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Research Article

Crucial Biomarkers for Pulmonary Arterial Hypertension (PAH) by Transcriptome Comparison with Idiopathic Pulmonary Fibrosis with and without PH and Identification of Essential Signaling Pathways- A Meta Analysis and Bioinformatics Study

Bhuvnesh Rai*

Department of Molecular Medicine and Biotechnology, Sanjay Gandhi Postgraduate Institute of Medical Sciences,Lucknow, 226014, India

***Corresponding Author:** Bhuvnesh Rai, Department of molecular medicine and biotechnology, Sanjay Gandhi Postgradutae Institute of Medical Sciences, Lucknow-226014, India

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Abstract

The development of pulmonary arterial hypertension (group I PH) complicates many interstitial lung diseases, including idiopathic pulmonary fibrosis (IPF) mostly present with underlined pulmonary hypertension, is suspected to be an independent risk factor for mortality in chronic lung diseases. This meta-analysis of transcriptomics study of pulmonary arterial hypertension and pulmonary fibrosis associated with and without pulmonary hypertension aims to utilize current evidences to extract novel genetic identifiers specifically for PAH in order to identify it among all 5 groups of pulmonary hypertension in IPF patients using pre existing vast number of observational experimental databases freely accessible publicly to facilitate early diagnosis of PAH and thereby improving its therapeutics. This meta-analysis framework extracts expression intensity features from each study, corresponding to genes that are consistently among the highly significant differentially expressed genes (DEGs) in PAH and IPF (with and without PH).

Comparative study with a relevant effect size, highly précised confidence interval establishing global ties between transcriptomics studies without any publication bias using review manager tool (Revman 5.4) and further microarray analysis of specified groups done by transcriptome analysis console (TAC 4.0).

Keywords: Pulmonary arterial hypertension; Idiopathic pulmonary fibrosis; Microarray; DEGs; Biomarker; Transcriptomics; Databases

1. Introduction

Many types of diffuse parenchymal lung diseases may cause pulmonary arterial hypertension (PAH) to develop. Arcasoy and associates identified PAH in one quarter of patients who were referred for transplantation with various kinds of advanced lung diseases [1]. Pulmonary arterial hypertension is characterised as a persistent elevation of pulmonary arterial pressure at rest to more than 25 mm Hg or with exercise to more than 30 mm Hg, with a mean pulmonary-capillary wedge pressure and a left ventricular end-diastolic pressure of less than 15 mm Hg, being used as the diagnostic criteria as in the National Institutes of Health (NIH) registry [2]. Pulmonary Arterial Hypertension (PAH) refers to Category I PH containing idiopathic or heritable sources in which the lung vasculature is compromised but not the lung parenchyma. Among the environmental factors associated with increased risk of pulmonary arterial hypertension production, three-hypoxia, anorexigens, and stimulants to the central nervous system-have possible mechanistic underpinnings. PAH has been correlated with some of the coexisting conditions too. Those with possible mechanical references include scleroderma, HIV infection, human herpesvirus (HHV), portal hypertension, thrombocytosis, hemoglobinopathy, and hereditary hemorrhagic telangiectasis. In all of these cases the histological presentation of lung tissue is similar: intimate fibrosis, increased medial thickness, pulmonary arteriolar occlusion and plexiform lesions predominate. Vasoconstriction, smooth muscle cell and endothelial cell proliferation, and thrombosis are the principal vascular modifications in pulmonary arterial hypertension. PAH has been identified as occurring in 5 to 38 percent of scleroderma patients, 4.3 to 43 percent of systemic lupus erythematosus patients, and 21 percent of rheumatoid arthritis patients [3, 4]. Sarcoidosis is also associated with PAH in 1 to 28 percent of cases, which is more common in more advanced disease patients [5].

PAH in patients with idiopathic pulmonary fibrosis (IPF) has been documented but the prevalence has not been well-defined. In an Unified analyses Organ exchange registry network, Shorr and colleagues found that about one-quarter of 2,000 IPF patients diagnosed for lung transplants, had PAH [6, 7]. Idiopathic pulmonary fibrosis (IPF), also known as cryptogenic fibrosing alveolitis, is a clinicopathologic term referring to an unexplained cause typically fatal condition characterised by varying degrees of inflammation and fibrosis in the parenchyma of the lungs [8]. Mean survival in IPF has been estimated to be 3 to 6 yr but with a variable clinical course. IPF patients' pathological analysis of lung specimens display a variety of histological patterns. Normal interstitial pneumonia (UIP) is a particular histological pattern of interstitial fibrosing pneumonia seen in most IPF-patients. UIP is the hallmark trait of IPF histopathology, characteristics include temporal and spatially heterogeneous fibrosis, clusters of fibroblasts and myofibroblasts (fibroblastic foci), and excessive deposition of disorganized collagen and extracellular matrix (ECM), resulting in distortion of normal lung morphology, with or without a cyst formation [9].

An important complication of chronic lung diseases here specifically IPF, that is strongly linked to the mortality, is the presence of pulmonary hypertension (PH). The prevalence of PH among patients with IPF depends on the IPF severity. PH affects < 10 per cent of patients with IPF in the early stages or when first diagnosed. However, the frequency of PH rises markedly as the IPF progresses. An occurrence of 32% was identified in one study of patients undergoing lung transplantation and thus in an advanced stage of IPF. Subsequent studies have provided significant support to percentage increase to between 32 and 50 percent [10-12]. It is important to note, though, that the signs for PH and IPF are very close (shortness of breath and exertional dyspnea) and, as such, under-diagnosis of PH in patients with IPF is practicable. PH is characterised by a mean pulmonary arterial pressure (mPAP) of approximately >25mmHg and a pulmonary artery coil pressure (PAWP) of <15mmHg and elevated pulmonary vascular resistance (PVR) > 3 wood units (WU) [10]. The pathological process in PH is characterised by extensive vascular remodelling including increased proliferation of smooth muscle cells (PASMC) in the pulmonary artery. [13, 14] This results in vessel lumen narrowing and obliterating resulting in improved vascular tone. About the same way, regardless of the elevated pressure of the pulmonary vasculature, the right ventricle (RV) helps to brace for remodelling, hypertrophy, inflammation and finally right-sided cardiac failure and death [15]. PH is subdivided into 5 comprehensive subsets of PH: Group I – Group V PH. Group I PH comprises idiopathic or heritable pulmonary arterial hypertension (PAH) where the lung vasculature is affected but not the lung parenchyma. Class II PH is related to left heart disease. Group III PH is associated with chronic lung diseases which affect parenchyma and hypoxemia in the lungs. Group IV PH is chronic pulmonary thromboembolic hypertension (CTEPH); Group V PH finally includes PH from unclear and multifactorial mechanisms.

We hypothesized that PAH is common in patients with more advanced IPF and may be an independent risk factor for mortality. We attempted to define this association using a cohort of patients respective for IPF and PAH who underwent lung biopsies as part of their evaluation using their biopsy tissue as the study sample. We propose a comprehensive meta-analysis for the overall study effect size Z score , P value (P<0.05) and hetrogenity (I2 <50%) method that establishes global relations between transcriptomics studies without publication bias by the use of review manager tool (Revman 5.4) and further, analysis of defined groups by the transcriptome analysis console (TAC 4.0). Our architecture uses this method to extract gene from any global data set function in research that is correlated with genes most commonly or differentially expressed in studies of PAH and PF (with and without PH) providing new insights into novel PAH genetic biomarkers and thereby improving its future therapeutics.

2. Methods and Materials

2.1 Data collection

Data-sets were searched pertaining to pulmonary arterial hypertension (PAH) and pulmonary fibrosis associated gene expression on gene expression omnibus (GEO, NCBI) databases. Two PAH data sets (gse113439, gse53408) [16, 17, 18] with lung biopsy tissue as the sample source were identified focusing primarily on PAH gene profiling. Additionally, two PF data sets (gse24988, gse19976) [19, 20] also with lungs biopsy samples were obtained, for cross reference and comparison. All the datasets shared a common platform (homosapiens & Affymetrix Human Gene 1.0 ST Array). The detailed information about the selected data sets is provided in (Table 1). In total, we

curated gene expression data from these four publicly available data sets keeping the study independent of age, gender, race and region.

S. no.	Study accession no.	Disease Acronym	Study description	No. of samples (disease specific)	Species and platform (GPL6244)
2	GSE113439	PAH	Gene expression profiling of pulmonary arterial hypertension	15	Affymetrix Human Gene 1.0 ST Array
3	GSE24988	1-PF with PH 2-PF with no PH	Gene expression profiles based on Pulmonary Artery Pressures in Pulmonary Fibrosis	62 30	Affymetrix Human Gene 1.0 ST Array
4	GSE19976	PF	Gene expression analysis of lung biopsies from patients with two different forms of pulmonary sarcoidosis	8	Affymetrix Human Gene 1.0 ST Array
5	GSE53408	PAH	Metabolomic heterogeneity of severe pulmonary arterial hypertension	12	Affymetrix Human Gene 1.0 ST Array
6	GSE113439, GSE24988, GSE19976, GSE53408	PAH vs.PF with PH and PF with no PH base line contr ol (healthy lung biopsy tissue)	Meta analysis with transcriptome study TAC 4.0 significant analysis microarray	PAH-22 PF with PH-22 PF with no PH-22 Healthy control-22 Total=88	Affymetrix Human Gene 1.0 ST Array

Table 1: Human datasets included for transcriptomic analysis.

2.2 Meta-analysis work flow

An equivalent protocol was used to evaluate all of the findings. Until doing the TAC analysis for the differentially expressed genes and the REACTOME pathway analysis, each study was pre-processed including quality management and standardization by REVMAN 5.4. Using uniform threshold condition false discovery rate (FDR) f-test < 1E-43, differentially expressed genes were incorporated by TAC 4.0 similarly, associated pathways were identified using threshold P value ($P<0.05$) and gene ontology of DEGs. in PAH was identified by PANTHER (Figure 1).

