

# Cardiopulmonary phenotypic variability and discordance in Duchenne muscular dystrophy

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## Abstract

Neuromuscular respiratory medicine has traditionally focused on mechanically assisted lung ventilation and mucus clearance. These therapies have prolonged survival for patients with Duchenne muscular dystrophy (DMD). However, the field is rapidly evolving in a new direction: it is being revolutionized by molecular and genetic therapies. A good correlation between a patient's dystrophin mutation and his cardiopulmonary phenotype would allow accurate prediction of patient prognosis and would facilitate the design of studies that assess new DMD therapies. Instead, patient prognosis and the design of valid therapeutic studies are complicated by cardiopulmonary phenotypic discordance and variability, by which a notable proportion of DMD patients have unexpectedly good or poor cardiopulmonary function. The likely cause of phenotypic variability and discordance is genetic modifiers. Once the modifiers that affect cardiopulmonary function are better understood, it should be possible to create a personalized genetic profile that accurately predicts the prognosis of each individual DMD patient. This would allow investigators to assess the effect of new therapies in the context of each patient's particular cardiopulmonary natural history. Amplification of beneficial cardiopulmonary genetic modifiers and blocking of detrimental modifiers is a promising strategy for creating new DMD therapies. When patients with chronic respiratory failure are treated with assisted ventilation, cardiac function determines their survival. Therefore, prioritizing new cardiac therapies is most likely to prolong patient survival. By focusing on these topics we aim to move neuromuscular respiratory medicine beyond assisted ventilation and coughing and into the age of translational medicine.

## Cardiopulmonary phenotypic variability and discordance in Duchenne muscular dystrophy: implications for new therapies

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## ABSTRACT

Neuromuscular respiratory medicine has traditionally focused on mechanically assisted lung ventilation and mucus clearance. These therapies have prolonged survival for patients with Duchenne muscular dystrophy (DMD). However, the field is rapidly evolving in a new direction: it is being revolutionized by molecular and genetic therapies. A good correlation between a patient's dystrophin mutation and his cardiopulmonary phenotype would allow accurate prediction of patient prognosis and would facilitate the design of studies that assess new DMD therapies. Instead, patient prognosis and the design of valid therapeutic studies are complicated by cardiopulmonary phenotypic discordance and variability, by which a notable proportion of DMD patients have unexpectedly good or poor cardiopulmonary function. The likely cause of phenotypic variability and discordance is genetic modifiers. Once the modifiers that affect cardiopulmonary function are better understood, it should be possible to create a personalized genetic profile that accurately predicts the prognosis of each individual DMD patient. This would allow investigators to assess the effect of new therapies in the context of each patient's particular cardiopulmonary natural history. Amplification of beneficial cardiopulmonary genetic modifiers and blocking of detrimental modifiers is a promising strategy for creating new DMD therapies. When patients with chronic respiratory failure are treated with assisted ventilation, cardiac function determines their survival. Therefore, prioritizing new cardiac therapies is most likely to prolong patient survival. By focusing on these topics we aim to move neuromuscular respiratory medicine beyond assisted ventilation and coughing and into the age of translational medicine.

## 1| INTRODUCTION

Neuromuscular respiratory medicine has traditionally focused on the use of devices for mechanically assisted lung ventilation and mucus clearance. These devices have prolonged patient survival, as reported for Duchenne muscular dystrophy (DMD).<sup>1-6</sup> However, the overall therapeutic approach of neuromuscular medicine is rapidly evolving in a very different direction. The field is being revolutionized by the development of molecular and genetic therapies, some of which have already been approved for patient use, with others in development.<sup>7-12</sup>

Neuromuscular respiratory medicine must now find a way to interface with these emerging therapies in order to advance into the age of translational medicine.

The central concept of this article is that it is essential to fully understand the natural history of cardiopulmonary function in patients with DMD. Natural history is the trajectory of cardiopulmonary function over time; this trajectory determines whether a patient has a beneficial or detrimental phenotype. Based on prior studies and clinical observations, there appear to be three DMD pulmonary phenotypes: typical, mild and severe.<sup>13,14</sup> Patients with the mild pulmonary phenotype have a forced vital capacity (FVC) that peaks at a higher level and at a later age compared to patients with the typical pulmonary phenotype. After peaking, the FVC declines more slowly over time. As a result, patients with the mild pulmonary phenotype have better pulmonary function through adulthood (Figure 1).<sup>14</sup> Similarly, a detrimental or beneficial cardiac phenotype is determined by the age at onset of left ventricular dysfunction (LVD). In the detrimental phenotype, LVD begins at an early age and then steadily declines, leading to an early onset of congestive heart failure (CHF). Once CHF occurs, the mean survival is just 8 months.<sup>15</sup> In the beneficial phenotype, the onset of LVD occurs later. While left ventricular dysfunction is progressive, the later onset of LVD also delays the onset of CHF, prolonging survival (Figure 2).<sup>15</sup>

