Inherited interstitial lung disease

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Interstitial lung diseases (ILDs) are a heterogeneous group of more than 100 different pulmonary diseases. As a group, many of the clinical, radiologic, and physiologic features of these diseases are similar. They are all the result of injury to the distal airspaces producing inflammation and fibrosis of the alveolar septa, which results in disrupted gas exchange across the alveolar septa. These diseases are associated with a diverse number of contributing causes, including granulomatous inflammation (e.g., sarcoidosis), systemic diseases (especially rheumatoid arthritis), environmental exposures (occupational, drug toxicities), and idiopathic interstitial pneumonias. Only in more modern times have the pathologic distinctions been made among the different idiopathic interstitial pneumonias [1]. These refined classifications are not only semantic; they also have been correlated with clinical course, response to therapy, and outcome. Idiopathic pulmonary fibrosis (IPF) is the most deadly of the idiopathic pneumonias, with no known cure and few treatments. Studying the genetics of IPF may reveal the underlying mechanisms for this human disease and offer the development of novel treatments.

Given the clinical heterogeneity of these disorders, it is not surprising that understanding the genetic predispositions has been elusive. Most progress in this area over the last 10 years has been with the interstitial lung disorders that occur as a clinical feature of a genetic disease with straightforward mendelian inheritance. Examples of these systemic diseases include tuberous sclerosis (TS), neurofibromatosis, Hermansky-Pudlak syndrome, and Gaucher disease. In this group, the interstitial disease is only one manifestation of the genetic disorder. The lung phenotype is usually the result of either an infiltrating cell type or the programmed response to alveolar or interstitial cell injury.

In some cases, no systemic disease or environmental exposure can be associated causally with the ILD—the lung injury is the dominant clinical feature of the disease. Familial clustering of these diseases is rare but has been described. Recent progress also has been made in the understanding of the genetics of familial ILDs, which include pediatric cases. Recently discovered mutations in surfactant proteins B and C begin to explain the molecular basis of congenital pulmonary alveolar proteinosis (PAP) and a small subset of familial interstitial pneumonias, respectively. Still other familial ILDs have been described, such as familial IPF, with presumed mendelian inheritance, for which no molecular explanation yet exists.

Although several environmental stimuli are associated with pulmonary fibrosis, only a subset of individuals exposed to these fibrogenic agents develops clinical manifestations of ILD. For example, only a small group of the many patients treated with amiodarone, an antiarrhythmic agent, develops pulmonary fibrosis. Researchers have proposed that inherent host susceptibility or genetic predispositions in conjunction with extrinsic factors contribute to the development of these diseases. The contributions of gene-gene interactions, gene-environment interactions, and epigenetic phenomena are unknown. Using the paradigm of other complex genetic diseases, the expression of the fibrotic lung phenotype has been proposed to be related to multiple genetic polymor-
phisms at different regions of the human genome, each exerting variable effects in conjunction with environmental exposures. Identification and dissection of these genetic effects is a considerable task.

Two broad approaches are used to determine the genetic underpinnings for a certain disease. In the first, families are studied and regions of the genome that are shared among individuals with the same phenotype are identified. The region is narrowed and the genes within the region are analyzed to determine the genetic mutation accounting for the disease phenotype. A large number of related individuals within one or a few families are more likely to yield statistically significant linkage with a genomic region. This approach is hampered by the rarity of familial cases of ILD, however, especially large families with multiple affected individuals. Even when such families are discovered, because the disease is usually expressed late in life and because the disease is usually diagnosed late in the course of the disease, many of the affected individuals have died before their family is brought to the attention of geneticists, so no source of genetic material is available for the affected individuals or their affected ancestors. In the second approach, one tests for the association of different genetic polymorphisms, genotypes, or haplotypes with the expression of a certain disease in unrelated individuals. Given the rarity of available families, most of the published reports on the genetics of ILD have been case-controlled association studies using single nucleotide polymorphisms (SNPs) within candidate genes. Interpreting these reports requires the understanding that genetic associations are reflections of the statistical likelihood that the disease and the polymorphisms occur more frequently together than would be expected by chance alone. Association studies have inherent problems of reproducibility, inadequate numbers of case-controlled pairs, and ethnic diversity within both groups. For these approaches, establishing the correct clinical phenotype is imperative, especially for a group of diseases as heterogeneous as the ILDs.

This article focuses on recent advances in the identification of genes and genetic polymorphisms that have been implicated in the development of human ILDs. It focuses on the inherited mendelian diseases in which pulmonary fibrosis is part of the clinical phenotype and the genetics of familial IPF and other rare inherited ILDs. The article also reviews the association studies that have been published to date regarding the genetics of sporadic IPF. The reader is directed to recent reviews on human genetic predisposition of sarcoidosis, environmental-related, drug-related, connective tissue–related pulmonary fibrosis, and genetic predisposition of fibrosis in animal models.

