DEFINING THE PULMONARY DISEASE AND DISABILITY FROM LUNG DYSFUNCTION IN CUTIS LAXA

by

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Cutis laxa is a heterogeneous group of disorders characterized by loose, lax, and inelastic skin. Other complications include lung, cardiovascular, musculoskeletal, gastrointestinal, and genitourinary disease. Cutis laxa (CL) can be congenital or acquired, with the congenital forms resulting from mutations in numerous genes. Chronic obstructive pulmonary disease (COPD) is a major contributor to morbidity and mortality in CL, making it a subject of public health importance. This purpose of this analysis is to determine quantitative differences between CL onset, congenital and acquired, and CL diagnosis, CL versus CL carrier in regard to pulmonary function and disability that results from lung dysfunction. An odds ratio determined that the cutis population has an increased risk for COPD and t-test analysis determined that COPD severity was the same between congenital and acquired subjects, but significantly different between CL and CL carriers. Multiple people with a previous asthma diagnosis were determined to actually have COPD. Additionally, the SGRQ total score was found to be highly correlated with FEV1/FVC and CT visual emphysema score. Further studies are warranted to more clearly determine genotype-phenotype correlations and assess longitudinal changes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would first like to thank all of the participants in this research study. Without their contributions and willingness, none of this would be possible. I would also like to thank Dr. Zsolt Urban for this opportunity to learn so much about cutis laxa and about clinical research.

I would also like to thank my thesis committee, Dr. Robin Grubs, Dr. John Wilson, and Juliann McConnell, who have helped me evolve as both a genetic counselor and a researcher. Also, I am incredibly grateful to the Human Genetics faculty, staff, my clinical supervisors, co-workers, and my classmates for all of their support.

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1.0 INTRODUCTION

Cutis laxa (CL) is an inherited or acquired connective tissue disease characterized by loose, lax, and inelastic skin. The inherited forms of CL are genetically heterogeneous, with significant variable expressivity seen in each genetic subtype. To date, mutations in the following genes have been shown to cause CL: fibulin-4 (*FBLN4*) or EGF containing fibulin-like extracellular matrix protein 2 (*EFEMP2*) gene, fibulin-5 (*FBLN5*), latent transforming growth factor beta binding protein 4 (*LTBP4*), ATPase, H^+ transporting, lysosomal V0 subunit a2 (*ATP6V0A2*), pyrroline-5-carboxylate reductase 1 (*PYCR1*), elastin (*ELN*), golgin, RAB6-interacting (*GORAB*), and Ras and Rab interactor 2 (*RIN2*) (Berk et al 2012, Morava et al 2009). All of these genes have a role in the extracellular matrix, with their disruption causing a highly variable phenotype.

The skin findings in cutis laxa are primarily loose, lax, redundant, and inelastic skin, giving an aged appearance to the individual. Pulmonary disease is highly variable, with the most common findings including tachypnea, recurring respiratory infections, and chronic obstructive pulmonary disease, specifically emphysema. Cardiovascular disease includes aortic dilation and aneurysm, arterial tortuosity, and stenosis. Other systems possibly affected include the musculoskeletal, and the gastrointestinal and genitourinary systems, with the most common features including hernias and joint laxity, and diverticula, respectively. Some individuals with cutis laxa also experience growth and/or developmental delay.

While the various forms of CL have been described in detail, there has not been a study to examine differences in lung function between congenital and acquired forms of CL. While COPD has been described as a feature in many subtypes of CL, no study has looked to formally assess what the increased risk for COPD is in CL. Additionally, the disability and morbidity associated with CL can be inferred for those familiar with the phenotype and by anecdotal communications, but has never been assessed quantitatively. This study will aim to address all of these gaps in the literature.

1.1 OBJECTIVE

The objective of this study is to better define the pulmonary phenotype and disability from lung dysfunction in cutis laxa. The pulmonary phenotype was assessed by cutis laxa onset classification as well as by genotype through pulmonary function tests (PFTs), chest computed tomography (CT) and by respiratory questionnaire analysis.

2.0 BACKGROUND

2.1 CUTIS LAXA AND THE EXTRACELLULAR MATRIX

Elastic fibers are the main component of the extracellular matrix, providing structure and elasticity to the skin. In the skin, elastic fibers are composed of a core of elastin that is surrounded by microfibrils, which contain fibrillins and microfibril-associated glycoproteins (Lewis et al 2004). In cutis laxa, mutations in various genes and/or environmental effects lead either to improper elastin and microfibril assembly or to the destruction of existing elastic fibers, which leads to compromised skin structure (Figure 1) and elasticity (Hu et al 2010).

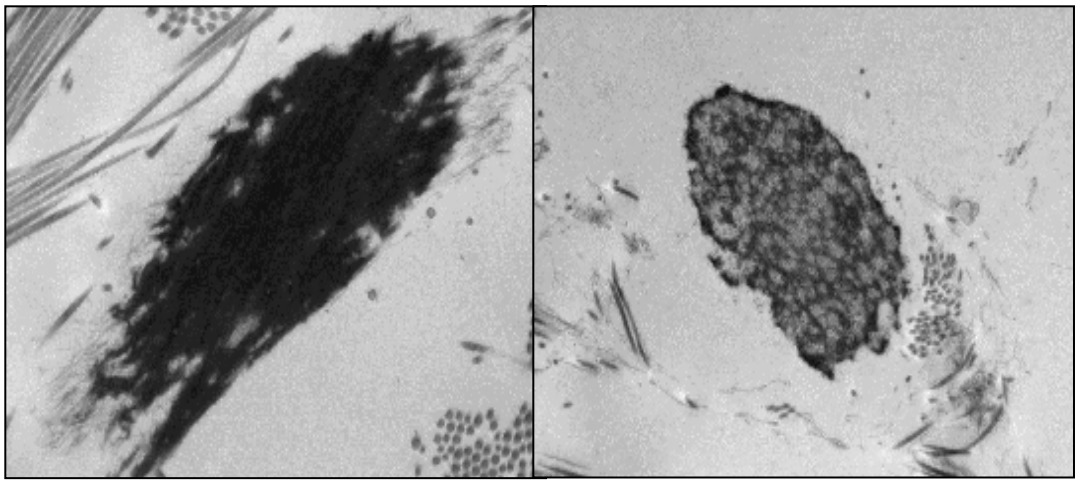

Figure 1. *left*, electron microscopy (EM) of an unaffected individual, showing normal elastin and microfibril ultrastructure, 1100x direct magnification; *right,* EM of an individual with CL, showing abnormal elastic fiber ultrastructure characterized by a moth-eaten appearance, 6800x direct magnification.

While the skin features are the hallmark of cutis laxa, connective tissue is found throughout the body, including the cardiovascular system, pulmonary system, gastrointestinal system, and genitourinary system (Badylak et al 2008). Abnormal biogenesis or damage to elastin and the extracellular matrix leads to manifestations in these body systems, although the specific expression and involvement is subtype-dependent, with different patient subsets showing different manifestations (Lewis et al 2004).

2.2 CHRONIC PULMONARY DISEASE

2.2.1 Chronic Obstructive Pulmonary Disease (COPD)

According to the GOLD criteria, an international NHLBI and WHO Initiative for COPD diagnoses and management summary, chronic obstructive pulmonary disease (COPD) is an airflow limitation that is not fully reversible (Pauwels et al 2001). COPD is also defined by Pauwels et al (2001) as a spirometry FEV1/FVC post-bronchodilation percent predicted value less than or equal to 70%. COPD is a multifactorial disease, influenced by many genetic factors, for example by alpha-1 antitrypsin (AAT) deficiency, and environmental factors (ALA 2008). The most common environmental risk factor is smoking, with other environmental factors including air pollution, second-hand smoke, and recurrent respiratory infections (ALA 2008). The World Health Organization (WHO) (2008) estimates that 64 million people worldwide had COPD in 2004, with COPD contributing 5% of all deaths worldwide in 2005. COPD has become a disease of international public health importance, and will only continue to become

more prevalent. In 2002 COPD was the fifth leading cause of death in the world, and is expected to become the third leading cause of death by the year 2030 (WHO 2008).

While COPD can be easily diagnosed and there are established treatments, many people do not realize they have COPD when it is in the early stages. Symptoms of COPD include recurrent respiratory infections, fatigue, dyspnea, and exercise intolerance (ALA 2008). COPD is most accurately diagnosed by performing spirometry, as part of a pulmonary function test (PFT) (Pauwels et al 2001). Spirometry is used to determine FEV_1/FVC (where FEV_1 is the forced expiratory flow in 1 second and FVC is the forced vital capacity), which, when less than 0.70, is diagnostic of COPD, with FEV_1 indicates the severity of the obstruction, as detailed in Table 1 (Mohamed Hoesein et al 2011, Pauwels et al 2001).

Table 1. FEV_1 determines severity of COPD (Pauwels et al 2001)

Stage of COPD	**FEV_1 % predicted**
Mild	$FEV_1 \geq 80\%$ predicted
Moderate	$30\% \leq FEV_1 < 80\%$ predicted
Severe	$FEV_1 < 30\%$ predicted

The NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, originally developed by Pauwels et al (2001) detailed management guidelines for COPD with four main components of management: Assess and Monitor Disease Diagnosis, Reduce Risk Factors, Manage Stable COPD, and Manage Exacerbations. Assessing and monitoring disease diagnosis is done through spirometry, monitoring symptoms, and reviewing risk factors and medical history. Successful ways to alter these risk factors include reducing or eliminating smoking and smoke exposure, exposure to air pollution, and any occupational

exposures. Managing stable COPD is done through health education, inhaled glucocorticosteroids and bronchodilators, both when indicated, oxygen treatment, and an exercise training program to improve exercise intolerance, dyspnea, and fatigue. Acute exacerbations, most commonly respiratory infections, are treated with antibiotics, glucocorticosteroids, and/or bronchodilators, depending on the nature of the infection. (Pauwels et al 2001)

The SGRQ, which was the respiratory questionnaire used for disability and lung dysfunction analysis in this study, is one of the most commonly used quality of life questionnaire in COPD studies, provides physical health-related quality of life scores caused by COPD lung dysfunction (Ferrer et al 2002). The SGRQ is a validated questionnaire with an available scoring manual (Ferrer et al 2002). The SGRQ assesses what pulmonary symptoms someone has, what activities are affected by one's lung disease, and the level of impact in day-to-day life from lung dysfunction (Ferrer et al 2002). The scores that are determined from the SGRQ questions are total score, symptom score, activity score, and impact score (Ferrer et al 2002). This is the first use of the SGRQ questionnaire in the cutis laxa population.