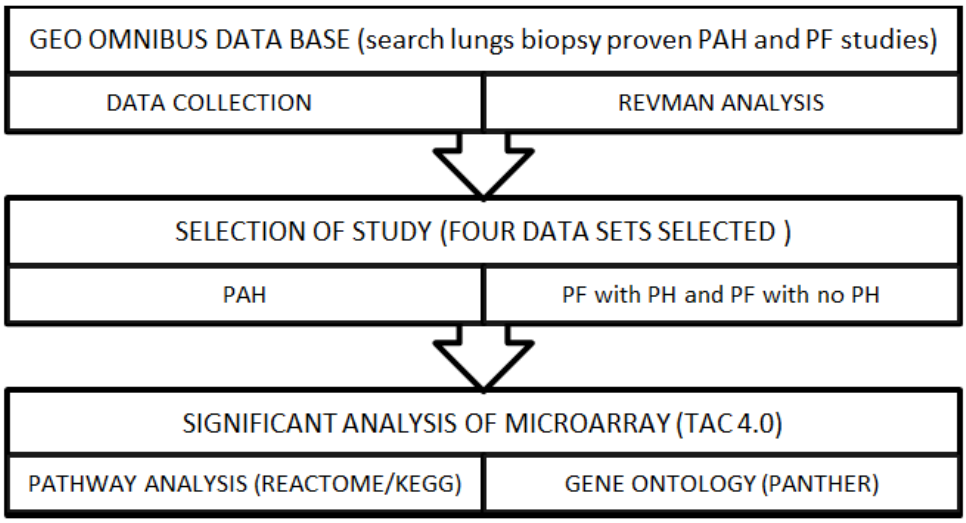


Figure 1: Work flow of meta-analysis process.

2.3 Statistical meta-analysis: Rev-man manager 5.4

The software RevMan 5.4 was used for statistical analysis of all datasets. Dichotomous data was analyzed using the statistical method Mental Haenszel and the model of fixed effect analysis. The data was measured with 95 percent analysis complete as well as total CI (Confidence interval) with effect measures OR (odds ratio). The heterogeneity was measured by ($I2 < 50\%$) with P value ($P< 0.05$) and overall effect size was measured by Z score with P value ($P<0.05$). Represented by Funnel and Forest plots.

2.4 Microarray analysis for identification of DEGs in PAH vs. IPF with and without PH

The analysis was performed by TAC 4.0 software along with background adjustment, quantile normalization, summarization, and log2 value transformation using RMA+DABG algorithm. At first principal component analysis (PCA) was executed to obtain the overall similarities and dissimilarities of the log-transformed expression ratios of genes between all the samples of all the three groups. Further, ANOVA was used for the statistical evaluation among groups (PAH, PF with PH, PF without PH and control for normalization). Subsequently all statistically evaluated genes were sorted to obtain the significant ones with a cut off condition FDR F Test ($f<1E-43$) for the differential gene expression study and generation of hierarchical clustering using distance metric (Euclidean distance). Distances between clusters of objects were computed using the complete linkage method. Sample used in each category were (PAH-22, PF with PH -22, PF without PH-22 and CONTROL-22).

2.5 Quality check through Box plot by TAC

Boxplot displays the distribution of data based on five parameters i.e. (minimum, first quartile (Q1), median, third quartile (Q3), and maximum). It tells about outliers and its values. It also tells symmetry of data, grouping of data, and skewing of data.

2.6 Pathway analysis of DEGs using REACTOME

The open source program Reactome [15, 21] was used to classify particular pathway correlated with separate and typical DEGs in benjamini and hochberg FDR (P < 0,05) classes in PAH and PF.

2.7 Gene Ontology of DEGs using PANTHER

In order to predict gene ontology for novel genes found in PAH and PF groups, PANTHER(Protein ANalysis THrough Evolutionary Relationships) was used, the systematic method integrating genomes, gene function classifications, pathways and methods of statistical analysis, was used to analyze the large-scale genome wide experimental results.

3. Results

3.1 PAH and IPF (with and without PH) microarray datasets

We identified, and included, 4 datasets from PAH and IPF that matched our criteria. Of the 4 datasets, two were from PAH sets (gse113439, gse53408) and 2 were from PF (with and without PH) (gse24988, gse19976) as detailed in table 1.

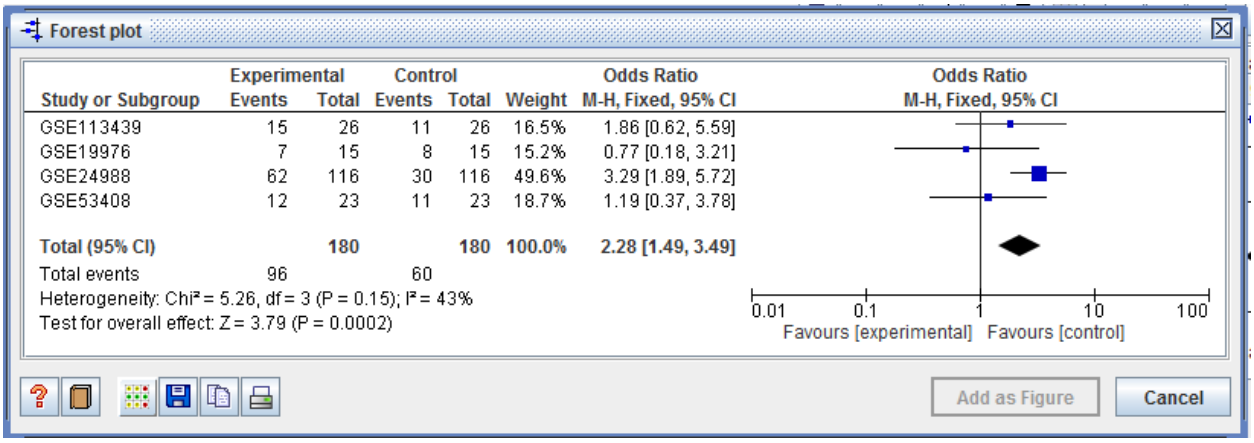


Figure 2: Forest plot shows test for overall effect size z score (Z=3.79) with P value (P=0.0002) and hetrogenity (I^2=43%).

3.2 Statistical analysis showing significance of all datasets by REVMAN 5.4

In the forest plots shown with two columns, the left column lists the names in chronological order of the studies wereas, the right column is a chart of the effect calculation (i.e. probability ratio) for each of the experiments, and the horizontal lines represent the intervals of confidence. The graph was plotted on a regular logarithmic scale using odds ratios, so that the confidence intervals are symmetrical around the mean of each sample, and excessive

importance is not given to odds ratios greater than 1 over less than 1. In meta-analysis, the area of each square represents the relative weight of the individual sample. The total meta-analyzed impact measure was depicted as a

diamond on the diagram, the lateral points of which suggested intervals of confidence for this calculation. Our plot shows test for overall effect Z score (Z=3.79), P value (P=0.0002) and heterogeneity (I2=43%) (Figure2).

A scatter plot of the impact estimation size from individual experiments is seen in the Funnel plot. The standard error of the impact calculation was used as the indicator of sample size and was plotted on the vertical axis with a reversed scale that put the bigger, more effective studies at the top. The impact results from smaller experiments scattered more uniformly at the right. The outer dashed lines indicated the triangular region within which 95% of studies are expected to lie in the absence of both biases and heterogeneity (fixed effect summary log odds ratio ±1.96× standard error of summary log odds ratio) (Figure3).

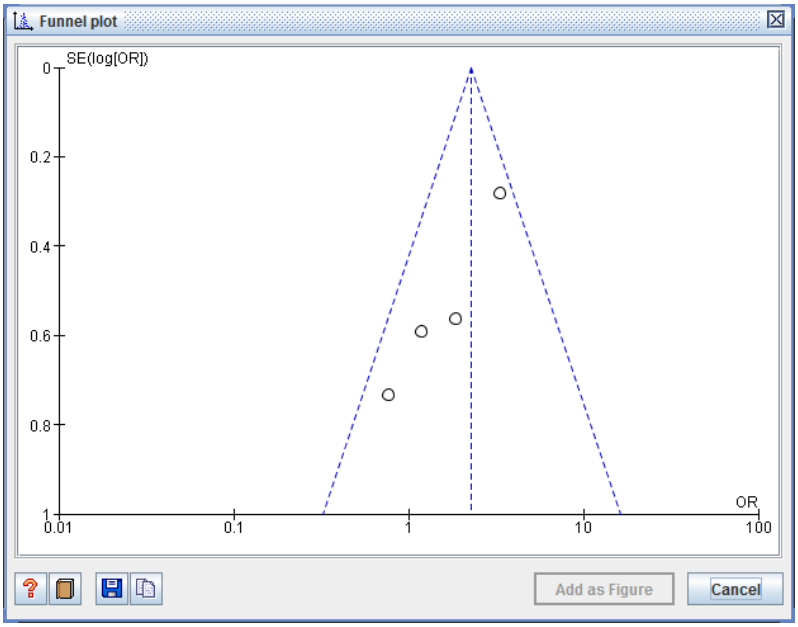


Figure 3: The outer dashed lines in the symmetrical funnel plot indicate the triangular region within which 95% of studies are expected to lie in the absence of both biases and heterogeneity. Funnel plot evaluates the standard error plotted on the vertical axis with a reversed scale showing the larger, most powerful studies towards the top and smaller studies scattered widely at the bottom.

3.3 Quality check of raw data (.CEL) files in PAH and PF (with PH and PF without PH)

Box plot of 88 samples showed the distribution of data to be homogenous in each group (PAH, PF with PH, PF without PH and control) with values significantly lying between (7.2-8) for all the groups. No outliers are present in our study (Figure 4).

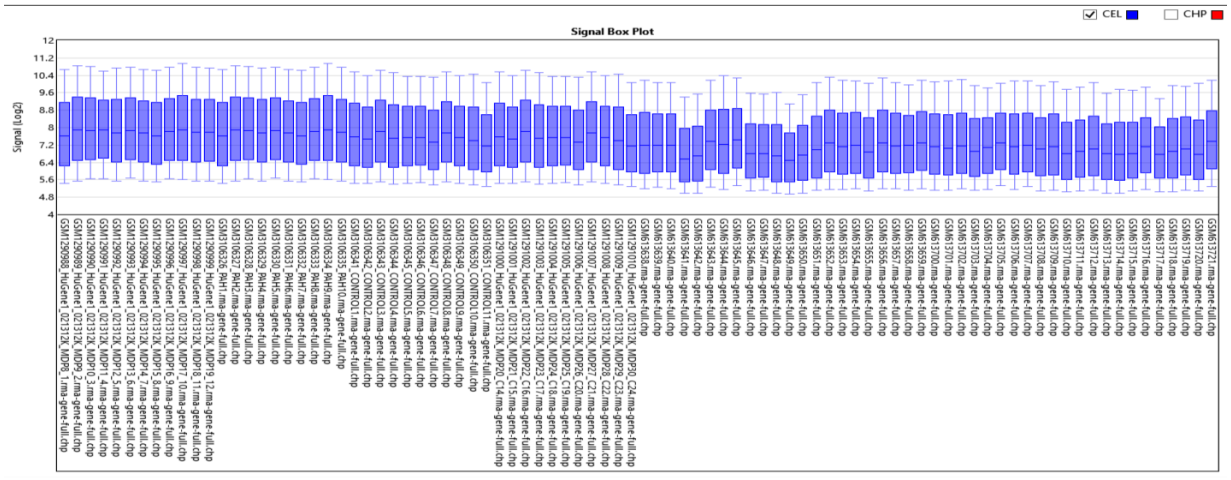


Figure 4: Box plot of 88 samples in PAH vs. PF. It shows that the input sample in each chip array is homogeneous significantly lying between (7.2-8.0).

