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Meeting Report

Parent Project Muscular Dystrophy Females with Dystrophinopathy Conference, Orlando, Florida June 26 – June 27, 2019

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INTRODUCTION

Duchenne muscular dystrophy (DMD), an X-linked recessive condition that results from mutations in the DMD gene encoding the dystrophin protein. DMD occurs in approximately 1 in 5000 live male births. While one-third of mutations are spontaneous in nature, two-thirds are inherited. Although elevations in serum CK are common in obligate female carriers in DMD, occurring in around two-thirds [1], most female carriers of *DMD* mutations remain clinically asymptomatic. Few studies have addressed the prevalence of symptoms in this population, as reviewed elsewhere [2], but estimates of symptomatic weakness in carriers range from 1.7–22 percent [3–7]. The weakness may differ in severity and distribution, resulting in phenotypes ranging from a Duchenne-like progression to a milder Becker-like course, with

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an age of symptom onset ranging from 1 to 50 years in two cohort studies [3, 5].

Although skeletal muscle weakness has historically often been used as the defining feature of such "manifesting carriers," they typically can have a mosaic pattern of dystrophin expression in both skeletal and cardiac muscle, with histopathologic lesions of myofiber necrosis and fibrosis typically seen in DMD. Symptoms of skeletal muscle and cardiac disease are seen together in some carriers [8], while others may show evidence of cardiomyopathy alone [9]. Several past studies have examined the cardiac involvement of females with both Duchenne and Becker dystrophin mutations [10–12], but surveillance recommendations based upon expert consensus have been no more specific than suggesting cardiac screening every five years [13, 14].

The females with dystrophinopathy conference (aka,"manifesting carrier pre-conference") was convened June 26-27, 2019 in Orlando, FL, prior to the start of the annual Parent Project Muscular Dystrophy (PPMD) Annual Conference. The goal of this conference was to initiate a dialogue that would ultimately lead to improved care for all individuals living with DMD, including females with dystrophinopathy (FD), more commonly termed "manifesting carriers." Participants in the conference included women with symptoms of a dystrophinopathy or having girls with symptoms, physicians from a variety of medical specialties who have an expertise in the care of boys and men with DMD/BMD, and basic science researchers in the field of dystrophinopathies

A specific aim was to define care guidelines for the diagnosis and treatment of FD at any age and disease severity. Pat Furlong, president and CEO of PPMD, opened the discussion as to how, in the absence of data, we can accomplish these goals.

DEFINING TERMS

The term "carrier" was discussed at length throughout the conference with an attempt to come to a consensus on the most appropriate term to use in future clinical settings and in research. The term "female with dystrophinopathy (FD)" was proposed to describe *all* females who carry a heterozygous pathogenic variant in the dystrophin gene, regardless of their degree of symptom manifestation.

Many of the working group considered the term FD to be appropriate, pointing out as one example that the actual lifetime risk of cardiac dysfunction is still under study, and that the term FD is useful in highlighting both the risk and the need for special attention to this population. Several in the working group considered that because many women carrying pathogenic variants never develop symptoms or signs of skeletal and/or cardiac dysfunction, the term "carrier" remained a useful term for females who have a genetic mutation but no manifesting symptoms, and FD a more accurate designation of females with DMD-related symptoms (equivalent to the historical usage of "manifesting carriers"). Some participants expressed concern that the widespread adoption of term "FD" for carriers without manifesting symptoms has the potential to establish a lifelong label associated with significant condition for females who might never develop signs or symptoms, with unknown social, emotional, and even financial impacts. The adult symptomatic women in the group felt that the term "carrier" did not accurately describe either their genotype or phenotype and that the use of this term may delay the diagnosis and treatment of conditions associated with DMD/BMD. They advocated for the use of FD to assure that appropriate monitoring and care was received. A consensus was not reached by the participants, and all agreed that further conversations, including assuring that the voice of the patient is included, is important. For the purposes of this paper, the term FD represents a female who is manifesting symptoms of dystrophinopathy.

Session 1: The patient and family experience

Marsha, mother to a daughter with FD, illuminated the lack of awareness from pediatric healthcare providers about the potential diagnosis of FD. Her daughter's most prominent symptom was frequent falls at school observed by her teacher who recommended a neurology evaluation. Initial visits with the primary care provider (PCP) and otolaryngology (ENT) doctors were non-diagnostic and the providers were not concerned that there was a significant problem; it was only upon visiting a neurologist that testing for FD was initiated. Marsha also related the hurdles faced getting genetic testing approved through her insurance provider.