2.2.1.1 Emphysema

Emphysema is one of the two main pathological hallmarks of COPD characterized by the destruction of the alveoli, leading to a reduction of the internal surface area of the lung available for gas exchange, an enlargement of the air spaces and hyperinflation of the lung (ALA 2010). Emphysema also leads to the collapse of bronchioles and bronchi during exhalation, leading to air trapping in the lungs (Pauwels et al 2001). While treatment may ease symptoms, current medications cannot reverse or stop lung damage from occurring (Pauwels et al 2001). Thus, even

after elimination of environmental and behavioral risk factors, such as smoking, emphysema (and COPD) tends to worsen with time (Pauwels et al 2001).

2.2.1.2 Chronic Bronchitis

Chronic bronchitis is the second main pathological manifestation of COPD characterized by inflammation of the bronchi, with the most common symptom being chronic cough that produces mucus or sputum (Pauwels et al 2001). Bronchodilators are the most commonly used treatment to counteract the inflammation and to relieve COPD symptoms (Pauwels et al 2001).

2.2.2 Other Pulmonary Disease

Other pulmonary diseases noted in cutis laxa patients are pneumonia, upper respiratory infections, and asthma. Viral and bacterial infections are known exacerbations of COPD that can lead to significant changes in quality of life (Wedzicha et al 2003). Asthma, which is defined as obstructive lung disease that is intermittent and completely reversible with bronchodilation, differs from COPD, which is considered progressive and irreversible (Zeki et al 2011).

2.3 TYPES OF CUTIS LAXA

Cutis laxa is a group of heterogeneous disorders with ten currently described causative genes for CL or CL-like disorders. A summary of all CL subtypes is described in Table 2.

Table 2. Summary of Cutis Laxa Subtypes

CL Type	Inheritance	Main Features
ARCL1A	AR (*FBLN4*)	Cutis laxa, arterial tortuosity, aortic aneurysm, emphysema, hernias, joint laxity
ARCL1B	AR (*FBLN5*)	Cutis laxa, supravalvular aortic stenosis, emphysema, hernias
ARCL1C (URDS)	AR (*LTBP4*)	Cutis laxa, emphysema, diverticula, intestinal dilation/tortuosity, hernias, joint laxity
ARCL2A	AR (*ATP6V0A2*)	Cutis laxa, delayed closure of the fontanelles, joint laxity, developmental delay, seizures
ARCL2B	AR (*PYCR1*)	Cutis laxa on the hands and feet, triangular shaped face, joint laxity, developmental and growth delay
ARCL3	AR (*ATP6V0A2, PYCR1, ALDH18A1*)	Cutis laxa, joint laxity, cornea opacities, cataracts, progeroid-like features
ADCL	AD (*ELN*)	Cutis laxa, aortic dilation and aneurysm, emphysema, hernias
OHS	XL (*ATP7A*)	Cutis laxa, occipital horns, hernias, bladder diverticula
GO	AR (*GORAB*)	Cutis laxa on dorsum of hands and feet, joint hyperextensibility, decreased bone density, dwarfism
MACS	AR (*RIN2*)	Macrocephaly, Alopecia, cutis laxa, scoliosis
Acquired	Likely multifactorial	Cutis laxa, emphysema, aortic dilation, hernias

2.3.1 Autosomal Recessive Cutis Laxa Type 1A (ARCL1A)

Autosomal recessive cutis laxa type 1A (ARCL1A) is caused by mutations in the *fibulin-4* (*FBLN4*) or EGF containing fibulin-like extracellular matrix protein 2 (*EFEMP2*) gene, as originally described by Hucthagowder et al (2006). The main features of *FBLN4*-related cutis laxa include loose, lax, and redundant skin, arterial tortuosity, aortic aneurysm, developmental emphysema, hernias, and musculoskeletal findings that include joint laxity, multiple fractures, and pectus excavatum (Hucthagowder et al 2006). Additional publications revealed some *FBLN4* mutations occurring with arterial tortuosity and aneurysm, but without the characteristic skin findings or emphysema (Renard et al 2010). The presence of significant heterogeneity

among individuals with ARCL1A was also noted by Dasouki et al (2007) and Hoyer et al (2009), with the children in both of these reports passing away from complications at less than one month of age.

2.3.2 Autosomal Recessive Cutis Laxa Type 1B (ARCL1B)

Autosomal Recessive Cutis Laxa type 1B (ARCL1B) is known to be caused by mutations in *fibulin-5 (FBLN5)*, a gene known to be part of the elastic fiber network, which is found in many different tissues, including the aorta, lungs, and skin (Loeys et al 2002). Fibulin-5 protein is necessary for proper assembly of microfibrils and elastin, which are interrupted by *fibulin-5* mutations, ultimately resulting in improper microfibril and elastin assembly, which in turn lead to loose, redundant skin, as well as emphysema and other complications in individuals with ARCL1B (Hu et al 2006). While the most common features of ARCL1B are skin laxity, supravalvular aortic stenosis (SVAS), emphysema, and hernias, the phenotype is heterogenous, with reports of variable expressivity among members of the same family (Loeys et al 2002 and Berk et al 2012). In addition to phenotypic heterogeneity, genetic heterogeneity is common in *FBLN5* related cutis laxa, with missense mutations and duplications as well as recessive and dominant inheritance being reported (Loeys et al 2002 and Markova et al 2003).

2.3.3 Urban-Rifkin-Davis Syndrome (URDS) or Autosomal Recessive Cutis Laxa Type 1C (ARCL1C)

In 2009, Urban et al found that mutations in the gene for the *latent transforming growth factor-β binding protein 4* (*LTBP4*) caused ARCL1C, primarily characterized by cutis laxa, emphysema,

gastrointestinal, genitourinary, and musculoskeletal findings. Mutations in *LTBP4* led to increased transforming growth factor-β (TGF-β) activity, which negatively affected the elastic fiber organization and ultrastructure (Urban et al 2009).

Improper elastic fiber formation due to mutations in *LTBP4* creates a wide range of complications. Craniofacial features noted in the first group of identified *LTBP4*-related cutis laxa included hypertelorism, micrognathia, and a long philtrum. Pulmonary findings were seen in all four of the original patients, with findings ranging from pneumonia, chronic respiratory infections, tachypnea, and generalized respiratory distress, which was severe enough in one patient to require a tracheotomy. Gastrointestinal and genitourinary findings included rectal and cervical prolapse, inguinal, umbilical, diaphragmatic, and pelvic floor hernias, dilation and/or tortuosity of the gastrointestinal tract, tortuous blood vessels, diverticula in the stomach, intestines, and bladder, as well as pyloric stenosis and hydronephrosis. (Urban et al 2009)

2.3.4 Autosomal Recessive Cutis Laxa Type 2A (ARCL2A)

Autosomal Recessive Cutis Laxa Type 2A (ARCL2A) is caused by mutations in *ATP6V0A2*, the gene encoding the a2 subunit of the vacuolar H^+ -ATPase, which is involved in pH regulation (Kornak et al 2008). ARCL2A is part of a larger class of disorders known as the congenital disorders of glycosylation (CDG), which is characterized by disturbed glycosylation in the Golgi apparatus

(Morava et al 2009). There is ubiquitous expression of *ATP6V0A2*, leading many possible tissues being affected (Guillard et al 2009). Hucthagowder et al (2009) demonstrated that the mechanism for *ATP6V0A2*-related CL is through loss of function mutations, which leads to reduced mRNA levels in fibroblasts. Loss of *ATP6V0A2* function in turn caused defective

secretion of the elastin precursor, tropoelastin (Hucthagowder et al 2009). Of note, Wrinkly Skin Syndrome (WSS) is an allelic syndrome that is characterized by wrinkled skin, microcephaly, intellectual disability, and musculoskeletal complications (Morava et al 2009, Kornak et al 2008).

ARCL2A is characterized by congenital cutis laxa, delayed closure of fontanelles, congenital eye anomalies, growth, and developmental delay, and characteristic facies that include downslanting palpebral fissures, broad flat nasal bridge, and anteverted nostrils, narrow face (Noordam et al 2009). Other features include low bone density, including osteopenia and osteoporosis, lax joints, hypotonia, cobblestone like brain dysgenesis, and hernias (Hucthagowder et al 2009, Morava et al 2009). While it was previously believed that there were no pulmonary or cardiovascular findings that are more commonly found in other types of CL, our data has shown abnormal pulmonary function test (PFT) results in one *ATP6V0A2* compound heterozygote, as well as physician reported aortic dilation in a young *ATP6V0A2* homozygote (Z. Urban unpublished data).

2.3.5 Autosomal Recessive Cutis Laxa Type 2B (ARCL2B)

Autosomal recessive cutis laxa type 2B (ARCL2B) is caused by mutations in *PYCR1*, a gene expressed highly in the bones and skin (Mohamed et al 2011). Unlike *ATP6V0A2*-related CL, *PYCR1*-related CL is not a CDG (Berk et al 2012). Nevertheless, the clinical features of the two disorders are highly similar, with characteristic features of ARCL2B including cutis laxa on the dorsum of hands and feet, low bone density, joint laxity, intellectual disability, speech delay, dysmorphic features, which include a triangular shaped face with large ears, large fontanelles, microcephaly, adducted thumbs, contractures, and intrauterine growth retardation (IUGR)

(Mohamed et al 2011, Kouwenberg et al 2011). While there are many overlapping features with other disorders, including ARCL2A, gerodermia osteodysplastica (GO), ARCL2B is a distinct subtype of CL.

2.3.6 Autosomal Recessive Cutis Laxa Type 3 (ARCL3) or De Barsy Syndrome

Autosomal recessive cutis laxa type 3 (ARCL3) or De Barsy Syndrome is characterized by cutis laxa, progeria-like appearance, dysmorphic features, significant ophthalmologic abnormalities, neurologic, and musculoskeletal anomalies (Kivuva et al 2008 and Morava et al 2009). Ophthalmologic abnormalities in ARCL3 include corneal opacities, cataracts, strabismus, and myopia (Kivuva et al 2008). While some eye abnormalities have been reported in other subtypes of ARCL, ARCL3 has the most severe findings (Berk et al 2012). ARCL3 overlaps both clinically and genetically with ARCL2. Mutations in patients diagnosed with ARCL3 have been found in ATP6V0A2 (Kornak et al. 2008), in PYCR1 (Reversade et al. 2009) and in the gene for another mitochondrial enzyme in the proline biosynthetic pathway, *ALDH18A1* (Bicknell et al. 2008, Skidmore et al. 2011).

2.3.7 Autosomal Dominant Cutis Laxa (ADCL)

Autosomal dominant cutis laxa (ADCL) is caused primarily by mutations in *elastin* (*ELN*), with the most common molecular findings being frameshift mutations in exons 28-34 (Callewaert et al 2011). There has also been a reported case of a heterozygous *FBLN5* mutation causing ADCL (Markova et al. 2003). *ELN* mutations leading to CL show increased TGF-β activity, similar to the findings in *LTBP4*-related CL (Hu et al 2010, Callewaert et al 2011).

