Original research

Pulmonary function and structure abnormalities in children and young adults with osteogenesis imperfecta point to intrinsic and extrinsic lung abnormalities

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ABSTRACT

Purpose Pulmonary disease is the major cause of morbidity and mortality in osteogenesis imperfecta (OI). We investigated the contribution of intrinsic lung factors to impaired pulmonary function in children and young adults with OI types III, IV, VI.

Methods Patients with type III (n=8), IV (n=21), VI (n=5), VII (n=2) or XIV (n=1) OI (mean age 23.6 years) prospectively underwent pulmonary function tests (PFTs) and thoracic CT and radiographs.

Results PFT results were similar using arm span or ulnar length as height surrogates. PFTs were significantly lower in type III than type IV or VI OI. All patients with type III and half of type IV OI had lung restriction; 90% of patients with OI had reduced gas exchange. Patients with *COL1A1* variants had significantly lower forced expiratory flow (FEF)25%–75% compared with those with *COL1A2* variants. PFTs correlated negatively with Cobb angle or age. CT scans revealed small airways bronchial thickening (100%, 86%, 100%), atelectasis (88%, 43%, 40%), reticulations (50%, 29%, 20%), ground glass opacities (75%, 5%, 0%), pleural thickening (63%, 48%, 20%) or emphysema (13%, 19%, 20%) in type III, IV or VI OI, respectively.

Conclusion Both lung intrinsic and extrinsic skeletal abnormalities contribute to OI pulmonary dysfunction. Most young adult patients have restrictive disease and abnormal gas exchange; impairment is greater in type III than type IV OI. Decreased FEF25%–75% and thickening of small bronchi walls indicate a critical role for small airways. Lung parenchymal abnormalities (atelectasis, reticulations) and pleural thickening were also detected. Clinical interventions to mitigate these impairments are warranted.

Trial registration number NCT03575221.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Osteogenesis imperfecta (OI) is a heterogeneous group of inherited bone dysplasias characterised by skeletal and extraskeletal manifestations.
⇒ Respiratory disease is the leading cause of morbidity and mortality in patients with OI.
⇒ Despite previous thought that lung disease in OI was secondary to scoliosis and other skeletal deformities, research in a cohort of young children with OI showed significant decline in pulmonary function with age, independent of scoliosis.
⇒ Genetically modified mice with collagen type 1 defects also display cardiopulmonary dysfunction independent of skeletal abnormalities.

WHAT THIS STUDY ADDS

⇒ Our findings expand the phenotype of respiratory disease in OI to young adults, demonstrating restrictive disease and impaired gas exchange in most patients independent of skeletal abnormalities, such as scoliosis, using pulmonary function tests (PFTs).
⇒ Analysis of chest computed tomography (CCT) and PFT—which showed highly prevalent small airway bronchial thickening and decreased FEF25%–75%, indicating reduced airflow through the small airways, respectively—indicate a role of small airway abnormalities in the pathobiology of OI respiratory disease.
⇒ We also provide the first demonstration that arm span and ulnar length measurements are comparable height surrogates for calculating and interpreting PFT results in patients with OI.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings of this study provide critical insight into origin, progression and manifestations of pulmonary disease in OI that may lead to improved treatment options and therapies for patients before they impact quality of life.

INTRODUCTION

Osteogenesis imperfecta (OI), a heterogeneous group of inherited skeletal dysplasias, is a collagen-related disorder.[1–3] The hallmarks of OI are bone fragility, leading to ease of fracture, skeletal deformities and growth deficiency.[1 2] OI is also a generalised connective tissue disorder with defects in extraskeletal organs.[1] Notably, respiratory disease is a leading cause of morbidity and mortality in OI, either directly or indirectly, and respiratory symptoms negatively impact quality of life.[4–7] An examination of patients with OI in a comprehensive Danish registry revealed cardiopulmonary dysfunction as the highest cause of morbidity and mortality in OI.[5]

For many years, the prevailing view was that restrictive lung disease in OI was secondary to scoliosis and chest wall deformity, and eventually led to cardiac failure. Pulmonary function tests (PFTs) in adult patients with OI dropped sharply when scoliosis curves exceeded 30°.[8] In addition to respiratory failure and chronic ventilatory defects

secondary to severe vertebral and rib cage deformities, individuals with OI are susceptible to respiratory infections and bronchiectasis.[1 4 5] The first clear indications of bone-independent pulmonary disease in OI came from both genetically modified mice with a dominantly inherited collagen defect, the *Aga2* mouse and children with types III or IV OI.[9] In a cohort of 46 children with OI, we reported that PFT-derived total lung capacity (TLC), vital capacity and forced vital capacity (FVC) decline with age. Importantly, when children without scoliosis were analysed separately, significant declines in PFTs with age occurred. Later, a murine study of recessive type VII OI demonstrated distortion of normal lung architecture in the *Crtap*$^{-/-}$ mouse.[10 11]

Thus, lung function measurements and an understanding of the mechanism of lung dysfunction in OI are important for the clinical management of patients with OI and as a basis for devising approaches to improve care. Given the presence of type I collagen in lung parenchyma and airways,[12] it is perhaps not surprising that intrinsic abnormalities would be part of OI lung pathology.

Here, we expand the pulmonary phenotype of children and extend our investigation to young adults with different OI types. Most patients in our cohort had classical OI, with moderately severe type IV (OMIM 166220) or severe progressive type III OI (OMIM 259420), which are characterised by defective structure or quantity of type I collagen.[1 13 14] Some participants had type VI (OMIM 613982), type VII (OMIM 610682) or type XIV (OMIM 610682) OI, recessive forms caused by null mutations in *SERPINF1, CRTAP* or *TMEM38B*, respectively.[1 3 15 16] Types VII and XIV have defects in collagen post-translational modification. We hypothesised that abnormalities of pulmonary function in OI are not entirely due to scoliosis or abnormal thoracic shape, but rather, that there is an intrinsic pulmonary parenchymal component to this clinically significant secondary feature of OI. We identified structural abnormalities in the lungs and pleura independent of bony thoracic defects. This prospective observational cohort study further defines functional and structural abnormalities of the airways, pulmonary tissue or pleura in patients with OI and broadens our understanding of clinical features of lung disease in OI.

