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Genetic variations in idiopathic pulmonary fibrosis and patient response to pirfenidone

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ABSTRACT

Background: Genetic variations in Idiopathic Pulmonary Fibrosis (IPF) affect survival and outcomes. Current antifibrotic agents are managed based on the patient's reported side effects, although certain single nucleotide polymorphisms (SNPs) might alter treatment response and survival depending on the antifibrotic administered. This study investigated variations in response and outcomes to pirfenidone based on patients-specific genetic profiles.
Methods: Retrospective clinical data were collected from 56 IPF patients and had blood drawn for DNA extraction between 7/2013 and 3/2016, with the last patient followed until 10/2018. Nine SNPs were selected for pharmacogenetic investigation based on prior associations with IPF treatment outcomes or implications for pirfenidone metabolism. Genetic variants were examined in relation to clinical data and treatment outcomes.
Results: Of the 56 patients, 38 were males (67.85%). The average age of IPF at diagnosis was 66.88 years. At the initiation of pirfenidone, the average percent predicted FVC was 70.7%, and the average DLCO percent predicted was 50.02% (IQR 40–61%). Among the genetic variants tested, the TOLLIP rs5743890 risk allele was significantly associated with improved survival, with increasing pirfenidone duration. This finding was observed with CC or CT genotype carriers but not for those with the TT genotype ($p = 0.0457$). Similarly, the TGF-B1 rs1800470 risk allele was also significantly associated with improved survival with longer pirfenidone therapy ($p = 0.0395$), even though it was associated with disease progression.
Conclusion: This pilot study suggests that in IPF patients, the TOLLIP rs5743890 genotypes CC and CT, as well as TGF-B1 rs 1800470 may be associated with increased survival when treated with pirfenidone.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a rare, progressive, and disabling lung disease of unknown etiology characterized by fibrosis. The prevalence of IPF varies between 14 and 43 per 100,000 people in the U.S. and approximately 5 million people worldwide [1,2].

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IPF is generally fatal, with a median survival of 2–5 years. The cause of IPF is thought to be a multifactorial interplay of genetics, infections, and environmental exposures, with a strong association with aspiration [2,3]; however, the specific etiology remains elusive. At least one-third of the risk for developing IPF is thought to be due to genetic variation [4]. Several single nucleotide polymorphisms (SNPs) were found to be associated with IPF susceptibility and survival [5]. These SNPs occur in genes associated with telomere length, surfactant production, fibroblast activation, and mucin production, including TOLLIP, TLR, CCL18, TERT, MUC5B, and surfactant A, C, and D [4,6–10]. More recently SNPs in other genes were identified to be associated with IPF, such as KNL1, NPRL3, STMN3, and RTEL1 [11]. Evidence suggests that survival in IPF patients may be affected by the genetic variation in some of the aforementioned genes. For example, in the MUC5B gene, certain alleles are associated with a 50% increase in survival compared to others, suggesting genetically distinct IPF disease patterns [4]. SNPs within TOLLIP also carry prognostic implications in IPF [4].

There are currently two approved treatments for IPF, pirfenidone, and nintedanib. Both of these agents slow the progression of the disease, but neither is curative nor do they reverse disease progression [12,13]. Although these agents are the standard of care today, they have many limitations, including significant costs and side effects, as well as fixed dosing regimens for patients regardless of their characteristics despite considerable inter-patient variation in pharmacokinetics. The dosage for both medications is currently adjusted based on side effects and liver function impairment in clinical practice. Although the metabolic pathways of these medications are known, no studies to date have examined if variations in metabolic genes alter the effectiveness of these medications in IPF.

