CHRONIC LUNG DISEASE IN CUTIS LAXA

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BACKGROUND: Cutis laxa (CL) is a group of disorders characterized by loose, inelastic, and redundant skin. The different types of CL are distinguished by clinical features, inheritance, and molecular findings. The objective of this study was to characterize the pulmonary phenotype of the different types of CL based on age of onset (congenital, acquired/late-onset, or unknown) and mutational status. The first aim of this study was to collect clinical data to better define the pulmonary involvement in cutis laxa. The second aim was to determine if heterozygous carriers of recessive types of cutis laxa are susceptible to chronic lung disease. **METHODS:** Clinical questionnaires, medical histories, and pulmonary function tests (PFTs) were used to collect clinical data on patients with a confirmed or suspected diagnosis of CL and unaffected first-degree relatives with a known mutation (carriers). The clinical data was then compared between the groups categorized by age of onset and between those with known mutations (mutational status). **RESULTS**: The clinical questionnaires and medical histories of 83 CL patients with 8 acquired/late-onset, 52 congenital, and 23 of unknown etiology and 5 carriers were analyzed. In addition, mutations have been identified in 27 of the 83 patients in *ELN, FBLN4*, *FBLN5, ATP6V0A2,* or *LTBP4,* and the 5 carriers had mutations in either *FBLN4* or *LTBP4*. The most common respiratory responses amongst the patients included pneumonia (24.1%), tachypnea (15.7%), and emphysema (12.0%). The only statistically significant finding was dyspnea in acquired/late-onset patients. Of those with a known mutation, 15 reported pulmonary involvement, with 10 of these individuals having at least one *LTBP4* mutation. For the 13 PFTs

collected, the 10 CL patients ranged from normal lung function to very severe obstruction, and 3 carriers were deemed to have normal function. An important new finding was the presence of pulmonary obstructive disease in those with *ATP6V0A2* mutations. **CONCLUSION:** These results demonstrate a high prevalence and significant heterogeneity of pulmonary complications in CL. Further research is needed to determine any correlation between specific pulmonary findings and genotypes. **PUBLIC HEALTH SIGNIFICANCE:** Chronic lung disease is a common cause of morbidity in the general population. Uncovering the genetic basis of chronic lung disease in inherited syndromes has the potential to identify key molecular targets for improved diagnosis and treatment of common respiratory ailments.

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PREFACE

I would like to thank, and I have a great amount of gratitude for, the individuals and families with cutis laxa who have participated in our research. Without their continued involvement and interest, much of our research, including this project, would not be possible.

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1.0 INTRODUCTION

Cutis laxa (CL) is a phenotypically and genetically heterogeneous group of conditions characterized by the presence of loose, lax, inelastic, and redundant skin. The different types of CL are classified by clinical presentation, inheritance, and molecular findings. To date at least nine different types of CL have been classified. These types demonstrate autosomal dominant and recessive inheritance, X-linked inheritance, and acquired onset. Mutations in several genes are known to cause cutis laxa: copper transporting adenosine triphosphatase (*ATP7A*), elastin (*ELN*), fibulin-4 (*FBLN4*), fibulin-5 (*FBLN5*), the A2 subunit of the vacuolar-type H^+ -ATPase (*ATP6V0A2*), pyroline-5-carboxylate reductase 1 (*PYCR1*), latent transforming growth factor-beta-binding protein 4 (*LTBP4*), and a guanine nucleotide exchange factor (GEF) for RAB5 (*RIN2*). The majority of these proteins are known components of or are involved in the formation of elastic fibers. Of the clinical findings there is an overlap between the different types with common features including cutis laxa, joint laxity, hernias, bladder and/or intestinal diverticulae, and developmental or growth delays. Other significant systemic involvement affects the cardiovascular and pulmonary systems. The most common cardiovascular findings include aortic dilation and aneurysm, vascular tortuosity, and stenosis. The most common pulmonary features include obstructive lung disease, emphysema, and recurrent respiratory infections. The cardiovascular and pulmonary complications cause significant morbidity for

cutis laxa patients. The objective of this study is to better characterize the pulmonary phenotype of the different types of CL based on age of onset and mutational status.

2.0 BACKGROUND

2.1 TYPES OF CUTIS LAXA

2.1.1 Occipital Horn Syndrome (OHS)

X-linked Cutis Laxa (XCL)

Previously known as Ehlers-Danlos Syndrome Type IX

OHS is an X-linked type of cutis laxa caused by mutations in the gene *ATP7A*. This type is characterized by the presence of skeletal anomalies that include occipital exostoses, skin laxity, joint laxity, hernias, bladder diverticulae, vascular tortuosity, dysautonomia, and low-normal intelligence (Lazoff et al 1975, Byers et al 1980, Kaler et al 1994). The clinical features of OHS are related to a decrease in the activity of the copper transporting adenosine triphosphatase (ATP7A). As a result there is a decrease in intestinal absorption of copper, leading to low serum copper and ceruloplasmin, and accumulation of copper in fibroblasts (Das et al 1995).

Mutations in *ATP7A* are also known to cause Menkes disease (MD) (Mercer et al 1993, Vulpe et al 1993, Chelly et al 1993). MD is a lethal disorder characterized by neurodegeneration and "kinky" hair. Those affected typically present with failure to thrive and developmental delay. There can be vascular, urinary, skeletal, and muscular involvement. A common skin

feature is seborrheic dermatitis. Mutations typically result in complete loss of function of ATP7A, thus causing a more severe phenotype.

Kaler et al (1994) demonstrated that those with OHS, or milder Menkes disease variants, have residual *ATP7A* expression. Splicing mutations in *ATP7A* have been found in a majority of OHS patients (Kaler et al 1994, Das et al 1995, Ronce et al 1997, Qi & Byers 1998). Other mutations reported include frameshift and missense (Tang et al 2006, Dagenais et al 2001, Levinson et al 1996). Several of these groups showed low levels of normal transcripts, and Moller et al (2000) & Gu et al (2001) found normal transcript levels of 2-5%. In these cases the presence of low levels of normal transcript or low protein function explains the milder features compared to Menkes disease.

2.1.2 Autosomal Dominant Cutis Laxa (ADCL)

ELN-Related Cutis Laxa

In 1972, Beighton provided a review of the autosomal dominant and recessive forms of cutis laxa. The review noted that skin laxity was the only finding in the majority of reported cases of dominant cutis laxa. Other health complications noted included pulmonary or cardiovascular features. Damkier et al (1991) reported additional systemic involvement in a family with three affected individuals (in five generations) with gastrointestinal diverticulae, hernias, genital prolapse, and muscle weakness. No pulmonary or cardiovascular findings were reported in this family. Since these publications a number of cases with variable systemic involvement have been described.

Mutations in the elastin gene (ELN) have been shown to cause autosomal dominant CL (ADCL) (Tassabehji et al 1998). Elastin is the main component of elastic fibers. It is secreted from the cell as tropoelastin monomers that are cross-linked to form polymers by a lysyl oxidase enzyme (Narayanan et al 1977). Elastin is thought to provide the elastic coil of the fiber (Partridge 1962). The other components of elastic fibers are microfibrils. Microfibrils are made up of fibrillins, latent transforming growth factor-beta-binding proteins (LTBPs) (Gibson et al 1995), microfibril associated glycoproteins (MAGPs) (Gibson et al 1996), and fibulins (Timpl et al 2003). Microfibrils are suggested to provide a scaffold, thus the shape, of the elastic fiber, and thought to position the tropoelastin monomers for cross-linking (Narayanan et al 1977). The greatest level of expression of elastic fiber proteins are primarily during the prenatal and neonatal periods, after about 21 days postnatal levels decrease and remain at a stable low rate during adulthood (Marianai, Reed, Shapiro 2002).

Tassabehji et al (1998) first detected a deletion in *ELN* in exon 32. The patient had cutis laxa, a cardiac murmur, and right ventricular hypertrophy. Elastin fibers in the patient's fibroblasts had abnormal structure and were present in low amounts. As a result, Tassabehji et al proposed that mutant tropoelastin was incorporated into the elastic fibers, and the monomers were packed abnormally. As a result, there was less elastic recoil due to a loose framework/abnormal architecture. They suggested that this could explain the cutis laxa, hernias, and pulmonary stenoses seen in those affected, and that cutis laxa was due to a dominant negative effect.

Abnormalities of the tropoelastin and the mature elastin have also been shown by Hu et al (2010) and Callewaert et al (2011). Using mouse models, Hu et al (2010) demonstrated that incorporation of mutant elastin into elastic fibers was needed for the development of cutis laxa.

In these mice, the lung elastic recoil was reduced. Callewaert et al (2011) showed poor integration of mutant tropoelastin into elastin fibers is a result of increased aggregation of the mutant tropoelastin and decreased binding to microfibrils. Similar findings have been shown in other types of cutis laxa (Hu et al 2006, Hucthagowder et al 2009).

The majority of mutations identified in *ELN* result in stable transcripts with a disruption of the C-terminus (Callewaert et al 2011, Urban et al 2005, Rodriquez-Revenga et al 2004, Zhang et al 1999). Most mutations in ADCL are short deletions or insertions within the last 4 exons of *ELN* (exons 30-34) causing a reading frame shift which replaces the C-terminus of tropoelastin with a missense peptide sequence. (Zhang et al 1999, Szabo et al 2006, Callewaert et al 2011, Rodriquez-Revenga et al 2004). In addition, a partial tandem duplication (Urban et al 2005) and a splicing mutation in exon 25 of *ELN* (Graul-Neumann et al 2008) were also reported in ADCL. In addition, a dominant mutation was found in the gene *FBLN5* (Markova et al 2003). As *FBLN5* mutations generally cause ARCL1, this finding blurs the distinction between genetics of ADCL and autosomal recessive CL (ARCL).

The clinical features of ADCL individuals with *ELN* mutations include pulmonary and cardiovascular manifestations. Cardiovascular involvement comprises redundant mitral and tricuspid valves, right ventricular hypertrophy (Zhang et al 1999), mitral valve stenosis and regurgitation, regurgitation of the aortic valve (Rodriquez-Revenga et al 2004), and aortic dilation, aneurysm, dissection and rupture (Callewaert et al 2011, Szabo et al 2006). Pulmonary involvement includes reduced expiratory flow and dyspnea (Zhang et al 1999), emphysema (Graul-Neumann et al 2008, Urban et al 2005, Rodriquez-Revenga et al 2004), obstructive pulmonary disease and recurrent respiratory infections (Callewaert et al 2011). Characteristic

facial features include coarse facies, long philtrum, hooked nose, and large dysplastic ears (Callewaert et al 2011).

Of these ADCL cases, a demonstrative example is the tandem duplication in *ELN* identified in a mother-daughter pair (Urban et al 2005) previously described by Corbett et al (1994). Both had cutis laxa and pulmonary complications. Due to severe COPD, the mother received a double lung transplant. Prior to lung transplant she had frequent lung infections and was diagnosed with bronchiectasis, and emphysema was confirmed at age 36 years. She also had an inguinal hernia. Her daughter was found to have emphysema based on lung function tests. Of relevance both had a history of smoking and were M/Z heterozygotes for alpha-1-antitrypsin. However, only a minority of such individuals will develop emphysema (Dahl et al 2002), typically with onset after the fourth decade of life. Evidence of emphysema was present in the second to third decades of life in these patients.

The cases described by Szabo et al (2006) suggest that *ELN* mutations can involve cardiac abnormalities. These cases were from two families. In family 1 all affected members had either aortic dilatation or severe aneurysm or dissection, only two members had cutis laxa, and two members had inguinal hernias. Family 2 had one affected individual with cutis laxa and aortic dilatation. This group of researchers demonstrated that *ELN* mutations that are associated with cutis laxa may also cause aortic disease.

2.1.3 Autosomal Recessive Cutis Laxa Type 1 (ARCL1)

FBLN4 (EFEMP2)-Related Cutis Laxa

FBLN5-Related Cutis Laxa

Autosomal recessive CL type 1 (ARCL1) is caused by mutations in the *FBLN4 (EFEMP2)* and *FBLN5* genes. Those with *FBLN4* mutations were found to have generalized cutis laxa, emphysema, respiratory infections, arterial tortuosity and aortic dilation or aneurysm, hernias, joint laxity, and arachnodactyly, and bone fragility. Those with *FBLN5* mutations were found to have cutis laxa, emphysema, respiratory infections, supravalvular aortic stenosis (SVAS), and hernias. To date three cases have been reported with *FBLN4* mutations, and eight cases have been reported with *FBLN5* mutations.

Fibulins (FBLNs) are a family of proteins associated with elastic fiber formation. To date seven FBLNs have been identified, and each has calcium-binding EGF-like domains and a conserved C-terminal domain (Argraves et al 2003, Timpl et al 2003). Fibulins are involved in organogenesis and expressed in a variety of systems, primarily in blood vessels (Argraves et al 2003).

The gene for the EGF-containing fibulin-like extracellular matrix protein 2 (*EFEMP2*), is also known as fibulin 4 (*FBLN4)*. Fibulin 4 is primarily expressed in the large veins and arteries and some capillaries (Argraves et al 2003). Mouse models have shown that fibulin 4 directly interacts with tropoelastin and may be involved in its deposition onto fibrillin microfibrils (McLaughlin et al 2006). In addition, loss of fibulin 4 may affect the cross-linking of tropoelastin monomers. The primary findings in these mouse models are vascular anomalies,

which include arterial tortuosity, dilation, and aneurysms (Horiguchi et al 2009, McLaughlin et al 2006).

Fibulin-5 is encoded by the *FBLN5* gene, also called *DANCE* (developmental arteries and neural crest EGF-like). Fibulin 5 has been shown to be expressed in the arterial vasculature (Argraves et al 2003). Mouse models have demonstrated that fibulin 5 binds to tropoelastin, directly interacts with elastic fibers, and is involved in the maturation of the elastic fiber (Nakamura et al 2002, Yanagisawa et al 2002). Fibulin 5 also promotes adhesion of elastic fibers to cells (Nakamura et al 1999). Fibulin-5 deficient mice present with cutis laxa (loose skin), arterial and aortic tortuosity, and emphysema (Nakamura et al 2002).

2.1.3.1 Mutations in FBLN4

Hucthagowder et al (2006) reported the first case of fibulin-4 (FBLN4) related cutis laxa. The patient was born with multiple fractures, and at 9 months was diagnosed with generalized cutis laxa, transparent skin, hypotonia, emphysema, arterial tortuosity, inguinal and diaphragmatic hernias, joint laxity, and pectus excavatum. At 2 years of age the patient was diagnosed with aortic root aneurysm. Mutational analysis revealed homozygosity for a *FBLN4* mutation (c.169G>A, p.E57K). Evaluation of fibroblasts revealed reduced levels of FBLN4 in the extracellular matrix and underdeveloped elastic fibers. These results demonstrated that the mutation E57K severely impaired the secretion of the FBLN4.

Dasouki et al (2007) reported a second case. The patient was noted to have long hands, mild generalized skin laxity, pulmonary hypertension, dilation of the ascending aorta and dissection of the wall of the main braches of the pulmonary arteries, dilated great vessels, tortuous abdominal aorta, enlarged right ventricle and right atrium, multiple episodes of bradycardia, and development of pneumonia. The patient passed away at 27 days of age.

Mutational analysis revealed compound heterozygous mutations in FBLN4 (c.835C>T, p.R279C; c.1070_1073dupCCGC). Histological evaluation revealed similar findings as Hucthagowder et al (2006).

Hoyer et al (2009) reported a third case of FBLN4 related cutis laxa. The patient was noted to have cutis laxa, arachnodactyly, medial rotation of the feet, spina bifida, joint laxity, facial dysmorphism, and microcephaly. Autopsy revealed hypoplastic diaphragm, collapsed lungs, emphysematous lung changes, and vascular tortuosity. Mutational analysis revealed homozygosity for a FBLN4 mutation (c.800G>A, p.C267Y). Histological evaluation revealed fragmentation of elastic fibers.

Mutations in FBLN4 have also been found in three patients with arterial tortuosity, aneurysms, and/or stenosis (Renard et al 2010). Of these three patients none had cutis laxa, and one had velvety skin but normal scarring. As Renard et al mentions, these patients have a clinical overlap with Loeys-Dietz syndrome caused by mutations in *TGFBR1* and *TGFBR2*, and arterial tortuosity syndrome caused by mutations in *SLC2A10 (GLUT10)*. Therefore, screening of *FBLN4* should be considered in cases with these types of features when mutations are not found in *TGFBR1, TGFBR2,* or *SLC2A10*.

2.1.3.2 Mutations in FBLN5

A large consanguineous family was identified by Loeys et al (2002) and was found to be homozygous for a mutation in *FBLN5* (c.998T>C, p.S227P). Within the family, four individuals were noted to have cutis laxa. The proband was diagnosed with supravalvular aortic stenosis and emphysema, and had recurrent lower respiratory tract infections. Three siblings from a separate branch of the family were all noted to have cutis laxa, emphysema, and recurrent respiratory infections. In addition, one had pulmonary hypertension due to peripheral pulmonary artery

hypoplasia, and another had bladder diverticulae. All three passed away between the ages of 6 months to 22 years due to cardiorespiratory failure.

Elahi et al (2006) reported a consanguineous family with two affected children. One sibling was reported to have cutis laxa, umbilical hernia, emphysema, supravalvular aortic stenosis, and a hoarse voice. Evaluation of fibroblasts revealed abnormal and reduced elastic fibers. The patient passed away at the age of 14 years. The second sibling was reported to have similar skin findings, and passed away at the age of 2 due to pulmonary infections. Mutational analysis revealed homozygosity for a *FBLN5* mutation (679T>C, p.S227P).

Claus et al (2008) reported a consanguineous family with two affected children. The first sibling was noted to have cutis laxa, facial dysmorphism, bilateral inguinal hernias, large and delayed closure of the anterior fontanel, dilated right ventricle, and joint laxity. The second sibling was noted to have cutis laxa, facial dysmorphism, bilateral inguinal hernias, kyphoscoliosis, small ostium secundum, delayed bone maturation, and severe emphysema. Fibroblasts from both patients were analyzed and revealed absence of elastic fibers. Mutational analysis revealed homozygosity for a FBLN5 mutation (649T>C, p.C217R).

