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Natural history of pulmonary function in collagen VI-related myopathies

A. Reghan Foley,[1] Susana Quijano-Roy,[2] James Collins,[3] Volker Straub,[4] Michelle McCallum,[4] Nicolas Deconinck,[5,6] Eugenio Mercuri,[7] Marika Pane,[7] Adele D'Amico,[8] Enrico Bertini,[8] Kathryn North,[9] Monique M. Ryan,[10] Pascale Richard,[11,12] Valérie Allamand,[12,13,14] Debbie Hicks,[4] Shireen Lamandé,[15] Ying Hu,[16] Francesca Gualandi,[17] Sungyoung Auh,[18] Francesco Muntoni[1] and Carsten G. Bönnemann[16]

1 Dubowitz Neuromuscular Centre, University College London Institute of Child Health and Great Ormond Street Hospital for Children, London, WC1N 1EH, UK
2 Garches Neuromuscular Centre (GNMH), Raymond Poincaré University Hospital (UVSQ), Garches, 92380, France
3 Neurology Division, Cincinnati Children's Hospital Medical Centre, Cincinnati, Ohio, 45229, USA
4 Institute of Genetic Medicine, International Centre for Life, University of Newcastle, NE1 3BZ, UK
5 Department of Neurology, Queen Fabiola University Children's Hospital, Free University of Brussels, ULB, Brussels, B-1000, Belgium
6 Neuromuscular Reference Centre, Ghent University Hospital, Ghent, B-9000, Belgium
7 Department of Paediatric Neurology, Catholic University, Rome, 00168, Italy
8 Laboratory of Molecular Medicine, Bambino Gesù Children's Hospital, Rome, 00165, Italy
9 Institute for Neuroscience and Muscle Research, Children's Hospital at Westmead, University of Sydney, Westmead, NSW 2145, Australia
10 Department of Neurology, Royal Children's Hospital, Murdoch Childrens Research Institute, University of Melbourne, Parkville, VIC 3052, Australia
11 AP-HP, Pitié-Salpêtrière Hospital Group, Cardiogenetics and Myogenetics Functional Unit, Metabolic Biochemistry Division, Paris, 75013, France
12 UPMC University of Paris 06, IFR14, Institute of Myology, Paris, 75013, France
13 CNRS, UMR7215, Paris, 75013, France
14 Inserm, U974, Paris, 75013, France
15 Muscular Dystrophy Research, Cell Biology, Development and Disease, Murdoch Childrens Research Institute, The Royal Children's Hospital, University of Melbourne, Parkville, VIC 3052, Australia
16 Neuromuscular and Neurogenetic Disorders of Childhood Section, Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, 20892, USA
17 Department of Medical Science, Section of Medical Genetics, University of Ferrara, Ferrara, 44100, Italy
18 Biostatistics Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, 20892, USA

Correspondence to: Carsten G. Bönnemann, MD
Chief, Neuromuscular and Neurogenetic Disorders of Childhood Section,
National Institute of Neurological Disorders and Stroke/NIH,
Bethesda, Maryland 20892 USA
E-mail: carsten.bonnemann@nih.gov

The spectrum of clinical phenotypes associated with a deficiency or dysfunction of collagen VI in the extracellular matrix of muscle are collectively termed 'collagen VI-related myopathies' and include Ullrich congenital muscular dystrophy, Bethlem myopathy and intermediate phenotypes. To further define the clinical course of these variants, we studied the natural history of pulmonary function in correlation to motor abilities in the collagen VI-related myopathies by analysing longitudinal forced vital capacity data in a large international cohort. Retrospective chart reviews of genetically and/or pathologically confirmed collagen VI-related myopathy patients were performed at 10 neuromuscular centres: USA ($n = 2$), UK ($n = 2$), Australia ($n = 2$), Italy ($n = 2$), France ($n = 1$) and Belgium ($n = 1$). A total of 486 forced vital capacity measurements obtained in 145 patients were available for analysis. Patients at the severe end of the clinical spectrum, conforming to the original description of Ullrich

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congenital muscular dystrophy were easily identified by severe muscle weakness either preventing ambulation or resulting in an early loss of ambulation, and demonstrated a cumulative decline in forced vital capacity of 2.6% per year ($P < 0.0001$). Patients with better functional abilities, in whom walking with/without assistance was achieved, were initially combined, containing both intermediate and Bethlem myopathy phenotypes in one group. However, one subset of patients demonstrated a continuous decline in pulmonary function whereas the other had stable pulmonary function. None of the patients with declining pulmonary function attained the ability to hop or run; these patients were categorized as intermediate collagen VI-related myopathy and the remaining patients as Bethlem myopathy. Intermediate patients had a cumulative decline in forced vital capacity of 2.3% per year ($P < 0.0001$) whereas the relationship between age and forced vital capacity in patients with Bethlem myopathy was not significant ($P = 0.1432$). Nocturnal non-invasive ventilation was initiated in patients with Ullrich congenital muscular dystrophy by 11.3 years (± 4.0) and in patients with intermediate collagen VI-related myopathy by 20.7 years (± 1.5). The relationship between maximal motor ability and forced vital capacity was highly significant ($P < 0.0001$). This study demonstrates that pulmonary function profiles can be used in combination with motor function profiles to stratify collagen VI-related myopathy patients phenotypically. These findings improve our knowledge of the natural history of the collagen VI-related myopathies, enabling proactive optimization of care and preparing this patient population for clinical trials.

