Allergic asthma biomarkers using systems approaches

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INTRODUCTION

Asthma is characterized by lung inflammation caused by complex interaction between the immune system and environmental factors such as allergens and inorganic pollutants. Recent research in this field is focused on discovering new biomarkers associated with asthma pathogenesis. This review illustrates updated research associating biomarkers of allergic asthma and their potential use in systems biology of the disease. We focus on biomolecules with altered expression, which may serve as inflammatory, diagnostic and therapeutic biomarkers of asthma discovered in human or experimental asthma model using genomic, proteomic and epigenomic approaches for gene and protein expression profiling. These include high-throughput technologies such as state-of-the-art microarray and proteomics Mass Spectrometry (MS) platforms. Emerging concepts of molecular interactions and pathways may provide new insights in searching potential clinical biomarkers. We summarized certain pathways with significant linkage to asthma pathophysiology by analyzing the compiled biomarkers. Systems approaches with this data can identify the regulating networks, which will eventually identify the key biomarkers to be used for diagnostics and drug discovery.

Keywords: allergic asthma, biomarker, DAAB, TH-2 cytokines and ROS pathway

MOLECULAR BIOMARKERS IN ALLERGIC ASTHMA

Genomic studies have reported large number of candidate biomarkers through both high and low throughput techniques. Experiments were done on human, mouse, monkey and rat model systems by comparing the expression of genes through challenging them with inhalant allergens and monitoring at
different time intervals or by using resistant and susceptible strains of animals. Microarray based experiments reported hundreds of differentially expressed genes and hence plethora of information. Different samples like bronchial epithelial cells, eosinophils, CD4+ T-cells, mouse lungs have been employed in the experimental designs. The genes which showed significant differential expression were found to be linked with airway remodeling, production of mucus, macrophages and shifting the immune response toward Th2 phenotype thus enhancing asthma exacerbation (Laprise et al., 2004; Woodruff et al., 2007; Siddiqui et al., 2013). In most microarray experiments the differentially expressed genes were further validated either by RT-PCR or western blot. Genome Wide Association Study and Candidate gene approach have identified several regions on human chromosome which are linked to asthma phenotype. Nucleotide substitution in promoter region and ORF of IL4 receptor, IL13, HLA-II alleles, RANTES and CC-chemokine ligands were found to be strongly associated with asthma (Toda and Ono, 2002). We have compiled fifteen biomarkers from Database of Allergy and Asthma Biomarkers (DAAB)1 having more than two citations and listed in Table 1, out of which 11 were obtained from genomics.

In genomic studies of asthma several genes have been found to be significantly induced, of which some significant biomarkers are Chemokine ligands (CCL8, CCL5, CCL11, and CCL24), SERPINS (SERPINB2, SERPINB4, and SERPIN1A) and CarboxypeptidaseA3. These three genes have not been studied earlier in detail however they have the potential of being used as asthma biomarkers. Chemokine ligands are potent attractants of Th2 lymphocytes at the site of lung inflammation in atopic asthma (Lukacs, 2001). SERPINS are members serine protease inhibitors family which inhibit neutrophil protease cathepsin G and mast cell chymase and protects the lower respiratory tract from damage caused by proteolytic enzymes. Thus, it can be used as potent diagnostic marker of asthma attack (Zou et al., 2002). Carboxypeptidase A3 is an asthma associated protease identified in lung epithelium and is a significant mast cell marker and was found to be upregulated in 42 non-smoking asthma patients (Woodruff et al., 2007). Retnla, also known as Fizz (found in inflammatory zone) protein is an inducible product of bronchial epithelial cell. This is considered as a marker of alternatively activated macrophages and highly polarized Th2 responses. In Retnla deficient mice the severity of atopic response is increased dramatically, whereas the IL13 response is suppressed by Retnla in airway hyper-responsiveness (Pesce et al., 2009). NOS2A is a gene that encodes inducible nitric oxide synthase, iNOS which produce nitric oxide (NO) from T lymphocytes in response to proinflammatory cytokines in an asthma model (Ricciardolo et al., 2004). This NO assists in the development of reactive nitrogen species such as peroxynitrites leading to cellular injury in the airways (Gabazza et al., 2003). NOS2A was found to be upregulated in bronchial biopsies in a microarray study (Laprise et al., 2004) and a (CCTTT)9 polymorphism in the promoter region was associated with asthma phenotype studied in White population (Pascual et al., 2008) and some SNP’s were found on asthmatic children having Latino and Caucasian ancestry (Islam et al., 2010). These genes together with other mediators contribute to epithelial cell activation and dysfunction (Dougherty et al., 2010).

