Gene Expression in Peripheral Blood can Discriminate Early from Dual Responses in Asthmatic Individuals Undergoing Allergen Inhalation Challenge

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Introduction

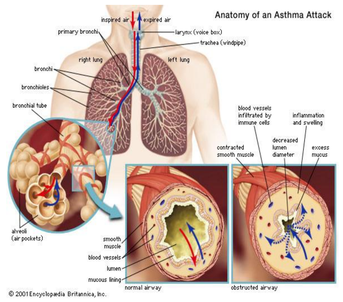


Figure 1. Asthmatic Response. A. Anatomy of an Asthma Attack. B. Lung function of early and dual responders undergoing allergen inhalation challenge.

- Upon allergen inhalation, the airways of asthmatic individuals contract; a reversible contraction (early phase) that resolves within 30min to 1h after inhalation.
- 50-60% of individuals that exhibit the early response go on to develop a later more severe airway contraction (late phase) 3-4h after allergen inhalation. This is characterized by mucus secretion, increase in vascular permeability and immune cell infiltration (Figure 1).
- Asthmatic individuals that exhibit only the early phase response are called early responders (ERs), whereas those exhibiting both the early and late phases are called dual responders (DRs).

Hypothesis

- Gene expression changes in peripheral blood of asthmatic individuals undergoing allergen inhalation challenge can discriminate ERs from DRs.

Materials and Methods

- Eight ERs and six DRs participated in a cat allergen inhalation challenge.
- Whole blood was collected immediately prior to challenge (pre) and 2 hours post-challenge; complete blood cell counts and differentials were also obtained.
- Whole blood transcriptome profiling was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays.
- The Robust Multiarray Average (RMA) was used to preprocess the microarray data, using the 'affy' package.
- Differential gene expression analysis in whole blood was carried out using moderated robust regression (LIMMA: Linear Models for Microarray), and moderated univariate t-tests (SAM: Significance Analysis of Microarrays) .
- Cell-specific Significance Analysis of Microarrays[1] (csSAM); a moderated multiple regression approach was used to deconvolute whole blood gene signatures to the five white blood cell-types.
- A Benjamini Hochberg false discovery rate (FDR) of 5% was used to assess differential gene expression.
- Gene set enrichment analysis (Erminej[2], and LRpath[3]) and Ingenuity Pathway Analysis was used for biological interpretability.

Results

Asthma Cohort

Patient ID	Age (yr)	Sex (M:F)	Pre PC20 (mg/ml)	Post PC20 (mg/ml)	Allergen Induced Shift	% Fall in FEV_1 Early	% Fall in FEV_1 Late
ERs							
Mean ± SE	32.63 ± 2.19	3:5	2.84[a]	7.50[a]	0.99 ± 0.40	-29.55 ± 3.15	-5.13 ± 1.40
DRs							
Mean ± SE	33.8 ± 5.31	1:5	1.27[a]	0.51[a]	2.97 ± 0.77	-34.73 ± 3.24	-21.28 ± 3.23*

Table 1. Subject demographics.
ND - Not determined; a: geometric mean (PC_{20} values are measured on a log scale); *p<0.05 versus ER group

Allergen Inhalation Challenge

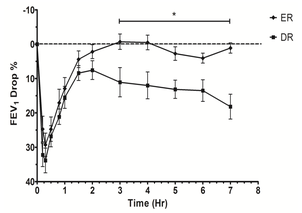


Figure 2. FEV_1 of individuals undergoing allergen inhalation challenge.

- 14 adult subjects (Table 1) with allergic asthma participated in the ethically approved cat allergen inhalation challenge.
- The two response classes had significant differences in their lung function (Figure 2).
- Inclusion criteria included non-smokers with stable, mild atopic asthma, free of other lung diseases.
- Leukocyte-enriched (buffy coat) and plasma fractions (multiple aliquots) were freshly prepared from the blood and frozen at -80ºC.

Statistical Analyses

Model Selection:

Model 1: Y=α + βType;
Model 2: Y=α + β_1Type + β_2Age;
Model 3: Y=α + β_1Type + β_3Sex;
Model 4: Y=α + β_1Type + β_2Age + β_3Sex;
Y = post gene expression minus pre gene expression
Type = 0- early responders, 1- dual responders

	Model 1	Model 2	Model 3	Model 4
AIC	16588	6503	3901	**1740**
BIC	20526	5482	3232	**1094**

Note: lowest information criterion score is the best.

Differential Gene Expression Analysis

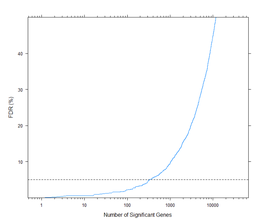


Figure 3. Differentially expressed genes at an FDR of 5%.

- 332 (99 over and 233 under-expressed) genes significantly changed (FDR=5%) in dual responders relative to early responders during the allergen inhalation challenge (Figure 3).
- These genes can be used to cluster individuals into their respective phenotypes (Figure 4A).
- Figure 4B shows the change in expression of (under and over-expressed) genes in DRs relative to ERs.

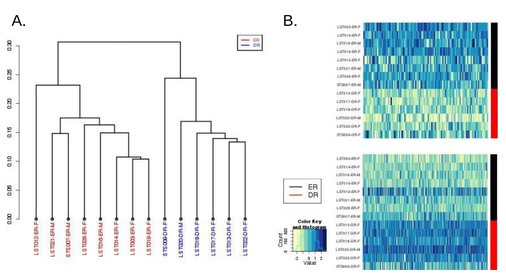


Figure 4. Dendrogram and Heatmaps. A. Clustering of individuals using 332 significant genes. B. Heatmaps of under and over-expressed genes in DRs relative to ERs.