## 2| PHENOTYPIC VARIABILITY AND DISCORDANCE

A good correlation between dystrophin genotypes and cardiopulmonary phenotypes would be useful. If the natural history of a patient's cardiopulmonary function could be predicted from the patient's genotype, then variations from the patient's predicted clinical course could be attributed to effect of a particular therapy under investigation. Correction of the dystrophin mutation would be an effective therapeutic strategy.

Instead, even patients who share a common dystrophin genotype may have disparate pulmonary and cardiac phenotypes, making cardiopulmonary natural history highly unpredictable. For example, one DMD patient's forced vital capacity (FVC) may peak at a high absolute value and remain favorable over time, while his brother's FVC peaks at a markedly lower level, and subsequently declines at a rapid rate (Figure 3).<sup>16</sup> Furthermore, unrelated patients with the same or similar dystrophin genotype can have highly divergent pulmonary function.<sup>17</sup> With regard to cardiac natural history, a DMD patient may experience favorable heart function into adulthood, while his brother experiences early-onset cardiomyopathy.<sup>16</sup> Moreover, patients with identical dystrophin genotypes have significant variability both in their age at onset of LVD and in the subsequent rate of deterioration of their cardiac function over time (Figure 4, Table 1).<sup>15,18</sup> These are examples of cardiopulmonary phenotypic variability – defined as unexpected differences in cardiopulmonary function among patients with identical dystrophin genotypes. A related concept is cardiopulmonary phenotypic discordance, in which an individual DMD patient has a cardiac phenotype that is the diametric opposite of his pulmonary phenotype; specifically, surprisingly good heart function in a patient with very poor pulmonary function, or surprisingly poor heart function in a patient with good pulmonary function.<sup>17</sup> With phenotypic discordance, the skeletal muscles controlling pulmonary function and the cardiac muscles controlling heart function express phenotypes that are unexpectedly divergent.

Cardiopulmonary phenotypic variability and discordance have important implications for determining patient prognosis and for assessing the effectiveness of DMD therapies. The genetic basis of phenotypic variability and discordance also provides a promising strategy for designing new DMD treatments.

## 3| PHENOTYPIC DISCORDANCE AND PATIENT PROGNOSIS

With regard to patient prognosis, it seems self-evident that patients with better cardiopulmonary function should experience better survival. For example, in one study of DMD patients *who were not treated with assisted ventilation*, 5-year survival was just 8% when the patients' FVC fell below 1 liter.<sup>19</sup> Instead, our studies of phenotypic discordance suggest a more complex reality. When we studied our DMD patients who could be classified into two groups—prolonged survivors (alive, at mean age 34.3 years) and those who experienced early death (at mean age 21.7 years),<sup>20</sup> – we discovered that the patients who experienced early death had unexpectedly poor cardiac function (mean ejection fraction (EF) 29.2%) despite comparatively good pulmonary function (mean FVC 804 mL); i.e., the patients had cardiopulmonary phenotypic discordance. The patients who experienced prolonged survival also had phenotypic discordance, but their discordance was the opposite of patients experiencing early death: the prolonged survivors had unexpectedly good cardiac function (mean EF 42.2%) despite their highly impaired pulmonary function, requiring 24 hour/day assisted ventilation due to an FVC of 0 mL (Figure 5).<sup>20</sup> We concluded that when DMD patients with poor pulmonary function are treated with assisted ventilation, *cardiac function determines their survival*. Viewed another way, in patients treated with contemporary respiratory management, poor cardiac function is the main risk factor for early death, while favorable cardiac function makes them eligible for prolonged survival. These results suggest that it is crucial to assess the effect of new DMD therapies on heart function and that cardiac outcome measures should be prioritized. Instead, most DMD studies report on skeletal muscle strength, such as timed function tests, the Northstar Ambulatory Assessment, upper extremity function and the 6-minute walk test,<sup>7,21-23</sup> and data on the cardiac effects of new DMD therapies are sparse.

These observations related to phenotypic discordance and patient survival also have important implications with regard to study design. It is incorrect for studies of emerging therapies to state or infer<sup>7,8,24,25</sup> that if a therapy preserves pulmonary function it will definitely prolong patient survival. If a patient has a detrimental cardiac phenotype, his survival is likely to be shortened, even if that patient has good pulmonary function.