This article also focuses on the more recent studies that have advanced the field of inherited ILDs. The discussion is subdivided into three topics: (1) inherited genetic diseases in which pulmonary fibrosis is part of the clinical phenotype, (2) genetics of inherited ILDs, and (3) association studies between genetic polymorphisms and idiopathic pulmonary fibrosis.

**Known mendelian disorders associated with pulmonary fibrosis**

ILD and specifically pulmonary fibrosis have been associated with several known genetic diseases that are inherited in a mendelian fashion. For all of these genetic diseases, the lung is secondarily injured as part of the systemic derangements. The extent of pulmonary involvement may range from incidental, being described in case reports, to being a consistent clinical feature of the disease. The known mendelian disorders associated with pulmonary fibrosis are listed in Table 1 and are described in the following section.

**Tuberous sclerosis and lymphangioleiomyomatosis**

TS is a dominantly inherited disease of variable penetrance; it is characterized pathologically by the presence of hamartomas in multiple organ systems. The well-known clinical features include epilepsy, mental retardation, and skin lesions, such as facial angiofibromas, shagreen patches, and ash-leaf hypopigmented macules. Other clinical features include renal angiomyolipomata, renal cysts, central nervous system tubers, nodules, giant cell astrocytomas, cardiac rhabdomyomas, retinal astrocytomas, pulmonary lymphangioleiomyomatosis (LAM), gingival fibromas, tooth enamel pits, rectal polyps, and bone cysts.

ILD occurs in only 1% of patients with TS, usually in subjects with little or no mental retardation. When it occurs, it generally affects patients older than 30 years and it disproportionately affects women [2]. The usual presenting symptoms include exertional dyspnea, recurrent pneumothoraces, and hemoptysis. The pulmonary involvement is indistinguishable from LAM. High-resolution CT scans of the chest reveal diffuse thin-walled cysts smaller than 2 cm in diameter and a diffuse reticulonodular infiltrate [3]. Pathologically the pulmonary findings are also identical to that of LAM [4,5]. There is an abnormal proliferation of smooth muscle cells around lymphatic vessels, which results in a dilatation of the lymphatic spaces.
The obstruction of lymph flow can lead to the development of chylous pleural effusions. The smooth muscle proliferation also can cause distortion of small airways, arterioles, and venules, which can explain the other pulmonary manifestations. Disruption of the small airways leads to air trapping and the radiographic appearance of preserved or increased lung volumes in the presence of interstitial infiltrates. Intermittent hemorrhage caused by vascular involvement accounts for occasional hemoptysis. Recurrent parenchymal hemorrhage leads to hemosiderin deposition and interstitial pulmonary fibrosis.

Most families with TS have been found linked to two different loci: TSC1 at 9q34 and TSC2 at 16p13 [6]. Mutations in the TSC1 gene product, also known as hamartin [7], and mutations in the TSC2 gene product, tuberin [8], which causes disease, have been reviewed recently [9].

LAM is a rare disease that occurs almost exclusively in women. Most cases exclusively involve the lung, but involvement of retroperitoneal, pelvic, and perirenal lymph nodes has been reported [10]. The similarity between the pulmonary manifestations of TS and LAM led to the early speculation that LAM was a forme fruste of TS [4,11]. Support for this hypothesis on a molecular level recently was obtained. LAM that occurs in association with TS is caused by mutations in the TSC1 or TSC2 gene. Sporadic LAM usually results from somatic mutations in the TSC2 gene [12,13]. A small fraction of sporadic LAM (less than 5% in one study) is caused by germ-line mutations in TSC1 [14]. Identification of identical somatic mutations in affected lung and kidney tissue in patients has led to the speculation that the smooth muscle cells from the kidney lesions can migrate to the lung [13,14].

Neurofibromatosis

Neurofibromatosis is one of the most common autosomal-dominant disorders that affects all ethnic groups, both sexes, and all age groups. Type 1, with mutations of the NF1 gene at 17q11, is the more common form of the disease and is known as von Recklinghausen’s disease or classic neurofibromatosis. This disease is characterized clinically by the