The present study explored how genetic variations related to IPF disease risk and drug metabolism may be associated with patient response to pirfenidone treatment. We hypothesized that genetic variants associated with disease risk and those associated with increased metabolism of pirfenidone would be associated with lower symptom response or worse outcomes throughout treatment. As an exploratory pilot study to establish proof of principle and rationale to conduct future comprehensive pharmacogenomic studies, we conducted an examination of IPF patients taking pirfenidone. Combining an understanding of the genetic underpinnings of IPF with pharmacogenomics may allow for the transformation, personalization, and advancement in treatment. Such identification has already been applied in similar scenarios, such as for clopidogrel in coronary artery disease, where identification of CYP2C19 genetic variants predicts reduced clopidogrel activity, guiding clinicians to prescribe alternative medications in these patients [14]. Other examples where guidelines have been implemented for gene-drug pairs include CYP2D6 and codeine, TPMT and azathioprine, SLCO1b1 and simvastatin, and CFTR and ivacaftor [15]. These genetic variations affect drug metabolism at different stages. In the azathioprine (active drug) example, a reduced TPMT function places the patient at risk of hematological toxicity. While in the codeine (prodrug) example, a reduced CYP2D6 predicts lower morphine (active drug) concentration [15].

2. Methods

2.1. Participants

Utilizing our Interstitial Lung Disease database previously collected blood samples from IPF patients at the University of Minnesota (IRB # 1104M98418), 56 IPF patients who had taken pirfenidone were identified. IPF was diagnosed according to published criteria [1,16]. These criteria were confirmed in each patient in our multidisciplinary meetings and documented in the medical record. Clinical data collected included IPF diagnosis date, medication start and stop dates, transplant status, death date (if applicable), demographics, and smoking status. Additionally, supplemental oxygen use and pulmonary function tests were collected at 6, 12, and 24 months from

Table 1
SNPs analyzed.

Gene	dbSNP ID	SNP Genotyping Taqman Assay ID	Nucleotide Coordinate (GRCh37)	SNP Description	Non-Risk Genotype	Risk Allele	Genetic consequence	Clinical consequence of risk allele in IPF	Ref.
CYP1A2	rs35694136	C_60142977_10	Chr15:75039613	c.-1635 delT	TT	Any Del	Upstream variant	Increases CYP1A2 activity	[30]
	rs762551	C_8881221	Chr15:75041917	c.-9-154C > A	CC	Any A	Intron variant		
	rs2069514	C_15859191	Chr15:75038220	g.75038220 G > A	GG	Any A	Upstream variant		
	rs2069526	C_16017734	Chr15:75041341	c.-10 + 103 T > G	TT	Any G	Intron variant		
	rs12720461	c_30634146	Chr15:75041351	c.-10 + 113C > T	CC	Any T	Intron variant		
TERT	rs2736100	C_1844009	Chr5:1286516	c.1574-3777 G > T	CC	Any A	Intron variant	Increased IPF risk	[31]
TOLLIP	rs5743890	C_31456252	Chr11:1325829	c.33 + 4867 A > G	TT	Any C	Intron Variant	Decreased risk of IPF, increased mortality	[23]
MUC5B	rs35705950	C_1582254	Chr11:1241221	g.1241221 G > T	GG	Any T	Upstream Variant	Increased risk of IPF, but decreased mortality	[23]
TGFB1	rs1800470	C_22272997	Chr19:41858921	c.29C > T; Pro-10-Leu	GG	Any A	Missense variant	Lower PaO2 and worse prognosis	[32]

the medication start date, if available.