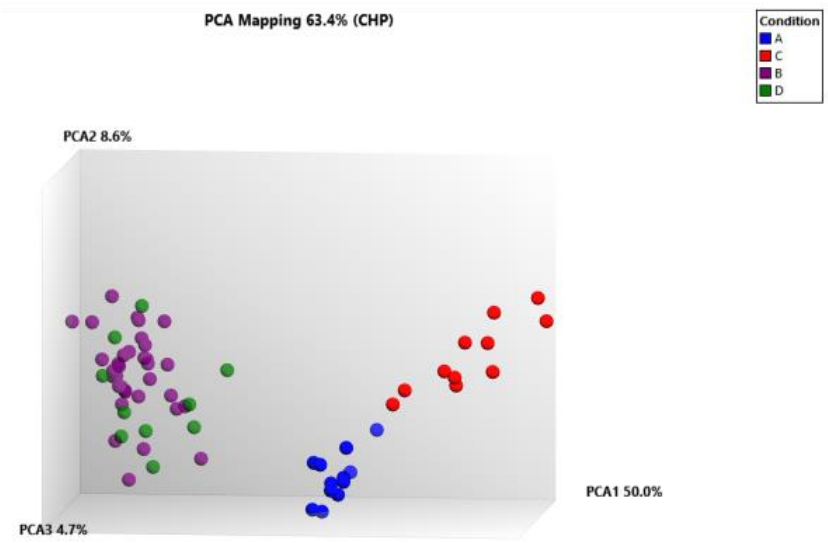


Figure 5A: Three-dimensional principal component analysis (PCA) plot of gene set mapping shows distinction between the 88 samples,.Total of 63.4% variance between PAH (n=22), PF with PH (n=22), PF with no PH and healthy lung tissue samples (n=22) is shown using component 1 (PCA1, 50.0%), component 2 (PCA2, 8.6%), and component 3 (PCA3, 4.7%).The three axes represent the first three principal components identified by the analysis, Each red (C) spot represents a control sample, PAH tissue sample (A), blue spots, and purple spots sample from PF with PH tissue (B) and PF with no PH sample (D), green spots.

3.4 PCA and Heatmap

Principal component analysis plot of gene set mapping showed distinction between the total 88 samples. Total of 63.4% variance between PAH (n=22), PF (with PH) (n=22), PF (without PH) (n=22), and healthy control (n=22)

was shown by component 1 (PCA1, 50.0%), component 2 (PCA2, 8.6 %), and component 3 (PCA3, 4.7%). (Figure 5A)

Hierarchical cluster analysis of the significant 353 DEGs filtered out by applying false discovery rate (FDR) f-test < 1E-43 was obtained excluding unassigned genes with log2 fold expression and transcripts. (Figure 5B)

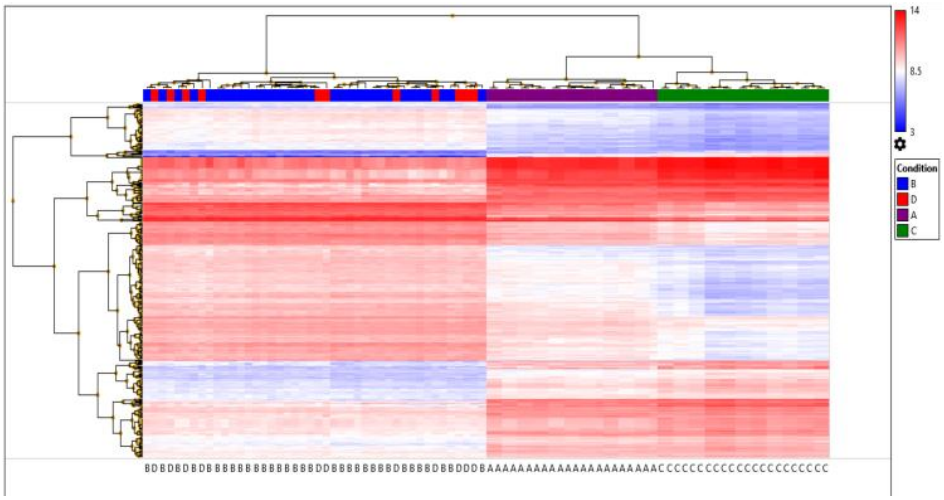


Figure 5B: Heat map and dendogram shows, hierarchical cluster analysis of the significant 353 DEGs. filtered out on giving FDR condition F test (F < 1E-43) excluding unassigned genes with log2 fold expression and transcripts in four sets of samples; A, B, C and D. The clustering was performed through (TAC) 4.0, Distance metric used between objects was the Euclidean distance. Distances between clusters of objects were computed using the complete linkage method.

3.5 Total number of DEGs differentially regulated in PAH and PF (with PH and PF without PH)

For an outline of expression profiles in lung disorder with PAH in human subjects, we used TAC4.0 software to spot differentially expressed genes (DEGs). For the analysis samples from two PAH datasets were merged giving 27 samples in total. Similarly on merging the 2 PF datasets a total of 62 samples were obtained belonging to PF with PH and 22 samples of PF without PH. Merging control samples from all 4 datasets gave a total of 29 samples. Finally 22 samples from each group (PAH, PF with PH, PF without PH and Control for normalization) were analyzed for DEGs.

On comparing PAH vs. PF with PH and PF without PH group we obtained a total of 353 DEGs on applying significant filters i.e. condition F Test (f <1E-43) out of which all 353 DEGs were assigned with gene symbol and included in the study (Table 2).

S.no.	Study accession no.	Disease acronym	Significantly gene upregulated Condition fdr f<1E-43	Significantly gene downregulated Condition fdr f<1E-43
1	GSE113439+ GSE24988+ GSE19976+ GSE53408	PAH vs.PF with PH and PF with no PH base line control (healthy lung biopsy tissue) Commonly regulated	198	145
2	GSE113439+ GSE24988+ GSE19976+ GSE53408	PAH vs.PF with PH and PF with no PH base line control (healthy lung biopsy tissue) differentially regulated	5	5

Table 2: Shows significantly differentially expressed gene which are commonly and differentially up and downregulated in PAH vs. PF with PH and without PH.

From a total of 353 our analysis indicated 145 DEGs commonly downregulated in PAH vs. PF (with PH and PF without PH) (Table 3i) and 198 DEGs were commonly upregulated (Table 3ii). However, on comparing the three groups 10 DEGs. showed differential regulation significantly in PAH (Table 4).

S.no.	Gene Symbol	A vs C Fold Change	B vs C Fold Change	D vs C Fold Change	Condition FDR F-Test
1	AKT1	-1.28	-2.94	-2.85	8.83E-44
2	ARF5;	-1.96	-3.17	-3.29	1.40E-45
3	ATP5G1	-1.34	-9.28	-8.1	8.83E-44
4	ATRAID	-1.71	-3.85	-3.86	2.80E-45
5	BRK1	-1.38	-3.93	-3.9	1.40E-45
6	BRK1	-1.17	-3.42	-3.65	1.40E-45
7	C12orf10	-1.56	-3.05	-3.03	1.40E-45
8	C20orf24	-1.09	-3.79	-3.42	1.40E-45
9	CCDC130	-1.45	-2.59	-2.42	1.40E-45
10	CCDC97	-1.25	-2.42	-2.31	1.40E-45
11	CCND3	-1.48	-4.02	-3.91	1.40E-45
12	CDC34	-1.65	-3.89	-3.56	1.40E-45
13	CEBPD	-1.41	-4.88	-4.74	1.40E-45
14	COX5B	-1.82	-8.54	-7.87	1.40E-45
15	CRTC3	-1.28	-2.72	-2.51	1.40E-45
16	DIRC2	-1.38	-3.05	-3.01	1.40E-45
17	ERH	-1.15	-3.05	-3.24	1.40E-45
18	FKBP1C	-1.28	-3.04	-2.88	1.40E-45
19	GABARAP	-1.39	-3.09	-3.24	8.41E-45