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<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Gene(s)</th>
<th>Locus(i)</th>
<th>Pathogenesis</th>
<th>Lung pathology</th>
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<tr>
<td>Tuberous sclerosis/lymphangioleiomyomatosis</td>
<td>AD</td>
<td>TSI</td>
<td>9q34</td>
<td>Proliferation of smooth muscle cells</td>
<td>Hemangiomas</td>
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<td></td>
<td></td>
<td>TSI</td>
<td>16p13.3</td>
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<td>Diffuse fibrosis with cysts and smooth muscle hyperplasia</td>
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<td>Hermansky-Pudlak syndrome</td>
<td>AR</td>
<td>HPS1–HPS7</td>
<td>Many</td>
<td>Abnormal cytoplasmic organelles</td>
<td>Ceroid-containing alveolar macrophages</td>
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<td>Gaucher’s disease</td>
<td>AR</td>
<td>GBA</td>
<td>1q21</td>
<td>Glucocerebroside accumulation</td>
<td>Alveolar Gaucher cells</td>
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<td>Niemann-Pick disease, types A and B</td>
<td>AR</td>
<td>SMPD1</td>
<td>11p15</td>
<td>Sphingomyelin accumulation</td>
<td>Interstitial fibrosis</td>
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<td>Lysinuric protein intolerance</td>
<td>AR</td>
<td>SLC7A7</td>
<td>14q11.2</td>
<td>Deficient transport of dibasic amino acids</td>
<td>Pulmonary hypertension</td>
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<td>Farber lipogranulomatosis</td>
<td>AR</td>
<td>ASAH</td>
<td>8p22–p21.3</td>
<td>Deficiency of acid ceramidase</td>
<td>Pulmonary fibrosis</td>
</tr>
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Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ASAH, N-acylsphingosine amidohydrolase; GBA, acid-beta glucosidase; SLC7A7, solute carrier family 7; member 7; SMPD1, sphingomyelin phosphodiesterase-1.
presence of two or more of the following: café-au-lait spots, neurofibromas, freckling in non–sun-exposed areas, optic glioma, Lisch nodules, distinctive bony lesions, and an affected first-degree family member [15]. Type 2 is rarer and is associated with bilateral acoustic neuromas.

Coexisting diffuse ILD has been reported in patients with type 1 neurofibromatosis. The incidence ranges from less than 7% [16] to 20% [17] of patients over the age of 30 years. Characteristic radiographic findings include bilateral lower lobe fibrosis, bullae and cystic changes, or both. Pathologically, there is interstitial fibrosis and alveolitis with thickening of the alveolar septa and a cellular infiltrate [18]. In addition to the fibrosis, neurofibromatosis may have other intrathoracic manifestations, including “dumbbell” neurofibromas, intercostal neurofibromas, and intrathoracic meningoceles.

**Familial hypocalciuric hypercalcemia**

Familial hypocalciuric hypercalcemia is an autosomal-dominant disease characterized by asymptomatic hypercalcemia and low urinary calcium excretion. At least three different genetic loci are linked to the disease, one of which has been identified as the gene for the calcium sensor receptor [19]. There have been few reports of affected siblings in families with familial hypocalciuric hypercalcemia with coexisting ILD, recurrent respiratory tract infections, and granulocyte dysfunction [20,21]. The pulmonary manifestations are characterized radiographically by a reticulonodular infiltrate that progresses to honeycombing, restrictive spirometry, and slow disease progression that usually begins in the fourth decade [22]. The nature of the molecular defect in families with familial hypocalciuric hypercalcemia with coexisting pulmonary fibrosis is currently unknown.

**Hermansky-Pudlak syndrome**

Hermansky-Pudlak syndrome is an autosomal-recessive disorder characterized by oculocutaneous albinism, bleeding, and ceroid inclusions in macrophages. The disease was first described in 1959 in two unrelated albinos with a lifelong bleeding tendency and peculiar pigmented reticular cells of the bone marrow, lymph nodes, and liver [23]. Although the most prominent symptoms usually are related to the bleeding diathesis, pulmonary fibrosis can occur and is often progressive and fatal. The first patient to be described was a 33-year-old farmer who developed chronic interstitial pulmonary fibrosis. The association between Hermansky-Pudlak syndrome and pulmonary fibrosis was noted by others [24–26]. The clinical, physiologic, and radiologic features are similar to those of IPF. The onset of the pulmonary disease is usually in the third or fourth decade, however, and the incidence in women is twice that in men. Dyspnea can develop abruptly, progress rapidly over several weeks or years and eventually result in end-stage pulmonary fibrosis and death. Pulmonary function tests reveal a restrictive lung defect with reduced diffusing capacity. Radiographically, a ground-glass pattern is noted early and typically evolves to a diffuse interstitial pattern with progression to honeycombing. Pathologically, there is a diffuse interstitial fibrosis; the presence of ceroid-like inclusions in the alveolar macrophages is not absolute.

Most patients are of Puerto Rican ancestry, although patients of diverse ethnic backgrounds have been reported. Besides albinism, bleeding tendency, and restrictive pulmonary defect, the other clinical features of the disease are inflammatory bowel disease, rare cutaneous malignant melanomas, kidney failure, and cardiomyopathy. Ceroid lipofusin-like inclusions found throughout the reticuloendothelial system are diagnostic of Hermansky-Pudlak syndrome.

In recent years the molecular basis of this disease has been elucidated. At least seven different genomic loci have been implicated in the pathogenesis of this disease. Two loci and their corresponding genes, HPS1 and HPS3, were found by genomic linkage, homozygosity mapping, and positional cloning using well-characterized families [27,28]. Four genes, HPS4, HPS5, HPS6, and HPS7, were found by homology to known mouse loci with mutant phenotypes similar to the human Hermansky-Pudlak syndrome [29–31]. HPS2 is caused by mutations in the gene that encodes the beta-3A subunit of the AP3 complex [32,33]. The known genes for Hermansky-Pudlak syndrome are either components of cytoplasmic organelles or function in the trafficking of organelar-specific proteins to melanosomes, lysosomes, and cytoplasmic granules.

