

# Genetic Risk Factors for Idiopathic Pulmonary Fibrosis: Insights into Immunopathogenesis

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**Abstract:** Idiopathic pulmonary fibrosis is an etiologically complex interstitial lung disease characterized by progressive scarring of the lungs with a subsequent decline in lung function. While much of the pathogenesis of IPF still remains unclear, it is now understood that genetic variation accounts for at least one-third of the risk of developing the disease. The single-most validated and most significant risk factor, genetic or otherwise, is a gain-of-function promoter variant in the *MUC5B* gene. While the functional impact of these IPF risk variants at the cellular and tissue levels are areas of active investigation, there is a growing body of evidence that these genetic variants may influence disease pathogenesis through modulation of innate immune processes.

**Keywords:** pulmonary fibrosis, interstitial lung disease, genetics, *MUC5B*, host defense, innate immunity

## Introduction

Idiopathic pulmonary fibrosis (IPF) is an etiologically complex interstitial lung disease (ILD) characterized by progressive lung scarring with a subsequent decline in function.<sup>1</sup> There have been major efforts over the past decade investigating IPF which have resulted in a more specific classification of the disease<sup>2</sup> and the approval of two disease-specific agents, pirfenidone<sup>3</sup> and nintedanib;<sup>4</sup> however, IPF diagnosis remains challenging and disease prognosis remains poor even with pharmacologic intervention.

While the pathogenesis of IPF remains an area of active investigation, it is generally accepted that disease pathobiology results from repeated injury to the airway and alveolar epithelia. These injuries result in an exaggerated and cyclical repair response with subsequent hallmark fibroblast activation and matrix deposition.<sup>5–7</sup> It is also now well understood that genetic susceptibility in addition to environmental risk factors, including cigarette smoking, has a major contribution to the risk of developing IPF.<sup>8–10</sup> Indeed, the single-most validated and strongest risk factor for IPF, genetic or otherwise, is the single nucleotide polymorphism rs35705950 in the promoter region of the mucin 5B (*MUC5B*) gene.<sup>11–21</sup>

What remains more debated in IPF pathogenesis are the roles of inflammation and immune-driven mechanisms. Initially, IPF was thought to be a disease primarily driven by chronic inflammation similar to other immunologic or autoimmune lung diseases. With accumulating evidence that immunomodulatory therapies were ineffective and potentially harmful in the treatment of IPF,<sup>22</sup> this viewpoint gradually shifted towards the model of aberrant tissue repair that is currently more accepted.

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However, there is emerging evidence that innate and adaptive immune processes may also contribute to pulmonary fibrogenesis.<sup>23–27</sup> Understanding the interface between immunologic dysfunction and known genetic risk variants may deepen the understanding of the complexity of IPF.

In this review, we provide a brief overview of the intrinsic immune mechanism in the distal airway. We describe how *MUC5B* and other genetic risk variants for IPF may modulate host defense and innate immune mechanisms towards furthering IPF pathology.

## Host Defense and Innate Immunity in the Airway

The human airway is constantly faced with threats in the way of inhaled pathogens and particles and thus has evolved a multilayered set of primary innate defenses<sup>28</sup> (Figure 1).

### Mucus Barrier

The mucus barrier is a viscoelastic gel layer designed to trap and remove offending agents and is composed of water, salts, and macromolecules held together by membrane-bound and secreted glycoproteins called mucins.<sup>29</sup> Airway mucins are secreted onto the apical surface of the epithelial layer by secretory cells, and through the coordinated beating of cilia on multi-ciliated airway cells, the mucus layer and trapped pathogens are swept proximally until ultimately being cleared

