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A Patient-Report Registry to Study Outcomes in a Rare Genetic Disease: DuchenneConnect

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A Patient-Report Registry to Study Outcomes in a Rare Genetic Disease: DuchenneConnect

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by

Cheri Alissa Silverstein

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ABSTRACT OF THE THESIS

A Patient-Report Registry to Study Outcomes
In a Rare Genetic Disease: DuchenneConnect

by

Cheri Silverstein

Master of Science in Clinical Research
University of California, Los Angeles, 2013
Professor Robert Elashoff, Chair

Background*:* Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy, but it is a rare disease, creating challenges for study design. Randomized trials have shown that steroids improve surrogate endpoints of skeletal muscle function, but many treatment questions lack robust data and necessitate innovative approaches. **Methods***:* DuchenneConnect is a novel patient-report registry and the largest US-based registry for DMD. We compared time from birth to loss of ambulation among three categories of steroid use: current, past and never. To control for possible confounding, we used a Cox proportional hazards model to estimate hazard ratios according to steroid use. **Results***:* Current steroid use is associated with longer time to fulltime wheelchair use in this population (HR 0.32, $p < 0.0001$). **Conclusion***:* A self-report registry can efficiently amass a large population for long-term follow-up and provides a useful adjunct to randomized trials in the study of a rare genetic disease.

The thesis of Cheri Alissa Silverstein is approved.

Katrina Dipple

Stanley Nelson

Ning Li

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University of California, Los Angeles

2013

This thesis is dedicated to my sweet daughter, Chloe Dannah Fadlon, who entered this world right in the middle of studies and to my loving husband, Yariv Fadlon, who commuted back and forth 2000 miles each week to support me in my research training

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Acknowledgments

Chapter two is a version of “Observations of therapeutic benefits in patient-report registry for Duchenne muscular dystrophy” a manuscript in preparation for submission.

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Cheri Silverstein: study design; statistical analysis and interpretation of data; primary drafting of the manuscript; critical revision of the manuscript for important intellectual content;

Ascia Eskin: study design, database management, statistical analysis; drafting of the manuscript

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Chapter 1: Background

Rare diseases generate special challenges for clinical research. Different organizations define rare diseases differently. The World Health Organization defines a rare disease as one that occurs in fewer than 6.5 to 10 in 10,000 people. The Food and Drug Administration of the United States defines a rare disease as one affecting fewer than 200,000 Americans[1]. Although each disease affects a relatively small number of individuals, the approximately 7,000 different disorders currently considered rare are thought to account for 10% of diseases that affect humans and impact 6-8% of people worldwide [2,3,4].

Although they cumulatively affect a substantial fraction of the population, many of these disorders have few or no treatments supported by evidence-based medicine. Rarity limits sample sizes and may necessitate a high threshold of effect size to reach adequate power. Inadequate observational data makes it harder to properly estimate effect sizes for sample size calculations. Since there are fewer specialists for these diseases, patients in many geographic areas lack access to centers conducting studies. Patients tend to be diagnosed later, when it may be too late to prevent, delay or reverse outcomes. Since rare diseases tend to have high morbidity and mortality and no standard treatments, patients and their families may be resistant to traditional randomization. Finally, although incentives provided by the Orphan Drug Act have greatly improved the interest of pharmaceutical companies in rare diseases, investment is still limited compared to many common diseases [2, 4, 5, 6].

About 80% of rare diseases are genetic and many affect children [2,3]. One such disease is Duchenne Muscular Dystrophy (DMD), which occurs in about 1 in 3500 live male births [7]. DMD is an X-linked recessive disorder caused by a mutation in the DMD gene at Xp21, and, therefore, it almost exclusively affects males. Most commonly, a

deletion in one or more exons results in a frameshift, which leads to a premature stop codon and a severely truncated protein. The cell quickly degrades this severely truncated protein, and dystrophin is absent or almost absent in muscle tissue [8]. Becker Muscular Dystrophy (BMD) is a related, but even more rare, disorder. In general, BMD is due to mutations in dystrophin that do not disrupt the reading frame so some dystrophin is present. The range of skeletal muscle impairment in BMD varies greatly, but it is overall milder than DMD.

Dystrophin is located within the dystrophin-glycoprotein complex, which forms a link between the cytoskeleton and the extracellular matrix. When this complex is disrupted, several downstream effects occur which may contribute to the pathophysiology of DMD. For example, the membrane becomes more permeable. With greater permeability, the cell loses its ability to control cytosolic calcium leading to cell death [9]. Without dystrophin, neuronal nitric oxide synthase (nNOS) is also no longer localized to the sarcolemma. This leads to loss of attenuation of reflex sympathetic vasoconstriction during exercise, leading to muscle ischemia. Other described alterations include increased inflammation and changes in gene regulation [10].

The loss of dystrophin ultimately leads to fibro-fatty replacement of muscle tissue. As a result, calf muscles appear pseudohypertrophied and creatine kinase levels rise. DMD may present as a delay in motor milestones in very early childhood, and will ultimately lead to progressive muscle weakness and loss of motor function. Untreated, almost all children are wheelchair dependent by 12 years of age and will die of respiratory muscle failure or cardiomyopathy by the early 20s. Due to the presence of dystrophin in neuronal tissue, some boys with DMD also have learning disabilities and/or behavioral problems [11].

Studies of rare diseases can be hampered by a lack of consistent diagnostic criteria, but formal diagnostic criteria for DMD exist. Current studies generally use criteria

adapted from those first proposed by the Belgian-Dutch Neuromuscular Club and the Dutch Foundation for Research of Neuromuscular Disease in 1991. These established nine elements of DMD: (1) symptoms present before the age of 5, (2) progressive symmetrical muscular weakness with proximal limbs affected more than distal and lower limbs affected first (often with calf pseudohypertrophy), (3) absence of fasciculations or sensory loss, (4) loss of unassisted ambulation before age 13, (5) at least a 10-fold increase in serum CK, (6) excessive variation in muscle fiber diameter (with both atrophic and hypertrophic fibers), necrotic and regenerative fibers, hyaline fibers and an increase in endomysial connective tissue and fat, (7) muscle biopsy with dystrophin in less than 5% of fibers, (8) frameshift mutation within the dystrophin gene and (9) a family history compatible with X-linked inheritance. For a case to be considered definite in those without a family history, the patient must meet the first five criteria as appropriate for the current age and must meet either muscle biopsy or genetic criteria. In those with a family history, muscle biopsy or genetic criteria are not required as long as they were met in an affected family member [12].

Only one class of pharmacologic therapy, glucocorticoid corticosteroids, is currently proven to benefit skeletal muscle outcomes [13, 14]. The American Academy of Neurology (AAN) formerly recommended corticosteroids as an essential component of quality care in DMD in 2005 [15]. Their practice guidelines referenced seven studies of prednisone and two studies of deflazacort which met their criteria for Class I evidence, which requires studies be prospective, randomized, controlled and blinded. Similarly, a Cochrane review from 2008 identified six randomized trials of either prednisone or deflazacort that met their criteria for inclusion in a meta-analysis. These studies provide consistent evidence of benefit for corticosteroids. In addition, these studies support an optimal dose of 0.75mg/kg/day for daily prednisone (equivalent to 0.9mg/kg/day of deflazacort) with a lesser effect at lower daily doses and increased side effects without

additional benefit at higher daily doses [7,15]. In 2010, the DMD Care Consideration Working Group reiterated the strong endorsement for steroid therapy [13].

Despite the substantial data to support steroids, use is not consistent. MD STARnet looked at steroid use from 1991 to 2005, when practice guidelines clearly endorsed steroid use, in their cohort of boys. Although the inclusion of some BMD patients may have decreased overall steroid use, only 44.1% of patients were on steroids in 2005. In addition, only 50.9% of patients had ever been on steroids. The reason for declining steroid use was unknown in most cases. Of 150 reports of discontinuation, 23.3% were due to weight gain, 19.1% were due to behavioral problems and 15.1% were due to reaching fulltime wheelchair use. In 20% of cases, the reason for discontinuation was not known[16].

Questions remain regarding optimal steroid course over the lifetime of boys with DMD. In general, steroids are started when motor function stops improving with age, usually sometime between age 4 and 6 [14]. Whether boys would benefit from starting earlier is not known. A recently published study started steroids in five 2- to 4-year old boys and four of the boys could still walk at ages 16-18 [17]. As shown in the MD STARnet study, continuation of steroids after loss of ambulation is variable. Studies have suggested that non-ambulatory boys may still benefit from improvements in upper extremity motor function, pulmonary function and cardiac function and reduced need for scoliosis surgery [18,19]. Data from randomized trials are still needed.

The relative benefit and safety of deflazacort and prednisone is another open topic. Studies have suggested that deflazacort may cause less loss of bone mineral density and less weight gain but increased risk of cataract, but only one published randomized study has directly compared deflazacort and prednisone[20]. This study found that there was no difference in motor function between the deflazacort and prednisone groups, but deflazacort was associated with less weight gain [21, 20]. This study, however,

enrolled only 18 patients and may not have been powered adequately to detect a clinically meaningful difference in motor function [7].

Due to side effects, many children are treated with alternative regimens that dose steroids less than daily. Various regimens have been studied and show beneficial effects on muscle function with suggestion of fewer side effects than with daily regimens[22-24]; however, few studies have addressed how these various less frequent regimens compare directly to daily dose treatment. One study took patients who recently completed a randomized trial and placed them on alternate day therapy. This trial found that those who had been on daily dose steroids lost muscle strength when switched to alternate day therapy, and those who had been on placebo initially improved but then declined after three months [25]. A more recent study, however, compared 0.75mg/kg/day of prednisone with 10mg/kg/week in 64 boys ages 4-10 followed for 12 months and found no difference in either muscle or side effect endpoints [26].

Although observational studies have suggested that steroids prolong ambulation by 2-5 years [27], boys still generally enter a wheelchair in their second decade and have markedly reduced life expectancies. Promising therapies such as exon-skipping are in development, but, in the meantime, some parents turn to various supplemental therapies supported by minimal data. Protandim is a dietary supplement reported to upregulate endogenous antioxidant enzymes. One study examined the effect of Protandim on oxidative stress markers, leg-muscle MRI, motor function and histology in mdx mice, which are dystrophin-deficient. They found a reduction in oxidative stress markers and MRI abnormalities but no change in histology or motor function [28]. Green tea extract is another antioxidant studied in the mdx mouse. Unlike Protandim, green tea extract improved muscle function and histology in mdx mice [29, 30]. Coenzyme Q10 is an antioxidant that has been studied in humans. The Cooperative International Neuromuscular Research Group recently published an open-label study of Coenzyme

Q10 in addition to prednisone and found improvements in muscle strength over six months of treatment [31]. Additional studies of these and other supplemental therapies are needed.

