Genetic Counselling

Mothers of boys affected with haemophilia, rated the genetic counselling services as either 'extremely' or 'very' useful (Sawyer et al., 2006). In light of this, it is expected that the uptake of genetic counselling services should be high. However, very few studies mention the decisions of at-risk individuals to undertake genetic counselling.

A study on the uptake of genetic counselling by individuals in families with breast/ovarian cancer, showed that individuals were less likely to have genetic counselling when the proband had cancer (Hagoel et al., 2000). Individuals were also less likely to have genetic counselling if they were referred by a doctor (and not self-referred) and if they were from the older generation in a family. Younger individuals and first-degree relatives were more likely to accept genetic counselling (Hagoel et al., 2000).

In families with colorectal cancer, younger, more highly educated individuals and individuals with more affected people in the family, were more likely to have genetic counselling (Glanz, Grove, Lerman, Gotay & Le Marchand, 1999). Other factors that positively influence the decision to undertake genetic counselling include increased anxiety and perception of risk, the availability of family social support, and the individual's need to obtain more information on the potential benefits and risk of genetic testing (Glanz et al., 1999). Factors that can negatively influence decisions to undertake genetic counselling include the cost of the service, the fear of the emotional impact on the family, the belief that the service is not beneficial, the distance from the genetic counselling clinic and the time commitments for the first and follow-up sessions (Geer, Ropka, Cohn, Jones & Miesfeldt, 2001).

1.4.3.1 In DMD/BMD families

The researcher found limited literature on DMD/BMD individuals and genetic counselling. A study on individuals that received genetic counselling showed that after a couple of years, mothers of affected boys could accurately recall their risks. It also showed that the risks were not relayed to the younger generation, especially in families with isolated cases of DMD (Zatz, 1983).

1.5 Motivation for research

It is well documented that having genetic testing and being a carrier of a genetic disease has a psychosocial impact on the lives of individuals (Williams & Schutte, 1997). Very few articles on the psychosocial aspects of DMD/BMD have been published, the most recent being in 1999 (Eggers et al., 1999) and none in South Africa. The researcher

proposed that the risk of being a DMD/BMD carrier, as in other conditions, may influence an individual's decisions regarding genetic counselling, carrier testing and reproduction.

The literature available on the influence of potential carrier risk on reproduction and carrier testing is controversial. The researcher felt that a retrospective study on a South African DMD/BMD population examining whether genetic counselling, carrier testing and reproductive decisions differ between at-risk individuals with varying risks would be valuable. This study is the first in South African to investigate the behaviour of individuals at risk of being DMD/BMD carriers. The findings of this study can assist in the improvement of genetic counselling services to a diverse population.

1.6 Aims

The aim of this study was to assess which members of DMD/BMD families came for genetic counselling and of those, who returned for subsequent counselling. The study also aimed to identify individuals in these families at risk of being carriers of DMD/BMD, to assign their individual risks and group them into different risk categories. Lastly, the study aimed to investigate the influence of risk on the carrier testing, genetic counselling and reproductive decisions of individuals in the different risk groups.

1.7 Study Objectives

- Part I:
1. To investigate the reasons individuals pursued genetic counselling.
 2. To establish whether at-risk individuals attend follow up genetic counselling sessions and the reasons for attending.

3. To establish whether the mutation of the affected individual in the family is known.

- Part II:
1. To determine the number of at-risk individuals from the family pedigree, assign carrier risks and categorise individuals according to their risk.
 2. To investigate the uptake of genetic counselling in the different risk categories.
 3. To examine the uptake of testing in the different risk categories.
 4. To compare the reproductive behaviour of the individuals in the different risk categories.

In the next chapter the methodology of the study will be discussed. This includes the data collection method, a description of the analysis used to test the significance of the data and also a description of the method used to determine the influence of different factors on the decisions of at-risk individuals.

2.0 METHODS

The design of the study is quantitative, descriptive and correlational. It is a retrospective study and data was obtained by reviewing the genetic counselling files in the Division of Human Genetics, NHLS/WITS. The study was unconditionally approved by the Human Research Ethics Committee at the University of the Witwatersrand, Johannesburg (Protocol number M080922, Appendix A).

2.1 Study Sample

The services offered by the Division of Human Genetics, NHLS/WITS, include genetic counselling, diagnostic and carrier testing. The sample size consisted of 79 DMD/BMD patient files of individuals that attended the genetic counselling clinics regarding DMD/BMD from 01/01/1995 to 31/12/2008. The study sample included the maternal female relatives of DMD/BMD affected individuals. They were identified from the genetic counselling files and are referred to as the ‘at-risk’ individuals.

2.2 File Collection

At the genetic counselling session a file is created for every individual consulted. The completed files contain demographic information, family history including family pedigree, medical history, information on the diagnosis, discussion points of the session and future management plans. The files and listed records are kept at the Division of Human Genetics, NHLS/WITS. The records were used by the researcher to identify all the DMD/BMD families seen between 1995 and 2008.

2.3 Data Collection

2.3.1 Identification of at-risk individuals

The at-risk individuals were women from DMD/BMD families that attended the genetic counselling clinic as well as maternal female relatives that had a risk of being a DMD/BMD carrier and who were identified from the family pedigrees. The family pedigrees were obtained from the genetic counselling files. The at-risk individuals were excluded or included based on the following criteria:

Inclusion criteria:

- Female relatives from the maternal side of affected individuals were included; the mothers, sisters, aunts, cousins, nieces and daughters (BMD families). Included too, were maternal grandmothers and more distant relatives categorized as '*other*'.
- Only females of reproductive ages, 15 to 49 years, were included in the study.

Exclusion criteria:

- In situations where it was unclear what the ages of at-risk individuals were and the ages did not fall comfortably in the 15-49 year age group, individuals of that generation were excluded.
- If an at-risk individual was 45 years of age, her older siblings or cousins were excluded from the study.
- Similarly, if an at-risk individual was 18 years old, her younger siblings and cousins were excluded from the study.

2.3.2 Risk allocation

2.3.2.1 *In families with a history of DMD/BMD*

The following scenarios describe women that can be at-risk of being carriers of the disease causing mutation (van Essen et al., 1997):

- A woman who has an affected son as well as another affected individual in the maternal family will be an obligate carrier of the disease causing mutation.
- A woman with one or more affected brothers but no other family history of the condition is at-risk because her mother is at risk of being a carrier of the DMD/BMD mutation or can have either somatic or germline mosaicism.
- A woman with affected family members and healthy children is still at-risk of being a carrier.

It is relatively easy to estimate the carrier risks of female relatives from the family pedigree. However, it is necessary to calculate their conditional risks on new information that becomes available by using Bayes' theorem. Figure 2.1 can be used to illustrate the risk calculations of at-risk individuals. In Figure 2.1, if I1 is an obligate carrier, her offspring (II1-5) are at 50% risk to inherit the disease causing mutation. Daughters that inherit the mutation will be carriers (II2) of DMD/BMD whereas sons that inherit the mutation will be affected (II4). The other siblings that did not inherit the mutation are not carriers, they are not affected and there is no risk to their children.

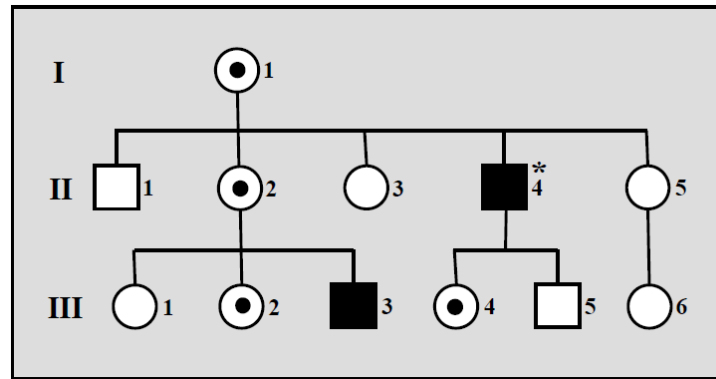


Figure 2.1: Pedigree of a family with a history of DMD/BMD.

**Affected individuals will only reproduce in the case of BMD.*

In Figure 2.1, both II3 and II5 are at 50% risk of being carriers. Their carrier risks can only be clarified with molecular genetic testing or by linkage analysis. In the absence of results, their assigned risk to be carriers would remain 50%. The children would therefore have half their mother's risk of inheriting the mutation because they only receive one of the two X-chromosomes. Thus II5's daughter (III6) has a 25% chance of inheriting the mutation and being a carrier and II5's sons would have a 25% chance of being affected with DMD/BMD.

Unlike DMD affected individuals, BMD affected individuals (Figure 2.1, II4) can reproduce. BMD affected individuals will always pass on the disease causing mutation to their daughters and they would thus be obligate carriers. The sons of BMD individuals will not be affected because they inherit the Y-chromosome from their affected father.

2.3.2.2 *In families with an isolated occurrence of DMD/BMD*

In a *de novo* or ‘isolated’ case where a woman has one affected son and no family history of the condition, the following scenarios describe women that can be at-risk of carrying the disease causing mutation (van Essen et al., 1997):

- A woman with an affected son can be a carrier if the mutation occurred in either her mother or father’s germline
- A woman with an affected son can have somatic mosaicism if the mutation occurred after she was conceived
- A woman with an affected son could have a germline mutation
- A woman with an affected son could *not* be a carrier if the new mutation arose in her ovum resulting in a somatic mutation in her son
- A woman with an affected son could also *not* be a carrier if the new mutation occurred after her son was conceived
- A woman with more than one affected son and no family history of the condition can be a carrier, have somatic mosaicism or germline mosaicism.
- Sisters of an affected male are at-risk of being carriers because their mother can be either a full carrier or have somatic or germline mosaicism

In apparent *de novo* cases with one affected male, it is essential to clarify the carrier status of the mother so that her daughters and female relatives can be informed of their possible risk of being carriers. This would be important for young mothers with DMD/BMD children who are planning subsequent pregnancies.

To calculate the risks of the female relatives of an isolated DMD/BMD case it is assumed that males and females have equal mutation rates in their germ cells (Harper, 1998). With *de novo* mutations, the risk of the mother being a carrier is $\frac{2}{3}$ (66.6%) and the risk that the mutation was transmitted from the grandmother is $\frac{1}{3}$ (33.33%) (Harper, 1998).

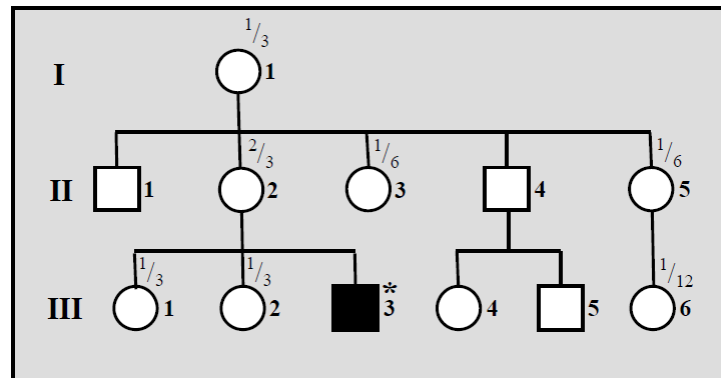


Figure 2.2: Pedigree of a family with no history of DMD/BMD.
**Affected individuals will only reproduce in the case of BMD.*

Using Figure 2.2 as illustration, the carrier risk of the mother (II2) of the affected individual will be $\frac{2}{3}$ and that of the grandmother (I1) will be $\frac{1}{3}$. The daughters of II2 will have half the risk of their mother which is $\frac{1}{3}$. The sisters of II2 will have half the risk of the grandmother (I1) which will be $\frac{1}{6}$. The daughter of II5 will have a $\frac{1}{12}$ risk of being a carrier of the *DMD* mutation (Harper, 1998).

Mothers of affected individuals that do not have a somatic mutation have an 8.6% recurrence risk based on likely germline mosaicism (Helderman-van den Enden et al., 2009). This means that brothers who inherit the same X-chromosome as their affected sib will have an 8.6% chance of being affected, and sisters that inherit the same X-chromosome, have an 8.6% chance of carrying the disease causing mutation.

The initial carrier risk assigned to an individual based on the family pedigree was adapted if testing information was available. The risks were also recalculated with the use of Bayes' theorem if an individual at-risk of being a carrier had unaffected sons. Finally, the carrier risks were halved to obtain an individual's reproductive risk, i.e. the risk to have an affected son.