Keywords: collagen VI-related myopathies; natural history; forced vital capacity; optimization of care; outcome measure

Abbreviations: CCC = concordance correlation coefficient; FVC = forced vital capacity

Introduction

The congenital muscular dystrophies are characterized by early-onset weakness, hypotonia and frequent joint contractures and are associated with dystrophic or myopathic-appearing muscle biopsy findings. Mutations in any of the three collagen-6 genes (*COL6A1*, *COL6A2* or *COL6A3*), coding for the three α-chains of collagen type VI, can affect the complex assembly and secretion of the resulting protein, leading to the absence or aberrant formation of collagen VI in the extracellular matrix of muscle and the associated spectrum of clinical phenotypes termed 'collagen VI-related myopathies'. Ullrich congenital muscular dystrophy [MIM 254090] is characterized by a combination of congenital onset muscle weakness, contractures of the proximal joints, hyperlaxity of the distal joints, progressive weakness and respiratory insufficiency (Ullrich, 1930) and results from recessive or dominantly acting mutations (Camacho Vanegas *et al.*, 2001; Pan *et al.*, 2003). Although the most severe patients do not achieve ambulation, the majority of patients with Ullrich congenital muscular dystrophy achieve ambulation but lose it by an average age of 10 years (Nadeau *et al.*, 2009; Brinas *et al.*, 2010). Patients with Ullrich congenital muscular dystrophy who did not achieve ambulation were also referred to as 'early severe' and those that did as 'moderate progressive' by Brinas *et al*. (2010). Bethlem myopathy (MIM 158810), a milder form of collagen VI-related myopathy, is characterized by slowly progressive muscle weakness and joint contractures. Although Bethlem myopathy typically follows autosomal dominant inheritance, autosomal recessive inheritance has also been described (Foley *et al.*, 2009; Gualandi *et al.*, 2009). Variable clinical manifestations of Bethlem myopathy range from a limb-girdle muscular dystrophy phenotype with proximal weakness in the absence of prominent contractures (Scacheri *et al.*, 2002) to 'myosclerosis', a condition of severe contractures with only mild weakness (Bradley *et al.*, 1973; Merlini *et al.*, 2008). The patients with collagen VI-related myopathy whose clinical phenotypes fall between Ullrich congenital muscular dystrophy and Bethlem myopathy can be categorized as having 'intermediate collagen VI-related myopathy' (Bonnemann, 2011), a category included within the 'mild early onset collagen VI myopathies' (Allamand *et al.*, 2010; Brinas *et al.*, 2010).

Robust natural history studies are essential for clarifying and validating phenotypic classifications, optimizing clinical care and preparing for clinical trials. The internationally recognized need for optimizing and standardizing care in the congenital muscular dystrophies (Wang *et al.*, 2010) along with the development of potential therapies for the collagen VI-related myopathies (Angelin *et al.*, 2007; Tiepolo *et al.*, 2009) and the recognition of the collagen VI-related myopathies as one of the most common forms of congenital muscular dystrophy (Okada *et al.*, 2007; Clement *et al.*, 2012) have highlighted the need for identifying relevant and viable outcome measures in this patient population.

Significant, progressive joint contractures complicate standardized assessments of motor function and muscle strength in patients with collagen VI-related myopathy (Bonnemann *et al.*, 2011). Previous reports and smaller case series have reported progressive respiratory failure as arguably the most important aspect of the natural history in this patient population given its relevance for disease progression, morbidity and mortality. Forced vital capacity (FVC) is a quantitative measure of pulmonary function, which can be reliably measured in patients over 6 years of age regardless of the severity of joint contractures. Thus serial FVC measurements have the potential to serve as a good tool for charting disease course, as demonstrated in the Duchenne muscular dystrophy population in whom respiratory insufficiency is also a leading cause of morbidity and mortality (Phillips *et al.*, 2001; Finder *et al.*, 2004). Furthermore, identifying distinct profiles of FVC decline may help in delineating the different phenotypes within the collagen VI-related myopathies.

Respiratory muscle function has not yet been studied in detail in a congenital muscular dystrophy population. However, a

correlation between FVC and volitionally as well as non-volitionally generated transdiaphragmatic and oesophageal pressures, as a measure of respiratory muscle strength, has been established in other neuromuscular disorders of childhood, indicating that FVC can serve as a reasonable indicator of global respiratory function (Nicot *et al.*, 2006).

A UK retrospective study of 13 patients with Ullrich congenital muscular dystrophy reported a pattern of early and invariable decline in pulmonary function beginning at 6 years of age (Nadeau *et al.*, 2009), and similar findings were recently reported from Japan in another 20 patients with Ullrich congenital muscular dystrophy (Yonekawa *et al.*, 2013). Nevertheless, a more comprehensive natural history study of respiratory insufficiency assessing a large cohort of patients with collagen VI-related myopathy has never been performed. Furthermore, the pulmonary function of patients with collagen VI-related myopathy falling in the mild-to-moderate end of the phenotypic spectrum has not been studied in detail. This group of patients is particularly important as they could be at high risk of both losing independent ambulation as well as developing respiratory insufficiency, which may not necessarily be linked to each other. This study thus evaluates longitudinal pulmonary function data (in the form of FVC) and correlates these to motor function to determine the natural history of pulmonary function and to clarify the clinical classifications within the collagen VI-related myopathies while consequently improving anticipatory care and outcome measure design.