20	GABARAPL1	-1.18	-3.93	-3.98	1.40E-45
21	GDI1	-1.46	-2.47	-2.4	1.40E-45
22	GIT1	-1.35	-2.47	-2.54	2.80E-45
23	GMPR	-1.07	-4.06	-4.2	1.40E-45
24	GNA11	-1.48	-3.36	-3.17	1.40E-45
25	GNAI2	-1.17	-1.95	-1.83	1.40E-45
26	GNB2	-1.29	-2.4	-2.33	1.40E-45
27	GSK3A	-1.14	-2.34	-2.34	1.40E-44
28	HEBP1	-1.11	-3.25	-3.28	1.40E-45
29	HINT2	-1.72	-5.98	-5.8	1.96E-44
30	HIST1H1E	-1.28	-4.63	-4.96	1.96E-44
31	HIST2H2BA	-1.45	-2.97	-3.02	2.10E-44
32	HNRNPUL1	-1.19	-2	-2	1.40E-45
33	ICAM2	-2.05	-6.76	-5.88	1.40E-45
34	IDH3G	-1.27	-3.05	-2.88	4.20E-45
35	INF2	-1.37	-2.74	-2.74	2.80E-45
36	LMAN2	-1.2	-4.52	-4.49	1.40E-45
37	LSM12	-1.16	-3.31	-3.15	4.20E-44
38	LSM12	-1.17	-3.26	-3.12	1.40E-45
39	MAD2L1BP	-1.18	-3.41	-3.11	2.80E-45
40	MALSU1	-1.07	-2.48	-2.41	6.87E-44
41	MAU2	-1.27	-1.88	-1.87	1.40E-45
42	MEA1	-1.36	-3.71	-3.68	1.54E-44
43	MRPL28	-1.38	-3.11	-3.18	1.40E-45
44	MRPL40	-1.07	-3.43	-3.38	1.40E-45
45	MRPS11	-1.24	-4.38	-4.2	1.40E-45
46	MRPS18A	-1.6	-4.22	-4.43	1.40E-45
47	NDUFAF3	-1.44	-4.7	-4.49	1.40E-45
48	NELFE	-1.11	-2.11	-2.28	3.78E-44
49	NELFE	-1.11	-2.11	-2.28	2.80E-45
50	OR7E14P	-1.68	-2.8	-2.89	1.40E-45
51	OR7E12P;	-1.68	-2.8	-2.89	1.40E-45
52	OR7E26P	-1.81	-3.05	-3.25	4.76E-44
53	FSCN3	-1.96	-3.17	-3.29	1.40E-45
54	OR7E12P	-1.71	-2.8	-2.89	2.80E-45
55	OR7E14P	-1.78	-2.77	-2.92	1.40E-45
56	TGIF2	-1.09	-3.79	-3.42	1.40E-45
57	OR7E37P	-1.75	-2.68	-2.76	1.40E-45
58	OR7E12P	-1.71	-2.86	-2.92	1.40E-45
59	OR7E14P	-1.7	-2.85	-2.92	1.68E-44
60	HIST2H2BC	-1.45	-2.97	-3.02	2.10E-44
61	OR7E55P	-1.43	-2.21	-2.12	1.40E-45
62	PEBP1	-1.19	-3.91	-3.49	4.76E-44
63	PSMB6	-1.03	-4.42	-3.86	1.40E-45
64	PSMD8	-1.27	-3.11	-2.86	1.40E-45
65	PSMG2	-1.05	-2.48	-2.49	1.40E-45
66	PXN	-1.47	-3.3	-3.19	1.40E-45

67	RAB5C	-1.22	-2.84	-2.78	1.40E-45
68	RNU2-1; WDR74	-1.56	-5.79	-5.89	1.40E-45
69	RNU2-1; WDR74	-1.47	-5.39	-5.25	3.08E-44
70	RNU2-1; WDR74	-1.42	-5.13	-5.01	1.40E-45
71	RPL27A	-1.13	-16.61	-16.21	3.36E-44
72	SNORD116@	-1.59	-6.69	-6.94	1.40E-45
73	SNORD13P3	-1.24	-8.33	-9.2	1.40E-45
74	SNHG1	-1.39	-9.65	-9.93	1.40E-45
75	RABGGTB	-1.64	-19.64	-20.13	1.40E-45
76	NOP56	-1.3	-3.79	-4.17	1.40E-45
77	COX16	-1.03	-3.33	-3.21	1.40E-45
78	SNHG12	-1.18	-7.14	-6.36	4.34E-44
79	RNU4-1	-1.64	-11.58	-10.52	1.40E-45
80	RNU4-2	-1.09	-13.03	-17.19	1.40E-45
81	RNU4ATAC	-1.61	-17.27	-15.58	1.96E-44
82	RNVU1-18	-1.82	-9.15	-8.04	1.40E-45
83	RNU1-3	-1.82	-9.15	-8.04	1.40E-44
84	RNU1-4	-1.81	-8.37	-7.42	1.40E-45
85	RNU1-2	-1.81	-8.37	-7.42	1.40E-45
86	RNU1-1	-1.81	-8.37	-7.42	4.06E-44
87	RNU1-28P;	-1.81	-8.37	-7.42	1.40E-45
88	RNU1-27P	-1.81	-8.37	-7.42	1.82E-44
89	RNU1-27P	-1.8	-8.26	-7.33	1.40E-45
90	RPL23AP5	-1	-2.22	-2.28	1.40E-45
91	RPL18A	-1.73	-3.29	-3.41	1.40E-45
92	RPL23A	-1	-2.22	-2.28	1.40E-45
93	RPL23A	-1	-2.32	-2.34	4.76E-44
94	RPL36	-1.19	-3.24	-3.22	5.61E-45
95	SCARNA4	-8.4	-20.56	-20.51	1.40E-45
96	SCYL1	-1.25	-2.33	-2.29	1.26E-44
97	SELPLG	-1.57	-6.29	-5.76	6.03E-44
98	SF3B5	-1.56	-3.65	-3.61	1.68E-44
99	SKI	-1.44	-2.88	-2.75	1.40E-45
100	SLC25A6	-1.25	-3.88	-3.73	1.40E-45
101	SLC25A6	-1.12	-3.4	-3.24	1.40E-45
102	SLC25A6	-1.12	-3.4	-3.24	8.41E-45
103	SNORA16A	-1.18	-7.14	-6.36	4.34E-44
104	SNORA20	-1.79	-9.5	-9.15	2.80E-45
105	SNORA22	-2.05	-8.32	-11.01	1.40E-45
106	SNORA23	-1.59	-10.99	-10.18	1.40E-45
107	SNORA38B	-1.82	-9.1	-8.16	2.52E-44
108	SNORA3A	-1.13	-16.61	-16.21	3.36E-44
109	SNORA60	-2.96	-14.61	-15.23	1.40E-45
110	SNORA71D	-1.39	-16.4	-15.62	1.40E-45
111	SNORD116-14	-1.59	-6.19	-6.1	1.40E-45
112	SNORD116-15	-1.27	-4.73	-4.96	1.40E-45
113	SNORD116-20	-1.59	-6.69	-6.94	1.40E-45

114	SNORD116-23	-1.75	-6.72	-6.61	1.40E-45
115	SNORD116-24	-1.58	-7.39	-7.98	1.40E-45
116	SNORD13	-1.24	-8.33	-9.2	1.40E-45
117	SNORD13P3	-1.39	-9.65	-9.93	1.40E-45
118	SNORD29	-1.22	-4.77	-4.89	1.40E-45
119	SNORD32B	-1.38	-3.22	-3.07	8.41E-45
120	SNORD41	-1.64	-19.64	-20.13	1.40E-45
121	SNORD45A	-1.59	-4.98	-5.21	1.40E-45
122	SNORD57	-1.3	-3.79	-4.17	1.40E-45
123	SYNJ2BP-	-1.03	-3.33	-3.21	1.40E-45
124	TMED4	-1.42	-3.08	-3.13	7.01E-45
125	TMEM14C	-1.31	-3.1	-2.94	1.40E-45
126	TMEM160	-1.47	-3.05	-3.12	1.40E-45
127	TMEM179B	-1.53	-4.35	-4.4	1.40E-45
128	TMEM248	-1.21	-2.67	-2.65	2.80E-45
129	TMEM261	-1.41	-4.49	-4.37	1.40E-45
130	TMEM50A	-1.21	-2.37	-2.41	1.40E-45
131	TMEM53	-1.66	-3.64	-3.81	1.40E-45
132	TPST2	-1.5	-3.01	-2.84	1.40E-45
133	TRIM39-RPP21; RPP21	-1.58	-4.66	-4.53	1.40E-45
134	TRIM39-RPP21; RPP21	-1.58	-4.66	-4.53	1.40E-45
135	TRIM39-RPP21; RPP21	-1.57	-4.11	-3.94	7.01E-45
136	UBE2A	-1.06	-2.11	-2.16	1.40E-45
137	UBE2Q1	-1.08	-1.99	-1.96	1.40E-45
138	UBE2R2	-1.18	-2.34	-2.3	9.81E-44
139	UCKL1	-1.37	-2.4	-2.38	1.40E-45
140	UFC1	-1.11	-3.49	-4	2.80E-44
141	URGCP-MRPS24; MRPS24	-1.34	-4.26	-4.2	8.41E-45
142	VTRNA1-1	-2.5	-51.35	-49.39	1.40E-45
143	WFS1	-2.04	-4	-4.08	8.41E-45
144	XRCC1	-1.47	-2.63	-2.79	2.24E-44
145	ZNF384	-1.16	-1.72	-1.74	1.40E-45

Table 3(i): 145 DEGs commonly downregulated gene out of total 353 in PAH, PF with PH and PF with no PH compared to healthy control with condition FDR F<1E-43.

S.no.	Gene Symbol	A vs C Fold Change	B vs C Fold Change	D vs C Fold Change	Condition FDR F-Test
1	ABCD3	1.78	5.36	4.82	1.40E-45
2	ABI1	1.25	2.3	2.24	1.40E-45
3	ADSS	1.7	3.16	2.99	1.40E-45
4	AGPS	2	4.41	4.36	1.40E-45
5	ANP32E	1.7	3.86	3.9	1.40E-45
6	ARMCX3	1.46	4.8	4.86	1.40E-44
7	ATL2	1.54	5.81	5.11	1.40E-44
8	ATP6V1C1	2.16	4.16	4.12	1.40E-44
9	ATRX	2.66	6.58	6.18	1.40E-45
10	AZIN1	1.64	4.34	4.06	1.40E-45
11	B3GALNT2	1.29	2.99	2.78	1.40E-45
12	BMS1	1.8	4.28	4.14	1.40E-45
13	BNIP2	1.7	3.13	3.15	1.40E-45
14	BRMS1L	1.72	4.23	4.26	1.40E-45
15	BZW1	2.34	3.86	3.85	1.40E-45
16	C11orf58	1.56	2.56	2.36	5.61E-45
17	C6orf62	1.62	2.99	2.94	8.41E-45
18	CAAP1	1.38	2.97	2.84	2.80E-44
19	CCDC186; MIR2110	3.84	6.89	6.22	1.40E-45
20	CCDC47	2.07	3.51	3.59	1.68E-44
21	CCDC82	1.6	3.69	3.72	7.01E-45
22	CEP290	2.47	5.25	4.81	8.41E-45
23	CLCN3	1.66	3.79	3.85	1.40E-45
24	CLPX	1.77	3.48	3.37	1.54E-44
25	CNBP	1.31	2.15	2.18	1.40E-45
26	COL4A3BP	1.81	3.38	3.04	1.40E-45
27	COPB1	2.7	3.58	3.65	1.40E-45
28	CSNK1A1	1.59	3.72	3.86	1.40E-45
29	CTR9	2.53	5.67	5.58	1.40E-45
30	CWC27	2.06	3.26	3.26	7.01E-44
31	DCUN1D1	1.47	3.57	3.6	1.40E-45
32	DDX3X	2.38	4.58	4.73	1.40E-45
33	DDX42	1.43	3.78	3.66	1.40E-45
34	DDX46	2.04	3.34	3.16	1.40E-45
35	DDX50	1.2	3.38	3.15	1.40E-45
36	DEK	2.04	3.92	3.6	1.40E-45
37	DLD	2.33	4.71	4.58	4.20E-44
38	DNAJA2	1.53	3.77	3.61	1.40E-45
39	DNAJC10	2.19	5.17	5.42	2.52E-44
40	DNAJC3	2.65	4.28	4.81	1.40E-45
41	EID1	1.15	3.2	2.92	1.40E-45
42	EIF4A2	1.81	6.18	6.67	1.40E-45
43	EIF5B	2.75	4.93	5.08	1.40E-45
44	ENOPH1	1.19	3.22	3.28	1.40E-45