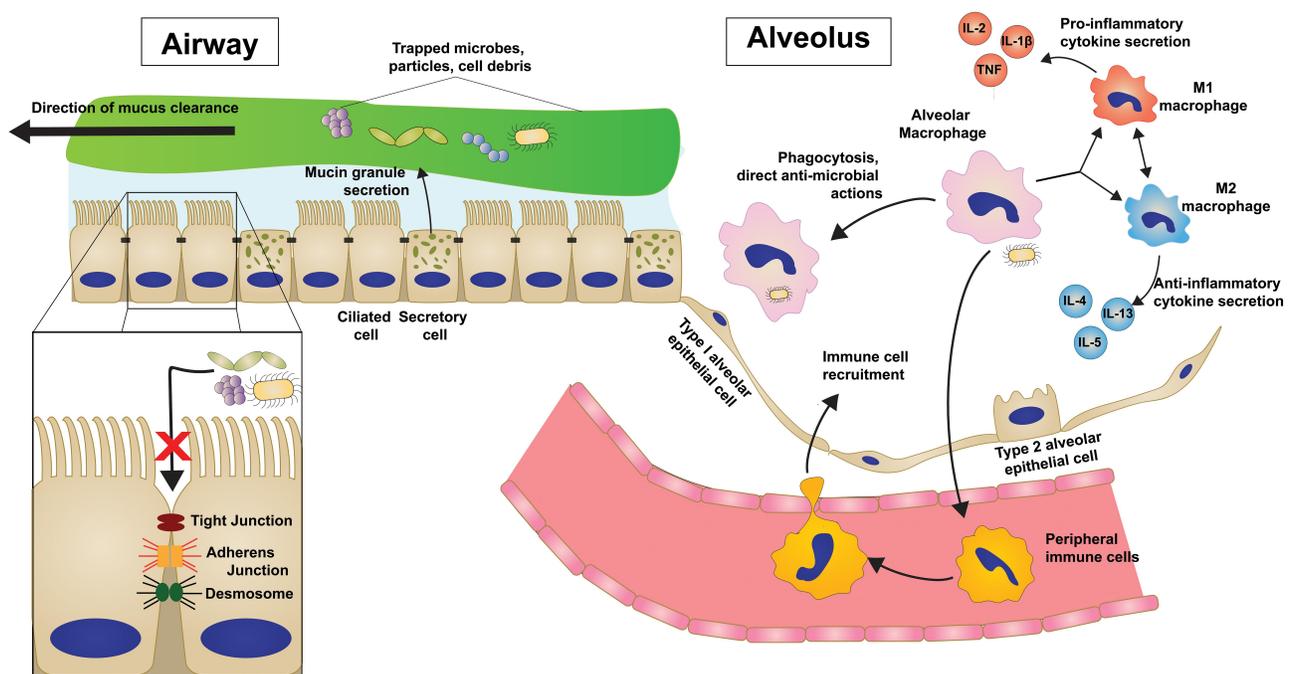
from the airway.<sup>30,31</sup> This process of mucociliary clearance (MCC) requires precise regulation of factors including mucin production, mucus composition, and coordinated ciliary movements in order to maintain effective defense.

Additionally, airway cells secrete products with antimicrobial properties,<sup>32</sup> such as lactoferrin, lysozyme, defensins, and surfactant proteins, into the lumen which remain in the mucus layer to combat potential pathogens during clearance.

### Epithelial Barrier

Beneath the mucus barrier, the airway and alveolar epithelial cells themselves participate in the innate airway defense by forming a physical barrier against harmful external substances.<sup>33</sup> The epithelial layer accomplishes this selective permeability through the expression and regulation of multiple cell-cell adhesion complexes.<sup>33–35</sup> Tight junctions are composed of claudins and occludins and are most involved in preventing paracellular passage of luminal material. Adherens junctions and desmosomes are involved in linking the actin cytoskeleton and intermediate filaments, respectively, on neighboring cells allowing the epithelium to remain intact. Loss or dysregulation of any of these complexes can result in disintegration of the protective epithelial barrier.

In addition to serving as a physical barrier, epithelial cells are closely involved in the innate response through recognition of uncleared pathogens via the expression of



**Figure 1** Innate immune and host defense mechanisms in the distal airway and alveolus are multi-tiered.

pattern recognition receptors (PRRs).<sup>28,36–39</sup> These PRRs, including Toll-like receptors (TLRs), are involved in surveying the environment for the presence of pathogens or dying cells by binding so-called pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) respectively. This vast diversity of PRRs allows the lung to recognize and differentially respond to innumerable environmental cues.

## Macrophages and Immune Cell Recruitment

Macrophages are the most predominant immune cells in the healthy lung and organize immune defense through a variety of mechanisms including direct antimicrobial and phagocytic activity.<sup>38–40</sup> Like other macrophage populations, AMs are traditionally classified as one of the two phenotypic states, classically activated (M1 macrophages) and alternatively activated (M2 macrophages), which are a result of their exposure to a milieu of cytokines, growth factors, and other signals. M1 macrophages are polarized by Th1 pro-inflammatory cytokines (TNF, IL-2, IFN- $\gamma$ ) and generally induce further inflammatory cytokine signaling and immune cell recruitment. M2 macrophages are induced by Th2 cytokines (IL-4, IL-5, IL-13) and are thought to have anti-inflammatory characteristics seen in chronic repair processes. AMs serve as scavengers in the airways that endocytose inhaled particles, cellular debris, and pathogens that cannot be cleared by the mucociliary escalator. AMs then play a major role in coordinating the subsequent immune response through the secretion of cytokines and chemokines to differentially activate or recruit immune cells. Additionally, AMs can bridge innate and adaptive responses through their role as antigen-presenting cells in the lung periphery.

## Genetic Variants Associated with IPF

Numerous familial studies and several larger genome-wide linkage and association studies have identified rare and common genetic variants associated with both familial interstitial pneumonia (FIP), which is characterized by familial clustering of IPF, and sporadic IPF risk (Table 1). These variants include the single nucleotide polymorphism (SNP) in the *MUC5B* gene<sup>11–17,19–21</sup> described in detail below as well as genes related to innate immune function (*TOLLIP*, *TLR3*, *IL1RN*, *IL8*, *TGFBI*), and epithelial barrier function (*DSP*, *DPP9*). Additional gene ontologies represented in those identified variants include telomere maintenance

(*TERT*, *TERC*, *OBFC1*, *TINF2*, *DKC1*, *RTEL1*, *PARN*), surfactant production (*SFTPC*, *SFTPA2*, *ABCA3*), and cell cycle regulation (*KIF15*, *MAD1L1*, *CDKN1A*).