Materials and methods

Patients

Clinical data of patients with genetically and/or pathologically confirmed diagnoses of collagen VI-related myopathies were reviewed at 10 different neuromuscular centres worldwide: USA ($n = 2$), UK ($n = 2$), Australia ($n = 2$), Italy ($n = 2$), France ($n = 1$) and Belgium ($n = 1$). Data were collected in accordance with ethical guidelines of each participating neuromuscular centre. Patients with pathogenic mutation/s in *COL6A1, COL6A2* or *COL6A3* and/or evidence of significantly decreased or mislocalized collagen VI on muscle biopsy immunohistochemical studies were included in this study. Spirometry techniques were performed according to international standards (Miller *et al.*, 2005).

The lead author (A.R.F.) collected data from the cohorts of patients followed at two participating centres (Great Ormond Street Hospital for Children, London, UK and The Children's Hospital of Philadelphia, Philadelphia, USA) and coordinated the collection of data from collaborators at the other eight centres.

Owing to the fact that FVC data were collected retrospectively from 10 different neuromuscular centres spanning six countries and three continents, spirometry machines varied. Per cent predicted FVC values were derived from the reference equations specific to the spirometry machines used. We attempted to gather 'raw' FVC data (FVC in litres) corresponding to each per cent predicted FVC value provided, with the goal of using the same formula for converting 'raw' FVC data into per cent predicted values. We discovered, however, that 'raw' FVC data (in litres) was not available for a large number of FVC measurements. Rather than discard a large number of FVC values, we decided to analyse the per cent predicted FVC values provided by each centre as derived from the respective spirometry machines.

Statistical analysis

Linear mixed models were used to examine the effect of an independent variable (or variables) of interest on FVC. The analysis was based on multiple FVC values per subject. A working covariance structure was assumed as intra-class correlation covariance structure to take into account correlations among different number of FVC values per subject. Analyses were implemented in SAS (SAS institute Inc.) using PROC Mixed (Littell, 1996) to conduct linear mixed models and PROC Lifetest to generate graphs for time to events data. A concordance correlation coefficient (CCC), proposed by Vonesh *et al*. (1996) for a goodness-of-fit measure in linear mixed model, was calculated to check the adequacy of the linear mixed models. Summary statistics for FVC were described by using mean $\pm$ standard deviation. All statistical tests were conducted with a significance level of 0.05.

Results

Demographics

Our cohort totalled 211 patients with collagen VI-related myopathy originating from 10 international neuromuscular centres. One hundred and forty-five patients had FVC measurements on record, ranging from 1 to 14 FVC measurements (mean = 3.35; median = 3) per patient and totalling 486 FVC measurements. Of these 145 patients, 80 (55%) were male, 65 (45%) were female (Supplementary material) and ranged in age from 4 years to 63 years (mean = 18 $\pm$ 11.6) at the time of evaluation. Three patients with longitudinal FVC measurements were deceased at the time of the study, having succumbed to respiratory infections at the ages of 10, 15 and 23 years (and with evidence of concomitant under-use of non-invasive ventilation in the younger two patients). Confirmed pathogenic mutations were identified in *COL6A1*, *COL6A2* or *COL6A3* in 138 patients (95%) (Supplementary material) and muscle biopsy immunohistochemical evidence of significantly decreased, mislocalized or absent collagen VI was found in seven patients (5%) (Supplementary material); pending molecular genetic confirmation. FVC data from 12 patients followed at the Dubowitz Neuromuscular Centre, London (UK) and 13 patients followed at the Neuromuscular Centre, Garches (France) have been described previously (Nadeau *et al.*, 2009; Brinas *et al.*, 2010).

Entire cohort analysis

We initially sought to evaluate data from the cohort as a whole. This analysis revealed that the relationship between age and FVC (in 145 patients) was highly significant ($P < 0.0001$), demonstrating a decline in FVC of 0.70% per year [95% confidence interval (CI): $-0.010 - -0.004$), $P < 0.0001$; CCC = 0.9542].

Correlation with motor function

Of the 486 FVC measurements analysed, corresponding maximal motor ability was available for 475 values (98%; recorded in 140 of

the 145 patients with FVC data). Even when analysed before clinical classification, the relationship between maximal motor ability and FVC was highly significant ($P < 0.0001$), with the distribution of FVC measurements demonstrating a direct relationship with motor ability (Fig. 1A). The relationship between age and FVC within maximal motor ability categories was also highly significant with those patients whose maximal motor ability was sitting demonstrating a decline in FVC of 4.2% per year (95% CI: −0.057–−0.027, $P < 0.0001$; CCC = 0.92), those whose maximal motor ability was walking only with assistance demonstrating a decline in FVC of 2.1% per year (95% CI: −0.032–−0.017, $P = 0.0003$; CCC = 0.90), those who achieved walking independently with a decline in FVC of 0.6% per year (95% CI: −0.009–−0.002, $P = 0.0016$; CCC = 0.95) and those who achieved running demonstrating a cumulative increase in FVC of 1.2% per year (95% CI: 0.003–0.022, $P = 0.0134$; CCC = 0.65) (Fig. 1B).