2.3.3 Recording information

The women who attended the genetic counselling clinic and other at-risk individuals identified from the pedigrees were assigned individual numbers thus ensuring anonymity and confidentiality. For each at-risk individual (women who attended the genetic counselling clinic and other identified at-risk women) the relevant information was collected from the genetic counselling file onto a data collection sheet (Appendix B).

The data collection sheet consisted of six categories; *Demographics*, *Family history of DMD/BMD*, *Genetic counselling*, *Risks*, *Carrier testing* and *Reproduction*. The *demographics* category included the ages and ethnicity of at-risk individuals. The *Family history of DMD/BMD* section depicted information on the relationship of the at-risk individual to the affected individual and also the clinical diagnosis and molecular confirmation of the diagnosis in the family. The section on *Genetic counselling* gathered information on the number of sessions or follow-up sessions attended by an individual and also the reasons for attendance as well as the referral sources.

The *Risks* section indicated information on the number of at-risk individuals in a family, whether the family mutation was *de novo*, and included pedigree information and risk

calculations. In this section, carrier risks were allocated to individuals and their reproductive risks were assigned. In the *Carrier testing* category it was shown if individuals had carrier testing and if they received results after testing. Results obtained meant that individuals' risks were refined with either molecular or by linked marker analysis.

In the *Reproduction* section, individuals were categorized into three groups based on their reproductive risks. The three groups were defined as; *Low risk* with a 0-9% reproductive risk, *Intermediate risk* with a 10-24% reproductive risk, and *High risk* with a $\geq 25\%$ reproductive risk. From the genetic counselling files, the researcher recorded the decisions of at-risk individuals in terms of the number of children they had, whether they knew that they were at-risk, and if they knew, whether they chose to have more children. When individuals chose to have further children, the decisions regarding prenatal testing and termination of pregnancy were recorded as well as the pregnancy outcome.

The data collected were entered from the data collection sheets into an Excel sheet that contained all the required study variables.

2.4 Data Analysis

A statistician from the Department of Public Health was consulted to validate the analysis method of the data. The two programs used with the analysis of data and presentation of results were STATISTICA Version 8.0 (StatSoft, 2008) and Excel. STATISTICA was used for descriptive statistics that included averages, counts, means, and standard deviations (SD). Contingency tables and the Pearson's Chi-square (χ^2) were used to

determine the significance of the relationships between variables. The Pearson's chi-square can be used for 'goodness-of-fit' or 'independence' tests. The 'goodness-of-fit' test determines the association between the observed and expected frequency distributions. The 'independence' test determines whether two paired variables independently affect an outcome, and is analysed with contingency tables (WikiDoc website, 2009).

The uni- and multivariate logistic regression functions in STATISTICA were used to determine if independent variables (e.g. risk) influenced decisions of individuals (e.g. uptake of testing). Logistic regression analysis is applied in studies where the outcome or dependent variable is measured in a categorical scale. The analysis also uses likelihood estimation, i.e. it tests the 'goodness-of-fit', proposes the probability for various outcomes and calculates the odds of one outcome occurring over another (Burns & Grove, 2001). The Excel program was used to generate pie charts, histograms and tables for the graphic illustrations of results obtained.

Most of the data in the *Demographics*, *Family history of DMD/BMD* and *Risk* categories were presented as counts and/or percentages. The ages and number of at-risk individuals per family was represented as the mean values with standard deviations. The referring individuals documented in the *Genetic counselling* section were presented with counts and histograms. The reasons why individuals attended genetic counselling and follow-up consultations were also represented with counts and histograms.

2.4.1 Analysis to determine the influences on individuals' behaviour

The behaviour of at-risk individuals in terms of genetic counselling was documented as a dichotomous outcome; they either attended genetic counselling or did not. The same was documented for behaviour around carrier testing. For reproductive decisions an individual's behaviour was measured as having children or not having children. Decisions regarding the above can be influenced by a variety of factors. The different variables that were thought to affect decisions regarding genetic counselling, carrier testing and reproduction are presented Table 2.1.

Table 2.1: Variables analysed to determine their contribution on behaviour of individuals.

	Genetic Counselling (GC)	Carrier Testing	Reproduction
Dichotomous outcome variable:	1. GC – Yes/No 2. GC follow-up – Yes/No	Testing – Yes/No	Children – Yes/No
Independent categorical variables:	Risk Relationship Ethnicity <i>De novo</i> mutation Age	Risk Relationship Ethnicity <i>De novo</i> mutation Mutation known Affected children Age	Risk Relationship Ethnicity <i>De novo</i> mutation Mutation known Age

The variable *risk* consists of the three risk groups, Low (0-9%), Intermediate (10-24%) and High ($\geq 25\%$) based on individuals reproductive risks. The variable *relationship* contains five groups; mothers, sisters, aunts, cousins and distant. The relationship data were initially captured in seven categories; mothers, sisters, aunts, cousins, daughters, nieces and other. The 'daughters' category was excluded from the *statistical analysis* of the study because of category size consisting of only one individual. The newly formed 'distant'

category is a combination of the ‘nieces’ and ‘other’ in order to increase the group numbers and to include the individuals in the analysis. The ethnicity variable consisted of three groups; Black, White and Indian. The *de novo* variable is a binary variable that documented a mutation in the family as *de novo* or not. ‘*Mutation known*’ and ‘*affected children*’ are also binary variables with yes/no answers.

2.4.2 Logistic regression

The first step in determining the influence of these variables on decisions of individuals was to do a univariate logistic regression analysis on each variable and determining the relationship it had with the outcome. The variables that showed a significant relationship to the outcome variable were then used to create a multivariate logistic regression model. The purpose of this model was to present contributions of the independent variables on the outcome simultaneously and to provide odds ratios for each variable to present the strengths of associations (Ostir & Uchida, 2000).

In the next chapter the findings of the study will be reported. This includes demographic information on at-risk individuals, information on who attended genetic counselling, the reasons for attendance and the referral sources. Results on the different mutations in the families will be reported. The extensive findings on factors influencing the decisions of at-risk individuals will be reported.

3.0 RESULTS

The first aim of this retrospective study was to identify the at-risk individuals in all the families that attended genetic counselling for DMD/BMD at the Division of Human Genetics, NHLS/WITS, and to assign a carrier risk (based on the pedigree) and a reproductive risk (risk to have an affected son). The next aim was to determine why at-risk individuals attended genetic counselling and follow-ups and also to establish who referred them for genetic counselling. Finally, the researcher investigated the influence of reproductive risk on the behaviour of the at-risk individuals in terms of genetic counselling, carrier testing and reproduction, as well as the possible contributions of other factors.

3.1 Study Sample

Initially, 100 DMD/BMD files were identified from the records, kept in the Division of Human Genetics, NHLS/WITS. Of the 100 files, 21 were excluded for various reasons: 9 files could not be found, 7 files were found but lacked a considerable amount of information e.g. family history, and with 5 files the diagnosis was a neither DMD or BMD but another type of muscular dystrophy. The study thus included 79 files; 62 DMD and 17 BMD genetic counselling files. From the 79 files, 237 at-risk individuals were identified that met the inclusion criteria. The file selection method is depicted in Figure 3.1.

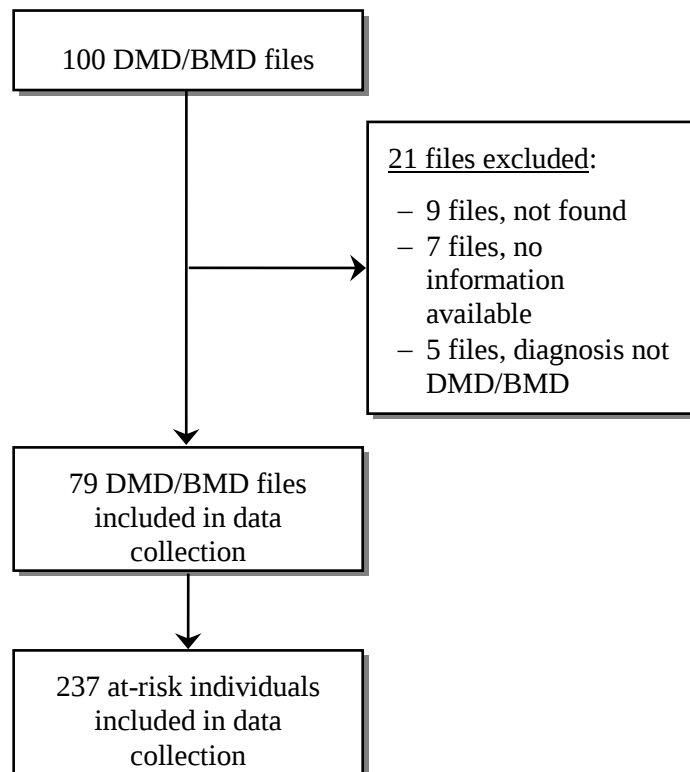


Figure 3.1: Selection process of at-risk individuals

3.2 At-risk individuals

The mean number of at-risk individuals per family was 3.5 ± 3.3 . Of the 237 at-risk individuals, 127 (53,6%) individuals were White, 96 (40.5%) were Black, 14 (6%) individuals were Indian but none were from the Coloured population. Ages were available for 116 at-risk individuals, their mean age was 29.4 ± 8.4 years.

3.2.1 Relationship to affected individual

The majority of at-risk individuals were the aunts of affected individuals, and mothers and sisters made up a large proportion of the relationship distribution in families.

Figure 3.2, illustrates the relationship distribution of the at-risk individuals in DMD/BMD families.

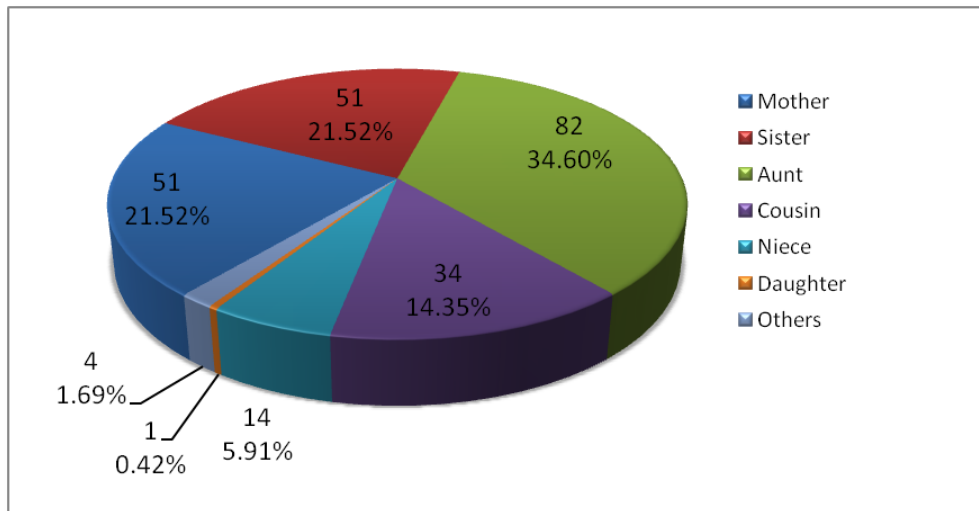


Figure 3.2: The relationship distribution of the at-risk individuals.

3.2.2 Risk Allocations

The 237 at-risk individuals were categorised into the three respective reproductive risk groups; Low, Intermediate and High (Table 3.1).

Table 3.1: The number of at-risk individuals in each risk group and the average carrier and reproductive risks of the groups.

	Number of at-risk individuals	Average Carrier risk	Average Reproductive risk
Low risk group	112	10.6±5.8%	5.3±2.9%
Intermediate risk group	39	31.1±4.0%	15.5±2.0%.
High risk group	86	74±19.8%	37±9.9%.

Table 3.2 summarises the relationship distribution of individuals in the three risk groups:

The Low risk group mainly consisted of the cousins (85.29%) and aunts (69.51%). The Intermediate risk group consisted of a high number of sisters (43.14%) and nieces

(42.86%). The High risk group consisted mainly of the mothers of affected individuals (92.16%).

Table 3.2: The relationships of the at-risk individuals in each risk group.

	Low		Intermediate		High		Total N (100%)
	N	%	N	%	N	%	
Mother	4	7.84	0	0.00	47	92.16	51
Sister	15	29.41	22	43.14	14	27.45	51
Aunt	57	69.51	8	9.76	17	20.73	82
Cousin	29	85.29	3	8.82	2	5.88	34
Niece	5	35.71	6	42.86	3	21.43	14
Daughter	0	0.00	0	0.00	1	100.00	1
Other	2	50.00	0	0.00	2	50.00	4
Total	112	47.26	39	16.46	86	36.29	237

3.2.3 Mutation in family

Table 3.3 summarises the specific diagnosis in families, shows whether the diagnosis was confirmed with mutation analysis, and also indicates the type of mutation involved. This is summarised in the three distinct risk groups. Most individuals (76.8%, 182/237) had a diagnosis of DMD in their families, 47 of the 237 (19.8%) had a diagnosis of BMD in their families and the diagnosis was unconfirmed (could be either DMD or BMD) in the families of a few individuals (3.4%, 8/237). With DMD/BMD the clinical symptoms of individuals can overlap and uncertainty of whether the condition is the milder DMD or more severe BMD arise; in these individuals the diagnosis would thus be unconfirmed.