Hu et al (2006) analyzed the functional affects of two reported *FBLN5* mutations, p.S227P and p.C217R, on fibulin-5 and elastin. They showed that the mutation S227P causes reduced synthesis and secretion of fibulin-5. In addition, they demonstrated that both mutations result in fibulin-5 mutant proteins that cannot interact with elastin fibers, therefore showing that they are null mutations with respect to elastin fiber formation.

2.1.4 Autosomal Recessive Cutis Laxa Type 2 (ARCL2)

ARCL type 2 (ARCL2) is characterized by the presence of a large fontanel with delayed closure, dysmorphic features, growth and developmental delays. Of those affected, some may develop seizures and mental deterioration (Morava et al 2005, Kornak et al 2008). Two types of ARCL2 have been described in literature, ARCL2 Type A (ARCL2A) and ARCL2 Type B (ARCL2B). These types are distinguished by clinical features, glycosylation anomalies, and molecular genetic findings.

2.1.4.1 Autosomal Recessive Cutis Laxa Type 2A

ATP6V0A2-Related Cutis Laxa

In a group of patients with ARCL2, Kornak et al (2008) analyzed the glycosylation of serum proteins. The results of this analysis revealed a defect of N-glycosylation and O-glycosylation, consistent with a congenital disorder of glycosylation (CDG) type 2 pattern. CDG is a class of disorders characterized by glycosylation defects at the level of the endoplasmic reticulum or Golgi apparatus (Freeze 2006). With these results, homozygosity mapping of 15 consanguineous families was pursued, and mutations in the gene ATP6V0A2 were found. This gene encodes the A2 subunit of the vacuolar-type H^+ -ATPase. Kornak et al (2008) identified 10 different mutations among 12 families. Hucthagowder et al (2009) identified 18 different mutations among17 patients. Some of the families in both studies were initially diagnosed with wrinkly skin syndrome (WSS) All of these mutations are predicted to result in a loss of function. It is proposed that loss of function of ATP6V0A2 leads to elevated vesicle pH and premature aggregation of tropoelastin. This then may impair tropoelastin secretory vesicle trafficking,

leading to accumulation within the cell (Hucthagowder et al 2009). Fibroblasts from affected patients demonstrated this accumulation.

ARCL2A is characterized by the presence of cutis laxa, large fontanels or delayed closure, microcephaly, eye anomalies including strabismus, myopia, and hypermetropia, joint laxity, and developmental delay, mental retardation (Hucthagowder et al 2009, Morava et al 2008, Van Maldergem et al 2008). Other relevant findings include partial pachygyria, and cardiac, urogenital, liver, and coagulation anomalies. Several patients have been reported to develop seizures later in life (Morava et al 2008). Of importance, growth delay was seen in low frequency among the reported patients, a previously reported characteristic finding of ARCL2.

2.1.4.2 Autosomal Recessive Cutis Laxa Type 2B (ARCL2B), Geroderma Osteodysplastic (GO), & Wrinkly Skin Syndrome (WSS)

Cutis Laxa with Global Developmental Delay (CLGDD)

Cutis Laxa with Progeroid Features

ARCL2B (previously called cutis laxa with global developmental delay) and GO are two conditions in which cutis laxa is primarily localized to the dorsum of the hands and feet. In the literature there has been much debate over whether these two conditions represent the same condition or are indeed two distinct conditions. In addition, the term wrinkly skin syndrome (WSS) has been used to describe individuals with both of these conditions, and as a separate condition. Even after the identification of the causative genes for ARCL2B and GO (*PYCR1* and *SCYL1BP1/GORAB* respectively) by Guernsey et al (2009), Reversade et al (2009), and Hennies et al (2009), there is still discussion about the classification of these conditions.

GO is characterized by the presence of lax and wrinkled skin predominantly on the hands and feet, dysmorphic facial features including a broad, prominent forehead, mandibular prognathism, and large protruding ears. Other findings include joint laxity, which results in hip dislocation in many cases, and some level of developmental delay. Common radiological features include osteoporosis, wormian bones, and multiple fractures (Al-Gazali et al 2001).

WSS is characterized by the presence of wrinkled skin on the dorsum of the hands and feet and abdomen, increased palmar and plantar creases, prenatal and postnatal growth delays, and developmental delays (Al-Gazali et al 2001, Boente et al 1999). Other noted features include musculoskeletal complications including joint laxity, hypotonia, microcephaly, and a prominent venous pattern on the chest. Due to the level of overlap, Zlotogora (1999) suggested that WSS and ARCL2B are the same syndrome before glycosylation and molecular genetic testing became available for distinguishing these patients.

In 2001, Al-Gazali et al described five patients from two families diagnosed with overlapping features of GO and WSS. Patients were described to have intrauterine growth retardation, growth and developmental delays, dysmorphic facial features, joint laxity, winged scapula, hip dislocation, wrinkly skin, prominent veins, prominent palmar/plantar creases, osteoporosis, and wormian bones. Al-Gazali et al. as well as others (Hamamy et al. 2005) suggested that the conditions GO, WSS, and cutis laxa with developmental delay represent the same syndrome. Differences between the three may represent the variability of the syndrome and lack of a full assessment when diagnosed. For instance, individuals with WSS and cutis laxa do not typically have radiological evaluations to determine the presence of osteoporosis or wormian bones.

Rajab et al (2008) described 22 patients, eight with GO and 14 with WSS. Based on clinical evaluations they disagreed with Al-Gazali et al (2001), and stated that GO and WSS are distinct syndromes. This is due to the extent of wrinkling present, where GO skin wrinkling is localized to the hands and feet, and WSS skin wrinkling can be more generalized. Rajab et al (2008) and Zlotogora (1999) suggested that there are no major clinical differences between WSS and ARCL2B (CLGDD).

Nanda et al (2008) described three patients and reviewed the literature. They suggested that not all cases of ARCL2B can be grouped with WSS. Individuals with CL presenting with developmental delay have soft doughy skin with redundant folds, and those with WSS have thin, inelastic, and wrinkly skin primarily affecting the hands and feet.

Genetics of ARCL2B

In 2009, Guernsey et al described five patients with lax, wrinkled, inelastic skin, joint laxity, and facial dysmorphism. The skin findings are prominent on the hands and feet, and the facial dysmorphism included microcephaly, and a broad, prominent forehead. Two of the five had congenital hip dislocation. All of the patients had some degree of prenatal and postnatal growth deficiency, and developmental delays. No radiological evaluations were performed. Homozygosity analysis revealed a potentially pathogenic variant in the gene *PYCR1* (c.797G>A, p.R226Q). Further analysis demonstrated that this mutation leads to a loss of function of *PYCR1*. They conclude that this loss of function is responsible for the phenotype seen in their patients.

Reversade et al (2009) detected 15 mutations in *PYCR1* in 35 affected individuals from 22 families initially diagnosed as WSS, GO or DBS. The generalized clinical features of these individuals included skin wrinkling primarily on the hands and feet, finger contractures, hernias,

osteopenia, and a triangular shaped face often with proganthism. Variable degrees of mental retardation were reported in all but one case.

PYCR1 encodes for the enzyme pyroline-5-carboxylate reductase 1. Protein expression analysis in fibroblasts demonstrates that PYCR1 is a mitochondrial protein. Mutations reported by Reversade et al were demonstrated to result in a loss of function leading to abnormal morphology of the mitochrondria and increased cell death.

Genetics of GO

Newman et al (2008) mapped GO to a 4 Mb locus on chromosome 1q24 in 10 individuals from five families. Then, Hennies et al (2008) identified nine different mutations in the gene *SCYL1BP1 (NTKLBP1/GORAB*) in 13 families with a GO phenotype. GO was diagnosed based on a lack of large fontane with delayed closure, facial dysmorphism with downslanting palpebral fissures, generalized skin wrinkling, and mental retardation or neurodegenerative deterioration. *SCY1BP1* encodes for SCY-like 1 binding protein 1, which is localized to the Golgi apparatus and is involved in vesicle transport. Fibroblast evaluation demonstrated that these mutations had a loss-of-function effect.

Al-Dosari & Alkuraya (2009) confirmed the findings of Hennies et al. Seven patients in four families were diagnosed with GO based on similar criteria as Hennies et al. Homozygosity and genomic analysis revealed two mutations (c.226_227insA, p.Q76QfsX20; c.658G>C, p.A220P) in *SCYL1BP1*. These mutations were proposed to be loss of function mutations.

However, Yildirim et al (2010) identified *PYCR1* mutations in four patients diagnosed with GO. The diagnosis was based on the presence of wrinkled skin predominantly on the hands and feet, broad, prominent forehead, prognathism, and variable degrees of osteopenia and wormian bones. Yildirim et al (2010) discusses that overall, the syndromes caused by mutations

in *SCYL1BP1* (GO) and *PYCR1* (ARCL2B) are clinically similar. The major differences between the two syndromes is mental retardation in ARCL2B and more severe osteoporosis in GO.

2.1.5 De Barsy Syndrome

De Barsy syndrome was first described by de Barsy et al. in 1967 and since that time 28 additional cases have been reported in literature. Kivuva et al (2008) provided a review of all cases and described the prominent features of this syndrome. The most common features include cutis laxa (97%), musculoskeletal complications (90%), mild to severe developmental delay (76%), dysmorphic facial features (up to 79%), hypotonia (72%), and corneal opacification (48%). Muscoloskeletal complications included joint laxity, joint dislocations and/or contractures, clubbed foot, pectus excavatum, delayed bone age, and scoliosis. Dysmorphic facial features included progeroid features, large, low-set ears, prominent forehead, large fontanels, thin lips or small mouth, and small nose. Reversade et al (2009) have found mutations in PYCR1 in several families diagnosed with DBS, suggesting that this disease is allelic with ARCL2B.

2.1.6 Urban-Rifkin Davis Syndrome (URDS)

Cutis laxa with severe pulmonary, gastrointestinal, and urinary abnormalities

LTBP4-Related Cutis Laxa

Urban et al (2009) reported a new type of autosomal recessive cutis laxa, called Urban-Rifkin-Davis syndrome (URDS) based on four patients with generalized cutis laxa. All had some degree of facial dysmorphism including long philtrum, flat midface, receding forehead, and microretrognathia. Major complications were due to pulmonary, gastrointestinal, and urinary abnormalities. Pulmonary complications included tachypnea or respiratory distress, emphysema, tracheomalacia, and diaphragmatic hernia. Three of the four passed away due to respiratory failure. Gastrointestinal and genitourinary complications included diverticulae, intestinal dilation or tortuosity, and umbilical and inguinal hernias. Other findings included pulmonary artery stenosis, joint laxity, hypotonia, and postnatal growth delay. The patients described by Urban et al were found to be either homozygous or compound heterozygous for mutations in *LTBP4*, which encodes latent transforming growth factor-beta-binding protein 4.

There are four different types of latent transforming growth factor-beta-binding proteins (LTBPs). These proteins vary in expression from wide to localized. All four are involved in the regulation of transforming growth factor beta (TGF-beta), which is involved in the growth and differentiation of various cell types (Oklo & Hesketh 2000). LTBP4 has been shown to be expressed in the heart and aorta, pancreas, small intestine, lung, liver, kidney, and muscular system (Giltay et al 1997, Saharinen et al 1998). Urban et al (2009) demonstrated the first association of the *LTBP4* gene with disease. They identified five mutations in the gene. Most of

the mutations (four out of five identified) reported were nonsense mutations leading to reduced amounts of LTBP4 in fibroblasts. In addition, four of the five identified mutations were located in domains that are known to affect fibrillin and LTBP conformation. Functional analysis demonstrated abnormal release and/or storage of TGF-beta, thus changing its availability in the extracellular matrix (ECM) and affecting its incorporation into the ECM. Therefore, LTBP4 deficiency, may lead to changes in the development of pulmonary, intestinal, and urinary systems where LTBP4 is primarily expressed in.

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

RIN2-Related Cutis Laxa

Six patients, from two families, were described by Basel-Vanagaite et al (2009) and Syx et al (2010) to have macrocephaly, receding hairlines and sparse hair (alopecia), progressive facial coarsening and swelling, joint laxity, scoliosis, high-pitched voices, and cutis laxa. The condition was mapped to chromosome 20p11.21-p11.23, and patients were found to be homozygous for a mutation in *RIN2* (c.1731delC, p.I578SfsX4; c.1914_1915delGC, p.Glu638AspfsX9). RIN2 is a guanine nucleotide exchange factor (GEF) for RAB5, a protein involved in endocytosis (Saito et al 2002). Functional analysis demonstrated decreased mRNA and protein levels in fibroblasts of the patients. In addition, deficiency of RIN2 was shown to be associated with a deficiency of fibulin-5 (FBLN5) and paucity of dermal microfibrils (Basel-Vanagaite et al 2009), which are involved in elastin fiber formation. Basal-Vanagaite et al proposed that the newly described condition be called MACS syndrome (macrocephaly,

alopecia, cutis laxa, and scoliosis). Syx et al (2010) proposed that this condition be called RIN2 syndrome.

2.1.8 Acquired Cutis Laxa

Acquired cutis laxa is a condition characterized by the presence of lax, redundant, and inelastic skin, which typically affects adults. However, several isolated cases of children and adolescents have been reported (Lewis et al 2004). The extent of skin involvement ranges from localized areas to generalized skin laxity. In approximately half of cases, an inflammatory phase precedes the onset of skin laxity. Other health complications associated with acquired cutis laxa include emphysema, cor pulmonale, aortic dilation and rupture, intestinal diverticulae, and inguinal and hiatal hernias (Lewis et al 2004, Hashimoto & Kanzaki 1975, Reed et al 1971). These additional health complications are proposed to be caused by a high rate of systemic elastolysis (Koch & Williams 1985).

The cause of acquired cutis laxa remains relatively unknown. Conditions associated with acquired cutis laxa include multiple myeloma (Gupta & Helm 2002, Nikko et al 1996, McCarty et al 1996, Ting et al 1984, Cho & Maguire 1980, Scott et al 1976), kidney disease (Tan et al 2003, Tsuji et al 1987), lymphoma (Chartier et al 1997, Machet et al 1995), celiac disease (Garcia-Patos et al 1996), and rheumatoid arthritis (Rongioletti et al 2002), and radiation therapy (Koch & Williams 1985). In addition, several studies have examined the histopathology of acquired cutis laxa. These studies have shown disarrangement of collagen fibers, fragmented, clumped and reduced elastic fibers, and absent or reduced amounts of oxytalanic fibers (de Almeida et al 2008, Bouloc et al 1999, Lebwohl et al 1994, Nanko et al 1979).

In 2006, Hu et al reported a patient with acquired cutis laxa who had missense mutations in *ELN* and *FBLN5*. The patient developed cutis laxa after a *Toxocara canis* parasitic infection. Other health complications that developed included aortic root aneurysm and B-cell lymphoma. Mutational analysis revealed compound heterozygosity for *ELN* mutations (p.A55V; p.G773D) and heterzygosity for a *FBLN5* mutation (p.G202R). Functional analysis determined that the *FBLN5* mutation G202R may increase FBLN5 binding potential with ELN, and that the *ELN* mutation G773D may be sufficient to cause cutis laxa. Therefore, the *FBLN5* mutation G202R may partially rescue the *ELN* mutation G773D. At this time, no other similar cases have been reported.

The case described by Hu et al raises an important question of whether the term "acquired" is appropriate for all later-onset cases of cutis laxa. Therefore, for patients described in this paper with later onset CL will be classified as "Acquired/Late-Onset Cutis Laxa."

Table 1: Summary Table of CL Types.

CL Type	Gene	Inheritance	Clinical Features
Occipital Horn Syndrome (OHS)	ATP7A	X-linked	Cutis laxa, joint laxity, occipital exostoses, hernias, vascular tortuosity, bladder diveritculae, dysautonomia, low-normal IQ
Autosomal Dominant CL (ADCL)	ELN	Autosomal Dominant	Cutis laxa, valve stenosis & regurgitation, aortic dilation, **emphysema, obstructive pulmonary disease, recurrent respiratory infections**
Autosomal Recessive CL Type 1 (ARCL1)	FBLN4	Autosomal Recessive	Cutis laxa, **emphysema, recurrent respiratory infections**, arterial tortuosity, aortic dilation, hernias, joint laxity, arachnodactyly
	FBLN5	Autosomal Recessive	Supravalvular aortic stenosis, **emphysema, recurrent respiratory infections**, hernias, cutis laxa
Autosomal Recessive CL Type 2A (ARCL2A)	ATP6V0A2	Autosomal Recessive	Cutis laxa, large and/or delayed closure of fontanelles, eye anomalies, joint laxity, developmental delay
Autosomal Recessive CL Type 2B (ARCL2B)	PYCR1	Autosomal Recessive	Cutis laxa (primarily on hands and feet), triangular shaped face, broad/prominent forehead, joint laxity, growth and developmental delay
De Barsy Syndrome	Unknown	Autosomal Recessive	Cutis laxa, joint laxity, joint dislocations, scoliosis, progeroid features, prominent forehead, large fontanelles, hypotonia, corneal opacification
Urban-Rifkin-Davis Syndrome (URDS)	LTBP4	Autosomal Recessive	Cutis laxa, **tachypnea, respiratory distress, emphysema**, diverticulae, intestinal dilation/tortuosity, hernias, joint laxity, hypotonia, pulmonary artery stenosis
MACS Syndrome	RIN2	Autosomal Recessive	Macrocephaly, alopecia, cutis laxa, scoliosis
Acquired/Late-onset	--	--	Adult onset cutis laxa, **emphysema**, aortic dilation, intestinal diverticulae, hernias

2.2 CHRONIC PULMONARY DISEASE

Chronic pulmonary disease is a common cause of morbidity in the general population. The different types are classified as either restrictive or obstructive. Restrictive disease is characterized by a decrease in total lung capacity (TLC). The two general types are chest wall disorders (e.g. neuromuscular disorders) and chronic interstitial and infiltrative disease (e.g. interstitial fibrosis) (Kuma et al 2009). Obstructive lung diseases include conditions such as chronic obstructive lung disease (COPD), emphysema, chronic bronchitis, bronchiectasis, and asthma.

2.2.1 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is progressive and poorly reversible (ALA 2010, Barnes 2004, Celli et al 2004). About 12.1 million adults 18 years of age or older are estimated to have a diagnosis of COPD. COPD is ranked as the fourth leading cause of death in the United States, with an age adjusted rate of 39.3 per 100,000 with whites 1.6 times more likely than blacks and 2.5 times greater than Hispanics to be affected (ALA 2010). A diagnosis, based on ICD-10 codes, includes COPD, chronic bronchitis, emphysema, and other lower chronic obstructive pulmonary diseases like bronchiectasis.