PROTEOMIC APPROACH

Proteomic approaches are widely used to identify the expression level and modification of proteins to understand the pathophysiology of asthma. Proteomic signatures of lung parenchyma, BAL fluid, Immune cells (CD3+ T cells or CD4+ T cells) from human or animal model have been used in different studies after experimental allergen challenge or after natural exposure to inhalant allergens. The advancement of proteomic techniques from earlier 2D gel based approach to recently more advanced LC-MS/MS based methodology resulted in precise identification of candidate proteins involved in asthma inflammation. In Table 1 we have listed six proteins identified in asthma proteomics studies, which can be analyzed in more detail to use them as clinical biomarkers. Similarly in asthma proteomics, a number of protein biomarkers have been identified, three of these potential biomarkers include AMCase (Chi3l3, Chi3l4, Chi3l1, and ChiT1), Calcium binding protein (S100A8 and S100A9), and Arginase (Arg1 and Arg2). These three proteins and their corresponding genes need further investigation at system level to reveal their use as potential diagnostic biomarkers.

AMCases are human chitinases induced via Th2 specific IL13 mediated pathway in aeroallergen challenged lung epithelium and macrophages as means of host defense. Th2 inflammation in asthma can be improved by targeted neutralization of these human chitinases (Zhu et al., 2004). A K(Lys)17R(Arg) polymorphism was identified in AMCase gene by genotyping study conducted on 322 pediatric asthma patients at University of Berlin and Freiburg (Bierbaum et al., 2005). Chi3l3 (Ym1) and Chi3l4 (Ym2) are other non-chitinolytic chitin binding proteins, have close linkage with asthma. Certain corticosteroids and leukotrienes receptor antagonist were shown to suppress the elevated pulmonary level of this protein (Zhu et al., 2004).

Calcium binding protein (S100A9/A8) form complex and inhibits macrophage activation and immunoglobulin synthesis by lymphocytes. Its homodimer also acts as a chemotactic agent for leukocytes and has pro-inflammatory activity on endothelial cell and inflammatory cells (Zhou et al., 2001). It is found in neutrophil cytoplasm and released upon cell activation (Cookson, 2002). This protein was found to be highly upregulated in endotoxin mediated response in non-smoking population challenged with endotoxin (Michel et al., 2013).

In asthmatic lung, Arginase expression is increased via Th2-induced, STAT6-dependent mechanism (Zimmermann et al., 2003). This affects arginine metabolism, and contribute to asthma pathogenesis through inhibition of NO generation and alterations of cell growth and collagen deposition (Shi et al., 2001). Association between four SNP’s in this gene and atopic asthma were identified by genotyping 433 asthmatic case-parent triads in a public hospital of Mexican city (Huiling et al., 2006).

BPIFA1 (also known as SPLUNC), is highly expressed in the upper airways and nasopharyngeal regions and thought to be involved in inflammatory responses to irritants in the upper 1http://bicresources.jcbose.ac.in/ssaha4/daab/1.
Table 1 | List of asthma biomarkers cited in two or more times in Database of Allergy and Asthma Biomarkers (DAAB).