Conversely, a patient with a beneficial cardiac phenotype is more likely to experience prolonged survival, even if he has poor pulmonary function, if that patient is treated with assisted ventilation.

#### 4| IMPLICATIONS FOR THERAPEUTIC TRIALS

When designing therapeutic trials, it is also important to consider that phenotypic variability and discordance create a population of “outlier” patients with surprisingly good or poor pulmonary and cardiac function. It is common for studies of new DMD therapies to use aggregate data, derived from a limited number of subjects.<sup>7,8,25</sup> This study design is based on the assumption that phenotypic outliers are rare. If, instead, cardiopulmonary phenotypic variability and discordance are common, then “outlier” patients with unexpectedly good cardiac or pulmonary function may comprise a significant proportion of the study population, confounding the results of studies based on aggregate data.

Our recent studies suggest that cardiopulmonary phenotypic discordance is indeed common. In one study, discordance occurred in one-third of our DMD patients who were alive and 18 years of age or older.<sup>17</sup> In a discordant subset of patients with good heart function and bad lung function, mean FVC was just 0.18 liters, but mean ejection fraction was 50% (Table 2).<sup>17</sup> Phenotypic discordance was common among patients in sub-groups with identical or similar dystrophin mutations and cardiopulmonary function was unpredictable, having no correlation with the dystrophin mutation (Table 3, Table 4, Figure 6). For DMD patients in clinical trials that are based on aggregate data, prolonged survival could be attributed to the effect of a new therapy, when instead a sizable proportion of the study subjects are simply expressing a beneficial cardiac or pulmonary phenotype. Additionally, grouping patients by their identical or similar dystrophin genotypes does not assure a homogeneous study population, as those patients may have dissimilar cardiopulmonary phenotypes.<sup>7,8</sup> These observations suggest that new DMD therapies should be assessed in the context of each patient’s particular cardiopulmonary natural history, rather than relying on aggregate patient data, even when the patients share a common dystrophin mutation.

In studies that focus on very young DMD patients as their subjects, phenotypic variability can be masked, as younger patients have not yet “declared” their phenotype. Instead, cardiopulmonary natural history diverges over time. For example, as previously discussed, we found that DMD patients with the detrimental cardiac phenotype had onset of LVD before age 18 years, while those with the beneficial cardiac phenotype manifested LVD at age 18 years or later, and had prolonged survival.<sup>15</sup> After LVD onset, cardiac function worsened over time in both groups, but survival did not diverge until the patients reached their late teens and early twenties (Figure 2).<sup>15,17</sup> Very young patients usually had normal cardiac function, regardless of whether they ultimately expressed a beneficial or detrimental cardiac phenotype (Figure 7).<sup>18</sup> Therefore, the effect of new DMD therapies should be assessed over an extended period of observation and the study design should include a significant proportion of “older” patients who have established phenotypes. For example, recent studies of eteplirsen and ataluren generally included younger patients (10-13 years at baseline) with a follow-up period of only 2 to 4 years,<sup>8,21</sup> during which pulmonary function remained fairly well preserved (as expected given the young age of the subjects and the relatively short duration of observation). Datasets such as these may demonstrate treatment-related benefits to respiratory function in the short term, but these benefits do not necessarily translate into reduction in the morbidity and mortality associated with long-term declines in respiratory function. Furthermore, drawing conclusions from datasets that include primarily younger subjects with short follow-up periods may incorrectly attribute “preservation” of pulmonary or cardiac function to treatment. Instead, beneficial cardiopulmonary function may be due to the patients’ natural history, independent of any treatment effect.

To summarize, the natural history of cardiopulmonary function in DMD requires an understanding of phenotypic variability and discordance, which can be used to optimize the design of therapeutic trials. However, in stark contrast to the recommendations for valid trial design outlined above, current DMD studies involve relatively short durations, study populations consisting of very young subjects, analyses based on aggregate patient data, and attempts to create homogeneous study populations by grouping together subjects who have similar or identical dystrophin mutations.<sup>7,8,24,25</sup> Results from such studies are not necessarily translatable to reducing cardiopulmonary morbidity or mortality in the long-term. Additionally, using pulmonary function

as a surrogate for survival is inaccurate; rather, cardiac function is the primary determinant of survival in people with DMD who are treated with ventilatory support.