## MUC5B Promoter Variant

The rs35705950 variant in the promoter region of the mucin 5B (*MUC5B*) gene was first identified in a 2011 genome-wide linkage study and is associated with an approximately 7-fold increased risk of developing IPF.<sup>11</sup> Since then this *MUC5B* variant has been validated in multiple independent studies and is still considered to be the most significant risk, genetic or otherwise, for IPF.<sup>12–17,19–21</sup> In addition to its being the strongest risk factor for disease, the rs35705950 risk allele is also strikingly common in both healthy and IPF populations (mean allele frequency of 9% and 38%, respectively). Interestingly, IPF patients who are heterozygous carriers of the minor allele have been reported to have a paradoxical survival benefit compared to noncarriers,<sup>41,42</sup> although this result has not been unanimously confirmed.<sup>43,44</sup> In other ILD populations, the rs35705950 variant has also been demonstrated to confer worse survival in interstitial pneumonia with autoimmune features<sup>45</sup> and a trend towards worse survival in connective tissue disease associated-ILD<sup>45</sup> and chronic hypersensitivity pneumonitis.<sup>46</sup> The interplay between *MUC5B* variant and transplant-free survival across ILDs and how these differences are specific to the IPF-related disease processes remains to be investigated.

The minor, disease-associated T allele at rs35705950 is a gain-of-function variant has been suggested to drive differential methylation of and transcription factor binding to the *MUC5B* leading to increased *MUC5B* expression.<sup>11,47</sup> Interestingly, this variant-dependent increase in *MUC5B* production has been demonstrated to be specific to IPF<sup>48</sup> and localized to the distal airway regions including terminal airways<sup>49,50</sup> and honeycomb cysts,<sup>51</sup> which are thought to be the end result of chronic airway. Examining the influences of a multitude of factors, including epigenetic and environmental factors, on the rs35705950 variant and *MUC5B* expression continues to be a focus of ongoing studies.

## Relationship Between Genetic Variants and IPF

### Immunopathogenesis

### Altered Mucociliary Clearance and Host Defense

As one of the two human primary mucins secreted in the airway, along with mucin 5AC (*MUC5AC*), *MUC5B*

**Table I** Common and Rare Genetic Variants Associated with IPF

Common variants associated with IPF	Gene Function	Gene	Risk Allele(s)	Reference(s)
	Airway mucin production	<i>MUC5B</i>		rs35705950
		<i>MUC2</i>	rs7934606	[13,20]
Cell-cell adhesion	<i>DSP</i>		rs2076295	[13,20,21,67]
		<i>DPP9</i>	rs12610495	[13,20,21]
Toll-like receptor signaling	<i>TOLLIP</i>		rs111521887, rs5743894 rs2743890	[13,14]
	<i>TLR3</i>		rs3775291 (L412F)	[97]
	<i>ATP11A</i>		rs1278769	[13,20,21]
Cytokine/growth factor signaling	<i>IL1RN</i>		VNTR*2 haplotype block	[89]
	<i>IL8</i>		rs4073, rs2227307	[124]
	<i>IL4</i>		rs2243250	[125,126]
	<i>TGFB1</i>		rs1800470	[127]
Telomere maintenance	<i>TERT</i>		rs2736100	[13,20,21,128]
	<i>OBFC1</i>		rs11191865	[13]
Cell cycle regulation	<i>KIF15</i>		rs78238620	[21]
	<i>MAD1L1</i>		rs12699415	[21]
	<i>CDKN1A</i>		rs2395655	[129]
	<i>TP53</i>		rs12951053, rs12602273	[129]
Rare variants associated with IPF	Gene Function	Gene	Mutation(s)	Reference(s)
Surfactant production/secretion		<i>SFTPA1</i>	T622C, W211R	[113,114]
		<i>SFTPA2</i>	G231V, F198S	[115]
		<i>SFTPC</i>	I73T, M71V, multiple others	[130,131]
		<i>ABCA3</i>	S1261G, R288K	[132]
Telomere maintenance		<i>TERT</i>	L55Q, R901W, T1110M, multiple others	[133–135]
		<i>TERC</i>	98G>A, 37A>G, multiple others	[133–135]
		<i>TINF2</i>	K280E, R282H, R282S	[136]
		<i>DKC1</i>	T405A, multiple others	[137]
		<i>RTEL1</i>	R213W, T49M, F964L	[138,139]
		<i>PARN</i>	A383V, multiple others	[140]

plays a critical role in maintaining effective mucociliary clearance (MCC) which serves in removing inhaled debris and pathogens.<sup>29,52,53</sup> Effective MCC involves the coordinated actions of mucus secretion and ciliary beating. This process is critical for the maintenance of normal lung

physiology by acting as the first-line innate defense mechanism in the airway. MCC dysfunction is seen in many chronic lung diseases including chronic obstructive pulmonary disease (COPD),<sup>54,55</sup> cystic fibrosis (CF),<sup>55–57</sup> and asthma.<sup>52</sup>

Genetic manipulation of Muc5b expression in mice has revealed pathologic consequences of both deficient and excessive Muc5b in the airways. Complete knockout of Muc5b in mice (Muc5b<sup>-/-</sup>) results in impaired MCC and chronic lower respiratory infections. Muc5b<sup>-/-</sup> mice also demonstrate dramatic changes in resident alveolar macrophage (AM) populations with increased AM apoptosis, reduced activation, and impaired phagocytosis.<sup>53</sup> Conversely, transgenic Muc5b overexpression, specifically when localized to the distal airway, results in diminished MCC and enhanced fibrosis after intra-tracheal bleomycin instillation.<sup>58</sup> Collectively these results demonstrate that host defense and MCC rely on the well-regulated expression of MUC5B and that disturbances can lead to significant pathologic sequela including increased fibroproliferative responses to injury.