The major pathological changes involve the central airways, peripheral airways, lung parenchyma, and pulmonary vasculature (Barnes 2004, Celli et al 2004). Large airway changes

include hyperplasia of goblet cells and mucus gland enlargement (Shapiro & Ingenito 2005). In addition, the alveolar attachments are disrupted, which causes airway closure during expiration. This leads to gas trapping in alveoli and hyperinflation (Barnes 2004). Differences in mechanism and presenting anatomic changes dictate the type of obstructive disease (Kuma et al 2009).

2.2.1.1 Emphysema

Emphysema is characterized by irreversible airspace enlargement with destruction of the alveolar walls (Kuma et al 2009). In 2008, 3.8 million Americans reported a diagnosis of emphysema, with 94% of individuals older than 45 years of age (ALA 2010). In 2009, 4.9 million (2.2%) noninstitutionalized adults reported ever being diagnosed with emphysema (CDC 2009). The two types of emphysema most commonly seen in those with COPD are centrilobar and panacinar emphysema. Centrilobar emphysema is characterized by the presence of microbullae in the center of the secondary lung lobule, and predominates in the upper lung zones. This is a result of inflammation of the terminal and respiratory bronchioles, which leads to enlargement of the airspaces. Panacinar emphysema is characterized by uniform destruction and enlargement of the airspaces, and predominates in the lower lung zones (Snider 2000). Of note, clinical symptoms typically do not appear until at least one third of the parenchyma is damaged; symptoms include dyspnea, cough or wheezing (often confused with asthma).

2.2.1.2 Chronic Bronchitis

Bronchitis is characterized by the presence of hypersecretion of mucus (Hogg et al 2004, Kuma et al 2009). Bronchitis is classified as chronic when hypersecretion of mucus and a persistent cough are present for at least three months in at least two consecutive years (Kuma et al 2009).

An estimated 3.8 million Americans were diagnosed with chronic bronchitis in 2008, with women twice as likely as men to be diagnosed. In 2009, 9.9 million (4.4%) of noninstitutionalized adults reported ever being diagnosed with chronic bronchitis (CDC 2009). Onset is associated with inflammation and/or hyperplasia of the epithelium, gland ducts, and glands of the large central airways (Hogg et al 2004).

2.2.1.3 Other Chronic Pulmonary Diseases

Asthma

The main signs and symptoms of asthma include episodic wheezing, a cough, chest tightness and dyspnea. These symptoms are associated with bronchoconstriction that is reversible either with a bronchodilator or spontaneously. Asthma primarily involves inflammation in all airways and mucus production, and does not involve the lung parenchyma (Kuma et al 2009, Barnes 2004).

Asthma is considered a complex genetic disease with different causes and mechanisms, with genetic factors contributing the most to susceptibility. Genome-wide linkage studies have identified over 100 genes associated with asthma and allergy. Environmental risk factors include allergen sensitization, tobacco exposure, exposure to animals, and viral or bacterial infections (Subbarao et al 2009). Factors in childhood that contribute to a poorer lung function in adulthood include early onset and/or more severe respiratory disease symptoms, persistent wheezing, low FEV_1 values, and bronchial hyperresponsiveness. It has been reported that longer duration of asthma is associated with more severe obstruction, indicating that duration is more important than age for the amount of obstruction in adulthood (Eisner et al 2010).

Bronchiectasis

Bronchiectasis is characterized by the presence of large volumes of sputum and the presence of bronchial dilation and wall thickening. The dilation is often permanent and caused by a breakdown of elastic tissue. Symptoms and onset are commonly associated with respiratory tract infection (Celli et al 2004, Kuma et al 2009). Symptoms include a cough, sputum, and fever.

2.3 CUTIS LAXA & OBSTRUCTIVE PULMONARY DISEASE

2.3.1 Elastin Threshold Hypothesis

Shifren & Mecham (2006) presented data from elastin deficient mice. They found that mice with at least 50% the amount of normal elastin are able to have normal lung development. Mice with lower levels of elastin are at risk to develop severe lung disease, suggesting that susceptibility to lung disease or injury is influenced by the amount of normal elastin in the lung. Finally, mice with 30% the amount of normal elastin have congenital emphysema. Based on this data, they propose the elastin threshold hypothesis of lung injury.

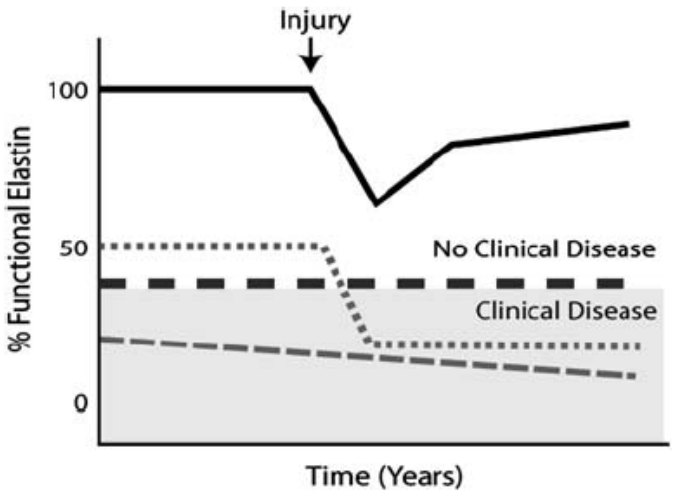

Figure 1: Elastin Threshold Hypothesis of Lung Injury (Shifren & Mecham 2006).

The threshold hypothesis is shown in Figure 1. Because mice heterozygous for elastin mutations ($ELN^{+/-}$) have normal lung development, the assumption is that the threshold of injury-to-disease progression is lower than 50% (thick dashed line). Those with normal elastin levels (top solid line) have the ability to repair lung injury. When injury occurs in those with normal lung development and elastin deficiency, such as the $ELN^{+/-}$ mice (middle dotted line), the injury drops them below the threshold. As a result they develop clinical disease, as seen in 10-15% of smokers who develop COPD. Finally, those with a functional elastin level below the threshold (bottom dashed line), lung development is impaired causing congenital lung disease. Shifren & Mecham (2006) suggest that this hypothesis may provide a model and explanation for the relationship between the extent of injury and amount of functional elastin.

2.3.2 Cutis Laxa & Pulmonary Disease

Of the nine types of cutis laxa described, ADCL, ARCL1, URDS, and acquired/late-onset CL are noted to have significant pulmonary involvement. The pulmonary involvement includes emphysema, history of pneumonia, recurrent respiratory infections, tachypnea, and respiratory distress. Emphysema is noted to be a common pulmonary feature in all four of these types of cutis laxa. The genes associated with these types include *ELN*, *FBLN4,* and *LTBP4*. All three of these are components of elastic fibers. Mutations in these genes have been shown, as previously discussed, to affect tropoelastin cross-linking and aggregation, conformation of other related elastic fiber components, and the stability of the elastic fiber. Therefore, based on the discussion of lung development and threshold hypothesis, mutations in these genes may decrease the amount of available functional elastin causing congenital pulmonary disease or increase the risk of obstructive pulmonary disease like emphysema in cutis laxa patients.

3.0 SPECIFIC AIMS

3.1 SPECIFIC AIM 1: DEFINE THE NATURAL HISTORY OF PULMONARY PHENOTYPES ASSOCIATED WITH CL.

The purpose of this aim is to better characterize the pulmonary involvement within cutis laxa. This will allow for comparisons between the different ages of onset and between the different types of cutis laxa. Such comparisons can lead to better classification, and for the determination of genotype-phenotype correlations.

3.2 SPECIFIC AIM 2: DETERMINE IF HETEROZYGOTE CARRIERS OF SEVERE RECESSIVE CL MUTATIONS ARE SUSCEPTIBLE TO CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD).

The genes known to cause cutis laxa are components of or involved in the formation of elastic fibers. The loss of elasticity due to a break down or improper formation of elastic fibers is proposed to cause COPD. As a result, loss of function of these involved proteins can lead to significant pulmonary disease in cutis laxa patients. Due to this association, the purpose of this aim is to determine if mutations in one copy of a cutis laxa gene increase the risk for pulmonary disease.

4.0 METHODS

The data used for this study was collected at University of Pittsburgh, Washington University of St. Louis, and University of Hawaii (previous IRB approval letters in Appendix A). The protocol is currently funded by the National Heart, Lung, and Blood Institute. The protocol is approved by University of Pittsburgh's Institutional Board of Review (Appendix A). Data was collected from 1999 until present.

4.1 CLINIC PROTOCOL

4.1.1 Recruitment

Patients are primarily referred to our study by their treating physician, a genetic counselor, a member of the cutis laxa online support group, or they are self-refered. Upon referral we invite these patients to participate. First-degree relatives, specifically parents and children, are recruited through the participants already enrolled.

4.1.2 Screening

Potential participants are contacted by phone to confirm their own or a first degree relative's cutis laxa diagnosis. They are asked if they or a first degree relative have (1) loose, lax skin, (2) skin in redundant folds, (3) inelastic (doughy) skin, (4) premature aging of the skin, (5) excessive premature wrinkling, or (6) another family member affected by cutis laxa. If an individual has three or more of these features, their or their first degree relative's diagnosis will be considered to be confirmed. We also ask whether the cutis laxa is congenital (present since birth or developed in early childhood) or acquired/late-onset (developed after early childhood).

4.1.3 Informed Consent Process

Individuals are able to participate either by attending a research clinic at University of Pittsburgh Medical Center or through their treating physician. For individuals who attend one of the research clinics, prior to their study visit a letter explaining the study and the procedures that will be performed is sent. At the study visit informed consent is obtained. For individuals who will not attend one of the research clinics, the study will be explained to the subject over the phone. During this process, the information included in the consent form is orally communicated.

4.1.4 Research Activities

4.1.4.1 Research Clinic Involvement for Affected Individuals

The research clinic is a multidisciplinary clinic. A genetic evaluation and physical exam is performed on all individuals who attend the clinic. Other evaluations and testing involved in the clinic includes cardiovascular, pulmonary, skeletal, and hearing assessments.

The genetic evaluation includes measurements, such as the patient's height and weight, as well as general observations about the subject's overall health and appearance. As part of this evaluation a clinical questionnaire is completed. The questionnaire (Appendix B) collects information regarding a patient's medical and family history. Responses indicate the presence or absence of common cutis laxa clinical features, and features of related disorders. This allows for confirmation of a cutis laxa diagnosis. All questionnaires are entered into a database after the clinic. Finally samples are collected, which include 30 ml of blood and two 3 mm skin biopsies. If blood draw is unsuccessful or declined by the patient, a saliva sample may be collected instead of a blood sample.

A cardiovascular evaluation consisting of an echocardiogram is performed on those age 13 years or older. The echocardiogram is performed by a trained technician and the results are interpreted by a physician. A report is generated which includes impressions and measurements.

The pulmonary evaluation involves a self-administered respiratory health questionnaire, pulmonary function test, and chest CT. All patients complete the respiratory health questionnaires, and assistance is provided if needed. The questionnaires ask about the patient's history of breathing problems and environmental exposures. The pulmonary function tests are only performed in patients older than 5 years of age. In addition, only adult patients without cardiovascular disease are tested for bronchodilator responses. Finally, only adult patients, those

18 years of age and older, have a chest CT. All three aspects are evaluated by members of the research team.

A hearing test is performed on patients older than 6 years of age. The test involves the following: otoscopy, tympanometry, behavioral hearing, frequency-sweep distortion-product otoacoustic emissions (DPOAE) (measure of cochlear integrity), DPOAE Input/Output (measure of cochlear compression), and contralateral-suppression DPOAE (measure of lower-brainstem integrity).

The skeletal evaluation consists of a DEXA scan. The DEXA scan is only performed on adult patients, those 18 years of age or older. The report generated provides images, measurements, z-scores, and indicates if whether the patient has normal bone density, osteopenia, or osteoporosis.

Recent additions to the protocol include 3D facial image and elasticity testing. The facial imaging involves a portable 3D stereophotogrammetry system that uses six overlapping cameras to capture the face at different angles. This helps to better characterize the facial features and structure of those with cutis laxa. The elasticity testing involves a measurement of skin elasticity and an estimate of blood vessel elasticity. The skin elasticity is measured on the arms of each patient. The blood vessel estimate is obtained through pulse wave analysis and pulse wave velocity.

This is a biannual research clinic for individuals affected with cutis laxa and individuals who have a first-degree relative affected with cutis laxa. The clinic takes place over several days, and each participant receives individual appointments for the above evaluations.

4.1.4.2 Non-Research Clinic Involvement for Affected Individuals

For affected individuals who do not attend the research clinic, the following describes the process for their involvement. Patients with the help of their treating physician are asked to fill out the clinical questionnaire. In addition a dermatological and/or genetic evaluation with clinical photographs is performed by the patient's referring or primary care physician as part of their standard of care. Any of the following materials (blood, skin biopsy, saliva, banked DNA, tissue sample removed as part of surgical treatment) that have already been collected by the participant's physician will be requested and subsequently sent to our laboratory. If these samples, specifically blood, saliva, and/or skin biopsy, have not been collected as part of standard of care, we ask physicians to collect these.

Additionally, if patients have had an echocardiogram or pulmonary function test within the year prior to enrollment, we ask for copies of these reports. When these tests have not been performed within the year prior to enrollment, we ask their treating physician order these tests and to send copies of these reports. Pulmonary function tests will only be asked to be performed in subjects older than 5 years.

4.1.4.3 Research Activities for Unaffected First Degree Relatives

When a first-degree relative is deemed unaffected (does not meet the inclusion criteria for affected cutis laxa participants, see section "Screening") either a blood or saliva sample will be collected for genetic testing. A first-degree relative found to have a mutation in a cutis-laxa-causing gene is asked to participate in the research activities as already described above for those who attend a research clinic or for those who do not attend the research clinic. When a mutation is not identified, the relative is not asked to participate in additional research activities.

4.1.5 Follow-up

As part of the current protocol participants are contacted yearly for at least five years to obtain updated medical information on the individual's condition. The purpose is to follow the progression of disease. The annual follow-ups will be conducted over the phone. The principal investigator or one of the study coordinators ask about any changes in the research subject's health status. If any additional evaluations or exams have been performed, copies of the reports will be requested. Patients are also encouraged to contact a member of the research team with questions or if they wish to provide more often updates on changes in their health.

In addition to annual phone calls, patients are welcome to participate in future research clinics. If they attend additional research clinics we ask them to participate in the same testing that they participated at their initial visit, and to participate in any new testing. This allows us to monitor if there has been any progression of disease if the initial test was abnormal, also if new symptoms have presented, or if the patient has remained stable.

4.1.6 Mutational Analysis

Sources of DNA for mutational analysis include peripheral blood, skin fibroblasts, or saliva samples. A standard DNA isolation method used which involves lysis of nuclei and deproteination of DNA. For mutational analysis, gene specific primers were developed for PCR amplification. PCR reactions are carried out using the thermal cycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). All PCR products are purified with ExoSAP-IT, and then prepared for sequencing using ABI BigDye Terminator Kit (Applied Biosystems, Foster City, CA, USA). Sequences of both sense and antisense strands are

compared with reference gene sequences with UCSB Genomic Brower or Sequencher software (GeneCodes, Ann Arborm MI, USA). Confirmation is obtained by identification of a mutation in at least two independent amplification products.

4.2 PULMONARY DATA

For this study, information collected from the clinical questionnaire and pulmonary function tests (PFTs) were used. Due to limited numbers of chest CTs, they were excluded from this analysis. Inclusion was limited to patients and first-degree relatives with completed clinical questionnaires or detailed medical histories.

4.2.1 Patient Classifications

4.2.1.1 Onset Classification

All affected patients were classified as congenital, acquired/late-onset, and unknown. Requirements for each classification are based on age of onset and mutational status:

(1) Congenital: (A) Skin features noted to be present at birth; (B) Clinical questionnaire completed or medical history obtained under the age of 5 years, with confirmation of cutis laxa based on the study inclusion criteria; (C) Known gene mutation in a cutis laxa causative gene.

(2) Acquired/Late-onset: (A) Skin features developed in adolescence or adulthood; (B) No gene mutation; (C) No affected family members.

(3) Unknown: (A) Clinical questionnaire or medical history obtained above the age of 5 years and did not indicate age of onset of skin features; (B) Multiple affected family members in at least two generations with onset of skin features after the age of 5 years, and no gene mutation.

Classifying patients by onset allows for comparison of type, severity, and/or progression of pulmonary complications.

4.2.1.2 Cutis Laxa Type

If a mutation was identified in a cutis laxa causative gene in a patient, they were classified as having the associated type of cutis laxa. Unaffected first-degree relatives with an identified mutation were classified as carriers for the related type of cutis laxa. Table 2 lists the genes and types of cutis laxa. For patients with no identified mutation, they were classified only by onset (congenital, acquired/late-onset, unknown) as previously described.

Table 2: Cutis Laxa Types & Genes.

Type	OMIM#	Gene	OMIM#
OHS	304150	*ATP7A*	300011
ADCL	123700	*ELN*	130160
ARCL1	219100	*FBLN4* *FBLN5*	604633 604580
ARCL2A	219200	*ATP6V0A2*	611716
ARCL2B	612940	*PYCR1*	179035
URDS	613177	*LTBP4*	604710
MACS	613075	*RIN2*	610222

4.2.2 Clinical Questionnaires & Medical Histories

Clinical questionnaires and detailed medical histories have been collected from patients for the past 12 years. Clinical questionnaires were completed by the patient's referring physician or genetic counselor, a member of our research group, or the patient. Detailed medical histories were obtained from either medical records or the patient. The questionnaire responses and information from medical histories were entered into a database.

Over the past 12 years, the clinical questionnaire has evolved to include additional questions. Figure 2, Figure 3, and Figure 4 represent the progression of the respiratory section of the questionnaire (See Appendix B for the complete 2011 Cutis Laxa Questionnaire). Additions consisted of common answers written next to the respiratory section or additional comments section.