## 5| IMPLICATIONS FOR PATIENT PROGNOSIS AND THE DESIGN OF NEW THERAPIES

The genetic basis of cardiopulmonary phenotypic variability and discordance provides a strategy to more accurately assess patient prognosis and for designing new DMD treatments. The unpredictability of cardiopulmonary phenotype in patients with identical dystrophin genotypes is likely due to the presence of genetic modifiers. These modifiers may include different isoforms of the dystrophin gene product, or separate genes that change the expression and products of the mutated dystrophin gene. Thus, when modifiers are present, the phenotype cannot be predicted from the dystrophin mutation alone; i.e., by the degree to which a dystrophin mutation is predicted to alter the gene's reading frame, disrupting the production of functional dystrophin protein.

While some modifiers appear to affect skeletal muscle and cardiac muscle synchronously, there are examples of tissue-specific modifiers, such as a modifier that is detrimental to skeletal muscle but is associated with later onset of cardiomyopathy.<sup>26</sup> Thus, modifiers have the potential to explain both phenotypic variability (divergent phenotypes among groups of patients who share a common dystrophin mutation) and phenotypic discordance (individual patients who have a cardiac phenotype that is diametrically opposite to their pulmonary phenotype). If the most common and most potent modifiers are identified and characterized, it might be possible to predict an individual patient's long-term cardiopulmonary natural history early in life, from his extended genetic profile, consisting of his dystrophin mutation and his relevant genetic modifiers. That would allow clinicians to predict patient prognosis sooner and more accurately and would allow investigators to assess DMD therapies in the context of each patient's particular cardiopulmonary natural history.

A promising strategy for identifying those modifiers is the use of Whole Exome Sequencing in a study population consisting of patients with "extreme" cardiopulmonary phenotypes. Recent work utilizing such a strategy in DMD patients with early loss of ambulation identified variants in the modifier gene *TCTEX1D1* that were associated with earlier loss of ambulation, and were also noted in patients with earlier and more severe onset of cardiomyopathy.<sup>27</sup> These "extreme phenotypes" are equivalent to the phenotypic "outliers" we have identified in our studies of phenotypic discordance. As discussed previously in this article, we showed that DMD patients who experience early death and those who experience prolonged survival both manifest unexpected levels of heart function: unexpectedly poor cardiac function in the patients who experience early death and unexpectedly good cardiac function in the prolonged survivors. Thus, Whole Exome Sequencing of patients with phenotypic discordance may be a way to identify the modifier genes that cause phenotypic outliers and "extreme phenotypes," allowing creation of extended genotypic profiles that have a high degree of prognostic accuracy.

There are numerous candidates for modifiers of the dystrophin gene and the latest candidates have been described in various publications.<sup>26,28,29</sup> Most of the studies describe how modifiers affect skeletal muscle, with a few reports focusing on modifiers of pulmonary and cardiac function.<sup>29-31</sup> While there is an association between pulmonary phenotype and age at loss of ambulation (for example, patients with the most severe pulmonary phenotype experience loss of ambulation at the earliest age),<sup>14</sup> the relationship between skeletal muscle deterioration and cardiopulmonary phenotypes is not well-studied. Further complicating the picture, exogenous factors such as administration of glucocorticoids may have a differential effect in patients with certain genetic modifiers. For example, increasing doses of glucocorticoid were shown to increase expression of the modifier gene *SPP1* in patients who possessed the G allele of this genotype,<sup>32</sup> suggesting a mechanism for paradoxical increases in muscle inflammation in the presence of glucocorticoids.

With these limitations in mind, amplifying beneficial modifiers and downregulating deleterious ones could be a promising therapeutic strategy. For example, Yamamoto and colleagues reported on 181 patients with DMD (all of whom had exonic mutations causing depletion of the Dp427 isoform).<sup>28</sup> Those patients who also had a deficiency of the Dp116 isoform experienced substantially longer cardiac dysfunction-free survival compared to those who did not have a mutation affecting Dp116, despite all patients having similar cardiac

function at initial evaluation. This is a potential cause of the divergent cardiac phenotypes we described earlier in this review and suggests the possibility that depletion of the Dp116 dystrophin isoform could improve cardiac function and, thus, patient survival.

Overall, cardiopulmonary modifier genes have great potential for use as therapeutic agents. Therapies that amplify beneficial modifiers and block detrimental modifiers could positively alter the key clinical features of DMD, including cardiopulmonary function. Our data implicating cardiac function as the main determinant of survival<sup>17,20</sup> suggest that a therapeutic strategy focused on beneficial cardiac modifiers is most likely to prolong lifespan in patient with DMD.