MATERIALS AND METHODS

Study population

All subjects were diagnosed with OI as described.[17] Genotyping was performed for all subjects (see online supplemental table 1). Thirty-seven individuals with moderate-to-severe OI were evaluated prospectively at the NIH Clinical Center from 2018 through 2022 (Clinical Trials NCT03575221; A Natural History of the Collagen-Related Disorder Osteogenesis Imperfecta and the Genotype-Phenotype Correlation). A respiratory questionnaire (see online supplemental material) was administered by SKT to each subject or parents of children orally or in writing about histories of smoking, prior respiratory infections, chronic respiratory disease diagnoses, cough, use of walking aids or dyspnoea. Since these were patient-reported results, they were necessarily subjective and reflected the voices of the patients about their respiratory quality of life. Key questionnaire results were correlated with the presence or absence of restrictive lung disease in patients with type IV OI, who comprised the largest subgroup. Due to the complexity and variability of questionnaire responses, correlations were not performed with CT scans.

Pulmonary function testing

Spirometry, lung volumes by nitrogen washout and diffusion capacity studies were performed in accordance with American Thoracic Society (ATS)/European Respiratory Society (ERS) standards as described.[18 19] PFT results were obtained by an experienced examiner (MB), who determined whether values met standard criteria. Our pulmonary function lab follows ERS/ATS standards (https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.thoracic.org%2Fstatements%2Fresources%2Fpft%2FDLCO.pdf&data=05%7C01%7Cmarinij%40cc1.nichd.nih.gov%7C1d9545c8116546cdc26608db40348560%7C14b77578977342d58507251ca2dc2b06%7C0%7C0%7C638174366537393808%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=7jYePwKKEc%2FfzYk%2Fs8BC1mPKMg%2BJ2a24%2F38SzQzx9YU%3D&reserved=0) .

We grade diffusion capacity efforts A–D where:

Grade A: Inspitatory volume/vital capacity (VI/VC) ≥90% (or VI/VC >85% and alveolar volume (VA) is within 200 mL and 5% (whichever is greater) of the largest VA from another acceptable effort.

Grade B: VI/VC ≥85%.

Grade C: VI/VC ≥80%.

Grade D: VI/VC ≤80%, breath-holding time (BHT) <8 s or >12 s.

If no grade A manoeuvre is obtained, manoeuvres of grades B–D might still have clinical utility. For these reasons, there may have been tests where an effort <90% of best VC was reported. PFT data determined to be unreliable or unreproducible were not reported. Specifically, five individuals (one with type III OI, two with type IV OI, one with type VI OI and one with type VII OI) were unable to perform some or all PFTs; only reliable values from these individuals were analysed.

Patients with OI typically have significant short stature secondary to bony deformities, such as scoliosis. Short or bowed lower extremities exert less effect on trunk size. Because height is a parameter used to calculate PFT predicted values, OI skeletal deformities can distort PFT calculations that use patient height. To determine whether arm span or ulnar length measurements are comparable surrogates for height for PFT calculations in patients with OI, we compared percentages of predicted PFTs calculated using these height surrogates. Arm span (ie, longest distance measured between tip of third digit in each hand, accounting for upper extremity deformity) and ulnar length (ie, mean distance of three measurements performed with a tape measure between the olecranon process and the midpoint of the styloid process of the same forearm) were used as height surrogates for PFT calculations. Percentages of predicted values for FVC, forced expiratory volume in 1 s (FEV1), forced expiratory flow at 25%–75% of FVC (FEF25%–75%), TLC and adjusted diffusion capacity (DLCOa) were calculated using standardised equations. By convention, normal percentages of predicted values for FVC, FEV1, TLC and DLCOa are >80% of predicted, while for FEF25%–75%, values >65% of predicted are considered to be normal.

Radiographic imaging

Chest CT scans without intravenous contrast were performed as described.[20] CT images were reviewed by a pulmonologist with expertise in rare lung diseases who was blinded to each subject's OI type (BRG). CT images were evaluated for bronchiectasis,

atelectasis, reticulations or interstitial lung disease, ground glass opacities, infiltrates, lung nodules, emphysema (ie, low attenuation lung parenchyma), lung cysts, bony chest wall deformities, pleural thickening, pleural effusions or presence of vertebral rods. Thickness of airway walls and outer airway diameters at levels of the trachea, bronchus intermedius and lower lobar subsegmental bronchi were measured using electronic callipers. Lung tissue density was measured in 10 pixels, each with an approximate area of 10 mm^2, randomly selected per lobe from regions without visible airways or blood vessels. Hounsfield units in each pixel were used to calculate mean lung tissue density for each lobe and for whole lungs in each individual. Cobb angles were measured from anteroposterior thoracic spine radiographs by two investigators (ANDD and JCM) using the angle tool in the Picture Archiving and Communication System as described (https://raduopaedia.org/articles/cobb-angle?lang=us). Scoliosis was defined as Cobb angle $\geq 10^{o}$.

Statistical analysis

Data are shown as mean±SD. By convention, normal percentages of predicted values for FEV1, FVC, TLC and DLCOa are >80% of predicted, while for FEF25%–75%, values >65% of predicted are considered to be normal.

Statistical analyses were conducted on data from OI types with sufficient n, that is, OI types III versus IV, OI types III versus VI and OI types IV versus VI. Statistical significance was calculated using a two-tailed, unpaired Student's t-test (GraphPad Prism, Dotmatics, Boston, Massachusetts, USA). Pairwise comparisons for patients with type IV OI with and without restrictive lung disease were performed using Fisher's exact test. Best-fit lines using simple linear regression analysis to correlate pulmonary function values (FEV1, FVC, FEF25%–75%, TLC, DLCOa percentages of predicated values) with Cobb angle or age were generated (GraphPad Prism). A p value of <0.05 was considered to be statistically significant.