2.2. Laboratory studies

DNA was extracted from whole blood samples using the Quick DNA Miniprep kit (Zymo Research, Irvine, CA) following the manufacturer's protocol, followed by quantitative assessment using the NanoDrop-8000 spectrophotometer (ThermoFisher Scientific, Waltham, MA) and the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific). DNA samples were standardized to 10 ng/μl concentration prior to variant detection using TaqMan SNP Genotyping assays (ThermoFisher Scientific). Quantitative real-time polymerase chain reaction (PCR) amplification was performed on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using TaqPath ProAmp Master Mix (ThermoFisher Scientific) following the manufacturer's protocol. Each assay plate contained two negative control samples, two CEPH DNA control samples, and 12 samples randomly selected for duplication to assess congruency. Allele calling was performed using allelic discrimination on the Applied Biosystems 7500 Real-Time PCR System software. We employed a candidate gene approach to focus this first pharmacogenetic investigation of pirfenidone on nine SNPs with previous evidence supporting associations with either IPF disease risk or pirfenidone metabolism (Table 1). Rs1800470, which is found within the hydrophobic core of the TGF-beta gene, has been linked to susceptibility to IPF [17–19]. Rs35705950 in the MUC5B promoter region is present in approximately 30% of IPF patients and is associated with IPF susceptibility and improved survival [9,20,21]. Rs2736100 within the TERT gene has been shown to be associated with IPF in prior patient cohorts [22]. Several TOLLIP SNPs, including rs5743890, rs5743894, and rs3750920 have also been associated with IPF susceptibility and increased mortality [23–25]. Seventy to eighty percent of pirfenidone metabolism occurs via the cytochrome p450 CYP1A2 gene [26]. Several CYP1A2 SNPs have been previously found to have significant effects on the metabolism of other medications or be associated with cancer risk, including rs35694136, rs762551, and rs206951 [27–29]. Assay IDs corresponding to nine targeted variants across five genes are provided in Table 1.

2.3. Data analysis

Within our sample, each SNP was correlated with clinical data and outcomes using Wald testing, permutation testing, and LRT testing. Associations were explored between SNPs and outcome measures using both additive and dominant genetic models for the disease risk SNPs. Genotypes for CYP1A2 were each examined using additive models, with some of the genotype-associated impacts on enzyme metabolism included in Table 1. None of the SNPs examined significantly deviated from Hardy-Weinberg equilibrium (HWE). SNP minor allele frequencies were compared to expected minor allele frequencies from the 1000 Genomes populations via the Hardy-Weinberg principle.

3. Results

3.1. Demographics

Our study included 56 subjects, 38 of whom were male (67.85%) (Table 2). Most of the study population were Caucasians (52 subjects, 94.54%). Smoking status was known in 55 subjects, with 37 being former smokers (67.28%) and 18 (32.72%) having never smoked. Among smokers, the average pack-years smoked was 23.57 (IQR 10–30). The average age of IPF at diagnosis was 66.88 years (IQR 61.99–72.33). At the initiation of pirfenidone, the average percent predicted FVC was 70.7%, and the average DLCO percent predicted was 50.02% (IQR 40–61%). FVC changes prior to our study were lacking in many subjects, especially prior to the diagnosis.

Table 2
Demographic characteristics.

Gender, n = 56			
Male	38		
Female	18		
Smoking status, n = 56			
Former	37		
Never	18		
Unknown	1		
	Average	**Min**	**Max**
Age at diagnosis, n = 55 (years)	66.9	49.3	79.7
Pack year history of former smokers, n = 37	23.6	1	109
Walk distance, n = 32 (m)	395.6	127	670
Oxygen use, n = 14, LPM	3.9	2	10
PFT data (n = 52)			
FVC (L)	2.63	1.35	5.09
FVC Percent Predicted	70.73	40	116
DLCO (mL/min/mmHg)	12.5	3.6	23.6
DLCO Percent Predicted	50.	14	85
FVC/FEV1 ratio	82.98	59	94

3.2. Clinical outcomes

Several SNPs we tested had significant associations with survival. The TERT CA genotype was associated with worse survival in patients treated with pirfenidone ($p = 0.042$) (Table 3). On the other hand, the MUC5B promoter SNP and the CYP1A2 SNPs that were tested did not show a statistically significant association with survival (data not shown).

The TOLLIP rs5743890 risk allele was significantly associated with improved survival, with increasing pirfenidone duration, for CC or CT genotype carriers but not for those with the TT genotype ($p = 0.0457$). Similarly, the TGF-B1 rs1800470 risk allele was also significantly associated with improved survival, with longer pirfenidone therapy ($p = 0.0395$) (Fig. 1A and B). Interestingly, the GA genotype of the TGF-B1 rs1800470 was also significantly associated with disease worsening.