DuchenneConnect was established in 2007 with the goal of creating a centralized registry and information portal for all patients with DMD and BMD. In only five years, it has become the largest US-based dystrophinopathy registry. DuchenneConnect is unique among several DMD cohorts because it is a patient-report registry. Any patient or his family member can elect to join the registry by visiting an online portal and entering data directly. DuchenneConnect staff then "curate" the data by verifying clinical status and genetic testing and assisting registrants with interpretation of relevant medical records, such as echocardiograms or MRI reports, as necessary [32]. This design facilitates enrollment of patients with DMD living across the globe regardless of proximity to a specialized neuromuscular disease clinic. It provides a large cohort for ongoing updates to natural history studies as standards of care change. It also facilitates study planning by investigators planning randomized trials. We reviewed DuchenneConnect to test the following primary hypothesis: glucocorticoid corticosteroids are associated with longer time from birth to fulltime wheelchair use in patients with DMD.

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Chapter 2: Manuscript to be adapted for publication

INTRODUCTION

Duchenne muscular dystrophy (DMD; OMIM #310200) is the most common form of muscular dystrophy, affecting about 1/3,500 live male births.[1] DMD is a genetic disorder caused by mutations in the *DMD* gene on the X chromosome that result in the loss of expression of the dystrophin protein. The lack of dystrophin protein, an essential component of the dystrophin associated glycoprotein complex that stabilizes the muscle membrane, results in progressive muscle damage and muscle loss. The progressive weakness ultimately leads to the inability to ambulate in the second decade of life and death, most commonly from pulmonary or cardiac failure, in the third decade of life.

Although it is clear that the proximate cause of DMD is the loss of functional dystrophin, the downstream consequences of loss of dystrophin protein are numerous, providing a host of possible therapeutic targets to mitigate the disease process. Based on the tremendous knowledge about the disease pathophysiology, there are a number of potentially effective therapies for DMD in development and clinical trials [2]. There are also various alternative therapies popular with patients and their parents but supported by little or no data. At this time, glucocorticoid corticosteroids are the only pharmacologic therapy for skeletal muscle weakness in DMD proven to be effective in randomized controlled clinical trials [1].

Since DMD is a rare disease primarily affecting children, the challenges associated with recruiting an adequate sample size limit the feasibility of studying many therapies, and particularly their effects on long-term functional outcomes, with classical research methods such as clinical trials or natural history studies. As a result, registry

based approaches have important potential advantages. DuchenneConnect is a patient-report registry established in 2007. Patients or family members of patients elect to enroll and enter data through an internet-based portal. Collected data includes information about therapies used and the severity of the disease process, including lung function, cardiac function and age at fulltime wheelchair use [3]. As of the time of data extraction for our study, there were 2,285 registrants in DuchenneConnect, around three times as many patients as surveyed in the Muscular Dystrophy Surveillance, Tracking, and Research Network population, a large dystrophinopathy cohort [4].

We sought to determine whether glucocorticoid corticosteroids are associated with protection of skeletal muscle function in the DuchenneConnect population. This analysis serves both to establish the feasibility of using a patient-report registry design to assess patient outcomes in DMD and to describe the impact of steroids on DMD in typical use, outside the controlled environment of traditional research studies. A key advantage of the DuchenneConnect registry is the collection of data on how care varies in general clinical practice. Since some of this variation reflects treatment controversies not yet adequately addressed by other studies, we also explored some of these questions in DuchenneConnect. In particular, we reviewed whether steroid type, steroid dosing frequency or certain nutriceuticals and off-label uses of medications have a measurable impact on skeletal muscle progression. We also sought to determine whether we could identify any factors that affect cardiac dysfunction in DMD.

METHODS

Data Source

We retrospectively reviewed data collected by DuchenneConnect as of August 3rd, 2011. DuchenneConnect has been described previously and is an online registry that compiles patient-reported data on individuals with Duchenne or Becker muscular dystrophy [3]. Responses for more than 150 different variables are collected via a web-based questionnaire, and additional data are obtained by staff review of submitted genetic testing reports. Although DuchenneConnect asks patients to update questionnaires periodically, this analysis is based on entries at a single point in time, the date of last login. All data were downloaded without personal identifiers, and the UCLA IRB issued a letter of exemption from IRB approval.

Study Sample

We selected a subset of DuchenneConnect participants for this study. Criteria included: a diagnosis of Duchenne muscular dystrophy, male gender, and residence in a developed country, defined as one of 34 members of the Organization for Economic Co-operation and Development (OECD) [5]. Of 2285 individuals self-reported in DuchenneConnect at the time of data extraction, 1396 reported a diagnosis of DMD. Twenty-four of those did not provide a gender and 28 are female, leaving 1344 male DMD patients. Of the 1344 males, 1164 were from OECD countries; 941 of those were from the United States and another 223 were from 25 of the other 33 OECD countries. 34 patients were excluded due to inconclusive data for the primary outcome variable. Furthermore, in an effort to reduce recall bias as well as confounding by general improvements in medical care over decades, we excluded 29 patients who were known to have first required a wheelchair greater than 20 years ago. We also excluded 6 outlier subjects who reported that they were still walking but were age 39 or above, which is 20 years older than the next oldest still ambulatory subject. The remaining sample size was 1095. Of those, 384 had reached the primary endpoint [**Figure 2-1**].

Subjects were defined as having DMD by a response of “Duchenne” to the question “What diagnosis was given by the doctor?” DuchenneConnect collects patient reported information on whether muscle biopsy or genetic testing was performed and the identified mutation; however, we did not require these for inclusion because it was our goal to evaluate outcomes according to patient self-report. Of 1095 patients in our sample, 1037 reported having had muscle biopsy, genetic testing or both. 294 had genetic testing results verified by DuchenneConnect.

Primary Endpoint

The primary outcome measure was time from birth to fulltime wheelchair use. A major functional milestone in disease progression, age at fulltime wheelchair use (Age_{WC}) is consistently reported in this database and should be recalled reliably. Subjects were considered to be wheelchair dependent if they entered an age in response to the question “If a wheelchair is used all the time to move around, at what age did this become necessary?”

Those without an entered age were considered ambulatory. In addition, responses to two additional questions were reviewed for consistency. If a participant entered a wheelchair age but also responded that he walked with or without assistance in response to the question “Are any devices used to help/assist with walking?” and did not select “Use a wheelchair fulltime” in response to “If strollers, wheelchairs, or scooters are used, then check all that apply below,” wheelchair status was felt to be inconclusive and the patient was excluded. Similarly, a participant was excluded if he left wheelchair age blank but selected “no, do not walk.”

Primary Analysis

The primary analysis is a comparison of wheelchair-free survival by corticosteroid use. We first compared survival curves for the three categories of steroid use, current, past or never, using the Kaplan-Meier method and log-rank test. Those still ambulatory were censored at the time of their last login to the database. To control for possible confounding, we used a Cox proportional hazards model to estimate hazard ratios according to steroid use. Variables for the steroid use categories and the additional covariates, including insurance status, calcium use, vitamin D use, alternative therapy use, age of diagnosis, whether the patient was from the United States or not, and whether the patient had the diagnosis confirmed by either genetic testing or biopsy or not, were entered into the model and backward stepwise selection with a threshold of $p = 0.1$ was used to select the model. Variables for the interaction between current steroid use and either Vitamin D or calcium use were also tested in the model. All variables remaining in the final model were tested for compliance with proportionality assumptions.

Explanatory variables were defined as follows. For steroids use, subjects were grouped by whether they "never", "previously" or "currently" use steroids as this is how steroid use data is collected by DuchenneConnect. For the proportional hazards model, steroid use was entered as two dummy variables for current and previous use with never as the reference variable. For all other pharmacologic interventions, subjects could only indicate whether they currently use a listed medication or supplement by selecting that medication or not. We defined a response as use and no response as non-use, which is consistent with guidance provided by DuchenneConnect to registrants. Because vitamin D and calcium are generally used to treat osteoporosis induced by corticosteroids, these supplements were considered individually [6]. The other twenty-three supplements listed under the question "Are the following vitamins, supplements, or other medications used?" were grouped into a single variable, use of alternative therapy, for this analysis.

Insurance status was divided into those with any type of health insurance and those without health insurance. Age at diagnosis was stratified by diagnosis at age less than or equal to four or age greater than four based on the overall median age of diagnosis in the population. The Cox analysis included 938 subjects with data for both the outcome and explanatory variables.

Exploratory Analyses

Steroid regimen and wheelchair-free survival in current steroid users

We evaluated how two factors in steroid dosing, type of steroid (prednisone versus deflazacort) and frequency of steroid dosing (daily versus less than daily), relate to wheelchair-free survival in the subset of 657 individuals currently on steroids. In DuchenneConnect, patients indicate steroid frequency by choosing daily, one of four listed less frequent regimens or "other" with a text response. Since the number of individuals in each less than daily group was small, we combined them into a single category for less than daily, and we reviewed text responses and assigned the patient to daily or less than daily as appropriate. We initially compared wheelchair-free survival between daily and less than daily steroid users and between deflazacort and prednisone users using Kaplan-Meier. A Cox proportional hazards model with backward stepwise selection was then used to estimate hazard ratios after controlling for steroid dosing frequency, steroid type and the additional covariates listed for the primary analysis. The interaction between steroid frequency and steroid type was also tested. All variables remaining in the final model were tested for compliance with proportionality assumptions. The Cox analysis included 555 subjects with data for both the outcome and explanatory variables.

Steroid regimen and fracture history

Given the suggestions in the literature that deflazacort has a less negative effect on bone mineral density than prednisone [7], we further assessed whether steroid regimen was associated with likelihood of either vertebral fracture or any kind of fracture using Fisher's exact test or chi-square analysis, respectively. We then conducted a logistic regression to assess the association between steroid type and steroid dosing frequency and risk of having any type of fracture after controlling for calcium and vitamin D use. The regression analysis included 634 subjects with data for both the outcome and explanatory variables.

Effects of other therapies

Given the number of different largely unstudied supplements used by patients, we sought to determine whether we could identify an association between use of any individual supplemental therapy and improvement in wheelchair-free survival after controlling for steroid use. The steroid categories, nine supplements taken by at least twenty subjects overall, calcium and vitamin D and the additional covariates found to be significant in the primary analysis were entered into a Cox proportional hazards model and backward stepwise selection with a threshold of $p = 0.1$ was again used to select a final model. All variables remaining in the final model after backward stepwise selection were tested for compliance with proportionality assumptions and the model was stratified by the non-proportional variable. The Cox analysis included 938 subjects with data for both the outcome and explanatory variables.

Predictors of cardiomyopathy

Since human studies have suggested that steroids also benefit cardiovascular function in DMD whereas studies in the mdx mouse model have shown corticosteroids may be harmful to cardiac function[8], we also sought to review the relationship between steroid

use and cardiomyopathy in DuchenneConnect. In our sample, 173 patients reported being told that they have cardiomyopathy. In addition, 279 patients reported taking an ACE inhibitor, and 294 reported taking any one of eighteen listed types of prescription cardiovascular medications. A large number of participants, 803, reported having had an assessment of cardiac function by echocardiogram or magnetic resonance imaging (MRI). Of those, however, only 121 provided a value for an ejection fraction (EF), and only 157 provided a value for either EF or fractional shortening. Of those who reported a diagnosis of cardiomyopathy, 95% reported having had an assessment of cardiac function, but only 29% reported a value for either EF or fractional shortening. Of those who reported not having been diagnosed with cardiomyopathy, 865 reported having had an assessment of cardiac function, and only 12% reported a value for either EF or fractional shortening.