RESULTS

Patient characteristics

Our study involved 29 children and young adults with types III and IV OI, 5 children with type VI OI, 2 children with type VII OI and one young adult with type XIV OI. Median age was 23.6 years (range 3.1–49.6), with types III, IV, VI, VII or XIV OI median age was 26.7 (range 19.6–49.6), 26.2 (range 3.1–42.5), 12.7 (range 4.5–31.7), 7.0 (range 3.9–10.1) or 32.9 years, respectively (table 1). Sixteen of 37 individuals with OI were male; 21 were female. Genotyping identified *COL1A1* variants in 17 individuals (7 with type III OI, 10 with type IV OI) or *COL1A2* variants in 12 individuals (1 with type III OI, 11 with type IV OI). Questionnaire responses for behaviours potentially affecting respiration revealed that 2 of 8 individuals with type III and 3 of 21 with type IV OI smoked tobacco. Histories of significant respiratory infections were elicited from both children with type VII OI and 3 of 8 (37%), 4 of 21 (19%) and 1 of 5 (20%) individuals with types III, IV or VI OI, respectively. Chronic respiratory disease was self-reported in 2 of 8 (25%), 4 of 21 (19%), 1 of 5 (20%) and 1 of 2 (50%) of individuals with types III, IV, VI or VII OI, respectively. One of 8 (12.5%) individuals with type III and 9 of 21 (43%) type IV OI experienced chronic cough. Dyspnoea with activity was reported by 3 of 8 (37%), 7 of 21 (33%) and 1 of 5 (20%) individuals with types III, IV or VI OI, respectively. Walking aids were used by 7 of 8 (87%), 10 of 21 (48%) and 2 of 5 (40%) of individuals with types III, IV or VI OI, respectively, both children with type VII, and one adult

Table 1 Clinical characteristics and pulmonary function measurements of subjects with OI

	III OI (n=8)	IV OI (n=21)	VI OI (n=5)	VII OI (n=2)	XIV OI (n=1)	P value*
Age: median (range)	26.7 (19.6–49.6)	26.2 (3.1–42.5)	12.7 (4.5–31.7)	7.0 (3.9–10.1)	32.9	
Male/Female	3/5	10/11	2/3	0/2	1/0	
COL1A1/COL1A2 variant	7/1	10/11	NA	NA	NA	
Smoking history, n (%)	2 (25)	3 (14)	0	0	0	
Prior respiratory infection, n (%)	3 (38)	4 (19)	1 (20)	2 (100)	0	
Chronic respiratory disease, n (%)	2 (25)	4 (19)	1 (20)	1 (50)	0	
Cough, n (%)	1 (13)	9 (43)	0	0	0	
Use of walking aid, n (%)	7 (88)	10 (48)	2 (40)	2 (100)	1 (100)	
Dyspnoea with activity, n (%)	3 (38)	7 (33)	1 (20)	0	0	
Dyspnoea at rest, n (%)	1 (13)	3 (14)	0	0	0	
Restrictive lung disease, n (%)	7 of 7 (100)	11 of 19 (58)	1 of 5 (20)	1 of 1 (100)	0	
Obstructive lung disease, n (%)	1 of 7 (14)	0	1 of 5 (20)	0	0	
FEV1%±SD	35.3±14.5	76.0±19.3	81.8±18.2	56†	103	<0.001, 0.006, NS
FVC%±SD	37.9±13.8	75.4±20.1	86±11.6	69†	98	<0.001, 0.002, NS
FEV1/FVC±SD	0.80±0.06	0.86±0.06	0.84±0.2	0.91†	0.85	NS, NS, NS
FEF25%–75%±SD	31.4±17.5	87.1±28.7	73.3±41.7	NA	112	<0.001, 0.041, NS
TLC%±SD	41.0±13.4	76.1±19.3	137±74.6	NA	98	<0.001, 0.007, NS
DLCOa%±SD	33.1±14.2	58.4±15.7	59.8±16.6	NA	73	0.001, 0.020, NS

Chronic respiratory disease, included diagnoses of restrictive lung disease, asthma, sleep apnoea with or without use of CPAP. Sleep apnoea in OI is generally secondary to soft connective tissue in the upper airway and can be a significant respiratory system problem for these patients. Although sleep apnoea is an upper airway condition, it has a complex bidirectional relationship with chronic lung disease.[33] Prior respiratory infections, included pneumonia with or without recurrence, bronchitis with or without recurrence, influenza, Scarlet fever with respiratory component.

*P values of type III OI versus type IV OI, type III OI versus type VI OI, type IV OI versus type VI OI.

†Data available from one individual with type VII OI.

CPAP, continuous positive airway pressure; DLCOa%, diffusion capacity percent predicted; FEF25%–75%, forced expiratory flow at 25%–75% of forced vital capacity percent predicted; FEV1%, forced expiratory volume in 1 s percent predicted; FVC, forced vital capacity; FVC%, forced vital capacity percent predicted; NA, data not available or not applicable; NS, not significant (ie, p>0.05); OI, osteogenesis imperfecta; TLC%, total lung capacity percent predicted.