Results continued

Gene set Enrichment Analysis

Erminej: Over Representation Analysis (GO Ontology)

Name	ID	Probes	NumGenes	Raw Score	P-val	Corrected P-value
alkaloid metabolic process	GO:0009820	11	9	1.85144	2.70E-040	0.52002
keratin filament	GO:0045095	97	78	1.09434	1.90E-04	0.54891
regulation of neutrophil chemotaxis	GO:0090022	20	19	1.43131	3.80E-04	0.54891
extracellular matrix structural constituent	GO:0005201	79	77	1.04413	7.40E-04	0.71262
hormone biosynthetic process	GO:0042446	67	57	1.10232	6.20E-04	0.71647
positive regulation of neutrophil chemotaxis	GO:0090023	16	15	1.58437	1.30E-04	0.75114

LRpath (KEGG pathways)

Category ID	Category Description	# of genes	coeff	Odds ratio	P value	FDR
hsa03010	Ribosome	87	-0.93	0.003	0.0003	0.03
hsa00591	Linoleic acid metabolism	28	0.70	76.17	0.0005	0.03
hsa00565	Ether lipid metabolism	34	0.64	52.55	0.0007	0.03
hsa04512	ECM-receptor interaction	83	0.44	15.85	0.0010	0.04
hsa00190	Oxidative phosphorylation	114	-0.63	0.02	0.0013	0.04
hsa04146	Peroxisome	77	-0.68	0.01	0.0053	0.12
hsa00590	Arachidonic acid metabolism	57	0.45	16.02	0.0056	0.12
hsa03430	Mismatch repair	23	-1.66	3.22E-05	0.0086	0.15
hsa00592	alpha-Linolenic acid metabolism	18	0.65	57.23	0.01	0.15

- The gene set enrichment analyses above indicate differential neutrophil chemotaxis using GO Ontology (Erminej) and linoleic and arachidonic acid metabolism using KEGG pathways (LRpath) between early and dual responders undergoing allergen challenge.

Ingenuity Pathway Analysis

- Figure 5 shows a representative network displaying the interaction between linoleic and arachidonic metabolites with the granulocyte receptor (IL5RA).

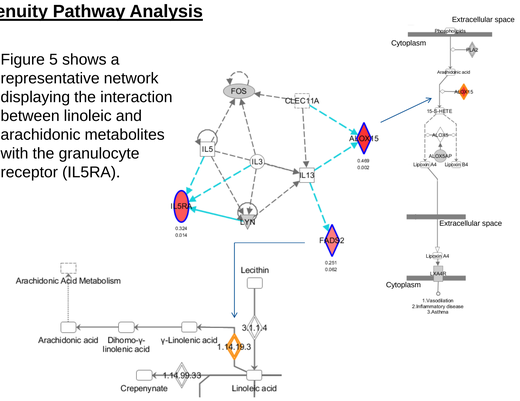
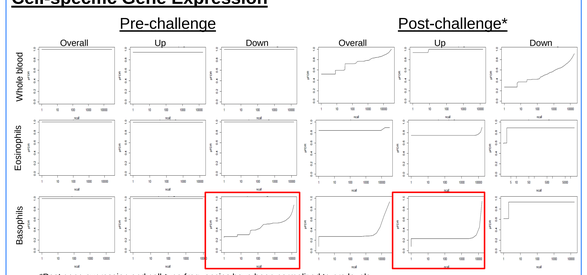


Figure 5. Network Analysis.

Cell-specific Gene Expression



*Post gene expression and cell-type frequencies have been normalized to pre levels.

Figure 6. Deconvolution of whole blood to the five white blood cells.

- Although no significant differences in cell-type frequencies between responders was found, deconvolution to various leukocyte frequencies, indicated a change in gene expression that was highly correlated with a change in basophil count in dual responders relative to early responders (Figure 6).

Discussion

- Genomic analysis in the peripheral blood can be used to discriminate early from dual responses in asthmatic subjects undergoing allergen inhalation challenge.
- A total of 332 genes were found to discriminate early from dual responders, with the capability of clustering individuals into their respective phenotypic classes (Figure 4A).
- Gene set analyses indicated genes enriched in neturophil chemotaxis and linoleic and arachidonic acid metabolism.
- Arachidonic acid metabolism induces the production of proinflammatory mediators such as prostaglandins and leukotrienes which lead to bronchoconstriction, increased endothelial membrane permeability leading to airway edema, and enhanced secretion of mucus.[4]
- Cysteinyl leukotrienes-1 receptor (CysLT1R) is expressed on both eosinophils and CD34+/IL-5Rα+ eosinophil progenitors; attracting these cells to the site of inflammation.[5]
- In addition, arachidonic acid metabolites are produced by eosinophils and mast cells and promote eosinophil survival.[6,7]
- Increased activation of pro-inflammatory pathways in dual responders relative to early responders may explain the clinical symptoms associated with the late response.

Limitations

- Reduced power due to small sample size. Increased sample sizes will be required for classification analysis.
- Misclassification of subjects in to their respective classes based on FEV_1 and PC_{20} concentrations may increase noise in the data and make it harder to detect phenotypic differences.

Future Directions

- Use appropriate pre-filtering methods in an effort to extract biological signal from technical noise, in order to achieve greater significance for our gene list.
- Verifying gene expression by using methods such as qPCR.
- Measure the protein and metabolite composition of the blood plasma, pre- and post-challenge in the same subjects.

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