Clinical classifications

Patients with Ullrich congenital muscular dystrophy were easily identified from the cohort by a congenital onset of symptoms including a severe degree of muscle weakness and proximal contractures either preventing ambulation or resulting in an early loss of ambulation (by an average age of 10 years). Seventy-five patients (52%) were categorized as having Ullrich congenital muscular dystrophy, encompassing those patients whose maximal motor function was sitting (corresponding to 'early severe type'; Brinas *et al.*, 2010), walking with assistance or walking independently with early loss of ambulation (corresponding to 'moderate progressive type'; Brinas *et al.*, 2010). Due to differences among neuromuscular centres in clinically categorizing patients as intermediate collagen VI-related myopathy versus Bethlem myopathy, the patients designated to either of these collagen VI-related myopathy subtypes were initially studied as one group. When FVC measurements were plotted in this group to evaluate FVC patterns over time, two clear subgroups emerged: one group whose FVC values demonstrated continuous decline beginning at ~7 years of age and another group whose FVC values either remained stable or improved over time (without a pattern of progressive decline). Correlating motor functional abilities with pulmonary function, none of the patients with progressive decline in pulmonary function (as measured by FVC) achieved the ability to run or hop; these patients were then consistently assigned to the phenotypic category of intermediate collagen VI-related myopathy. The group of patients with either stable or improving FVC values all achieved the ability to walk independently and typically achieved the ability to run or hop and were assigned to the phenotypic category of

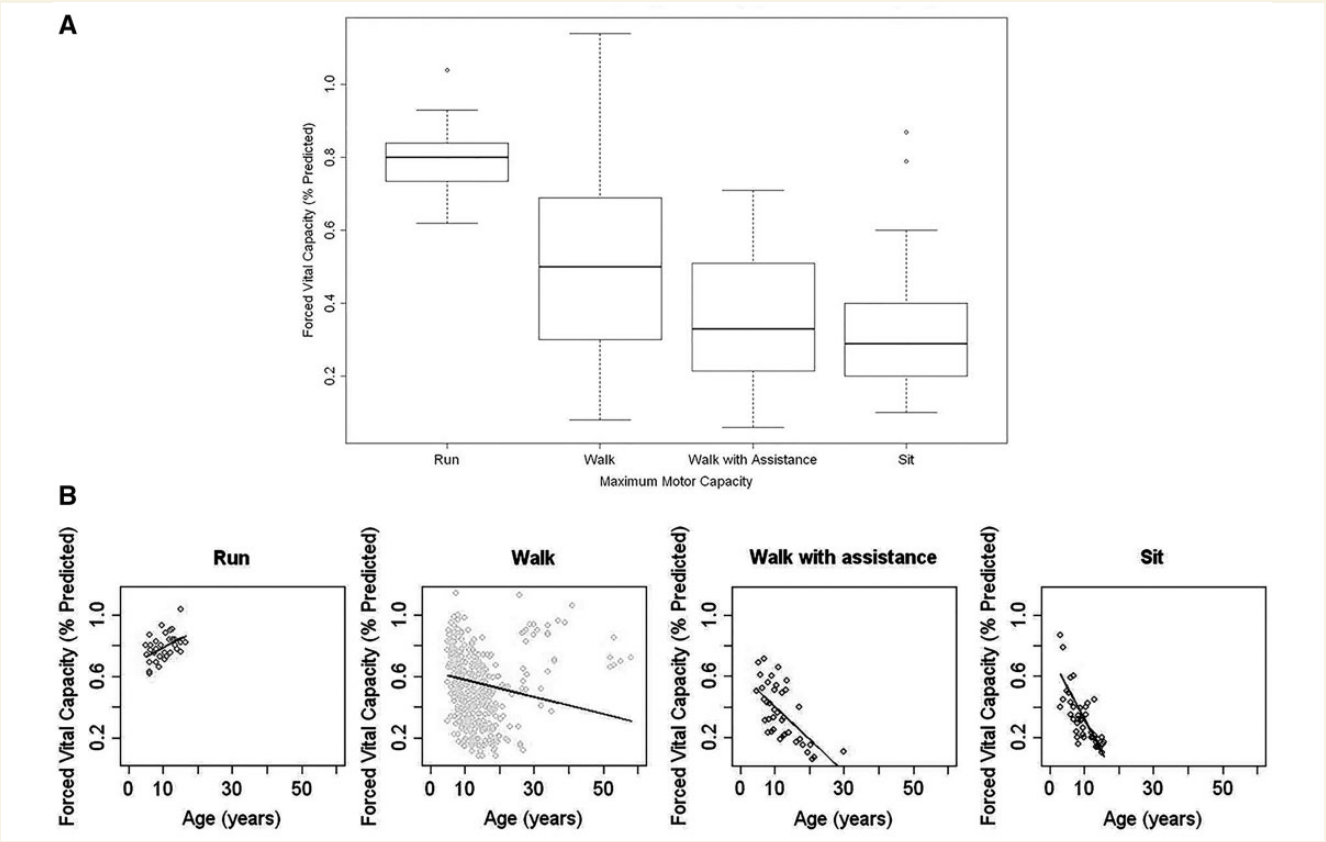

Figure 1 Correlation with motor function. (**A**) Box-plot demonstrating distribution of FVC measurements corresponding to different maximum motor functional abilities. Run ($n = 13$): box range: 0.73–0.84; whiskers: 0.62–1.04. Walk ($n = 105$): box range: 0.30–0.69; whiskers: 0.08–1.14. Walk with assistance ($n = 11$): box range: 0.21–0.51; whiskers: 0.06–0.71. Sit ($n = 11$): box range: 0.20–0.40; whiskers: 0.10–0.87. (**B**) Profiles of decline of FVC corresponding to different maximum motor functional abilities.

Bethlem myopathy. In total, 27 patients (19%) were categorized as intermediate and 43 (30%) as Bethlem myopathy.