The prevalence of *de novo* mutations was high with the majority (71.3%, 169/237) of individuals being part of families with *de novo* mutations. Only 27% (64/237) of at-risk

individuals had the mutation in their family confirmed by molecular analysis. Of these mutations 93.7% (60/64) were deletions and 6.3% (4/64) were duplications.

Table 3.3: The diagnosis, presence and nature of *DMD* mutations in families of at-risk individuals.

	Low		Intermediate		High		Total
	N	%	N	%	N	%	N (100%)
Diagnosis in family							
DMD	84	46.15	30	16.48	68	37.36	182
BMD	22	46.81	9	19.15	16	34.04	47
Unconfirmed (DMD/BMD)	6	75.00	0	0.00	2	25.00	8
Total	112	47.26	39	16.46	86	36.29	237
New or familial mutation							
Familial	18	26.47	11	16.18	39	57.35	68
De Novo	94	55.62	28	16.57	47	27.81	169
Total	112	47.26	39	16.46	86	36.29	237
Mutation confirmed with molecular testing							
Mutation Confirmed	31	48.44	8	12.50	25	39.06	64
Mutation not confirmed	81	46.82	31	17.92	61	35.26	173
Total	112	47.26	39	16.46	86	36.29	237
Type of mutation							
Deletion	30	50.00	6	10.00	24	40.00	60
Duplication	1	25.00	2	50.00	1	25.00	4
Unknown	81	46.82	31	17.92	61	35.26	173
Total	112	47.26	39	16.46	86	36.29	237

3.3 Genetic Counselling

3.3.1 Referral source

Of the 237 at-risk individuals identified in the genetic counselling files who met the inclusion criteria, 30.4% (72/237) attended Genetic Counselling (GC). The individuals were mostly referred by medical professionals or were self referred. Medical professionals that referred most patients were neurologists, who referred 25.0% (18/72) and paediatricians, who referred 19.4% (14/72) of the at-risk individuals that attended GC. A

large proportion of individuals, 22.2% (16/72) were self-referred. Figure 3.3 illustrates the referral sources and the number of individuals per risk group that were referred.

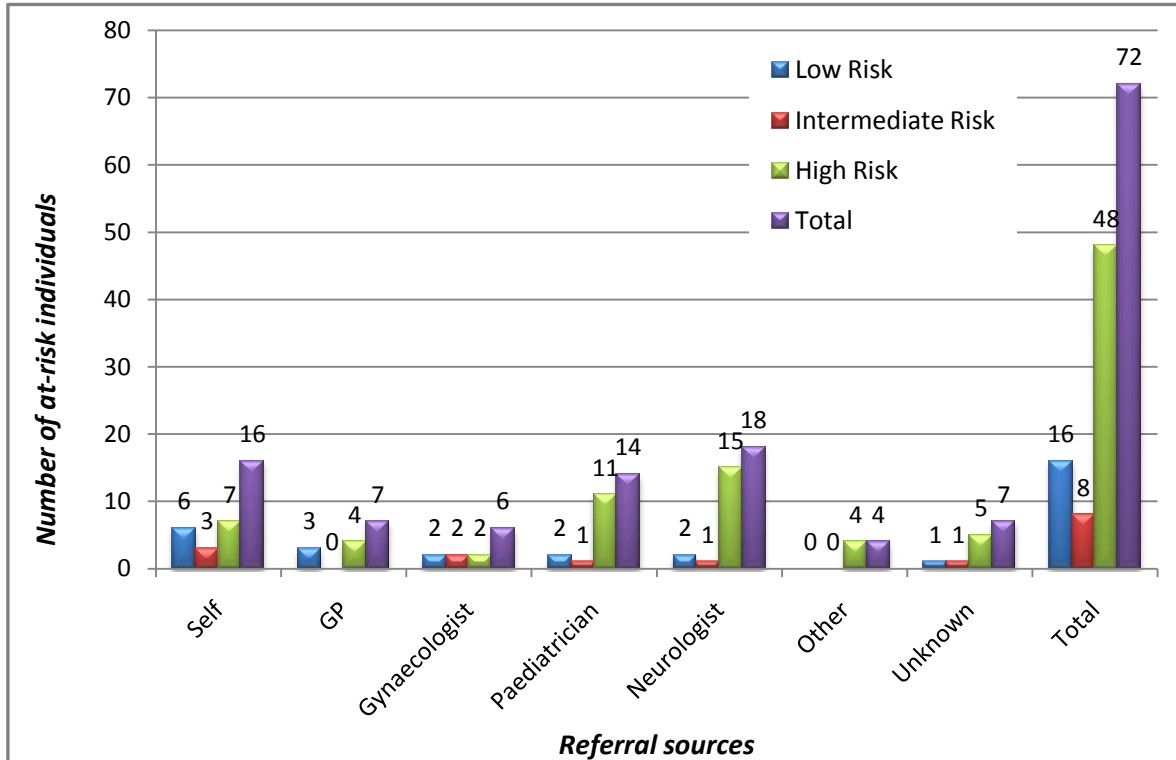


Figure 3.3: A representation of the number of at-risk individuals in the different risk groups that attended genetic counselling and their referral sources.

3.3.2 Reasons to attend Genetic Counselling and Genetic Counselling follow-up sessions

Of the 30.4% (72/237) at-risk individuals that attended GC, 22.2% (16/72) of the individuals belonged to the Low risk group, 11.1% (8/72) to the Intermediate risk group and 66.7% (48/72) to the High risk group. Individuals attended GC for different reasons; 50.0% (36/72) of individuals were referred for GC because they had an affected son, 2.8% (2/72) because of the concern that their son might be affected with DMD/BMD, 25.0%

(18/72) for reasons around family planning, and 15.3% (11/72) at-risk individuals attended GC because they were pregnant and wanted prenatal testing (Figure 3.4).

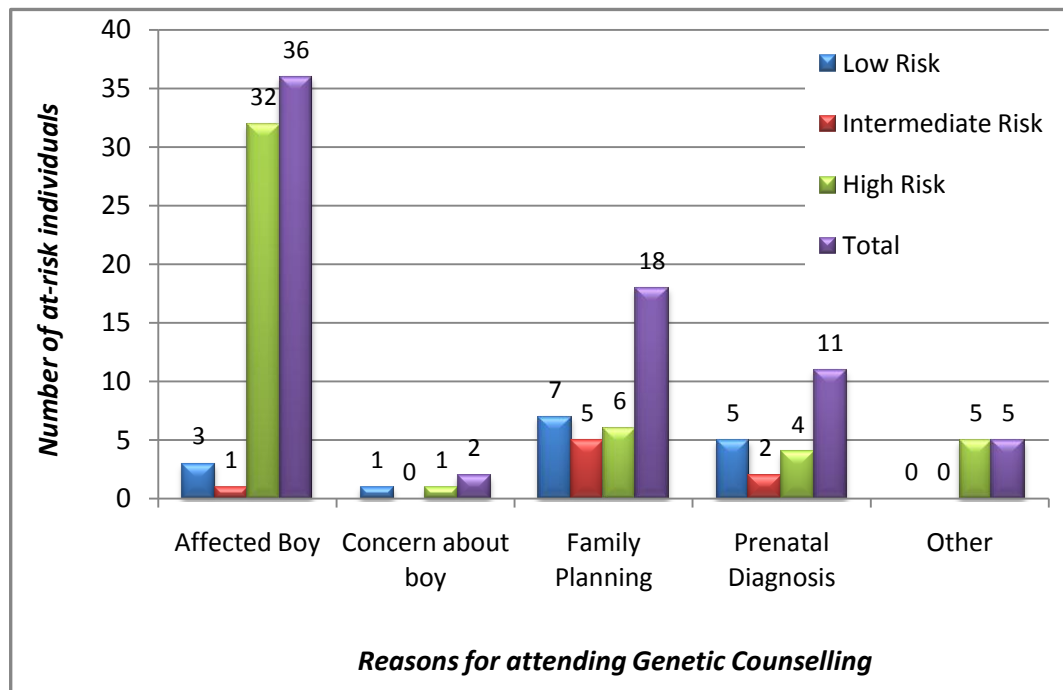


Figure 3.4: The number of at-risk individuals per risk group that attended genetic counselling and their reasons for attending.

Individuals in the High risk group attended GC mostly because they had an affected son, 66.7% (32/48). Individuals in the Intermediate and Low risk groups attended GC mainly for family planning; 62.5% (5/8) and 43.7% (7/16) respectively. Individuals in the Low risk group were also more concerned with prenatal diagnosis, 31.3% (5/16).

Only 31 (43.06%) at-risk individuals of the 72 that attended the first GC session attended a follow-up session. As some individuals attended more than one follow-up session, the reasons for attending follow-up sessions were more than the number of individuals that attended follow-up GC. Of the follow-up GC visits 46.3% (19/41) sought test results.

Other reasons for attending GC follow-up sessions were for family planning, prenatal and postnatal testing (Figure 3.5)

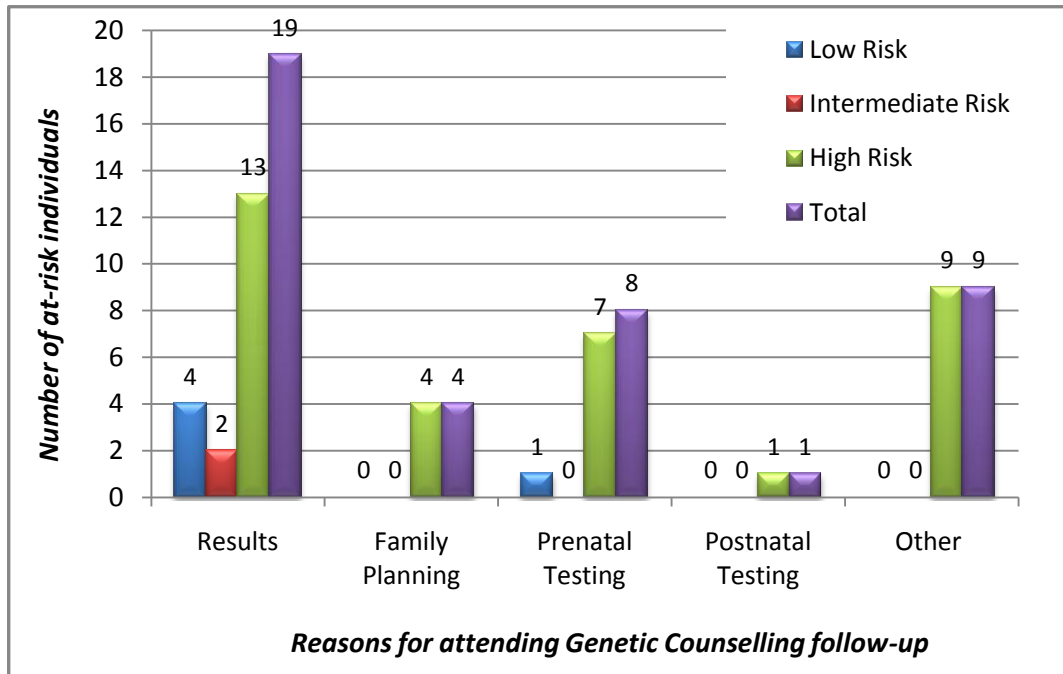


Figure 3.5: The number of at-risk individuals in each risk group and their reasons for attending genetic counselling follow-up sessions.

3.3.3 Factors influencing decisions to attend Genetic Counselling

To establish what factors influenced individuals' decisions to either attend or *not* to attend GC, the chi-square and logistic regression analysis were performed on 236 of the 237 ('daughter' category was excluded from the analysis as there was only one individual in this category) at-risk individuals identified in the GC files. The variables identified that may influence an individual's decision to go for GC were mentioned in section 2.4.1, Table 2.1. These variables were reproductive *risk*, *relationship* to proband, *ethnicity*, *de novo* mutation and *age*. Using Chi-squared (χ^2) analysis, it was shown that independently, *risk* ($\chi^2=41.81$, $P<0.0001$), *relationship* ($\chi^2=112.09$, $P<0.0001$), *de novo* mutations

($\chi^2=4.32$, $P<0.05$) and *age* ($\chi^2=11.78$, $P<0.005$) significantly contributed to the decision of an individual to go for GC. However, *ethnicity* did not significantly influence GC decisions ($\chi^2=3.05$, $P>0.1$).