45	EPRS	3.3	6.55	6.68	1.40E-45
46	ESF1	2.25	7.17	6.95	1.40E-45
47	ETNK1	1.42	3.85	3.45	7.01E-45
48	EWSR1	1.01	2.87	2.96	2.80E-45
49	EXOC5	2.28	3.73	3.72	4.20E-45
50	FAM133B; FAM133DP	2.27	4.15	4.3	1.40E-45
51	FAM133DP; FAM133B	2.32	4.05	4.08	1.40E-45
52	FAM133DP; FAM133B	2.41	4.49	4.53	3.50E-44
53	FAM208A	1.36	3.14	3.08	1.68E-44
54	FAM3C	1.56	4.46	4.5	2.80E-45
55	FAM3C; FAM3C2	1.59	4.46	4.46	5.61E-44
56	FAR1	1.67	3.7	3.52	3.78E-44
57	FBXO11	1.81	3.56	3.52	2.80E-45
58	FBXO28	1.67	2.58	2.52	1.40E-45
59	FGFR1OP2	1.49	3.3	3.53	1.96E-44
60	FKBP3	1.12	2.66	2.59	9.25E-44
61	FXR1	2.38	6.17	5.76	1.40E-45
62	FYTTD1	1.65	2.87	2.96	1.40E-45
63	GBE1	2.11	3.6	3.7	1.40E-45
64	GCC2	3.48	7.6	6.91	1.40E-45
65	GGNBP2	2.07	3.63	3.56	1.40E-45
66	GLOD4	1.06	2.87	2.81	1.40E-45
67	GNAI3	1.7	2.91	3.13	1.40E-45
68	GOLGA4	2.86	4.12	4.25	1.40E-45
69	GOLGA6L17	1.35	3.94	3.5	1.40E-45
70	GOLGA6L9	1.31	4.03	3.6	1.40E-45
71	GOLGB1	2.73	5.13	5.08	1.40E-45
72	GOLT1B	1.66	4.72	4.98	1.40E-45
73	GTF3C3	2.11	3.93	3.89	1.40E-45
74	HERC4	1.53	3.78	3.44	1.40E-45
75	HNRNPA1P10	2.11	3.88	3.82	7.01E-45
76	HNRNPA1P1	2.27	4.07	3.93	1.40E-45
77	HNRNPA3	2.37	5.28	4.83	1.40E-45
78	HNRNPA3	2.37	5.39	4.88	1.40E-45
79	HNRNPH1	1.6	4.1	4.16	1.40E-45
80	HNRNPH2; RPL36A-HNRNPH2	1.59	2.96	3	1.40E-45
81	HNRNPM	1.26	3.04	3.03	1.40E-45
82	HNRNPR	1.75	3.38	3.35	1.40E-45
83	HNRNPU	1.67	3.39	3.41	1.40E-45
84	HS2ST1	1.47	2.65	2.66	1.40E-45
85	HTATSF1	1.63	5.02	4.5	1.40E-45
86	IFT80	1.92	6.11	5.54	8.41E-45
87	INSIG2	1.25	5.06	4.82	2.80E-45
88	ITCH	1.43	2.35	2.4	1.40E-45
89	KTN1	3.08	6.1	5.57	1.40E-45
90	LBR	1.19	3.06	3.2	1.40E-45

91	LEO1	1.84	4.59	4.2	1.40E-45
92	LRPPRC	2.78	5	4.99	1.96E-44
93	LUC7L3	2.14	6.7	6.27	1.40E-45
94	MED4	1.64	2.94	2.64	1.40E-45
95	MFN1	1.97	3.78	3.6	1.40E-45
96	MIB1	1.62	3.8	3.42	5.61E-45
97	MIER1	1.61	2.63	2.76	1.40E-45
98	MOB1A	1.25	2.59	2.6	1.40E-45
99	MPHOSPH10	2.15	5.84	5.58	5.61E-45
100	MRFAP1	1.2	2.55	2.53	1.40E-45
101	MRPL1	1.59	3.47	3.47	1.40E-45
102	MSANTD4	2.35	5.11	4.73	8.55E-44
103	NAP1L1	1.82	3.01	2.92	1.40E-45
104	NBPF20	1.89	2.82	2.78	7.01E-45
105	NBPF1	1.97	3.1	3.05	2.80E-45
106	NBPF14	2.03	3.08	3.02	2.80E-45
107	NBPF14	1.93	2.9	2.84	1.40E-45
108	NDUFA5	1.17	4.92	3.84	1.40E-45
109	NEMF	2.03	3.25	3.34	1.40E-45
110	NFYB	1.05	4.49	4.4	1.40E-45
111	NMD3	1.85	4.87	4.9	1.40E-45
112	NUP107	1.76	4.82	4.98	2.80E-45
113	OPA1	2.68	4.85	4.81	1.40E-45
114	PAFAH1B1	1.42	2.73	2.66	1.40E-45
115	PDIA3	1.9	3.65	3.53	1.40E-45
116	PDIA3	1.88	4.32	4.11	1.40E-45
117	PDIA6	1.89	4.64	4.68	1.40E-45
118	PHF14	1.92	3.4	3.25	1.40E-45
119	PI4K2B	1.51	3.52	3.31	1.40E-45
120	PITPNB	1.68	3.99	4.05	1.40E-45
121	PLRG1	1.97	4.58	4.65	1.40E-45
122	PNN	2.17	6.05	5.67	1.40E-45
123	POLR2B	2.23	5.36	5.43	1.40E-45
124	PPP3R1	1.34	1.97	1.96	1.40E-45
125	PPP4R2	2.17	4.78	4.54	1.40E-45
126	PPP4R3A	1.52	2.33	2.4	1.40E-45
127	PRKAR1A	1.29	2.1	1.97	1.40E-45
128	PRPF38B	1.71	2.94	3.06	1.40E-45
129	PRPF39	1.6	4.02	3.89	1.40E-44
130	RAB18	1.84	3.24	3.11	1.40E-45
131	RAB1A	1.8	3.37	3.38	1.40E-45
132	RABEP1	2.25	3.29	3.3	5.61E-45
133	RABGGTB; ACADM	1.63	3.36	3.27	1.40E-45
134	RAD21	2.04	4.31	4.07	2.80E-45
135	RANBP2	2.04	4.04	4.03	1.40E-45
136	RB1CC1	2.21	4.39	4.2	2.80E-44
137	RBM34; ARID4B	1.48	2.89	3.15	1.40E-45

138	RCHY1	1.57	3.52	3.38	1.40E-45
139	RECQL	2.15	4	4.14	9.81E-45
140	RNF219	1.99	4.86	5.22	1.40E-45
141	RNF6	2.56	4.01	3.87	9.25E-44
142	RNMT	2.02	4.42	4.47	1.40E-45
143	ROCK1	3.18	4.77	4.68	8.13E-44
144	RSF1	2.23	4.78	4.3	1.40E-45
145	RSL24D1	1.98	3.35	3.12	1.40E-45
146	SBNO1	2.27	5.62	5.34	5.89E-44
147	SEC23IP	1.54	3.52	3.7	1.40E-45
148	SEC62	1.75	3.25	3.06	1.40E-45
149	SESN3	1.67	5.69	5.22	1.26E-44
150	SKIV2L2	2.24	4.62	4.79	8.41E-45
151	SLC33A1	1.25	3.34	3.27	2.80E-45
152	SLC35A3	1.16	3.34	3.17	1.40E-45
153	SLC35D1	1.27	3.88	3.81	1.40E-45
154	SLTM	2.49	4.31	4.26	1.40E-45
155	SLU7	3.09	6.21	6.25	1.40E-45
156	SMARCA5	2.04	3.87	3.78	9.81E-45
157	SNX4	1.77	3.98	3.68	1.40E-45
158	SREK1	2.31	4.79	4.68	1.40E-45
159	SRSF10	1.79	3.21	3.17	1.40E-45
160	SRSF4	1.67	2.82	2.81	1.40E-45
161	STAG1	2.06	4.02	3.87	1.40E-45
162	STXBP3	1.67	3.02	2.86	3.08E-44
163	SYF2	1.73	3.56	3.41	1.40E-45
164	TAX1BP1	2.83	4.29	4.03	1.40E-45
165	TBC1D23	1.96	2.58	2.65	1.40E-45
166	TCEA1	1.74	3.15	2.88	1.40E-45
167	TCEA1	1.85	3.66	3.29	8.41E-45
168	THUMPD1	2	3.48	3.29	1.40E-45
169	TM9SF3	1.58	2.71	2.64	2.94E-44
170	TMEM167A	1.62	3.47	3.1	4.20E-45
171	TMF1	2.77	7.61	7.81	1.40E-45
172	TMX3	1.62	3.68	3.66	5.47E-44
173	TOP2B	2.41	5.69	5.1	1.40E-45
174	TPR	3.18	4.85	4.73	1.40E-45
175	TRAM1	1.28	3.07	3	1.40E-45
176	TRAPPC8	1.81	4.02	4.01	1.40E-45
177	TSN	1.42	2.42	2.32	1.40E-45
178	TSPAN3	1.23	2.63	2.53	1.40E-45
179	TTC3	2.55	4.71	4.32	1.40E-45
180	TVP23B	1.56	3.01	2.75	1.40E-45
181	TYW3	1.79	3.54	3.49	1.40E-45
182	UBA5	1.82	3.31	3.07	5.61E-45
183	UBE2V2	1.29	2.74	2.59	1.40E-45
184	UBE3A	1.46	2.95	2.88	2.66E-44

185	UBXN4	2.32	3.69	3.65	1.12E-44
186	UHMK1	1.31	3.55	3.43	1.40E-45
187	UHRF1BP1L	2.26	2.95	2.94	1.40E-45
188	USP1	1.89	3.59	3.34	9.81E-45
189	USP16	2.92	3.41	3.55	1.40E-45
190	USP33	1.32	3.62	3.51	8.69E-44
191	USP47	2.29	6.47	6.4	4.20E-45
192	VPS4B	1.56	3.02	2.84	1.40E-45
193	WDR35	1.64	4.4	4.13	4.76E-44
194	YTHDC1	1.89	3.3	3.29	1.40E-45
195	ZC3H13	2.7	4.25	4.4	8.41E-45
196	ZFYVE16	2.05	3.47	3.38	7.43E-44
197	ZNF23	1.11	2.54	2.55	9.67E-44
198	ZNF841	2.28	6.81	7.29	1.40E-45

Table 3(ii): 198 DEGs. commonly upregulated genes out of total 353 in PAH, PF with PH and PF with no PH compared to healthy control with condition FDR F<1E-43.