## 7| CONCLUSIONS

In this article we have discussed the complexities of cardiopulmonary natural history in patients with DMD, including the key concepts of cardiopulmonary phenotypic variability and discordance, which cause patients to have unexpectedly good or poor cardiopulmonary function (“phenotypic outliers”). A true understanding of cardiopulmonary natural history is necessary in order to accurately determine patient prognosis and to design studies that accurately assess the effectiveness of new therapies. With this in mind, current studies of new DMD therapies are flawed for several reasons: they focus on timed tests and other measures of skeletal muscle function, rather than cardiopulmonary outcome measures; their results are based on aggregate data without accounting for the sizable prevalence of phenotypic “outliers”; and their study populations are composed primarily of younger patients with good cardiopulmonary function, i.e., subjects who have not yet declared their cardiopulmonary phenotypes. We have discussed how the dystrophin mutation alone is a poor predictor of cardiopulmonary function in an individual patient. However, once the genetic modifiers that beneficially and detrimentally affect cardiopulmonary function are better understood, it should be possible to create a personalized genetic profile that accurately predicts the prognosis of each individual DMD patient. This would allow investigators to design better treatment studies by assessing the effect of new therapies in the context of each patient’s particular cardiopulmonary natural history. Amplification of beneficial cardiopulmonary genetic modifiers and blocking of detrimental modifiers is a promising strategy for creating new DMD therapies. Moreover, our data suggest that, when patients with chronic respiratory failure are treated with assisted ventilation, cardiac function determines their survival. Therefore, prioritizing the development of effective cardiac therapies is most likely to prolong patient survival. By focusing on these topics we aim to move neuromuscular respiratory medicine beyond assisted ventilation and coughing and into the age of translational medicine.

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### Figure Legends

Figure 1: Natural history of forced vital capacity (FVC) with age in boys with DMD. (a) shows FVC as % predicted (mean + 95% confidence interval). (b) shows FVC in absolute values (mL; mean + 95% confidence interval). Group A included patients with loss of ambulation at <8 years of age. Group B included patients with loss of ambulation between 8-11 years of age. Group C included patients with loss of ambulation between 11 and 16 years of age. Adapted from Humbertclaude et al.,<sup>14</sup> and reproduced with permission.

Figure 2: Cumulative survival for DMD patients with onset of left ventricular dysfunction (LVD) <18 years versus > 18 years. Patients with onset of LVD at a later age lived significantly longer than those with earlier onset of LVD. Adapted from Wang et al.,<sup>15</sup> and reproduced with permission.

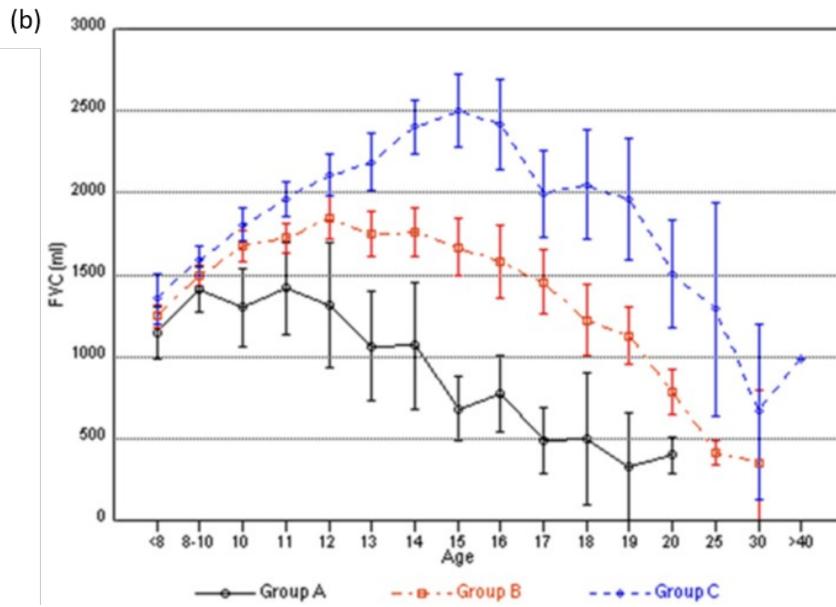
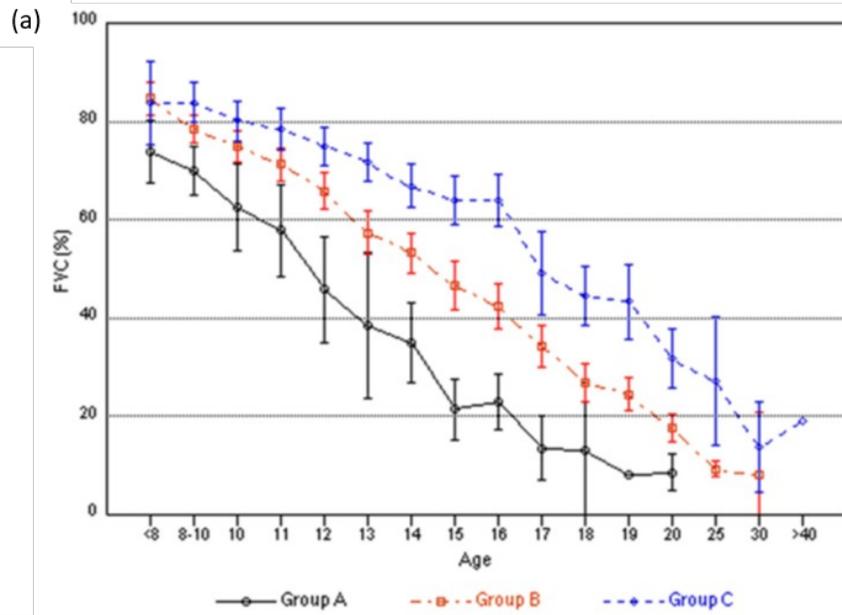
Figure 3: Forced vital capacity (FVC, in liters) with age in two brothers with DMD and variable pulmonary function. Each reached peak FVC around the age of 18, but patient 1 maintained FVC near the peak level, whereas his brother experienced a progressive decline after reaching peak FVC. Adapted from Birnkrant et al.,<sup>16</sup> and reproduced with permission.