Using Chi-squared (χ^2) analysis, the different factors; *risk*, *relationship*, *de novo* mutations, *ethnicity* and *age*, were shown not to be significantly associated with an individual's decision to attend the follow-up GC session (all P values are greater than 0.05).

The odds ratios calculated for the different variables were deducted from the univariate logistic regression analysis, where each variable was tested independently for significance surrounding the decision to go for GC (Table 3.4). In order to look at the combination of factors and what most significantly influences GC decisions, multiple logistic regression analysis was performed. Two multiple regression models were constructed using the variables that significantly influenced decisions to go for GC (Table 3.4). The first model included variables; *risk*, *relationship* and *de novo* mutations. The second model included the variables used in the first model as well as the *ages* of individuals. *Age* was not considered in the first model because the ages for only 116 individuals were known; this reduced the sample size by more than half. However, *age* plays a significant role in the decision to attend GC and was therefore included in model 2 for comparative reasons. The results for both the univariate and multiple logistic regression analysis are discussed in the following sections:

Table 3.4: Logistic regression analysis for the different variables with genetic counselling as the dependent variable.

Factor	Univariate Logistic Regression		Multiple Logistic Regression (Model 1)		Multiple Logistic Regression (Model 2)	
			<i>Pearson χ^2=247.57</i>		<i>Pearson χ^2=165.01</i>	
	OR ^y (95% CI*)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Risk Group	$\chi^2=41.81$					
High	3.33 (2.18 - 5.09)	0.00	1.37 (0.65 - 2.92)	0.40	0.65 (0.23 - 1.82)	0.41
Intermediate	0.68 (0.38 - 1.20)	0.18	0.68 (0.32 - 1.42)	0.31	0.66 (0.28 - 1.59)	0.36
Low	Reference		Reference		Reference	
Relationship	$\chi^2=112.09$					
Other	0.24 (0.05 – 1.26)	0.09	0.32 (0.06 - 1.79)	0.19	0.75 (0.08 – 6.85)	0.80
Cousin	0.70 (0.28 – 1.77)	0.46	0.72 (0.26 - 1.99)	0.53	0.24 (0.06 - 0.96)	0.04
Aunt	0.10 (0.03 – 0.35)	0.00	0.09 (0.03 - 0.33)	0.00	0.08 (0.02 - 0.39)	0.00
Sister	2.64 (1.29 – 5.35)	0.01	2.80 (1.30 – 6.09)	0.01	2.29 (0.72 – 7.20)	0.16
Mother	Reference		Reference		Reference	
De Novo	$\chi^2=4.32$					
Yes	1.42 (1.02 – 1.98)	0.04	1.24 (0.75 - 2.07)	0.39	0.64 (0.30 - 1.38)	0.25
No	Reference		Reference		Reference	
Age (N=116)	$\chi^2=11.78$		Excluded			
Group 1 (15 to 25 yrs)	0.39 (0.22 – 0.68)	0.00			0.59 (0.26 - 1.36)	0.22
Group 2 (26 to 35 yrs)	1.53 (0.89 – 2.63)	0.12			1.81 (0.82 - 4.02)	0.14
Group 3 (36 to 49 yrs)	Reference				Reference	
Total N			236		115	

^y Odds Ratio

* Confidence Interval

3.3.3.1 Reproductive risk

Table 3.4, shows that individuals in the High risk group differed significantly in their decision to go for GC compared to the Low risk group individuals. The odds ratio or likelihood for the High risk group to go for GC was 3.33 *more* than for the Low risk group (95% CI 2.18 – 5.09, $P < 0.0001$).

Correcting the association between *risk* and the decision to go for GC, by taking into account the influence of *relationship*, *de novo mutation* and *age*, the association was no longer significant (Table 3.4).

3.3.3.2 Relationship to proband

The following relationships were documented: mothers, sisters, aunts, cousins, nieces, daughters and other. The category ‘other’ included individuals that were more distantly related. For the analysis to test for an association between relationship and GC, the category ‘daughters’ was excluded, as this group contained only one individual. The more distantly related individuals in categories ‘nieces’ and ‘other’ were grouped together in order to increase the number of individuals in the group for analysis, and the new group was referred to as ‘distant’. Figure 3.6 depicts the 236 at-risk relatives of affected individuals that attended GC and follow-up GC.

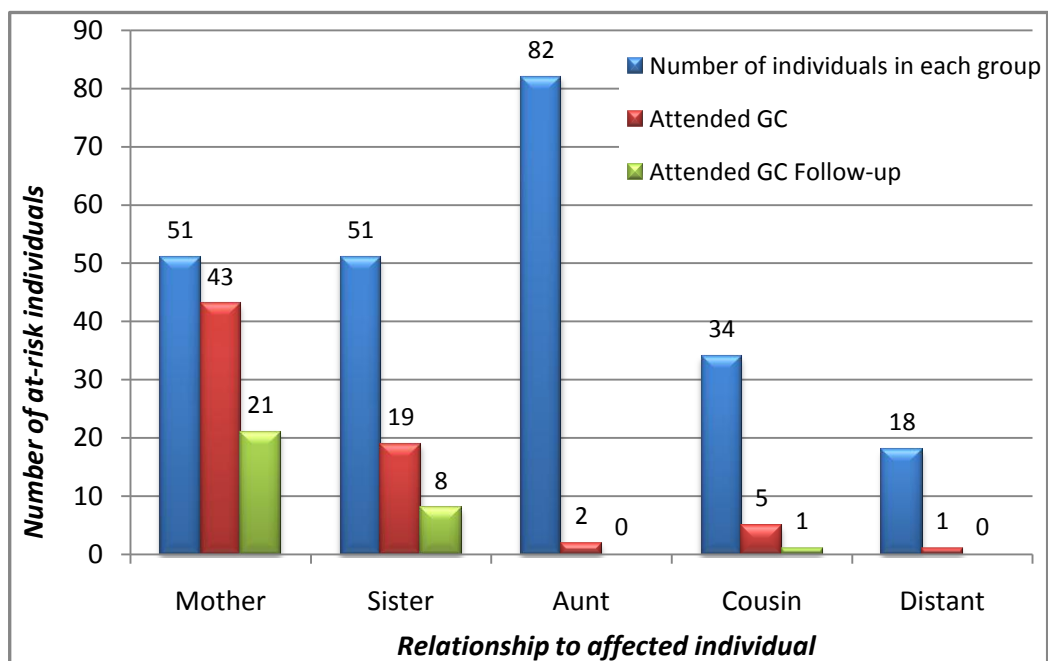


Figure 3.6: The relationship distribution of family members of DMD/BMD affected individuals, who attended genetic counselling and follow-up genetic counselling.

More mothers of affected individuals attended GC (Figure 3.6). The aunts of affected individuals were 0.10 times as likely to go for GC as the mothers (95% CI 0.03 – 0.35, $P < 0.001$). However, the sisters of affected individuals were 2.64 times *more* likely to attend GC than the mothers of these individuals (95% CI 1.29 – 5.35, $P < 0.05$). The analysis also showed that cousins and more distantly related individuals were *less* likely to attend GC compared to the mothers (Table 3.4).

Multiple logistic regression showed that the relationship to the affected individual plays a significant role in decisions surrounding GC. In Model 1, the odds ratios for aunts and sisters did not change even after the contribution of *relationship* was corrected for the variables; *risk* and *de novo mutation*. The relationships continued to play a significant role in the GC decision. However, in Model 2, after the odds ratios were adjusted for *risk*,

relationship, *de novo mutation* and *age*, being a ‘sister’ did not have a significant influence on the GC decision. The ‘aunt’ relationship was still highly significant and the ‘cousin’ relationship seemed to be significant when *age* was taken into consideration.

3.3.3.3 *De novo mutations*

Another significant association is the relationship between *de novo* mutations in a family and the likelihood that individuals in those families will attend GC. The results in Table 3.4 show that individuals in *de novo* families were 1.42 times *more* likely to attend GC than their counterparts (95% CI 1.02 – 1.98, $P < 0.05$). The contribution of *de novo* mutations proved not to be significant when the other factors (*risk* and *relationship*) were taken into account.

3.3.3.4 *Age*

The age of individuals also played a significant role in making decisions about GC. The decision of Group 1 (15 to 25 years) individuals differed significantly from those of Group 3 (36 to 49 years) in that they were 2.56 times *more* likely (95% CI 1.47 – 4.55, $P < 0.001$) *not* to attend GC than the older individuals (or 0.39 times as likely to attend GC). The contribution of *age* proved not to be significant when the other factors (*risk*, *relationship* and *de novo mutation*) were taken into account.

3.4 Carrier Testing

The decision of individuals to have carrier testing was analysed for 236 of the 237 (‘daughter’ category was excluded from the logistic regression analysis as there was only

one individual in this category) at-risk individuals identified from the GC files. The ages of 116 individuals were known. The group that did not have testing consisted of 54 individuals with a mean age of 27.3 ± 9.03 . The group electing to have carrier testing, consisted of 62 individuals, with a mean age of 31.1 ± 7.4 . The High risk group had the largest percentage of individuals that chose to have carrier testing 59.3% (51/81), the Low risk group had 20.5% (23/112) who had testing, and the Intermediate risk group had the lowest percentage with 5% (2/39) of the individuals in the group having had carrier testing. Figure 3.7 graphically represents the individuals in the different risk groups that had carrier testing and the number of individuals that obtained results.

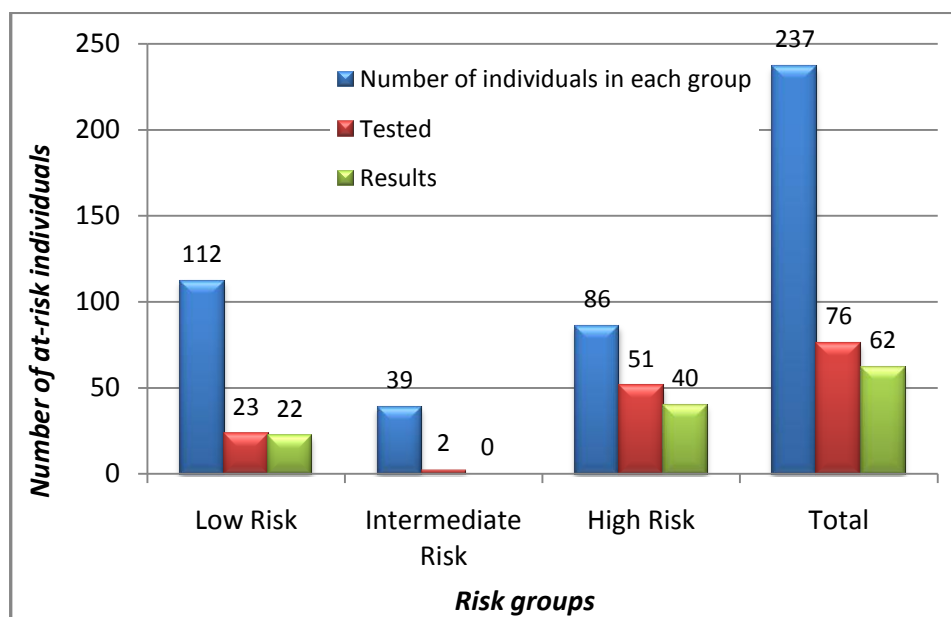


Figure 3.7: Carrier testing and results for individuals in the different risk groups.

3.4.1 Factors influencing decisions to take up carrier testing

The variables identified that could influence an individual's decision to take up carrier testing were mentioned in section 2.4.1, Table 2.1. These variables were reproductive *risk*, *relationship* to proband, *ethnicity*, *de novo* mutation, *mutation known*, *affected children* and *age*.

The variables that independently had a significant influence on individuals' decision to undertake carrier testing were *risk* ($\chi^2=49.11$, $P<0.0001$), *relationship* to the proband ($\chi^2=68.08$, $P<0.0001$), whether an individual had an *affected child* ($\chi^2=38.48$, $P<0.0001$) and *age* ($\chi^2=10.89$, $P<0.005$). Variables that did not have a significant influence on decisions to undertake carrier testing were *ethnicity* ($\chi^2=3.95$, $P>0.1$), whether the *mutation was known* ($\chi^2=1.97$, $P>0.1$) or if the mutation was *de novo* ($\chi^2=0.97$, $P>0.2$). Only the variables with significant P values were included in the multiple logistic regression models (Table 3.5).