In order to explore observed frequencies of disease risk genes examined in our study sample with the broader population, the minor allele frequencies (MAF) of the 11 SNPs tested in our cohort were compared to the 1000 Genomes Population (Table 4). There was a significant difference in the frequency of the TERT allele rs2736100, which is consistent with the literature, while the C allele was less common in our cohort (0.393 *vs.* 0.485, $p = 0.009$). There was a trend observed in the MUC5B rs35705950 SNP, with the T allele being more common in our cohort (0.429 *vs.* 0.047, $p = 0.073$).

4. Discussion

We conducted, to our knowledge, the first pharmacogenetic study of pirfenidone in IPF. Despite the rarity of these conditions, improving treatment precision for these patients is desperately needed, with the potential to impact overall survival, drug selection, dosing, and the cost-effectiveness of treatment. As a first step in translating findings from prior disease risk studies in IPF to determine if they impact treatment outcomes, we explored a potential pharmacogenetic relationship with pirfenidone, a common first-line medication used to treat IPF. We found two genetic variants, TOLLIP rs5743890 and TGF-B rs1800470, to be associated with significantly improved survival when the duration of pirfenidone was longer. As the natural history of IPF can vary significantly from one individual to the other, it is possible that such variation has, coincedentally, presented itself as the association at hand. However, it is not possible to accurately predict the natural history of IPF early in its course [33]. Some predictive models for IPF are available, such as the one developed in the 1980s termed the Clinical, Radiographic and Pathologic (CRP) predictive model. Unfortunately, it lacks external validation and involves parameters rarely captured in clinical practice. Most of these parameters, such as clubbing and pulmonary artery enlargement, do not become pronounced until the disease is advanced, limiting its utility to predict IPF course in its early stages [33].

TOLLIP regulates the innate immune response by inhibiting Toll-like receptor (TLRs) signaling. TLRs have been implicated in other fibrotic diseases, such as liver cirrhosis [34] In patients with IPF, bronchoalveolar lavage cells express higher mRNA levels of multiple TLRs [35]. Higher levels of TLR9 mRNA expression levels in IPF lung fibroblasts predict a rapid disease course [36].

Previous work suggests that genetic mutations can influence response to medications in IPF. In the PANTHER trial of NAC for IPF, patients carrying the *TOLLIP* rs3750920 TT genotype benefitted from NAC [37]. Among 154 patients, a significant reduction in composite endpoint risk was found (hazard ratio, 0.14; 95% CI, 0.02–0.83; P value = 0.03) in those with a TT genotype. This was the first analysis that linked genes to treatment response.