ADCL is characterized by the typical facial appearance with loose, lax skin, large ears, emphysema, aortic aneurysm, and hernias (Urban et al 2005, Callewaert et al 2011, and Szabo et al 2006). While it was once thought that there were few internal organ complications, it is now apparent that aortic aneurysm and emphysema are consistent with ADCL. Callewaert et al (2011) described four of six ADCL cases cause by mutations in *ELN* had some type of aortic dilation, one of six had chronic obstructive pulmonary disease (COPD), and an additional two of six individuals had more minor pulmonary findings, which included recurrent upper airway infections and dyspnea. Szabo et al (2006) described two families with *ELN*-related ADCL, with some affected family members having aortic dilation, but detailed lung function testing was not reported.

2.3.8 Occipital Horn Syndrome (OHS)

Occipital Horn Syndrome (OHS) or X-linked recessive CL (XLCL) is a type of cutis laxa allelic to Menkes disease, with OHS being the mildest form of the Menkes spectrum of disease (Tumer et al 2010). OHS is caused by mutations in *ATP7A*, a copper-transporting adenosine triphosphatase (Tumer et al 2010). The most distinguishable feature in OHS is occipital horns, which become more prominent with age (Tumer et al 2010). There is also skin laxity, bladder diverticula, hernias, and generalized connective tissue problems (Tumer et al 2010). While there are neurologic deficits in Menkes disease, OHS does not typically have these features (Berk et al 2012). Additionally, low serum copper and ceruloplasmin levels may be present in OHS, helping to distinguish OHS from the other forms of CL (Tumer et al 2010, Kaler et al 1998, Borm et al 2004).

2.3.9 Gerodermia Osteodysplastica (GO)

Gerodermia Osteodysplastica (GO) is a rare connective tissue disorder caused by mutations in *GORAB,* previously known as *SCYL1BP1* (Yildirim et al 2011). GO has many overlapping features of CL, especially with ARCL2, which include skin wrinkling on the dorsum of the hands and feet, hyperextensible joints, decreased bone density with frequent fractures, dwarfism (Kouwenberg et al 2011, Yildirim et al 2011). Differentiating GO from ARCL2B, is a lack of intellectual and speech delay, frequently seen in those with *PYCR1* mutations (Kouwenberg et al 2011). Unlike ARCL and ADCL, GO does not have significant cardiovascular or pulmonary involvement (Noordam et al 2009).

2.3.10 Macrocephaly, Alopecia, Cutis Laxa, and Scoliosis (MACS) Syndrome

Macrocephaly-alopecia-cutis laxa-scoliosis (MACS) syndrome, caused by recessive mutations in *RIN2*, is characterized by macrocephaly, sparse hair, cutis laxa, scoliosis, facial dysmorphism, joint hypermobility, and dental anomalies (Basel-Vanagaite et al 2009). There can also be gingival hyperplasia and hernias (Berk et al 2012). Mutations in *RIN2* lead to low amounts of dermal microfibrils and abnormal endocytic trafficking, which ultimately leads to the disease phenotype (Basel-Vanagaite et al 2009).

2.3.11 Late-Onset/Acquired Cutis Laxa

Late-onset or acquired cutis laxa (ACL) is considered in individuals who have developed cutis laxa signs and symptoms after childhood. While the exact mechanism of acquired cutis laxa is

not known, it is believed to be multifactorial in nature. It is known that a variety of environmental factors can trigger the development of acquired CL, including: inflammatory disease, multiple myeloma and lymphoma, autoimmune diseases, and allergies to certain medications (Berk et al 2012). While it has been suggested that there is a genetic component to acquired CL (Hu et al. 2006), the nature of this genetic predisposition is not yet clear.

The phenotype of ACL is highly variable, with symptoms usually progressing with time. Skin findings are similar to the inherited forms of CL, with systemic findings including COPD, aortic dilations, gastrointestinal and genitourinary diverticula and prolapse, and hernias (Berk et al 2012, Kim et al 2011, New et al 2011, Mehta et al 2011).

2.4 CLINICAL FEATURES

2.4.1 Skin Findings in Cutis Laxa

Loose, lax, and redundant skin is the hallmark of cutis laxa, but the severity of the skin findings and whether the skin findings are generalized or localized, varies greatly among the different subtypes of CL. ADCL, ARCL1A, ARCL1B, ARCL1C, ARCL3, OHS, and MACS usually have generalized skin findings, although the severity can be highly variable (Berk et al 2012 and Morava et al 2009). ARCL2B and GO are differentiated by skin findings being mostly localized to the dorsum of the hands and feet (Yildirim et al 2011 and Mohamed et al 2011).

2.4.2 Pulmonary Disease in Cutis Laxa

The most common pulmonary manifestation in CL is COPD, which can be seen in ADCL, ARCL1A, ARCL1B, ARCL1C, ARCL2A, and acquired CL (Hucthagowder et al 2006, Loeys et al 2002, Urban et al 2009, Z. Urban unpublished data, Berk et al 2012, Urban et al 2005, Callewaert et al 2011). COPD can be diagnosed by spirometry, which is performed as part of a PFT (Pauwels et al 2001). While COPD cannot be cured or even partially reversed, it is possible to treat the symptoms in order to improve quality of life and day-to-day activities (Pauwels et al 2001).

2.4.3 Cardiovascular Disease in Cutis Laxa

The most common cardiovascular complication in CL is aortic dilation and aneurysm, with other complications including arterial tortuosity, supravalvular aortic stenosis (SVAS), pulmonary artery stenosis, and regurgitation (Berk et al 2012). Aortic dilation is most commonly found in ARCL1A, ADCL, and acquired CL (Hucthagowder et al 2006, Urban et al 2005, Callewaert et al 2011). Arterial tortuosity is most commonly seen in ARCL1A and can affect any of the arteries, including the aorta (Hucthagowder et al 2006). SVAS, which is the narrowing of the ascending aorta near the aortic valve, is most commonly seen in ARCL1B and ADCL (Urban et al 2005, Callewaert et al 2011, Loeys et al 2002). Pulmonary artery stenosis is most common in ARCL1C and can lead to shortness of breath, fatigue, and a fast heart rate as oxygenation is poor when the pulmonary artery is narrowed (Urban et al 2009).

2.4.4 Musculoskeletal Disease in Cutis Laxa

Musculoskeletal disease is a common feature of CL, with the most common musculoskeletal features including joint laxity or hypermobility, bone fractures, and decreased bone density found in ARCL1A, ARCL1C, ARCL2A, ARCL2B, OHS, and MACS (Urban et al 2009, Hucthagowder et al 2009, Morava et al 2009, Mohamed et al 2011, Kouwenberg et al 2011, and Yildirim et al 2011). An increased rate of bone fracture is seen in ARCL1A and GO, with decreased bone density most common in ARCL2A and ARCL2B (Hucthagowder et al 2006, Kouwenberg et al 2011, and Yildirim et al 2011). Less common musculoskeletal findings include pectus excavatum in ARCL1A and scoliosis in MACS (Hucthagowder et al 2006, Basel-Vanagaite et al 2009).

2.4.5 Diverticula and Hernias in Cutis Laxa

Hernias and diverticula in cutis laxa are highly variable in location and severity. Numerous types of hernias can be seen in all subtypes of ARCL1, ARCL2A, ADCL, OHS, and acquired CL (Berk et al 2012, Urban et al 2009, Hucthagowder et al 2006, Loeys et al 2002). Types of hernias seen include inguinal, hiatal, umbilical, and diaphragmatic. Diverticula can be seen in ARCL1C and OHS, with most diverticula occurring in the bladder and intestines, but has been reported throughout the gastrointestinal tract as well (Urban et al 2009 and Tumer et al 2010).

3.0 HYPOTHESIS AND SPECIFIC AIMS

Genetic, clinical and pathological information available to date support the notion that cutis laxa is a key feature of a group of diseases caused by elastic fiber dysfunction (Berk et al. 2012). Whereas congenital cutis laxa is mostly inherited and characterized by an abnormality of elastic fiber formation, in individuals with acquired or late-onset cutis laxa the elastic fibers appear to be formed properly but are destroyed by inflammatory or immune-mediated processes. Thus, comparative studies of congenital vs. acquired cutis laxa allow for the dissection of the importance of elastic fibers in the development vs. the maintenance of organ function. Based on current understanding of the role of elastic fibers in lung development and physiology (Shifren et al 2006) and previous case reports on cutis laxa, we hypothesized that both congenital and acquired CL patients have increased risk of developing COPD. Furthermore, we hypothesized that COPD in patients with cutis laxa has a significant impact on their quality of life. To test these hypotheses, we had the following specific aims:

3.1.1 Specific Aim 1

Determine the nature of the pulmonary disease and clinical differences in a variety of patients with congenital or acquired cutis laxa through PFT analysis.

3.1.2 Specific Aim 2

Assess the significance of pulmonary dysfunction and disability in the cutis laxa population in attendance of the University of Pittsburgh clinics.

4.0 METHODS

The data collected for this study was obtained under IRB approval at University of Pittsburgh, Washington University at St. Louis, and University of Hawaii, with IRB approval letters included in Appendix A. This study is currently funded through NIH by NHLBI.

4.1 CLINIC PROTOCOL

4.1.1 Recruitment

Research subject recruitment is primarily by self- or physician-referral. Other ways subjects are recruited include through the online patient support group, through a genetic counselor, or through family members already enrolled in the study.

4.1.2 Screening

Screening was performed on each individual to determine eligibility for participation, with eligibility consisting of a confirmed or suspected diagnosis in the individual or one of his/her first-degree relatives. All individuals were asked if he/she or his/her affected first-degree relative had (1) loose, lax skin, (2) skin in redundant folds, (3) inelastic or doughy skin, (4) premature

aging of the skin, (5) excessive premature wrinkling, or (6) another family member affected with cutis laxa. Any individual answering "yes" to at least three of more of the aforementioned features was considered eligible to participate in the study. At this point other information regarding demographics and diagnosis were gathered including patient contact information, cutis laxa onset, age of onset, and name and contact information of the physician who made the diagnosis.

4.1.3 Informed Consent

If the participant attended a research clinic at University of Pittsburgh Medical Center, information regarding the study and research activities was provided before arrival to the clinic, with informed consent occurring in person, but before any research activities were started. If the subject was participating through his or her treating physician, the entire informed consent process had occurred over the phone, and the consent form had been signed before any research activities were performed.

4.1.4 Research Activities

Research activities varied based on whether the participant was coming to a research clinic in Pittsburgh or if he/she was participating through his/her local, treating physician. It was possible for subjects to participate in both sets of activities.

4.1.4.1 Participation through Research Clinics for Affected Individuals

For individuals participating through a Pittsburgh research clinic, activities after informed consent included: personal medical history, pedigree, CL questionnaire, blood and/or saliva sample, genetics physical exam and evaluation and skin biopsy, PFT and chest CT, respiratory questionnaires, echocardiogram, DEXA scan, skin elasticity testing, vascular elasticity testing, hearing testing, craniofacial imaging, and lymphedema measurements. All of these tests were completed through the Montefiore Hospital Clinical and Translational Research Center (MUH CTRC).

