CARRIER SCREENING FOR DUCHENNE MUSCULAR DYSTROPHY

INTRODUCTION

Duchenne Muscular Dystrophy (DMD), an X-linked condition, is the most common muscular dystrophy in children and affects families of all ethnicities. Approximately 2/3 of clinically diagnosed cases of DMD are attributable to a carrier mother, who is likely unaware that she is a carrier. In addition to providing information about reproductive risks, carrier screening can identify women who are, themselves, at risk of health effects caused by defects in the DMD gene. The first commercial broad population carrier screening program available has already, in its first several months, shown to be effective in identifying carriers of mutations in the DMD gene, both in women with and without family history.

Although to date there have not been screening guidelines for DMD, the condition aligns with recommendations made in a 2015 Joint Statement by The American College of Medical Genetics and Genomics, The American Congress of Obstetricians and Gynecologists, the National Society of Genetic Counselors, the Perinatal Quality Foundation, and the Society for Maternal-Fetal Medicine regarding expanded carrier screening in reproductive medicine. These recommendations state that conditions for which there is cognitive disability, a need for medical intervention, and that have an effect on quality of life should have screening available.

THE DMD (DYSTROPHIN) GENE

The DMD gene, which encodes the protein dystrophin, is located on the X chromosome (Xp21.2-p21.1) and is the largest protein-coding gene (FIGURE 1). Dystrophin is essential to the integrity of muscle fibers; it is part of a protein complex that provides protection to the muscles over time. Dystrophin helps to connect muscle cells to surrounding proteins, and may help to facilitate cell signaling between muscle cells. Mutations in the gene lead to an absence or decreased expression of dystrophin, causing progressive damage to muscle cells. Mutations are associated with Duchenne and Becker Muscular Dystrophy as well as DMD-associated dilated cardiomyopathy.

FIGURE 1: THE DMD GENE
**DMD Incidence and Phenotype**

DMD affects approximately 1/3500 male births. DMD has a similar male population incidence to Fragile X Syndrome (1/3600 males)\(^3\) and to Cystic Fibrosis (1/3700 births)\(^4\) (FIGURE 2).

![Figure 2: Incidence per 100,000 Male Births](image)

Boys with DMD present in early childhood with delayed milestones, such as sitting and standing. There is progressive symmetrical proximal muscle weakness and atrophy, calf hypertrophy, persistent toe walking, scoliosis, and an unsteady gait. Boys with DMD demonstrate a positive Gowers' sign, a maneuver used by individuals with proximal muscle weakness to rise from a seated position. Blood creatine kinase (CK) is elevated. Cognitive impairment is seen in varying degrees\(^5\), in particular related to verbal working memory skills\(^6\). The incidence of attention deficit hyperactivity disorder and autism spectrum disorders are increased in this population\(^6\). Boys with DMD are typically wheelchair dependent by approximately age 12. Cardiomyopathy has clinical onset in the early teen years or younger, is present in patients in the second decade of life, and is often the cause of death in early adulthood, along with respiratory failure\(^7\).

Current treatments for DMD are limited, but include supportive therapies and corticosteroid therapy to improve muscle strength and respiratory function and to prevent scoliosis\(^8\). There are several gene therapies under investigation, including Ataluren to promote ribosomal read-through of stop codons, and antisense oligonucleotides that induce exon skipping, which increases the expression of dystrophin and reduces the severity of disease\(^6\).

**Becker Muscular Dystrophy**

Becker Muscular Dystrophy (BMD) is similar to DMD, and is less prevalent, affecting approximately 1/20,000 - 1/30,000 males. Clinical features are generally similar to DMD; however, they are later in onset and are frequently less severe. Wheelchair dependency occurs after age 16, and the neck flexor muscle strength is generally preserved, which differentiates it from DMD. Very mild cases include men who remain ambulatory even into their 60s\(^8\). Cardiomyopathy is a common cause of morbidity and mortality in BMD, with the mean age of death being in the mid-40s\(^9\).

**Inheritance Patterns**

Females have two copies of the DMD gene, one on each X-chromosome, and so DMD and related conditions are inherited in an X-linked pattern. A mutation in one copy of the DMD gene is generally not sufficient to cause disease (although carrier women may be at risk of some health effects, see next page). If a woman is a carrier of a mutation in the DMD gene, there is a 50% chance in each pregnancy that the fetus will inherit the DMD gene mutation. If the fetus is female, there is a 50% chance that she will be a carrier and if male a 50% chance that he will be affected (FIGURE 3).
Given the DMD gene’s large size of 79 exons, it is vulnerable to mutations. Approximately 60-70% of mutations causing DMD are deletions of one or more exons, and approximately 5-7% are duplications. Most of the remainder of patients have point mutations within the coding region of the gene, although approximately 7% do not (FIGURE 4).