As the rs35705950 SNP leads to increases in MUC5B accumulation and presumably mucociliary stasis, this could result in chronic exposure of the distal airway to inhaled particles and pathogens. Environmental and occupational factors including cigarette smoke, silica, and metal dusts have repeatedly been shown to be associated with IPF and other ILDs strongly suggesting that repeated exposure of the airway to inhaled particles is implicated in fibrotic lung diseases.<sup>59–61</sup> In genetically susceptible individuals with impaired MCC, it might be expected that exposure to inhaled pro-fibrotic particles could have an even more drastic effect in initiating epithelial cell injury and the fibroproliferative response.

In addition to the clearance of particulate matter, MCC is essential for effectively removing pathogens from distal airspaces. Recent evidence has implicated the lung microbiome in IPF pathogenesis.<sup>62–64</sup> Lung dysbiosis characterized by increased bacterial burden and loss of microbiotic diversity has been reported in bronchoalveolar lavage (BAL) specimens from both IPF patients and bleomycin-treated mice.<sup>62,64,65</sup> Interestingly, IPF patients with the *MUC5B* variant had a lower bacterial burden than noncarriers. This may in part help to explain the paradoxical survival benefit that *MUC5B* variant carriers have, as a bacterial burden has been shown to have an independent correlation with mortality in IPF patients.<sup>66</sup>

## Disrupted Epithelial Barrier Function

In conjunction with the mucous layer, the airway and alveolar epithelial cells themselves serve as a key defense mechanism in the lung by forming a protective

barrier from luminal pathogens, cellular debris, and inhaled particles.

A 2013 genome-wide association study (GWAS) identified variants in two cell-cell adhesion-related genes, *DSP* (desmoplakin) and *DPP9* (dipeptidyl peptidase 9), associated with IPF.<sup>13</sup> The *DSP* variant rs2076395 has been further shown to cause a reduction in desmoplakin expression in epithelial cells.<sup>67,68</sup> Furthermore, complete knockout of *DSP* in an airway epithelial cell line was associated with impaired epithelial barrier function and an aberrantly increased wound repair response.<sup>68</sup>

While the in vivo consequences of DSP loss are areas of active investigation, loss-of-function mutations in other desmosomal genes including *DSG1* have been shown to upregulate NF- $\kappa$ B signaling with increased production of pro-inflammatory cytokines and phagocyte recruitment.<sup>69</sup> Cytokines including IL-1 $\beta$ , IL-6, and IL-8, which are produced by both injured epithelial cells and activated alveolar macrophages, have been shown to further disrupt epithelial barrier integrity potentiating this process of cyclic damage.<sup>70,71</sup> Additionally, as the epithelial layer loses its ability to maintain a barrier either through genetic predisposition or inflammatory signals, innate immune receptors that are normally localized to the protected basolateral membrane, such as TLR2 and TLR6, are exposed to PAMPs and DAMPs.<sup>72</sup> Active TLR2 signaling in mouse airway cells has been shown to induce TGF- $\beta$  expression, which is the most well-studied pro-fibrotic mediator.<sup>73</sup>

Collectively, these associations between epithelial dis-integrity as a consequence of genetic variants and the pro-inflammatory and fibroproliferative environment created by impaired epithelial barriers could reveal important insights into IPF pathobiology.

## Autoinflammatory Toll-Like Receptor Family Signaling

As a link between innate and adaptive immune responses, TLR signaling dysregulation has been described in IPF patients<sup>74,75</sup> though the exact contribution of these signaling cascades to the fibroproliferative response remains mostly undefined.

In humans, 10 functional TLRs have been identified which have distinct receptor-ligand associations.<sup>76</sup> TLRs are either localized to the cell membrane (TLR1, 2, 4, 5, 6) or endosomal compartments (TLR3, 7, 8, 9) in order to recognize various extracellular and intracellular signals,

respectively. The majority of TLRs signaling through a MyD88-dependent pathway with the ultimate result of NF-κB activation and proinflammatory cytokine gene transcription. TLR3 signaling, as well as alternative TLR4 signaling, occurs through a MyD88-independent mechanism whereby recruitment of TRIF subsequently leads to transcription of type I interferon (IFN) genes by IRF3 or IRF7.<sup>76</sup>

The genetic risk variants affecting TLR family signaling which are associated with IPF are described below (Figure 2).

