Preliminary Analysis: Breath Biopsy® Discovery of Biomarkers for IPF and ILA

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1. Background and Objectives

Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease of unknown cause associated with the development of progressive and irreversible fibrosis of the lung parenchyma. IPF affects approximately 3 million people worldwide[1], with a mean survival rate of 2-3 years after diagnosis[2].

Interstitial lung abnormalities (ILA) have been postulated as an early-stage imaging finding of pulmonary fibrosis; it shares common genetic variants with IPF.

Metabolic changes associated with both IPF and ILA are thought to produce unique volatile organic compounds (VOCs) detectable on breath that could be applied as diagnostic biomarkers.

Study Aims:

- Identify new VOC biomarkers for Control vs. ILA or IPF
- Identify VOC biomarker's similarities and differences in IPF vs. ILA groups.
- Identify VOC changes pre- and post- six-minute walk test (SMWT) linked to ILA or IPF
- Determine VOCs associated with IPF-related clinical variables (DLCO, FVC, TLC, FEV1, O2 saturation)

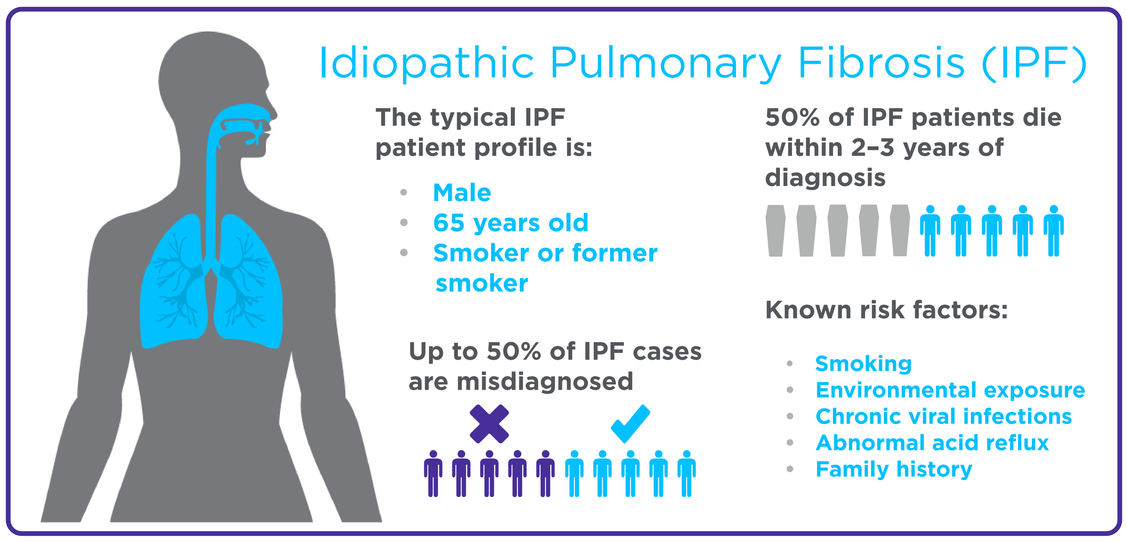

Figure 1: A selection of information and statistics relating to IPF.[3]

2. Methods

Subjects with IPF, ILA and controls were enrolled at Brigham and Women's Hospital, Boston, MA.

Breath Biopsy samples were collected before and after a six minute walk (SMWT) using the ReCIVA® Breath Sampler **(Figure 2)**, developed by Owlstone Medical, and analyzed with thermal desorption gas chromatography mass spectrometry (TD-GC-MS) using the Breath Biopsy Platform.

	Controls	ILA	IPF	Full Cohort
N°of Subjects	47	19	58	124
Female	30	11	25	66
Male	17	8	33	58
Age	60.0±6.0	62.0±4.0	72.0±4.0	N/A
BMI	27.7±3.60	27.4±2.89	26.8±2.98	N/A

Table 1: Test subject groups and demographic overview. Each group of subjects, contained a mixture of former smokers and subjects that had never smoked.