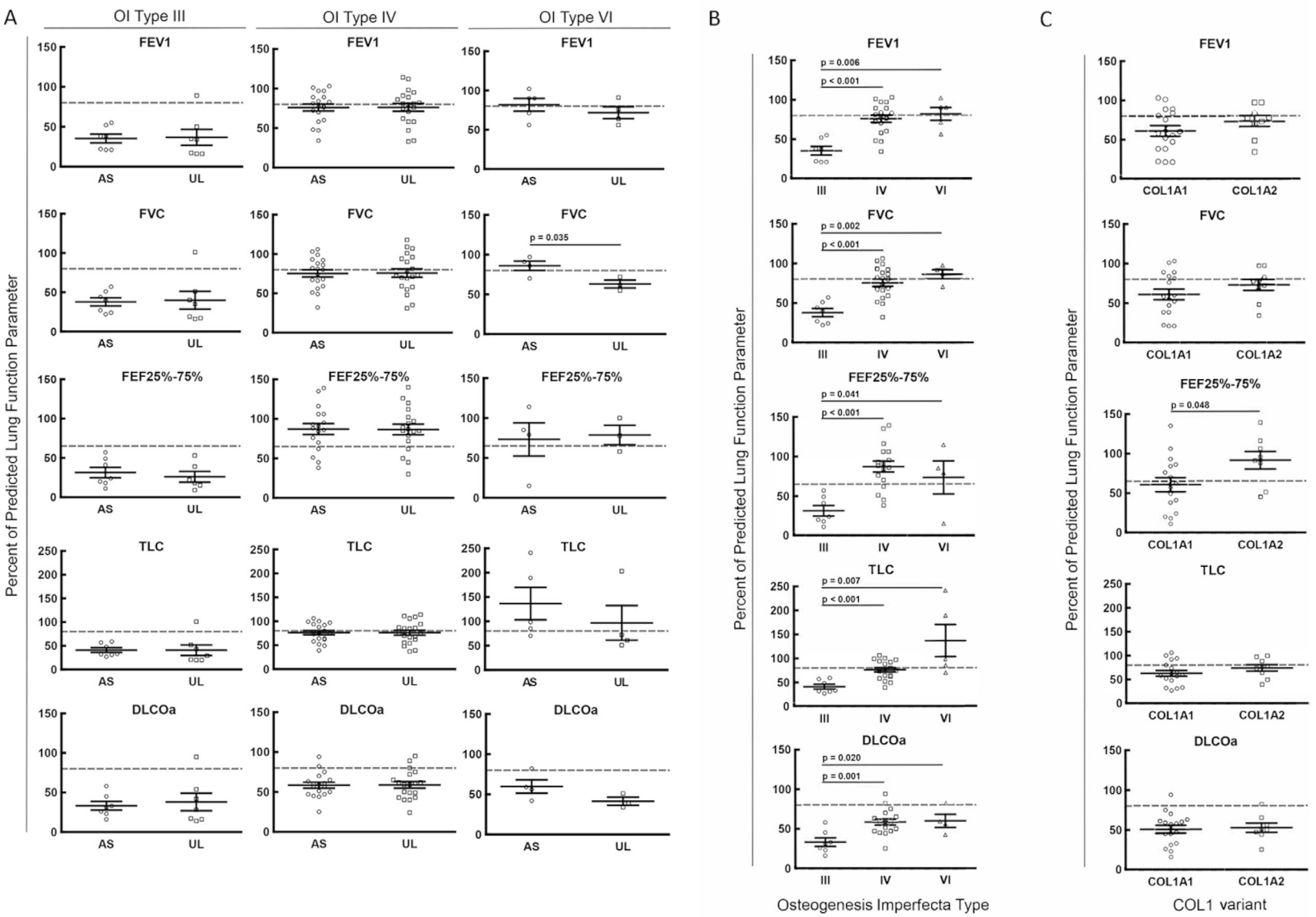

Figure 1 Pulmonary function values in individuals with type III, IV or VI osteogenesis imperfecta (OI). (A) Comparison of pulmonary function test (PFT) results for forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), forced expiratory flow at 25%–75% of FVC (FEF25%–75%), total lung capacity (TLC) or diffusion capacity (DLCOa) in individuals with type III, IV or VI calculated using arm span (AS) or ulnar length (UL) as surrogate measurements for height. No significant differences were found, except individuals with type VI OI had higher FVC calculated using AS versus UL as surrogate for height. (B) Percentages of predicted FEV1, FVC, FEF25%–75%, TLC or DLCOa values calculated using AS as surrogates for height were significantly lower in type III OI compared with either type IV or VI OI. (C) In individuals with type III or type IV OI, percentage of predicted FEF25%–75% values calculated using AS as surrogates for height were significantly lower in those with *COL1A1* variants compared with those with *COL1A2* variants. FEV1, FVC, TLC and DLCOa normal range: 80%–120% predicted; FEF25%–75% normal range: ≥65% predicted.

Table 2 Clinical characteristics of subjects with type IV OI with and without restrictive lung disease

	Type IV OI with restrictive lung disease (n=11)	Type IV OI without restrictive lung disease (n=8)	P value
Age: median (range)	32.2 (14.5–42.5)	20.9 (9.4–32.4)	
Male/Female	6/5	4/4	NS
COL1A1/COL1A2 variant	5/6	5/3	NS
Smoking history, n (%)	2 (18)	1 (13)	NS
Prior respiratory infection, n (%)	4 (36)	0	NS
Chronic respiratory disease, n (%)	3 (27)	0	NS
Cough, n (%)	6 (56)	1 (13)	NS
Use of walking aid, n (%)	8 (73)	1 (13)	0.02
Dyspnoea, n (%)	5 (45)	2 (25)	NS
Cobb angle, degrees	28.3±20.9	11.1±9.7	0.06
Thoracic bone deformities or fractures, n (%)	7 (64)	2 (25)	NS
Vertebral rods, n (%)	3 (27)	1 (13)	NS

NS, not significant (ie, p>0.05); OI, osteogenesis imperfecta.

with type XIV OI. Overall, more than one respiratory behaviour or symptom was reported by six individuals (one with type III OI and five with type IV). Five of these six individuals had a *COL1A1* variant, and one had a *COL1A2* variant.

Height surrogate comparison for PFT calculation in individuals with OI

We compared percentages of predicted PFTs calculated using both arm span and ulnar length as height surrogates. No significant differences in percentages of predicted FEV1, FEF25%–75%, TLC or DLCOa were found using either height surrogate in individuals with type III, IV or VI OI (figure 1A). Only FVC percent predicted values calculated using arm span measurements as surrogates for height were significantly higher than those calculated using ulnar length in individuals with type VI OI.