The genetics evaluation consisted of a targeted physical exam, which was completed by Dr. Madan-Khetarpal and Juliann McConnell, MS, CGC. If permitted by the participant, clinical photographs were also obtained. Also completed during the genetics evaluation was the CL questionnaire (see Appendix B), which details the personal medical history, pedigree, as well as phenotypic information by body system. A skin biopsy was also performed on willing participants, which could be used for EM, histology, and growing fibroblasts. Additionally, blood samples were collected for DNA, plasma, and serum. If a blood sample could not be obtained, a saliva sample was collected for DNA isolation.

Pulmonary function testing was performed both at Washington University in St. Louis and at University of Pittsburgh. Testing was offered to any affected individual or known carrier of *LTBP4* mutations over age 5 who attended a clinic. Due to the lengthy test that required more complicated commands, it could not be completed on younger participants. The pulmonary function test included spirometry, lung volumes, DLCO testing, and blood gas testing. Respiratory questionnaires were completed by the subjects or his/her adult parents. The St. George's Respiratory Questionnaire (SGRQ) was completed to assess how lung disease

impacted daily living, the Beck Depression Inventory (BDI) to assess level of depression, Shortness of Breath questionnaire to assess what level of activity leads to dyspnea, MRC questionnaire to assess how an individual's lung disease affected activity level, the Stanford Brief Activity Survey determined level activity at work and in free time, and the Smoking History Form looked at the amount of direct and indirect smoke exposure over the lifetime.

Echocardiograms were obtained at both University of Washington in St. Louis and at University of Pittsburgh. These could be obtained on any individual around three years of age or older who was able to lie still for approximately 30 minutes. Echocardiography was completed on any eligible affected individual or known *LTBP4* carrier in attendance.

DEXA scans, to assess bone density, were performed on all affected individuals and known *LTBP4* carriers over the age of 18 who were not pregnant at the time of testing. Women of childbearing potential were required to take a urine pregnancy test. Reports generated included z-scores and t-scores on the spine and hip, with WHO classification of normal, osteopenia, or osteoporosis, as well as body fat composition analysis.

Two types of elasticity testing were performed: skin elasticity and vascular elasticity. Skin elasticity testing was performed on all affected individuals and known *LTBP4* carriers of any age. We used an investigational device, DermaLab, which assesses skin elasticity and skin moisture, on a research basis. Moisture testing began in 2012, while the skin elasticity testing began in 2011. Vascular elasticity testing was performed at the University of Pittsburgh by the Vascular CTRC and is a non-invasive test that uses ultrasound to measure pulse wave velocity which depends on how elastic a person's blood vessels are.

Hearing testing was performed, when available, on any affected individual or known *LTBP4* heterozygote over the age of 6. The testing included otoscopy, tympanometry,

behavioral hearing, frequency-sweep distortion-product otoacoustic emissions (DPOAE), DPOAE input/output, and contralateral-supression DPOAE. DPOAE testing looked at cochlear integrity, DPOAE input/output measured cochlear compression, and contralateral-supression DPOAE measured lower-brainstem integrity.

Craniofacial imaging was performed using a 3-dimensional stereophotogrammetry system that consists of six cameras that generate a 3D image of the face. This is performed on all affected individuals and known *LTBP4* carriers who were able to sit still for at least five minutes.

Lymphedema measurements were added in October 2011 and were performed in three different ways. Numerous measurements were taken along the extremities. Perometry was also performed, which used light beams and sensors to determine the extremity volume. This was done to determine any asymmetry, which may be attributed to lymphedema. Bioelectric impedance measured the electric properties of the extremities, with the goal of determining total water composition.

4.1.4.2 Participation through Treating Physician for Affected Individuals

For individuals participating through his/her treating physician, activities after informed consent included obtaining personal medical history, pedigree, blood or saliva sample, CL questionnaire, and a skin biopsy if the treating physician was able to collect one. Additionally, medical record information, including echocardiograms, PFTs, chest CT, and other imaging performed to assess arterial tortuosity, diverticula, or hernias were collected and used in this analysis.

4.1.4.3 Participation for Unaffected First-Degree Relatives

For unaffected first-degree relatives who were not known *LTBP4* heterozygotes, participation involved informed consent, followed by a blood draw or saliva sample. DNA was used to confirm genetic results in the proband or in whole exome sequencing and new gene discovery.

4.1.5 Follow-up

Follow-up consisted of yearly phone calls to note any changes in medical history, update the pedigree, and collect any new medical records of interest. Additionally, some participants called more regularly for updated genetic testing information. When a mutation was found, a participant was asked if he/she would like to know the results. For those participants who wanted to know his/her mutation, results were shared over the phone and then a research mutation report was mailed to the participant or guardian.

4.2 LABORATORY PROTOCOLS

4.2.1 Mutation Analysis

Mutation analysis was performed on DNA mostly from peripheral blood, or occasionally on saliva or fibroblasts cultured from a skin biopsy. DNA was isolated using a phenol chloroform extraction of Qiagen's PureGene System (Qiagen ©) for blood samples, and Genotek's Oragene protocol (DNA Genotek) for saliva samples. The concentration of the DNA preparation was

measured by UV spectrophotometry and working solutions were prepared at standard 50 ng/µl concentration. Exons and flanking intronic sequences of target genes (*ELN, FBLN4, FBLN5, LTBP4, ATP6V0A2, PYCR1*) were amplified by PCR. ExoSAP-IT and ABI BigDye Terminator Kit (Applied Biosystems) is used for PCR product cleaning and sequencing reaction. Sequencing of sense and antisense strands was done using the University of Pittsburgh Genomics and Proteomics core facility and analyzed using Sequencher (Gene Codes Corporation) and the UCSC Genomic Browser. Confirmation of identified mutations was done on separate amplification products and sometimes through RNA studies.

4.2.2 Electron Microscopy

Skin biopsies obtained from the University of Pittsburgh research clinics or from a treating physician were fixed in glutaraldehyde, stained, and embedded in Epon embedding resin, sectioned, and examined with a Tecnai 12 transmission electron microscope (Urban et al 2009).

4.3 PULMONARY ANALYSIS

4.3.1 Respiratory Questionnaire Scoring

The St. George's Respiratory Questionnaire (SGRQ) (Jones et al 1991), Beck Depression Inventory (BDI) (Beck et al 1996), the MRC breathlessness scale (MRC) (Stenton 2008), and Stanford brief activity survey (Taylor-Piliae et al 2006) were scored according to the protocols

provided in the questionnaire manuals. The Shortness of Breath questionnaire is scored by summing all responses for a total score.

4.3.2 Statistical Analysis

Statistical analysis was performed using SPSS version 19. Two PFT datasets, congenital CL versus acquired CL and carriers of CL versus all CL, were analyzed. Each dataset was first checked for normality, and then 2-tailed t-tests were run using α=0.05. After this was performed, bivariate correlation was performed to determine the most correlated disability questionnaire scores to the most used PFT variables in COPD testing, both pre-bronchodilation (pre-BD) and post-bronchodilation (post-BD): FEV1 percent predicted, FVC percent predicted, and FEV1/FVC percent predicted. The Odds Ratio was calculated using The MedCalc software (www.medcalc.org/calc/odds_ratio.php), the exact binomial test for goodness of fit was calculated using the University of Tennessee Health Center binomial program (http://biostat-server.uthsc.edu/pt600/bintestframe.html), and the relative risks were calculated using The MedCalc software (http://www.medcalc.org/calc/relative_risk.php).

5.0 RESULTS

The congenital subset consisted of 11 individuals, all of whom had congenital cutis laxa, with some individuals excluded from the post-BD analysis if bronchodilation was not indicated by the pre-bronchodilation results. No bronchodilation is performed if FEV1/FVC≥0.80 or if the subject was a minor, and therefore not permitted to receive bronchodilation per the study protocol. One individual with CL who had a PFT was excluded from the congenital versus acquired CL as it was unclear what onset of CL she had. The acquired group consisted of 8 individuals, all of whom had acquired cutis laxa, with some of the individuals excluded from the post-BD analysis if bronchodilation was not indicated by the pre-bronchodilation results. The CL group consisted of 20 individuals, all of whom had cutis laxa, with some individuals excluded from the post-BD analysis if bronchodilation was not indicated by the pre-bronchodilation results or if the subject was a minor. The individual with unknown CL onset was included in the CL group. The unaffected carrier group consisted of 4 *LTBP4* heterozygotes, who acted as controls. Two of the carriers did not receive bronchodilation and were therefore excluded from the post-BD analysis. Demographic information, including CL affection status, age at PFT, sex, and gene status are summarized for all participants that were analyzed in Table 11 (Appendix B).

Table 3. Demographic Information by CL onset

CL onset subgroup	N	Mean Age	Age Range	t-test 2-tailed p-value for age	% Male	% Female	Exact binomial test for goodness of fit
Congenital	11	25	3-58	0.000	45.45	54.55	P=0.500
Acquired	8	55.75	39-68		25	75	P=0.145
Carrier	4	36.5	8-50	N/a	75	25	--

Data on age among the congenital and acquired subgroups was determined to have a normal distribution (see Table 18 in Appendix B). As expected, the acquired group was older than the congenital group (Table 3), but was not older at a statistically significant level. One possible reason for having more women in the acquired group is that acquired cutis laxa can be precipitated by autoimmune diseases, which are more common in women.

Table 4. CL COPD and Asthma classification

Pedigree ID	Patient ID	FEV1/FVC percent	FEV1 percent predicted	COPD classification	Previously diagnosed with asthma?	Smoking pack years
CL-004	7166	87	118	No COPD	Yes	0
CL-028	7034	52*	71*	Moderate COPD*	Yes	0
CL-032	7040	49	34	Moderate COPD	No	0
CL-036	7044	50	41	Moderate COPD	Yes	0.165
CL-050	7065	84*	78*	No COPD	Don't know	0
CL-060	7078	36	39	Moderate COPD	No	0
CL-065	7093	55*	69*	Moderate COPD*	No	0.572

Table 4 continued

CL-068	7099	51	61	Moderate COPD	Yes	0
CL-094	7159	77	62	No COPD	Don't know	13.5
CL-094	7160	61	87	Mild COPD	Yes	0
CL-095	7161	70	99	No COPD	Yes	4
CL-096	7162	63	73	Mild COPD	No	0
CL-109	7183	76*	104*	No COPD	No	12.5
CL-114	7196	82*	85*	No COPD	No	0
CL-115	7197	68	58	Moderate COPD	Don't know	0
CL-116	7198	45	31	Moderate COPD	Don't know	0
CL-117	7202	81*	77*	No COPD	Yes	0
CL-120	7207	76*	78*	No COPD	No	0
CL-121	7209	74	98	No COPD	No	2.975
CL-125	7226	82*	133*	No COPD	No	0
Carriers						
CL-015	7006	81	89	No COPD	Don't know	--
CL-015	7007	85	132	No COPD	Don't know	0
CL-015	7192	86*	104*	No COPD	Yes	0
CL-028	7035	84*	99*	No COPD	No	0

*Values or categorization based on pre-bronchodilation values

COPD classification was determined for all individuals with post-bronchodilation PFT values, indicated in Table 4, with 50% of this CL population showing mild to moderate COPD (Table 5). Table 5 also shows a higher rate of asthma and COPD in this CL population compared to the general US population. The general US population data was obtained through the National Health Interview Survey, which contained 229,505 individuals in the 2010 survey, with 48.20%

of the individuals in the 18-44 year old range, 35.00% in the 45-64 year old range, and 16.8% in the 65 and older range (CDC 2012).