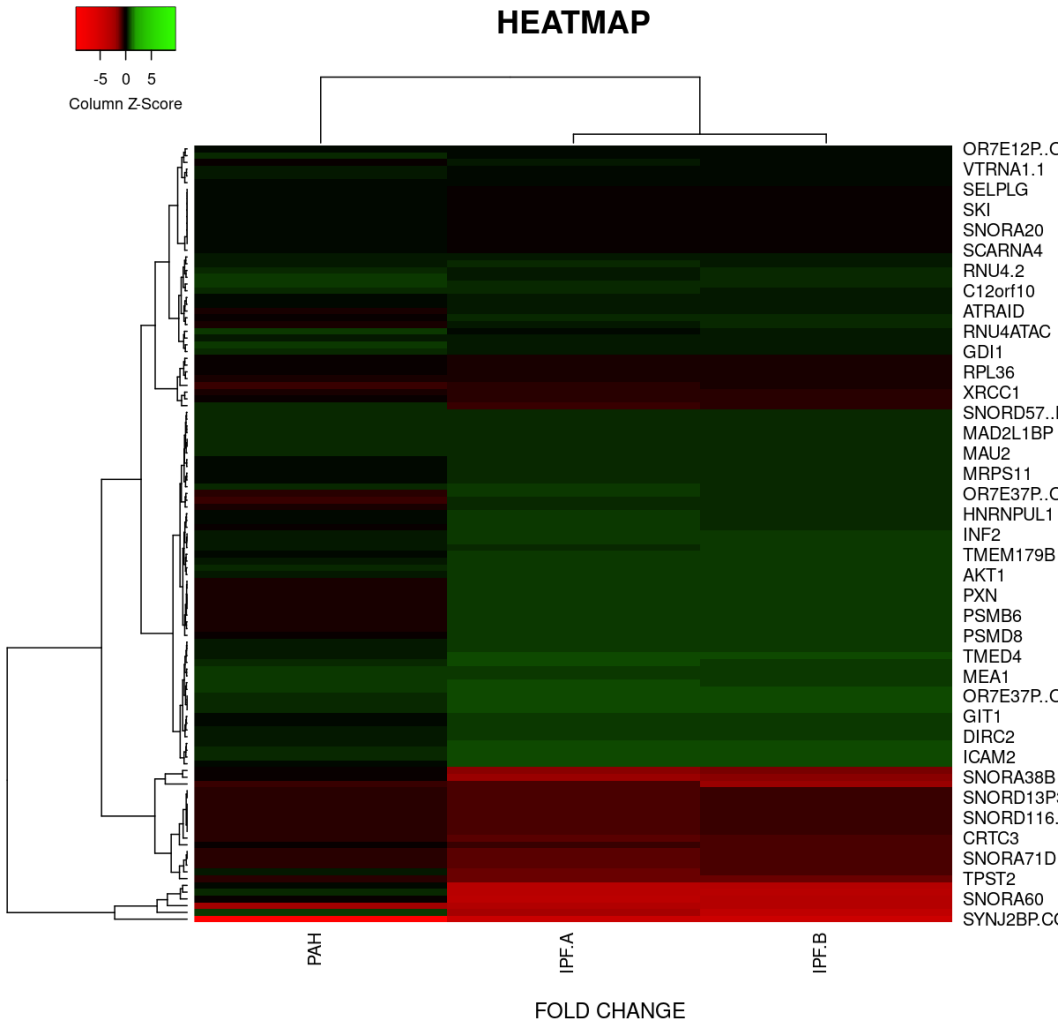
S.NO.	Gene Symbol	A vs C Fold Change	B vs C Fold Change	D vs C Fold Change
1	ATMIN	1.34	-1.84	-1.88
2	MAP1LC3B2	1.34	-2.36	-2.28
3	POMP	1.04	-3.11	-2.89
4	PPP6C	1.22	-1.66	-1.59
5	PTMAP3	1.07	-1.66	-1.64
6	CDK5RAP3	-1.03	3.06	2.86
7	CREBZF	-1.12	2.69	2.43
8	ND6	-1.11	11.08	10.05
9	SCARNA17	-1.26	3.51	3.55
10	SOD1	-1.11	2.07	1.94

Table 4: Differentially up and down regulated 10 significant genes in PAH with respect to PF with and without PH base line control with condition FDR F test value F<1.40E-45.

3.6 DEGs in PAH and PF (with PH and PF without PH)

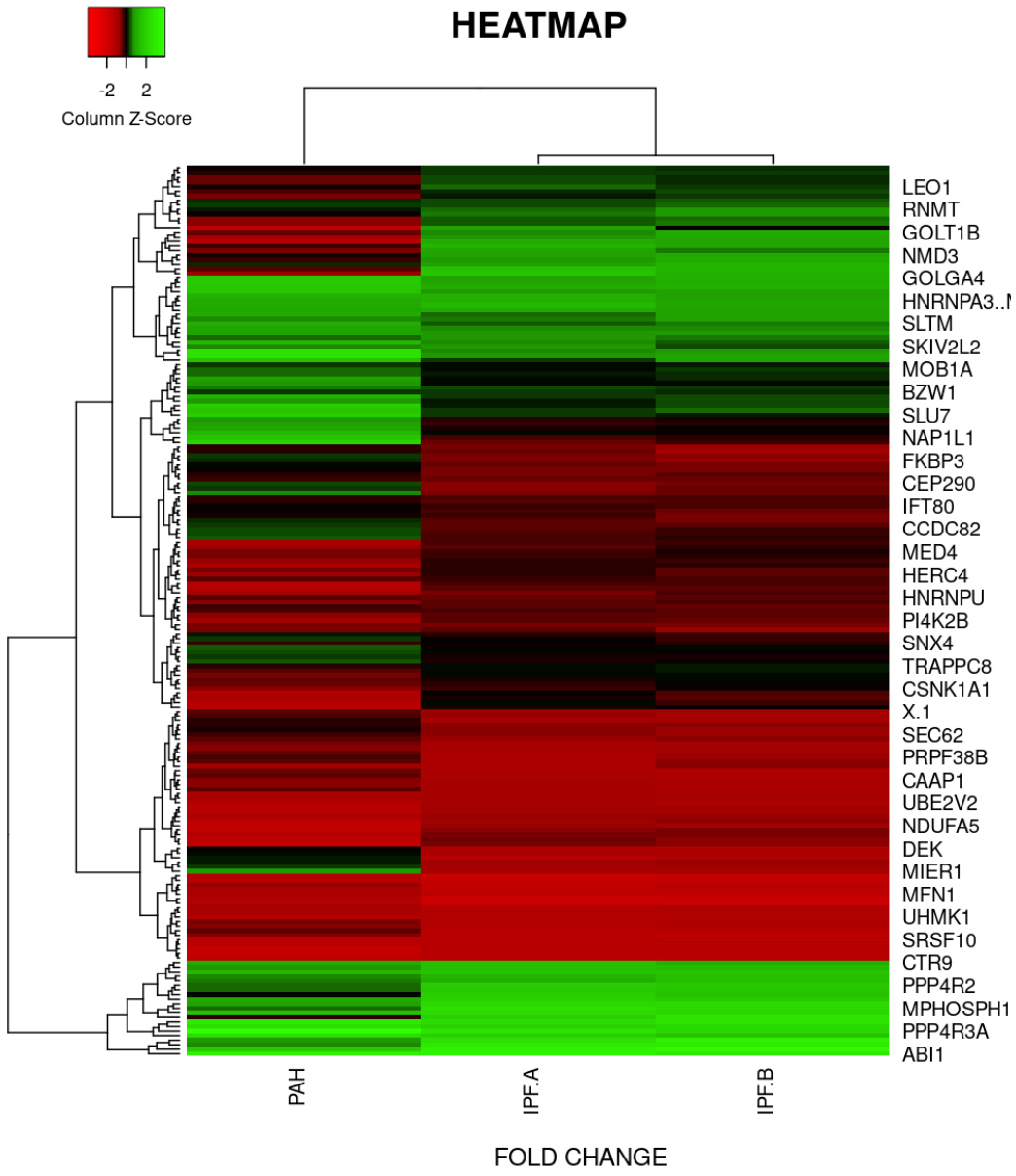
On the basis of fold change as shown in the heatmap (Figure 6) top 5 DEGs among the total commonly downregulated in PAH and PF both were namely (OR7E12P, VTRNA1.1, SELPLG, SKI, SNORDA20) and among the commonly upregulated, top 5 DEGs were (LEO1, RNMT, GOLT1B, NMD3, GOLGAH) as shown in (Figure 7). Whereas, PAH showed differential regulation in 10 DEGs) (Figure 8) on comparison of PAH and PF (with PH and PF without PH) including 5 upregulated DEGs. in PAH (PTMAP3, PPP6C, ATMIN, MAPILC, POMP) and 5 downregulated DEGs. in PAH (CREBZF, CDK5RA, SOD1,SCARNA, ND6. Differentially regulated top 5

upregulated DEGs in PAH were plotted on log2 gene expression level to observe the intensities of each gene expression (Figure 9) (Table 5).