Deb is a mother who described similar struggles in receiving a diagnosis. Deb has a family history of DMD and recognized the Gower's maneuver when her twin daughters were 2 years old. Despite obvious muscle weakness, it took an additional two years to have her daughters receive genetic testing and a diagnosis. Although the twins are believed to be identical,

the severity of their phenotype differs - one with mild weakness and fatigue and one with more significant weakness and developmental delays. Deb expressed frustrations with the current clinical trial landscape in DMD with a lack of clinical trial availability for girls with a severe phenotype.

Jen is an adult with FD who began to exhibit weakness in her 20 s. At the time of her brother's diagnosis of DMD, doctors opted to examine creatine kinase (CK) levels on her entire family and found hers to be high. She had classic calf hypertrophy as a child with some fatigue but was not limited in activity. Jen was able to have genetic testing in 2011 which confirmed her FD status. Following childbirth, Jen required assistance carrying her child and cannot currently climb stairs unassisted.

Elizabeth is an adult with FD who is non-ambulatory, diagnosed at age 11. Several of her first-degree relatives share the same genetic mutation though other affected women in her family do not manifest symptoms of skeletal muscle weakness. Elizabeth began using a wheelchair at age 14, and began using glucocorticoids for symptoms at age 17, but eventually required a reduction in the dosage to lessen side-effects. She expressed concerns about steroid use in females as well as the chronic use prescribed for boys with DMD. Elizabeth also shared a general lack of medical advice she has received from physicians throughout her life living with FD, noting she has always been the "expert."

All these case presentations demonstrate a lack of understanding and recognition of DMD in the primary care setting, as well as a significant lack of understanding of manifesting symptoms in FD. In addition, the variability of FD manifestations, even amongst family members with an identical diagnosis, further complicates identification and referral.

In addition, several case studies were presented by clinicians caring for FD children in their practices (Drs. Susan Apkon, Vamshi Rao, Brenda Wong and Mena Scavina). These cases included:

- Patient 1: A 10-year-old girl presented at age 5 to orthopedics for a gait abnormality. Given a family history of DMD (maternal cousin), the CK level was examined and was found markedly elevated with subsequent genetic testing confirming the diagnosis of FD. She walked at 20 months of age and had delayed speech development.
- Patient 2: An 8-year-old girl presented at age 3 years with a viral illness and elevated liver enzymes. A CK was obtained and was also elevated. Given a family history of DMD (brother and maternal cousin), genetic testing was obtained which confirmed a mutation in the dystrophin gene. She walked at 15 months and presents now with cognitive delays, attention deficit hyperactivity disorder (ADHD), and dyslexia.
- Patient 3: A 10-year-old girl presented at age 6 with an elevated CK during a hospitalization for influenza. She had a history of sensitivity with food texture and other minor sensory issues and dark urine. Her physical examination revealed and calf pseudohypertrophy and a Gower's maneuver. Her family history was remarkable for her mother having a history of falls and as a child difficulty keeping up with her peers and a maternal grandmother with poor endurance. These findings led to full exome sequencing which confirmed the diagnosis of FD.
- Patient 4: A 19-year-old girl presented at age 5 with sensory issues, speech delay, and delayed potty-training. Laboratory testing demonstrated elevated transaminases which led to a liver biopsy, which was normal. Follow-up testing included a CK which was markedly elevated prompting a referral to neurology. On the initial examination, this patient had calf pseudohypertrophy, toe-walking and a Gower's maneuver. Genetic testing was obtained and confirmed a specific mutation in the dystrophin gene.
- Patient 5: A 7-year-old girl who initially presented at age 3 with elevated CK level in the 1700–2400 U/L range obtained as part of a work-up for gastrointestinal issues related to constipation. A neurological assessment revealed mild muscle weakness and a modified Gower's maneuver. Muscle biopsy showed a mosaic pattern of dystrophin expression consistent with a dystrophinopathy. Genetic testing demonstrated a deletion amenable to exon 51 skipping and an X-inactivation ratio of 54:46. A follow-up CK obtained at age 7 following a viral illness and changes in mood, behavior, and motor function revealed a CK in the 10,000 range.

