Author's response to reviews

Title: Incorporating genetics into the identification and treatment of Idiopathic Pulmonary Fibrosis

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Author's response to reviews: see over
June 20, 2015

To: Editors of *BMC Medicine*

Re: Response to review of manuscript 1811422580170482: “Incorporating genetics into the identification and treatment of Idiopathic Pulmonary Fibrosis”

Dear Editors:

Thank you for the reviewers’ comments and suggestions on our manuscript. We have edited our manuscript in response to the concerns, questions, and suggestions from the reviewers. We have also provided a point by point response to each of the suggestions as outlined below.

On behalf of all of the authors, thank you for your consideration.

Sincerely,

Susan K. Mathai, MD
University of Colorado Denver

Comments from Reviewer 1:

1. Although the authors comment that “the genotyping of patients is not a generally accepted strategy”, for evaluating IPF patients, could it useful in some circumstances? For example, is genotyping for TERT/TERC mutations useful in patients with a syndrome suggestive of a telomeropathy (early greying of hair, lung fibrosis, macrocytosis, etc) being considered for lung transplantation? The purpose would be that if the mutations were present, the patient may be at risk for post-transplant cytopenias. Knowing their genotype could assist with patient management.

   We agree that preliminary findings by other groups might suggest that genotyping for mutations (such as TERT/TERC) could be useful for patients being considered for transplantation. However, large-scale studies examining this particular subset of patients and their outcomes for transplantation have not yet been done, and as such it may be premature to recommend that screening as part of routine clinical care. To this end, we have added a sentence at the end of page 10 alluding to this: “However, discussion of these higher complication rates with IPF patients who have confirmed telomerase mutations and corresponding telomere syndromes (characterized by lung fibrosis, premature graying of the hair, cytopenias, and liver disease) is warranted to allow patients to make informed decisions regarding transplantation.”

2. Risk for family members section: The authors comment regarding patients in the 1986 Bitterman study “....whether these individuals progressed to development of pulmonary fibrosis themselves was not studied” is incorrect. El-Chemaly et al reported 27-year follow-up for two of the patients in a 2011 publication in Chest. Both of the individuals
developed pulmonary fibrosis. Although the sample size is limited, this report should be cited in the MS.

We appreciate this correction and have made changes accordingly. Please see the following addition on page 13: “Twenty-seven years later, El-Chemaly and colleagues evaluated two of the patients in that initial FIP study. They showed that though their initial evaluations showed alveolar inflammation without radiographic or physiologic evidence of pulmonary fibrosis, nearly three decades later, HRCT imaging revealed interim development of pulmonary fibrosis, both subjects reported symptomatic respiratory impairment, and physiologic testing showed evidence of restriction and impaired gas exchange [49]. Though this study was limited by its small sample size, it illustrates that alveolar inflammation in first-degree relatives of FIP patients can progress to overt pulmonary fibrosis and that these patients can experience a long duration of preclinical disease.”

3. Page 7 of document: “The association of the MUC5B promoter polymorphism appears to be specific to pulmonary fibrosis”. Should probably read “..... specific to idiopathic pulmonary fibrosis”.

This change has been made, “The association of the MUC5B promoter polymorphism appears to be specific to IPF.”

4. Figure 1. Listing TERT and TERC as both very rare and common variants is confusing and contradictory. Are they rare or common?

TERT and TERC are listed in both common and rare variant groups because there have been common variants in or near these genes (please see Fingerlin et al. Nature Genetics 2013) as well as rare mutations in these genes described by various authors associated with pulmonary fibrosis. A sentence explaining this has been added to the figure legend: “TERT and TERC are listed in both common and rare variant groups because there have been common variants in or near these genes, as well as rare mutations in these genes, associated with pulmonary fibrosis.”

Comments from Reviewer 2:

The manuscript titled “Incorporating genetics into the identification and treatment of Idiopathic Pulmonary Fibrosis” by Mathai et al, has two main sections, the first is a summary of prior work in the field, and the latter a discussion of the implications of these findings for patient care. The manuscript is written by experts in the field and the perspective provided in the latter part of the manuscript will be of interest to readers.