### Pro-Fibrotic TLR Signaling

A 2013 genome-wide association study (GWAS) identified three common variants (rs111521887, rs5743894, rs574389) in the Toll-interacting protein (*TOLLIP*) gene which were associated with IPF and one of which (rs5743894) is associated with decreased risk of IPF but increased mortality in those with the disease.<sup>14</sup> Expression of *TOLLIP* occurs primarily in lung macrophages and epithelial cells,<sup>77</sup> and each

of these variants is associated with a 20–50% reduction in *TOLLIP* mRNA expression.<sup>14</sup> Since *TOLLIP* and *MUC5B* are adjacent genes at the chromosome 11p15.5 region, there has been conflicting evidence as to whether variants in these are in linkage disequilibrium or provide independent associations with IPF.<sup>20,21</sup> Regardless, *TOLLIP* has been shown to suppress TLR activation, particularly TLR2 and TLR4, by inhibiting IL-1 receptor-associated kinase (IRAK) phosphorylation.<sup>77–80</sup> Both TLR2 and TLR4 activation occur in response to many different microbial signals and have been shown to be elevated in IPF epithelia, possibly due to chronic exposure to pathogenic microbes.<sup>81</sup> Reduced *TOLLIP* expression has been shown to increase pro-inflammatory cytokine (IL-6 and TNF) production by macrophages after TLR stimulation.<sup>79</sup> *TOLLIP* induces the production of IL-10,<sup>80</sup> which has protective effects against a bleomycin-induced fibrosis model in mice via TLR4-dependent signaling.<sup>82</sup> Additionally, *TOLLIP* antagonizes TGF-β signaling by degrading TGF-β1 receptors through

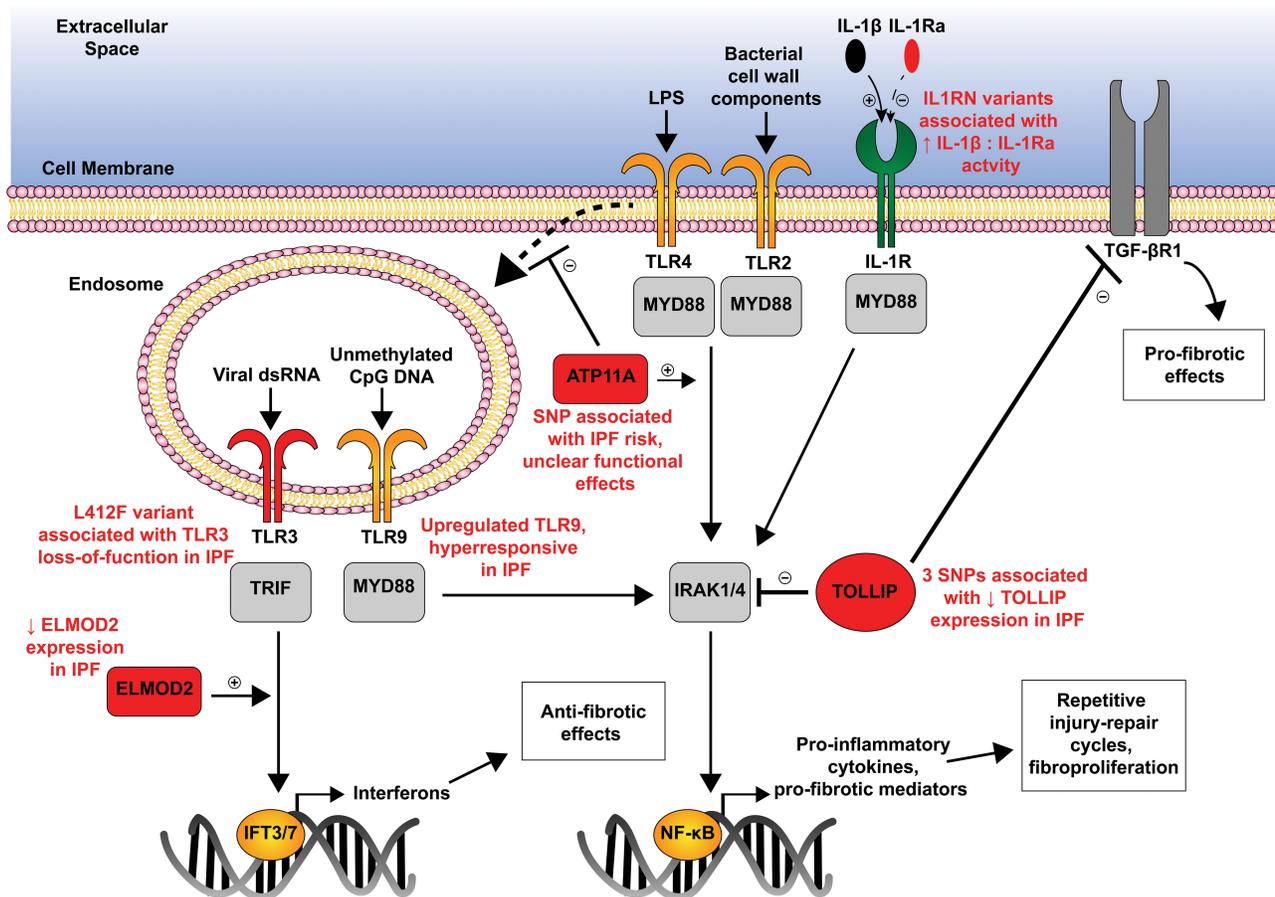


Figure 2 Genetic variants disrupt TLR/IL-1R family signaling in IPF.