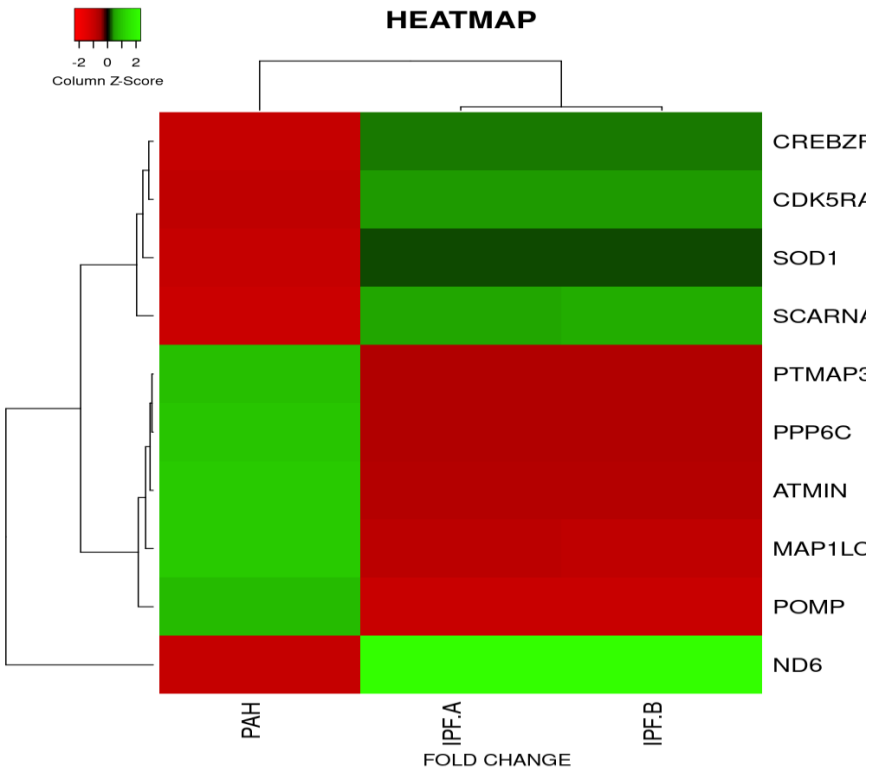
PAH*- PAH, **PF-A***- PF WITH PH , **PF-B***- PF WITH NO PH

Figure 6: Heat map and dendogram shows, Hierarchical Cluster analysis of the top 39 DEGs. commonly downregulated in both groups (PAH and PF) out of 353 total DEGs. ,with FDR condition F test (F< 1E-43). Distance metric used between objects is the Euclidean distance. Distances between clusters of objects are computed using the complete linkage method.



PAH*- PAH, **PF-A***- PF WITH PH , **PF-B***- PF WITH NO PH

Figure 7: Heat map and dendogram shows, hierarchical cluster analysis of the top 39 DEGs. commonly upregulated in both groups (PAH and IPF) gene out of 353 with FDR condition F test (F< 1E-43). Distance metric used between objects is the Euclidean distance. Distances between clusters of objects are computed using the complete linkage method.



PAH*- PAH, **PF-A***- PF WITH PH , **PF-B***- PF WITH NO PH

Figure 8: Heat map and dendogram shows, hierarchical cluster analysis of the top 10 Differentially regulated DEGs. in PAH out of 353 genes with FDR condition F test (F < 1E-43). Distance metric used between objects is the Euclidean distance. Distances between clusters of objects are computed using the complete linkage method.

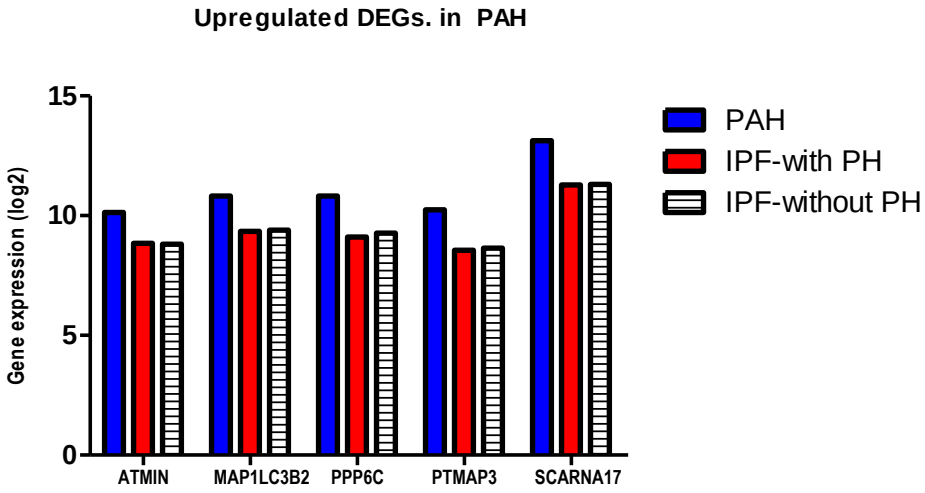


Figure 9: Gene expression level in log2 manner for 5 differentially upregulated genes in PAH.

Gene Symbol	PAH(log2)	IPF-A(log2)	IPF-B(log2)
ATMIN	10.13	8.84	8.81
CDK5RAP3	8.53	7.29	7.42
CREBZF	10.05	7.81	7.93
MAP1LC3B2	10.82	9.34	9.4
ND6	10.46	11.19	11.14
POMP	9.87	10.81	10.77
PPP6C	10.82	9.1	9.27
PTMAP3	10.24	8.55	8.65
SCARNA17	13.13	11.28	11.31
SOD1	9.78	10.82	10.88

Table 5: Differentially up regulated 10 significant genes expression (log 2) in PAH with respect to PF with and without PH base line control with with condition FDR F<1E-43.

3.7 Pathway and gene ontology associated with PAH and PF (with PH and PF without PH)

On submitting a total of 343 DEGs commonly regulated in PAH and PF, REACTOME gave 48 significant pathways () showing involvement of 14 genes (Table 6). And among 10 differentially expressed genes in PAH, 3 genes were respectively involved in 6 pathways such as (Interleukin-12 signaling, Complex I biogenesis etc) (Table 7). Gene ontology by PANTHER for 10 differentially regulated genes gave its associated biological processes (such as cellular component biogenesis, metabolic processes, biological regulation) (Figure 10).

s.no.	Submitted entities found	Pathway name	Entities pValue
1	ABI1;AKT1;BRK1	VEGFA-VEGFR2 Pathway	2.05E-04
2	ABI1;AKT1;BRK1	Signaling by VEGF	2.82E-04
3	CEBPD;AKT1	Interleukin-4 and Interleukin-13 signaling	0.001405
4	AKT1	CTLA4 inhibitory signaling	0.001571
5	AKT1	CD28 dependent PI3K/Akt signaling	0.001696
6	CCND3;CEBPD	Transcriptional regulation of white adipocyte differentiation	0.002012
7	AKT1	G beta:gamma signalling through PI3Kgamma	0.002101
8	AKT1	Constitutive Signaling by AKT1 E17K in Cancer	0.002547
9	AKT1	CD28 co-stimulation	0.003745
10	AKT1	G-protein beta:gamma signalling	0.003745
11	ABI1;BRK1	RHO GTPases Activate WASPs and WAVEs	0.004127
12	AKT1	VEGFR2 mediated vascular permeability	0.004733
13	CEBPD	Defective SLC24A1 causes congenital stationary night blindness 1D (CSNB1D)	0.009227
14	ABI1;AKT1;BRK1;ATP6V1C1	Signaling by Receptor Tyrosine Kinases	0.013647

15	AKT1	AKT-mediated inactivation of FOXO1A	0.01381
16	CEBPD;AKT1;ARF5	Signaling by Interleukins	0.015185
17	AKT1	Costimulation by the CD28 family	0.021312
18	ARF5	Nef Mediated CD4 Down-regulation	0.022913
19	AKT1	PTK6 Regulates RTKs and Their Effectors AKT1 and DOK1	0.025176
20	ARF5	COPI-dependent Golgi-to-ER retrograde traffic	0.025563
21	ARF5	COPI-mediated anterograde transport	0.025563
22	AKT1	AKT phosphorylates targets in the nucleus	0.027434
23	AKT1	Regulation of localization of FOXO transcription factors	0.031935
24	AKT1	RUNX2 regulates genes involved in cell migration	0.031935
25	AKT1;COX5B	TP53 Regulates Metabolic Genes	0.034
26	CEBPD	Sodium/Calcium exchangers	0.034178
27	AKT1	AKT phosphorylates targets in the cytosol	0.036416
28	AKT1	Downregulation of ERBB2:ERBB3 signaling	0.036416
29	AKT1	PI3K/AKT Signaling in Cancer	0.038573
30	AKT1	Negative regulation of the PI3K/AKT network	0.038573
31	CCND3	Defective binding of RB1 mutants to E2F1,(E2F2, E2F3)	0.038648
32	CCND3	Aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects	0.038648
33	AKT1	Regulation of TP53 Activity through Association with Co-factors	0.038648
34	AKT1	Activation of BAD and translocation to mitochondria	0.043099
35	AKT1	Butyrate Response Factor 1 (BRF1) binds and destabilizes mRNA	0.043099
36	AKT1	KSRP (KHSRP) binds and destabilizes mRNA	0.045316
37	CEBPD	Activation of the phototransduction cascade	0.045316
38	ARF5	Golgi-to-ER retrograde transport	0.046124
39	COX5B;ATP5G1	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.	0.047245
40	ARF5	Nef-mediates down modulation of cell surface receptors by recruiting them to clathrin adapters	0.049737

Table 6: Rectome pathway analysis for commonly regulated DEGs out of which 12 significant genes regulate 40 significant pathways.

S.NO.	GENE NAME	Pathway name	Entities pValue
1	SOD1	Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	0.001321316
2	SOD1	Interleukin-12 signaling	0.001741671
3	SOD1	Interleukin-12 family signaling	0.002263701
4	PPP6C	Telomere Extension By Telomerase, EGF receptor signaling pathway, FGF signaling pathway (Panther)	0.025195724
5	ND6	Complex I biogenesis	0.041911153
6	SOD1	Detoxification of Reactive Oxygen Species	0.047663852

Table 7: Rectome pathway analysis for 10 differentially regulated DEGs out of which 3 significant genes regulate 06 significant pathways.