3.4.1.1 Reproductive Risk

Using univariate logistic regression analysis it was shown that individuals in the High risk group were 5.34 times (95% CI 3.00 – 9.48, $P<0.0001$) *more* likely to undertake carrier testing than the Low risk group. Analysis also showed that the Intermediate risk group was 0.20 times (95% CI 0.07 – 0.52, $P=0.001$) as likely to undertake carrier testing when compared to the Low risk group.

Using multiple logistic regression analysis it was shown that the *reproductive risk* of an individual significantly contributed to carrier testing decisions even after all the other variables were taken into account. After correcting for *relationship* and *age*, individuals in the High risk group were 2.53 times (95% CI 1.01 – 6.32, $P<0.05$) *more* likely to have carrier testing than the Low risk group. The Intermediate risk group were 0.09 times (95% CI 0.03 – 0.34, $P<0.001$) as likely to have carrier testing.

Table 3.5: Logistic regression analysis for the different variables with carrier testing as the dependent variable.

Factor	Univariate Logistic Regression		Multiple Logistic Regression	
			<i>Pearson $\chi^2=108.91$</i>	
	OR ^y (95% CI*)	P value	OR (95% CI)	P value
Risk Group	$\chi^2=49.11$			
High	5.34 (3.00 - 9.48)	0.00	2.53 (1.01 - 6.32)	0.05
Intermediate	0.20 (0.07 - 0.52)	0.00	0.09 (0.03 - 0.34)	0.00
Low	Reference		Reference	
Relationship	$\chi^2=68.08$			
Other	0.35 (0.10 - 1.16)	0.09	2.68 (0.33 - 21.79)	0.36
Cousin	0.72 (0.34 - 1.54)	0.40	0.29 (0.08 - 0.99)	0.05
Aunt	0.26 (0.13 - 0.53)	0.00	0.17 (0.05 - 0.60)	0.01
Sister	2.11 (1.17 - 3.80)	0.01	3.49 (1.03 - 11.83)	0.04
Mother	Reference		Reference	
Age (N=116)	$\chi^2=10.89$			
Group 1 (15 to 25 yrs)	0.45 (0.26 - 0.78)	0.00	0.53 (0.23 - 1.25)	0.15
Group 2 (26 to 35 yrs)	2.00 (1.18 - 3.36)	0.01	2.49 (1.26 - 4.90)	0.01
Group 3 (36 to 49 yrs)	Reference		Reference	
Affected Children (N=131)	$\chi^2=38.48$		Excluded	
Yes	3.42 (2.26 - 5.18)	0.00		
No	Reference			
Total N			115*	

[‡] Odds Ratio

* Confidence Interval

3.4.1.2 Relationship to affected individual

Univariate logistic regression showed that the relationship to an affected individual significantly contributed to the decision to undertake carrier testing. The aunts of

DMD/BMD affected individuals were 0.26 times (95% CI 0.13 – 0.53, $P<0.001$) *as* likely to have carrier testing as the mothers of affected individuals. In other words the aunts were 3.84 times *less* likely to have carrier testing when compared to the mothers. The sisters of affected individuals were 2.11 times (95% CI 1.17 – 3.80, $P=0.01$) *more* likely to have carrier testing than the mothers.

After adjusting the odds ratios for *risk* and *age* with multiple logistic regression, *relationship* still had a significant influence on the decision to have carrier testing. Cousins and aunts were *less* likely to have carrier testing than mothers with odds ratios of 0.29 (95% CI 0.08 – 0.99, $P<0.05$) and 0.17 (95% CI 0.05 – 0.60, $P<0.01$) respectively. Sisters were 3.49 times (95% CI 1.03 – 11.83, $P<0.05$) *more* likely to have carrier testing than mothers.

3.4.1.3 Age

Individuals in the youngest reproductive group (15 – 25 years), were 0.45 times (95% CI 0.26 – 0.78, $P<0.01$) *as* likely to have carrier testing as individuals of older reproductive age (36 – 49 years). The age group 26 – 35 years of age were 2 times (95% CI 1.18 – 3.80, $P=0.01$) *more* likely to have carrier testing than the oldest group.

Adjusting the relationship between *age* and the decision to have carrier testing by taking into account the reproductive *risk* and *relationship* to the affected individual, it was found that age-group 2 (26 – 35 years) were 2.49 times (95% CI 1.26 – 4.90, $P<0.01$) *more* likely to have carrier testing than the older individuals (36 – 49 years).

3.4.1.4 Affected child

Individuals with DMD/BMD affected children were 3.42 times (95% CI 2.26 – 5.18, $P<0.0001$) *more* likely to have carrier testing when compared to individuals that did not have affected children.

The variable *affected children*, was not included in the multiple logistic regression model because of the limited overlap of information available on the *ages* of individuals and whether they had *affected children*. With the inclusion of affected children in the model, the number of individuals in the data was reduced to N=56 and the model was no longer significant.

3.5 Reproduction

The analysis on reproduction was performed on a sample size of 218 at-risk individuals, identified from the GC files. Information on whether individuals had children was available for only 218 individuals, ages were available for 114 of the 218. Individuals with children (69/114) had a mean age of 32.8 ± 7.5 and individuals without children (45/114) had a mean age of 23.8 ± 6.7 . Of the individuals in the Low risk group 69.5% (66/95) had children, 38.5% (15/39) in the Intermediate risk group and 78.6% (66/84) in the High risk group (Figure 3.8).

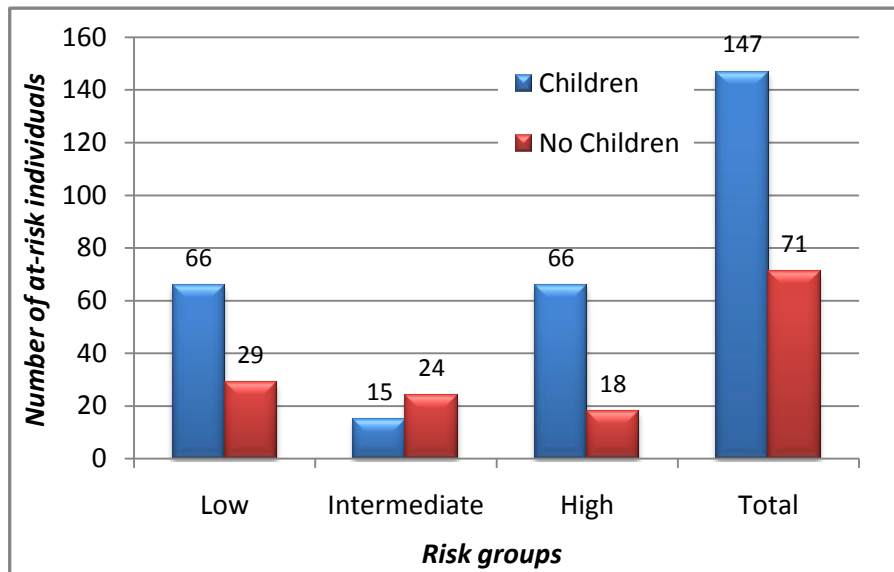


Figure 3.8: The number of at-risk individuals in the different risk groups with and without children.

Of the individuals in the different risk groups that had children, the following percentages of the group had children affected with DMD/BMD: The Low risk group consisted of 7.1% (4/56) individuals that had affected children, the Intermediate risk group did not have affected children, and the High risk group had the largest percentage of individuals with affected children, 78.1% (50/64) (Figure 3.9).

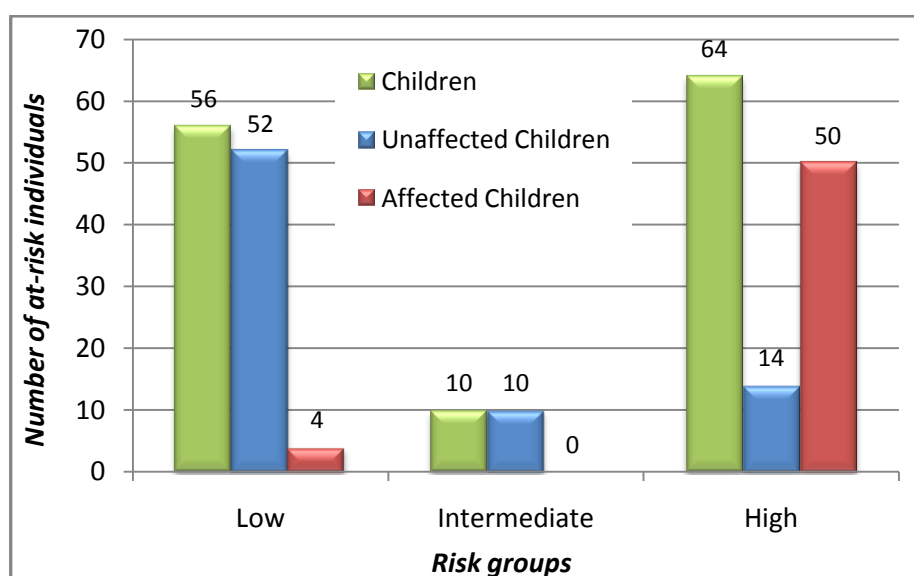


Figure 3.9: The number of at-risk individuals in the different risk categories with children and those with affected and unaffected children.

3.5.1 Factors influencing decisions to reproduce

The variables identified that could have influenced an individual's decision to have children were mentioned in section 2.4.1, Table 2.1. These variables were reproductive risk, relationship to proband, ethnicity, de novo mutation, mutation known and age.

The variables that independently had a significant influence on an individual's decision to have children were risk ($\chi^2=19.83$, $P<0.0001$), relationship to the proband ($\chi^2=37.32$, $P<0.0001$), ethnicity ($\chi^2=14.22$, $P<0.001$), de novo mutation ($\chi^2=5.16$, $P<0.05$) and age ($\chi^2=25.27$, $P<0.0001$). Whether the mutation in the family was known did not have a significant influence on the decision to have children ($\chi^2=0.067$, $P>0.5$). Only the variables with significant P values were included in the multiple logistic regression models (Table 3.6).

3.5.1.1 Reproductive Risk

Univariate logistic regression showed that individuals in the High risk group were 2.11 times (95% CI 1.37 - 3.26, $P<0.0001$) more likely to have children than the Low risk group. Analysis also showed that the Intermediate risk group was 0.36 times (95% CI 0.22 - 0.59, $P<0.0001$) as likely to have children when compared to the Low risk group. However, after adjusting the relationship between risk and children for the other variables (relationship, de novo mutation and ethnicity) no significant influence on the decision to have children was found.

Table 3.6: Logistic regression analysis for the different variables with having children as the dependent variable.

Factor	Univariate Logistic Regression		Multiple Logistic Regression (Model 1)	
			<i>Pearson $\chi^2=169.94$</i>	
	OR ^y (95% CI*)	P value	OR (95% CI)	P value
Risk Group	$\chi^2=19.83$			
High	2.11 (1.37 - 3.26)	0.00	0.91 (0.46 - 1.81)	0.79
Intermediate	0.36 (0.22 - 0.59)	0.00	0.67 (0.36 - 1.24)	0.20
Low	Reference		Reference	
Relationship	$\chi^2=37.32$			
Distant	0.70 (0.22 - 1.09)	0.08	0.67 (0.27 - 1.70)	0.40
Cousin	0.32 (0.37 - 1.42)	0.35	0.58 (0.28 - 1.21)	0.14
Aunt	1.63 (2.87 -9.13)	0.00	4.10 (2.19 - 7.67)	0.00
Sister	Reference		Reference	
De novo	$\chi^2=5.16$			
Yes	1.42 (1.05 – 2.85)	0.02	1.07 (0.65 - 1.75)	0.80
No	Reference		Reference	
Ethnicity	$\chi^2=14.22$			
Black	2.04 (1.21 - 3.46)	0.01	1.86 (0.98 - 3.53)	0.06
White	0.60 (0.37 - 0.96)	0.03	0.74 (0.40 - 1.35)	0.32
Indian	Reference		Reference	
Age (N=116)	$\chi^2=25.27$		Excluded	
Group 1 (15 to 25 yrs)	0.22 (0.12 - 0.42)	0.00		
Group 2 (26 to 35 yrs)	1.07 (0.58 - 1.98)	0.83		
Group 3 (36 to 49 yrs)	Reference			
Total N			166*	

^y Odds Ratio

* Confidence Interval

3.5.1.2 Relationship to proband

In this study, the majority of individuals had children (62%, 147/237). The ‘aunts’ of affected individuals was the group with the largest proportion 83.8% (62/74) of individuals that had children. Of the cousins 42% (11/26), of the sisters 35.4% (17/48) and of the more distant relatives 33.3% (6/18) had children (Figure 3.10).