SMAD7-dependent interactions.<sup>83</sup> Together, these data suggest that TOLLIP expression may be protective against IPF through dampening of pro-inflammatory, pro-fibrotic cascades.

While less functionally defined, the rs1278769 variant in *ATP11A* identified in the 2013 GWAS may impact TLR4 signaling.<sup>13</sup> *ATP11A* codes for a phospholipid flipase and has been shown to enhance MyD88-dependent NF- $\kappa$ B activation and production of proinflammatory cytokines through inhibition of TLR4 endocytosis.<sup>84</sup> It remains unclear if this IPF-associated variant enhances this signaling cascade.

While not specifically associated with any genetic variant, dysregulated TLR9 activation drives fibroblast-to-myofibroblast differentiation and has been associated with a more aggressive IPF phenotype.<sup>85</sup> TLR9 recognizes hypomethylated CpG nucleotides typically from microbial genomic material.<sup>76,86</sup> As mitochondrial DNA released from injured cells is also recognized through TLR9, it is possible that cell injury and non-apoptotic cell death that is seen in the IPF distal airway could also be a potent driver of TLR9-mediated fibrosis. Understanding the full extent of these associations between irregular TLR signaling and etiology and progression of IPF will undoubtedly be pivotal in understanding the overlap between innate immunity and fibroproliferation.

The IL-1 receptor (IL-1R) is a member of the same receptor family as TLRs with a shared intracellular Toll/IL-1R (TIR) domain that activates MyD88-dependent signaling.<sup>87,88</sup> IL-1R is activated by multiple ligands, of which IL-1 $\alpha$  and IL-1 $\beta$  are the best studied, and competitively inhibited by IL-1 receptor antagonist (IL-1Ra). While several variants in the IL-1Ra (*IL1RN*) gene have been investigated, the most convincing evidence from a meta-analysis demonstrated that a haplotype block of a variable number tandem repeat (VNTR\*2) and two minor alleles (rs408392 and rs419598) in *IL1RN* gene was significantly associated with IPF disease.<sup>89</sup> These risk alleles result in a reduction in IL-1Ra expression, leading to unrestricted IL-1R activation in airway/alveolar epithelial cells and innate immune cells. IL-1R signaling has a well-established role in animal models of pulmonary fibrosis with adenoviral-mediated IL-1 $\beta$  overexpression being used as a comparative model to the traditional bleomycin model.<sup>90</sup> Alveolar macrophages from IPF patients have an elevated IL-1 $\beta$ :IL-1Ra ratio indicative of this proinflammatory state.<sup>91</sup> Both genetic and pharmacologic targeting of the IL-1R/

MyD88 axis has been shown to attenuate fibrosis in bleomycin- and silica-induced fibrosis.<sup>92,93</sup>

Many of these overactive TLR signaling pathways, including TLR2 and TLR4, are directly responsive to both nonpathogenic and pathogenic bacterial signals. As discussed previously, dysregulation of commensal bacterial populations has been suspected to play a contributing role in IPF pathogenesis.<sup>62–64,66</sup> TLR-dependent signaling has been shown across multiple systems to tightly maintain homeostasis between host and commensal organisms, and disruption of these signaling pathways can drive disease processes.<sup>94,95</sup> In the mouse lung, in particular, it has been demonstrated that dysregulation of commensal bacterial populations can promote fibrosis through TLR2- and TLR4-dependent production of IL-17B; however, this pro-fibrotic phenotype can be diminished through genetic ablation of these pathways.<sup>96</sup> The full spectrum of these relationships between pathogen-induced TLR signaling remains unstudied in the context of pulmonary fibrosis.

Taken together these genetic variants along with the general environment in the IPF distal airway may create a pro-inflammatory environment that disrupts the normal homeostasis necessary for repair through hyper-responsiveness to small chronic insults.

### Anti-Fibrotic TLR Signaling

A loss-of-function variant in the *TLR3* gene (L412F) has been associated with more rapid lung function decline and greater mortality in IPF patients.<sup>97</sup> This variant was shown to increase human lung fibroblast proliferation and resistance to apoptosis in culture. Additionally, a TLR3-knockout mouse (*TLR3*<sup>-/-</sup>) model of pulmonary fibrosis demonstrated an increase in TGF- $\beta$  and pro-inflammatory cytokine production.

A TLR3-related gene *ELMOD2* has also been identified as a potential candidate gene in FIP and IPF pathobiology with decreased gene and protein expression seen in alveolar epithelial tissue and pulmonary macrophages in IPF patients.<sup>98,99</sup> *ELMOD2* is necessary for TLR3 signal transduction with targeted knockdown of *ELMOD2* resulting in a decreased IFN-dependent response.<sup>99</sup> IFNs have been shown to have strong anti-fibrotic characteristics in animal models,<sup>100</sup> yet these results have not been observed in patients with established IPF,<sup>101</sup> thus suggesting their role in affecting the initiation of the fibrotic cascade rather than reversing established fibrosis.

