**METHODS**

– Gene modules were characterised by the enrichment of Gene Ontology (GO) terms that are useful in predicting clinically meaningful pulmonary function decline in the future.

However, there is still a need for robust, validated biomarkers in IPF that are able to predict clinically meaningful pulmonary function decline in the future.

– Hierarchical clustering of patients was performed based on the expression levels and absolute changes in %FVC measurements over time.

Biomarker performance was initially assessed with the following analyses:

- Spearman rank correlation
- Logistic regression analysis

Approach 1: WGCNA constructed 51 gene co-expression network modules.

One module was enriched for genes involved in reproduction, oxygen and hypoxia response and protein oligomerisation; another module was enriched for genes involved in the regulation of receptor biosynthetic process.

**RESULTS**

**Patient Characteristics and Clinical Outcomes**

- 132 unselected patients with IPF completed the study evaluation.

- The candidate biomarkers between functional outcomes in patients with IPF were selected using a forward stepwise process of the ANCOVA (GEE).

- The candidate biomarkers identified in these analyses merit further scrutiny.

**Further Evaluation**

- The candidate biomarkers identified in these analyses merit further scrutiny and should be validated in a larger cohort of patients with IPF.

**Table 1: Baseline Demographics and Clinical Outcomes of All 132 Patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Body Proportions (kg/m²)</th>
<th>Age (median, IQR), years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index</td>
<td>25.4 (21.9-30.4)</td>
<td>65 (46-75)</td>
</tr>
<tr>
<td>Baseline Score</td>
<td>97 (85-112)</td>
<td>72 (60-83)</td>
</tr>
<tr>
<td>Search for Further Tests</td>
<td>48 (39-59)</td>
<td>55 (46-64)</td>
</tr>
</tbody>
</table>

**Gene Ontology**

- Gene Ontology terms for biological processes that were enriched in the gene co-expression network module.

- The candidate biomarkers identified in these analyses merit further scrutiny.

**Approach 2: MMRM using gene expression as a continuous variable**

- The candidate biomarkers identified in these analyses merit further scrutiny.

**Evaluation of Selected Candidate Biomarkers Associated With %FVC Decline**

- Identification of candidate biomarkers associated with %FVC decline.

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 5: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 6: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 7: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 8: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 9: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 10: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 11: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 12: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 13: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 14: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 15: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).

**Figure 16: Weighted Gene Co-Expression Module Heat Map**

- Gene expression scores were calculated by the enrichment of Gene Ontology terms for biological processes.

**Figure 17: Hierarchical Clustering of Patients With IPF Based on Gene Expression Levels of Selected Candidate Biomarkers**

- Hierarchical clustering of patients with IPF based on gene expression levels of the selected candidate biomarkers associated with %FVC decline identified 2 distinct clusters of patients (Figure 4: A, B, C, D, E, F).