Table 5. Pulmonary Statistics for CL versus General Population

	Frequency of COPD	**Percent COPD**	**Frequency of asthma**	**Percent asthma**	**Percent current smokers**
Cutis laxa	10 of 20	50	7 of 20	35	15 (N=20)
General population	4,314 of 229,505 *source: CDC 2012*	1.88	29,057 of 229,505 *source: CDC 2012*	12.66	19.3 *source: CDC 2010*

Table 6. Odds Ratio and Relative Risk for CL and the General Population

	Odds Ratio	**Relative Risk**
COPD	52.20 (21.72-125.48), z: 8.839, p<0.0001	27.10 (17.47-42.05), z: 14.723, p<0.0001
Asthma	3.71 (1.48-9.31), z: 2.799, p=0.0051	3.11 (1.71-5.66), z: 3.728, p=0.0002

An odds ratio calculation (Table 6) with 95% CI was performed from the data in Table 5 and determined that the cutis laxa cohort had a COPD odds ratio of 52.20 and a relative risk of 27.10. For asthma, the odds ratio was 3.71 and the relative risk was 3.11.

Table 7. Age and Lung Function Differences in Asthma Diagnoses in CL

	N	**Mean**	**Std. Error Mean**	**2-tailed t-test p-value**
Age of individuals who were diagnosed with asthma	9	22.02	5.23	.002
Age of individuals who were not diagnosed with asthma	15	46.87	4.10	
Pre-BD FEV1 % predicted	5	74.40	15.08	.025
Post-BD FEV1 % predicted	5	81.20	13.65	

There is a statistically significant difference in age of individuals who have been diagnosed with asthma and those who have not (Table 7). For the individuals who have been diagnosed with asthma and have pre- and post-BD PFT information, the post-BD FEV1 % predicted goes to 81.20%. As asthma is diagnosed as a fully reversible lung obstruction, none of the individuals in this group had full reversal of FEV1, indicating that none of these subjects actually have asthma.

5.1.1 Lung Function Analysis of Congenital versus Acquired/Late-Onset CL

Shapiro-Wilk analysis of PFT data in congenital and acquired CL (Table 19, Appendix B) was performed. Given that all p-values in Table 19 were greater than 0.05, the null hypothesis was not rejected, meaning the data could be assumed to be normally distributed.

Table 8. Congenital versus Acquired CL Pre- and Post-BD

	CL onset	N	Mean	Std. Error Mean	t-test 2-tailed p-value	Mean % change pre- to post-BD
FVC pre-BD percent predicted	Congenital	11	86.27	8.310		N/a
	Acquired	8	78.50	7.447	.496	N/a
FEV1/FVC pre-BD percent predicted	Congenital	7	72.29	7.539		N/a
	Acquired	6	76.33	6.677	.695	N/a
FEV1 pre-BD percent predicted	Congenital	11	67.73	9.465		N/a
	Acquired	8	68.00	9.046	.984	N/a
FVC pre-BD percent predicted	Congenital	6	82.33	11.83		N/a
	Acquired	5	74.40	10.75	.631	N/a
FEV1/FVC pre-BD percent predicted	Congenital	5	65.00	8.47		N/a
	Acquired	5	71.60	5.77	.540	N/a
FEV1 pre-BD percent predicted	Congenital	6	52.83	11.63		N/a
	Acquired	5	55.40	10.25	.872	N/a
FVC post-BD percent predicted	Congenital	6	86.67	11.254		6.52
	Acquired	5	82.00	9.236	.756	12.5
FEV1/FVC post-BD percent predicted	Congenital	5	70.40	8.600		9.82
	Acquired	4	70.25	6.486	.989	3.45
FEV1 post-BD percent predicted	Congenital	6	60.33	11.152		17.13
	Acquired	5	64.20	10.888	.810	3.45
DLCO percent predicted	Congenital	9	72.89	9.932		N/a
	Acquired	8	55.88	5.765	.163	N/a

A 2-tailed t-test comparing congenital CL and Acquired CL with an $\alpha=0.05$ had a p-value >0.05 for all variables tested, indicating there was not a statistically significant difference between congenital and acquired CL in any of the PFT variables tested (Table 8). P-values used did not assume equal variances. Mean % change from pre- to post-BD (Table 8) shows that with bronchodilation, the population is not showing a full reversal of bronchodilation, which is indicative of COPD.

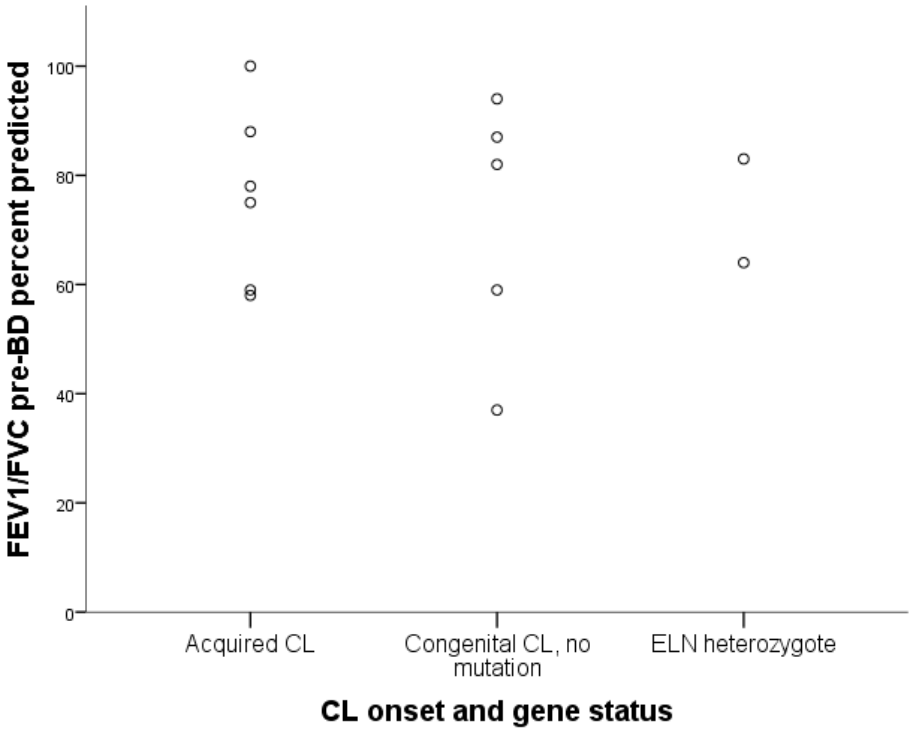

Figure 2. Plot of FEV1/FVC pre-BD percent predicted versus CL onset and gene status.

Figure 2 is a visual representation of the lung function pre-bronchodilation for all CL subtypes. Acquired CL, Congenital CL with no known mutation, and with ELN mutations appear to follow similar trends.

5.1.2 Lung Function Analysis of CL versus Unaffected Carriers

Shapiro-Wilk analysis of PFT data in unaffected carriers and individuals with cutis laxa (Table 21, Appendix B) was performed. Given that all p-values in Table 21 were greater than 0.05, the null hypothesis was not rejected, meaning the data could be assumed to be normally distributed.

Table 9. Affected CL versus Unaffected Carrier Pre-BD Group Statistics

	CL onset	N	Mean	Std. Error Mean	t-test 2-tailed p-value
FVC pre-BD percent predicted	Carrier	4	99.2500	7.80358	.174
	Cutis Laxa	20	84.6000	5.60892	
FEV1/FVC pre-BD percent predicted	Carrier	3	101.0000	3.46410	.002
	Cutis Laxa	14	76.0714	4.94304	
FEV1 pre-BD percent predicted	Carrier	4	102.5000	9.66523	.032
	Cutis Laxa	20	70.2500	6.61731	
DLCO percent predicted	Carrier	3	88.0000	7.02377	.054
	Cutis Laxa	18	65.6667	5.82366	

A 2-tailed t-test comparing unaffected carriers and cutis laxa with a 95% CI has a p-value ≤0.05 for FEV1 pre-BD and FEV1/FVC pre-BD, and a p-value >0.05 for FVC pre-BD, and DLCO variables (Table 9). Statistical analysis was not done for post-BD values as only two carriers had post-BD data available. P-values used did not assume equal variances.

5.1.3 Measures of Disability versus Lung Function Analysis

Bivariate correlation was performed using disability assessing questionnaire scores in addition to all PFT variables included in previous analyses. All disability raw data is in Table 16 (Appendix B), with results from the bivariate correlations included in Tables 22 and 23 (Appendix B). This analysis indicated the SGRQ total score had the most significant correlations with the FEV1/FVC pre-BD PFT variable and the chest CT visual emphysema score.

A linear regression comparing SGRQ total score to CT visual emphysema score, pre-BD FEV1/FVC, sex, and age indicated a p-value ≤0.05 for the CT visual emphysema score and pre-

BD FEV1/FVC (Table 10). Sex and age each had a p-value >0.05 when compared to the SGRQ total score (Table 10).

Table 10. Disability and Pulmonary Function Correlation (n=5)

		SGRQ total score	**CT visual emphysema score**	**FEV1/FVC pre-BD percent predicted**	**Sex**	**Age at PFT**
Pearson Correlation	SGRQ total score	1.000	.903	-.930	.118	.334
	CT visual emphysema score	.903	1.000	-.853	-.089	-.092
	FEV1/FVC pre-BD percent predicted	-.930	-.853	1.000	-.130	-.360
	Sex	.118	-.089	-.130	1.000	.441
	Age at PFT	.334	-.092	-.360	.441	1.000
Sig. (1-tailed)	SGRQ total score	.	.018	.011	.425	.291
	CT visual emphysema score	.018	.	.033	.443	.441
	FEV1/FVC pre-BD percent predicted	.011	.033	.	.417	.276
	Sex	.425	.443	.417	.	.229
	Age at PFT	.291	.441	.276	.229	.