TLR3 is classically associated as a receptor for viral dsRNA, and conflicting evidence exists as to the role of

viral infections, including Epstein-Barr virus (EBV),<sup>102,103</sup> herpes simplex virus 1 (HSV-1),<sup>104</sup> and cytomegalovirus (CMV)<sup>105</sup> as a contributing source driving the progressive fibrosis. A multitude of viruses have been shown to increase fibroproliferative responses both in vitro and in vivo models, particularly in the setting of absent IFN-dependent responses.<sup>106–110</sup> Therefore, viral-related TLR signaling likely acts as a blockade to fibroproliferation, and underlying TLR3-signaling deficiencies could prime the airway for deficient responses to viral pathogens priming the distal lung for chronic injury-repair cycles that are thought to be central to IPF pathology.

## Surfactant Protein Changes

Pulmonary surfactant is a phospholipid-rich substrate produced in the distal airway and alveolus which provides essential roles in preventing alveolar collapse and host defense.<sup>111,112</sup> Approximately 10% of surfactant is composed of surfactant proteins which are produced and secreted by alveolar epithelial type II (AE2) cells and terminal airway secretory cells. Of the four surfactant proteins, surfactant proteins A (SP-A) and D (SP-D) are members of a specific family of innate immune protein termed collectins, named for calcium-binding C-terminal lectin domain that recognizes motifs on microbial surfaces.<sup>111,112</sup> SP-A and SP-D have both been shown to opsonize common bacterial and viral pathogens and enhance phagocytic-killing by innate immune cells including macrophages and neutrophils.

Rare variants in the two adjacent SP-A coding genes, *SFTPA1* and *SFTPA2*, have been described in several cases of FIP.<sup>113–115</sup> While the role that these and other surfactant-related variants play in contribution to sporadic IPF remains less clear, IPF patients have been reported to have reduced BAL concentrations of SP-A compared to healthy patients, and SP-A levels are inversely correlated with survival.<sup>116,117</sup> Mice deficient in SP-A (SP-A<sup>-/-</sup>) show dramatically decreased bacterial clearance, impaired macrophage phagocytosis, and increased production of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF which are also associated with fibroblast activation.<sup>118</sup> SP-A<sup>-/-</sup> mice have also increased mortality and collagen deposition after induction of fibrosis with intratracheal bleomycin, suggesting a direct link between the pro-inflammatory state created by SP-A deficiency and subsequent fibroproliferative remodeling.<sup>119</sup> Additionally, it has been shown that an FIP-associated loss-of-function mutation in *SFTPA1* leads to increased necroptosis in AE2 cells in

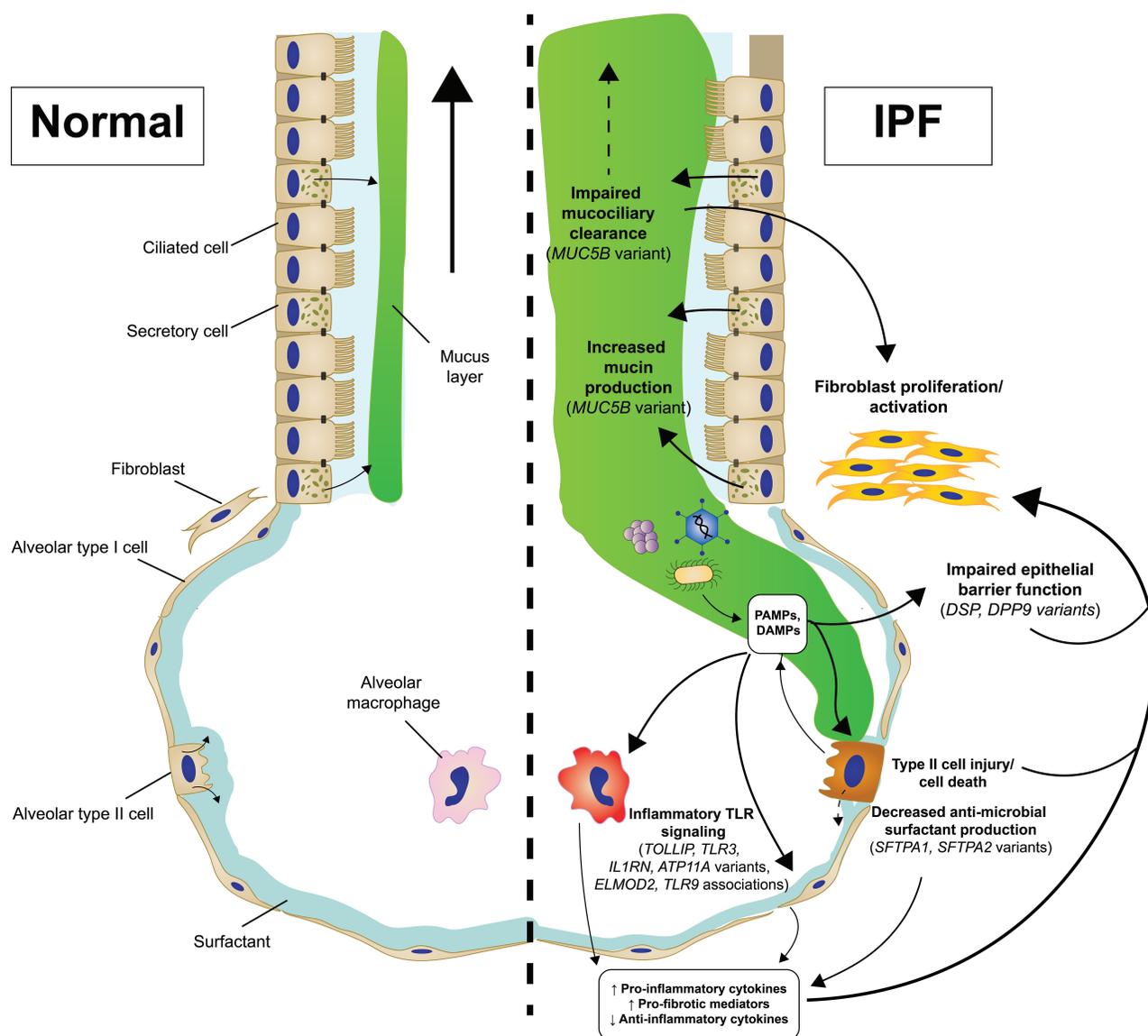
mice.<sup>113</sup> Necroptotic cell death leads to the release of highly immunogenic intracellular proteins and has been increasingly been implicated in IPF pathogenesis.<sup>120,121</sup> Treatment of mice with exogenous SP-A has been shown to reduce the expression of Th2 cytokines including IL-4 and IL-5, which are involved in chronic tissue repair responses and are linked to the development of fibrosis.<sup>122,123</sup> Taken together these findings suggest that the defective host defense and highly inflammatory state created by a lack of SP-A, either due to *SPFTA2* variants or generally in IPF, may directly contribute to pro-fibrotic environment in the alveolar space.