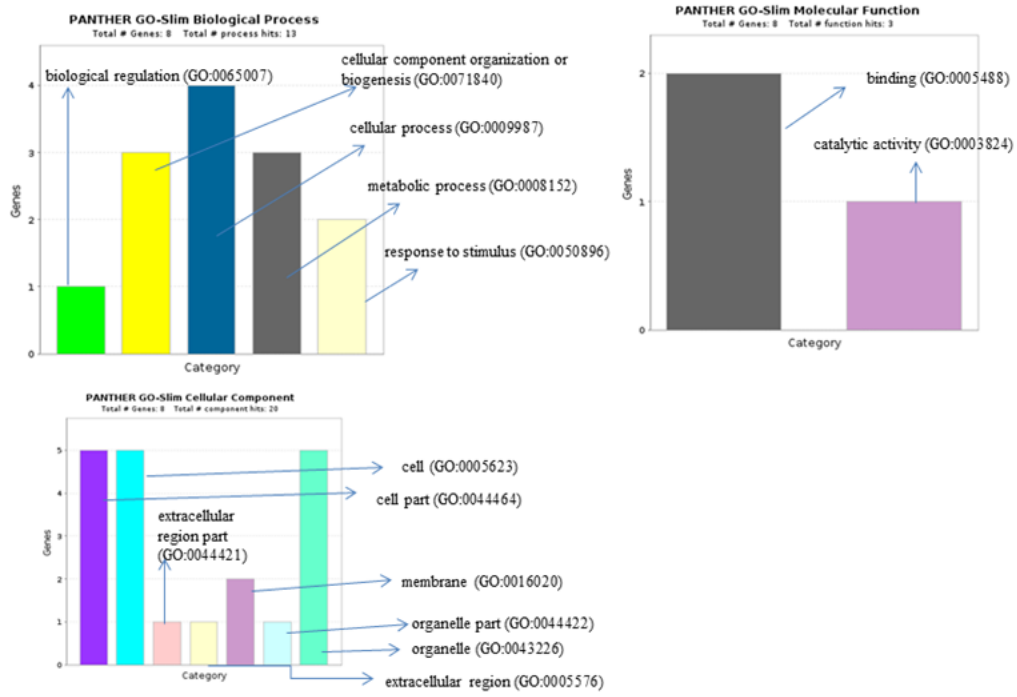


Figure 10: Panther analysis giving biological process, cellular component and molecular function of 10 differentially regulated DEGs. in PAH.

4. Discussion

The primary objective of a microarray experiment is to classify the gene expression trends in living organisms due to disease, tolerance against pathogen, a chemical compound or a certain relevant condition. For every gene, the microarray analysis tests the intensities, which means its relative degree of expression. Nevertheless, in order to

eliminate low-quality measurements, correct calculated intensities, simplification of comparisons and screening of genes that are substantially differentially expressed between samples is carried out on the data before properly associating these rates [21]. Thus, normalization is the first conversion step applied to expression data to fine-tune the individual hybridization intensities in order to be able to interpret significant biochemical associations [22].

In the present study, TAC4.0 microarray analysis revealed a total of 353 differentially expressed genes assigned with gene symbol on analyzing all the 88 lung biopsy samples with filter condition FDR F TEST (F<1E-43) within 3 groups, which are commonly or differentially regulated. 145 genes out of the total 353 get commonly downregulated and 198 gets commonly upregulated when comparing the PAH and the PF groups, proving as common markers for lung disease were as, the other 10 genes are differentially expressed in PAH between the three groups indicating group specific biomarkers. Pathway analysis through REACTOME tool for all the commonly regulated genes, with P value (P<0.05) identified 14 highly significant genes involved in 48 associated pathways and top 10 differentially regulated genes in PAH submitted to REACTOME showed output of 3 significant DEGs. involved in 06 significant pathways.

Among the top 10 differentially expressed genes, 2 (SOD1,SCARNA17) have already been reported depicting their association in idiopathic lung fibrosis and pulmonary arterial hypertension, i.e. the methyl transferase inhibitor EZH2, EPZ005687 substantially inhibits the production of TAC-induced PAH depending upon EZH2-SOD1-ROS signaling [23]. SCARNA17 (Small Cajal Body-Specific RNA 17) an RNA Gene, affiliated with the lncRNA class recently identified by microRNA expression profiling of bronchoalveolar lavage fluid cells from patients with idiopathic pulmonary fibrosis and sarcoidosis is known to be involved in IPF [24].

Going with our findings and exploring our 8 novel genetic PAH identifiers, the first one being CREBZF (CREB/ATF BZIP Transcription Factor) a coding gene is involved in multiple processes such as (negative regulation of gene expression, epigenetic modulation, negative transcription regulation, virus response, DNA-dependent regulation). Disease associated with CREBZF includes Acute Necrotizing Encephalitis. Although this gene is well explored in liver in, lipogenesis, liver regeneration and lipogenic pathway [25, 26, 27] however, we are reporting it for the first time to be involved in the genetic cause of PAH .CDK5RAP3 (CDK5 Regulatory Subunit Associated Protein 3) encodes a protein that has been reported to function in signaling pathways governing transcriptional regulation and cell cycle progression. It is known to play role in tumorigenesis and metastasis as reported in various cancers like as a tumour suppressor, CDK5RAP3 negatively controls self-renewal and invasion, and is regulated by ERK1/2 in human gastric cancer [28]. CDK5RAP3 Participates in Regulation on Autophagy and is Downregulated in Renal Cancer [29]. Lung adenocarcinoma falls under the umbrella of non-small cell lung cancer

(NSCLC) and has a strong association with previous smoking. CDK5RAP3, CCNB2, and RAGE Genes are already being researched to be involved in Lung Adenocarcinoma diagnostics [30]. We thereby indicate its sole involvement in causing PAH. POMP protein (Proteasome Maturation Protein) is a molecular chaperone that binds components of 20S preproteasome, and is necessary for the creation of 20S proteasome. The 20S proteasome is the active

proteolytic portion of the 26S proteasome complex. POMP is already studied in Extracellular Alveolar Proteasome involved in Lung Injury and Repair [31] however its genetic cause in PAH is being reported by us.

ATMIN (ATM Interactor) protein plays a key role in the development of cell survival and RAD51 foci in response to damage of methylating DNA. It is majorily involved in the regulation of ATM's activity in the absence of DNA damage. ATM Signaling Network in Development and Disease are its associated pathways. Gene Ontology annotations relating to ATMIN gene includes DNA binding for the transcription regulatory region. Till now this gene is well studied in lung morphogenesis [32] and ciliogenesis, lung adenocarcinoma [33, 34], lung cancer [35], however our analysis has shown its involvement in PAH cause. MAP1LC3B2 (Microtubule Associated Protein 1 Light Chain 3 Beta 2),ubiquitin-like modifier involved in autophagosomal vacuole formation (autophagosomes). Plays a role in mitophagy that helps to control mitochondrial quantity and efficiency by removing the mitochondria at a baseline level to meet cellular energy requirements and avoid excess development of ROS. MAP1LC3B2 is studied in various studies involved in lung epithelial cell autophagy; Elastase causes autophagy of the lung epithelial cells by a placental growth factor: a new perspective into emphysema pathogenesis [36] and also in lung fibroblast studies [29]. Our results have also shown its significant expression in PAH. PTMAP3 (Prothymosin Alpha Pseudogene 3) is a pseudogene till now studied in corneal dystrophy a group of rare genetic eye disorders in which abnormal material builds up in the cornea most corneal dystrophies affect both the eyes, this progress slowly and runs in the families [37]. This gene is not much studied and our analysis gives insight to further explore this gene in being one of the genetic causes of PAH. PPP6C (Protein Phosphatase 6 Catalytic Subunit) gene encodes the protein phosphatase catalytic subunit, a component of the signalling pathway which regulates the progression of the cell cycle. PPP6C-related disorders include Pineal Parenchymal Tumor with Intermediate Differentiation and Crouzon Syndrome of Acanthosis Nigricans. Also it is studied in human glioma cells, the expression AEG-1 is correlated with levels of CD133 and PPP6c in human glioma tissue [38]. Its involvement in the PAH disease is indicated by our analysis. MT-ND6 (Mitochondrially Encoded NADH: Ubiquinone Oxidoreductase Core Subunit 6), Core subunit of NADH dehydrogenase (Complex I), the mitochondrial membrane respiratory chain, which is assumed to belong to the minimum assembly necessary for catalysis. Complex I acts for electron transport from NADH into the respiratory chain. Commonly associated diseases with MT-ND6 include Leber Optic Atrophy, Dystonia and Leber Optic Atrophy.

5. Conclusion

We proposed a method for the meta-analysis of transcriptomics studies in this article using overall effect size z score (Z=3.79), P value (P=0.0002), hetrogenity I2=43%) and confidence interval of 95%, which provides increase power for precession. Study reflects broad spectrum of PAH and its significant early biomarkers. In conclusion, a

significant of 198 DEGs. commonly upregulated and 145 commonly downregulated genes with 5 up and 5 down differentially regulated DEGs. were identified from a total of 353 DEGs. including all three groups PAH, IPF with and without PH. Reactome pathway analysis for 343 commonly regulated DEGs out of total 353 gave 14 significant genes which regulate 48 significant pathways (such as-VEGFA-VEGFR2 Pathway, Interleukin-4 and Interleukin-13 signaling etc..) whereas, for 10 differentially regulated DEGs out of total 353, 3 genes show regulation of 06

significant pathways (such as- Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation, Interleukin-12 signaling et.). Gene ontology of 10 differentially regulated genes in PAH through Panther were involved in biological processes (such as-cellular component organization or biogenesis, metabolic pathways etc..), molecular function (such as-binding, catalytic activity) and cellular component (such as-cell and organelle etc..). Among the differential significant 10 genes a total of 2 (SOD1, SCARNA17) are already reported in PAH and IPF proving pivotal in PAH pathogenesis as indicated by our results also. (CREBZF, CDK5RAP3, POMP, ATMIN, MAP1LC3B2) genes are previously explored and reported in various lung disorders (not in PAH or IPF) but are novel identifiers in PAH as per our analysis .However, (MAP1LC3B2, PPP6C, MT-ND6) are totally novel genes obtained giving future prospects for these findings to contribute in better understanding of PAH pathogenesis, and provide a theoretical basis for further experimental studies.

Disclosure

All the authors declared no competing interests.

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