6.0 DISCUSSION

The purpose of this study was to better characterize the risk for COPD, validity of asthma diagnoses, pulmonary phenotype between congenital CL, acquired CL, and CL carrier, and disability from COPD. The current literature does not provide information on lung disease outside of case series' and review articles, making these findings novel to the CL community. Particularly lacking was a quantitative measurement of the risk of COPD in CL patients. The most well known genetic risk factor for COPD is alpha-1 antitrypsin deficiency (Molfino 2007). Environmental risk factors include smoke exposure, either through tobacco smoke or second-hand tobacco smoke exposure, occupational exposures, and air pollution (Eisner et al 2005 and Johannessen et al 2012).

6.1.1 Increased Risk for COPD and Asthma Diagnoses

The percentage of individuals with CL who had COPD by PFT and asthma by report, was notably higher than the general population rates of disease, with the COPD odds ratio showing those with CL had a 52.20 greater odds to have COPD than those of the general population. The relative risk (RR) for the CL population when compared to the general population was 27.10, indicating that individuals with CL were 27.10 times more likely as people in the general population to develop COPD. The risk for COPD in someone with an alpha-1-antitrypsin

deficiency was described by Dahl (2002) to have an odds ratio of 22 and relative risk of 12 for the ZZ genotype. A study done by Johannessen et al (2012) showed that women who were exposed to environmental tobacco smoke (ETS) in childhood had an odds ratio of 1.9 at a 95% CI when compared to women who were not exposed to ETS. Smokers also have an increased risk for developing COPD, with a study by Lundback et al (2003) showing that ex-smokers had an odds ratio of 2.14, people who smoked less than 5 cigarettes a day had an odds ratio of 4.39, people who smoked 5 to 14 cigarettes a day had an odds ratio of 6.44, and people who smoked more than 14 cigarettes a day had an odds ratio of 8.04, all with a 95% CI. This is a lower odds ratio than determined for this CL cohort, demonstrating that CL has a higher risk for COPD then most of the well known COPD risk factors, which is something not previously reported in the literature.

Additionally, 3 of 8 individuals who have COPD by PFT also reported having been diagnosed with asthma. Since asthma is defined as being fully reversible with bronchodilation, the PFTs from these 3 individuals indicate that they have COPD, and do not have asthma. The significantly different age in those who reported a previous asthma diagnosis and those that did not suggests that younger individuals with CL are more likely to be misdiagnosed as having asthma. Asthma diagnoses in the CL population should be follow-up with a more extensive examination. Further investigations should assess the number of individuals being misdiagnosed with asthma when COPD is the correct diagnosis on a larger scale.

6.1.2 Congenital versus Acquired CL Pulmonary Function

Two-tailed t-test analysis indicated that there was no statistically significant difference between any PFT variables tested. This suggests that the severity of COPD is not significantly different

between the two groups. The separate analysis of the pre-BD variables in those who also had post-BD data also found no statistical difference between the congenital and acquired groups. This indicates that not only is there generally no difference found in lung dysfunction, but there is also no significant difference in those who specifically had an obstruction found on PFT. However, there was a statistically significant difference in the age between the congenital and acquired groups, with the congenital group being significantly younger. While additional participants should be tested to verify this finding with a larger sample size, the results are still important for cutis laxa management and our basic understanding of the connection between elastic fiber abnormalities and lung function. As lung function decreases with age, additionally analyses should include age-adjusted statistics. While there was no statistically significant difference in the sex distribution for either the congenital or acquired CL groups, the increase in females in the acquired group could be explained by the higher prevalence of autoimmune disease in women, which are a known trigger for acquired CL.

Since congenital cutis laxa is characterized by an abnormality of elastic fiber formation, which is lung development in the context of COPD, and acquired cutis laxa is characterized by elastic fiber destruction, or lung fiber homeostasis in the context of COPD, lung dysfunction that is not significantly different in the congenital and acquired CL groups shows that elastic fiber formation and elastic fiber homeostasis are equally important. This is the first study indicating that elastic fiber formation is equally important for lung development and lung homeostasis.

The significance found in pre-BD PFT variables that was not seen in post-BD variables is not completely clear. It is likely however, that the small number of subjects who were eligible for bronchodilation made our statistical analysis less powerful. This will be important to re-analyze after additional subjects are tested with bronchodilation.

6.1.3 Unaffected Carrier versus CL Pulmonary Function

Two-tailed t-test analysis indicated that there was a statistically significant difference between unaffected carriers and subjects with CL for pre-BD FEV1 and pre-BD FEV1/FVC. This shows that individuals with CL have significantly worse pulmonary function than carriers, which demonstrates that CL patients have an increased risk of COPD compared to carriers. Additional PFTs on other subjects will help to better define this difference with a larger population.

There were no statistically significant differences for any of the other PFT variables tested indicating that FEV1 and FEV1/FVC are the two most sensitive measures of lung function. None of the LTBP4 carriers had COPD and in fact both the average FEV1 and FEV1/FVC values for this group were >100%, suggesting that heterozygous LTBP4 mutations do not have a detectable negative effect on lung function.

6.1.4 Disability in CL

The SGRQ, which looks at how much someone's lung disease affects daily living, was found to be highly correlated with multiple lung function variables, specifically chest CT visual emphysema score and FEV1/FVC pre-BD. This indicates that COPD has a significant impact on the morbidity and disability in the cutis laxa population and that it can be captured with the SGRQ. This finding should be confirmed with additional subjects. FEV1 was not significantly correlated with SGRQ total score. The Pearson correlation was -0.230 (Table 22), suggesting that the lack of statistical significance was from lack of correlation and not small sample size. Additional data collection may be helpful in determining whether the SGRQ could be used to

measure the impact of COPD severity on disability in cutis laxa and also if lung disease-related disability shows any differences based on genotype, CL onset, or other comorbidities.

None of the other questionnaires were as highly correlated with lung function assessments as the SGRQ, suggesting that the SGRQ is the most sensitive disability questionnaire we currently have in the context of cutis laxa. However, the other questionnaires should be included in any analysis with additional subjects as with increased power other questionnaires may also show significance.

6.1.5 Public Health Significance

COPD is a major public health issue and one of the top causes of morbidity and mortality across the world. Not only does COPD contribute to healthcare costs, but the associated quality of life issues, like disability, inability to work, and depression, increase the cost to the individual and to society. Understanding how all of these factors are related is imperative to decreasing the burden of disease.

6.1.6 Future Directions

Future studies should include longitudinal data on existing subjects, additional subjects with lung function and disability data, and analyses on other complications of CL. Longitudinal data on existing subjects would allow for the analysis of lung function and disability changes over time. Increasing the number of subjects with pulmonary function and disability data would not only increase the power of this analysis, but could lead to additional genotype-phenotype information that is not currently possible given the number of individuals included. It would also be

important to understand the effect of other CL complications on disability. While pulmonary function alone has a detectable effect, understanding disability in the full context of CL is also critical.

7.0 CONCLUSION

We show significantly increased prevalence of COPD in cutis laxa patients compared to the general population and compared to *LTBP4* mutation carriers. COPD in cutis laxa is a frequently debilitating complication, which is now understood to be similar in severity in both congenital and acquired cutis laxa. Additionally, the SGRQ total score is highly correlated with FEV1/FVC and CT visual emphysema score, indicating that the presence of COPD and the severity of emphysema significantly impact the morbidity and disability of CL patients. This is significant, as no previously published studies have quantitatively assessed the differences in lung dysfunction or disability in the cutis laxa population. Additional studies are required to further delineate the contribution of other manifestations of cutis laxa to the morbidity of affected individuals.

APPENDIX A: IRB APPROVAL LETTER

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Zsolt Urban, PhD
From: Sue Beers, PhD, Vice Chair
Date: 6/11/2012
IRB#: REN12050212 / PRO10020125
Subject: Genetics of Extracellular Matrix in Health and Disease

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The Renewal for the above referenced research study was reviewed and approved by the Institutional Review Board, Committee D, which met on 6/7/2012.

Please note the following information:

The risk level designation is Greater Than Minimal.

Approval Date: 6/7/2012
Expiration Date: 6/6/2013

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX B: SUPPLEMENTARY DATA

B.1 PARTICIPANT DEMOGRAPHICS FOR PFT ANALYSIS

Table 11. Participant Demographics for PFT Analysis

Pedigree ID	Patient ID	CL Affection status	Type of CL	Age at PFT	Sex	Gene	Mutation status
CL-004	7166	Affected	Unknown	34	F		
CL-028	7034	Affected	Congenital	14	F	LTBP4	Compound heterozygous
CL-032	7040	Affected	Congenital	58	F		
CL-036	7044	Affected	Congenital	23	F	LTBP4	Compound heterozygous
CL-050	7065	Affected	Acquired	66	F	COL8A1	Heterozygous
CL-060	7078	Affected	Congenital	27	M		
CL-065	7093	Affected	Congenital	23	F	ATP6V0A2	Compound Heterozygous
CL-068	7099	Affected	Acquired	49	M		
CL-094	7159	Affected	Congenital	43	F	ELN	Heterozygous
CL-094	7160	Affected	Congenital	16	M	ELN	Heterozygous
CL-095	7161	Affected	Congenital	40	M		
CL-096	7162	Affected	Acquired	42	F		
CL-109	7183	Affected	Acquired	68	F		
CL-114	7196	Affected	Acquired	56	F		
CL-115	7197	Affected	Acquired	64	F		
CL-116	7198	Affected	Acquired	39	M		
CL-117	7202	Affected	Congenital	11	M		
CL-120	7207	Affected	Congenital	17	F		
CL-121	7209	Affected	Acquired	62	F		
CL-125	7226	Affected	Congenital	3	M	ATP6V0A2	Compound heterozygous
CL-015	7006	Carrier	N/a	45	F	LTBP4	Heterozygous
CL-015	7007	Carrier	N/a	43	M	LTBP4	Heterozygous
CL-015	7192	Carrier	N/a	8	M	LTBP4	Heterozygous
CL-028	7035	Carrier	N/a	50	M	LTBP4	Heterozygous

B.2 PULMONARY FUNCTION RAW DATA

Table 12. FEV1 measurements from PFT testing

Pedigree ID	Patient ID	Age	Ref value (L)	Pre-BD (L)	Pre-BD % predicted	Post-BD (L)	Post-BD % predicted	Post-BD % change
CL-004	7166	34	2.35	2.73	116	2.78	118	2
CL-028	7034	14	2.63	1.88	71			
CL-032	7040	58	2.51	0.78	31	0.86	34	9
CL-036	7044	23	3.19	0.90	28	1.31	41	46
CL-050	7065	66	2.33	1.82	78			
CL-060	7078	27	4.39	1.19	27	1.70	39	43
CL-065	7093	23	3.38	2.32	69			
CL-068	7099	49	3.67	2.10	57	2.25	61	7
CL-094	7159	43	2.67	1.59	60	1.66	62	5
CL-094	7160	16	3.47	2.72	78	3.02	87	11
CL-095	7161	40	3.80	3.52	93	3.78	99	7
CL-096	7162	42	3.38	2.08	62	2.47	73	19
CL-109	7183	68	2.38	2.48	104			
CL-114	7196	56	3.22	2.73	85			
CL-115	7197	64	2.35	1.03	44	1.36	58	32
CL-116	7198	39	4.45	1.14	26	1.39	31	21
CL-117	7202	11	2.81	2.17	77			
CL-120	7207	17	3.52	2.74	78			
CL-121	7209	62	22.48	2.19	88	2.43	98	13
CL-125	7226	3	0.42	0.56	133			
CL-015	7006	45	3.24	2.73	80	2.89	89	6
CL-015	7007	43	3.99	5.06	127	5.26	132	4
CL-015	7192	8	1.80	1.88	104			
CL-028	7035	50	2.95	2.93	99			