**Metabolic diseases**

Several rare diseases seen predominantly in the pediatric population are associated with diffuse ILD. All of these diseases are inherited in an autosomal-recessive manner, with well-characterized molecular and metabolic defects.

Gaucher disease is a lysosomal glycolipid storage disorder characterized by the accumulation of glucosylceramide (glucocerebroside), a normal intermediate in the catabolism of gangliosides. This disease is
common in the Ashkenazi Jewish population. Three types have been clinically delineated, but all result from a deficiency of the enzyme, acid-beta glucosidase, GBA. The GBA gene is located at 1q21. Type 1, also called the “adult” form, is the most common and is distinguished from types 2 and 3 by its lack of central nervous system involvement. Clinical features include hepatosplenomegaly, hematologic abnormalities, skin pigmentation, and occasional pulmonary involvement. A large series of type 1 patients noted pulmonary function abnormalities in 68% [34], but only a fraction of them had overt pulmonary disease. A recent evaluation of 150 consecutive patients with type 1 Gaucher disease found that less than 5% have evidence of clinical ILD [35]. In contrast, autopsy reports of almost all patients with type 2 disease reported pulmonary involvement [36]. Three patterns of pulmonary pathology have been noted: (1) interstitial infiltration by Gaucher cells with fibrosis, (2) alveolar consolidation and filling of alveolar spaces by Gaucher cells, and (3) capillary plugging by Gaucher cells and resultant secondary pulmonary hypertension [37]. Active research in enzyme replacement therapy, bone marrow transplantation, and gene therapy offers the promise of clinical cure.

Niemann-Pick disease, types A and B, is a rare lipid storage diseases characterized by the accumulation of sphingomyelin in cells because of lack of the enzyme sphingomyelinate. Types A and B are caused by mutations in the sphingomyelin phosphodiesterase-1 (SMPD1) gene located at 11p15. Type A is a fatal disorder of infancy characterized by failure to thrive, hepatosplenomegaly, and a rapidly neurodegenerative course. Patients with type B have no neurologic involvement, may survive into adulthood, and may have prominent involvement of the spleen, liver, and lungs. The histologic hallmark of these diseases is the pathologic “foam” cell” or “Neimmann-Pick cell.” This cell is a histiocyte with multiple, uniformly sized lipid droplets or particles within the cytoplasm. Lung involvement is seen in both types with variable extent. In patients with type B, the lung pathology is characterized by the infiltration of the characteristic “foam” cell throughout the pulmonary lymphatics, the pulmonary arteries, and the pulmonary alveoli [38].

Lysinuric protein intolerance results from deficient transport of all cationic amino acids, especially lysine. There is excess urinary clearance of these amino acids and deficient intestinal absorption, which lead to low plasma concentrations and depleted body pools. The predominant symptoms are vomiting and diarrhea after weaning, poor appetite, failure to thrive, hyperammonemia, hepatosplenomegaly, hypotonia, osteoporosis, and variable mental development. Two thirds of affected patients have interstitial changes on high-resolution CT scans and mild defects in pulmonary function tests [39]. Multiple associations between acute or chronic respiratory failure and the development of PAP have been described [40].

Farber lipogranulomatosis is a rare disorder of lipid metabolism that usually presents in the first few months after birth with infiltration by lipid-laden macrophages in joints, skin, and larynx and variable involvement of the liver, spleen, lung, heart, and central nervous system. All severely affected infants have lung involvement with infiltration of the alveoli and septa with massive numbers of macrophages, foam cells, and granuloma formation [41]. The disease is caused by mutations in the acid ceramidase gene on chromosome 8p22-p21.3, which leads to a deficiency of lysosomal acid ceramidase and accumulation of ceramide within lysosomes [42].

**Genetics of inherited interstitial lung disease**

This heterogeneous group of diseases is characterized by a predominant pulmonary phenotype and transmission within families consistent with mendelian inheritance. As a group, less is known about the molecular mechanism of these disorders compared with the genetic disorders discussed above. The recent discovery of abnormalities in surfactant composition in the lungs of affected individuals has highlighted the contribution of surfactant metabolism in diffuse lung diseases. A summary of the diseases reviewed in this section is listed in Table 2.