We limited our sample in this analysis to the 803 patients who reported having had an assessment of cardiac function. Given the limited availability of quantitative information in the database, we selected whether or not the patient reported having been given a diagnosis of cardiomyopathy as the outcome. We used logistic regression to determine whether steroid use was a predictor of receiving a diagnosis of cardiomyopathy after controlling for the additional covariates used in the primary analysis. Since cardiomyopathy in DMD is highly age dependent, we also controlled for age at last follow-up. Linear, quadratic, cubic and categorical relationships between age and the outcome were considered in the model. This analysis included 680 subjects with data for both the outcome and explanatory variables.

Since angiotensin converting enzyme (ACE) inhibitors are used primarily to treat cardiomyopathy in DMD and more than 100 more patients were taking ACE inhibitors than indicated a diagnosis of cardiomyopathy, we also assessed predictors of

taking an ACE inhibitor as an additional outcome. This may serve as a better proxy for cardiomyopathy if some patients to not recognize they carry this diagnosis. For the outcome, we created an aggregate category that included a positive response to any ACE inhibitor or angiotensin receptor blocker (ARB), which are often used as substitutes for those who cannot tolerate ACE inhibitors. This analysis included 698 subjects with data for both ACE inhibitor or ARB use and the explanatory variables. Finally, since cardiomyopathy often presents after the age of fulltime wheelchair and since some have hypothesized that increase in skeletal muscle function my actually increase cardiac demand and worsen cardiac function, we performed an additional analysis with age at fulltime wheelchair use as a predictor of having received a diagnosis of cardiomyopathy [9, 10]. This analysis included 282 subjects with data for both the outcome and explanatory variables.

Statistical significance for all analyses was accepted at $p < 0.05$. Since additional analyses were considered exploratory, adjustment was not made for multiple comparisons. All analyses were done in SAS, version 9.1 or 9.3 or R, version 2.10.1.

RESULTS

General characteristics of DuchenneConnect

Patients in our sample reported years of birth between 1976 and 2010 with a median year of birth of 2000. Of those in a wheelchair fulltime, the reported years of birth range from 1976 to 2003 with a median year of birth of 1993.5. Age of diagnosis is symmetrically distributed with a mean of 4.0 (±2.3) and a median of 4 (IQR 2-6). Among

the subset of 768 who indicated that they did not have a known family history of DMD, the mean age of diagnosis was 4.2 (±2.1). Among the subset of boys who have already progressed to fulltime wheelchair use and who indicated that they did not have a known family history, the mean age of diagnosis was 4.4 (±2.2). The distribution of Age_{WC} is right skewed with a median age of fulltime wheelchair use among those who report already using a wheelchair of 10 (IQR 9-12) and a mean age of 10.5 (±2.5) [**Figure 2-2].** In our cohort, 18 of 1095 were reported to have died. Sixteen of those were reported to have required a wheelchair fulltime at the time of death. Of the 17 patients who had died and whose age at death was known, the mean age at death was 18.2(±2.1) and the median age at death was 19 (IQR 16-22). The youngest died at 7, and the oldest died at 25.

In order to determine if the 1095 subject DuchenneConnect sub-sample was comparable to other large natural history data or registry reports, we compared the mutation spectrum of our sample with that in the literature. Mutation category was available for 688 of 1095 subjects [**Figure 2-3**]. Among those 688 subjects, 73% had a deletion of one or more exons and another 3% had a smaller deletion. Approximately 10% had a duplication of one or more exons, 8% had a nonsense mutation, and about 3% had a splice site mutation. Small duplications, insertions, combined frameshift insertion and deletion mutations and either “other” or “no result provided” accounted for the remaining 3%. The distribution of mutations was almost identical for the 200 of 384 subjects who had already progressed to fulltime wheelchair use, and is consistent with other large registries of DMD mutations [11,12].

Corticosteroid use and wheelchair-free survival in DuchenneConnect

In the full set, steroids were the most commonly used medication, but they were currently used by only 60% of patients. Among those using a wheelchair fulltime, current steroid use fell to 41%. The guidelines clearly support use of steroids between the plateau phase (usually sometime between ages 4 and 6) and loss of ambulation (around age 10). Nevertheless, even among boys in this age range, steroids were underused. A histogram of steroid use by age is shown in **Figure 2-4**.

Table 2-1 summarizes the baseline characteristics of our sample by steroid use groups. Other than percent with insurance, each of the characteristics was significantly different among never, past and current steroid users. The past steroid users are overall older. The range of ages among the never steroid users is wide, with both older patients and many of the youngest patients, some of who may not yet have progressed sufficiently to justify steroid initiation. We could not control for birth year because of 100% correlation with the outcome in our censored subjects; however, we entered each of the other characteristics into the model as covariates.

Using the Kaplan-Meier method, we compared time from birth to progression to fulltime wheelchair use among the 633 patients currently on steroids, 144 patients previously on steroids and 280 patients who had never taken steroids who had wheelchair free survival data available. Current steroid use was associated with significantly longer time to fulltime wheelchair use when compared with past and never steroid users ($p < 0.0001$) [**Figure 2-5**]. After controlling for potential confounders, current steroid use remained a significant predictor of time to fulltime wheelchair use. Current steroid use has a hazard ratio of 0.32 ($p < 0.0001$), meaning that current steroid users enter a wheelchair at approximately 1/3 the rate per unit time when compared to those not currently using steroids. Having insurance, use of vitamin D and age at diagnosis were also significant predictors of wheelchair-free survival. Increased age at

diagnosis is associated with reduced hazard and longer survival to fulltime wheelchair, implying that age at diagnosis primarily reflects age of symptom onset, which is marker of overall disease severity [**Table 2-2**].

Explanatory Analyses

Steroid regimen and wheelchair-free survival

We assessed the influence of steroid regimen in the 657 subjects currently using steroids. Typically, steroids are prescribed at a dose of 0.75mg/kg/day of prednisone, which has been shown to be the optimal daily dose, or an equivalent daily dose of deflazacort, 0.9mg/kg/day[13]. Consistent with this, approximately 85% of current steroid users in DuchenneConnect report taking steroids daily. Existing data has suggested that deflazacort is associated with less weight gain than prednisone [14], and, in DuchenneConnect, deflazacort was more common than prednisone, used by approximately 58%. A 2x2 table is shown in **Table 2-3**.

Using Kaplan-Meier, deflazacort is significantly associated with longer time to fulltime wheelchair use [**Figure 2-6**]. For daily dosing overall, there is a trend toward longer time to fulltime wheelchair use [**Figure 2-7**]. When the survival curves of all four subgroups are compared, less than daily prednisone has the lowest wheelchair free survival **[Figure 2-8].** On pairwise comparison of each of the subgroups, daily deflazacort has significantly longer time to fulltime wheelchair use than less than daily prednisone (p=0.0004 for the direct comparison). There was no significant difference when daily deflazacort was compared to less than daily deflazacort [**Figure 2-9**] and the survival curves of less than daily deflazacort and daily prednisone are almost completely superimposed [**Figure 2-10**]. In the multivariate model, deflazacort was significantly associated with longer time to fulltime wheelchair use (HR = 0.64, p = 0.01) but

frequency of steroids was not a significant predictor. Age at diagnosis and use of vitamin D were also significant predictors of wheelchair-free survival in this analysis [**Table 2-4**].

Steroid regimen and fractures

Although were unable to demonstrate a significant difference by Fisher's exact test ($p = 0.15$), it is interesting to note that zero fractures were reported in the patients on less than daily dosing [**Tables 2-5a/b**]. Patients on deflazacort tended to have more vertebral fractures (3.1% vs 1.1%) and deflazacort was associated with more of any kind of fracture, even after controlling for steroid frequency, vitamin D and calcium use (OR for deflazacort 1.76, 95% CI 1.13-2.73, c = 0.589) [**Tables 2-6a/b and Table 2-7**].

Effects of other therapies

The most frequent supplement (other than calcium and vitamin D) was coenzyme Q10. 31% of patients did not report using steroids, vitamin D, calcium or any alternative supplements. Most subjects using alternative supplements took those supplements in addition to steroids [**Figure 2-11**]. After controlling for current or past steroids, vitamin D, insurance, and age at diagnosis, no alternative therapy remained significantly associated with later age at fulltime wheelchair use. There was a trend toward longer time to fulltime wheelchair use in those on CoenzymeQ10 [**Table 2-8**].

Predictors of Cardiomyopathy

Of the 680 patients included in the analysis, 140 indicated that they had received a diagnosis of cardiomyopathy and 537 indicated that they had not. Using logistic regression, we determined that only age at follow-up was a significant predictor of having a heart diagnosis [**Table 2-9**]. This model has a c statistic of 0.811. When we

instead look at predictors of taking an ACE inhibitor, current and past steroid use are both predictors of ACE inhibitor use (OR 2.62, 95%CI 1.44-4.76 for current and OR 3.095, 9%CI 1.61-5.96 for past, n= 698, c = 0.801) in addition to age at follow-up. In the subset of those who have progressed to fulltime wheelchair use, again only age at follow-up was a significant predictor. (OR 1.21, 95%CI 1.14-1.28, $p < 0.0001$, n=282, c = 0.754; p for Age_{WC} = 0.54).

DISCUSSION

The above findings demonstrate that DuchenneConnect can serve as a unique and vital tool in ongoing research on DMD. Of the 1095 patients in our sample, 87% indicated that they had not previously participated in a clinical trial, observational study or registry. The fraction was slightly higher, 91%, for the subset who reported already using a wheelchair fulltime. This indicates that these data represent a population not previously reported in any study. One challenge in research is recruitment bias. Although an internet based design may have its own bias toward recruiting those with better access to technology, this cohort may better reflect how DMD is treated in general practice than many traditional studies.

There are limitations to the analysis. Although DuchenneConnect engages in ongoing efforts to verify submitted data, these data are primarily based on patient self-report, which may be subject to more inaccuracies than studies based on data collected directly or through chart review. To assess the potential for recall bias distorting results, we compared general characteristics of DuchenneConnect and the effect of a well-studied therapy, steroids, to what was previously described in studies using more

traditional designs. In all cases, the DuchenneConnect gathered data matches well to expectations.

The distribution of mutations in DuchenneConnect is similar to that described in both a previously published small clinical cohort from a single MDA clinic and some large registries [15,11,12], suggesting this cohort is biologically similar to the general DMD population. Furthermore, the close agreement of the mutation distribution in the fulltime wheelchair subset to that in full sample suggests that the wheelchair subset is overall older but otherwise consistent with the larger group.

Age at diagnosis should reflect how access to care and severity of disease in our population compares to those previously described. The mean age of diagnosis among patients without a family history was 4.2. This result is somewhat younger than the mean age of definitive diagnosis of 4.9 reported for boys without a known family history of DMD in the MD STARnet cohort[16]. This may partly reflect the fact that DuchenneConnect does not specify "definitive" diagnosis; nevertheless, we consider this encouraging since DuchenneConnect is a younger cohort that would have benefited from advances in awareness and diagnosis with time.