Table 13. FVC measurements from PFT testing

Pedigree ID	Patient ID	Age	Ref value (L)	Pre-BD (L)	Pre-BD % predicted	Post-BD (L)	Post-BD % predicted	Post-BD % change
CL-004	7166	34	2.74	3.14	115	3.21	117	2
CL-028	7034	14	2.95	3.59	122			
CL-032	7040	58	3.24	1.67	52	1.76	54	5.10
CL-036	7044	23	3.66	2.19	60	2.65	72	21
CL-050	7065	66	3.05	2.16	71			
CL-060	7078	27	5.34	4.01	75	4.62	87	15
CL-065	7093	23	3.91	4.22	108			
CL-068	7099	49	4.71	4.43	94	4.41	94	-0.50
CL-094	7159	43	3.18	2.27	71	2.17	68	-4
CL-094	7160	16	4.04	4.96	123	4.93	122	-1
CL-095	7161	40	4.60	5.20	113	5.37	117	3
CL-096	7162	42	4.19	3.39	81	3.92	94	16
CL-109	7183	68	3.13	3.26	104			
CL-114	7196	56	4.13	3.34	81			
CL-115	7197	64	3.07	1.71	56	2.00	65	17
CL-116	7198	39	5.60	2.40	43	3.06	55	27
CL-117	7202	11	3.07	2.69	88			
CL-120	7207	17	3.98	3.59	90			
CL-121	7209	62	3.22	3.17	98	3.28	102	3
CL-125	7226	3	1.45	0.68	47			
CL-015	7006	45	4.04	3.53	87	3.55	88	1
CL-015	7007	43	5.05	6.14	122	6.21	123	1
CL-015	7192	8	2.00	1.91	96			
CL-028	7035	50	3.75	3.48	92			

Table 14. FEV1/FVC measurements from PFT testing

Pedigree ID	Patient ID	Age	Pre-BD %	Pre-BD % predicted	Post-BD %	Post-BD % predicted	Post-BD % change
CL-004	7166	34	87	101	87	101	0.00
CL-028	7034	14	52				
CL-032	7040	58	47	59	49	61	4.10
CL-036	7044	23	41		50		
CL-050	7065	66	84				
CL-060	7078	27	30	37	36	43	20.00
CL-065	7093	23	55				
CL-068	7099	49	47	58	51	63	7.80
CL-094	7159	43	70	83	77	91	9.00
CL-094	7160	16	55	64	61	72	12.00
CL-095	7161	40	68	82	70	85	4.00
CL-096	7162	42	61	75	63	78	3.00
CL-109	7183	68	76	100			
CL-114	7196	56	82				
CL-115	7197	64	60	78	68		
CL-116	7198	39	48	59	45	56	-4.00
CL-117	7202	11	81	94			
CL-120	7207	17	76	87			
CL-121	7209	62	69	88	74	84	7.00
CL-125	7226	3	82				
CL-015	7006	45	77	95	81	81	5.00
CL-015	7007	43	82	101	85	105	4.00
CL-015	7192	8	86				
CL-028	7035	50	84	107			

Table 15. DLCO measurements from PFT testing and Chest CT results

Pedigree ID	Patient ID	Age	DLCO: Pre- or Post-BD	DLCO ref value (mL CO/ min/mmHg)	DLCO (mL CO/min /mmHg)	DLCO % predicted	Chest CT Visual emphysema score
CL-004	7166	34	Pre-BD	26.88	21.12	79	
CL-028	7034	14	Pre-BD	20.60	13.00	63	
CL-032	7040	58	Post-BD	24.20	9.60	39	4
CL-036	7044	23	Post-BD				1
CL-050	7065	66	Pre-BD	23.20	10.90	47	
CL-060	7078	27	Post-BD	40.50	22.90	57	0
CL-065	7093	23	Pre-BD	30.60	22.70	74	0
CL-068	7099	49	Post-BD	34.50	21.00	61	4
CL-094	7159	43	Pre-BD	37.51	22.03	59	
CL-094	7160	16	Pre-BD	21.70	29.94	138	
CL-095	7161	40	Pre-BD	31.83	30.22	96	
CL-096	7162	42	Post-BD	29.90	17.20	58	
CL-109	7183	68	Pre-BD	23.80	14.80	62	0
CL-114	7196	56	Pre-BD	28.90	11.60	40	1
CL-115	7197	64	Post-BD	23.40	12.40	53	0
CL-116	7198	39	Post-BD	40.80	15.10	37	3
CL-117	7202	11	Pre-BD	20.30	16.50	81	
CL-120	7207	17	Pre-BD	31.90	15.50	49	
CL-121	7209	62	Post-BD	24.20	21.40	89	2
CL-125	7226	3	N/a	7.80			
CL-015	7006	45	Post-BD	3.49	25.80	74	1
CL-015	7007	43	Post-BD	37.00	34.90	94	0
CL-015	7192	8	Pre-BD				
CL-028	7035	50	Pre-BD	28.00	26.90	96	0

B.3 DISABILITY RAW DATA

Table 16. Disability Questionnaire Scores

Pedigree ID	Patient ID	SGRQ symptom score	SGRQ activity score	SGRQ impact score	SGRQ total score	Shortness of breath total	BDI score	Stanford brief activity score	MRC total score
CL-004	7166								
CL-028	7034	38.87	0.00	0.00	6.58	0	0	2	
CL-032	7040	66.30	85.80	36.00	56.10	59	3	1	4
CL-036	7044	0.00	11.20	1.90	4.60	3	1	1	1
CL-050	7065	53.00	93.30	58.40	68.90	50	18	1	3
CL-060	7078								
CL-065	7093								
CL-068	7099	85.40	29.60	29.40	38.80	12	12	2	2
CL-094	7159								
CL-094	7160								
CL-095	7161								
CL-096	7162	15.80	50.60	10.30	24.00				
CL-109	7183								
CL-114	7196	0.00	6.20	4.60	4.30	120	0	3	0
CL-115	7197	38.18	23.72	1.90	15.15	6	6	3	2
CL-116	7198								
CL-117	7202	40.38	0.00	0.00	5.85				
CL-120	7207	9.40	12.20	0.00	5.70	6	0	0	1
CL-121	7209								
CL-125	7226	60.49	0.00	30.30	24.48	0	0	0	0
CL-015	7006	18.40	0.00	0.00	2.65	0	2	1	0
CL-015	7007	0.00	0.00	0.00	0.00	2	3	1	0
CL-015	7192	21.69	0.00	8.34	7.47	0	0	1	0
CL-028	7035	0.00	0.00	0.00	0.00	0	2	3	0

B.4 DISABILITY AND PFT CORRELATIONS

Table 17. PFT T-test analysis for Congenital versus Acquired CL

	Equal Variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	95% Confidence Interval of the Difference	
									Lower	Upper
FVC pre-BD % predicted	Assumed	1.24	.28	.66	17	.514	7.77	11.65	-16.82	32.37
	Not assumed			.69	16.92	.496	7.77	11.15	-15.77	31.32
FVC post-BD % predicted	Assumed	.45	.51	.31	9	.762	4.67	14.97	-29.21	38.54
	Not assumed			.32	8.93	.756	4.67	14.55	-28.30	37.63
FEV1 pre-BD % predicted	Assumed	.05	.81	-.02	17	.984	-.27	13.54	-28.84	28.29
	Not assumed			-.02	16.70	.984	-.27	13.09	-27.93	27.38
FEV1 post-BD % predicted	Assumed	.41	.53	-.24	9	.812	-3.87	15.76	-39.53	31.80
	Not assumed			-.24	8.93	.810	-3.87	15.58	-39.16	31.43
FEV1/FVC pre-BD % predicted	Assumed	.56	.46	-.39	11	.700	-4.05	10.23	-26.58	18.48
	Not assumed			-.40	10.99	.695	-4.05	10.07	-26.21	18.12
FEV1/FVC post-BD % predicted	Assumed	.55	.47	.01	7	.990	.15	11.29	-26.55	26.85
	Not assumed			.01	6.87	.989	.15	10.77	-25.41	25.71
DLCO % predicted	Assumed	1.74	.20	1.43	15	.173	17.01	11.87	-8.30	42.33
	Not assumed			1.48	12.65	.163	17.01	11.48	-7.86	41.89

Table 18. Congenital versus Acquired CL Age Normality Test

		Shapiro-Wilk		
	CL onset	Statistic	df	Two-tailed p-value
PFT age	Congenital	.935	11	.459
	Acquired	.900	8	.289

Table 19. Congenital versus Acquired CL Normality Test

	CL onset	Shapiro-Wilk		
		Statistic	df	P-value
FVC pre-BD percent predicted	Congenital	.914	5	.490
	Acquired	.847	4	.216
FVC post-BD percent predicted	Congenital	.918	5	.518
	Acquired	.776	4	.066
FEV1 pre-BD percent predicted	Congenital	.918	5	.520
	Acquired	.972	4	.856
FEV1 post-BD percent predicted	Congenital	.914	5	.494
	Acquired	.994	4	.976
FEV1/FVC pre-BD percent predicted	Congenital	.910	5	.468
	Acquired	.883	4	.350
FEV1/FVC post-BD percent predicted	Congenital	.961	5	.812
	Acquired	.936	4	.631
DLCO percent predicted	Congenital	.906	5	.441
	Acquired	.959	4	.775