The relationship between FVC and collagen VI-related myopathy phenotype was highly significant ($P < 0.0001$) with the distribution of FVC values demonstrating a direct relationship with severity of clinical phenotype (Fig. 2A). The relationship between age and FVC for Ullrich congenital muscular dystrophy and intermediate patients was also highly significant ($P < 0.0001$) with patients with Ullrich congenital muscular dystrophy demonstrating a decline in FVC of 2.6% per year (95% CI: −0.031–−0.021, $P < 0.0001$; CCC = 0.92) and intermediate patients a decline in FVC of 2.3% per year (95% CI: −0.030–−0.015, $P < 0.0001$; CCC = 0.92]. In contrast, the relationship between age and FVC in patients with Bethlem myopathy was not significant ($P = 0.1432$) (Fig. 2B).

Although the FVC data within the Ullrich congenital muscular dystrophy and intermediate phenotypic subtypes demonstrated patterns of continued decline (without evidence of stepwise decline), we felt that studying the rates of decline in FVC corresponding to 5–15 years of age, a clinically relevant age range for decline in both respiratory and motor function, would provide data which could be instrumental for future clinical trial planning. This subanalysis revealed that between 5 and 15 years of age FVC declined in patients with Ullrich congenital muscular dystrophy by 3.5% per year (95% CI: −0.044–−0.028, $P < 0.0001$; CCC = 0.92) whereas FVC declined in patients with intermediate collagen VI-related myopathy by 1.7% per year (95% CI: −0.031–−0.002, $P = 0.0260$; CCC = 0.91). Again, the relationship between age and FVC in patients with Bethlem myopathy was not significant ($P = 0.7261$). Further age stratification analyses revealed statistically significant trends only in patients with Ullrich congenital muscular dystrophy, revealing a decline in FVC of 4.2% per year for age ⩾5 years and ⩽10 years ($P < 0.0001$), 2.9% per year for age ⩾11 years and ⩽15 years ($P < 0.0001$) and 2.5% per year for age ⩾16 years and ⩽20 years ($P = 0.0164$).

Non-invasive ventilation and loss of ambulation

Of the 75 patients with Ullrich congenital muscular dystrophy evaluated, 44 (59%) had initiated non-invasive bilevel positive pressure ventilation at the time of this study. The average age at initiation of nocturnal non-invasive ventilation was 11.3 years (±4.0 years) with an average FVC of 34% just before non-invasive ventilation initiation. Of 27 patients with intermediate collagen VI-related myopathy evaluated, three (11%) had started nocturnal non-invasive ventilation at the time of the study at an average age of 20.7 years (±1.5 years) with corresponding FVC