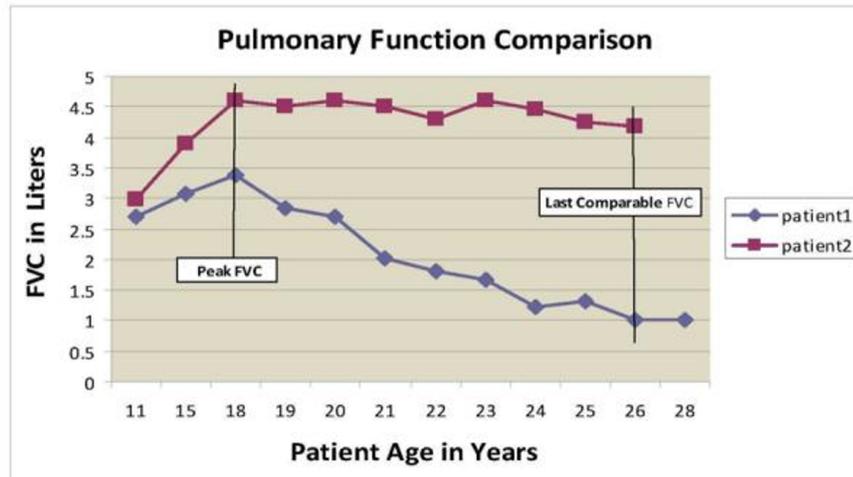
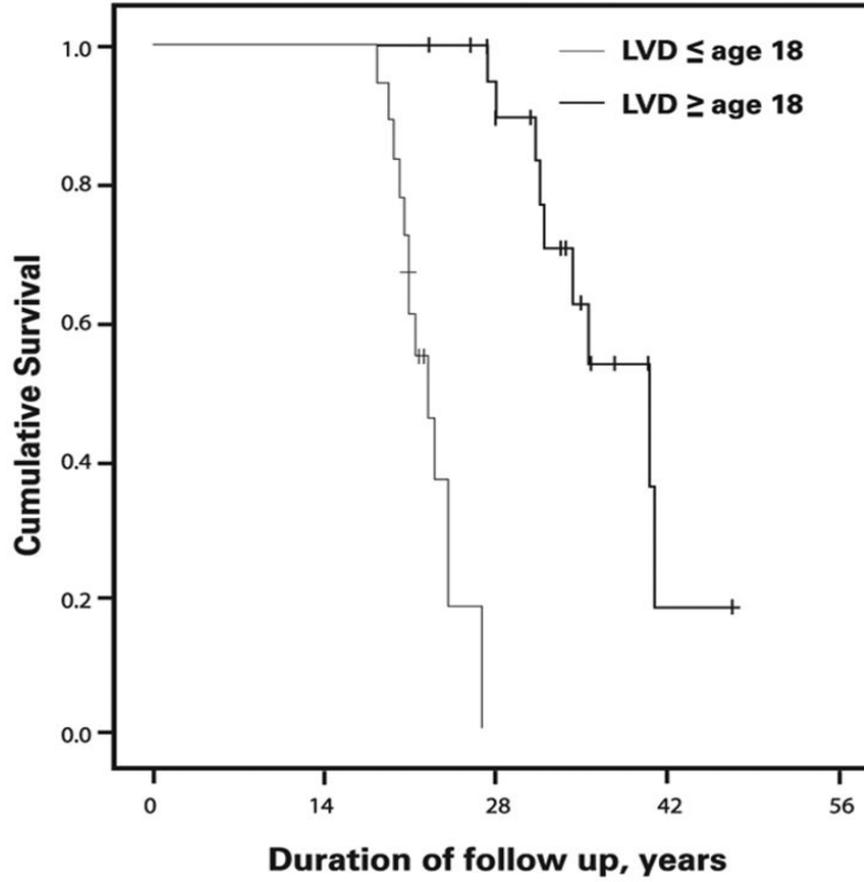
Figure 4: Changes in cardiac shortening fraction with age for patients with deletion of exon 44 (solid lines) and deletion of exon 51 (dashed lines). Despite identical dystrophin genotypes, patients experience variable timing in rates of decline in shortening fraction, but once the shortening fraction falls below 29% (solid horizontal line), the decline becomes steadier and more uniform. Adapted from Wang et al.,<sup>15</sup> and reproduced with permission.