RESPIRATORY:			
Bronchiectasis:	Yes [ ]	No [ ]	? [ ]
Hypoplastic lungs (newborn):	Yes [ ]	No [ ]	? [ ]
Emphysema:	Yes [ ]	No [ ]	? [ ]
Tachypnea:	Yes [ ]	No [ ]	? [ ]
Pneumonia:	Yes [ ]	No [ ]	? [ ]

Figure 2: 1999-2004 CL Questionnaire: Respiratory Section.

RESPIRATORY:

Bronchiectasis:	Yes [ ]	No [ ]	? [ ]
Hypoplastic lungs (newborn):	Yes [ ]	No [ ]	? [ ]
Emphysema:	Yes [ ]	No [ ]	? [ ]
Tachypnea:	Yes [ ]	No [ ]	? [ ]
Pneumonia:	Yes [ ]	No [ ]	? [ ]

Chest CT findings and date: _____

Current smoker: Yes [ ] No [ ]

Cigarette packs per day: _____

Years smoked: _____

Figure 3: 2004-2010 CL Questionnaire: Respiratory Section. Additions include chest CT findings and date, smoking status, and smoking history.

RESPIRATORY:

Bronchiectasis:	Yes [ ]	No [ ]	? [ ]
Hypoplastic lungs (newborn):	Yes [ ]	No [ ]	? [ ]
Emphysema:	Yes [ ]	No [ ]	? [ ]
Tachypnea:	Yes [ ]	No [ ]	? [ ]
Pneumonia:	Yes [ ]	No [ ]	? [ ]
Asthma:	Yes [ ]	No [ ]	? [ ]

Chest CT findings and date: _____

Pulmonary function test (PFT) findings and date: _____

Current smoker: Yes [ ] No [ ]

Cigarette packs per day: _____

Years smoked: _____

Other respiratory findings:

Figure 4: 2011 CL Questionnaire: Respiratory Section. Additions include asthma, PFT findings and date, and other respiratory findings.

Due to differences in the clinical questionnaires collected, responses other than those asked on the first questionnaire (Figure 2: bronciectasis, hypoplastic lungs, emphysema, tachypnea, and pneumonia) were only counted if more than two patients noted a history of the condition. In addition, if the response "?" was checked, it was counted as an absence of a diagnosis or history of the condition.

4.2.3 Pulmonary Function Tests (PFTs)

PFTs were primarily obtained at research clinics, which have been held at Washington University of St. Louis in 2008 and University of Pittsburgh in 2010. All other PFTs came from received medical records. At this time PFTs have only been obtained on a subset of the patients and unaffected first degree relatives.

4.2.3.1 Pulmonary Function Classification

To assess pulmonary function, the algorithm defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force was used (Pellegrino et al 2005). Determination and diagnosis of pulmonary function abnormality is based on the spirometry parameters forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC ratio, and total lung capacity (TLC). The types of pulmonary function abnormalities include normal, obstruction, restriction, and mixed defect. The diagnosis of the three types is based on lower limits of normal (LLN; parameter measurement less than the 5^{th} percentile of predicted), see Figure 5:

(1) Normal: $FEV_1/FVC \geq LLN$ & $FVC \geq LLN$

(2) Obstruction: (A) $FEV_1/FVC < LLN$ & $FVC \geq LLN$; (B) $FEV_1/FVC < LLN$, $FVC < LLN$, & $TLC \geq LLN$; or (C) $FEV_1/FVC \geq LLN$, $FVC < LLN$, & $TLC \geq LLN$

(3) Restriction: $FEV_1/FVC \geq LLN$, $FVC < LLN$, & $TLC < LLN$

(4) Mixed defect (Obstruction & Restriction are present): $FEV_1/FVC < LLN$, $FVC < LLN$, & $TLC < LLN$

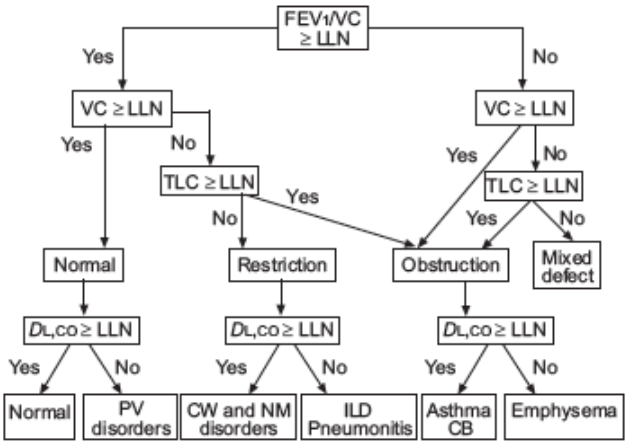

Figure 5: ATS/ERS algorithm to assess lung function in clinical practice (Pellegrino et al. 2005).

To determine the LLN for the spirometry parameters, published reference values were used. Reference equations developed by Hankinson, Odencrantz, and Fedan (1998) from the third National Health and Nutrition Examination Survey (NHANES III) were used for FVC, FEV_1, and FEV_1/FVC (Appendix C). Reference values and LLNs are applicable for those aged 8-80 years of age, and are specific for gender (male vs. female), and three race/ethnicity groups (Caucasian, African-American, and Mexican-American). For TLC, reference values and equations developed by Stocks & Quanjer (1995) from published data were used for determination of LLN (Appendix C). Equations and values are specific for gender (male vs. female), and by age (Children & adolescence: age 8-18 years; Adult: age 18-70 years).

4.3 STATISTICAL ANALYSIS

Descriptive statistics were used to determine the frequencies for the categorical data. Categorical data includes types of responses (presence or absence of diagnosis), lung function classification for type of CL onset, and mutational status. To compare the categorical data, either Fisher's exact test or a Chi-square analysis was performed. The PFT parameters FVC (% predicted), FEV_1 (% predicted), and FEV_1/FVC (%) between the types of CL onset were compared using analysis of variance (ANOVA). A significance of 0.05 was used for Fisher's exact test, Chi-square, and ANOVA.

5.0 RESULTS

A total of 94 questionnaires and medical histories have been collected over the past 12 years. Questionnaires and medical histories were obtained on individuals with a confirmed or suspected diagnosis of CL (patients), and unaffected first-degree relatives. Of the 94 questionnaires and medical histories, 6 were excluded from this analysis. Reasons for exclusion included an unconfirmed diagnosis of CL, unaffected first-degree relatives with no mutation (thus, not carriers), or incomplete respiratory responses. Therefore, for analysis of respiratory responses and histories, 88 were used. This included 83 patients with confirmed or suspected CL and 5 unaffected first-degree relatives with mutations (carriers).

5.1 GENERAL PULMONARY RESPONSES

The types and frequency of respiratory responses and histories (referred to as pulmonary condition) were analyzed (Appendix D shows responses for each patient). As described, the core respiratory questions asked about the presence or absence of bronchiectasis, hypoplastic lung, emphysema, tachypnea, or pneumonia. Other responses included in the analysis were a history of obstructive apnea, respiratory distress, bronchitis, and dyspnea. These were included because at least three patients noted a diagnosis or history of these pulmonary conditions. The most common responses amongst all participants were a history of pneumonia (24.1%), diagnosis of

tachypnea (15.7%), or a diagnosis of emphysema (12.0%). All other responses had a frequency of 3.61-7.23% (Table 3 and Figure 6).

Table 3: Pulmonary Responses for All Participants (N = 83)

Pulmonary Condition	Response	Frequency	Percent (%)
Bronchiectasis	Yes	3	3.61
	No	80	96.4
Hypoplastic lung	Yes	3	3.61
	No	80	96.4
Emphysema	Yes	10	12.0
	No	73	88.0
Tachypnea	Yes	13	15.7
	No	70	84.3
Pneumonia	Yes	20	24.1
	No	63	75.9
Asthma	Yes	6	7.23
	No	77	92.8
Obstructive Apnea	Yes	4	4.82
	No	79	95.2
Respiratory Distress	Yes	3	3.61
	No	80	96.4
Bronchitis	Yes	3	3.61
	No	80	96.4
Dyspnea	Yes	4	4.82
	No	79	95.2

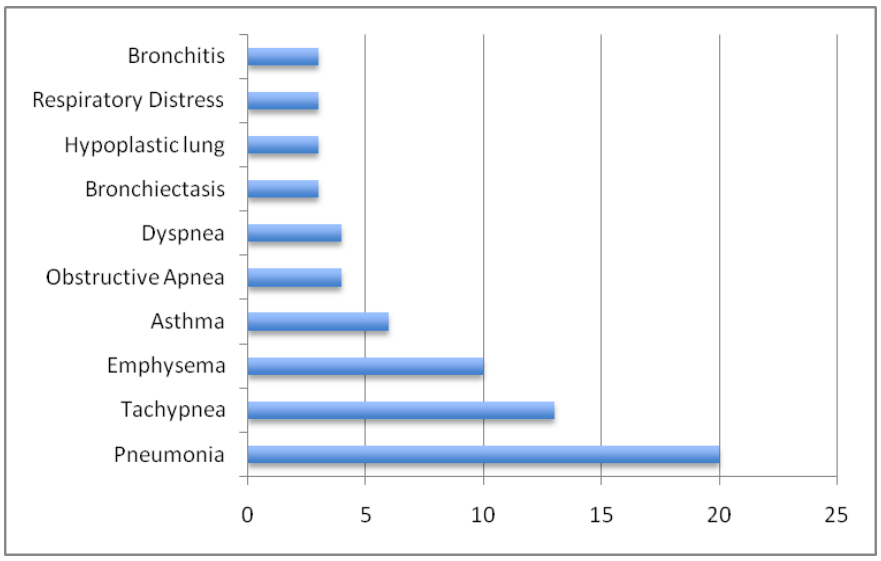

Figure 6: Pulmonary Responses for All Patients.

The prevalence of emphysema in our CL cohort was then compared to the prevalence of emphysema in the general population. Data for the prevalence in the general population was obtained from the 2009 National Health Interview Survey (NHIS) conducted by the Centers for Disease Control and Prevention (CDC). As indicated in Table 4, the frequency of emphysema was 12.0% in our CL cohort, compared to 2.2% in the general population. Chi-square analysis revealed a p-value of 5.47E-10, indicating that the prevalence of emphysema in our CL cohort is significantly higher than the general population.

Table 4: Comparison of Emphysema Prevalence Between CL & General Population.

Pulmonary Condition	Response	CL Cohort		General Population		p-value
		Frequency	Percent (95% CI)	Frequency	Percent (95% CI)	
Emphysema	Yes*	10	12.0% (5.0-19.0)	4895	2.2% (2.09-2.21)	**5.47E-10**
	No	73		222,476		
	Total	83		227,371		

* "Yes" indicates that the individual has been diagnosed with emphysema.

5.2 ONSET CLASSIFICATION AND PULMONARY RESPONSES

Classification of onset was determined as previously described. Of the 83 patients, 8 (9.64%) were acquired/late-onset, 52 (62.7%) were congenital, and 23 (27.1%) were unknown. The types of respiratory responses and histories are shown in Table 5 and Figure 7. At least one congenital patient indicated a history of a pulmonary condition for each response, with 27/52 (51.9%) congenital patients individually reporting a diagnosis or history of at least one pulmonary condition. The most common response of congenital patients was a history of pneumonia (23.1%), which is the most common response overall. The other common response for congenital patients was tachypnea (21.1%). Congenital patients accounted for 11/13 (84.6%) of all tachypnea responses. Patients classified as unknown provided similar responses to the congenital group, with 10/23 (43.5%) reporting at least one response. For acquired/late-onset, the only responses were emphysema (12.5%), pneumonia (25.0%), and dyspnea (37.5%), with 4/8 (50.0%) patients accounting for all responses.

For comparison of responses between the three groups of onset (acquired/late-onset, congenital, and unknown), only dyspnea was of statistical significance ($p = 0.0023$) (Table 5). Acquired/late-onset patients reported a significantly increased prevalence of dyspnea compared to other groups, accounting for 3/4 (75.0%) of all dyspnea responses.

Table 5: Pulmonary Responses by Onset Classification (N = 83).

Pulmonary Condition	Response	Acquired / Late-onset (%)*	Congenital (%)*	Unknown (%)*	p-value
Bronchiectasis	Yes	0	3 (5.77)	0	0.6681
	No	8	49	23	
Hypoplastic lung	Yes	0	2 (3.85)	1 (4.35)	1.000
	No	8	50	22	
Emphysema	Yes	1 (12.5)	6 (11.5)	3 (13.0)	1.000
	No	7	46	20	
Tachypnea	Yes	0	11 (21.2)	2 (8.70)	0.2598
	No	8	41	21	
Pneumonia	Yes	2 (25.0)	12 (23.1)	6 (26.1)	0.9269
	No	6	40	17	
Asthma	Yes	0	4 (7.69)	2 (8.70)	1.000
	No	8	48	21	
Obstructive Apnea	Yes	0	3 (5.77)	1 (4.35)	1.000
	No	8	49	22	
Respiratory Distress	Yes	0	3 (5.77)	0	0.6681
	No	8	49	23	
Bronchitis	Yes	0	2 (3.85)	1 (4.35)	1.000
	No	8	50	22	
Dyspnea	Yes	3 (37.5)	1 (1.92)	0	**0.0023**
	No	5	51	23	

*Percentages represent the percent of patients within the type of onset who responded "Yes" to the type of pulmonary condition.

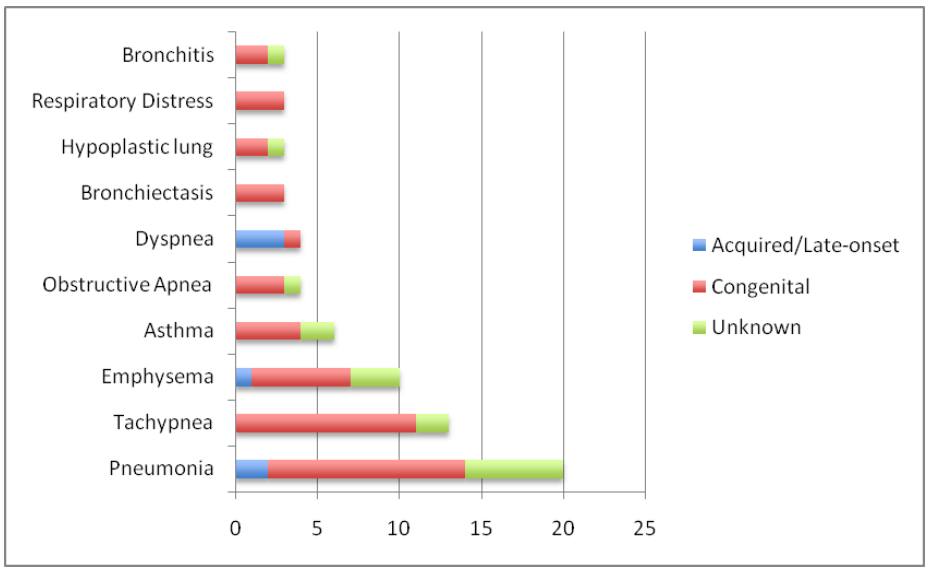

Figure 7: Pulmonary Responses by Onset Classification.

5.3 MUTATIONAL ANALYSIS

Mutations have been identified in 27 patients and 5 unaffected first-degree relatives (carriers) of the 88 questionnaires and medical histories analyzed. Mutational analysis identified mutations in *ELN*, *FBLN4*, *FBLN5*, *ATP6V0A2*, and *LTBP4* (Table 6). Table 7 indicates the type of CL, gene, mutational status, type of mutation(s), and if the case has previously been described in literature. For patients with mutations, 7 have ADCL (*ELN* mutation), 2 have ARCL1 (*FBLN4* or *FBLN5* mutation), 5 have ARCL2A (*ATP6V0A2* mutation), and 13 have URDS (*LTBP4* mutation). For *LTBP4*, only one mutation has been identified in 6 of the 13 patients. Even though only one mutation has been identified, based on clinical findings, these patients have been classified as having URDS. For unaffected first-degree relatives, 3 are URDS carriers and 2 are ARCL1 carriers.

Table 6: Pulmonary Responses by CL Type (Mutational Status) (N = 32).

Pulmonary Condition	Response	ADCL (%)*	ARCL1 (%)*		ARCL2A (%)*	URDS (%)*		p-value
		ELN	FBLN4 / FBLN5		ATP6V0A2	LTBP4		
		Affected	Affected	Carrier	Affected	Affected	Carrier	
Bronchiectasis	Yes	0	0	0	1 (20.0)	2 (15.4)	0	0.7982
	No	7	2	2	4	11	3	
Hypoplastic lung	Yes	0	0	0	0	2 (15.4)	0	0.8165
	No	7	2	2	5	11	3	
Emphysema	Yes	0	1 (50.0)	0	0	0	0	0.3608
	No	7	1	2	5	13	3	
Tachypnea	Yes	1 (14.3)	0	0	0	7 (53.9)	1	0.1675
	No	6	2	2	5	6	2	
Pneumonia	Yes	2 (28.6)	0	1 (50.0)	1 (20.0)	4 (30.8)	2 (66.7)	0.7354
	No	5	2	1	4	9	1	
Asthma	Yes	2 (28.6)	0	0	0	1 (7.69)	1 (33.3)	0.4686
	No	5	2	2	5	12	2	
Obstructive Apnea	Yes	0	0	0	0	1 (7.69)	1 (33.3)	0.5282
	No	7	2	2	5	12	2	
Respiratory Distress	Yes	0	0	0	0	1 (7.69)	0	1.000
	No	7	2	2	5	12	3	
Bronchitis	Yes	0	0	0	0	1 (7.69)	1 (33.3)	0.5282
	No	7	2	2	5	12	2	
Dyspnea	Yes	0	0	0	0	1 (7.69)	0	1.000
	No	7	2	2	5	12	3	

* Percentages represent the percent of patients within the CL type/mutational status who responded "Yes" to the type of pulmonary condition.

Table 7: Mutational Status of Patients and Carriers.