PFT results in types III, IV and VI OI

Since arm span and ulnar length measurements yielded comparable results as height surrogates, PFTs calculated using arm span were analysed in this cohort with OI. PFTs showed restrictive lung disease, indicated by TLC <80% predicted, in more than half of these young patients, including 7 of 7 (100%), 11 of 19 (58%), 1 of 5 (20%) and 1 of 1 individuals with type III, IV, VI or VII OI, respectively. Obstructive lung disease, indicated by FEV1/FVC ratio <70%, was found in two individuals, one with type III OI and one with type VI OI (table 1). It is notable that gas exchange or diffusion capacity was abnormal in 90% of all individuals with OI.

Among individuals with type IV OI, mean age and use of walking aids of patients with restrictive lung disease (31.6 years and 8 of 11 individuals, respectively) were significantly higher than those without restrictive lung disease (21.0 years and 1 of 8 individuals, respectively) (table 2). In addition, mean thoracic Cobb angle tended to be greater in patients with type IV OI with restrictive lung disease (28.3°) compared with those without restrictive lung disease (11.1°) (p=0.06).

PFT results correlate with severity of OI types

PFT results were compared in three OI types. Percentages of predicted FEV1, FVC, FEF25%–75%, TLC or DLCOa in individuals with type III OI were significantly lower than in individuals with type IV or type VI OI (figure 1B) (table 1). No significant differences were found between individuals with type IV or type VI OI.

Aggregate data showed that individuals with type III OI had small airways airflow obstruction (ie, FEF25%–75% <65% predicted), severe restriction and severe reduction of diffusion capacity. Individuals with type IV OI had mild restriction and moderate reduction of diffusion capacity, and individuals with type VI OI had hyperinflation and moderate reduction of diffusion capacity. One child with type VII OI had spirometry values suggestive of restriction. One individual with type XIV OI had mild reduction of diffusion capacity.

PFT results in individuals with COL1 genetic variants

We compared PFTs in individuals with *COL1A1* vs *COL1A2* pathological variants. We found that FEF25%–75% was significantly lower in individuals with *COL1A1* variants compared with those with *COL1A2* variants, whose mean FEF25%–75% was normal (figure 1C). In contrast, mean FEF25%–75% measurement in individuals with *COL1A1* variants was 60.6% predicted, indicating that this group of patients with OI had small airways airflow obstruction.

Pulmonary and extrapulmonary structure in individuals with OI

Chest CT scans identified structural abnormalities of the airways, lung parenchyma or extrapulmonary tissue in OI (figure 2) (table 3). The most common pulmonary finding was small bronchial thickening at the level of subsegmental bronchi. Abnormal bronchial thickening was defined as a ratio of airway wall thickness to airway diameter of 0.20 or greater.[21 22] Small bronchial wall thickening was found in all individuals, except 3 of 21 individuals with type IV OI. These three individuals had *COL1A2* variants; two of these three individuals had no detectable thoracic bone disease. The small bronchi ratio was significantly higher in individuals with type VI OI than those with type IV OI (p<0.001). No significant differences in trachea or large bronchi ratios were found between groups. Bronchiectasis was found in 3 of 8 (37.5%) and 2 of 21 (9.5%) of individuals with type III or IV OI, respectively.

Abnormal findings affecting lung parenchyma

Atelectasis, reticulation, ground glass opacities and emphysema were detected in this OI cohort. Atelectasis was found in many cases, including 7 of 8 (87.5%), 9 of 21 (43%), 2 of 5 (40%) individuals with type III, IV or VI, respectively, as well as both individuals with type VII OI. Reticulations were detected in 4 of 8 (50%), 6 of 21 (29%), 1 of 5 (20%) and 1 of 2 individuals with type III, IV, VI or VII OI, respectively. Ground glass opacities were found in 6 of 8 (75%) and 1 of 5 (20%) individuals with type III or IV OI, respectively, and both individuals with type VII OI.

Regions of emphysema were visualised in 1 of 8 (12.5%), 4 of 21 (19%) and 1 of 5 (20%) individuals with type III, IV or VI OI, respectively, and in one adult with type XIV OI. A threshold of −910 Hounsfield units was used to identify emphysema.[23 24] At least one pixel consistent with focal emphysema was identified in 4 of 8 (50%), 12 of 21 (57%), 2 of 5 (40%) individuals with type III, IV or VI OI, respectively, and one patient with type XIV OI. Mean Hounsfield unit measurements were consistent with emphysema in one lobe in 2 of 21 (9.5%) individuals with type IV OI and in three lobes in the individual with type XIV OI, whose whole lung tissue density was consistent with emphysema (data not shown). Whole lung tissue density in type III OI was significantly lower than in those with type VI OI (p=0.045). Furthermore, whole lung tissue density in individuals with *COL1A2* variants was significantly lower than in