Age_{WC} in our population is similar to the age at fulltime wheelchair use reported in the literature. For instance, Magri, *et al*. recruited a population of 205 DMD patients from neuromuscular disease clinics and found a mean age of loss of ambulation of 10.3 (±1.9) years [17]. Similarly, Bach *et al*. found a mean age of wheelchair dependence of 10.8 (±1.3) in a retrospective review of patients from their single center neuromuscular disease clinic [18]. Both of these results are similar to our finding of a median of 10 and mean of 10.5 among those already in a wheelchair. Of note, this does not represent the true median survival to loss of ambulation among the full DuchenneConnect population

since fewer than 50% of patients in our dataset have progressed to fulltime wheelchair use.

Older natural history studies, prior to the relatively common practice of chronic steroid usage, reported an average age at loss of ambulation of 9.5 [19,20]. We found a median Age_{WC} of 9.5 (±1.8) and median Age_{WC} of 9 (IQR 8-11) in the 54 boys in our analysis who had never taken steroids and who were not taking any other supplemental therapies. Despite the passage of more than forty years, we found the same course of skeletal muscle disease in the absence of pharmacologic intervention.

Steroid therapy has been well documented to slow the skeletal muscle disease progression of DMD since the effect was first reported in 1974 [21]. The authors of a Cochrane systematic review from 2008 identified fifty studies of glucocorticoid corticosteroids in DMD and performed a meta-analysis of six randomized controlled trials published in peer-reviewed journals. They found that steroids consistently improve a variety of muscle strength measures, such as time to rise from the floor (Gowers' time) and four-stair climbing time, over 6 months to 2 years of follow-up [1]. We, therefore, assessed the effect of chronic steroid therapy on ambulation as both an initial measure of the ability of the DuchenneConnect registry data to recapitulate a well-established effect and an appraisal of how the surrogate measures assessed in randomized trials translate to differences in a meaningful long-term endpoint in general use. As expected, we found that current steroid use is associated with longer time to fulltime wheelchair use. We found that there was no significant difference in wheelchair free survival ($p = 0.64$) between those previously and those never on steroids. Since DuchenneConnect only asks whether corticosteroids were used in the past and does not specify the length of past use or age of onset of use, this implies that many past steroid users in DuchenneConnect took steroids for an insufficient duration for skeletal muscle benefit.

Since multiple prior studies have shown a benefit for steroids, it is likely that this is a true effect. Since this is an observational study, however, we cannot determine cause and effect and our finding could be affected by confounding. Our baseline table demonstrated several differences between our current, past and never steroid users, likely driven primarily by the different age distributions of these groups. For example, age at diagnosis was of particular concern because the never steroid users have an earlier age at diagnosis. Since earlier age at diagnosis is associated with increased hazard, likely because it is a marker of earlier onset of symptoms and more severe disease, the never steroid users may just do worse because they happen to have more severe disease. Nevertheless, we are reassured by the fact that current steroid use remains significant after controlling for age at diagnosis. In addition, when we plot age at fulltime wheelchair against age at diagnosis, the scatter plot demonstrates that. although the correlation between age at fulltime wheelchair use and age of diagnosis is statistically significant, it is not strong (n = 379, correlation = 0.13, p = 0.01) [**Figure 2-12**]. Nevertheless, we cannot exclude the possibility that are our results are affected by inadequate control for confounding by age at diagnosis or other factors for which we do not have information in the DuchenneConnect database, such as socioeconomic status.

We found that deflazacort was associated with longer time to fulltime wheelchair use than prednisone, but daily dosing was not associated with longer time to fulltime wheelchair use than less than daily dosing. We also found that deflazacort was associated with a higher likelihood of having had a broken bone. The result for dosing frequency must be interpreted with caution because the number of participants taking steroids less than daily was quite small, limiting our power to detect a difference. The results also do not prove that deflazacort either prolongs wheelchair free survival or increases bone fractures. First, we performed several exploratory analyses without

adjusting for multiple comparisons. Therefore, at least one significant finding could be due to chance. Furthermore, since this is an observational study, we cannot determine cause and effect. Deflazacort is more expensive and, therefore, may correlate with access to top quality care. We have controlled for insurance, but this cannot completely control confounding by socioeconomic status and quality of care. We also do not know if patients have switched steroid type. Those who have had fractures may switch to deflazacort after a fracture because of the suggestion that it has less effect on bone mineral density. Nevertheless, we do feel these data raise the possibility that less than daily deflazacort may be the optimal balance of muscle protection and reduction in side effects. Therefore, we feel ongoing and/or future randomized trials that address optimal steroid regimen, such as the FOR-DMD trial, should consider including a less than daily dose deflazacort arm.

Although we were unable to show a consistent benefit for ambulation for any supplemental therapy, we would refrain from drawing any firm conclusions regarding the potential of these therapies based on our results. Our analysis of many of the supplements is underpowered given the relatively few registrants taking those therapies at this time. As the database grows, there may be increased power to detect an effect from the supplemental therapies.

We found that only age at last login to the database was significantly associated with receiving a diagnosis of cardiomyopathy. When we looked at predictors of ACE inhibitor use, on the other hand, steroids were a significant predictor. This likely reflects adherence to evidence-based medicine guidelines. Parent and providers who enthusiastically adopt steroid recommendations may be quicker to employ ACE inhibitors in earlier stages of heart dysfunction. Although we did not observe a benefit for receiving a diagnosis of cardiomyopathy, steroids may still be cardioprotective.

Unfortunately, the analysis of cardiac outcomes is DuchenneConnect is limited by patient access to technical reports and awareness of changes in heart function that may be asymptomatic. Those taking steroids may just be more likely to undergo cardiac function assessment. More quantitative data will be needed to better assess this.

CONCLUSION

The data presented above establish DuchenneConnect as framework for further growth in patient self-report data and interpretation. Although registry based study design is not new, there are few studies that use data gathered from patients who self-register on internet-based portals, making DuchenneConnect a major innovation in the study of, not only Duchenne Muscular Dystrophy, but rare diseases in general. In only five years, DuchenneConnect has accumulated the largest DMD cohort with age at wheelchair use and treatment information published to date. Although the treatments are not performed nor recorded in a controlled clinical environment, these data provide a unique insight into current clinical practice for DMD. In the coming years, we expect the registry to grow even further. With many current registrants still due to pass major milestones and new registrants, we will have an ongoing resource for observation of current DMD clinical care, exploring genotype/phenotype correlations and exploring unexpected disease relationships.

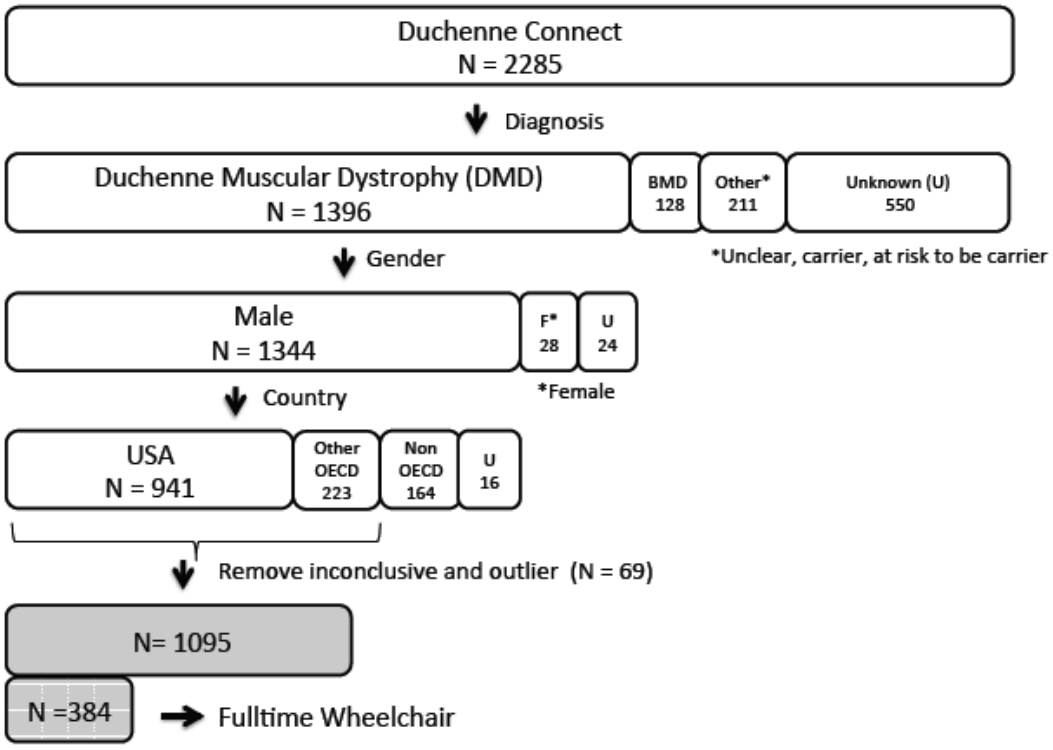

Figure 2-1. Sample selection

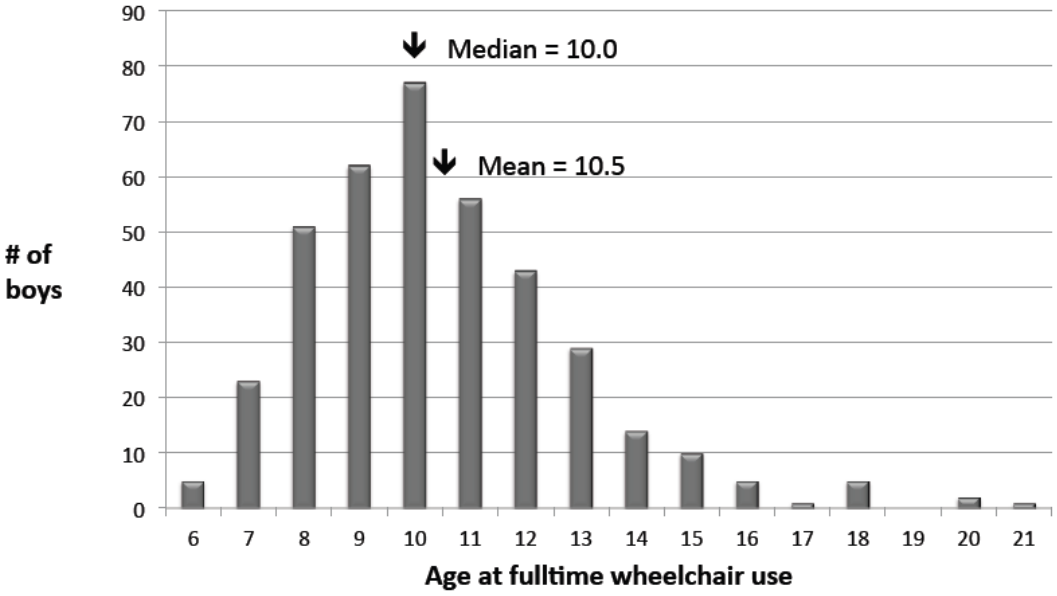

Figure 2-2. Histogram of age at fulltime wheelchair use (n = 384)