CL Onset Classification	Type of CL	Gene	Status	Genomic	cDNA	Protein	Type of Mutation(s)	Publication
Carrier	URDS Carrier	LTBP4	Heterozygote	g. + / 12666+1G>T	c. + / 883+1G>T	p. + / ?	+ / Intronic	
Carrier	URDS Carrier	LTBP4	Heterozygote	g. + / 18711C>T	c. + / 2161C>T	p. + / R721X	+ / Nonsense	
Carrier	URDS Carrier	LTBP4	Heterozygote	g. + / 12666+1G>T	c. + / 883+1G>T	p. + / ?	+ / Intronic	
Carrier	ARCL1 Carrier	FBLN4	Heterozygote		c. + / 169G>A	p. + / E57K	+ / Missense	Hucthagowder et al. (2006)
Carrier	ARCL1 Carrier	FBLN4	Heterozygote		c. + / 169G>A	p. + / E57K	+ / Missense	Hucthagowder et al. (2006)
Congenital	ADCL	ELN	Heterozygote		+ / 2159delC		Deletion	Szabo et al. (2006)
Congenital	ADCL	ELN	Heterozygote		+ / 2112_2136del		Deletion	Szabo et al. (2006)
Congenital	ADCL	ELN	Heterozygote		+ / 2112_2136del		Deletion	Szabo et al. (2006)
Congenital	ADCL	ELN	Heterozygote		c. +/2318G>A	p. +/G773D	Missense	
Congenital	ADCL	ELN	Heterozygote		c. +/2318G>A	p. +/G773D	Missense	
Congenital	ADCL	ELN	Heterozygote		c. +/2177delC		Deletion	
Congenital	ADCL	ELN	Heterozygote		c. +/2177delC		Deletion	
Congenital	ARCL1	FBLN4	Homozygote		c. 169G>A / 169G>A	p. E57K / E57K	Missense	Hucthagowder et al. (2006)
Congenital	ARCL1	FBLN5	Homozygote		c. 649T>C / 649T>C	p. C217R / C217R	Missense	Claus et al. (2008)
Congenital	ARCL2A	ATP6V0A2	Homozygote	g. 40006_40084dup / 40006_40084dup	c. 2096_2174dup / 2096_2174dup	p. E725fsX745 / E725fsX745	Frame-shift	Hucthagowder et al. (2009)
Congenital	ARCL2A	ATP6V0A2	Homozygote	g. 31487_31488insC / 31487_31488insC	c. 1058_1059insC / 1058_1059insC	p. I353fsX432 / I353fsX432	Frame-shift	Hucthagowder et al. (2006)
Congenital	ARCL2A	ATP6V0A2	Homozygote	g. 42220C>T / 42220C>T	c. 2293C>T / 2293C>T	p. Q765X / Q765X	Nonsense	Kornak et al. (2008)
Congenital	ARCL2A	ATP6V0A2	Compound Heterozygote	g. 326_327insC / 10097C>T	c. 78_79insC / 260 C>T	p.S27fsX54 / P87L	Frame-shift / Missense	Hucthagowder et al. (2006)
Congenital	ARCL2A	ATP6V0A2	Compound Heterozygote	g. 12439_12440insCATGCTGA / 45608A>G	c. 397_398insCATGCTGA / 2466_2470delGGTAG	p. R133fsX135 / W822X	Frame-shift / Nonsense	Hucthagowder et al. (2006)
Congenital	URDS	LTBP4	Compound Heterozygote	g. 12666+1G>T / 18711C>T	c. 883+1G>T / 2161C>T	p. ? / R721X	Intronic / Nonsense	

Table 7 (Continued).

Congenital	URDS	LTBP4	Compound Heterozygote	g. 30850T>A / 19015insA	c. 3856T>A / 2377insA	p. C1286S / G793fsX797	Missense / Frame-shift	
Congenital	URDS	LTBP4	Compound Heterozygote		c. 1242C>T/ 4114dupC	p. R448X/ A1372fsX2	Nonsense / Frame-shift	
Congenital	URDS	LTBP4	Compound Heterozygote	g.12574 / 20287_20288delGCinsAA	c. 791delC / 2570_2571delGCinsAA	p. P264fsX300 / C857X	Frame-shift / Nonsense	Urban et al. (2009)
Congenital	URDS	LTBP4	Homozygote	g. 12603T>G / 12603T>G	c. 820T>G / 820T>G	p. C274G / C274G	Missense	Urban et al. (2009)
Congenital	URDS	LTBP4	Compound heterozygote	g. 20287_20288delGCinsAA / 33861insC	c. 2570_2571delGCinsAA / 4128insC	p. C857X / P1376fsX1403	Nonsense / Frame-shift	Urban et al. (2009)
Congenital	URDS	LTBP4	Homozygote	g. 29481delA / 29481delA	c. 3554delA / 3554delA	p. Q1185fsX1211 / Q1185fsX1211	Frame-shift	Urban et al. (2009)
Congenital	URDS	LTBP4	Heterozygote	g. ? / 17384C>G	c. ? / 1792C>G	p. ? / R598G	? / Missense	
Congenital	URDS	LTBP4	Heterozygote	g. ? / 18229G>A	c. ? / 2143G>A	p. ? / D715N	? / Missense	
Congenital	URDS	LTBP4	Heterozygote	g. ? / 23820C>G	c. ? / 2099C>G	p. ? / P1032R	? / Missense	
Congenital	URDS	LTBP4	Heterozygote	g. ? / 33861insC	c. ? / 4128insC	p. ? / P1376fsX1403	? / Frame-shift	
Congenital	URDS	LTBP4	Heterozygote	g. ? / 36305C>T	c. ? / 4685C>T	p. ? / S1562F	? / Missense	
Congenital	URDS	LTBP4	Heterozygote	g. ? / 33841C>G	c. ? / 4108 C>G	p. ? / P1370A	? / Missense	

5.3.1 Mutational Analysis and Pulmonary Responses

Within ADCL, ARCL1, ARCL2A, and URDS and carriers of ARCL1 and URDS at least patient reported a diagnosis or history of at least one pulmonary condition (Table 7, Figure 8). Of ADCL patients, 3/7 (42.9%) reported pulmonary complications and the responses were tachypnea, pneumonia, and asthma. For ARCL1, the patient with *FBLN4* mutation was noted to have emphysema. This was already known, due to this case being previously described by Hucthagowder et al. (2006). One of the carriers of ARCL1, who is a parent of the *FBLN4* patient, reported a history of pneumonia. For ARCL2A, 1 patient had a history of bronchiectasis and 1 patient had a history of pneumonia, therefore 2/5 (40.0%) noted pulmonary complications. Finally, for URDS, all but emphysema was reported as a pulmonary complication for URDS patients. In addition, 10/13 (76.9%) of URDS patients reported a diagnosis or history of at least one pulmonary condition. Of the 3 URDS carriers, 1 had a history of bronchitis, obstructive apnea, asthma, tachypnea, and pneumonia. Comparison of pulmonary responses between the CL types, including patients and carriers, indicated no statistically significant associations. Comparison of pulmonary responses with the exclusion of carriers also did not indicate any statistically significant associations (Data available in Appendix D).

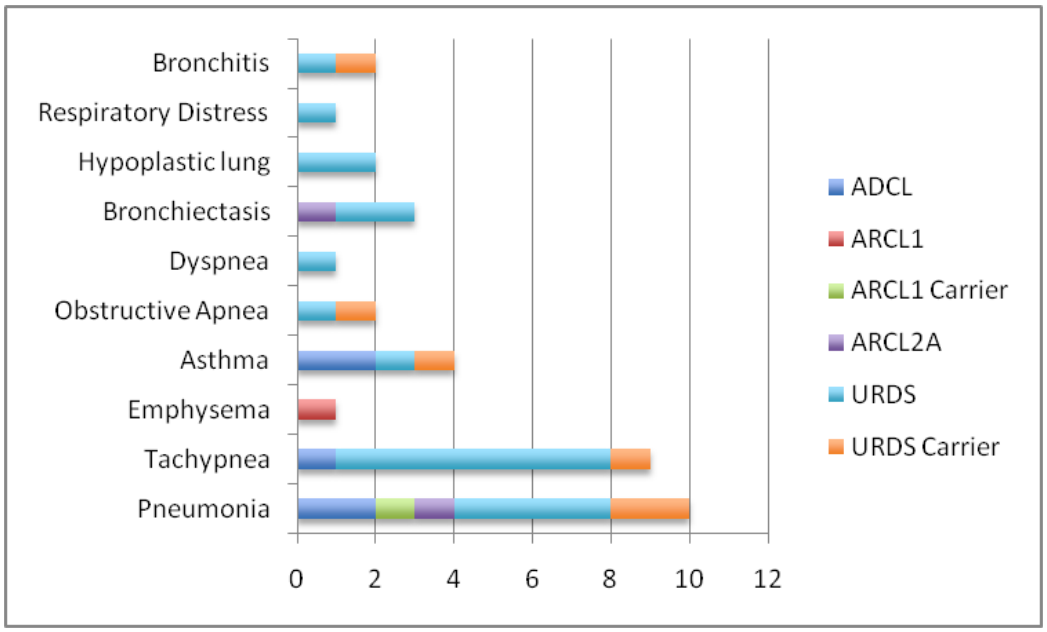

Figure 8: Pulmonary Responses by CL Types (Mutational Status).

5.4 PULMONARY FUNCTION TEST (PFT) RESULTS

PFTs have been obtained on 13 of the 88 patients and carriers with questionnaires or medical histories. Of the 13, 4 are acquired/late-onset, 5 are congenital, 1 is unknown, and 3 are carriers (Table 8). The 3 carriers are URDS carriers, 3 of the congenital patients have an *ELN* mutation, and 1 of the congenital patients has an *ATP6V0A2* mutation. The type of airway limitation (pulmonary function classification: obstruction, restrictive, mixed defect) was determined for each patient and carrier using the ATS criteria already described. Based on these criteria, which uses the PFT parameters FVC, FEV_1, FEV_1/FVC, and TLC, obstruction was the only type of airway limitation (PFT parameter results available in Appendix D). Within the 4 groups, 4/4 (100.0%) of acquired/late-onset patients have obstruction, 3/5 (60.0%) of congenital patients have obstruction, and the unknown patient and all carriers had normal pulmonary function (Figure 9). Of note, these 4 acquired/late-onset patients were the same 4 patients who account for all pulmonary responses on questionnaires and medical histories. Comparison of the normal pulmonary function vs. obstruction between the three groups with Fisher's exact test indicated no statistically significant difference. In addition, comparison of means of the parameters FVC (% predicted), FEV_1 (% predicted), and FEV_1/FVC (%) between acquired/late-onset, congenital, and unknown indicated no statistically significant differences.

Table 8: Pulmonary Function Classification

Onset Classification	Gene	Pulmonary Function Classification	Degree of Severity
Acquired/Late-onset	--	Obstruction	Mild
Acquired/Late-onset	--	Obstruction	Mild
Acquired/Late-onset	--	Obstruction	Severe
Acquired/Late-onset	--	Obstruction	Very Severe
Congenital	ELN	Normal	--
Congenital	ELN	Obstruction	Moderate
Congenital	ELN	Obstruction	Mild
Congenital	ATP6V0A2	Obstruction	Moderate
Congenital	--	Normal	--
Unknown	--	Normal	--
Carrier	LTBP4	Normal	--
Carrier	LTBP4	Normal	--
Carrier	LTBP4	Normal	--

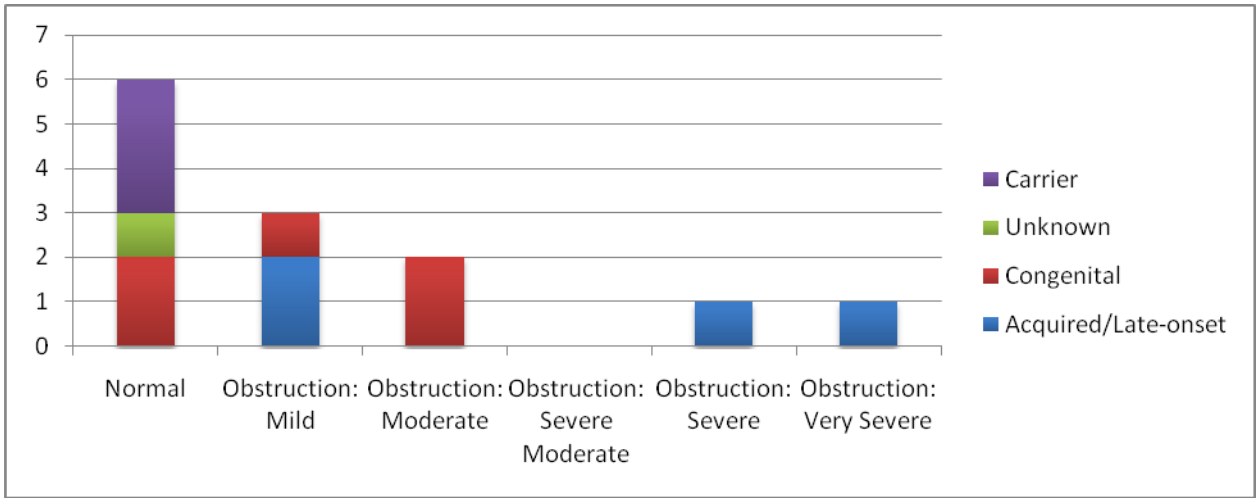

Figure 9: Pulmonary Function Classification.

6.0 DISCUSSION

The purpose of this study was to better characterize the pulmonary phenotype of CL. Previously, the pulmonary complications associated with CL have only been described in case studies of patients and reviews of specific CL types (as described in the "Background"). The majority of these reports have indicated obstructive lung disease and recurrent infections as the common findings. This study provides a preliminary analysis of the pulmonary involvement within and among the different types of CL. For this, clinical data collected from clinical questionnaires and medical histories on 83 patients with a confirmed or suspected diagnosis of CL was analyzed. In addition, 13 pulmonary function tests (PFTs) were collected on 10 patients and 3 unaffected first-degree relatives with a known mutation (carriers). Of importance, this is the largest group of CL patients described to date. Even though there are few statistically significant findings, this study helps better define the pulmonary disease found in CL. This study demonstrated that there is clinical overlap of the pulmonary complications between the different types of CL based on onset and mutational status.

6.1 GENERAL PULMONARY RESPONSES

The most common reported respiratory diagnoses amongst all of the CL patients were pneumonia (24.1%), tachypnea (15.7%), and emphysema (12.0%). Pneumonia and emphysema are both common causes of morbidity within the United States. For instance, 2.2% of noninstitutionalized adults reported a diagnosis of emphysema (CDC 2009). This is in comparison to our group of CL patients in which 12.0% reported a diagnosis of emphysema.

The difference in prevalence of emphysema between our CL cohort and the general population was determined to be statistically significant. Therefore, this suggests that individuals with CL are more likely to develop emphysema compared to the general population. In respect to individuals with CL, this indicates the importance of pulmonary evaluations and continual screening due to the health complications associated with emphysema. In addition, the higher prevalence exhibits the potential involvement of CL causative genes in the pathogenesis of emphysema. Therefore, determining the etiology of emphysema in CL may provide valuable information on emphysema for the general population.

A history of respiratory infections like pneumonia is important because it has been shown to be associated with obstructive lung disease (Gracia-Vidal et al. 2009). With COPD, it has been shown to predispose people to community acquired pneumonia, and those with COPD are more likely to have recurrent infections. In addition, patients with COPD who have chronic bronchitis with recurrent respiratory infections were shown to have lower levels of the complement components C3 and C4 compared to healthy subjects (Kosmas et al. 1997). A low level of complement components is indicative of sustained activation due to the presence of infection. An infection and sustained activation may be related to decrease in ability to clear

infection (Meyer 2004). Cough and mucociliary clearance are lower respiratory tract mechanical defenses against infectious agents and help to remove aspirated or inhaled agents. Inflammation, breakdown of the alveoli, and/or a decrease in elastic recoil (due to loss or lack of elasticity) may interfere with the lungs ability to perform these clearance mechanisms (Kosmas et al. 1997). The incidence of pneumonia among CL patients may be related to a similar mechanism of decreased ability to clear infections. Whether this is related to obstruction or a decrease in the elastic recoil is unknown at this time.

Tachypnea, or increased respiratory rate, is most often caused by hypoxemia. Conditions that can cause hypoxemia, thus tachypnea, include, but are not limited to, pneumonia, asthma, COPD, pulmonary edema, pulmonary embolism, and anemia (Goldman & Ausiello 2007). Of the CL patients who reported a history of tachypnea, 12/13 (92.3%) reported a diagnosis or history of at least one other pulmonary condition. Therefore, it is suggested that those reporting a history of tachypnea may actually have an underlying condition causing the changes in breathing rate.

A limitation of the clinical questionnaires and medical histories is a lack of no consistency between who reports and fills out the information. Referring physicians, patients, and members of our research group have reported and filled out the information. The concern is the difference in level of knowledge of medical terminology, and bias in respect to what type of information is reported. Also, for conditions and symptoms that are determined by quantitative measurements, such as tachypnea which is determined by the breathing rate per minute, these are not defined.

6.2 ONSET CLASSIFICATION & PULMONARY INVOLVEMENT

The common pulmonary responses between the different groups of onset were similar to the general responses. For congenital onset patients, pneumonia (23.1%) and tachypnea (21.2%) were the two most common responses. For unknown onset patients, a history of pneumonia (26.1%) and emphysema (13.0%) were the two most common responses. These answers were expected after determining that all three responses were the most common as a whole. For acquired/late-onset patients the only responses were emphysema, pneumonia, and dyspnea. Even though dyspnea, shortness of breath, was determined to be statistically significant, it most likely is a symptom of other pulmonary conditions. For instance, one of the patients with dyspnea also reported emphysema, which is a predominant reported symptom of obstructive lung disease. A potential explanation includes that adults may be more likely to report symptoms like dyspnea than other age groups. In general, the results for the three groups of onset demonstrate a clinical overlap of pulmonary involvement.

In respect to the PFTs collected, patients either had normal lung function or obstruction. No patients had restrictive or mixed defects of obstructive and restrictive lung function. Of the 4 patients with acquired/late-onset, pulmonary function ranged from mild to very severe obstruction. These 4 acquired/late-onset patients were the same 4 patients who accounted for all acquired/late-onset pulmonary responses on questionnaires and medical histories. For congenital patients, pulmonary function ranged from normal to moderate obstruction, and the one unknown patient had normal function. As described, obstructive lung disease can include diagnoses such as COPD, emphysema, chronic bronchitis, asthma, or bronchiectasis. Only 2 out of 7 (28.6%) of the patients with obstruction found on the PFT reported a related diagnosis (emphysema and

asthma). When related symptoms like dyspnea are included, then 4 out of 7 (57.1%) of the patients have a related diagnosis or history.