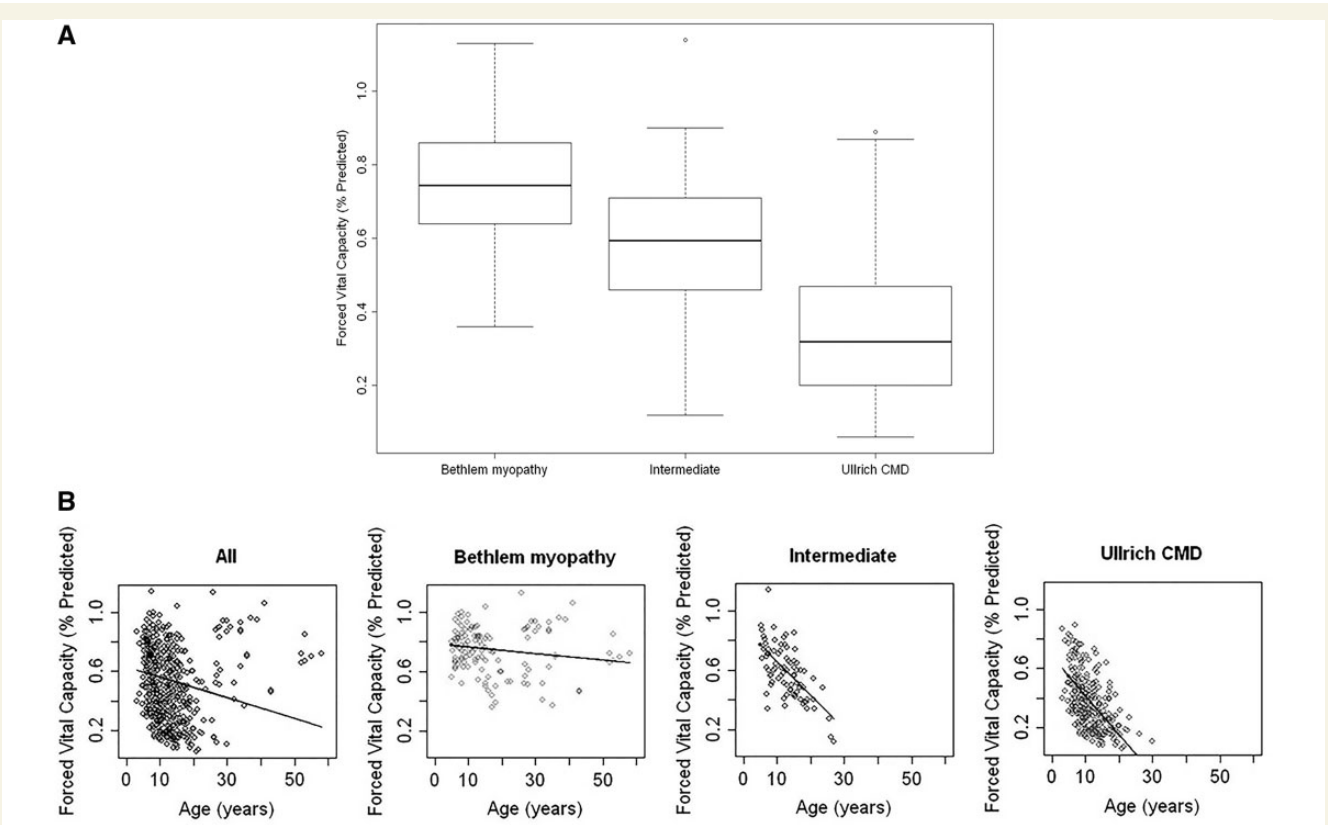

Figure 2 Correlation with clinical classifications. (**A**) Box plot demonstrating the distribution of FVC measurements corresponding to the different collagen VI-related myopathy phenotypic categories. Bethlem myopathy: box range: 0.64–0.86; whiskers: 0.36–1.13. Intermediate: box range: 0.46–0.71; whiskers: 0.12–1.14. Ullrich congenital muscular dystrophy: box range: 0.20–0.47; whiskers: 0.06–0.89. (**B**) Profiles of decline of FVC for all collagen VI-related myopathy patients studied and within the collagen VI-related myopathy phenotypes of Bethlem myopathy, intermediate collagen VI-related myopathy and Ullrich congenital muscular dystrophy.

values of 41%, 50% and 60% values just before non-invasive ventilation initiation. Only 1 (2%) of 43 patients with Bethlem myopathy evaluated required nocturnal non-invasive ventilation, which was initiated at age 41 years. Kaplan Meier curves depicting ventilation-free probability demonstrated a statistically significant ($P = 0.006$) difference between Ullrich congenital muscular dystrophy and intermediate patients, with 50% of patients with Ullrich congenital muscular dystrophy on nocturnal non-invasive ventilation by 11.0 years of age and 50% of patients with intermediate collagen VI-related myopathy on nocturnal non-invasive ventilation by 21.5 years of age (Fig. 3).

Loss of ambulation as defined by full-time wheelchair dependence in those patients who had attained independent ambulation occurred in 50 patients at the time of this study (44 patients with Ullrich congenital muscular dystrophy and 6 intermediate). Kaplan Meier curves depicting the probability of independent ambulation revealed that 50% of patients with Ullrich congenital muscular dystrophy lost ambulation by 10 years of age whereas 50% of patients with intermediate collagen VI-related myopathy lost ambulation by 19 years of age (Fig. 4).

Discussion

Of the two patients described by Otto Ullrich in his 1930 original report of what later became known as Ullrich congenital muscular dystrophy, one died of respiratory complications at 2 years of age (Ullrich, 1930). McMenamin *et al.* (1982) reported that in their series of 24 patients with congenital muscular dystrophy without subtype classification, six (25%) died of respiratory failure. In the ensuing years, respiratory decompensation during childhood or adolescence, even in the setting of relatively stable muscle weakness, has become a well-recognized clinical feature of the congenital muscular dystrophies (Wallgren-Pettersson *et al.*, 2004) with appropriate anticipation of this possible decompensation of great importance for clinical care (Wang *et al.*, 2010).

This study of longitudinal FVC measurements in 145 patients with genetically and/or pathologically confirmed collagen VI-related myopathy is the most extensive data set of its kind obtained in any congenital onset muscle disorder. The findings of this study substantially add to available natural history data of pulmonary function in this patient population in which respiratory insufficiency is a leading

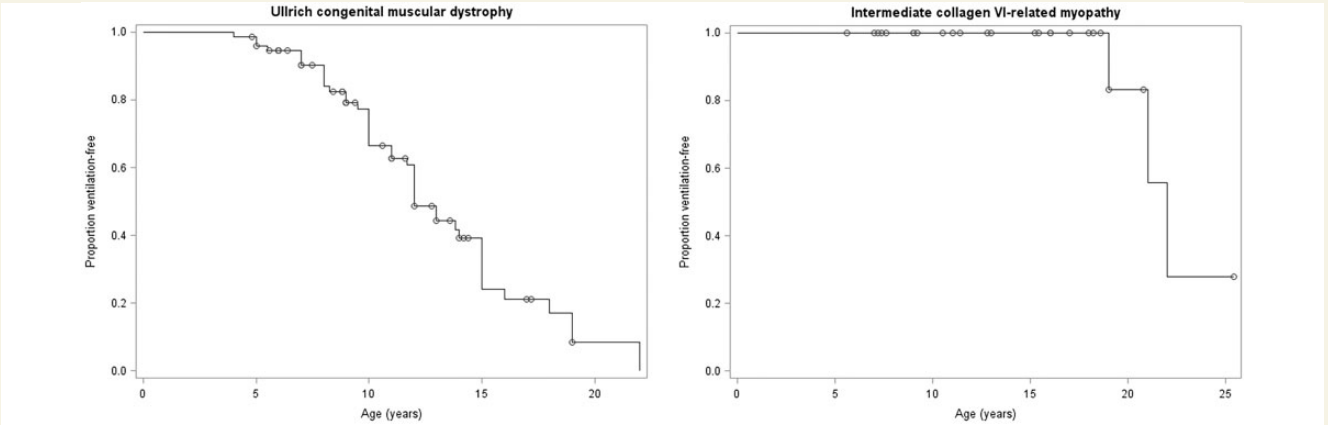

Figure 3 Kaplan-Meier curves depicting ventilation-free status in patients with Ullrich congenital muscular dystrophy (*left*) and patients with intermediate collagen VI-related myopathy (*right*).