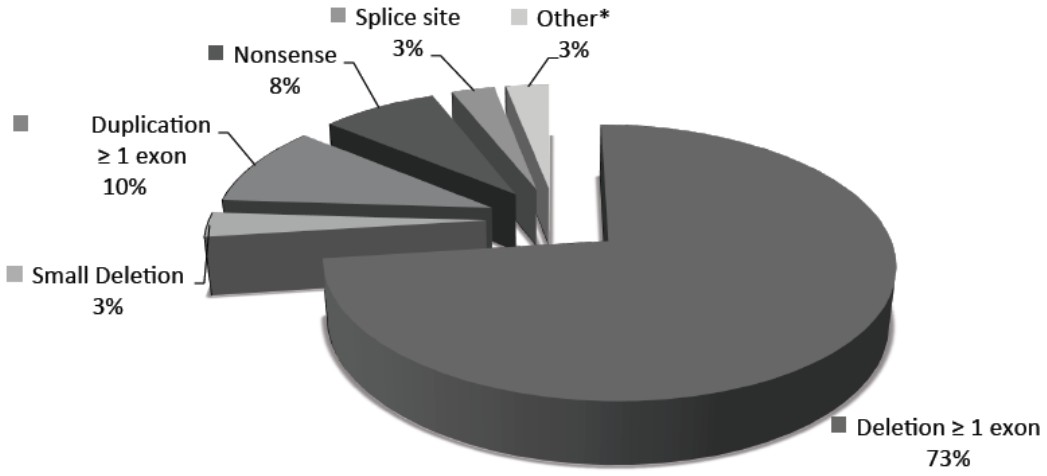

Figure 2-3. Mutation types reported by participants with DMD in DuchenneConnect.*Small duplications, Insertions insertion/deletions and "other" or "no result provided" together account for less than 3% of mutation types reported (n = 688).

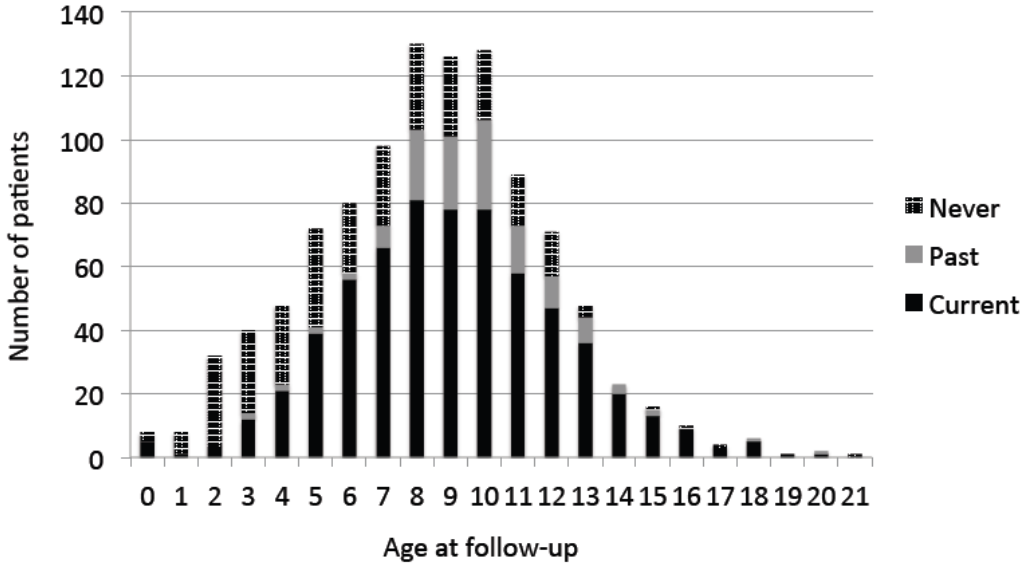

Figure 2-4. Steroid use by age

		Steroid Use			
Characteristic	**Overall** (n=1095)	**Never** (n=288)	**Past** (n=130)	**Current** (n=657)	**P value**
Birth year*	2000 (1995-2003)	2002.5 (1994-2006)	1994 (1990-1998)	2000 (1997-2002)	P < 0.0001
Age at diagnosis*	4 (2-6)	3 (2-5)	4 (3-5)	4 (3-6)	P = 0.0092
Genetic testing±biopsy-no. (%)	1037 (94.7)	263 (91.3)	121 (93.1)	639 (97.3)	P = 0.0002
From USA-no. (%)	890 (81.3)	223 (77.4)	115 (88.5)	535 (81.4)	P = 0.0274
Have insurance-no. (%)	947 (97.7)	241 (96.4)	122 (97.6)	573 (98.5)	P = 0.1769
Vitamin D-no. (%)	313 (28.6)	30 (10.4)	28 (21.5)	254 (38.7)	P < 0.0001
Calcium–no. (%)	246 (22.5)	12 (4.2)	19 (14.6)	214 (32.6)	P < 0.0001
Any alternative therapy-no.(%)	284 (25.9)	39 (13.5)	23 (17.7)	220 (33.5)	P < 0.0001

Table 2-1. Baseline characteristics overall and by steroid status. *Medians and interquartile ranges (IQR) with comparison using Kruskal-Wallis .

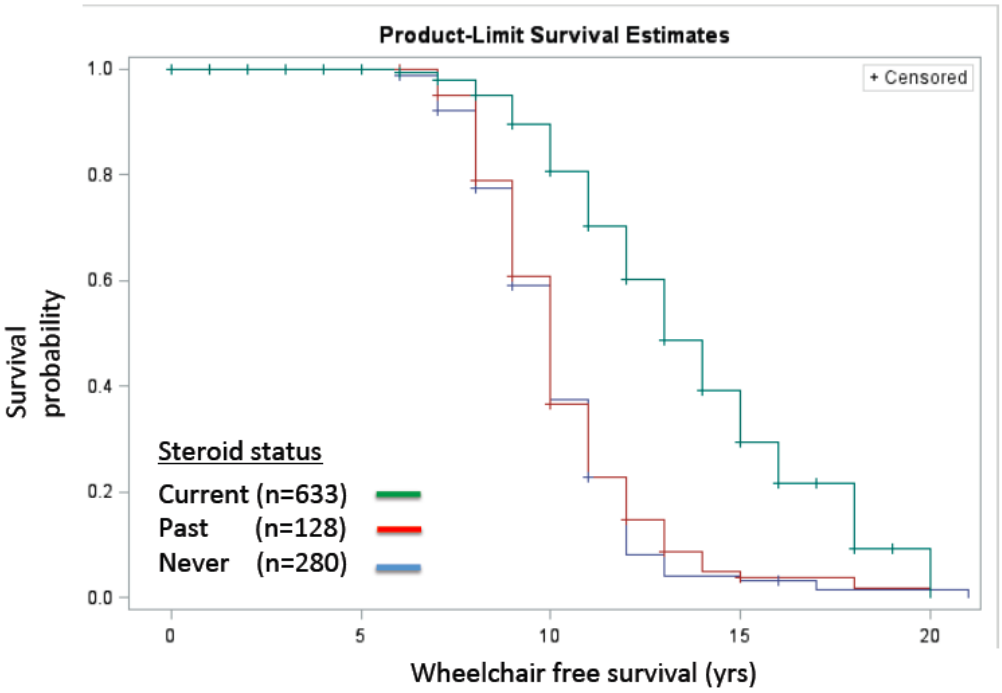

Figure 2-5. Kaplan-Meier curves of wheelchair free survival in current, past and never steroid users. (Log-rank $p < 0.0001$)

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Current steroid use	-1.13	< 0.0001	0.32	0.25	0.43
Past steroid use	-0.15	0.30	0.86	0.65	1.14
Has insurance	-0.82	0.02	0.44	0.23	0.86
Vitamin D use	-0.39	0.003	0.68	0.52	0.88
Age at diagnosis > 4	-0.26	0.02	0.77	0.62	0.96

Table 2-2. Final Cox proportional hazards model of time to fulltime wheelchair use by steroid use category (n = 938). Variables with p > 0.1 were eliminated from the model

	Deflazacort-no.(%)	Prednisone-no.(%)	
Daily–no.(%)	341 (52.9)	205 (31.8)	546 (84.65)
< Daily-no. (%)	34 (5.3)	65 (10.1)	99 (15.35)
	375 (58.1)	270 (41.9)	645 (100)

Table 2-3. Number of subjects on each steroid regimen

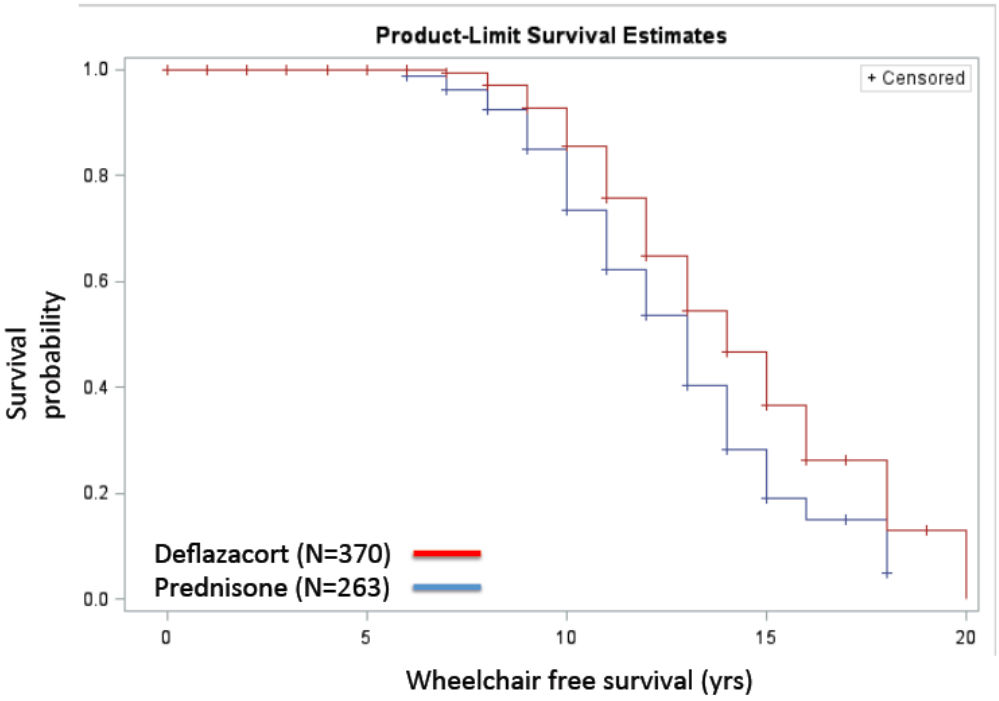

Figure 2-6. Kaplan-Meier analysis of wheelchair free survival in deflazacort versus prednisone. (Log-rank p = 0.0013)