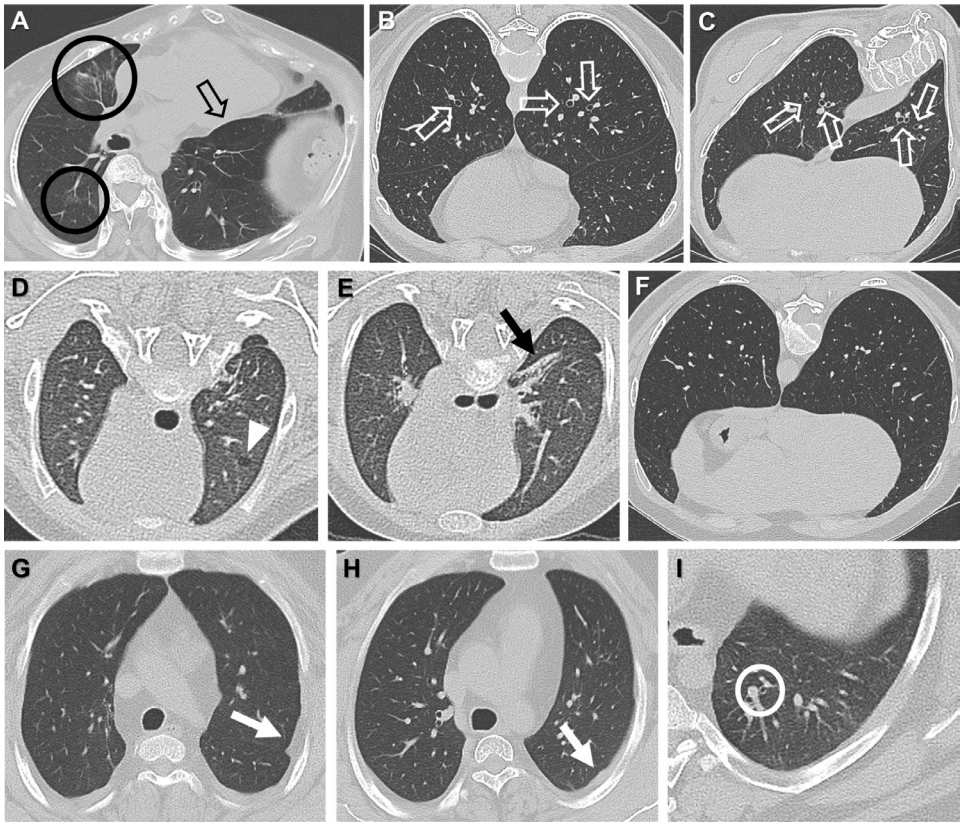

Figure 2 Chest CT scan findings in individuals with osteogenesis imperfecta (OI). Representative chest CT scan images are shown. (A) Supine CT scan image demonstrates focal emphysema (open black arrow) in the left lower lobe, ground glass opacities (black circles) and bony chest wall deformities in an individual with type III OI. (B) Prone high-resolution CT (HRCT) scan image shows bilateral lower lobe bronchiectasis (open white arrows) in an individual with type IV OI without significant chest wall defects. (C) Bilateral lower lobe bronchiectasis is detected in a prone HRCT scan image from an individual with type IV OI with chest wall deformities, gracile ribs and severe rotary kyphoscoliosis. (D and E) A small right upper lobe lung cyst (solid white arrowhead) and right lower lobe atelectasis (solid black arrow) are found in prone HRCT scan images from a child with type VII OI. (F) Diffuse low lung tissue attenuation in bilateral lobes is observed in a prone HRCT scan image from an individual with type XIV OI. (G and H) Pleural thickening (solid white arrow) is found in supine CT scan images from individuals with type III (G) or type IV OI (H). (I) Small bronchial airway thickening (white circle) is detected in an individual with type IV OI.

those with *COL1A1* variants ($p<0.001$) (online supplemental figure 1). A small lung cyst was detected in 1 of 2 children with type VII OI. Pulmonary infiltrates or abnormal lung nodules were not found.

In extrapulmonary tissue, small areas of pleural thickening were found in 5 of 8 (62.5%), 10 of 21 (47.6%), 1 of 5 (20%) individuals with type III, IV or VI OI, respectively, and both children with type VII OI. Pleural effusions were not identified. Multiple rib or vertebral deformities or fractures were detected in type III, IV, VI or VII OI; no bony deformities or fractures were found in the adult with type XIV OI. Scoliosis was found in 26 of 37 (70%) individuals with OI, including 7 of 8 (87.5%), 15 of 21 (71%), 1 of 5 (20%) individuals with type III, IV or VI OI, respectively, both individuals with type VII OI, and the one adult with type XIV OI. Surgical rods were present in 5 of 8 (62.5%), 4 of 21 (19%) and 1 of 5 (20%) individuals with type III, IV or VI OI, respectively.

Pulmonary function correlations in individuals with OI

Our data on this patient cohort also supported the current understanding correlating decline in pulmonary function with severity of scoliosis. We found that percentages of predicted FEV1, FVC, FEF25%–75% or DLCOa correlated negatively with Cobb angle in all individuals with OI (figure 3). Given the wide age range of 46 years in this cohort, we explored correlations between lung function and age. Percentages of predicted FEV1, FVC or TLC correlated negatively with age in all individuals with OI.

DISCUSSION

In the present study, we extended our prior examination of intrinsic lung disease in OI[9] to 37 children and young adults with OI, most of whom have classical OI with collagen structural abnormalities, and who underwent chest CT and PFTs. Because of the significant and disproportionate short stature of OI, calculation of PFTs using patient height is inaccurate.[25] Our data demonstrate that using arm span or ulnar length measurements as surrogates for height yield comparable results in patients with OI.

In the PFTs of our OI cohort, TLC is reduced below 80% predicted, indicative of restrictive disease, in all patients with type III OI and about half of patients with moderate type IV OI. Notably, mean TLC in type III OI is already markedly reduced to 40% of predicted at this relatively young age. Within type IV OI, those without restrictive disease were younger and did not use walking aids compared with those with restrictive disease. Thus, restrictive lung disease generally corresponds with severity of OI, with restrictive disease more severe in type III than in those type IV OI who are older and less mobile, who in turn have greater severity than the type IV who are younger and more mobile. Illustrating involvement of both intrinsic and