Interestingly, 2 out of 3 congenital patients with obstruction did not note any pulmonary conditions or symptoms on their clinical questionnaires. Both of these patients when evaluated were in late adolescence/early adulthood. This suggests a difference in likelihood of reporting symptoms such as difficulty breathing or dyspnea. One possible explanation is that acquired/late-onset patients are more attuned to the changes occurring due to developing symptoms of cutis laxa later in life. Congenital patients may potentially be used to their health complications, and be unaware that things, like their ability to breath, should be different.

6.3 CL TYPES & PULMONARY INVOLVEMENT

Mutations were identified in 27 of the 83 patients, and in 5 carriers. Based on the gene mutation, it was determined that these 27 patients represent cases of ADCL, ARCL1, ARCL2A, and URDS. The absence of mutations in *ATP7A, PYCR1*, and *RIN2* does not necessarily mean that none of our patients have a mutation in one of these three genes. If available, serum copper and ceruloplasmin levels are recorded for patients. This is an indirect genetic assessment for *ATP7A.* For the other genes it is most likely attributable to the fact that our laboratory currently does not screen patients for mutations in these genes.

In respect to mutational status and age of onset, pulmonary complications have previously been described for ADCL, ARCL1, URDS, and acquired/late-onset. This study confirms these findings. However, of note, several of the patients with mutations were

previously described in literature. Therefore, the reported pulmonary involvements did not confirm previous findings, but restated previously reported findings. For ADCL, 3 of our 7 of our cases were previously described by Szabo et al. (2006) and were noted to not have any pulmonary involvement. For ARCL1, both cases used were previously described by Hucthagowder et al. (2006) and Claus et al. (2008). However, for URDS and acquired/late-onset, the majority of cases described here are new cases not previously described; thus confirming and expanding on previous reports.

Comparison of pulmonary responses by mutational status, thus between the CL types with known genetic causes, demonstrated no statistically significant associations. This may suggest an overlap of pulmonary phenotype between the CL types and does not support a phenotype-genotype correlation for pulmonary involvement. However, it is important to consider that the small sample sizes limit the ability to draw firm conclusions.

An important finding, regardless of statistical significance, is the presence of pulmonary involvement in ARCL2A. In previous reports, patients with ARCL2A were not noted to have any diagnosis or history of a pulmonary condition. In our cohort, of the 5 patients with ARCL2A, 2 (40%) reported a history of infectious related pulmonary disease. These included bronchiectasis, in which onset is typically related to a bacterial or viral infection, and pneumonia. As already discussed, association between respiratory infections and obstructive lung disease has been shown. In addition, a third ARCL2A patient was found to have moderate obstruction on the PFT. Collection of more complete pulmonary data on patients with ARCL2A is needed to better define the pulmonary involvement and to determine if pulmonary complications are part of the clinical spectrum for ARCL2A.

6.4 URDS (LTBP4-RELATED CL)

Of the gene mutations identified, 48.1% (13/27) were identified to have *LTBP4* mutations. Therefore, patients with *LTBP4* mutations, or URDS, represent the largest type of CL within our CL cohort. This study presents 9 new cases in which patients have at least one *LTBP4* mutation; therefore presenting the largest group of URDS patients. To date, only 4 patients have previously been reported to have *LTBP4* mutations (Urban et al. 2009). Based on the frequency of *LTBP4* mutations within our CL cohort, it is suggestive that URDS may be the most prevalent type of autosomal recessive CL. As a result, screening of *LTBP4* should be considered first for patients with multi-systemic involvement and are suggestive of autosomal recessive inheritance.

As noted, only one *LTBP4* mutation has been identified in 6/13 of these patients. Of the patients with compound heterozygous or homozygous mutations, 5/6 had at least one frame-shift or nonsense mutation. For patients with only one identified mutation, 5/6 have a missense mutation. There is a question as to whether patients with only one mutation have different phenotypic features and if the presence of a missense mutation is associated with unique features. However, at this time the significance of mutation type between the two groups of URDS patients is unclear. For pulmonary involvement, comparison of the responses between the two groups indicated no significant associations (p-values ranged from 0.3500 to 1.000).

6.5 HETEROZYGOUS CARRIERS OF RECESSIVE CL MUTATIONS

Five carriers were included in this study. All 5 individuals were first-degree relatives of patients and presented with no cutaneous involvement. These individuals either had a *FBLN4* mutation (ARCL1 carrier) or a *LTBP4* mutation (URDS carrier). Two carriers, 1 ARCL1 carrier and 1 URDS carrier, reported a history of pneumonia. Pneumonia is a common disease in the general population and consequently, the history of pneumonia is most likely an incidental finding. One URDS carrier was reported to have multiple pulmonary conditions. This carrier was the child of parents (the other two URDS carriers) who had previously lost a child with URDS. Therefore, it is unclear whether this is a relevant association or an incidental finding. Finally the 3 URDS carriers had PFTs performed, and all were found to have normal lung function. Due to the small number of carriers, it is not possible to ascertain whether carriers are more likely to develop pulmonary disease or are more susceptible to conditions like COPD. To better determine the risk for pulmonary disease, a study with a larger number of carriers who are followed over time to monitor their pulmonary status would be necessary.

6.6 PUBLIC HEALTH SIGNIFICANCE: CHRONIC LUNG DISEASE

Chronic lung disease is a common cause of morbidity in the general population. There have been a number of studies and reviews that have discussed genetic causes, susceptibilities, and associations of COPD and related conditions. Examples include, and are not limited to, *AAT, AACT, EPHX1, GSTs, HMOX1, TNF-alpha, CFTR, SFTPD, TGFB1, LTBP4, STAT1, NFKBIB*,

and *GC* (Bakke et al. 2011, Hersh et al. 2006, Sandford & Silverman 2004). However, the associations identified have not always been replicated.. Uncovering the genetic basis of chronic lung disease in inherited syndromes identifies key molecular targets for improved diagnosis and treatment of common respiratory ailments.

The best described genetic susceptibility for chronic lung disease, specifically early-onset emphysema, is alpha-1 antitrypsin (AAT) deficiency. About 1 in 3000 to 1 in 5000 are affected with AAT deficiency (Silverman & Sandhaus 2009). The majority of individuals with AAT deficiency have a PI ZZ or PI ZNull genotype (two copies of the PI*Z allele or one copy of PI*Z allele and one copy of PI*Null allele). A diagnosis is most commonly made by measurement of plasma AAT level and either protease inhibitor (PI) typing or detection of mutation. The common clinical presentation of those with AAT deficiency is early-onset panacinar emphysema in adults, however individuals may present with other types of emphysema. Other features and symptoms may include bronchiectasis, dyspnea, chronic cough, or wheezing (Silverman & Sandhaus 2009). However, AAT deficiency only accounts for less than 1% of all emphysema cases (Silverman & Sandhaus 2009, Barnes 2004).

Identifying mutations or variants in genes associated with conditions in which patients are known to have chronic obstructive pulmonary involvement, like CL, and determining the functional and physiological impact may allow for a better understanding of the causes and susceptibilities to COPD. Examples of studies that found associations with CL-related genes include Cho et al. (2009) and Hersh et al. (2006). Cho et al. (2009) found the elastin variant G773D in 1-2% of individuals in a severe COPD cohort. However, they were unable to find other elastin variants that demonstrated a strong association with COPD in their cohorts. Hersh et al. (2006) looked at COPD-related phenotypes and found four genes to be associated, which

included *LTBP4*. Additional research on carriers of autosomal recessive CL types may allow for identification of associations of CL-related genes and chronic lung diseases.

6.7 FUTURE DIRECTIONS

6.7.1 Additional Pulmonary Data

Analyzing subsets of the clinical data collected on our CL cohort allows for better characterization of specific systemic involvement. In addition, it is possible to further define the clinical presentation of the different types of CL. For instance, the pulmonary involvement in ARCL2A was identified due to comparing respiratory responses and classification of pulmonary function. Continued collection of clinical data will aid this process. It will also allow for more statistical power, and the potential identification of genotype-phenotype correlations. Also, it will be important to follow-up with patients to monitor progression of disease.

Additional pulmonary data could be collected for individuals withARCL2A and heterozygous carriers for recessive types of CL. With respect to the pulmonary complications in those with ARCL2A, it would be important to follow-up on the findings from this study and determine whether other patients have similar respiratory responses and/or lung function. This could possibly involve contacting other research groups, physicians, and patients and suggesting that these patients have a pulmonary evaluation. For CL heterozygous carriers, based on the data collected on the 3 *LTBP4* and 2 *FBLN4* carriers it is unclear whether the reported pulmonary conditions and symptoms are related to the mutational status or are incidental findings. To

clarify these results clinical questionnaires and PFTs on other carriers could be collected. To ascertain the risk for chronic lung diseases like COPD, follow-up could include monitoring for any changes in lung function.

Finally, to account for confounders of pulmonary disease additional information should be considered when analyzing future pulmonary data. Confounders include such things as occupational or environmental exposures, genetic susceptibilities like AAT deficiency, age, gender, and race/ethnicity. For exposures, respiratory questionnaires that specifically ask about environmental and occupational exposure histories will be used. In regards to AAT deficiency, determining a patient's status would allow for better characterization of the impact of CL causative genes on the development of chronic lung disease. Finally, consideration of such factors would aid in determining whether a patient's pulmonary presentation was related solely to their CL, or a combination of CL and other susceptibility factors.

6.7.2 Chest CT Collection and Analysis

Finally, in this study to determine the type of pulmonary function, only PFTs were used. A benefit of PFTs is the availability of standardized protocols published by the American Thoracic Society (ATS) that are used by most centers to evaluate patients. PFT parameters such as FEV_1, FVC, and FEV_1/FVC are used to determine the presence, type, and severity of disease affecting pulmonary function. However, these measurements, as discussed by Han et al (2010), only explain a fraction (10-25%) of the impact of the disease on the patient. Chest computed tomography (CT) scans have been shown to provide more specific information regarding the cause and progression of lung disease (Han et al. 2010, Kim et al. 2009). Therefore, to

determine the cause or type of chronic lung disease, it would be valuable to collect chest CT scans on patients.

7.0 CONCLUSION

Cutis laxa is a group of conditions characterized by the cutaneous features. Other systemic involvement includes genitourinary, gastrointestinal, pulmonary, cardiovascular, and neurobehavioral. This study aimed to better define the pulmonary involvement. Through analysis of clinical questionnaires, medical histories, and PFTs, this study provided a qualitative and quantitative assessment of types of pulmonary conditions seen in CL. In our cohort, emphysema and pneumonia were two of the most common responses. Therefore, the primary findings confirmed previous reports indicating obstructive lung disease and recurrent respiratory infections as the primary pulmonary involvement. Even though there were no significant differences in the type of pulmonary conditions between onset groups (acquired/late-onset, congenital, unknown) or types of CL based on mutational status, the information provided here demonstrates the heterogeneity and clinical overlap of CL.

APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL LETTERS

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 MD
From: Frank Lieberman MD, Vice Chair
Date: 8/25/2010
IRB#: PRO10020125
Subject: Genetics of Extracellular Matrix in Health and Disease

---

At its full board meeting on 8/11/2010, the University of Pittsburgh Institutional Review Board, Committee E, reviewed the above referenced research study and approved it pending minor modifications. Your responses to these comments have been reviewed and the research submission, in its currently modified form, adequately addresses the concerns of the IRB and is therefore approved.

Please note the following information:

The risk level designation is Greater Than Minimal Risk.

Approval Date: 8/25/2010
Expiration Date: 8/10/2011

For studies being conducted in UPMC facilities, no clinical activities can be undertaken by investigators until they have received approval from the UPMC Fiscal Review Office.

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)]. The IRB Reference Manual (Chapter 3, Section 3.3) describes 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.

Washington University in St.Louis

Human Research Protection Office

Barnes-Jewish Hospital
St. Louis Children's Hospital
Washington University

September 17, 2008

Zsolt Urban, PhD
Genetics
Box 8208

RE: 04-0966

Genetic Determinants: Elastin Quality and Quantity (SVAS and CL)
-Fibulin-4 in Vascular Development
-Fibulin-4 in Cardiovascular and Connective Tissue Development

Dear Dr. Urban:

The above-stated protocol was reviewed and approved by the Human Research Protection Office (HRPO). Following please find specifics of the approval:

Approval Date:	8/21/2008
Date released for accrual:	9/17/2008
Expiration Date:	8/20/2009
Research Risk Level:	Greater than Minimal
Type of Review:	Greater Than Minimal Risk (Full Board)
Reviewing Committee:	03 CRC
HIPAA Compliance:	Compliant with Authorization

WU HRPO has eleven duly appointed Committees established in accordance with 45 CFR 46.107 that review protocols for faculty and staff at Washington University School of Medicine, Barnes-Jewish Hospital, and Saint Louis Children's Hospital. The Committees have 20 – 25 members with varying backgrounds to promote complete and adequate review of research activities commonly conducted at WU. The names and qualifications of the members are on file with the Office of Human Research Protections.

The WU HRPO complies with the regulations outlined in 45 CFR 46, 45 CFR 164, 21 CFR 50, and 21 CFR 56. The OHRP Federal Wide Assurance numbers for WU, BJH, and SLCH are FWA00002284, FWA00002281, and FWA00002282 (respectively).

If further information is necessary, please contact the HRPO office at (314) 633-7400.

Sincerely,

Ed Casabar, PharmD
03 CRC Chair
CC:

Medical Center Office: 660 South Euclid Ave., Campus Box 8089, St. Louis, MO 63110 Phone: (314) 633-7400, FAX: (314) 367-3041

OMB No. 0925-0418
Approved for use through 01/31/2001

Protection of Human Subjects
Assurance Identification/Certification/Declaration
(Common Federal Rule)

Policy: Research activities involving human subjects may not be conducted or supported by the Departments and Agencies adopting the Common Rule (56FR28003, June 18, 1991) unless the activities are exempt from or approved in accordance with the common rule. See section 101(b) the common rule for exemptions. Institutions submitting applications or proposals for support must submit certification or appropriate Institutional Review Board (IRB) review and approval to the Department or Agency in accordance with the common rule.

Institutions with an assurance of compliance that covers the research to be conducted on file with the Department, Agency, or the Department of Health and Human Services (HHS) should submit certification of IRB review and approval with each application or proposal unless otherwise advised by the Department or Agency. Institutions which do not have such an assurance must submit an assurance and certification of IRB review and approval within 30 days of a written request from the Department or Agency.

1. Request Type
☐ ORIGINAL
☒ FOLLOWUP
☐ EXEMPTION

2. Type of Mechanism
☒ GRANT ☐ CONTRACT ☐ FELLOWSHIP
☐ COOPERATIVE AGREEMENT
☐ OTHER:

3. Name of Federal Department or Agency and, if known, Application or Proposal Identification No.
NIH/NIAMS

4. Title of Application or Activity
"Elastin Gene Mutations in Skin and Vascular Diseases"

5. Name of Principal Investigator,Program Director, Fellow, or Other
Zsolt Urban, Ph.D.

6. Assurance Status of this Project *(Respond to one of the following)*

☒ This Assurance, on file with Department of Health and Human Services, covers this activity:
Assurance identification no. M- 1217 IRB identification no. 01

☐ This Assurance, on file with *(agency/dept)*_____, covers this activity.
Assurance identification no._____ IRB identification no._____ *(if applicable)*

☐ No assurance has been filed for this project. This institution declares that it will provide an Assurance and Certification of IRB review and approval upon request.

☐ Exemption Status: Human subjects are involved, but this activity qualifies for exemption under Section 101(b), paragraph_____.

7. Certification of IRB Review (Respond to one of the following IF you have an Assurance on file)

☒ This activity has been reviewed and approved by the IRB in accordance with the common rule and any other governing regulations or subparts on June 18, 2002 by: ☒ Full IRB Review or ☐ Expedited Review

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by the common rule will be reviewed and approved before they are initiated and that appropriate further certification will be submitted.

8. Comments

CHS #10467

9. The official signing below certifies that the information provided above is correct and that, as required, future reviews will be performed and certification will be provided.

10. Name and Address of Institution
University of Hawaii at Manoa
Office of the Chancellor
2444 Dole Street, Bachman Hall
Honolulu, HI 96822

11. Phone No. *(with area code)*
(808) 956-5007

12. Fax No. *(with area code)*
(808) 539-3954

13. Name of Official
William H. Dendle

14. Title
Compliance Officer

15. Signature
William H. Dendle

16. Date
6/19/02

Authorized for local Reproduction

OPTIONAL FORM 310 (Rev. 1-98)

Sponsored by HHS/NIH

APPENDIX B

2011 CLINICAL QUESTIONNAIRE

CUTIS LAXA

Patient/Physician questionnaire

Family ID:_____

Participant ID:_____

RACE:

American Indian or Alaskan [ ]

Asian [ ]

Black or African American [ ]

Native Hawaiian or Other Pacific Islander: [ ]

Other race (please specify): _____

Two or More Races (please specify): _____

White: [ ]

ETHNICITY:

Hispanic or Latino [ ]

Other ethnicity (please specify): _____

Definitions:

American Indian or Alaska Native: A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliation or community attachment.

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

Black or African American: A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or Other Pacific Islander: A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," can be used in addition to "Hispanic or Latino".