Session 2: The clinician perspective – Diagnosis–

Genetic testing

Stanley Nelson, MD, UCLA, gave a presentation on X-inactivation, covering the biological mechanism, its importance in aiding discovery of the Duchenne gene location, and testing methodologies [15]. An important group of females fully

manifesting a Duchenne phenotype were reported between Xp21 and an autosome, and de novo balanced translocations should be considered in isolated females manifesting Duchenne muscular dystrophy [16]. For female relatives of males affected by DMD with the same mutations within the DMD locus, X-inactivation is often cited as a factor in the prognosis of disease severity of FD, however the standard methodology analyzing blood for X-inactivation is insufficient in FD [17, 18]. Because dystrophin expression varies between muscles in FD [19], multiple muscles would need to be assessed for X-inactivation skewing ratios and ideally validated with biopsies to truly assess dystrophin expression. Dr. Nelson expressed that the X-linked inactivation test should not be relied on. Muscle biopsy (preferably from the vastus lateralis) should be obtained and tested for dystrophin expression when indicated.

During the case presentations there were several themes that emerged which require additional exploration. The consistent point throughout the cases was a delay in the diagnosis of a dystrophinopathy with several girls presenting with delays in speech or elevated transaminases obtained for incidental reasons, something that is commonly seen in boys diagnosed with a dystrophinopathy [20]. Providers shared that the lack of a standard approach to the monitoring and treatment of a FD can lead to under or over monitoring and treatment of conditions associated with the dystrophinopathy. Given the variability of the clinical cases that were presented all participants agreed for a need to further characterize the spectrum of FD phenotype. The hope is that with better characterization and earlier detection, this will allow for earlier and better treatment. Participants agreed that while there are FDA approved drugs to treat specific genetic mutations, females with dystrophinopathy were not included in the basic science research or clinical trials that led to the approval of such drugs. While FD could have access to approved drugs when their genotype and phenotype fit criteria, extreme caution needs to be used.

Session 3: The clinician perspective – Cardiology

May Ling Mah, MD, Nationwide Children's Hospital, provided an overview of the current literature regarding incidence of cardiomyopathy in FD. The prevalence of cardiomyopathy appears to be impacted by the imaging modality, with ranges from 5% to 100% of FD having cardiac abnormalities. Clinical testing of the women participating in the Nationwide Carrier, demonstrated that changes in cardiac function and strain on MRI preceded changes in function seen with echo, validating the importance of MRI in this patient population.

Dr. Mah reported that, in her clinic, she has seen improvement in cardiac function with implementation of heart failure treatment. She reiterated that fibrosis, rather than function, is a marker of disease progression. Her recommendations for adult FD include MRI with LGE starting at the 3rd decade, and no restriction for exercise.

Session 4: What are we learning – Animal models

Dongsheng Duan, PhD, University of Missouri, presented on both the mouse and dog FD models [21]. Dr. Duan gave an overview of the mdx mouse and heterozygous models [22, 23]. The carrier mouse diaphragm shows uniform strong dystrophin expression at terminal age (21-m-old). Consistently, no histological lesions such as fibrosis, degeneration and inflammation were detected in the diaphragm. The carrier mouse heart shows a mosaic pattern of dystrophin expression from 3 to 21 months. Specifically, 50% of cardiomyocytes express dystrophin at the wild-type level with the remaining 50% cardiomyocytes not expressing dystrophin but showing utrophin upregulation. Despite mosaic expression, the carrier mouse heart shows normal histology and anatomy. Furthermore, cardiac function (ECG and hemodynamics) is completely normal in the carrier mice. Ultimately, for FD, the mouse is not an ideal model due to the carrier mouse having no skeletal muscle or heart involvement. Dr. Duan also highlighted the phenotypic differences between male and female mdx mice. Male mdx mice show more severe skeletal muscle disease while female mdx mice show more severe cardiomyopathy [24, 25]. This suggests that sex hormone may play a role.

Dr. Duan highlighted the carrier dog as a potential model. The carrier dog shows elevated CK at birth and a reduced growth rate during the 1st week [26]. The newborn carrier dog has near-uniform dystrophin expression in skeletal muscle although the level is much lower than that of the normal puppy. In the heart, the newborn carrier dog shows a mosaic pattern of dystrophin expression. Dr. Duan commented that he expects that boys receiving gene transfer therapy may have a similar mosaic dystrophin distribution in the heart. Therefore, lessons learned from the carrier dog may be relevant for studies beyond FD. Aged carrier dogs show progressive decline of heart function

as measured by fraction shortening using echocardiography [27].