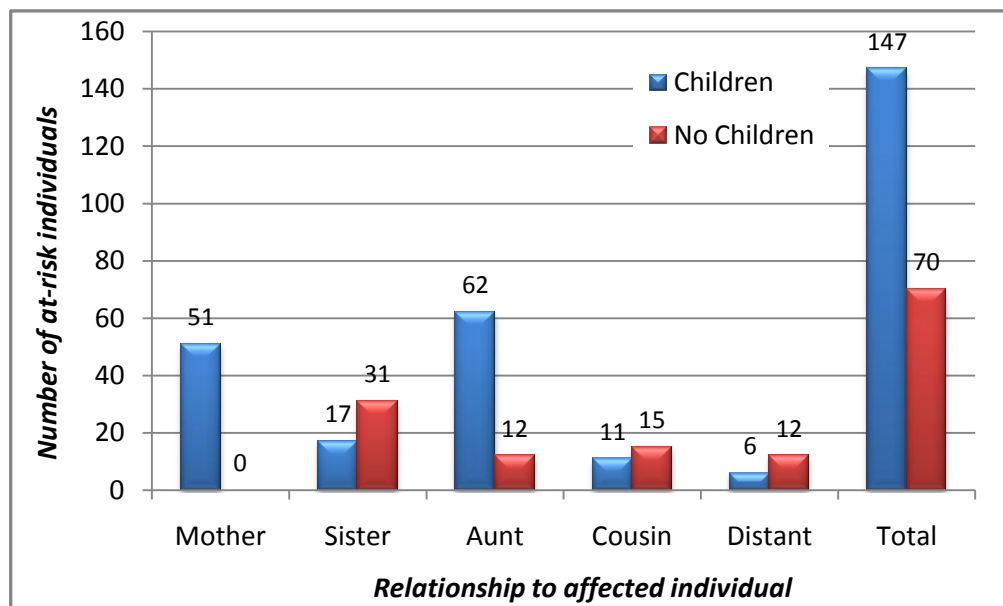


Figure 3.10: Relatives of the affected individual with the number of individuals in each group that had children and did not have children.

The relationship ‘mother’ was not included in the analysis because the relationship was not predictive of whether an individual would have children or not, as all mothers had children (Figure 3.10). The ‘daughter’ category was also excluded from the logistic regression analysis as this category consisted of one individual.

Univariate logistic regression showed that the relationship to an affected individual significantly contributed to the decision to have children. The aunts of DMD/ BMD

affected individuals were 5 times (95% CI 2.87 – 9.13, $P<0.0001$) *more* likely to have children than the sisters of affected individuals.

After adjusting the odds ratios for *risk*, *ethnicity*, and *de novo mutation* with multiple logistic regression, *relationship* still had a significant influence on the decision to have children. Aunts were 4.1 times (95% CI 2.19 – 7.67, $P<0.0001$) *more* likely to have children than the sisters of affected individuals.

3.5.1.3 Ethnicity

Using univariate logistic regression it was shown that individuals from the Black ethnic group were 2 times (95% CI 1.21 - 3.46, $P<0.01$) *more* likely to have children than Indian individuals. White individuals were 0.60 times (95% CI 0.37 - 0.96, $P<0.05$) as likely to have children as Indian individuals (or 1.7 times more likely *not* to have children).

However, after adjusting the relationship between *ethnicity* and children for the other variables (Table 3.6), no significant influence on the decision to have children was noticed.

3.5.1.4 De novo mutation

Using univariate logistic regression it was shown that individuals from families with *de novo mutations* were 1.42 times (95% CI 1.05 – 2.85, $P<0.05$) *more* likely to have children than individuals with previous affected individuals in their families. However, after adjusting the relationship between *de novo mutation* and children for the variables, *risk*, *relationship* and *ethnicity*, the *de novo mutation* did not have a significant influence on the decision to have children.

3.5.1.5 Age

Individuals in the youngest reproductive group (15 – 25 years) were 0.22 times (95% CI 0.12 - 0.42, $P < 0.0001$) as likely to have children as individuals of older reproductive age (36 – 49 years). In other words, Group 1 (15 – 25 years) individuals were 4.5 times more likely *not* to have children than individuals in Group 3 (36 – 49 years).

Age was initially excluded from the multiple logistic regression model because of the limited information available and the fact that the sample size decreased if age was included. *Age* was included in a second multiple logistic regression model, however the model did not show any significance and was therefore not included in the results.

3.5.2 Knowledge about potential carrier risk

Of the 237 individuals in the study, limited information was available on the knowledge individuals had about their risk. The information available showed that 46 individuals knew that they were at risk of carrying the disease causing gene, of whom 50% (23/46) chose to have children, 4.3% (2/46) chose not to have children, 6.5% (3/46) delayed having children, but for 39% (18/46), the decision to have children could not be obtained from the counselling files. Figure 3.11 graphically represents the number of individuals in each risk group that knew about their potential risk and their decisions regarding reproduction, prenatal testing, termination of pregnancy and also the pregnancy outcome.

In the High risk group, 22 of the 86 individuals (25.6%) were aware of their potential risk to be carriers. Of 22 individuals, 16 (72.7%) decided to have children and 13 of the 16 (81.3%) had prenatal testing. Limited information was available on decisions to terminate

a pregnancy and on the pregnancy outcomes. Of the 16 High risk individuals that had children, information on termination of pregnancy was available on 10 individuals (45.5%); four of the individuals would have terminated a pregnancy and 6 would not. The pregnancy outcomes for the 16 individuals in the High risk group that knew about their potential risk and proceeded to have children was as follows; 4 affected boys were born (25%), 5 unaffected boys (31.3%), 1 daughter (6.3%), 1 pregnancy was terminated (6.3%) but the outcome for 5 pregnancies was unknown.

In the Intermediate risk group, 7 of the 39 individuals (17.9%) were aware of their potential risk to be carriers. Of these individuals, 1 (14.3%) decided to have children and did not have prenatal testing. Information on the decision to terminate a pregnancy and on the pregnancy outcome was unavailable.

In the Low risk group, 17 of the 112 individuals (15.2%) were aware of their potential risk to be carriers. Of 17 individuals, 9 (52.9%) decided to have children and 5 (55.6%) had prenatal testing. Information on whether individuals would terminate an affected pregnancy was not available. Limited information was available on the pregnancy outcomes, 2 individuals (22.2%) had unaffected boys, the other pregnancy outcomes were unknown.

The results obtained in the different focus areas of the study and the limitations encountered during the process of the study will be discussed in detail in Chapter Four.

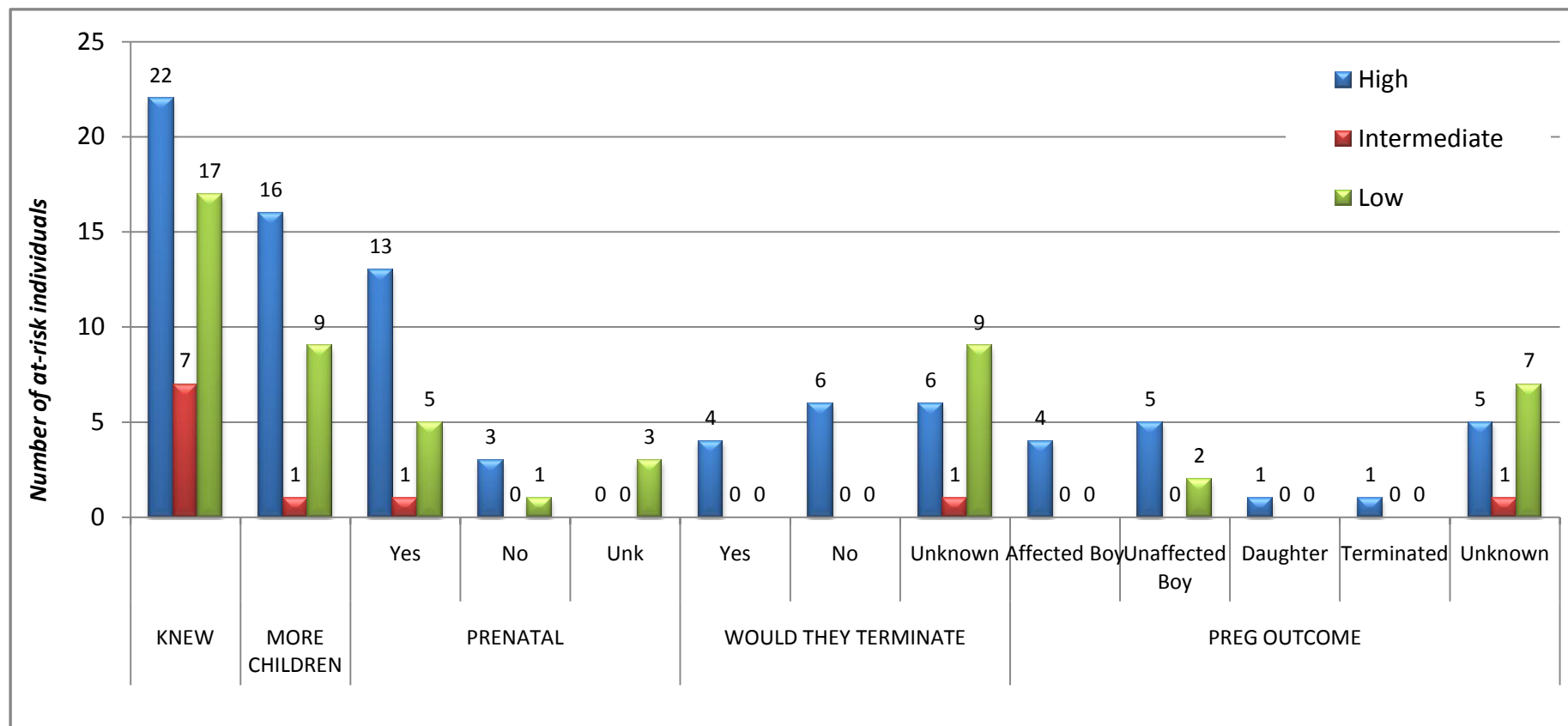


Figure 3.11: At-risk individuals in the different risk groups who were aware of their potential risks, their decisions to have children and prenatal testing, their decisions about termination of pregnancy and the outcomes of their pregnancies.

4.0 DISCUSSION

The first aim of this study was to assess which members of DMD/BMD families came for genetic counselling and of those, who returned for subsequent counselling. The study also aimed to identify individuals in these families at risk of being carriers for DMD/BMD, to assign their individual risks and group them into different risk categories. Lastly, the study aimed to investigate the influence of risk on the carrier testing, genetic counselling and reproductive decisions of individuals in the different risk groups. After careful consideration, the researcher realised that *risk* was not the only influential factor when having to decide about testing, reproduction and genetic counselling and the study therefore included other contributing factors.

4.1 At-risk individuals

From the files included in the study, 237 at-risk individuals were identified. The mean number of at-risk individuals per family was 3.5 ± 3.3 . The at-risk individuals were mostly Black and White individuals with a minority of the individuals from Indian ethnicity and none from the Coloured population. This result is therefore not representative of the South African population.

The at-risk individuals identified from the pedigrees were predominantly closely related individuals, mainly the aunts, mothers and sisters. The cousins and more distantly related individuals made up the rest of the at-risk group of individuals (Figure 3.1, p36). The researcher considered that perhaps more information was available on the closely related

individuals because, the genetic counsellor did not ask about the more distantly related individuals or the individuals who attended GC did not have the required information.

The at-risk individuals were categorised into the three risk groups; the Low risk group consisted mainly of the cousins and aunts (Table 3.2, p38), the Intermediate risk group of sisters and also nieces, whereas the High risk group comprised of mainly the mothers of affected individuals. This result is consistent with the X-linked recessive inheritance pattern where the more distantly related individuals have a lower risk of carrying the mutation and the closely related individuals, a higher risk. In this study, the Low and High risk groups contained individuals for whom risks could be further refined. Factors that were used in Bayes' theorem to refine the at-risk individuals' risks were the number of healthy sons they had, and also whether they had carrier testing done. The Intermediate risk group consisted of individuals where less information was available in terms of children and carrier testing, and their risk calculations therefore, were more dependent on the pedigree; thus the Intermediate risk group consisted of both closely and distantly related individuals.