PAST MEDICAL HISTORY

Birth Data:

Date of birth (mo/day/year): __/__/__ Male: [ ] Female: [ ]

Gestational age at birth: _____ weeks

Birth weight: _____ Length: _____ Head circumference: _____

Amniotic fluid:	Oligohydramnios: [ ]	Normal: [ ]	Polyhydramnios: [ ]
Fetal movements:	Reduced: [ ]	Normal: [ ]	Increased: [ ]
Fetal ultrasound:	Normal: [ ]	Abnormal: [ ]	Not performed: [ ]

Ultrasound abnormalities Gestational age

Death Data:

If deceased, date of death (mo/day/year): __/__/__

Cause of death:

Previous Surgical History:

History of abdominal vascular surgery: Yes [ ] No [ ] ? [ ]

History of any facial surgeries: Yes [ ] No [ ] ? [ ]

If yes, type(s) of surgeries:

Cosmetic surgeries:

Face lift: Yes [ ] No [ ] ? [ ]

Date(s):_____

Other:_____ Date(s):_____

_____ Date(s):_____

Previous Hospitalizations:

History of any trauma that affected the facial structure: Yes [ ] No [ ] ? [ ]
If yes, document here:

Medications:

Allergies: Yes [ ] No [ ] ? [ ]
Allergens:

Skin affected: Yes [ ] No [ ] ? [ ]

3 GENERATION PEDIGREE

Patient:_____
Historian:_____
Completed By:_____ Date:_____
Updated By:_____ Date:_____

Paternal Ethnicity:_____
Maternal Ethnicity:_____
Consanguinity:_____

Family Review of Systems (Please note findings on pedigree):

LD/DD/MR	Yes	No	Gastrointestinal Problems	Yes	No	Diverticulae	Yes	No
Respiratory Problems	Yes	No	Genitourinary Problems	Yes	No	Hernias	Yes	No
Cardiovascular Problems	Yes	No	Skeletal Problems	Yes	No	Growth Delay	Yes	No
Skin: At least 2-3 of the following (i) Loose, lax; (ii) Redundant; (iii) Inelastic; (iv) Premature aging of the skin; (v) Skin wrinkling							Yes	No

CLINICAL DESCRIPTION

Age at examination: _____

Age when symptoms began: _____

SKIN:

Lax: Yes [ ] No [ ] ? [ ]

Redundant: Yes [ ] No [ ] ? [ ]

Inelastic: Yes [ ] No [ ] ? [ ]

Loose facial skin: Yes [ ] No [ ] ? [ ]

Premature aging of the skin: Yes [ ] No [ ] ? [ ]

Skin wrinkling: Yes [ ] No [ ] ? [ ]

Affected skin: Generalized [ ] Localized [ ]

If localized: Symmetric [ ] Asymmetric [ ] Flexural [ ] Focal [ ]

Transparent skin: Yes [ ] No [ ] ? [ ]

Inflammatory skin disease: Yes [ ] No [ ] ? [ ]

Other skin findings:

CRANIOFACIAL:

Facial drooping: Yes [ ] No [ ] ? [ ]

Wizened appearance: Yes [ ] No [ ] ? [ ]

Large fontanels: Yes [ ] No [ ] ? [ ]

Delayed closure of fontanels: Yes [ ] No [ ] ? [ ]

Oxycephaly: Yes [ ] No [ ] ? [ ]

Frontal bossing: Yes [ ] No [ ] ? [ ]

Reversed-V eyebrows: Yes [ ] No [ ] ? [ ]

Downward slanting palpebral fissures: Yes [ ] No [ ] ? [ ]

Hooked nose: Yes [ ] No [ ] ? [ ]

Long philtrum: Yes [ ] No [ ] ? [ ]

Occipital horns: Yes [ ] No [ ] ? [ ]

Other craniofacial findings:

ORAL:

Vocal cord laxity: Yes [ ] No [ ] ? [ ]

Hoarse voice: Yes [ ] No [ ] ? [ ]

Dental caries: Yes [ ] No [ ] ? [ ]

Other oral findings:

EYES:

Retinovascular tortuosity: Yes [ ] No [ ] ? [ ]

Corneal opacities: Yes [ ] No [ ] ? [ ]

Corneal arcus: Yes [ ] No [ ] ? [ ]

Cataract: Yes [ ] No [ ] ? [ ]

Other eye findings:

NEUROBEHAVIORAL:

Hypotonia: Yes [ ] No [ ] ? [ ]

Congenital bilateral athetosis: Yes [ ] No [ ] ? [ ]

Mental retardation: Yes [ ] No [ ] ? [ ]

IQ Test type:_____ Value:_____

Education history:

Social history:

Other neurobehavioral findings:

RESPIRATORY:

Bronchiectasis: Yes [ ] No [ ] ? [ ]

Hypoplastic lungs (newborn): Yes [ ] No [ ] ? [ ]

Emphysema: Yes [ ] No [ ] ? [ ]

Tachypnea: Yes [ ] No [ ] ? [ ]

Pneumonia: Yes [ ] No [ ] ? [ ]

Asthma: Yes [ ] No [ ] ? [ ]

Chest CT findings and date: _____

Pulmonary function test (PFT) findings and date: _____

Current smoker: Yes [ ] No [ ]

Cigarette packs per day: _____

Years smoked: _____

Other respiratory findings:

CARDIOVASCULAR:

Cor pulmonale: Yes [ ] No [ ] ? [ ]

Right ventricular hypertrophy: Yes [ ] No [ ] ? [ ]

Infundibular stenosis: Yes [ ] No [ ] ? [ ]

Pulmonary valve stenosis: Yes [ ] No [ ] ? [ ]

Pulmonary artery stenosis: Yes [ ] No [ ] ? [ ]

Murmur (6 scale):_____

Pressure gradient (Hgmm):_____

Peak velocity (m/s):_____

Peripheral pulmonary stenosis: Yes [ ] No [ ] ? [ ]

Aortic stenosis: Yes [ ] No [ ] ? [ ]

Murmur (6 scale):_____

Pressure gradient (Hgmm):_____

Peak velocity (m/s):_____

Aortic dilatation: Yes [ ] No [ ] ? [ ]

Level:_____

Diameter:_____

Arterial tortuosity: Yes [ ] No [ ] ? [ ]

Which arteries:_____

Arterial aneurysms: Yes [ ] No [ ] ? [ ]

Which arteries:_____

Raynaud's phenomenon: Yes [ ] No [ ] ? [ ]

Venous varicosity: Yes [ ] No [ ] ? [ ]

Echocardiogram (Echo) findings and date:_____

Other cardiovascular findings:

GASTROINTESTINAL:

Diverticulae: Esophageal: Yes [ ] No [ ] ? [ ]

Duodenal: Yes [ ] No [ ] ? [ ]

Rectal: Yes [ ] No [ ] ? [ ]

Other:_____

Other gastrointestinal findings:

GENITOURINARY:

Bladder diverticulae: Yes [ ] No [ ] ? [ ]

Genital prolapse: Yes [ ] No [ ] ? [ ]

Obstructive uropathy: Yes [ ] No [ ] ? [ ]

Bladder neck obstruction: Yes [ ] No [ ] ? [ ]

Other genitourinary findings:

MUSCULOSKELETAL:

Hernias: Inguinal: Yes [ ] No [ ] ? [ ]

Diaphragmatic: Yes [ ] No [ ] ? [ ]

Hiatal: Yes [ ] No [ ] ? [ ]

Umbilical: Yes [ ] No [ ] ? [ ]

Hip dislocation: Yes [ ] No [ ] ? [ ]

Thumbs and/or toes dislocated: Yes [ ] No [ ] ? [ ]

Joint laxity: Yes [ ] No [ ] ? [ ]

Pectus carinatum: Yes [ ] No [ ] ? [ ]

Pectus excavatum: Yes [ ] No [ ] ? [ ]

Scoliosis: Yes [ ] No [ ] ? [ ]

Flat feet: Yes [ ] No [ ] ? [ ]

Short broad clavicles: Yes [ ] No [ ] ? [ ]

Fused carpal bones: Yes [ ] No [ ] ? [ ]

Other musculoskeletal findings:

GROWTH:

Growth deficiency/delay:

Prenatal:	Yes [ ]	No [ ]	? [ ]
Postnatal	Yes [ ]	No [ ]	? [ ]
Dwarfism	Yes [ ]	No [ ]	? [ ]

Physical data:

Age	Weight	Height	Head circumference

OTHER ABNORMALITIES NOT LISTED ABOVE:

_____

_____

_____

LABORATORY:

Reduced elastin synthesis:	Yes [ ]	No [ ]	? [ ]
Abnormal elastic fibers:			
Histology:	Yes [ ]	No [ ]	? [ ]
EM:	Yes [ ]	No [ ]	? [ ]
Reduced lysyl oxidase activity:	Yes [ ]	No [ ]	? [ ]

Serum ceruloplasmin:_____

Serum copper:_____

ADDITIONAL INFORMATION:

Clinical photographs available:	Yes [ ]	No [ ]
DNA available:	Yes [ ]	No [ ]
Cultured cells available:	Yes [ ]	No [ ]
Pathology slides available:	Yes [ ]	No [ ]
Frozen tissue available:	Yes [ ]	No [ ]
Patient available for re-examination:	Yes [ ]	No [ ]

CUTIS LAXA

Contact Information

PARTICIPANT

Family ID: _____

Participant ID:_____

Participant's Name:_____

Parent's or Guardian's Name (if participant is minor):_____

Address:_____

_____

_____

Phone:_____

Fax:_____

Email:_____

REFERRING PHYSICIAN

Physician's Name:_____

Address:_____

_____

_____

Phone:_____

Fax:_____

Email:_____

APPENDIX C

PFT REFERENCE VALUES

Table 9: Prediction and Lower Limit of Normal Equations for Spirometric Parameters for Male Subjects

Male Subjects		Intercept (b0)	Age (b1)	Age^2 (b2)	Ht LLN (cm)^2 (b3)
Caucasian < 20 yr of age	FEV1	-0.7453	-0.04106	0.004477	0.00011607
	FEV6	-0.3119	-0.18612	0.009717	0.00015323
	FVC	-0.2584	-0.20415	0.010133	0.00015695
	PEF	-0.5962	-0.12357	0.013135	0.00017635
	FEF 25-75	-1.0863	0.13939	0	0.00005294
Caucasian ≥ 20 yr of age	FEV1	0.5536	-0.01303	-0.000172	0.00011607
	FEV6	0.1102	-0.00842	-0.000223	0.00015323
	FVC	-0.1933	0.00064	-0.000269	0.00015695
	PEF	1.0523	0.08272	-0.001301	0.00017635
	FEF 25-75	2.7006	-0.04995	0	0.00005294
African-American < 20 yr of age	FEV1	-0.7048	-0.05711	0.004316	0.00010561
	FEV6	-0.5525	-0.14107	0.007241	0.00013499
	FVC	-0.4971	-0.15497	0.007701	0.0001367
	PEF	-0.2684	-0.28016	0.018202	0.00018938
	FEF 25-75	-1.1627	0.12314	0	0.00004819

Table 9 (Continued).

African-American ≥ 20 yr of age	FEV1	0.3411	-0.02309	0	0.00010561
	FEV6	-0.0547	-0.02114	0	0.00013499
	FVC	-0.1517	-0.01821	0	0.0001367
	PEF	2.2257	-0.04982	0	0.00018938
	FEF 25-75	2.1477	-0.04238	0	0.00004819
Mexican-American < 20 yr of age	FEV1	-0.8218	-0.04248	0.004291	0.0001267
	FEV6	-0.6646	-0.1127	0.007306	0.00015029
	FVC	-0.7571	-0.0952	0.006619	0.00014947
	PEF	-0.9537	-0.19602	0.014497	0.00021833
	FEF 25-75	-1.3592	0.10529	0	0.0000902
Mexican-American ≥ 20 yr of age	FEV1	0.6306	-0.02928	0	0.0001267
	FEV6	0.5757	-0.0286	0	0.00015029
	FVC	0.2376	-0.00891	-0.000182	0.00014947
	PEF	0.087	0.0658	-0.001195	0.00021833
	FEF 25-75	1.7503	-0.05018	0	0.0000902

Table is adapted from Hankinson, Odencrantz, & Fedan (1999).

The equation used to determine the lung function parameter is: $LLN = b0 + b1 * age + b2 * age^2 + b3 * height^2$.

Table 10: Prediction and Lower Limit of Normal Equations for Spirometric Parameters for Female Subjects

Female Subjects		Intercept (b0)	Age (b1)	Age^2 (b2)	Ht LLN (cm)^2 (b3)
Caucasian < 18 yr of age	FEV1	-0.871	0.06537	0	0.00009283
	FEV6	-1.1925	0.06544	0	0.00011827
	FVC	-1.2082	0.05916	0	0.00012198
	PEF	-3.6181	0.60644	-0.016846	0.00012148
	FEF 25-75	-2.5284	0.5249	-0.015309	0.00002302
Caucasian ≥ 18 yr of age	FEV1	0.4333	-0.00361	-0.000194	0.00009283
	FEV6	-0.1373	0.01317	-0.000352	0.00011827
	FVC	-0.356	0.0187	-0.000382	0.00012198
	PEF	0.9267	0.06929	-0.001031	0.00012148
	FEF 25-75	2.367	-0.01904	-0.0002	0.00002302
African-American < 18 yr of age	FEV1	-0.963	0.05799	0	0.00008546
	FEV6	-0.637	-0.04243	0.003508	0.00010848
	FVC	0.6166	-0.04687	0.003602	0.00010916
	PEF	-1.2398	0.16375	0	0.0001216
	FEF 25-75	-2.5379	0.43755	-0.012154	0.0000338

Table 10 (Continued).

African-American ≥ 18 yr of age	FEV1	0.3433	-0.01283	-0.000097	0.00008546
	FEV6	-0.1981	0.00047	-0.00023	0.00010848
	FVC	-0.3039	0.00536	-0.000265	0.00010916
	PEF	1.3597	0.03458	-0.000847	0.0001216
	FEF 25-75	2.0828	-0.03793	0	0.0000338
Mexican-American < 18 yr of age	FEV1	-0.9641	0.0649	0	0.0000989
	FEV6	-1.241	0.07625	0	0.0001148
	FVC	-1.2507	0.07501	0	0.0001157
	PEF	-3.2549	0.47495	-0.013193	0.00014611
	FEF 25-75	-2.1825	0.42451	-0.012415	0.00004594
Mexican-American ≥ 18 yr of age	FEV1	0.4529	-0.01178	-0.000113	0.0000989
	FEV6	0.2033	0.0002	-0.000232	0.0001148
	FVC	0.121	0.00307	-0.000237	0.0001157
	PEF	0.2401	0.06174	-0.001023	0.00014611
	FEF 25-75	1.7456	-0.01195	-0.000291	0.00004594

Table is adapted from Hankinson, Odencrantz, & Fedan (1999).

The equation used to determine the lung function parameter is: $LLN = b0 + b1 * age + b2 * age^2 + b3 * height^2$.

Table 11: Prediction and Lower Limit of Normal Equations for FEV1/FEV6% and FEV1/FVC% for Male and Female Subjects

Male Subjects		**Intercept LLN (b0)**	**Age (b1)**
Caucasian	FEV1/FEV6%	78.372	-0.1382
	FEV1/FVC%	78.388	-0.2066
African-American	FEV1/FEV6%	78.979	-0.1305
	FEV1/FVC%	78.822	-0.1828
Mexican-American	FEV1/FEV6%	80.81	-0.1534
	FEV1/FVC%	80.925	-0.2186
Female Subjects			
Caucasian	FEV1/FEV6%	81.307	-0.1563
	FEV1/FVC%	81.015	-0.2125
African-American	FEV1/FEV6%	81.396	-0.1558
	FEV1/FVC%	80.978	-0.2039
Mexican-American	FEV1/FEV6%	83.034	-0.167
	FEV1/FVC%	83.044	-0.2248

Table is adapted from Hankinson, Odencrantz, & Fedan (1999).

The equation used to determine the lung function parameter is: LLN = b0 + b1 * age.

Table 12: CL Patient Reference Values for FEV1.

Family ID	Patient ID	Gender	Race	Age	Height	Intercept (b0)	Age (b1)	Age^2 (b2)	Ht LLN (cm)^2 (b3)	LLN FEV1 Reference Value
CL-04	7166	Female	Hispanic	34	157.48	0.4529	-0.01178	-0.000113	0.0000989	2.374467095
CL-15	7006	Female	Caucasian	45	171	0.4333	-0.00361	-0.000194	0.00009283	2.59244203
CL-15	7007	Male	Caucasian	43	175	0.5536	-0.01303	-0.000172	0.00011607	3.22992575
CL-15	7192	Male	Caucasian	8	129.54	-0.7453	-0.04106	0.004477	0.00011607	1.160473588
CL-16	7009	Male	Caucasian/Asian	11	158	-0.7453	-0.04106	0.004477	0.00011607	2.24232848
CL-50	7065	Female	Caucasian	58	160	0.4333	-0.00361	-0.000194	0.00009283	1.947752
CL-50	7065	Female	Caucasian	63	161.29	0.4333	-0.00361	-0.000194	0.00009283	1.850806702
CL-50	7065	Female	Caucasian	65	161.29	0.4333	-0.00361	-0.000194	0.00009283	1.793922702
CL-65	7093	Female	Caucasian	20	167.64	0.4333	-0.00361	-0.000194	0.00009283	2.892317234
CL-94	7159	Female	Caucasian	43	160.02	0.4333	-0.00361	-0.000194	0.00009283	2.296406149
CL-94	7160	Male	Caucasian	16	165.1	-0.7453	-0.04106	0.004477	0.00011607	2.907689221
CL-95	7161	Male	Caucasian	40	172.72	0.5536	-0.01303	-0.000172	0.00011607	3.219823268
CL-96	7162	Female	Caucasian	39	173.99	0.4333	-0.00361	-0.000194	0.00009283	2.807634041
CL-115	7197	Female	Caucasian	64	160	0.4333	-0.00361	-0.000194	0.00009283	1.784084
CL-116	7198	Male	Black / African American (Bahamian)	40	181.5	0.3411	-0.02309	0	0.00010561	2.896531023
CL-117	7202	Male	African American / American Indian	11	149	-0.7048	-0.05711	0.004316	0.00010561	1.53387361

See Table 1 & Table 2 for reference values and the equation used to determine the lower limit of normal (LLN) for FEV1.

Table 13: CL Patient Reference Values for FVC.

Family ID	Patient ID	Gender	Race	Age	Height	Intercept (b0)	Age (b1)	Age^2 (b2)	Ht LLN (cm)^2 (b3)	LLN FVC Reference Value
CL-04	7166	Female	Hispanic	34	157.48	0.121	0.00307	-0.000237	0.0001157	2.820762261
CL-15	7006	Female	Caucasian	45	171	-0.356	0.0187	-0.000382	0.00012198	3.27876718
CL-15	7007	Male	Caucasian	43	175	-0.1933	0.00064	-0.000269	0.00015695	4.14343275
CL-15	7192	Male	Caucasian	8	129.54	-0.2584	-0.20415	0.010133	0.00015695	1.390628991
CL-16	7009	Male	Caucasian/Asian	11	158	-0.2584	-0.20415	0.010133	0.00015695	2.6401428
CL-50	7065	Female	Caucasian	58	160	-0.356	0.0187	-0.000382	0.00012198	2.56624
CL-50	7065	Female	Caucasian	63	161.29	-0.356	0.0187	-0.000382	0.00012198	2.479186331
CL-50	7065	Female	Caucasian	65	161.29	-0.356	0.0187	-0.000382	0.00012198	2.418794331
CL-65	7093	Female	Caucasian	20	167.64	-0.356	0.0187	-0.000382	0.00012198	3.293224628
CL-94	7159	Female	Caucasian	43	160.02	-0.356	0.0187	-0.000382	0.00012198	2.865250721
CL-94	7160	Male	Caucasian	16	165.1	-0.2584	-0.20415	0.010133	0.00015695	3.34739267
CL-95	7161	Male	Caucasian	40	172.72	-0.1933	0.00064	-0.000269	0.00015695	4.084063539
CL-96	7162	Female	Caucasian	39	173.99	-0.356	0.0187	-0.000382	0.00012198	3.484920002
CL-115	7197	Female	Caucasian	64	160	-0.356	0.0187	-0.000382	0.00012198	2.398816
CL-116	7198	Male	Black / African American (Bahamian)	40	181.5	-0.1517	-0.01821	0	0.0001367	3.623105575
CL-117	7202	Male	African American / American Indian	11	149	-0.4971	-0.15497	0.007701	0.0001367	1.7649277

See Table 1 & Table 2 for reference values and the equation used to determine the lower limit of normal (LLN) for FVC.