With this said the former part of the paper is poorly written and will require substantial editing. For example, in page 6 multiple sequential grammatical errors become very distracting: ...Patients with these variants had profound shortening of telomeres in peripheral blood mononuclear cells (PBMCs), though the mechanism by which loss of PARN affects telomere length is unknown. These newly described rare variants further
point to telomere length as important in the pathogenesis of IPF; ... The previous studies focused on ...; The authors determined utilizing a genome-wide linkage analysis follow by sequencing ...

We have reviewed and edited the early part of the manuscript to improve the style and grammar.

I have provided additional comments, unfortunately the grammatical errors are so frequent that they become distracting, please carefully edit the first section of the manuscript.

Comments:

1. Consider using throughout the manuscript standard nomenclature for IPF- not sporadic IPF, familial interstitial pneumonia- not familial IPF; there are multiple iterations in the manuscript

We have reviewed the text of the manuscript and clarified definitions of familial interstitial pneumonia (FIP) versus familial pulmonary fibrosis—these definitions are specific to the study in which they are used, and so we have clarified how different studies defined each term and which term was used for each key study. We have removed “sporadic” to refer to IPF whenever it is possible to do so without changing meaning.

2. Background section, as the authors know the statement “fibroblastic foci, a structure unique to IPF” is inaccurate please correct accordingly.

Fibroblastic foci are unique to the UIP pattern, and so this was misstated in the text. This statement has been changed to: “This is thought to lead to the formation of interstitial fibroblastic foci, a structure characteristic of IPF, and the accumulation of extracellular matrix and lung remodeling.”

3. Background, the statement “Initial investigations distinguished between familial and sporadic forms of IPF, though there is increasing evidence that genetic risk factors play a significant role in both forms of the disease”, is vague and somewhat unrelated to the rest of the paragraph, rephrase or remove.

This section has been edited to read as follows:

“Recent evidence has shown that there is an inherited risk of developing IPF, and specific genetic variants have been identified that are strongly associated with disease. Initial investigations distinguished between familial pulmonary fibrosis and IPF, but there is increasing evidence that genetic risk factors play a significant role both in familial forms of interstitial pneumonia as well as in IPF. These genetic risk factors are similar [4–6], prompting us to question the diagnostic distinction between familial forms of pulmonary fibrosis and IPF, classically thought of as a sporadic disease.
Although investigators continue to uncover new genetic risk factors for disease and to probe their connections to IPF pathophysiology, the full clinical implications of these genetic discoveries remain unknown. Here we briefly summarize the current knowledge regarding genetic risk and the development of IPF, describe how these genetic findings may influence the clinical management of patients with IPF, and suggest avenues for further investigation into the clinical implications of genetic risk in this disease.”

4. Page 5, Dyskeratosis Congenita, not congenital, please correct.

This has been corrected.

5. Page 5, Dyskeratosis Congenita 1, not dyskeratotis, please correct.

This has been corrected.

6. Page 7, what is the message for the reader with the following statement: MUC5B has also been found in honeycomb cysts, one of the hallmark pathologic findings of IPF.

We have added information this this statement in order to clarify its relevance to the paragraph: “MUC5B has also been found in honeycomb cysts, one of the hallmark pathologic findings of IPF [25]. This finding and the differences in gene expression between diseased and normal lung suggest that MUC5B expression may play a role in the development of characteristic IPF lesions.”

7. Page 7, I read this paragraph several times and unclear how the quoted frequencies justify the conclusions of gene by environment interactions, please be more precise: the frequency of the risk allele is 9.1% in the general non-Hispanic white population that comprised the control group, which implies interplay between genetic risk and environmental exposure in the development of IPF.

We have amended this section of the paragraph to read as follows: “In the initial study describing the association between rs35705950 and IPF, the minor allele frequency was 33.8% in FIP cases, 37.5% in IPF cases, and 9.1% in control subjects [6]. This highlights two important points: (1) the frequency of the risk allele is the same in FIP and in IPF and (2) the frequency of the risk allele is 9.1% in the general non-Hispanic white population that comprised the control group, suggesting incomplete penetrance of this risk allele. It is likely that the rs35705950 strongly predisposes an individual to development of IPF, but that additional factors, such as other genetic variants or environmental exposures, are necessary in order for development of clinically evident disease. Further studies in large populations will be necessary to understand the gene by gene and gene by environment interactions that influence disease development and phenotype.”
8. Page 8, “The association of the MUC5B promoter polymorphism appears to be specific to pulmonary fibrosis.” In cohorts with systemic sclerosis and interstitial lung disease, asbestosis, sarcoidosis ... be precise, pulmonary fibrosis encompasses all the non-IIP conditions listed; are you referring to idiopathic pulmonary fibrosis.