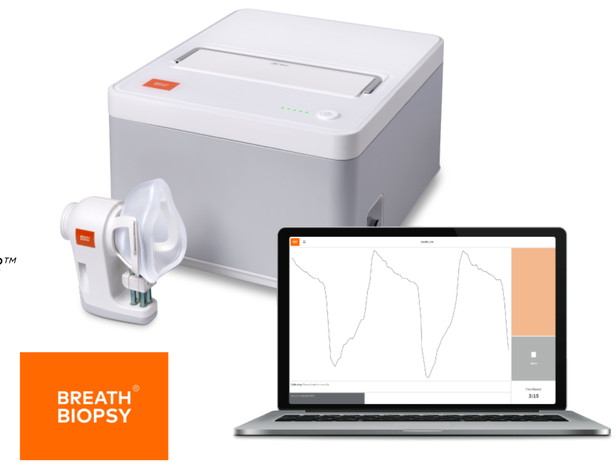

Figure 2: The Breath Biopsy® Collection Station, consisting of ReCIVA® Breath Sampler (left), CASPER™ Portable Air Supply (top) and Breath Biopsy Collect Software (lower right).

3. Results

129 distinct molecular features (MFs) were detected by TD-GC-MS, of which 22 MFs were shown to differ (unadjusted P<0.05*) between IPF patients and controls, pre-SMWT **(Figure 4A)**. In contrast, 27 MFs showed evidence of difference post-SMWT test, 3 of which are robust predictors of controls vs. IPF in multivariate analysis **(Figure 4B)**.

In both cases, pre- and post-SMWT, the sample size was too small for comparison of adjusted p-values. When pre- and post-SMWT analysis was combined a total of 34 MFs had evidence of difference between IPF and control (unadjusted p < 0.05*) **(Figure 4C)**.

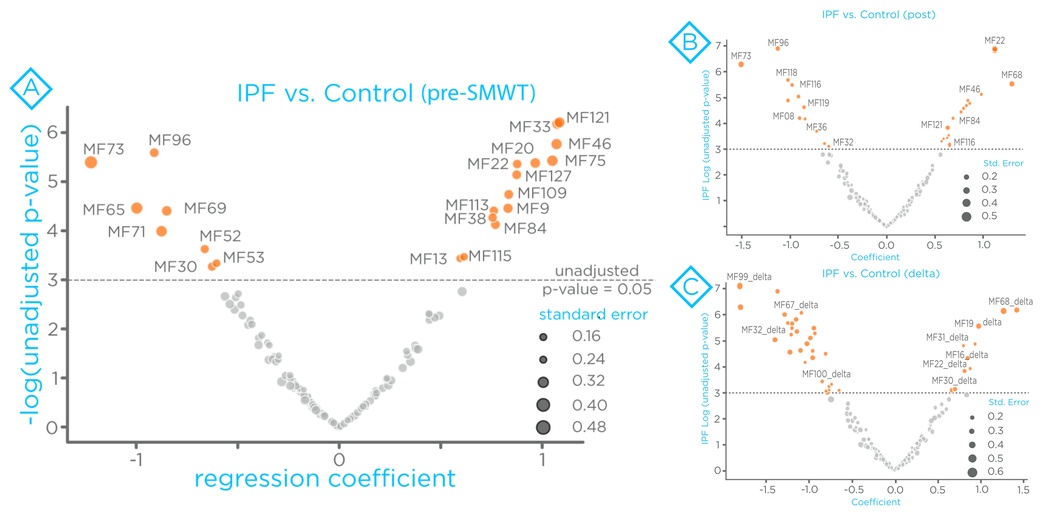

Figure 4 A, B & C: Volcano plots of molecular features plotted by -log (unadjusted p-value) and regression coefficient between IPF cases and controls. Features are colored by significance thresholds and sized by standard error. Left is pre-SMWT (A), top-right is post-SMWT (B) and bottom-right is the change between pre- and post-SMWT (C).

ILA vs. controls analysis, 4 MFs pre-SMWT and 6 MFs post-SMWT showed evidence of change. Combining pre- and post- analysis revealed 2 MFs with evidence of difference between ILA and Control (unadjusted p < 0.05*).

Most of the MFs that showed a statistically significant difference in IPF vs. control also showed a similar trend in ILA vs. control (although not significant) **(Figure 5).** These MFs showed potential as predicters of which ILA subjects might develop IPF.