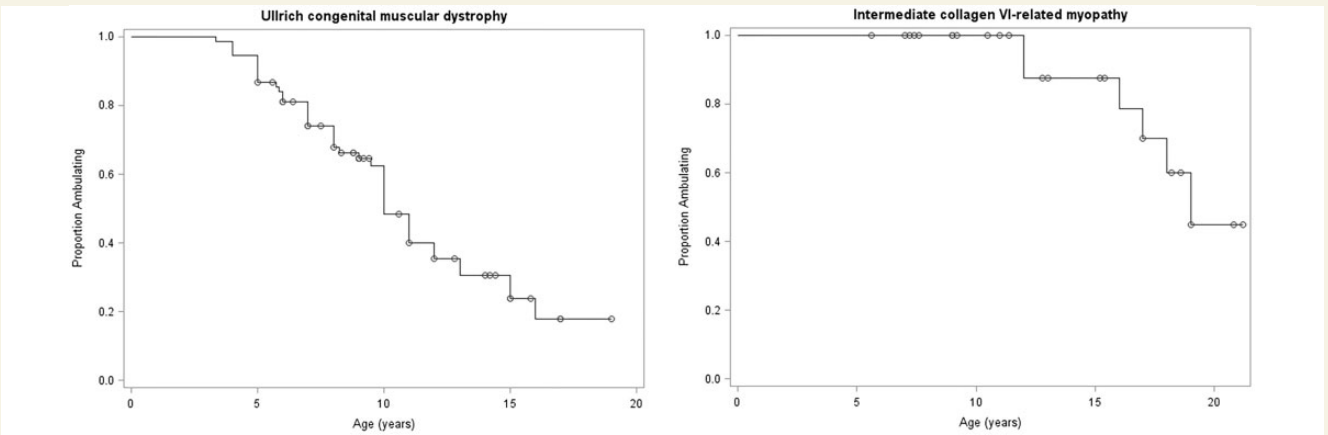

Figure 4 Kaplan-Meier curves depicting independent ambulation in patients with Ullrich congenital muscular dystrophy (*left*) and patients with intermediate collagen VI-related myopathy (*right*).

Table 1 Phenotypic stratification of the collagen VI-related myopathies

	Ullrich congenital muscular dystrophy	Intermediate collagen VI-related myopathy	Bethlem myopathy
Ambulation	Never walk or lose ambulation by ~10 years	Ambulation lost by ~19 years	Walk until adulthood. May need walking aid by 40s/50s
Pulmonary function	Decline in pulmonary function is early and invariable: 2.6% per year with an average age of onset of non-invasive ventilation of 11 years	Decline in pulmonary function starts slightly later than in Ullrich congenital muscular dystrophy, but proceeds at a similar rate: 2.3% per year with an average age of onset of non-invasive ventilation of 21 years	Decline in pulmonary function is variable and does not occur until adulthood (typically after age 40 years)

cause of morbidity and mortality. Furthermore, this study indicates that profiles of decline in FVC in combination with motor function profiles can be used to stratify the phenotypes of patients with collagen VI-related myopathy into the categories of Ullrich congenital muscular dystrophy, intermediate collagen VI-related myopathy and Bethlem myopathy in a clinically meaningful way (Table 1). The resulting proposed clinical classification system of collagen VI-related myopathy is unique in that it is based on the integration of both motor function and pulmonary function criteria.

Our data suggest that among patients with collagen VI-related myopathy, pulmonary function declines in a fashion parallel to decline in motor function/muscle strength. Within the Ullrich congenital muscular dystrophy group, primarily non-ambulant children (the 'early severe type' in Brinas *et al*., 2010) had a worse decline in FVC (4.2% per year) compared with the children who achieved ambulation with assistance (the 'moderate progressive type' in Brinas *et al*., 2010) (2.1% per year), who in turn had a worse decline in FVC compared with all independent ambulators. Furthermore, the observation that patients with intermediate collagen VI-related myopathy demonstrate an onset of decline in pulmonary function—and ultimate dependence on nocturnal non-invasive ventilation—later than patients with Ullrich congenital muscular dystrophy, follows the motor profiles of these patients in whom loss of ambulation also occurs later in patients with intermediate collagen VI-related myopathy compared to patients with Ullrich congenital muscular dystrophy (Fig. 4). It is important to note that for patients with intermediate collagen VI-related myopathy and some with Ullrich congenital muscular dystrophy, a significant decline in pulmonary function and the necessity for nocturnal non-invasive ventilation can occur while patients remain ambulant. This pattern is in contrast to patients with Duchenne muscular dystrophy, in whom a significant decline in pulmonary function with the need for the initiation of non-invasive ventilation occurs after the loss of ambulation (Rideau *et al.*, 1981; Bushby *et al.*, 2010), and underscores how clinical findings established for one form of muscular dystrophy cannot automatically be extrapolated to another because of relative differences in muscle involvement (including the degree of diaphragmatic, thoracic and paraspinal muscle involvement) and pathophysiology.

The parallel profiles of decline in pulmonary and motor function within the Ullrich congenital muscular dystrophy phenotype and within the intermediate phenotype suggest that the decline of pulmonary function is primarily driven by progressive muscle weakness and likely further aggravated by resulting spinal and thoracic deformities. This assumption would be consistent with the restrictive pulmonary function pattern seen in the collagen VI-related myopathies (Laghi and Tobin, 2003). Studies of pulmonary function in neuromuscular diseases have demonstrated that once respiratory muscle strength has been reduced by >50%, loss in vital capacity can be exacerbated by decreased compliance of the chest wall and the lungs (Misuri *et al.*, 2000), thereby also increasing the so-called 'load' against which the already weak respiratory muscles have to work (Panitch, 2010) and eventually leading to respiratory failure in patients with muscular dystrophy (Smith *et al.*, 1987). An additional factor contributing to progressive pulmonary function decline in patients with congenital muscular dystrophy is thoracic deformity related to severe scoliosis (Takaso *et al.*, 2010), as is frequently seen in patients with collagen VI-related myopathy. Furthermore, costovertebral joint stiffening can occur in neuromuscular patients, as a result of weakened inspiratory muscles and may also contribute to pulmonary function decline.