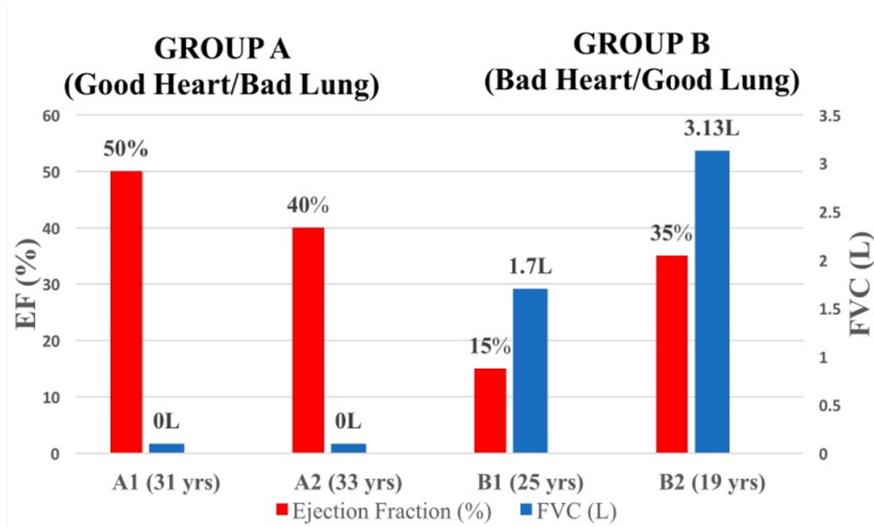
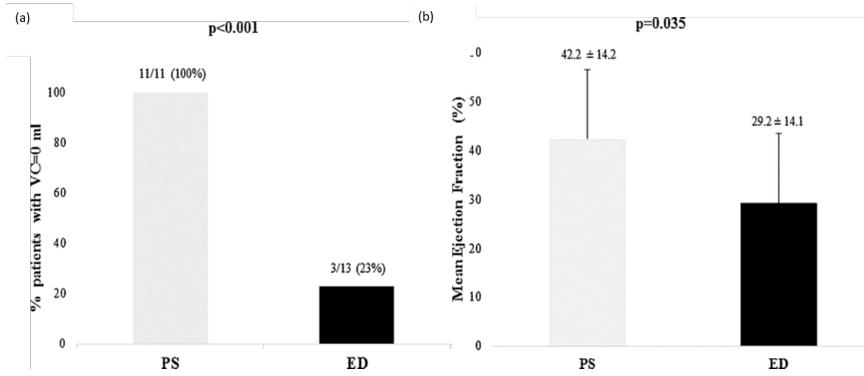
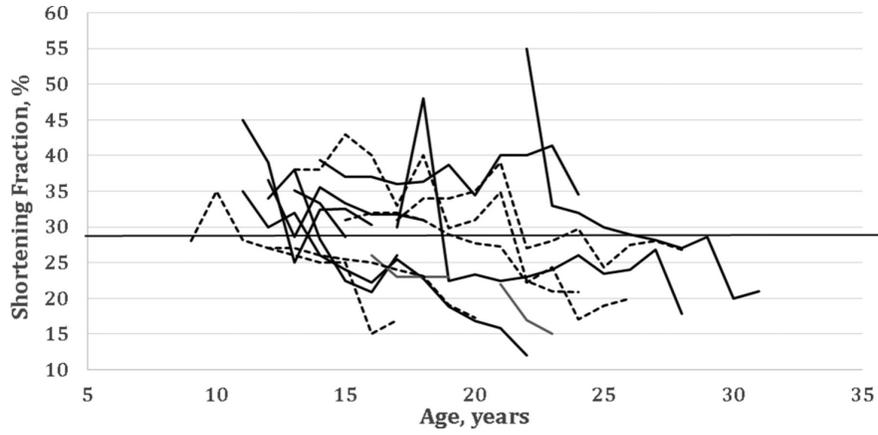
Figure 5: Cardiopulmonary phenotypic differences in patients with DMD who were prolonged survivors (PS) versus those who experienced early death (ED). (a) shows percentage of patients with a vital capacity of 0mL who experienced PS versus ED. (b) shows the mean ejection fraction in those with PS and ED. Adapted from Birnkrant et al.,<sup>20</sup> and reproduced with permission.