4.2 Diagnosis and mutations in families of at-risk individuals

4.2.1 Diagnosis

Most individuals in the study had a diagnosis of DMD in their families (Table 3.3, p39). This was expected because the prevalence of DMD is much higher (1 in 3,500) than the BMD prevalence (1 in 18,000). A few individuals did not have a specific diagnosis in their families; the familial condition was either severe BMD or milder DMD.

Interestingly, 71.3% of the at-risk individuals were part of families with a *de novo* mutation. This figure was much higher than expected and one might consider why more *de novo* families were seen for genetic counselling - the researcher speculates that these individuals could have had less knowledge of the condition and the inheritance thereof, and were therefore more likely to seek information.

4.2.2 Mutations

Only 27% of at-risk individuals had a confirmed mutation in their family, of which a large proportion had a deletion and a smaller proportion a duplication (Table 3.3, p39). The number of individuals with a confirmed mutation in their families was of concern.

However, until 2008 in South Africa only the ‘hotspot’ regions were screened for deletions and duplications. Screening the ‘hotspot’ regions with multiplex PCR detects almost all of the DMD/BMD causing deletions, which account for 65% of the DMD causing mutations and 85% of the BMD mutations. The new MLPA method was only introduced in 2008 and is used to screen all 79 exons for deletions and duplications. The MLPA method has greater specificity and a better detection rate of the DMD/BMD causing deletions and duplications, thus more individuals will learn their family specific mutation. The researcher considered that more individuals will have carrier testing if the family mutation is known.

4.3 Who refer individuals for genetic counselling?

Medical professionals who referred individuals for GC were mostly neurologists and paediatricians. A large proportion of individuals were also self-referred and low numbers of individuals were referred by general practitioners (GPs) and gynaecologists (Figure 3.3,

p40). This corresponds to another South African study on CF, where the author found individuals were mostly referred by paediatricians and much less by physicians and gynaecologists (Macaulay, 2008). By implication, only the individuals with affected children or where a concern about a child was raised would have been seen for GC. Also, only individuals who were aware of their potential risk would have been seen for GC. This is concerning as the researcher feels that more individuals should be seen regarding family planning and less because of affected children, and feels that more individuals could be referred by GPs and gynaecologists. Seeing individuals before they have children would allow them the opportunity to gain knowledge of their risk and reproductive options, which could promote informed decisions.

4.4 Why do individuals attend genetic counselling?

Individuals attended GC mainly because they had a child affected with DMD/BMD or were worried that a child may be affected. A large proportion of at-risk individuals attended GC for family planning reasons and some individuals pertaining to antenatal testing (Figure 3.4, p41). Considering the referring individuals (section 4.3), it is not unexpected that most individuals were seen for GC because of an affected child.

Individuals attended the GC follow-up sessions mainly to get results for either carrier testing or confirmation of the condition. This result was expected as the genetic counsellors and clinical geneticists at the Division of Human Genetics, NHLS/WITS prefer to give results face-to-face. Individuals also attended the follow-up sessions for family planning, prenatal and postnatal testing (Figure 3.5, p42).

4.5 Factors influencing the decision to attend Genetic Counselling

In this study the following independent factors were found to influence the decision to have GC significantly; the *reproductive risk* of at-risk individuals, the *relationship* to the affected individual, *de novo mutation* and *age*.

The researcher found that individuals in the High risk group were *more* likely to attend GC than individuals in the Low risk group (Table 3.4, p44). Considering that the High risk group consisted mostly of mothers of affected individuals and the Low risk group mainly of aunts and cousins the result was not unanticipated. Using logistic regression, it was shown that both the aunts and cousins were *less* likely to attend GC than the mothers (Table 3.4, p44). However, the sisters of affected individuals were *more* likely to attend GC than the mothers. In Chapter 1, it was discussed that in families with cancer, the first-degree relatives were *more* likely to accept genetic counselling (Hagoel et al., 2000). This correlates with this study where the mothers and sisters of affected individuals were *more* likely to attend GC. It is well known that first-hand experience of a condition, either being affected oneself or having an affected family member, provides an individual with information on the burden of the condition on both the affected individuals as well as the rest of the family. This lived experience affects individuals' decisions in terms of reproduction, prenatal testing and termination of pregnancy (Weil, 2000). The researcher postulates that growing up with an affected sibling and witnessing the everyday reality of the condition also motivated the decision of sisters to attend GC.

Another factor that impacted on individuals' decisions to attend GC was *age*. When, comparing the younger individuals (15 – 25 years) with the older individuals (36 – 49

years), it was shown that the younger individuals were much less likely to attend GC. This finding did not correlate with the study of Hagoel, et al. (2000) that found the younger individuals more likely to accept GC. However, Zatz (1983) showed that younger individuals in DMD/BMD families were rarely informed by other family members about their risk, which could explain why they did not seek GC. This researcher feels that younger individuals may have been less informed about their risk as well as the availability of the service. However, a large proportion of the current study sample consisted of mothers of affected individuals and the age group 36 – 49 years consisted of more mothers that were referred for GC compared to the youngest (15 – 25 years) age group (results not reported in study).

Using, multiple logistic regression, the researcher found that if all the factors that significantly influenced the decision to attend GC were considered simultaneously, the single most important predicting factor of whether an individuals would attend GC was the *relationship* to the affected individual. The first degree relatives would seek GC *more* and the aunts and cousins would be *less* likely to attend GC. This result indicates that first-degree relatives were possibly more aware of their risk, had more knowledge of the condition and were more informed about the availability of genetic counselling. This also indicates that the family communication around DMD/BMD was lacking. Similarly, Hagoel, et al. (2000) found that first degree relatives in families with breast/ovarian cancer were more likely to attend GC.

This study did not identify any factors that could predict the decision of an individual to attend the GC follow-up session. It seems that the most important factor in attending the follow-up session was to obtain results.

4.6 Factors influencing the decision to have carrier testing

The following variables were investigated for their contribution to the behaviour of individuals with regards to carrier testing: reproductive *risk*, *relationship* to proband, *ethnicity*, *de novo* mutation, *mutation known*, *affected children* and *age*. It was shown that reproductive *risk*, *relationship* to the affected individual, having an *affected child* and *age* influenced the decision to have carrier testing significantly.

Individuals in the High risk group were much *more* likely to be tested than individuals in the Low risk group. The result does not correspond with the study by Eggers, et al. (1999) which found that women with a high risk did *not* differ in their testing decisions from those women with lower genetic risks ($\leq 24\%$). The researcher also found that individuals in the Intermediate risk group were *less* likely to have carrier testing than the Low risk group. A study by Chen and Goodson (2007) showed that people with more knowledge are more likely to be tested. The researcher questioned whether or not individuals in the Intermediate risk group had been aware of their potential carrier risk, as their actions did not indicate this.

Eggers, et al. (1999) demonstrated that the sisters of affected individuals did not request more DNA tests than their at-risk aunts, cousins and nieces. In contrast, this study showed that the aunts of affected individuals were much *less* likely to have carrier testing than the

mothers, and the sisters were *twice* as likely to have carrier testing than the mothers. This study also showed that the more distantly related individuals were *less* likely to have carrier testing (although this association was not significant using Chi-square analysis). This finding is in keeping with a study by Varekamp, et al. (1990) who found that more distantly related individuals are less informed about the availability of carrier testing and therefore have less testing.

Individuals with DMD/BMD affected children were a great deal *more* likely to have carrier testing compared to individuals that did not have affected children. This finding is comparable to a study that found at-risk individuals with unaffected children were *less* likely to have carrier testing (Chen & Goodson, 2007). The authors of that study postulated that individuals with unaffected children perceived themselves being at a lower risk. This researcher believes that mothers of affected children also had more testing because they were referred more to the GC clinics by their neurologists and paediatricians (section 4.3, p63).

In this study the researcher found that individuals in the youngest reproductive group (15 – 25 years) were *less* likely to have carrier testing than individuals of older reproductive age (36 – 49 years). Also, the group, 26 – 35 years of age, were *twice* as likely to have carrier testing than the oldest group. In section 3.3.3.4 (p47), it was found that younger individuals were *less* likely to attend GC and Zatz (1983) established that younger individuals were less informed about their risk than the older individuals. This speculation can also explain why younger individuals were less likely to have carrier testing. However, in this study the oldest group of individuals were also *less* likely to have carrier

testing than the age group 26 – 35 years. Although the reproductive ages used were 15 to 49 years of age, the researcher feels that it can safely be assumed that the youngest (15 – 25 years) and oldest (36 – 49 years) groups had fewer individuals that were actively planning a family and that the decision to start a family would influence decisions around carrier testing.

Factors that were investigated for their contribution to the decision to have carrier testing and were shown not to be influential were *ethnicity*, whether the family *mutation was known* or if the mutation was *de novo*. A study by Chen and Goodson (2007) showed that ethnicity influenced decisions surrounding carrier testing and that White individuals were more likely to be tested. This does not correlate with the current study which showed that ethnicity did not significantly influence the decision to have carrier testing.

4.7 Factors influencing the decision to have children

The factors that significantly influenced the decision to have children were *risk*, *relationship* to the proband, *ethnicity*, *de novo mutation* and *age*. The factor whether the family *mutation was known*, did not have a significant influence on the reproductive decision.

One could infer that the *risk* of having an affected child would influence the decision to have children. Studies that examined the relationship between carrier risk and reproductive outcome differ in their findings. In some studies, risk does not influence the decision to have children (Eggers et al., 1999; Tedgård et al., 1999). The study by Tedgård, et al. (1999) on haemophilia carriers reported that the decision to have children did not differ

between at-risk haemophilia carriers and individuals who did not have a risk of being a carrier. Similarly, Eggers, et al. (1999) reported that women with a high reproductive risk for DMD did not differ from individuals at low risk in their decision to have children. However, other studies reported that fewer individuals at high risk of having affected children made the decision to reproduce compared to low risk women (Hutton & Thompson, 1976), and that women at high risk also chose not to have children or deliberately delayed having children (Norman et al., 1989). The findings of the current study did not correlate with the above. The results showed that individuals in the High risk group were *more* likely to have children than the Low risk group. This suggests that risk is not the ultimate deciding factor when it comes to reproduction and that other demographic and/or psychosocial influences can contribute to the decision making process. Two of the most important predictors of reproductive decisions are 1) experience with an affected individual, and 2) the desire to have children (Weil, 2000). Studies have shown that individuals who intended to have children prior to genetic counselling were nearly 29 times *more* likely to have children after genetic counselling than those individuals who did not have the intention or who were unsure of their reproductive plans (Weil, 2000).

Eggers, et al. (1999) found that the sisters of affected individuals did not behave differently in their reproductive decisions compared to their aunts, cousins and more distantly related family members. In contrast, the current study showed that sisters of DMD/ BMD affected individuals were *less* likely to have children than the aunts of affected individuals (result significant) but *more* likely to have children than their cousins and more distant relatives (results not significant). It was previously shown that reproductive behaviours are strongly linked with an individual's experience with an affected person or their sense of disease burden (Sawyer et al., 2006). One can surmise that sisters had more experience with the

everyday reality of the condition and were therefore *less* likely to reproduce than the more distant aunts. Even after multivariate logistic regression (Table 3.6, p55) where all influential factors were taken into account, *relationship* to the affected individual was still significantly linked to the decision to have children ($\chi^2=169.94$).

As expected, the results show that individuals from families with *de novo mutations* were *more* likely to have children than individuals with previous affected individuals in their families. The researcher thinks it probable that most individuals in *de novo* families did not know about their potential carrier risk, did not have previous experience with affected individuals or were most likely not aware of the disease burden. This is supported by Zatz (1983) who reported that risk communication was especially poor in families with *de novo* mutations. Also, Tedgård, et al. (1999) reported that individuals with a family history of haemophilia were more likely to have children in later life. In addition, studies on at-risk haemophilia carriers (Kadir et al., 2000) and confirmed CF carriers (Henneman et al., 2001) reported that a large proportion of individuals chose *not* to have children/have further children, and that their decisions were mainly influenced by not wanting to pass on the defective gene, not wanting to terminate an affected pregnancy and having previous experience with an affected individual.

Other factors that were shown to influence the decision to have children were *ethnicity* and *age*. The results from this study showed that Black individuals were twice as likely to have children and the White individuals were twice *less* likely to have children when they were both compared to Indian individuals. The multiple logistic regression results (Table 3.6, p55) showed that Black individuals were still *more* likely to have children. The result

was not significant ($P=0.058$) but the researcher believes this would become significant with an increase in sample size and shows that *ethnicity* certainly plays a role in reproductive decisions in the South African context. This can also be justified by Statistics South Africa's 2006 mid-year census, that showed the fertility rate of Black females to be 2.92 children per women per year, the fertility rate for Whites, 1.73, and for Indians 1.88 (Statistics South Africa., 2006). The results also showed that younger individuals (15 – 25 years) were *less* likely to reproduce than the older individuals (36 – 49 years).