Table 20. PFT T-test analysis for CL versus CL Carrier

	Equal Variances	Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff	Std. Error Diff	95% Confidence Interval of the Difference	
									Lower	Upper
FVC pre-BD % predicted	Assumed	1.939	.178	1.114	22	.277	14.65	13.15	-12.63	41.93
	Not Assumed			1.524	6.621	.174	14.65	9.61	-8.34	37.64
FVC post-BD % predicted	Assumed	.115	.741	.979	12	.347	18.25	18.63	-22.35	58.85
	Not Assumed			.968	1.346	.475	18.25	18.86	-115.32	151.82
FEV1 pre-BD % predicted	Assumed	1.062	.314	2.072	22	.050	32.25	15.56	-.025	64.52
	Not Assumed			2.753	6.255	.032	32.25	11.71	3.87	60.63
FEV1 post-BD % predicted	Assumed	.036	.852	1.991	12	.070	43.75	21.97	-4.13	91.63
	Not Assumed			1.899	1.314	.260	43.75	23.03	-125.83	213.33
FEV1/FVC pre-BD % predicted	Assumed	3.260	.091	2.257	15	.039	24.93	11.04	1.39	48.47
	Not Assumed			4.130	11.257	.002	24.93	6.04	11.68	38.18
FEV1/FVC post-BD % predicted	Assumed	.127	.729	1.433	10	.182	19.60	13.68	-10.88	50.08
	Not Assumed			1.480	1.477	.317	19.60	13.25	-61.88	101.08
DLCO % predicted	Assumed	.835	.372	1.511	19	.147	22.33	14.78	-8.60	53.27
	Not Assumed			2.448	5.395	.054	22.33	9.12	-.61	45.28

Table 21. Affected CL versus Unaffected Carrier Normality Test

	CL onset	Shapiro-Wilk		
		Statistic	df	P-value
FVC pre-BD percent predicted	Carrier			
	Cutis Laxa	.956	10	.744
FVC post-BD percent predicted	Carrier			
	Cutis Laxa	.909	10	.276
FEV1 pre-BD percent predicted	Carrier			
	Cutis Laxa	.939	10	.544
FEV1 post-BD percent predicted	Carrier			
	Cutis Laxa	.941	10	.565
FEV1/FVC pre-BD percent predicted	Carrier			
	Cutis Laxa	.966	10	.854
FEV1/FVC post-BD percent predicted	Carrier			
	Cutis Laxa	.983	10	.980
DLCO percent predicted	Carrier			
	Cutis Laxa	.897	10	.201

Table 22. Pearson Correlations for PFT Data Compared to Disability and PFT variables

		CT visual emphysema score	FVC pre-BD % pred	FVC post-BD % pred	FEV1 pre-BD % pred	FEV1 post-BD % pred	FEV1/FVC pre-BD % pred	FEV1/FVC post-BD % pred	DLCO % pred
CT visual emphysema score	Pearson Correlation	1	-.412	-.370	-.427	-.376	-.490	-.338	-.451
	Sig. (2-tailed)		.162	.327	.145	.319	.151	.459	.141
	N	13	13	9	13	9	10	7	12
FVC pre-BD percent predicted	Pearson Correlation	-.412	1	.981**	.508*	.882**	.468	.620*	.723**
	Sig. (2-tailed)	.162		.000	.011	.000	.058	.031	.000
	N	13	24	14	24	14	17	12	21
FVC post-BD percent predicted	Pearson Correlation	-.370	.981**	1	.842**	.862**	.428	.555	.852**
	Sig. (2-tailed)	.327	.000		.000	.000	.145	.061	.000
	N	9	14	14	14	14	13	12	13
FEV1 pre-BD percent predicted	Pearson Correlation	-.427	.508*	.842**	1	.992**	.840**	.901**	.536*
	Sig. (2-tailed)	.145	.011	.000		.000	.000	.000	.012
	N	13	24	14	24	14	17	12	21
FEV1 post-BD percent predicted	Pearson Correlation	-.376	.882**	.862**	.992**	1	.825**	.872**	.708**
	Sig. (2-tailed)	.319	.000	.000	.000		.001	.000	.007
	N	9	14	14	14	14	13	12	13
FEV1/FVC pre-BD percent predicted	Pearson Correlation	-.490	.468	.428	.840**	.825**	1	.951**	.323
	Sig. (2-tailed)	.151	.058	.145	.000	.001		.000	.206
	N	10	17	13	17	13	17	12	17
FEV1/FVC post-BD percent predicted	Pearson Correlation	-.338	.620*	.555	.901**	.872**	.951**	1	.437
	Sig. (2-tailed)	.459	.031	.061	.000	.000	.000		.155
	N	7	12	12	12	12	12	12	12
DLCO percent predicted	Pearson Correlation	-.451	.723**	.852**	.536*	.708**	.323	.437	1
	Sig. (2-tailed)	.141	.000	.000	.012	.007	.206	.155	
	N	12	21	13	21	13	17	12	21

Table 22 Continued

SGRQ symptom score	Pearson Correlation	.846**	-.308	-.373	-.201	-.480	-.848**	-.879*	-.373
	Sig. (2-tailed)	.008	.264	.410	.473	.276	.004	.049	.232
	N	8	15	7	15	7	9	5	12
SGRQ activity score	Pearson Correlation	.770*	-.427	-.570	-.477	-.612	-.827**	-.714	-.603*
	Sig. (2-tailed)	.025	.113	.182	.072	.144	.006	.176	.038
	N	8	15	7	15	7	9	5	12
SGRQ impact score	Pearson Correlation	.962**	-.445	-.368	-.068	-.528	-.881**	-.864	-.491
	Sig. (2-tailed)	.000	.097	.416	.809	.223	.002	.059	.105
	N	8	15	7	15	7	9	5	12
SGRQ total score	Pearson Correlation	.896**	-.401	-.298	-.230	-.501	-.928**	-.931	-.520
	Sig. (2-tailed)	.006	.155	.567	.429	.311	.001	.069	.101
	N	7	14	6	14	6	8	4	11
Shortness of breath	Pearson Correlation	.255	-.235	-.580	-.174	-.537	-.703	-.645	-.644*
	Sig. (2-tailed)	.543	.440	.228	.570	.272	.078	.355	.044
	N	8	13	6	13	6	7	4	10
BDI total	Pearson Correlation	.514	-.116	.131	-.212	-.096	-.630	-.466	-.180
	Sig. (2-tailed)	.193	.705	.805	.488	.856	.129	.534	.618
	N	8	13	6	13	6	7	4	10
Stanford brief activity survey	Pearson Correlation	-.310	.140	-.233	-.231	-.201	.051	-.473	.111
	Sig. (2-tailed)	.455	.647	.657	.448	.702	.914	.527	.760
	N	8	13	6	13	6	7	4	10
MRC total score	Pearson Correlation	.734*	-.463	-.739	-.687*	-.755	-.897**	-.810	-.624
	Sig. (2-tailed)	.038	.130	.093	.014	.082	.006	.190	.073
	N	8	12	6	12	6	7	4	9

* Correlation is significant at the 0.01 level (2-tailed)

**Correlation is significant at the 0.05 level (2-tailed)

Table 23. Pearson Correlations for Disability Scores Compared to Disability and PFT variables

		SGRQ symptom score	SGRQ activity score	SGRQ impact score	SGRQ total score	Shortness of breath	BDI total	Stanford brief activity survey	MRC total score
CT visual emphysema score	Pearson Correlation	.846**	.770*	.962**	.896**	.255	.514	-.310	.734*
	Sig. (2-tailed)	.008	.025	.000	.006	.543	.193	.455	.038
	N	8	8	8	7	8	8	8	8
FVC pre-BD percent predicted	Pearson Correlation	-.308	-.427	-.445	-.401	-.235	-.116	.140	-.463
	Sig. (2-tailed)	.264	.113	.097	.155	.440	.705	.647	.130
	N	15	15	15	14	13	13	13	12
FVC post-BD percent predicted	Pearson Correlation	-.373	-.570	-.368	-.298	-.580	.131	-.233	-.739
	Sig. (2-tailed)	.410	.182	.416	.567	.228	.805	.657	.093
	N	7	7	7	6	6	6	6	6
FEV1 pre-BD percent predicted	Pearson Correlation	-.201	-.477	-.068	-.230	-.174	-.212	-.231	-.687*
	Sig. (2-tailed)	.473	.072	.809	.429	.570	.488	.448	.014
	N	15	15	15	14	13	13	13	12
FEV1 post-BD percent predicted	Pearson Correlation	-.480	-.612	-.528	-.501	-.537	-.096	-.201	-.755
	Sig. (2-tailed)	.276	.144	.223	.311	.272	.856	.702	.082
	N	7	7	7	6	6	6	6	6
FEV1/FVC pre-BD percent predicted	Pearson Correlation	-.848**	-.827**	-.881**	-.928**	-.703	-.630	.051	-.897**
	Sig. (2-tailed)	.004	.006	.002	.001	.078	.129	.914	.006
	N	9	9	9	8	7	7	7	7
FEV1/FVC post-BD percent predicted	Pearson Correlation	-.879*	-.714	-.864	-.931	-.645	-.466	-.473	-.810
	Sig. (2-tailed)	.049	.176	.059	.069	.355	.534	.527	.190
	N	5	5	5	4	4	4	4	4
DLCO percent predicted	Pearson Correlation	-.373	-.603*	-.491	-.520	-.644*	-.180	.111	-.624
	Sig. (2-tailed)	.232	.038	.105	.101	.044	.618	.760	.073
	N	12	12	12	11	10	10	10	9

Table 23 Continued

SGRQ symptom score	Pearson Correlation	1	.467	.702**	.713**	.008	.535	-.136	.648*
	Sig. (2-tailed)		.079	.004	.004	.980	.060	.657	.023
	N	15	15	15	14	13	13	13	12
SGRQ activity score	Pearson Correlation	.467	1	.791**	.914**	.422	.704**	-.107	.933**
	Sig. (2-tailed)	.079		.000	.000	.151	.007	.727	.000
	N	15	15	15	14	13	13	13	12
SGRQ impact score	Pearson Correlation	.702**	.791**	1	.952**	.314	.725**	-.278	.674*
	Sig. (2-tailed)	.004	.000		.000	.296	.005	.357	.016
	N	15	15	15	14	13	13	13	12
SGRQ total score	Pearson Correlation	.713**	.914**	.952**	1	.306	.769**	-.230	.841**
	Sig. (2-tailed)	.004	.000	.000		.334	.003	.472	.001
	N	14	14	14	14	12	12	12	11
Shortness of breath	Pearson Correlation	.008	.422	.314	.306	1	.141	.307	.270
	Sig. (2-tailed)	.980	.151	.296	.334		.646	.308	.396
	N	13	13	13	12	13	13	13	12
BDI total	Pearson Correlation	.535	.704**	.725**	.769**	.141	1	.091	.611*
	Sig. (2-tailed)	.060	.007	.005	.003	.646		.767	.035
	N	13	13	13	12	13	13	13	12
Stanford brief activity survey	Pearson Correlation	-.136	-.107	-.278	-.230	.307	.091	1	-.025
	Sig. (2-tailed)	.657	.727	.357	.472	.308	.767		.938
	N	13	13	13	12	13	13	13	12
MRC total score	Pearson Correlation	.648*	.933**	.674*	.841**	.270	.611*	-.025	1
	Sig. (2-tailed)	.023	.000	.016	.001	.396	.035	.938	
	N	12	12	12	11	12	12	12	12

* Correlation is significant at the 0.01 level (2-tailed)

**Correlation is significant at the 0.05 level (2-tailed)

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