In young patients with collagen VI-related myopathy with degrees of muscle weakness either entirely preventing ambulation or enabling only assisted ambulation by the age of 7 years, a diagnosis of Ullrich congenital muscular dystrophy can be made based on motor function alone. Given the invariable progressive decline in pulmonary function demonstrated by our data in this patient group, careful surveillance of pulmonary function should directly follow the establishment of this diagnosis. In patients with collagen VI-related myopathy who achieve independent ambulation, however, the phenotypic distinction between Ullrich congenital muscular dystrophy, intermediate collagen VI-related myopathy and Bethlem myopathy can be challenging, particularly in young children whose future motor function can be difficult to predict or in teenagers who remain ambulant. While ambulant patients with Ullrich congenital muscular dystrophy typically lose ambulation by an average age of 10 years [ranging from as early as preschool years up to puberty; and 18% never acquire ambulation Brinas *et al.* (2010)], patients with intermediate collagen VI continue ambulating into the late teenage years and early adult years, and patients with Bethlem myopathy continue ambulating into older adult years. As our study demonstrates, FVC continues to decline in intermediate collagen VI-related myopathy patients at an average rate of 2.3% per year with the an average age of nocturnal non-invasive ventilation dependence of 21 years.

Accordingly, in patients with collagen VI-related myopathy of early onset and prolonged ambulation who do not achieve the ability to

hop or run, a diagnosis of intermediate phenotype should be established and pulmonary function carefully monitored prospectively. However, patients with Bethlem myopathy do not follow a clear pattern of decline in pulmonary function over time. While some patients with Bethlem myopathy demonstrate a progressive decline in pulmonary function, ultimately necessitating the initiation of nocturnal non-invasive ventilation, this study demonstrates that this is rare and does not occur until after 40 years of age.

A recent study of 49 patients with 'early onset' collagen VI-related myopathy used motor function alone to classify patients as 'early severe', 'moderate progressive' and 'mild' (Brinas *et al.*, 2010). In the analysis reported here, we have maintained one Ullrich congenital muscular dystrophy category encompassing both non- and transient ambulators to focus on delineating between patients with Ullrich congenital muscular dystrophy, intermediate collagen VI-related myopathy and Bethlem myopathy, as seen in our phenotypic stratification based on both pulmonary function and motor function (Table 1). FVC measurements for patients categorized as 'mild' in the French study ranged from 35% to 82% (Brinas *et al.*, 2010), which compared with our data indicates that this category may contain both patients with intermediate collagen VI-related myopathy and patients with Bethlem myopathy.

The criteria for initiation of non-invasive ventilation for 'progressive neuromuscular disease' proposed by the American College of Chest Physicians include 'maximal inspiratory pressures <60 cm/H_2O or FVC $<50\%$ predicted' (1999). Our study demonstrates an average FVC value of 34% just before the onset of non-invasive ventilation in patients with Ullrich congenital muscular dystrophy at 11.3 years (± 4.0 years; based on 44 patients with Ullrich congenital muscular dystrophy). Of note, a recent questionnaire-based study from Japan reported markedly similar findings (based on 13 patients from an identified cohort of 33 patients with Ullrich congenital muscular dystrophy, 19 of whom had molecular confirmation in addition to pathological confirmation), with an estimated average per cent predicted vital capacity of 36% at the time of initiation of non-invasive ventilation at 11.2 ± 3.6 years (Yonekawa *et al.*, 2013). This supports a remarkable consistency of the Ullrich congenital muscular dystrophy pulmonary phenotype across studies and populations, even accounting for different approaches to data acquisition. The number of patients with intermediate collagen VI-related myopathy who initiated non-invasive ventilation in our cohort was relatively small (three patients) and had FVC measurements preceding non-invasive ventilation of 41%, 50% and 60%.

Polysomnography with CO_2 monitoring is an essential complement to spirometric pulmonary function testing, and we propose that it should be performed to confirm the presence of sleep hypoventilation/the need for non-invasive ventilation in patients with FVC values $<50\%$ predicted or with symptoms and signs of sleep disordered breathing, and repeated annually so that non-invasive ventilation parameters can be adjusted as needed. Continuous prospective data acquisition in this patient population will be necessary to refine this approach by, for instance, taking the differential between upright and supine FVC into account.

If indeed skeletal muscle weakness is the primary aetiology of the relentless decline in FVC observed in patients with Ullrich congenital muscular dystrophy and patients with intermediate collagen VI-related myopathy, then therapeutic interventions aimed at slowing the progression of muscle weakness, and the resulting thoracic/pulmonary changes, might help to reduce the decline in pulmonary function in this patient population. Future clinical trials in collagen VI-related myopathies should be designed to include an age range during which time pulmonary function demonstrates significant change. As our study demonstrates, patients with Ullrich congenital muscular dystrophy experience their steepest rate of decline in FVC between 5–10 years of age (4.2% per year). By defining the natural history of pulmonary function in the collagen VI-related myopathies, this study promotes an optimization of care and a potential reduction in respiratory morbidity. Furthermore, by identifying FVC as a relevant and viable outcome measure in this patient population, this study improves clinical trial readiness for the collagen VI-related myopathies.

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Supplementary material

Supplementary material is available at *Brain* online.

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