4.8 Reproductive behaviour of at-risk individuals who knew about their potential risk

In section 4.7, it was discussed that individuals in the High risk group were *more* likely to have children than individuals in the Low risk group. The result also showed that individuals in the Intermediate risk group were *less* likely to have children than the Low risk group individuals. These results did not correlate with other studies and the researcher questioned whether if one knew the potential carrier risk, whether this might affect decisions around reproduction. Of the 237 individuals in the study, knowledge about 46 individuals who were aware of their potential risk was available (Figure 3.11, p60). More individuals in the High risk group that were aware of their potential carrier risks chose to have children than the Low risk group. The difference however, was that more of the High risk individuals who chose to have children, elected to have prenatal testing compared to the Low risk group. The decision to have prenatal testing depends on the presence of a family history and the individual's belief about termination of pregnancy. Individuals in favour of termination of an affected pregnancy are more likely to have prenatal testing (Tedgård et al., 1999). The researcher could not comment on the individuals' decisions to terminate a pregnancy because of the limited information available.

4.9 Limitations of this study

The study was a retrospective, file based study and only the information documented in the genetic counselling files could be used for the study. This resulted in a number of limitations;

- Pedigrees were not always constructed as a three generational pedigree and information mostly on the more distant relatives were inaccurate or incomplete. The pedigrees contained a lot of information on the mothers, sisters and aunts of individuals but decreased with an increase in relationship distance.
- Only 116 individuals of the 237 had documented ages which created a great restriction in the multivariate logistic regression analysis of the data.
- Another limitation to the sample size was that 9 files could not be found and 7 files lacked all the required information.
- The study focussed only on families seen in the Division of Human Genetics at the NHLS/WITS. Some of the at-risk individuals could have been seen at other centres in South Africa. This is evident from the demographic results that described the ethnicities of the at-risk individuals. Although DMD/BMD affects all ethnic groups similarly, no individuals from the Coloured population have been seen at the genetic counselling clinics held from 1995 to 2008, by the Division of Human Genetics, NHLS/WITS. The ethnic groups in this study were thus not representative of the South African population.
- The information in the reproduction section was difficult to obtain from the genetic counselling files;
 - It was difficult to deduce from the counselling file whether individuals had affected children because some children were younger than the age of onset for DMD/BMD.

- Limited information was available on whether individuals were aware of their carrier risks and were mostly available for the at-risk individuals that attended GC.
- Limited information was available on decisions to have more children, prenatal testing, attitudes about termination of pregnancy, and the pregnancy outcomes as the decisions and outcomes were not always followed up.
- A retrospective study in nature is static and can only record decisions made up to a certain point in time. This is a limitation as one would not know what individuals may have decided after receiving genetic counselling, i.e. after receiving their risks, information about the disease, testing and reproductive options. It is known that decisions about reproductive choices are usually dynamic (Sawyer et al., 2006) and change either with new information that becomes available or with the coping strategies of the at-risk individuals and their partners.

4.10 Future recommendations

- The researcher proposes that more awareness to the public and to the medical community should be created with regards to DMD/BMD. Public awareness can be achieved by giving talks to the general community, through radio and television interviews, and publishing articles in the Muscular Dystrophy Foundation of South Africa magazine, as well as medical and nursing publications. To raise awareness of the genetic services in the medical community, talks could be presented especially to general practitioners and gynaecologists for CPD points. These actions may contribute to the referral of more at-risk individuals.
- It is evident from this study that more distant relatives attended *less* GC and had *less* carrier testing. A cascade letter could be introduced with the aim to improve

knowledge about the familial condition, information about risk and the availability of genetic counselling and testing services. Cascade letters are usually given to the individuals that attend GC, and they are asked to forward the letter to their at-risk relatives.

- Individuals that attended GC should be followed up at regular intervals. The researcher feels that continued psychosocial support is important to the at-risk individuals, especially when they have to make difficult decisions regarding carrier testing, children, prenatal testing and termination of pregnancy.
- In the time period 1995 to 2008, the South African Institute for Medical Research (SAIMR)/NHLS molecular laboratory performed ± 526 molecular DMD/BMD tests. From the relatively high number of tests performed and the few families seen for GC, it can be assumed that either individuals and/or doctors are not aware of the GC service provided by the Division of Human Genetics, NHLS/WITS, doctors chose not to refer individuals or individuals were referred but chose not to attend GC. It is thus recommended that the doctors ordering the tests and the individuals tested should be informed and invited to make use of the GC service. This can be achieved by posting information booklets to both doctors and at-risk individuals.

The significant findings of this study and suggestions by the researcher for future research will be summarized in Chapter Five.

5.0 CONCLUSION AND FUTURE RESEARCH

The researcher feels that this study revealed important insights pertaining to genetic counselling attendance, genetic counselling referral sources, knowledge and awareness around GC and testing services. The study also provided additional understanding into what influences individuals' decisions regarding genetic counselling, carrier testing and reproduction.

Of the 237 at-risk individuals in the study, roughly a third attended GC. This could be contributed to a number of factors for example, poor family communication of the information, individuals not knowing that they were at risk, and individuals and/or medical specialists being unaware of the availability of services. GC attendance could therefore be improved with awareness campaigns to both the public and health sectors as well as an improvement in family communication.

Most individuals in this study who attended GC were either referred by neurologists, paediatricians or were self-referred. Individuals also attended GC for reasons regarding affected children or where concerns about a child were raised. Informing gynaecologists and GPs about the genetic counselling and testing services, might shift the reasons for attendance towards family planning and prevention, rather than concerns about affected children. As many individuals were self-referred, it can be speculated that individuals will seek information if they are aware of their risks and that cascade letters might increase the number of self-referrals.

Very few individuals knew the disease causing mutation in their families. This could be attributed to the limitations of the molecular testing methods used until 2008. It can be expected that the newly introduced, MLPA method will increase the number of mutations detected as it is a more efficient and sensitive method and detects mutations in the full *DMD* gene. Knowing the family specific mutation may also increase the number of individuals who would choose to have carrier testing.

The researcher found the following factors influence individuals' decisions regarding GC, carrier testing and reproduction:

- Factors found to influence an individual's decision **to attend GC** were *reproductive risk, relationship* to the affected individual, whether the family mutation was *de novo*, and *age* of the at-risk individual. Individuals with a high reproductive risk, sisters of affected individuals and individuals from *de novo* families were *most* likely to attend GC. Younger individuals and aunts were *least* likely to attend GC.
- Factors found that influenced an individual's decision **to have carrier testing** were *reproductive risk, relationship* to the affected individual, *age* and whether an individual had an *affected child*. Individuals with high reproductive risks, sisters of affected individuals, individuals between 26 and 35 years of age and individuals with affected children were *most* likely to have carrier testing. Individuals *least* likely to have carrier testing were those of the Intermediate risk group (10-24%), aunts and individuals between 15 and 25 years of age.

- Factors found that influenced an individual's decision **to have children** were *reproductive risk*, *relationship* to the affected individual, whether the family mutation was *de novo*, *ethnicity* and *age*. Individuals in the High risk group, aunts of affected individuals, individuals from *de novo* families and Black individuals were *most likely* to have children.

Using multivariate logistic regression to analyse the combined influence of all the different factors on the decisions of at-risk individuals, the researcher found that:

- The most significant factor in an individual's decision **to attend GC**, was the *relationship* of that individual to the affected individual.
- The decision **to have carrier testing** was dependent on the *reproductive risk*, *relationship* to the affected individual and *age*.
- The factor that was the most significant predictor **of reproductive decision** was *relationship* to the affected individual.

Although *reproductive risk* plays a significant role in making decisions regarding GC, carrier testing and reproduction, *relationship* to the affected individuals had the most significant influence on the decisions of individuals after all the other factors were taken into account. This illustrates the importance of family communication in the field of genetic counselling.

A couple of important conclusions of the study and the practical implications thereof are discussed below:

- The study found that decisions are complex and influenced by numerous factors. Decisions regarding GC, carrier testing and reproduction are dynamic and individual. The researcher feels strongly that genetic counsellors should provide continued support and promote informed decision making. Genetic counsellors should focus on individuals' risk perception, coping styles, beliefs, knowledge and experiences to get an idea of what affects their decisions.
- The study also revealed the need for DMD/BMD awareness to the public and medical sector and recommendations were discussed in section 4.10, p74.
- Lastly, this study demonstrated the need to improve risk communication within families. The consensus in the genetic counselling field is that individuals who attended GC and who received risk and disease information should be responsible for conveying the information to the rest of the family and that information should *not* be passed on by the genetic counsellor/clinical geneticist (Gaff et al., 2007). In the systemic review by Gaff, et al. (2007), it was reported that individuals felt it their responsibility to inform the family, but they preferred continuous psychosocial support from their counsellors to facilitate communication. The authors also proposed that there is not a proven approach to improve communication in families but that all families require an individual approach. In summary, genetic counsellors can help improve family communication by providing continued psychosocial support and to explore the dynamics and communication patterns of individual families. The researcher also proposes that it would be ideal if medical doctors ask about familial conditions, identify at-risk individuals in the family and inform individuals at-risk, and also refer them for genetic counselling.

5.1 Future Research

As discussed in the limitations of this study, a retrospective study is very restricted in the amount of information available. Decision-making around GC, carrier testing and whether to have children or not is a complicated, dynamic process that is dependent on a variety of factors, of which personal values and beliefs play important roles. This study was based on information recorded in GC files and the researcher had no insight into the individual decision-making processes and thoughts. The researcher proposes that a prospective study using interviews, should address the qualitative aspect of these issues. Such a study could explore individuals' different coping styles, beliefs, thoughts about disease burden, risk perception and other psychosocial influences that may influence decisions. It could also focus on a small sample that consists of individuals in the process of planning a family, explore individuals' decisions at the time of counselling as well as follow-up on GC, carrier testing and reproductive behaviour.

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Appendix A: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

R14/49 Erasmus

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M080922

PROJECT

Duchenne and Becker Muscular
Dystrophy: Implications for At-Risk
Individual

INVESTIGATORS

Ms S Erasmus

DEPARTMENT

Division of Human Genetics

DATE CONSIDERED

08.09.26

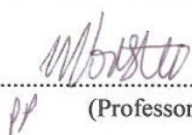
DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.09.29

CHAIRPERSON


(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Ms M Glass

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Data Collection Sheet

Number assigned to at risk individual

Demographics

Age of at risk individual

Ethnicity

Family history of DMD/BMD

Relationship of the at-risk individual to the affected individual

Mother
Sister
Aunt
Cousin
Niece
Other

Clinical diagnosis in family?

DMD
BMD
Unconfirmed

Confirmed with molecular testing?

Yes
No

If no, what was the reason for no results?

What type of mutation?

Deletion
Duplication
Point mutation
Unknown

Genetic Counselling

Did the at-risk individual attend genetic counselling?

Yes
No

If yes, What was the reason for attending genetic counselling?

Affected boy
Concern about boy
Family planning
Prenatal
Other

If other, what was the reason?

If yes, Who referred the at-risk individual?

Self referral
Relative
GP
Gynaecologist
Paediatrician
Other

If other, who referred the at-risk individual?

If yes, How many follow -up genetic counselling sessions did the at-risk individual have?

If yes, What was the reason for follow-up consultation

Results
Family Planning
Prenatal testing
Postnatal testing
Other

If other, what was the reason?

Risks

How many at-risk individuals in the family?

Is the family mutation *de novo*?

Yes
No

The family pedigree of the at-risk individual

--

Risk calculation

--

Assigned carrier risk

Reproductive risk

Carrier testing

Did the at-risk individual pursue genetic testing?

Yes

No

Results obtained?

Yes

No

If no, what was the reason for no results?

Reproduction

High, low or intermediate reproductive risk?

High (>25%)

Intermediate (10-24%)

Low (0-9%)

Did the at-risk individual have any children?

Yes

No

If yes, how many children did the at-risk individual have?

Does the at-risk individual have affected DMD/BMD children?

Yes

No

Did the individual know that she was at risk?

Yes

No

Unknown

If yes, Did the at-risk individual choose to have/have more children?

Yes

No

Delayed decision

Unknown

If yes, Did the at-risk individual pursue antenatal testing?

Yes

No

Would the at-risk individual terminate an affected pregnancy?

Yes

No

Unknown

The outcome of the pregnancy?

Affected boy

Unaffected boy

Daughter

Terminated