Other rare variants in surfactant-related genes, including genes encoding surfactant protein C (SFTPC) and surfactant transporters (ABCA3), are linked to FIP and IPF and are extensively reviewed elsewhere.<sup>9,10</sup>

MUC5B has been shown to be present at increased levels in the distal airway in IPF patients and at even more significant levels with the presence of the rs35705950 variant. Additionally, it has been reported that MUC5B is co-expressed with other surfactant proteins in AE2 cells to some degree in IPF lungs.<sup>58</sup> These data suggest that mucin-surfactant mixing is nearly inevitable in the IPF alveolus, and consequently may interfere with the normal antimicrobial effects of SP-A and SP-D. Furthermore, as MUC5B expression has been shown to occur in both surfactant-producing cell populations in the airway, AE2 cells and club cells, it remains unexplored whether the *MUC5B* variant negatively impacts the ability of these cells to produce surfactant proteins leaving the airway more susceptible to pathogen-related damage.

## Summary

Our working model of IPF pathogenesis involves chronic epithelial injury in the terminal airway which ultimately leads to an uncontrolled response that overwhelms the repair mechanisms of the distal lung. The interplay between inflammation, immune mechanisms, and this aberrant cascade of epithelial–mesenchymal crosstalk remains elusive, yet genetic variants clearly suggest a role of impaired innate defense in this process. While innate immune responses are a critical component to the lung's defense against offending agents and some level of the inflammatory response is necessary for many repair mechanisms, these processes need to be tightly regulated in order to restore the lung to homeostatic conditions. We suggest that these genetic variants associated with IPF which cause mucociliary dysfunction, impaired removal



**Figure 3** Genetic-driven innate immunity changes contribute to IPF pathogenesis.

of pathogenic substances, and persistent inflammatory signaling create an environment in which proper repair becomes impossible (Figure 3).

Genetic variants, such as the rs35705950 *MUC5B* variant, almost certainly interplay with other features of IPF pathobiology aside from impacts on innate host defense including distal airway cell homeostasis and repair, yet these exact relationships continued to be explored and are outside of the scope of this review. Additionally, studying the interactions between these genetic variants and both other genetic and environmental factors remains a key step in understanding IPF. Importantly, little is understood about the impact these genetic risk variants have on each other and how the

presence of multiple genetic variants influences disease pathogenesis.

Together, our current understanding of IPF as a disease driven by environment injuries to a genetically susceptible lung raises the possible role of clinical genetic testing. While there are no current recommendations for the role of genome sequencing for sporadic IPF patients, further research into the relationship that genetic variants play on clinical outcomes is certainly necessary.

## Disclosure

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VAMC Merit Review (IO1BX005295), NIH-NHLBI (P01-HL0928701), NIH-NHLBI (UH2/3-HL123442), and NIH-NHLBI (X01-HL134585), during the conduct of the study; personal fees from Eleven P15, Inc. (a company with the mission of early detection and early intervention of IPF), outside the submitted work; In addition, Dr David A Schwartz has patents 14/813.559, 62/624500 pending, and a patent 8,673,565 issued. The authors report no other conflicts of interest in this work.

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