Table 3 Chest CT scan findings of subjects with OI

	III OI (n=8)	IV OI (n=21)	VI OI (n=5)	VII OI (n=2)	XIV OI (n=1)	P value*
Bronchial thickening, n (%)	8 (100)	18 (86)	5 (100)	2 (100)	1 (100)	
Small bronchi ratio±SD	0.27±0.06	0.25±0.04	0.33±0.03	0.28±0.01	0.25	NS, NS, <0.001
Large bronchi ratio±SD	0.12±0.02	0.11±0.02	0.13±0.02	0.15±0.0	0.09	NS, NS, NS
Trachea ratio±SD	0.10±0.02	0.10±0.02	0.12±0.02	0.13±0.02	0.07	NS, NS, NS
Bronchiectasis, n (%)	3 (38)	2 (10)	0	0	0	
Atelectasis, n (%)	7 (88)	9 (43)	2 (40)	2 (100)	0	
Reticulations, n (%)	4 (50)	6 (29)	1 (20)	1 (50)	0	
Ground glass, n (%)	6 (75)	1 (5)	0	2 (100)	0	
Emphysema, n (%)	1 (13)	4 (19)	1 (20)	0	1 (100)	
Focal lung tissue density ≤ −910 Hounsfield units, n (%)	4 (50)	12 (57)	2 (40)	0	1 (100)	
Whole lung tissue density±SD, Hounsfield units	−846±46.9	−843±61.3	−838±54.9	−763±56.3	−912±17.8	NS, 0.045, NS
Cysts, n (%)	0	0	0	1 (50)	0	
Pleural thickening, n (%)	5 (63)	10 (48)	1 (20)	2 (100)	0	
Rib deformities, n (%)	6 (75)	8 (38)	1 (20)	1 (50)	0	
Rib fractures, n (%)	6 (75)	4 (19)	5 (100)	2 (100)	0	
Vertebral deformities, n (%)	6 (75)	4 (19)	3 (60)	1 (50)	0	
Vertebral fractures, n (%)	5 (63)	5 (24)	3 (60)	2 (100)	0	
Scoliosis, n (%)	7 (88)	15 (71)	1 (20)	2 (100)	1 (100)	
Vertebral rods, n (%)	5 (63)	4 (19)	1 (20)	0	0	

*P values of type III OI versus type IV OI, type III OI versus type VI OI, type IV OI versus type VI OI.
NS, not significant (ie, p>0.05); OI, osteogenesis imperfecta.

extrinsic factors in OI lung pathology, those with restrictive disease tended to have greater mean Cobb angle to their scoliosis. Furthermore, it was striking that diffusion capacity for carbon monoxide was reduced in almost all classical patients with OI, again with type III significantly worse than type IV OI. This factor correlates with diffuse parenchymal lung disease and corroborates the CT scan findings. A recent study of 30 patients with OI with an average age of 39 years did not detect decreased diffusion capacity.[22] The reason for this difference between studies is not clear, although the technical challenges of measuring diffusion capacity must be considered (see 'Materials and methods' section), as well as differences in the genetic aetiology of OI in this cohort, which included 14 individuals whose causative mutation had not been determined, and 9 individuals with type I OI. Type I OI is a mild form caused by quantitative collagen deficiency rather than collagen structural abnormalities. The NIH cohort did not include individuals with type I OI.

For air flow, PFTs reveal significant reduction in FEV1, FVC and FEF25%–75% in individuals with type III OI. However, the FEV1/FVC ratio, which detects obstructive lung disease involving large airways, is abnormal in only two individuals. FEV1 decreased with age, whether measured by regression curve or by decades, so further longitudinal examination of air flow is warranted to determine whether progression to obstructive disease is part of the phenotype.

Comprehensive review of CT scans from 37 individuals with OI reveals a broad spectrum of abnormal pulmonary structure. We localised highly prevalent airway wall thickening to the small airways at the level of subsegmental bronchi in type III and IV OI. Another recent study also reported bronchial wall thickening as a common finding.[22] We also show that individuals with type VI, VII or XIV OI, which are uncommon OI types, have bronchial wall thickening. While it remains unclear whether bronchial wall thickening is associated with altered airflow in OI, we found small airway obstruction only in type III OI. Furthermore, FEF25%–75%, a measure of air flow in small airways, was the only PFT component that was significantly worse in individuals with *COL1A1* vs *COL1A2* variants, again indicating small airway flow is a component of a more severe OI respiratory phenotype. Bronchial wall thickening in OI may be directly related to abnormal collagen or a secondary inflammatory response. Measurement of fractional exhaled nitric oxide, a non-invasive biomarker of airway inflammation, may elucidate the potential role of airway inflammation in OI. Although Fractional Exhaled Nitric Oxide (FeNO) is commonly used to measure allergen-induced airway inflammation, FeNO can also measure airway inflammation of other aetiologies,[26] and thus may be useful in elucidating the pathophysiology of bronchial wall thickening in OI.

Our study also independently validates a prior report of ground glass opacities in patients with OI.[22] We identified ground glass infiltrates in most individuals with type III OI, one with type IV OI and both with type VII OI. The aetiology of ground glass opacities in OI is unknown, but data from the *Aga2* mouse, an animal model of type III OI with a *Col1a1* defect, suggest that lung haemorrhage or alveolar infiltration by neutrophils and macrophages may be contributing factors.[9] These lung findings in *Aga2* mice suggest that vascular fragility or defects in angiogenesis are features of these OI types. Further studies in animal models of OI are indicated to determine underlying mechanisms of lung disease in OI.

Interstitial reticulations and regions of emphysema were identified in some of our patients with OI. This dichotomy demonstrates that collagen-related disorders are associated with a wide phenotypic spectrum in alveolar tissue, ranging from deficient (emphysema) to excessive (interstitial lung disease/fibrosis) levels of collagen. Emphysema was reported in $Col1a1^{Jrt/+}$ and $Crtap^{-/-}$ mice, which are murine models of OI associated with collagen structural and post-translational modification defects, respectively.[10 11 27] Inhibition of transforming growth factor (TGF)-β in $Crtap^{-/-}$ mice ameliorated pulmonary disease, suggesting that TGF-β is involved in the pathobiology of emphysema in some types of recessive OI.[28] Only limited information is available regarding emphysema in patients with OI. Emphysema was reported in a patient with type III OI, and histological findings