Lack of cardiac tissue samples is a major challenge in studying FD in human patients. This limits in-depth comprehensive cellular and molecular studies. In light of the pathological and clinical similarities between human patients and affected dogs, University of Missouri has begun to develop a canine (normal, carrier and affected) tissue repository. This resource will be open to the entire Duchenne research community.

Joe Kornegay, DVM, PhD, Texas A & M, provided background for the golden retriever muscular dystrophy (GRMD) dog model and its potential uses in FD studies. Dr. Kornegay shared the foundational work on canine X muscular dystrophy (CXMD) dogs from Barry J. Cooper at Cornell University [28]. Dr. Cooper's work demonstrates that dystrophin expression is mosaic at a young age in both the skeletal and cardiac muscle in CXMD. As the animal matures to 24 weeks, the human equivalent of $\sim$ 10 years, the skeletal muscle expression is indistinguishable from normal. The cardiac expression remains mosaic throughout the lifespan. Dr. Kornegay shared his research on cardiomyopathy in GRMD and CXMD dogs. The work presented highlighted the potential use of CXMD to model pathology [29] of cardiomyopathy and acute cardiac death [30] analogous to disease progression in DMD. This model could also inform biomarker selection and identify potential risk factors [31]

Day 2

Opening discussions were aimed at closing out the animal model discussion from the previous afternoon. The group felt that gathering longitudinal natural history on dystrophic/carrier dogs would be beneficial, beginning with young animals, and collecting serum biomarkers, correlating MRI/echo data, and, potentially, obtaining endocardial biopsies. X-inactivation has not been studied in the GRMD/CXMD dog model and would likely present similar challenges in dogs as it does in humans, and would require assessment of X inactivation in muscle over years of life. Effects of exercise on the cardiac phenotype also has not been established in dogs.

Session 5: Care Implications

Kevin Flanigan, MD, Nationwide Children's Hospital, shared data collected through the United Dystrophinopathy Project and published in 2010 [6]. The publication reported on 15 manifesting carrier, who presented with muscle weakness by manual muscle testing or evidence of a dilated cardiomyopathy. X-inactivation was performed in all patients, identifying 2 women with 100:0 skewing and 5 additional women with non-random X-inactivation as defined as 80:20 ratio. Dr. Flannigan emphasized the value of biopsies, given that altered dystrophin expression in muscle may be seen even in the absence of X-inactivation in lymphocytes, as was demonstrated among the cohort he described. Compound heterozygotes may be missed as current genetic testing is focused on finding duplications or deletions.

In response to a question as to whether girls with symptomatic dystrophinopathy should be treated with steroids, Dr. Flannigan commented that in this context he recommends corticosteroid treatment analogous to males with dystrophinopathy. Those girls who present similarly to boys with DMD would likely be treated with steroids, whereas a girl with milder phenotype would be treated like a boy with Becker, for whom steroids are not typically recommended. The clinical use of this approach has been observed among females compiled in at least one national cohort [32]. He recommended that if girls present with a milder phenotype, evaluating the biopsy for dystrophin levels and degree of mosaicism may provide a guide for how aggressive the course of treatment needs to be, as even low levels of dystrophin expression may ameliorate disease severity and enter into a family's risk/benefit analysis for lifetime steroid use [33].

Linda Cripe, MD, Nationwide Children's Hospital, presented on cardiac risk in FD. Dr. Cripe regards adult FD as similar to BMD, a group that may manifest no or mild skeletal muscle weakness, but may be at risk to develop a clinically significant cardiomyopathy. She emphasized the need for natural history studies in order to identify who may be at risk and at what age we surveillance should be initiated.