In boys diagnosed with DMD who have no family history of the condition, approximately 67% of the mothers are subsequently found to be carriers. Because diagnosis is often delayed, and boys do not receive a definitive diagnosis until approximately age 5, these women often have more children without knowing they carry a DMD mutation. Approximately 33% of cases of DMD are de novo, i.e., having occurred in the affected male child, and not inherited from a carrier mother (FIGURE 5). These cases cannot be picked up by carrier screening of the mother. Of the apparently de novo cases, some are due to germline mosaicism in the mother.
**FEMALE CARRIERS**

Some female carriers may exhibit symptoms that range in severity. Up to 20% of carriers may have some degree of muscle weakness, ranging from mild to moderate. Approximately 8-10% of carriers are reported to have dilated cardiomyopathy that is progressive. Historically, skewed X-inactivation was the generally accepted mechanism for the development of symptoms in carriers, although newer studies have shown this is not necessarily the case, and the reason for variability in symptoms among carriers is not well known. In rare cases, presentation in carrier females can be very severe.

The American Academy of Pediatrics provides recommendations for education and surveillance for female carriers of DMD-related conditions. Recommendations include education about the signs and symptoms of heart failure, and that complete cardiac evaluations by a specialist begin in late adolescence or early adulthood, and be performed at least every five years. Treatment recommendations for symptomatic women are similar to recommendations for affected males. Carrier females should consider being evaluated for cardiac symptoms during pregnancy, and symptomatic women should be followed closely by a high-risk obstetrician.

**TESTING TECHNOLOGY**

With the introduction of next generation sequencing, the ability to investigate large genes such as the DMD gene (79 exons) has become more time and cost effective. Techniques for testing for deletions and duplications have also improved in recent years, making high-throughput population-based screening possible. To detect deletions and duplications, two effective techniques are used: qPCR, which amplifies and quantifies a region of DNA, and multiplex ligation-dependent probe amplification (MLPA), a similar technique to qPCR, which uses fluorescence to determine the relative quantity of DNA sequence. These techniques—next generation sequencing, qPCR, and MLPA—allow for a >90% detection rate for deletions, duplications, and point mutations in the DMD gene. Population-based carrier screening can identify >90% of women who are carriers of a mutation in the DMD gene, and therefore, at risk of having a child with DMD (FIGURE 6).

**FIGURE 6: DMD GENE IN AFFECTED MALES AND FEMALE CARRIERS**

![DMD Gene in Affected Males and Female Carriers](image)
DMD SCREENING RESULTS TO DATE

Data are now available from the initial seven months of the first commercial population-based, pan-ethnic carrier screening program for DMD/BMD. These data show the effectiveness of the program in identifying carriers of DMD/BMD, both with and without family history of the condition.

INCIDENCE IN THE SCREENED POPULATION

As of February 19, 2016, carrier screening of the DMD gene was performed in approximately 22,500 women. The number of positive results to date is 34, establishing an incidence of DMD mutation carriers of approximately 1/667 in the population screened to date (TABLE 1).

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Total Screened</th>
<th>Number of Positive Results</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>8562</td>
<td>14</td>
<td>1/625</td>
</tr>
<tr>
<td>African American</td>
<td>3445</td>
<td>6</td>
<td>1/588</td>
</tr>
<tr>
<td>Southeast Asian</td>
<td>557</td>
<td>4</td>
<td>1/139</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4037</td>
<td>4</td>
<td>1/1111*</td>
</tr>
<tr>
<td>East Asian</td>
<td>780</td>
<td>1</td>
<td>1/769</td>
</tr>
<tr>
<td>Mixed/Other</td>
<td>1876</td>
<td>2</td>
<td>1/909</td>
</tr>
<tr>
<td>Ethnicity not reported</td>
<td>3244</td>
<td>3</td>
<td>1/1111</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>22,501</strong></td>
<td><strong>34</strong></td>
<td><strong>1/667</strong></td>
</tr>
</tbody>
</table>

*155 individuals reported Hispanic as part of their mixed ethnic descent, so this may help to explain the lower than expected incidence in the Hispanic population

FEATURES OF THE SCREENED POSITIVE COHORT

Among the 34 positive results, fourteen (41.2%) reported Caucasian ethnicity, six (19.3%) reported African American ethnicity, four (11.8%) reported Southeast Asian ethnicity, four reported Hispanic ethnicity (11.8%), two (5.9%) reported mixed or other (in this case, mixed Ashkenazi Jewish/Northern European), three (8.8%) did not report ethnicity, and one (2.9%) reported East Asian ethnicity (FIGURE 7).