This has been corrected to read as follows: “The association of the MUC5B promoter polymorphism appears to be specific to IPF.”

9. Page 8, Please reference the following statement: Interestingly, the MUC5B polymorphism is not present in African populations.

We have amended this statement as follows: “Interestingly, the MUC5B polymorphism is not present in African populations (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=35705950).”

10. Page 9, please clarify, what you mean by “fibrotic IIP”; the idiopathic interstitial pneumonias are by definition a group of fibrotic disorders.

In response to concerns that the term “fibrotic” in this context would be confusing for readers, “fibrotic” has been removed from this paragraph. We used the term “fibrotic IIP” in our manuscript because the authors of the referenced study used the term “fibrotic IIP” in their 2013 manuscript to refer to cases of IIP that were associated with progressive fibrosis. Please see below for the table from supplementary material in the Fingerlin et al. *Nature Genetics* 2013 manuscript:

**Supplementary Table 2:** Specific IIP diagnosis information for GWAS discovery samples.

<table>
<thead>
<tr>
<th></th>
<th>Genotyped (N=1914)</th>
<th>Included after QC (N=1616)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sporadic(^a)</td>
<td>Familial(^b)</td>
</tr>
<tr>
<td>IPF</td>
<td>1055 (55%)</td>
<td>445 (24%)</td>
</tr>
<tr>
<td>NSIP</td>
<td>51 (3%)</td>
<td>60 (3%)</td>
</tr>
<tr>
<td>COP</td>
<td>3 (&lt;1%)</td>
<td>3 (&lt;1%)</td>
</tr>
<tr>
<td>RB-ILD</td>
<td>8 (&lt;1%)</td>
<td>3 (&lt;1%)</td>
</tr>
<tr>
<td>DIP</td>
<td>5 (&lt;1%)</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified</td>
<td>226 (12%)</td>
<td>55 (3%)</td>
</tr>
</tbody>
</table>

\(^a\)No known family history
\(^b\)At least 2 affected relatives (3\(^{rd}\) degree or closer)

QC: Quality control; IPF: Idiopathic pulmonary fibrosis; NSIP: Non-specific interstitial pneumonia; COP: Cryptogenic organizing pneumonia; RB-ILD: Respiratory bronchiolitis-associated interstitial lung disease; DIP: Desquamative interstitial pneumonia
11. Page 9, “These common variants associated with fibrotic IIP suggest that host defense (MUC5B, ATP11A), cell-cell adhesion (DSP and DPP9), and DNA repair (TERT, TERC, and OBFC1) may be important in disease pathogenesis”; do you mean that the ontology of the genes in which the common variants are found suggest that these biological functions are relevant to the pathogenesis of IPF? Please edit.

The text has been amended as follows: “In 2013, Fingerlin and colleagues published a case-control GWAS in 1616 non-Hispanic white IIP patients and 4683 controls, followed by a replication study of 876 cases and 1890 controls [4]. Fingerlin and colleagues confirmed disease associations between variants in TERT at chromosome5p15, MUC5B at 11p15, and the 3q26 region near TERC, but also identified seven new loci associated with disease, including: FAM13A (4q22), DSP (6p24), OBFC1 (10q24), ATP11A (13q34), DPP9 (19p13) and chromosomal regions 7q22 and 15q14-15 [4]. The functions of the genes in which common variants associated with IIP were found raise the hypothesis that host defense (MUC5B, ATP11A), cell-cell adhesion (DSP and DPP9), and DNA repair (TERT, TERC, and OBFC1) may be important in disease pathogenesis [4, 34, 38]. Studying the role of these genes in models of lung fibrosis will be necessary to determine which molecular pathways are important for the development of fibrosis and the lung’s response to injury.”