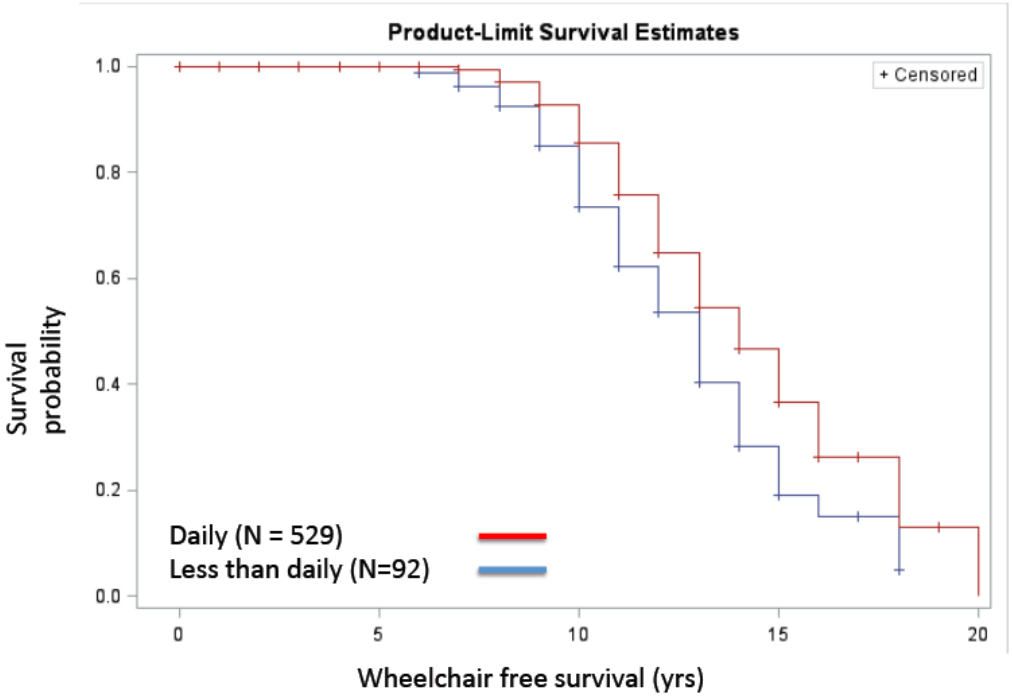

Figure 2-7. Kaplan-Meier analysis of wheelchair free survival in daily steroid users versus those who took steroids < daily. (Log-rank p = 0.09)

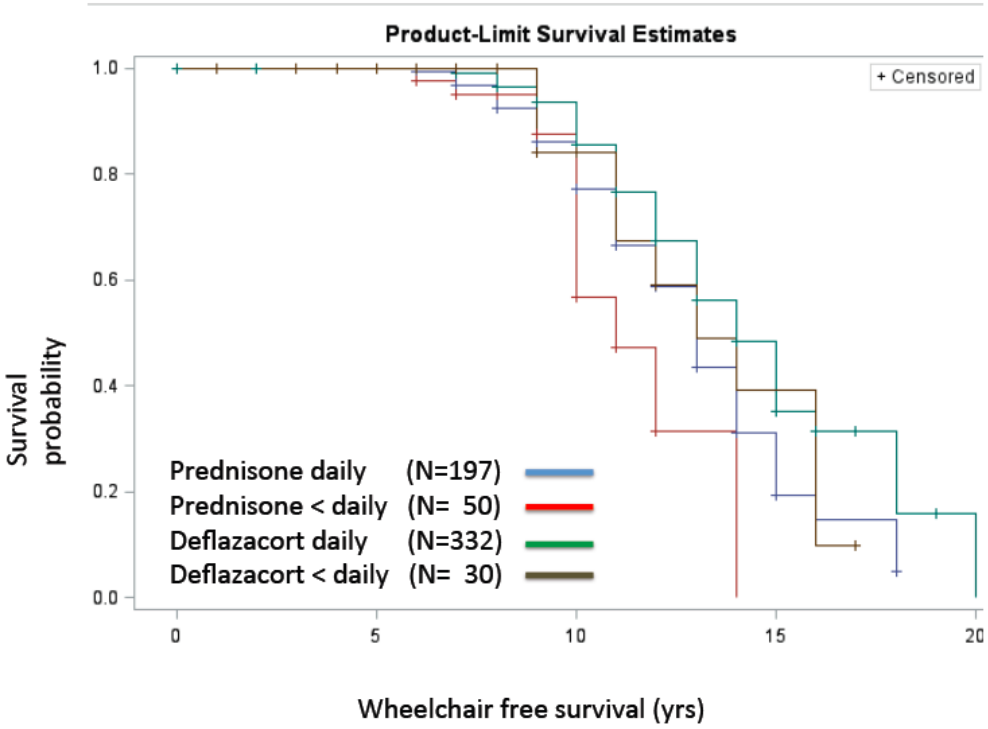

Figure 2-8. Kaplan-Meier analysis of wheelchair free survival in four steroid type/ frequency subgroups. (Log-rank p = 0.0017)

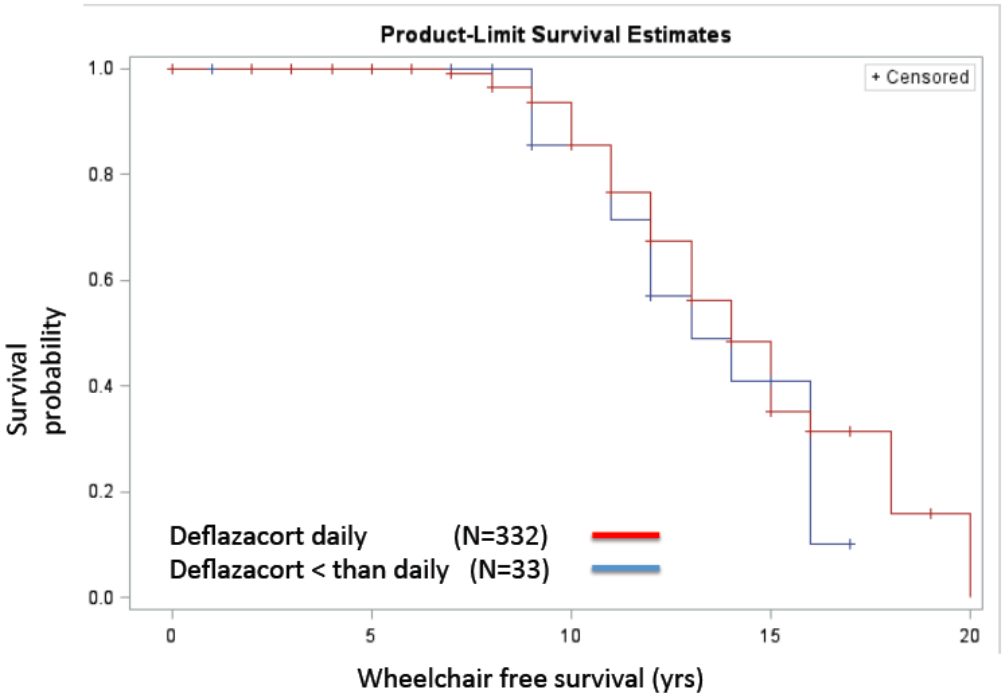

Figure 2-9. Kaplan-Meier analysis of wheelchair free survival in daily deflazacort users versus those who took deflazacort < daily. (Log-rank p = 0.36)

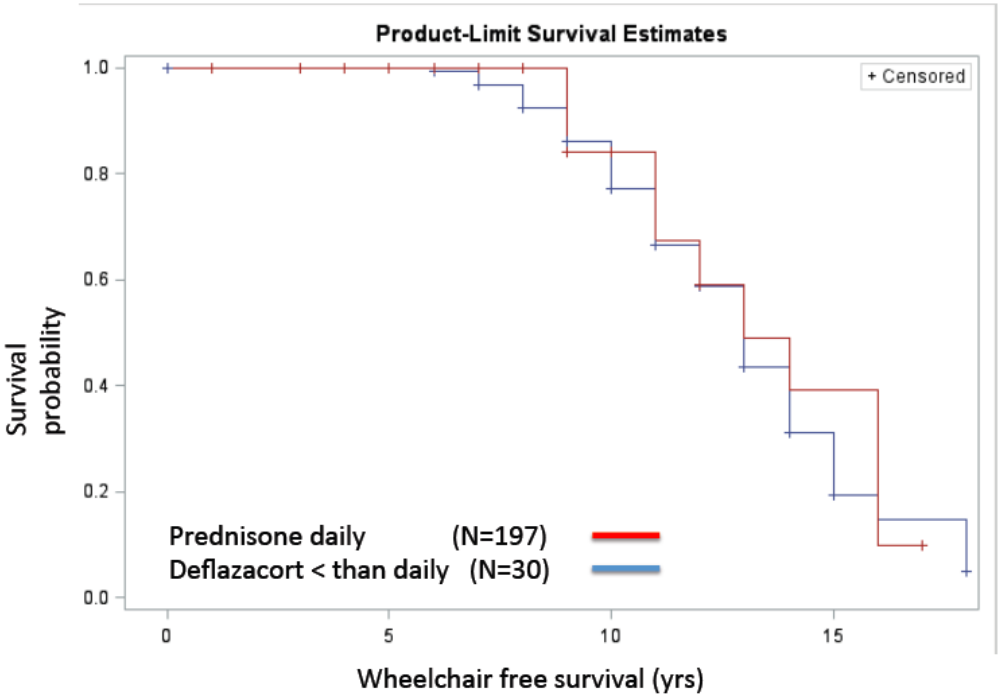

Figure 2-10. Kaplan-Meier analysis of wheelchair free survival in daily prednisone users versus those who took deflazacort < daily. (Log-rank p = 0.66)

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Deflazacort	-0.45	0.01	0.64	0.45	0.90
Age at diagnosis	-0.11	0.005	0.90	0.84	0.97
Vitamin D	-0.39	0.03	0.68	0.47	0.96

Table 2-4. Cox proportional hazards model of time to fulltime wheelchair use by steroid regimen in current steroid users (n=555). Variables with p > 0.1 were eliminated from the model.

a

		Steroid dosing		
		< Daily-No.(%)	Daily-No.(%)	
Vertebral Fracture*	No-No.(%)	109 (16)	551 (82)	660 (98)
	Yes-No.(%)	0 (0)	15 (2)	15 (2)
		109 (16)	566 (84)	N=675; P = 0.15

b

		Steroid dosing		
		< Daily-No. (%)	Daily-No.(%)	
Any Fracture+	No - No.(%)	90 (13)	450 (68)	540 (81)
	Yes-No.(%)	19 (03)	105 (16)	124 (19)
		109 (16)	555 (84)	N=664, P = 0.72

Tables 2-5a/b. Vertebral and fractures of any kind by steroid dosing regimen. *Fisher's exact, +Chi-square

a

		Steroid type		
		Deflazacort-No.(%)	Prednisone-No(%)	
Vertebral Fracture*	No-No.(%)	368 (55)	274 (41)	642 (96)
	Yes-No.(%)	12 (1.5)	3 (0.5)	15 (02)
		389	277	N=666, P = 0.11

b

		Steroid Type		
		Deflazacort-No.(%)	Prednisone-No.(%)	
Any Fracture+	No No.(%)	295 (46)	232 (36)	527 (82)
	Yes No.(%)	80 (12)	39 (06)	119 (18)
		375 (58)	271 (42)	N=646, P = 0.02