**Hereditary surfactant protein B deficiency**

Alveolar proteinosis is a rare pulmonary ILD that produces an alveolar-filling pattern on chest radiograph. It is characterized by the presence of amorphous, periodic acid-Schiff–positive proteinaceous, intra-alveolar exudates. Interstitial fibrosis complicates longstanding cases. Most cases of PAP are idiopathic or acquired [43]. Secondary PAP can develop in association with hematologic cancers, inhalation of inorganic dusts, or certain infections. The congenital form of PAP is inherited in an autosomal-recessive manner, affects infants and children, and is usually fatal. Mutations in three genes have been found in patients with congenital PAP; these genes encode for surfactant protein B, the ATP-binding cassette transporter A3 (ABCA3), and the beta chain of the receptor for the granulocyte-macrophage colony-stimulating factor.
Nogee et al [44] first described the case of an infant brother and sister with congenital PAP who died at 5 months and 1 month of age, respectively. An open lung biopsy of the boy showed characteristic findings of PAP, extensive interstitial fibrosis, and alveolar epithelial cell hyperplasia. Analysis of the lung tissue by immunologic and molecular methods revealed the absence of surfactant protein B protein and its mRNA. The affected children in the family were homozygous for the same frameshift mutation in the coding portion of the surfactant protein B gene (SFTPB) [45]. Since these initial reports, many more mutations in SFTPB have been described in patients with respiratory failure in the newborn period. It has been estimated that 10% of full-term infants who present with unexplained respiratory failure have SFTPB mutations [46].

Fatal surfactant deficiency

Mutations in the gene for the ATP-binding cassette transporter A3 (ABCA3) recently have been discovered in several patients of various ethnic groups, all of whom had neonatal severe respiratory disease [48]. All the infants were born after 36 weeks’ gestation, respiratory failure occurred within hours of birth, and there was no known cause for the hypoxic respiratory failure. All infants had clinical or radiographic findings that were consistent with surfactant deficiency. Most died within 1 month of birth. Missense, nonsense, frameshift, and splice-site mutations in ABCA3 were identified in 16 patients, including patients from five consanguineous families. Microscopic examination of lung tissue from 4 patients showed various amounts of proteinaceous material and interstitial thickening, consistent with infantile PAP and desquamative interstitial pneumonitis. In all patients, the lung tissue showed markedly abnormal lamellar bodies. Given the protein similarity of ABCA3 to transporters of phospholipids and cholesterol, it has been hypothesized that ABCA3 transports phospholipids critical for surfactant function in lamellar bodies.

Congenital pulmonary alveolar proteinosis and mutation of the CSF2RB gene

In other cases of infants with PAP with no deficiency of surfactant protein B, there has been reduced expression of the beta chain of the granulocyte-macrophage colony-stimulating factor receptor (CSF2RB), as measured by molecular techniques and flow cytometry [49]. The beta chain is common to the

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<th>Disease</th>
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<th>Gene(s)</th>
<th>Locus</th>
<th>Pathogenesis</th>
<th>Lung pathology</th>
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<tbody>
<tr>
<td>Congenital PAP/neonatal surfactant deficiency</td>
<td>AR</td>
<td>SFTPB</td>
<td>2p12–p11.2</td>
<td>Absence of surfactant protein B</td>
<td>Alveolar proteinosis</td>
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<td></td>
<td>AR</td>
<td>ABCA3</td>
<td>16p13.3</td>
<td>Abnormal surfactant phospholipids?</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td></td>
<td>AR</td>
<td>CSF2RB</td>
<td>22q12.2–q13.1</td>
<td>Reduced expression of the common b chain for the GM-CSF/IL-3/IL-5 receptor</td>
<td>Alveolar proteinosis</td>
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<tr>
<td>Familial interstitial pneumonitis</td>
<td>AD</td>
<td>SFTPC</td>
<td>8p21</td>
<td>Decreased amounts of surfactant protein C?</td>
<td>DIP</td>
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<td>Hamman-Rich</td>
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<td>AR?</td>
<td>?</td>
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<td>?</td>
<td>Multiple laminated calcium concretions</td>
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Abbreviations: ABCA3, ATP-binding cassette transporter A3; AD, autosomal dominant; AR, autosomal recessive; CPI, chronic pneumonitis of infancy; CSF2RB, beta chain of the granulocyte-macrophage colony-stimulating factor receptor; DIP, desquamative interstitial pneumonitis; GM-CSF, granulocyte-macrophage colony-stimulating factor; NSIP, nonspecific interstitial pneumonitis; SFTPB, surfactant pulmonary associated protein B; SFTPC, surfactant pulmonary associated protein C.
receptors of granulocyte-macrophage colony-stimulating factor and interleukin (IL)-3 and -5. Analysis of the CSF2RB gene revealed a homozygous C to A transversion at nucleotide 1135, which resulted in a proline to threonine change in the protein sequence in one patient. This patient was diagnosed with PAP at 20 months by open lung biopsy and bronchoalveolar lavage. The finding of a human mutation within the CSF2RB gene causing PAP is consistent with results obtained from genetically engineered mice. Mice generated by the targeted deletion of the genes for either the beta chain of the granulocyte-macrophage colony-stimulating factor receptor or granulocyte-macrophage colony-stimulating factor itself demonstrated pulmonary findings of PAP [43].

Familial interstitial lung disease associated with reduced surfactant protein C expression and surfactant protein C mutations

Several families with familial ILD including affected children and adults have been reported [50–53]. The lung pathology of ILDs in children usually presents with a wide spectrum of abnormalities that does not fit the classification for ILDs in the adult population. Recently, mutations in the surfactant protein C gene (SFTPC) involving affected children have been described in two families with familial pulmonary fibrosis.