Jamie L. Jackson, PhD, Nationwide Children's Hospital, presented preliminary psychosocial findings from the Carrier Study at Nationwide Children's Hospital in the context of the limited studies that have evaluated boys [34] with Duchenne or women with dystrophinopathy [35, 36]. She then discussed the psychosocial care considerations among FD in the overlapping realms of screening, treatment, and research. For screening, Dr. Jackson discussed considerations for who would administer screeners, which screeners to use (number of items, psychometric validity, cost, and availability of cutoff scores)

and the response to a positive screening (referral resources). Several screening tools were reviewed across multiple domains, such as anxiety and depressive symptoms (e.g., Patient Health Questionnaire [37] and Hospital Anxiety and Depression Scale) [38], obsessive-compulsive disorder (OCD), ADHD, and cognitive deficits. Next, Dr. Jackson discussed treatment options for FD who are reporting significant psychosocial concerns, including psychotherapy ("talk therapy") and medication for anxiety and depressive symptoms, OCD e.g., Obsessive Compulsive Inventory [39], ADHD (e.g., Conner's Adult ADHD Rating Scales [40]) and cognitive concerns. Lastly, Dr. Jackson reviewed considerations for assessing psychosocial concerns for research, including how to select questionnaires (i.e., age-appropriateness, self-report vs. other-report, and using instruments that have a suicidality item) and cognitive assessment (i.e., screeners vs. a neuropsychological battery, personnel needs, timing of testing, and theory-driven approach to instrument selection). She ended by highlighting the need for multidisciplinary research and care teams to span across these domains of psychosocial assessment.

Session 6: What do we do now?

A lengthy discussion was held around the necessity and ethics of performing carrier testing before a female can legally provide informed consent (typically at age 18). Questions were raised as to whether the psychological burden of genetic testing was worthwhile when there are limited actionable treatments and the severity of FD varies greatly. The American College of Medical Genetics and American Society of Human Genetics, currently, does not advocate for carrier testing in persons under age 18 years [41, 42]. However, this assertion has been challenged with some advocating for earlier screening, especially in the setting of those who may have symptoms [43].

Ensuring adequate time is given to education of the family was deemed important. The point was made that this education should be done outside of the initial visit of a newly diagnosed boy, so that the focus of that visit can be on the newly diagnosed child. Scheduling an additional visit would enable interaction with all, or more, female members of the family.

The following are a list of actionable items and care considerations that were discussed by the working group for FD. The clinicians stressed that at this time there is not enough data available to implement these items as guidelines. A significant portion of the discussion focused on trying to define the appropriate time for genetic testing for FD.

Next Steps:

- A patient preference survey should be conducted to evaluate how the patient population views the value of early genetic testing in at-risk females. Questions should include, but not limited to the following:
- Would females with dystrophinopathy (FD) want to have known earlier?
- Do parents want to know if their daughter may have dystrophinopathy and at what age?

Clinical Care

In order to help provide some guidance for clinicians and families, the following set of considerations were made for pre- and post-genotyping of FD.

Pre-Genotype

- Ensure that adequate time is spent by the physician or care team explaining the potential likelihood and risks of FD to the patient, her family and all first-degree females of a male patient diagnosed with a dystrophinopathy (Duchenne or Becker).
- For first-degree female relatives, the physician should gather history on learning behavior, cognitive issues, strength, balance and fatigue.
- If possible, this information should be gathered without minor female(s) present
- Should symptoms be identified through the history or on physical examination, CK testing along with genetic testing should be performed.
- Educate medical professionals involved in the diagnosis of children with speech delays or autism spectrum disorder that FD exists and a CK can be used as a screening tool to assure early diagnosis.

Confirmed FD by Genetic Testing

- Those females who are experiencing symptoms such as, fatigue, weakness, or cognitive disability, should be seen every 6 months by a neurologist, physical therapist, and cardiologist.
- Cardiac evaluation should follow guidelines previously established for individuals with DMD/BMD and preferentially include non-invasive imaging by cardiac MRI (using gadolinium contrast), as cardiac changes by MRI have been shown to precede those seen by echo. Cardiac

evaluation should be in place for all genetically at-risk individuals whether they have skeletal muscle or cognitive symptoms or not. Follow-up imaging, if normal, is recommended every 3 years. Abnormal imaging is repeated based on cardiology guidance for cardiomyopathy.

- CK may be a useful, but imperfect and highly variable measure, in assessing disease severity in FD. Repeat collection of CK at multiple visits was discussed as a starting point for defining the disease natural history and gathering more systematic data on this patient population. The patient's baseline CK should also be established so acute changes can be better monitored.

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DISCLOSURE

Author DD is a member of the scientific advisory board for Solid Biosciences and equity holders of Solid Biosciences.

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