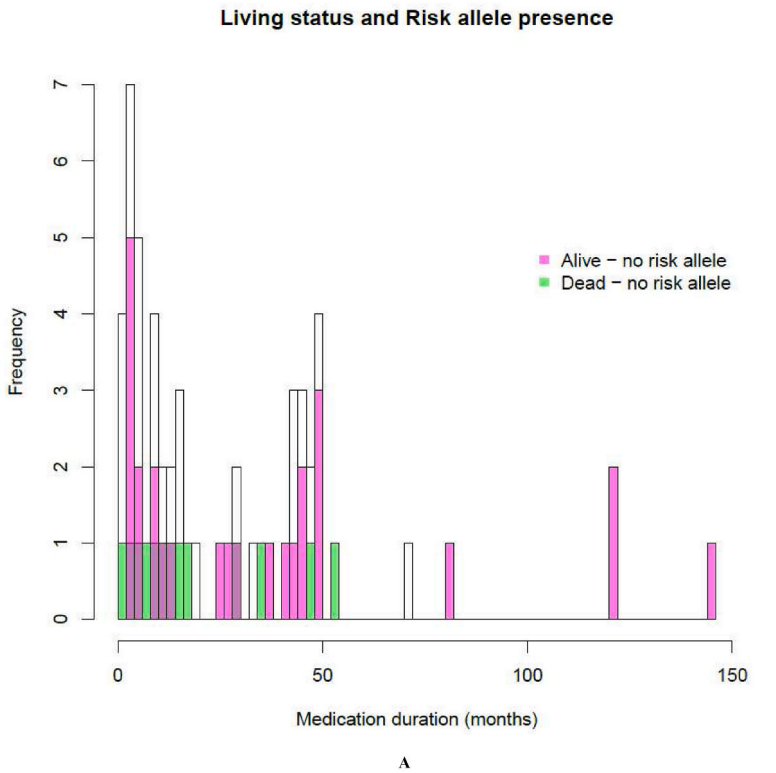
TGF-β is a cytokine that plays a major role in the development of IPF via the TGF-β/Smad pathway. This pathway was targeted and investigated in a mouse study, with several agents identified for inhibition. TGF-β induced collagen deposition and inhibited fibroblast migration. Collagen deposition in mice lung tissue was lessened more effectively when compared to nintedanib, as well as delaying lung destruction from bleomycin [38].

The absence of these mutations could also suggest that no survival benefit would be achieved with treatment, although slowing FVC deterioration might be of benefit to the patient [39]. If these observations are further confirmed by larger studies, providers may choose pirfenidone over other antifibrotic agents earlier in the disease course in patients with these mutations. In our study, the mutation frequency of the MUC5B promoter and TERT genes were within the same range of frequencies in some published IPF cohorts [21], although this was noted to widely vary in other cohorts. In addition, carriers of the TERT SNP CA genotype experienced worse survival, regardless of medication duration.

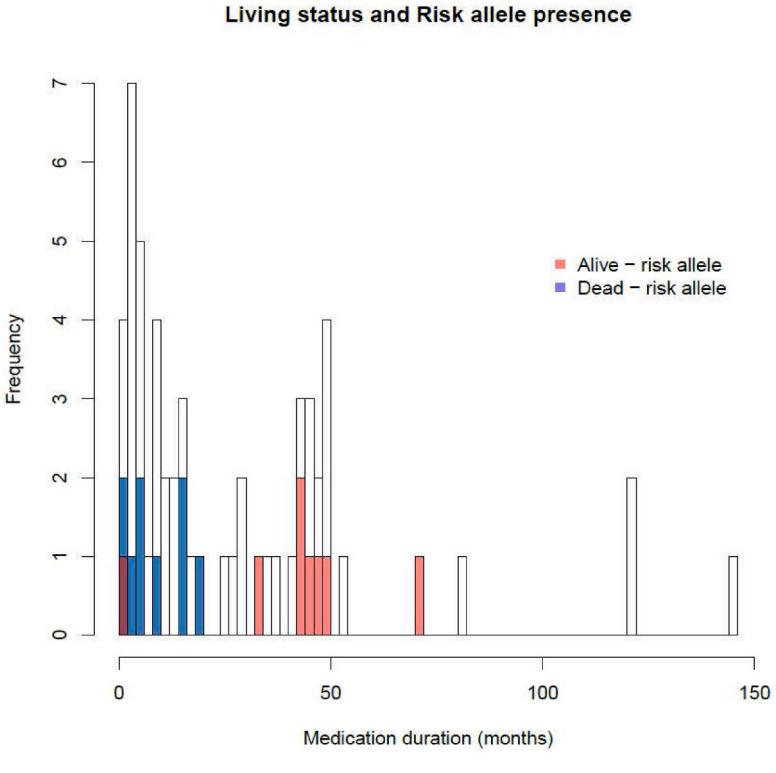
The findings reported herein need to be interpreted in the context of the limitations of our study. The sample size is small, which limits our ability to detect smaller effect sized associations and rigorously correct for multiple comparisons and quantify the impact of potential confounders and assess their interactions with genetic factors. We examined genetic variants based on our *a priori* hypotheses,

Table 3
Association of SNPs with survival.