FIGURE 7: REPORTED ETHNIC BACKGROUNDS OF 34 INDIVIDUALS WITH POSITIVE RESULTS
Nineteen (56.9%) of the mutations detected were found to be deletions or duplications, which is lower than expected given that more than 2/3 of the mutations in the population of DMD/BMD-affected individuals are deletions/duplications. Of those nineteen, five had a reported family history of DMD. The higher than expected rate of single nucleotide variants may be due to the utilization of next-generation sequencing. As more carriers are identified, it is possible that the distribution will more closely reflect published data (TABLE 2).

**TABLE 2: TYPES OF MUTATIONS WERE DETECTED IN 34 INDIVIDUALS WITH POSITIVE RESULTS**

<table>
<thead>
<tr>
<th>MUTATION TYPE</th>
<th>NUMBER</th>
<th>KNOWN FAMILY HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Duplication</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Premature Stop Codon</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Frameshift variant</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Splice Site Variant</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Fifteen of those who received positive results reported that they did not have any family history of a genetic disease. Nine individuals reported that they did have a family history of a DMD/BMD, either diagnosed in a family member, or family member was known to be a carrier. Two individuals reported family history of a genetic disease but did not specify which disease, or indicated a family history of a disease other than DMD/BMD. The remaining eight individuals did not report whether or not they had a family history of DMD/BMD (TABLE 3).

**TABLE 3: REPORTED FAMILY HISTORY OF DMD OF 34 INDIVIDUALS WITH POSITIVE RESULTS**

![Graph showing family history distribution](image)

Of those women with a positive result, 25 were reported to be pregnant, 8 were not pregnant, and pregnancy status was not reported for the remaining individual (TABLE 4).

**TABLE 4: PREGNANCY STATUS OF 34 INDIVIDUALS WITH POSITIVE RESULTS**

![Graph showing pregnancy status distribution](image)
SUMMARY OF DATA TO DATE

Twenty-five of the women screened were reported to be already pregnant at the time of screening. Mutations were seen across ethnic groups. There was a higher than expected number of single nucleotide variants, for which next generation sequencing may be an explanation.

For individuals with and without a family history of DMD/BMD, positive screen results allow for a range of reproductive options including pre-implantation genetic diagnosis (PGD) and prenatal diagnosis. In addition, those women found to be carriers can now engage in increased surveillance of their own health issues, for example, screening for cardiomyopathy.

A follow up study of those screened for DMD/BMD is currently underway. This study investigates the experience of individuals who received positive DMD carrier screening results. Information about personal and family health history, as well as pregnancy outcome data will also be collected as part of this study.

CONCLUSION

DMD, a severe, early onset disease, and its related conditions, are relatively common in the population. Historically, broad population DMD carrier screening was unavailable and many women who have children with DMD are unknowingly carriers of the condition. Carriers of DMD are also at risk of health problems, so carrier screening can be beneficial for a woman’s own healthcare by allowing the proactive monitoring of symptoms.

With newer technologies and reduced costs, DMD carrier screening makes it possible to detect >90% of carriers in advance of having an affected child. The recent data from broad population carrier screening for DMD outlined in this paper helps demonstrate the benefits of offering DMD carrier screening to individuals of all ethnicities with and without a family history.
REFERENCES


2. Edwards, Janice G. MS; Feldman, Gerald MD, PhD; Goldberg, James MD; Gregg, Anthony R. MD; Norton, Mary E. MD; Rose, Nancy C. MD; Schneider, Adele MD; Stol, Katie MS; Waagner, Ronald MD; Watson, Michael S. MD Expanded Carrier Screening in Reproductive Medicine—Points to Consider: A Joint Statement of the American College of Medical Genetics and Genomics. American College of Obstetricians and Gynecologists. National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine Obstetrics & Gynecology: March 2015 - Volume 125 - Issue 3 - p 653–662