Table 14: CL Patient Reference Values for FEV1/FVC%

Family ID	Patient ID	Gender	Race	Age	Height	Intercept LLN (b0)	Age (b1)	LLN FEV1/FVC Reference Value
CL-04	7166	Female	Hispanic	34	157.48	83.044	-0.2248	75.4008
CL-15	7006	Female	Caucasian	45	171	81.015	-0.2125	71.4525
CL-15	7007	Male	Caucasian	43	175	78.388	-0.2066	69.5042
CL-15	7192	Male	Caucasian	8	129.54	78.388	-0.2066	76.7352
CL-16	7009	Male	Caucasian/Asian	11	158	78.388	-0.2066	76.1154
CL-50	7065	Female	Caucasian	58	160	81.015	-0.2125	68.69
CL-50	7065	Female	Caucasian	63	161.29	81.015	-0.2125	67.6275
CL-50	7065	Female	Caucasian	65	161.29	81.015	-0.2125	67.2025
CL-65	7093	Female	Caucasian	20	167.64	81.015	-0.2125	76.765
CL-94	7159	Female	Caucasian	43	160.02	81.015	-0.2125	71.8775
CL-94	7160	Male	Caucasian	16	165.1	78.388	-0.2066	75.0824
CL-95	7161	Male	Caucasian	40	172.72	78.388	-0.2066	70.124
CL-96	7162	Female	Caucasian	39	173.99	81.015	-0.2125	72.7275
CL-115	7197	Female	Caucasian	64	160	81.015	-0.2125	67.415
CL-116	7198	Male	Black / African American (Bahamian)	40	181.5	78.822	-0.1828	71.51
CL-117	7202	Male	African American / American Indian	11	149	78.822	-0.1828	76.8112

See Table 3 for reference values and the equation used to determine the lower limit of normal (LLN) for FEV1/FVC%.

Table 15: CL Patient Reference Values for TLC.

Family ID	Patient ID	Gender	Race	Age	Reference table	Height (cm)	Height (m)	Reference value (Caucasian)	Reference value (African American)	LLN TLC Reference Value
CL-04	7166	Female	Hispanic	34	Adult	157.48	1.5748	4.60368		3.61368
CL-15	7006	Female	Caucasian	45	Adult	171	1.71	5.496		4.506
CL-15	7007	Male	Caucasian	43	Adult	175	1.75	6.9025		5.7525
CL-15	7192	Male	Caucasian	8	Child/Adolescent	129.54	1.2954	< 90		< 90
CL-16	7009	Male	Caucasian/Asian	11	Child/Adolescent	158	1.58	< 90		< 90
CL-50	7065	Female	Caucasian	58	Adult	160	1.6	4.77		3.78
CL-50	7065	Female	Caucasian	63	Adult	161.29	1.6129	4.85514		3.86514
CL-50	7065	Female	Caucasian	65	Adult	161.29	1.6129	4.85514		3.86514
CL-65	7093	Female	Caucasian	20	Adult	167.64	1.6764	5.27424		4.28424
CL-94	7159	Female	Caucasian	43	Adult	160.02	1.6002	4.77132		3.78132
CL-94	7160	Male	Caucasian	16	Child/Adolescent	165.1	1.651	< 90		< 90
CL-95	7161	Male	Caucasian	40	Adult	172.72	1.7272	6.720328		5.570328
CL-96	7162	Female	Caucasian	39	Adult	173.99	1.7399	5.69334		4.70334
CL-115	7197	Female	Caucasian	64	Adult	160	1.6	4.77		3.78
CL-116	7198	Male	Black / African American (Bahamian)	40	Adult	181.5	1.815	7.42185	6.531228	5.381228
CL-117	7202	Male	African American / American Indian	11	Child/Adolescent	149	1.49	< 90	79.2	< 79.2

References values for lung volumes are determined from Stocks & Quanjer (1995).

APPENDIX D

SUPPLEMENTAL RESULTS

Table 16: Clinical Questionnaire Responses.

Family ID	Patient ID	Onset Classification	Bronchiectasis	Hypoplastic lungs (newborns)	Emphysema	Tachypnea	Pneumonia	Asthma	Obstructive sleep apnea	Respiratory distress	Bronchitis	Dyspnea
CL-023	7026	Acquired/ Late-onset	No	No	No	No	No	No	No	No	No	No
CL-050	7065	Acquired/ Late-onset	No	No	Yes	No	No	No	No	No	No	Yes
CL-068	7099	Acquired/ Late-onset	No	No	No	No	No	No	No	No	No	No
CL-088	7149	Acquired/ Late-onset	No	No	No	No	No	No	No	No	No	No
CL-096	7162	Acquired/ Late-onset	No	No	No	No	Yes	No	No	No	No	No
CL-109	7183	Acquired/ Late-onset	No	No	No	No	No	No	No	No	No	No
CL-115	7197	Acquired/ Late-onset	No	No	No	No	Yes	No	No	No	No	Yes
CL-116	7198	Acquired/ Late-onset	No	No	No	No	No	No	No	No	No	Yes
CL-015	7006	Carrier	No	No	No	No	Yes	No	No	No	Yes	No
CL-015	7007	Carrier	No	No	No	No	No	No	No	No	No	No
CL-015	7192	Carrier	No	No	No	Yes	Yes	Yes	Yes	No	No	No
CL-044	7082	Carrier	No	No	No	No	No	No	No	No	No	No
CL-044	7083	Carrier	No	No	No	No	Yes	No	No	No	No	No
CL-013	7002	Congenital	No	No	No	No	No	No	No	No	No	No
CL-015	7005	Congenital	No	No	Yes	Yes	Yes	No	Yes	Yes	No	No
CL-016	7009	Congenital	No	No	No	No	No	No	No	No	No	No
CL-016	7010	Congenital	No	No	No	No	No	No	No	No	No	No
CL-018	7015	Congenital	No	No	No	No	No	No	Yes	No	No	No
CL-025	7028	Congenital	No	Yes	No	Yes	No	No	No	No	No	No
CL-026	7031	Congenital	No	No	No	No	Yes	No	Yes	No	No	No
CL-027	7032	Congenital	No	No	No	No	Yes	No	No	No	No	No

Table 16 (Continued).

CL-027	7033	Congenital	No	No	No	No	No	No	No	No	No	No
CL-028	7034	Congenital	No	No	No	No	Yes	No	No	No	No	No
CL-036	7044	Congenital	No	No	No	No	No	No	No	No	No	No
CL-037	7045	Congenital	No	No	No	No	No	No	No	No	No	No
CL-038	7046	Congenital	No	No	No	No	No	No	No	No	No	No
CL-039	7047	Congenital	Yes	No	No	No	No	No	No	No	No	No
CL-041	7051	Congenital	No	No	No	No	Yes	Yes	No	No	No	No
CL-041	7052	Congenital	No	No	No	No	No	No	No	No	No	No
CL-042	7053	Congenital	No	No	No	No	No	No	No	No	No	No
CL-043	7054	Congenital	No	No	No	No	No	No	No	No	No	No
CL-044	7055	Congenital	No	No	Yes	No	No	No	No	No	No	No
CL-045	7056	Congenital	Yes	No	No	Yes	No	No	No	No	No	No
CL-049	7064	Congenital	No	No	No	Yes	No	No	No	No	No	No
CL-051	7066	Congenital	No	No	No	No	No	No	No	No	No	No
CL-052	7067	Congenital	No	Yes	No	Yes	No	No	No	No	No	No
CL-053	7069	Congenital	No	No	No	Yes	No	No	No	Yes	No	No
CL-054	7070	Congenital	Yes	No	Yes	Yes	Yes	No	No	No	Yes	Yes
CL-055	7071	Congenital	No	No	No	No	No	No	No	No	No	No
CL-056	7072	Congenital	No	No	No	No	No	No	No	No	No	No
CL-059	7077	Congenital	No	No	No	No	No	No	No	No	No	No
CL-060	7078	Congenital	No	No	Yes	No	No	No	No	No	No	No
CL-061	7079	Congenital	No	No	No	No	No	No	No	No	No	No
CL-062	7081	Congenital	No	No	No	No	No	No	No	No	No	No
CL-062	7085	Congenital	No	No	No	No	No	No	No	No	No	No
CL-063	7086	Congenital	No	No	No	No	No	No	No	Yes	No	No

Table 16 (Continued).

CL-065	7093	Congenital	No	No	No	No	No	No	No	No	No	No
CL-067	7105	Congenital	No	No	No	Yes	Yes	No	No	No	No	No
CL-071	7102	Congenital	No	No	No	No	Yes	No	No	No	No	No
CL-072	7106	Congenital	No	No	No	Yes	Yes	No	No	No	No	No
CL-073	7112	Congenital	No	No	Yes	Yes	No	No	No	No	No	No
CL-075	7117	Congenital	No	No	No	No	No	No	No	No	No	No
CL-076	7118	Congenital	No	No	No	No	No	No	No	No	No	No
CL-078	7122	Congenital	No	No	No	No	Yes	No	No	No	No	No
CL-082	7132	Congenital	No	No	No	No	No	No	No	No	Yes	No
CL-086	7140	Congenital	No	No	No	No	Yes	No	No	No	No	No
CL-090	7153	Congenital	No	No	No	No	No	Yes	No	No	No	No
CL-094	7159	Congenital	No	No	No	No	No	No	No	No	No	No
CL-094	7160	Congenital	No	No	No	Yes	Yes	Yes	No	No	No	No
CL-098	7164	Congenital	No	No	Yes	No	No	No	No	No	No	No
CL-100	7167	Congenital	No	No	No	No	No	No	No	No	No	No
CL-102	7171	Congenital	No	No	No	No	No	No	No	No	No	No
CL-105	7178	Congenital	No	No	No	No	No	No	No	No	No	No
CL-113	7193	Congenital	No	No	No	No	No	No	No	No	No	No
CL-117	7202	Congenital	No	No	No	No	No	Yes	No	No	No	No
CL-004	7166	Unknown	No	No	No	No	No	Yes	No	No	No	No
CL-011	1391707	Unknown	No	No	No	No	No	No	No	No	No	No
CL-012	7001	Unknown	No	No	No	No	No	No	No	No	No	No
CL-012	7008	Unknown	No	No	No	No	Yes	No	No	No	No	No
CL-012	7013	Unknown	No	No	Yes	Yes	Yes	No	Yes	No	No	No
CL-012	7018	Unknown	No	No	No	No	Yes	No	No	No	No	No

Table 16 (Continued).

CL-012	7019	Unknown	No	No	No	No	Yes	No	No	No	No	No
CL-017	7014	Unknown	No	No	No	No	No	No	No	No	No	No
CL-046	7059	Unknown	No	No	No	No	No	No	No	No	No	No
CL-047	7060	Unknown	No	No	No	No	No	Yes	No	No	No	No
CL-048	7061	Unknown	No	No	No	No	No	No	No	No	No	No
CL-057	7073	Unknown	No	No	No	No	No	No	No	No	No	No
CL-058	7074	Unknown	No	No	No	No	No	No	No	No	No	No
CL-064	7092	Unknown	No	No	No	No	No	No	No	No	No	No
CL-066	7094	Unknown	No	No	No	No	No	No	No	No	No	No
CL-074	7116	Unknown	No	No	No	No	No	No	No	No	No	No
CL-077	7121	Unknown	No	No	Yes	No	No	No	No	No	No	No
CL-087	7141	Unknown	No	Yes	No	No	No	No	No	No	No	No
CL-089	7152	Unknown	No	No	No	No	No	No	No	No	No	No
CL-099	7165	Unknown	No	No	No	No	No	No	No	No	No	No
CL-101	7170	Unknown	No	No	Yes	Yes	Yes	No	No	No	Yes	No
CL-103	7173	Unknown	No	No	No	No	Yes	No	No	No	No	No
CL-104	7175	Unknown	No	No	No	No	No	No	No	No	No	No

Table 17: Pulmonary Responses by Cutis Laxa Type (No Carriers) (N = 27).*

Pulmonary Condition	Response	Cutis Laxa Type & Gene				p-value
		ADCL (%)**	ARCL1 (%)**	ARCL2A (%)**	URDS (%)**	
		ELN	FBLN4 / FBLN5	ATP6V0A2	LTBP4	
Bronchiectasis	Yes	0	0	1 (20.0)	2 (15.4)	0.6578
	No	7	2	4	11	
Hypoplastic lung	Yes	0	0	0	2 (15.4)	0.7407
	No	7	2	5	11	
Emphysema	Yes	0	1 (50.0)	0	0	0.1822
	No	7	1	5	13	
Tachypnea	Yes	1 (14.3)	0	0	7 (53.9)	0.0756
	No	6	2	5	6	
Pneumonia	Yes	2 (28.6)	0	1 (20.0)	4 (30.8)	1.000
	No	5	2	4	9	
Asthma	Yes	2 (28.6)	0	0	1 (7.69)	0.4267
	No	5	2	5	12	
Obstructive Apnea	Yes	0	0	0	1 (7.69)	1.000
	No	7	2	5	12	
Respiratory Distress	Yes	0	0	0	1 (7.69)	1.000
	No	7	2	5	12	
Bronchitis	Yes	0	0	0	1 (7.69)	1.000
	No	7	2	5	12	
Dyspnea	Yes	0	0	0	1 (7.69)	1.000
	No	7	2	5	12	

* This table only includes affected individuals (patients), thus does not include carriers.

**Percentages represent the percent of patients within the type of onset who responded "Yes" to the type of pulmonary condition.

Table 18: PFT Results (FEV1, FVC, FEV1/FVC, & TLC).

Family ID	Patient ID	Onset Classification	Gene	PFT Parameters	Pre-bronchodilator			Post-bronchodilator		
					Actual	Predicted	%Predicted	Actual	%Predicted	% Change
CL-50	7065	Acquired/ Late-onset		FVC (L)	2.24	3.09	72			
				FEV1 (L)	1.84	2.36	78			
				FEV1/FVC (%)	82	77				
				TLC (L)	3.87	4.96	78			
CL-96	7162	Acquired/ Late-onset		FVC (L)	4.1	3.87	106	4.32	112	6
				FEV1 (L)	2.82	3.21	88	2.97	92	4
				FEV1/FVC (%)	69	83	83	69	83	0
				TLC (Pleth) (L)	6.77	5.73	118			
CL-115	7197	Acquired/ Late-onset		FVC (L)	1.71	3.07	56	2	65	17
				FEV1 (L)	1.03	2.35	44	1.36	58	32
				FEV1/FVC (%)	60	77	80	68		
				TLC (L)		4.9		4.46	91	
CL-116	7198	Acquired/ Late-onset		FVC (L)	2.4	5.6	43	3.06	55	27
				FEV1 (L)	1.14	4.45	26	1.39	31	21
				FEV1/FVC (%)	48	81	59	45	56	-4
				TLC (L)		7.26		8.42	116	
CL-16	7009	Congenital	ELN	FVC	3.54	3.4	104			
				FEV1	2.74	2.83	97			
				FEV1/FVC (%)	77	83	93			
				TLC	5.06	4.45	113.6			
CL-65	7093	Congenital	ATP6V0A2	FVC (L)	3.98	3.91	102	4.11	105	3
				FEV1 (L)	2.22	3.36	66	2.65	79	20
				FEV1/FVC (%)	56	86	65	65	75	16
				TLC (Pleth) (L)	5.39	5.35	101			
CL-94	7159	Congenital	ELN	FVC (L)	2.27	3.18	71	2.17	68	-3
				FEV1 (L)	1.59	2.67	60	1.66	62	2
				FEV1/FVC (%)	70	84	83	77	91	12
				TLC (Pleth) (L)	4.43	4.9	90			

Table 18 (Continued).

CL-94	7160	Congenital	ELN	FVC (L)	4.96	4.04	123	4.93	122	-1
				FEV1 (L)	2.72	3.47	78	3.02	87	11
				FEV1/FVC (%)	55	85	64	61	72	12
				TLC (Pleth) (L)	6.67	5.13	130			
CL-117	7202	Congenital		FVC (L)	2.69	3.07	88			
				FEV1 (L)	2.17	2.81	77			
				FEV1/FVC (%)	81	86	94			
				TLC (L)	4.13	3.94	105			
CL-04	7166	Unknown		FVC (L)	3.14	2.74	115	3.21	117	2
				FEV1 (L)	2.73	2.35	116	2.78	118	2
				FEV1/FVC (%)	87	86	101	87	101	0
				TLC (Pleth) (L)	4.25	4.04	105			
CL-15	7006	Carrier	LTBP4	FVC (L)	3.53	4.04	87	3.55	88	1
				FEV1 (L)	2.73	3.24	80	2.89	89	6
				FEV1/FVC (%)	77	81	95	81	81	5
				TLC (L)		5.55		5.11	92	
CL-15	7007	Carrier	LTBP4	FVC (L)	6.14	5.05	122	6.21	123	1
				FEV1 (L)	5.06	3.99	127	5.26	132	4
				FEV1/FVC (%)	82	81	101	85	105	4
				TLC (L)		6.72		7.93	118	
CL-15	7192	Carrier	LTBP4	FVC (L)	1.91	2	96			
				FEV1 (L)	1.88	1.8	104			
				FEV1/FVC (%)	86					
				TLC (L)	2.37	2.59	92			

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