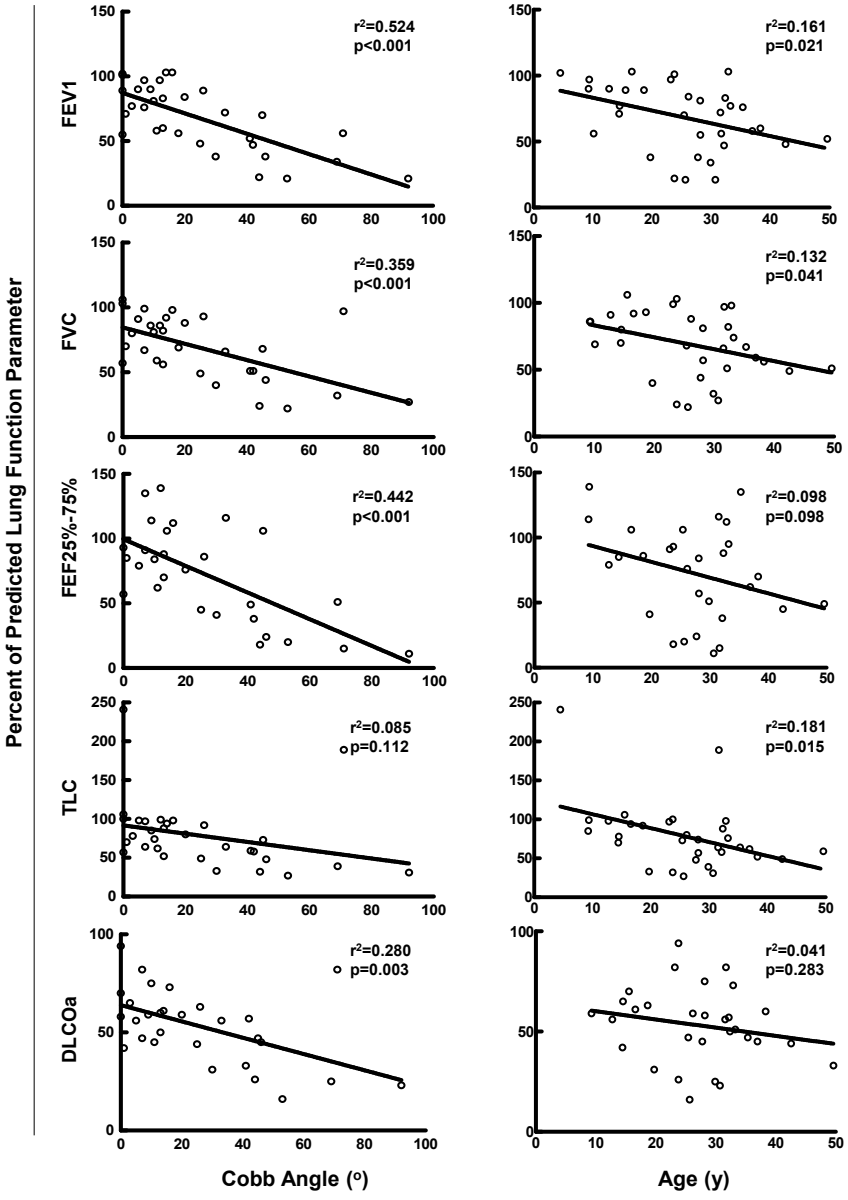

Figure 3 Correlation between scoliosis or age and pulmonary function in individuals with osteogenesis imperfecta (OI). Forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), forced expiratory flow at 25%–75% of FVC (FEF25%–75%) and diffusion capacity (DLCOa) percentage of predicted values correlated inversely with thoracic Cobb angle in all individuals with OI. FEV1, FVC and total lung capacity (TLC) percentage of predicted values correlated inversely with age in all individuals with OI.

of emphysema were reported in newborns with type II OI and a patient with OI aged 19 years.[6 22 29] In our study, emphysematous regions were visualised in CT scans of 7 patients with OI, and low lung tissue density measurements consistent with emphysema were found in 19 patients with OI. Notably, an adult non-smoker with type XIV OI with radiographic evidence of diffuse emphysema had corresponding impairment of diffusion capacity. Type XIV OI is an uncommon autosomal recessive OI type associated with variants in *TMEM38B*, which encodes trimeric intracellular cation channel type B, a ubiquitously expressed ER-membrane channel involved with intracellular calcium release.[30] Although few patients with type XIV OI were reported, an individual with type XIV OI aged 22 years developed lower airway obstruction and air trapping, which could be consistent with emphysema.[31] Furthermore, meta-analysis of genetic variants associated with chronic obstructive pulmonary disease identified *TMEM38B* as a leading gene.[32] Further studies are indicated to expand the understanding of emphysema in OI and to explore the potential pathobiological role of *TMEM38B*.

We identified pleural thickening in almost half of our cohort with OI. A paucity of information is available regarding pleural involvement in OI, with only one reported case of pleural thickening in a histopathological specimen from a patient with OI aged 19 years.[29] Lung reticulations and pleural thickening in OI may be consequences of abnormal fibrotic processes in alveolar interstitial and extrapulmonary tissue. Excessive TGF-β signalling was shown to contribute to bone disease and emphysema in the *Crtap*$^{-/-}$ murine model of OI and in the Pigment Epithelium-Derived Factor (PEDF) knockout

mouse for type VI OI.[15 28] Given the profibrotic effects of TGF-β, it is possible that the TGF-β pathway may also be involved in development of interstitial lung disease and pleural thickening in OI. Alternatively, pleural thickening in OI could be secondary to residual effects of prior respiratory infection or chest wall injury.

Overall, these findings expand the phenotype of intrinsic disease involving pulmonary parenchyma, lung airways and pleura in OI. While atelectasis can also be found in patients with scoliosis of other origins, bronchiectasis, reticulations, ground glass opacities, emphysema and bronchial thickening would not be expected to be secondary to scoliosis. Respiratory disease is a prominent cause of mortality in OI, and studies to identify therapeutic targets could lead to development of effective treatment for this aspect of OI.

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