Table 2-6a/b. Vertebral and fractures of any kind by steroid type. There are significantly more total fractures in the deflazacort group. *Fisher's exact, +Chi-square

Variable	Odd Ratio	95% Confidence Limits	P value
Deflazacort use	1.76	1.13 -2.73	0.01
Daily steroids	0.94	0.52-1.68	0.82
Vitamin D use	0.64	0.38-1.09	0.10
Calcium use	1.83	1.08-3.09	0.02

Table 2-7. Logistic Regression of predictors of having had any kind of fracture in current steroid users (n=634)

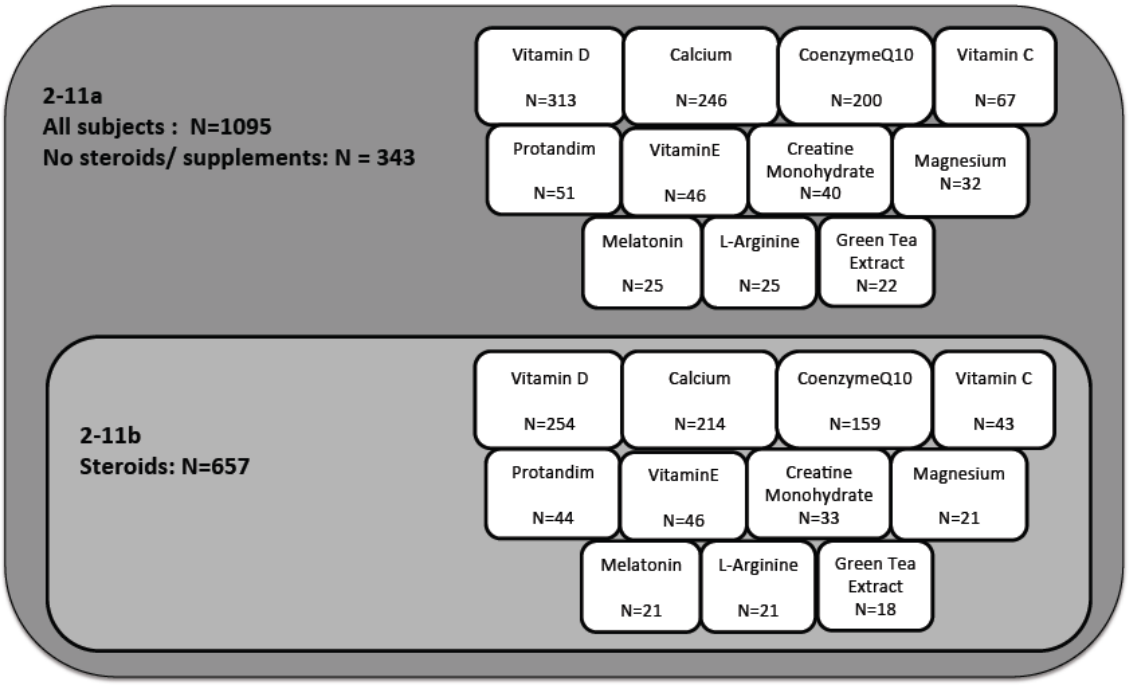

Figure 2-11a. # of subjects on alternative therapies (boxes) 2-11b. Number of subjects on steroids (left) and steroids plus each supplement (boxes)

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Past steroid use	-0.17	0.23	0.84	0.63	1.11
Insurance	-0.72	0.03	0.49	0.25	0.95
Vitamin D	-0.30	0.03	0.79	0.56	0.97
Age of diagnosis	-0.24	0.03	0.74	0.63	0.97
Coenzyme Q10	-0.33	0.05	0.72	0.52	1.01

Table 2-8. Cox proportional hazards model of time to fulltime wheelchair use. Effect of alternative therapies (n=938). Variables with $p > 0.1$ were eliminated from the model.

Variable	Odds Ratio	95% Confidence limit	P value
Current steroid use	0.84	0.47-1.50	0.56
Past steroid use	1.67	0.86-3.24	0.13
Age at follow-up	1.22	1.18-1.28	< 0.0001

Table 2-9 Logistic regression final model for predictors of cardiomyopathy. All variables eliminated from the model have a $p > 0.1$. ($n = 680$).

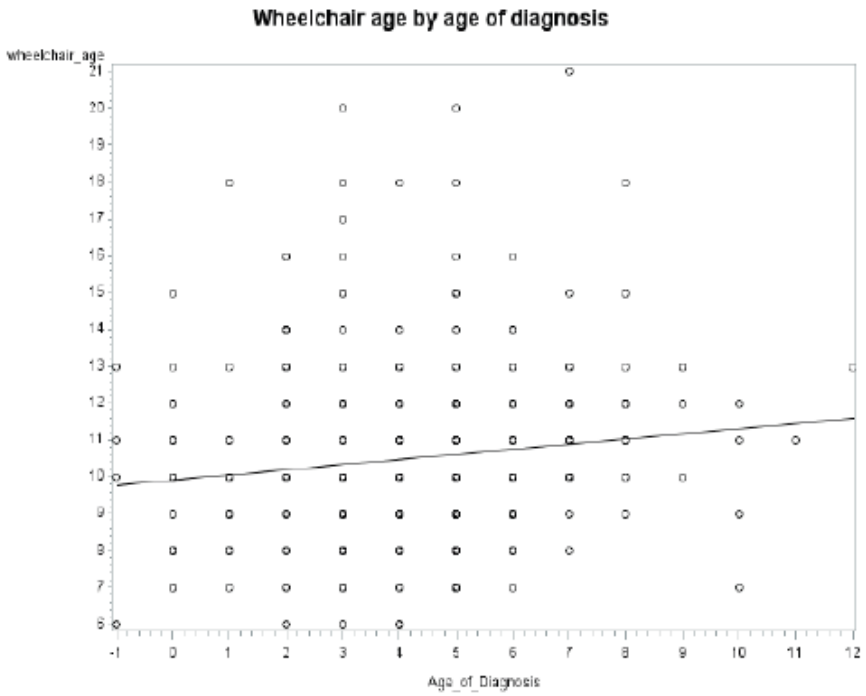

Figure 2-12. Correlation between age at fulltime wheelchair use and age at diagnosis (n = 379, correlation = 0.13, p = 0.01)

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Chapter 3: Expanded statistical methods

This study is a retrospective observational study that tests the following hypotheses:

(1) Primary hypothesis: Glucocorticoid corticosteroid use is associated with longer time from birth to fulltime wheelchair use in Duchenne Muscular Dystrophy (DMD)

(2) Secondary hypotheses:
    - Steroid type and dosing regimen are associated with time from birth to fulltime wheelchair use in DMD
    - Steroid type and dosing regimen are associated with risk of fractures in DMD
    - Alternative supplement are associated with time from birth to fulltime wheelchair use in DMD
    - Glucocorticoid corticosteroids are associated with risk of cardiomyopathy in DMD

Inclusion Criteria

Our inclusion criteria were a diagnosis of DMD, male gender and residence in a developed country, as defined by membership in the OECD. Male gender was required because DMD is X-linked and, therefore, generally only affects males. Females who carry a dystrophin mutation in one X chromosome can occasionally manifest symptoms to varying degrees; however, even in the most severely affected manifesting carriers,

presentation is still generally less severe than males [1]. Therefore, it would be inappropriate to include females in the analysis.

Only residents of OECD countries (mainly North American and European countries) registrants were included in an attempt to analyze subjects receiving similar standards of care. Among the sample of subjects from OECD countries, age of diagnosis was normally distributed with a mean of 4.0 (±2.3) and median of 4 (IQR 2-6). We note that this is very similar to a recent US based population survey [2]. Among subjects from non-OECD countries, the age of diagnosis was more broadly distributed and significantly higher ($p = 0.0005$, Kolmogorov-Smirnov test), with a mean of 5.0 (±2.5) and median of 5.0 (IQR 3-6) [**Figure 3-1**]. Most of the subjects were from the US, and the mean age of diagnosis in the US was not significantly different from the larger cohort of OECD countries. As a result, we have chosen to exclude those entries from non-OECD countries in order to reduce potential effects from variations in care between developed and developing nations.

As reviewed in Chapter 1, established criteria for making a diagnosis of DMD require either muscle biopsy or genetic analysis for a diagnosis to be considered definite. Because our aim was to analyze data according to patient self-report, we opted to include all patients who reported that DMD was the diagnosis given by the doctor. Nevertheless, we compared characteristics of the subgroup with muscle biopsy or genetic data verified and those without to assess the limitations of this approach. Out of our study sample of 1095, 1037 reported having had muscle biopsy, genetic testing or both. 294 had had genetic testing results verified by DuchenneConnect. Of note, 1 patient who had missing data for whether or not he had had muscle biopsy or genetic testing was listed as having had genetic testing results verified by DuchenneConnect. Since DuchenneConnect staff complete the verification status variable, we left this patient in our sample of those with verified genetic test results.

When I compare the mean age of diagnosis between those with verified genetic test results (mean = 4.0±2.2) and those without (mean = 4.1±2.2), there was no significant difference (p = 0.46). Student's t-test was used for this comparison since the age of diagnosis is normally distributed in these two groups. When I compare age of diagnosis between those with muscle biopsy and/or genetic testing to those without, there was a trend toward a higher age of diagnosis in those without (mean 4.6±2.6 and median of 5.0 IQR3-7 vs. mean of 4.0 and median of 4 IQR 3-5, one-sided p = 0.051 by Wilcoxon rank sum test). The distributions were not found to be unequal using a Kolmogorov-Smirnov test (p = 0.19). Non-parametric testing was selected for this comparison based on the appearance of the distributions. The one-sided p was selected because we would expect age of diagnosis to be higher in those who did not have genetic testing, since clinical signs appear over time.

When I compare Age_{WC} between those with verified genetic test results (mean 11.0 ±2.7, median 10 IQR 9-13) to those without (mean 10.4±2.4, median 10 IQR 9-12, there was no significant difference (p = 0.14). When I compare Age_{WC} between those who reported having had muscle biopsy and/or genetic testing (mean = 10.5±2.4, median 10 IQR9-12) to those who did not (mean 10.4±2.7, median 10 IQR9-11), there was also no significant difference (p = 0.77). Wilcoxon rank sum test was used for these comparisons because the distributions are not normal, but are similar between the two groups**.** Although there were some small, but not statistically significant, trends toward differences between groups, we felt that these differences were not consistent with significant misdiagnosis of another form of muscular dystrophy as DMD. Therefore, we opted to include all the patients with a reported diagnosis of DMD in the analysis. Since other forms of muscular dystrophy are generally less severe than DMD, incorrect inclusion of non-DMD participants would likely reduce our power to detect a significant result by decreasing the incidence of our primary outcome.