In one family, a mother was diagnosed with “desquamative interstitial pneumonitis” at 1 year of age and treated with glucocorticoids until age 15 [52]. Her father died from life-long lung disease of unknown cause. A child was born to the mother and was diagnosed with respiratory problems at 6 weeks of age. An open lung biopsy of the child’s lung revealed cellular nonspecific interstitial pneumonitis. The mother and child had a mutation of an invariant guanine at a splice-donor site, which resulted in the skipping of exon 4 and deletion of the terminal 37 amino acids of surfactant protein C. The finding of the mutation on only one allele of the infant and mother was consistent with autosomal-dominant pattern of inheritance in the family. An abnormal surfactant protein C precursor was detected, but no mature surfactant protein C was found in bronchoalveolar lavage and lung tissue from the child. The complete absence of mature protein was speculated to be attributed to aberrant folding and transport of the nascent protein. This mutation of one allele of the surfactant protein C gene presumably has a dominant negative effect with suppression of expression from the normal allele.

Amin et al [54] reported a family with an affected 11-year-old girl, her half-sister, and mother, who were given a diagnosis of chronic diffuse lung disease between the ages of birth and 26 years old. All three individuals had a marked decrease of the precursor of surfactant protein C in the alveolar epithelial cells and undetectable amounts of mature surfactant protein C in bronchoalveolar lavage fluid. Strong staining for surfactant protein SP-A, SP-B, and SP-D was found by immunohistochemistry of lung biopsy specimens. No DNA mutations in SP-C or SP-B genes were detected in the affected members of this family.

Another large family was reported to have mutations in the surfactant protein C gene [53]. Reports on the family had been published twice before as examples of familial fibrocystic pulmonary dysplasia [50,55]. Pathologic diagnoses of open lung biopsies from individuals in this family included Hamman-Rich disease, usual interstitial pneumonitis (UIP), and nonspecific interstitial pneumonitis. The age at diagnosis of family members ranged from 4 months to 57 years. DNA analysis revealed a heterozygous mutation in exon 5 of the gene in six of the affected individuals and two of the obligate heterozygous unaffected members of the family. A logarithm of the odds score of 4.3 at no recombination was highly significant and represented the odds of more than 20,000:1 that this gene was associated with the familial pulmonary fibrosis phenotype in this family. The mutation was not seen in 88 other chromosomes and thus was not likely to be a common polymorphism. Further proof that the mutation was causative was that it resulted in a substitution of a glutamine for a highly conserved leucine in a region of the protein crucial for proper intracellular folding. Mouse lung epithelial cells transfected with genetic constructs carrying the same SFTPC mutation were notable for electron microscopy evidence of cellular toxicity. Three interesting points arose from this study. First, two unaffected family members were found to be heterozygous for the mutation, which was consistent with incomplete penetrance that has been reported by others for familial pulmonary fibrosis [56,57]. Second, two pathologically different subtypes of pulmonary fibrosis (UIP and nonspecific interstitial pneumonitis) were associated with the same genetic mutation, which underscored the clinical heterogeneity associated with this one mutation. Third, the marked variability in age of onset, ranging from 4 months to 57 years, suggested strong environmental or genetic modifier effects.

All of these families demonstrated that the presence of abnormal surfactant protein C or the absence of surfactant protein C expression can lead to
inherited human lung disease. Support for this observation comes from animal studies. Transgenic mice in which a mutant surfactant protein C protein was expressed developed a lethal lung disorder [58]. Mice that were deficient in surfactant protein C that resulted from targeted deletion of its gene had altered stability in pulmonary surfactant and a severe progressive pneumonitis [46,59]. Surfactant protein C deficiency in the Belgian White and Blue cattle strain led to the respiratory distress of newborn calves [60]. Researchers have hypothesized that the mutations within the SFTPC gene cause improper folding of the highly hydrophobic precursor protein, toxic protein aggregates within the type 2 cells, cellular injury, inflammation, and an interstitial pneumonitis phenotype [61]. Accumulation of improperly folded proteins is a well-recognized cause of pulmonary diseases, including alpha-1-antitrypsin deficiency and cystic fibrosis [62].