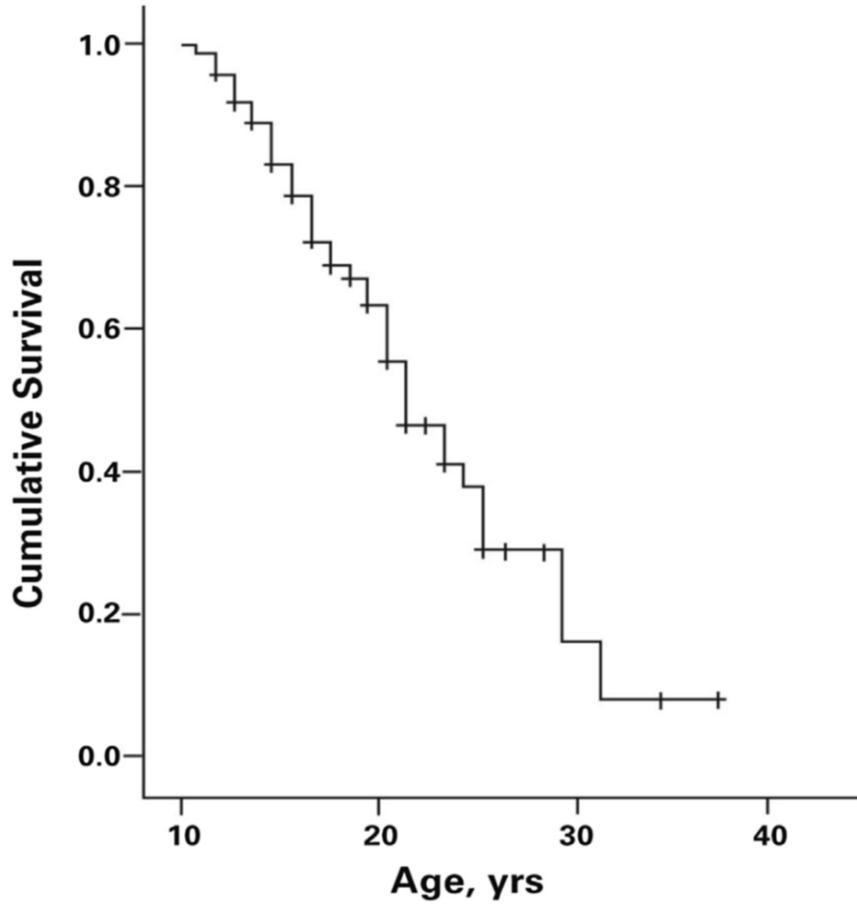
Figure 6: Phenotypic discordance in patients with identical dystrophin mutations. Four patients with deletion of exon 44 show diametrically different profiles in heart and lung function despite an identical dystrophin genotype. (A1, patient 1 in group A; A2, patient 2 in group A; B1, patient 1 in group B; B2, patient 2 in group B; EF, ejection fraction; FVC, forced vital capacity). Adapted from Jin et al.,<sup>17</sup> and reproduced with permission.

Figure 7: Survival curve showing time without cardiomyopathy on echocardiogram. Adapted from Ashwath et al.,<sup>18</sup> and reproduced with permission.









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