Selection of Primary Outcome

Our use of age at fulltime wheelchair use as our primary outcome measure was driven mainly by what was available within the DuchenneConnect database. DuchenneConnect collects information on whether or not patients can ambulate or sit without support and whether devices are use to assist with ambulation. Randomized trials in DMD have used surrogate endpoints [3]. The classical method of assessment in many neuromuscular diseases, including DMD, has been manual muscle testing of muscle strength using the Medical Research Council scale. This scale divides muscle strength into five grades: 0 for paralysis, 1 for minimally detectable muscle contraction, 2 for movement only with gravity eliminated, 3 for movement against gravity but not against resistance, 4 for reduced movement against resistance and 5 for normal strength. The key limitations of this method are that grading is subjective and that the scale is ordinal. In clinical practice, most patient variation falls within grade 4 [4].

Given the limitations of manual muscle testing, alternative quantitative measures of muscle strength and function have been developed. Quantitative strength tests such as hand-grip dynamometry provide more objective and linear measurements; however, they are complicated by the fact that the trajectory of muscle strength change is different for different muscle groups [5]. For example, hand-grip dynamometry tends to increase with age below age 10, and therefore, has limited sensitivity for overall functional disability in that age range [6].

The 6-minute walk test (6MWT), the Motor Function Measure (MFM), the North Star Ambulatory Assessment (NSAA) and the various timed tests, such as time to rise from the floor (Gowers' time), rise from a chair or climb stairs, are tests which aim to capture functional limitation directly [7, 8]. The 6MWT is used to assess exercise capacity in a variety of disorders in both children and adults and measures the distance the

individual can ambulate in six minutes [9]. The Motor Function Measure was developed in the last decade to assess motor function in patients with diverse forms of neuromuscular disease. In this tool, thirty-two tasks are judged on a four-point scale to create a total score as a percentage of the maximum [10]. The North Star Ambulatory Assessment was developed specifically for assessment of patients with DMD and provides a score based on 17 gross motor activities. Patients are given a score of 2 for each activity completed normally, 0 for activities they cannot complete and 1 if the activity is completed but with modification [11]. For example, if a boy can walk but walks on his toes rather than with heels on the floor, he would receive a score a 1 for that activity. The 6MWT, MFM and NSAA have all been found to be useful indicators of global motor function ability for use in following outcomes in clinical trials of DMD [5].

Recently, a group has even shown a correlation between muscle fat fraction by MRI and loss of ambulation. The authors studied at twenty boys ranging in age from five to twenty-three with DMD and examined relative fat fraction in the thigh muscles. In these boys, a mean fat fraction of 50% was 100% sensitive and 91% specific in identifying those with loss of ambulation[12]. This endpoint still needs to be validated in a separate longitudinal cohort to be adopted for more widespread use.

Nevertheless, the purpose of each of these surrogate endpoints is to predict clinically meaningful major functional milestones such as such as loss of ability to get up from the floor, climb stairs, walk independently, self-feed and sustain adequate overnight ventilation without support. Of these, loss of ambulation is widely considered the major functional milestone, but the follow-up required to assess impact on this outcome is felt to be too long to be feasible in randomized clinical trials [5]. Despite selecting loss of ambulation as the primary outcome in their meta-analysis of the effect of glucocorticoids corticosteroids on DMD, the Cochrane Neuromuscular Disease group only identified one trial that used this outcome and felt that analysis was done incorrectly in that trial. As a

result, the ability to analyze the effect of various factors on loss of ambulation rather than resort to use of surrogate endpoints as is done in clinical trials is strength of our study.

Finally, since a primary purpose of DuchenneConnect is to collect data through patient-report, having an endpoint that is easily reported by patients and their families, rather than by physicians, is paramount. Although quantitative muscle strength tests or functional scores such as the 6MWT, MFM and NSAA might be able reveal variation in motor function due to therapeutic interventions in the large number of patients in DuchenneConnect who are below the age of fulltime wheelchair use, these tests are not used universally in general clinical practice. Furthermore, even patients who completed these assessments as part of clinical practice or other studies may not have knowledge of their precise scores. Since patients or their families can consistently and precisely report age at fulltime wheelchair use, it is the most appropriate endpoint for this study.

Model Selection

The primary analysis was a survival analysis. In DMD, all patients ultimately progress to loss of ambulation and premature death usually due to loss of independent respiration and/or cardiac dysfunction. Variation among patients is the age at which, or length of survival until, these events occur. Furthermore, as discussed above, age at fulltime wheelchair use is the only motor endpoint collected in DuchenneConnect. As a result, survival analysis is most appropriate for this type of data.

Performing a survival analysis extrapolated from age data rather than using follow-up time from an initial study contact as in treatment trials does have limitations. Since patients are, by definition, different ages, patient years of birth span a few decades. Even a decade may lead to significant variation in general medical care and controlling for confounding from the variation using specific treatment variables in the database may be difficult; however, due the 100% correlation of progression free

survival time with age in the censored patients, we cannot control for age as a proxy for the intangible improvements in care over time.

An alternative model we considered was a linear regression using age at fulltime wheelchair as the outcome. This approach, however, would be subject to bias because we would not account for those who may have survived the same amount of time without reaching the endpoint. The other drawback is the need to address the non-normal distribution of age at fulltime wheelchair use. Use of a log-transformed outcome can make parameter estimates from the regression model harder for readers to interpret.

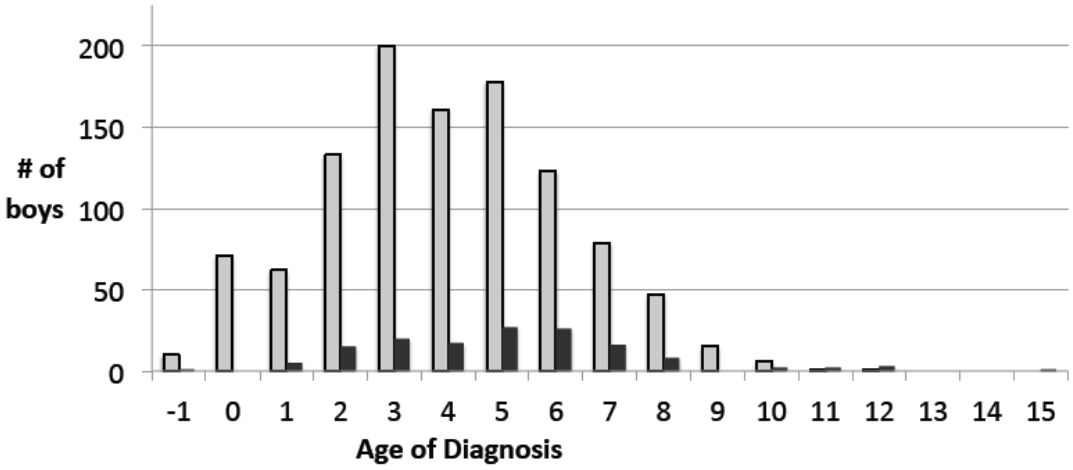
The covariate insurance status was defined as insurance or no insurance. A drawback of this approach is that the number without insurance in DuchenneConnect is quite small, limiting our ability to detect a difference according to insurance status. Nevertheless, Medicaid, Medicare and private or employer based insurance in the United States are all quite different from each other and the government run health programs of countries outside the United States. As a result, the categories are not easily combined in another way.

The Cox proportional hazards model assumes that the ratio of hazards over time remains constant, or proportional. The covariates remaining in the final primary model were tested for compliance with proportionality assumptions by entering time-dependent variables equal to variable*log(time) into the model. We initially included age of diagnosis as an interval variable. When variables remaining in the primary model after backward selection were tested for proportionality, age at diagnosis was found to be non-proportional ($p = 0.03$). As a result, age at diagnosis was converted to a categorical variable stratified by diagnosis at age less than or equal to four or age greater than four. Age four was selected because it is the overall median age at diagnosis in this population. The model with age of diagnosis as an interval variable is shown in **Table 3-1** for comparison with **Table 2-2**. When the proportionality test was run again for

variables remaining in the model after backward stepwise selection with age at diagnosis as a categorical variable, age at diagnosis met proportionality criteria ($p = 0.09$).

The primary explanatory variable, current steroids, was also non-proportional. This finding for current steroid use is consistent with the observation that the Kaplan-Meier survival curve for current steroid use is not parallel to the curves for past and never steroid use. Current steroid use was tested as a stratified variable and parameter estimates for the remaining variables are shown in **Table 3-2** for comparison with **Table 2-2**. The parameter estimates for the remaining variables did not change significantly. Therefore, current steroid use was felt to be sufficiently proportional, and we did not stratify on current steroid use.

The covariates remaining in the exploratory Cox analyses were also tested for proportionality. For the analysis of steroid type and dosing frequency, all covariates remaining in the model met proportionality criteria and age at diagnosis was left in the model as an interval variable. For the analysis of alternative therapies, current steroid use and age at diagnosis were found to be non-proportional. When the model was stratified by age at diagnosis, parameter estimates for the remaining variables were stable, so age at diagnosis remained in the model as a categorical variable. When the analysis was stratified by current steroid use, however, parameter estimates were not stable. As a result, the analysis stratified by current use was selected as the final model. The non-stratified model is presented in **Table 3-3** for comparison with the final model in **Table 2-8**.



	#*	Mean in years (SD)	Median in years (IQR)
OECD Countries	1087	4.0 (2.3)	4.0 (2-6)
Non OECD Countries	143	5.0 (2.5)	5.0 (3-6)

Kolmogorov-Smirnov P-value = p = 0.0005

*Based on data for DMD, Male, Age of Diagnosis age available, outliers removed

Figure 3-1. Age of Diagnosis in boys from OECD vs non OECD countries

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Current steroid use	-1.15	< 0.0001	0.32	0.24	0.42
Past steroid use	-0.16	0.26	0.85	0.64	1.13
Insurance	-0.78	0.02	0.46	0.24	0.89
Vitamin D	-0.42	0.002	0.66	0.88	0.96
Age at Diagnosis	-0.09	0.0001	0.92	0.51	0.86

Table 3-1. Cox proportional hazards model of time to fulltime wheelchair use by steroid use category with age at diagnosis as an interval variable

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Past steroid use	-0.15	0.30	0.86	0.65	1.14
Has insurance	-0.77	0.02	0.47	0.24	0.91
Vitamin D use	-0.38	0.005	0.69	0.53	0.89
Age at diagnosis > 4	-0.25	0.02	0.78	0.63	0.97

Table 3-2. Primary analysis Cox model stratified by current steroid use

Variable	Parameter Estimate	P value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Current steroid use	-1.20	<0.0001	0.30	0.23	0.39
Past steroid use	-0.19	0.18	0.83	0.62	1.09
Insurance	-0.89	0.009	0.41	0.21	0.80
Age of diagnosis	-0.27	0.01	0.76	0.62	0.95
Coenzyme Q10	-0.37	0.02	0.69	0.50	0.95
Magnesium	-0.82	0.01	0.44	0.23	0.83

Table 3-3. Alternative supplement Cox model prior to stratification by current steroid use

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