Familial idiopathic pulmonary fibrosis

IPF (also called cryptogenic fibrosing alveolitis) is a well-defined clinical entity that has characteristic clinical, radiographic, and physiologic characteristics. The lung histology is characterized by features of UIP [1]. The exact incidence of the disease is unknown but is estimated to be 13 to 20 cases per 100,000 persons [63]. The clinical features of familial IPF are indistinguishable from those of the nonfamilial form, except that the familial form may have an earlier age of onset [64]. Hamman and Rich [65,66] provided the first descriptions of progressive pulmonary fibrosis. Since then, clusters of families with familial IPF have been reported, including families with forms that were later discovered to be attributed to surfactant protein C gene mutations [50,55]. A large group of families with familial IPF was published by Marshall et al [64] in 2000. They identified 25 families that comprised 67 cases by surveying adult pulmonary physicians in the United Kingdom. They estimated that familial cases accounted for 0.5% to 2.2% of all cases of IPF, with a prevalence of 1.3 cases per million in the United Kingdom. The mean age at diagnosis was 55.5 years, and half of the patients were smokers. In comparison, the average age of onset for IPF was 67.4 years and 69.8 years, respectively, in two other UK studies on the epidemiology of IPF [67,68]. The male-to-female ratio of the cases was 1.75:1, different from an earlier review of familial cases that reported an inverted male-to-female ratio of 1:1.23 [69]. A compatible high-resolution CT was available in 93% of the cases, and an open lung biopsy was available in 32% of the cases. The open lung biopsies were consistent with a diagnosis of cryptogenic fibrosing alveolitis. No attempts were made to classify the lung pathology into subgroups according to the scheme of Katzenstein and Myer, however [1]. An exposure to a known fibrogenic agent was recorded by 36% of subjects, but in no family did all the affected members report the same exposure.

Additional reports of collections of families from the United States with two or more individuals affected with IPF have been published. One study reported a total of 38 families with 125 affected individuals [70]. Fifty-nine subjects met the criteria for IPF by using the revised international recommendations by the American Thoracic Society/European Respiratory Society [71]. The number of affected individuals per family varied from 2 to 7. Another study reported the identification of 76 families from a single institution, with up to 19% of subjects having a positive family history for the disease [72].

Using the same American Thoracic Society/European Respiratory Society recommendations for the classification of IPF [70], the prevalence of sporadic and familial IPF in Finland has been published [73]. In the study, hospital databases from all 29 respiratory clinics were examined from 1997 to 1998 to identify all International Classification of Diseases-10 coded cases of IPF. The prevalence of IPF was 16 to 18 per 100,000 individuals. Seventeen cases of familial IPF were identified, with 2 to 5 affected members per family. The familial form of the disease accounted for 3.3% to 3.7% of all cases, with a prevalence of 5.9 per million individuals. Geographic clustering of the familial cases suggested a founder effect.

A thorough review of the reported cases of familial IPF was compiled by Marshall et al [56]. Their conclusion was that the pattern of inheritance was not certain but consistent with autosomal dominance with variable penetrance. They also suggested that familial cases that occurred in infancy probably represented a different disease. The prediction was confirmed later by the identification of mutations in the SFTPC gene.

In three families with familial IPF, evidence of alveolar inflammation was found in half of the clinically unaffected family members [57]. Among the 17 individuals “at risk” for developing the disease, 8 nonsmokers had slightly increased proportions of neutrophils, activated macrophages, and increased levels of fibroblast growth factors in bronchoalveolar lavage fluid. Four of the 8 also had positive gallium-67 scans. These bronchoalveolar lavage fluid findings appeared consistently in 7 of the 8 subjects over 2 to 4 years. Whether the bronchoalveolar lavage fluid findings are a precursor to clinically evident
pulmonary fibrosis is unknown. No follow-up on the family has been published.

The largest family described to date included 15 affected members and three probable cases in four generations [74]. The family was previously described in three different reports [75–77]. There were no pediatric cases, and the patients’ age at death ranged from 38 to 54 years. The vertical transmission highly indicated one gene with mendelian inheritance. X-linked transmission is excluded by father-to-son transmission of the disease. The well-characterized family and others described in recent studies may reveal the genetic underpinnings of this deadly disease through the use of a linkage approach. Identification of a region of the genome that is shared in common by affected family members theoretically could reveal the genetic mutation that segregates with the IPF phenotype. The genetic and molecular basis of this familial disease is still unknown.

Pulmonary alveolar microlithiasis

This rare disease is characterized by multiple laminated calcium concretions within the interstitium and alveoli. Despite a striking radiographic appearance of a “sandstorm” of nodular calcifications, clinical symptoms are highly variable and often mild [78]. The disorder has been reported worldwide but is especially common in Turkey. A recent review of more than 300 cases noted the familial aggregations of cases and postulated autosomal-recessive inheritance. The cause of this disorder is unknown.

Association studies between genetic polymorphisms and idiopathic pulmonary fibrosis

No data are available on the heritability of IPF in the general population. Although only a few subjects have a familial form of ILD or a genetic disease associated with an ILD phenotype, most cases of IPF seem to be sporadic. IPF is a disease of older individuals and is usually diagnosed late in the course of the disease. Because of these two facts, family studies are difficult. Generally, most studies on the genetics of IPF have used a case-controlled association-based approach. In these studies, the frequency of a SNP in a biologically important candidate gene is calculated in populations of unrelated affected subjects and compared with unrelated but matched controls. A candidate gene is one that is speculated to be involved in the pathogenesis of pulmonary fibrosis. This approach requires knowledge of the mechanism of the disease to identify good candidate genes. In contrast to the linkage approach, large families are not required; one can study the polymorphism in subjects with the same disease. The detection and replication of the genetic polymorphism association are often difficult, however, because of the combination of multiple genes of modest individual effect, gene-environment interactions, and population heterogeneity. These problems are compounded for a clinically heterogeneous group of diseases such as ILDs. The rigorous phenotypic classification of subjects is important. Large numbers of individuals and independent cohorts of patients are needed to confirm or repudiate the proposed genetic associations.