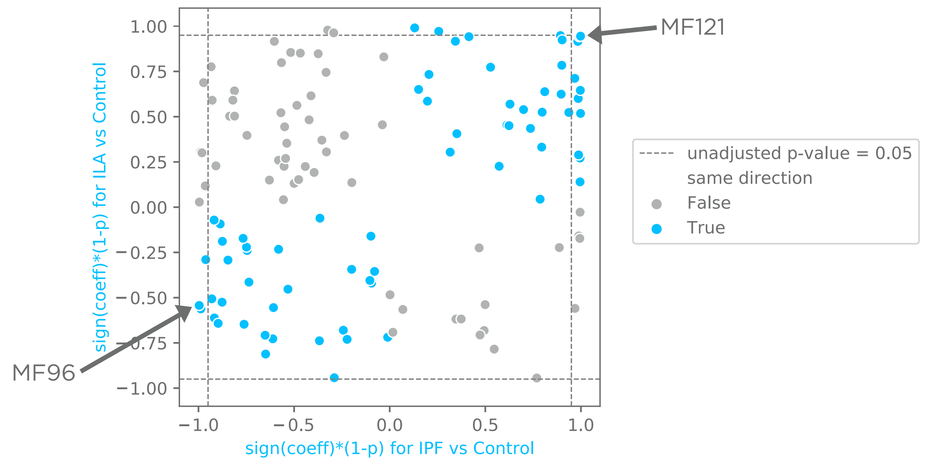

Figure 5: Scatter plot highlights trends observed in candidate MFs when IPF or ILA is compared to Control.

When compared with clinical variables, 4 MFs showed robust capacity to predict defusing capacity for carbon monoxide (DLCO) **(Figure 6)** and distance walked during the walk test (not shown).

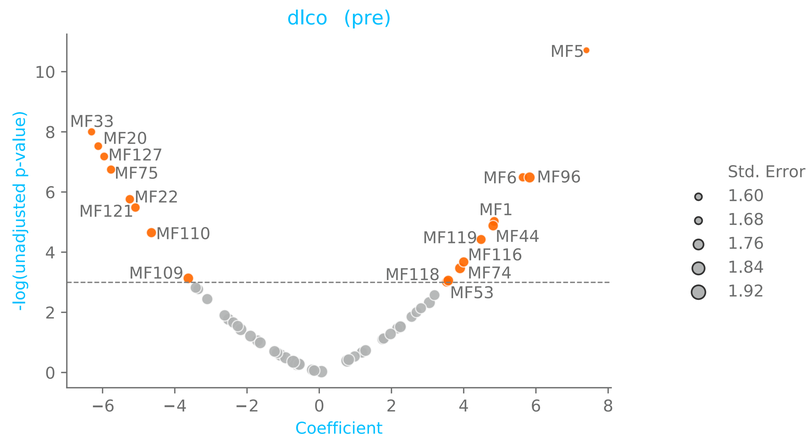

Figure 6: Volcano plot that demonstrates the abilty of MFs to predict DLCO.

4. Conclusions and Further Work

A robust relationship was found between MFs and IPF status (vs Control). MFs seem to be informative in predicting IPF.

Based on these results it may be possible to predict some ILA subjects to be more likely to develop IPF than others, however, follow-up diagnosis is needed to help confirm such a model.

A relationship was found between MFs and DLCO impairment.

There was some evidence to suggest that MFs are different between ILA and controls.

Interestingly, most of these MFs showed a similar correlation between IPF and controls.

Some MFs that differed in IPF vs ILA, likely reflecting biological mechanisms that are more different between IPF and ILA than between IPF/ILA and controls.

Our study was limited due to small ILA group size. Reopening recruitment of this study was unlikely to increase the statistical power due to increase sample variation. The next step for this work would therefore be a separate validation study to test all findings.

5. References

1. Nalysnyk L., et al., Incidence and Prevalence of Idiopathic Pulmonary Fibrosis: Review of the Literature. Eur, *Respir Rev.* 2012; 21 (126): 355-361.
2. Raghu G., et al., An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based, Guidelines for Diagnosis and Management. *Am J Respir Crit Care Med.* 2011; 183: 788–824.
3. Boehringer Ingelheim, https://www.boehringer-ingelheim.com/respiratory/ipf/ipf-video-infographic

Further Resources

Breath Biopsy: The Complete Guide (3rd Edition)
owlstonemedical.com/breath-biopsy-guide

Breath Biopsy for Respiratory Disease webinar
owlstonemedical.com/respiratory-webinar

Breath Biopsy Products & Services
owlstonemedical.com/products