SNP and Genotype	Association	Wald p-value	Permutation p-value	LRT p-value
TOLLIP rs5743890 (CC or CT)	Positive association between risk allele presence, medication duration, and survival	0.04566	0.0342	0.0995662
TOLLIP rs5743890 (TT)	Negative association of medication duration and survival	0.02344		
TGF-B1 rs1800470 (GA or AA)	Positive association between risk allele presence, mediation duration, and survival	0.0395	0.0098	0.03472
TGF-B1 rs1800470 (GA)	Positive association between GA genotype and disease progression	0.02558	0.007	0.03881
TERT rs2736100 (CA)	Negative association of CA genotype with survival	0.042	0.022	0.3319



A



B

Fig. 1. A& 1B: Frequency of subjects *vs.* medication duration in months. White bars indicate total frequency at each duration. Pink and red bars indicate living, and green and blue bars indicate deceased subjects. Shown for subjects without risk allele (Fig. 1A) and with risk allele present (Fig. 1B). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
SNP minor allele frequency.

Gene	dbSNP ID	MAF 1000 Genomes Population	MAF IPF Study Cohort	Hardy-Weinberg χ^2 Value	Hardy-Weinberg P Value
CYP1A2	rs35694136 T→del	0.38 [Del]	0.125 [Del]	0.023	0.879
CYP1A2	rs762551 C→A	0.37 [C]	0.32 [C]	0.017	0.896
CYP1A2	rs2069514 G→A	0.209 [A]	0.0446 [A]	0.122	0.727
CYP1A2	rs2069526 T→G	0.071 [G]	0.06 [G]	0.249	0.618
CYP1A2	rs12720461 C→T	0.001 [T]	0.009 [T]	0.005	0.946
TERT	rs2736100 C→A	0.485 [C]	0.393 [C]	6.766	0.009
TOLLIP	rs5743890 T→C	0.048 [C]	0.161 [C]	0.196	0.658
Intergenic/MUC5B	rs35705950 G→T	0.047 [T]	0.429 [T]	3.214	0.073
TGFB1	rs1800470 G→A	0.455 [G]	0.339 [G]	0.109	0.741

but were not powered to conduct a genome wide association study which precluded discovery of other genetic regions that may be associated with treatment outcomes. Our study sample was predominantly White, which limits the generalizability of the findings to other populations. A double-blind, randomized controlled trial comparing pirfenidone to other treatments such as nintedanib that incorporates pharmacogenetic information may help to determine whether there are genetic factors associated with the differential efficacy of these treatments.

Despite the limitations and exploratory nature of this study, reporting these results is important because we need to improve the precision with which we select and dose medications for patients with IPF. Medication dosing has been based on weight, body surface area, and renal and hepatic function for decades. Recent advances in pharmacogenetics have led to the translation of research findings to clinical applications for several medications across therapeutic areas, including, but not limited to, cardiology, infectious diseases, neurology, and pain management [40–42].

5. Conclusion

Although in a relatively small cohort, the preliminary findings reported in this study provide support for the continued investigation to confirm these associations and determine how genetic testing for these variants at the time of IPF diagnosis may be used to improve treatment precision and improve patient survival.

Resource availability

Lead contact:
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Materials Availability:
Nondisposable materials used as described in Methods section 2.2 are available in our lab upon request.

Data code and availability

This study did not generate/analyze data codes.

Ethical approval

IRB approval was acquired from our IRB committee at the University of Minnesota, reference # 1104M98418.

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Author contribution statement

Aahd Kubbara, William H. Amundson, Adam Herman: Analyzed and interpreted the data; Wrote the paper.
Adam M. Lee: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jeffrey R. Bishop: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hyun Kim: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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