Derangements in pulmonary surfactant expression can be found in rare cases of familial pulmonary fibrosis. Genetic polymorphisms of the genes that encode surfactant proteins A1 and B have been associated with IPF [79]. The SP-A1, 6A allele [4] and three of the five SNPs that distinguish it from other SP-A1 alleles seem to be statistically significant risk factors for nonsmokers. In contrast, one of the SP-B SNPs seems to be a genetic risk factor when only smokers are considered. The SP-C and SP-D SNPs did not associate with IPF. The subtle differences in the protein structure and function encoded by these SNPS may contribute to increased susceptibility to fibrosis under certain conditions. Further experiments are necessary to confirm these associations in case-controlled studies and determine the mechanisms by which these proteins contribute to IPF.

IPF has been proposed to be the result of persistent alveolar inflammation and interstitial fibrosis mediated by proinflammatory effects of IL-1 and the profibrotic effects of tumor necrosis factor-alpha. These cytokines are produced by the activated alveolar macrophage. The natural antagonist of IL-1 is the IL-1 receptor antagonist (encoded by the IL-1RN gene), which binds to the IL-1 signaling receptor but does not elicit a response. There is evidence of a protective role of the IL-1 receptor antagonist in two different mouse models of acute lung injury, including bleomycin-induced fibrosis in mice [80,81]. Genetic polymorphisms within the IL-1RN gene on chromosome 2 at position +2018 and within the promoter of the tumor necrosis factor-alpha gene (TNF-A) on chromosome 6 at position −308 have been associated with susceptibility to pulmonary fibrosis in subjects with IPF [82]. The polymorphism within IL-1RN reached statistical significance in two independent English and Italian populations; the polymorphism within TNF-A was statistically significant in only the Italian population. Investigators were unable to confirm the association between the either of these SNPs and different IPF cohorts [83,84].
Transforming growth factor-beta-1 is a cytokine that plays a role in the development of tissue fibrosis. Although no association between different genetic polymorphisms within this gene and IPF have been discovered, one polymorphism was significantly associated with worse disease progression [85]. SNPs within the erythrocyte complement receptor 1 gene reached statistical significance in subjects with IPF and sarcoidosis [86]. A certain allele within the angiotensin-converting enzyme gene approached statistical significance in yet another population [87]. In these association studies, additional experiments are necessary to either confirm or refute its association in independent populations and study the biologic impact of the genetic polymorphism.

The list of potential genetic associations is by no means complete. Additional candidate genes that remain to be studied include the genes that encode regulators of surfactant production, oxidant/antioxidant balance, proteases, and antiproteases. More studies are published monthly. A word of caution is advised in the interpretation of association studies as a whole. Two large reviews of genetic association studies have estimated that only 20% to 30% of all published genetic association studies are ultimately statistically significant [88,89]. Association studies are difficult; they rely heavily on precise pulmonary phenotypes, large numbers of case-controlled pairs, and control subjects that are ethnically and age matched (given the late onset of the disease). Statistical analysis cannot be too stringent or polymorphisms of modest effect will be buried. Conversely, the analysis cannot be too lax or an abundance of false-positive associations will be published. Confirmation of the association with an independent population is an excellent way to confirm its validity. For this to occur, we need collaborative efforts by expert clinicians and geneticists to create large banks of genetic material from patients with well-characterized interstitial lung disorders.

**Future directions**

The linkage analysis approach to studying the genetics of adult ILDs is hampered by the lack of large families with multiple affected individuals. Because ILD is usually a late-onset and terminal disease, availability of genomic DNA and precise phenotypic information from historic cases is often limited. Despite these limitations, family-based studies recently have shown that mutations in surfactant protein C are associated with inherited adult ILD at least in one family. Association studies have shown promise in identifying genetic polymorphisms that contribute to the genetic predisposition of developing IPF. This approach requires large numbers of well-characterized subjects and well-matched controls, replication by independent investigators, and stringent statistical analysis, however. This is an exciting time in the study of genetic predispositions of IPF. Additional candidate genes are suggested by new animal models for lung fibrosis, by increased understanding of basic mechanisms of lung fibrosis and repair, and by gene expression chips. The genes and nucleotide polymorphisms identified by a combination of these approaches may reveal important insights in the pathogenesis of IPF and possibly other pulmonary fibrotic disorders. A genetic marker of disease also may allow early identification of individuals at risk and the development of novel therapeutic interventions.

**References**


[78] Piguet PF, Vesin C, Grau GE, Thompson RC. Interleukin-1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. Cytokine 1993;5(1):57–61.


[81] Piguet PF, Vesin C, Grau GE, Thompson RC. Interleukin-1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. Cytokine 1993;5(1):57–61.