| Gene Symbol | Name of the genes/proteins | Sample | Organism | Approach | References |
|-------------|-----------------------------|--------|----------|----------|------------|
| ARG1 | Arginase 1 | BAL macrophages, BAL Fluid | Mouse, human | G, P | Siddiqui et al., 2013 [GH] Wu et al., 2005 [PH] Torrone et al., 2012 [PH] Cloots et al., 2013 [GL] North et al., 2009 [PL] |
| BPIFA1 | Palate lung nasal epithelial clone | BAL Fluid and nasal lavage fluid | Human | P | Wu et al., 2005 [PH] Ghafoori et al., 2006 [PH] Chu et al., 2007 [PH] |
| CPA3 | Carboxypeptidase A3 | Airway epithelial cells, bronchoscopy tissue sample | Human, mouse | G | Woodruff et al., 2007 [GH] Laprise et al., 2004 [GH] Balzar et al., 2011 [GL] |
| CCL8 | Chemokine (C-C motif) ligand 8 | Left lung tissue, BAL macrophages | Mouse | G | Park et al., 2008 [GH] Siddiqui et al., 2013 [GH] Fu et al., 2013 [GL] |
| Chi3l3 | Chitinase 3-like 3 | BAL Fluid | Mouse | P | Greenlee et al., 2006 [PH] Zhao et al., 2005 [PH] Luten et al., 2012 [PL] |
| Chi3l4 | Chitinase 3-like 4 | BAL macrophages, BAL Fluid, | Human, mouse | G, P | Siddiqui et al., 2013 [GH] Webb et al., 2001 [GH] Greenlee et al., 2006 [PH] Zhao et al., 2005 [PH] Luten et al., 2012 [PL] |
| CLCA3 | Calcium activated chloride channel -3 | Airway epithelial cells, left lung tissue | Mouse | G | Woodruff et al., 2007 [GH] Park et al., 2008 [GH] Zhou et al., 2001 [GH] |
| Cxcl15 | Chemokine (C-X-C motif) ligand 15 | BAL Fluid | Mouse | P | Greenlee et al., 2006 [PH] Zhao et al., 2005 [PH] |
| IL10 | Interleukin 10 | Lung tissue, CD4+ T Cell | Mouse, human | G | López et al., 2011 [GH] Hansel et al., 2008 [GH] Lyon et al., 2004 [GH] |
| IL13 | Interleukin 13 | CD4+ T Cell, | Human | G, E | Hansel et al., 2008 [GH] Durham et al., 2011 [GH] Kanoh et al., 2011 [GH] |
| MUC5AC | Mucin 5AC | Bronchoscopy tissue sample, Left lung tissue | Mouse | G | Laprise et al., 2004 [GH] Park et al., 2008 [GH] Ordonez et al., 2001 [GH] |
| NOS2A | Nitric oxide synthase | Bronchoscopy tissue sample | Mouse | G, E | Laprise et al., 2004 [GH] Torrone et al., 2012 [E] Pascual et al., 2008 [GL] |
| Retnla | Resistin like alpha | Lung eosinophil, BAL macrophage | Mouse | G | Siddiqui et al., 2013 [GH] Tumes et al., 2009 [GH] Doherty et al., 2012 [GL] |
| SERPINB | Serpin peptidase inhibitor, clade B | Bronchoscopy tissue sample, airway epithelial cells | Human, mouse | G | Woodruff et al., 2007 [GH] Laprise et al., 2004 [GH] Karaaslan et al., 2012 [GL] |
| S100A9 | Calcium binding protein A9 | CD3+ T cell | Human | P | Wu et al., 2005 [PH] Jeong et al., 2007 [PH] Lee et al., 2013 [PH] |

G, Genomics; P, Proteomics; E, Epigenetics; BAL, Broncho alveolar lavage; H, High-throughput; L, Low-throughput.
airways (Barnes et al., 2008). A Sialylated form of BPIFA1 was observed as post translational modification and was identified as being predominant in nasal lavage fluid (NLF) of allergy rhinitis patients (Ghafoori et al., 2006).

**EPIGENOMIC APPROACH**

Epigenomics has emerged as a promising field, and have addressed the gaps in our current understanding of the interaction between nature and nurture in the development of asthma. Epigenetic modification can alter the DNA structure (by methylation, acetylation), the chromatin structure (by altering the Scaffolding protein) and by small non-coding RNAs. It was found that reduced Histone Deacetylase (HDAC) activity and increased Histone acetyl transferase (HAT) activity jointly promotes the expression of multiple inflammatory genes associated with asthma, however inhaled steroids reduce HAT activity to the normal level (Ito et al., 2002). External stimuli such as allergen exposure, cigarette smoke, traffic exhaust and folate rich diet cause methylation mediated silencing of genes like IFNγ, FoxP3, IL2, iNOS and hypomethylation mediated activation of genes like IL6, IL4, IL8, and Acryl CoA thus increasing the Th2 phenotype associated in the development of asthma (Durham et al., 2011). Usually IFN-γ and FOX-P3 undergo H4 acetylation and demethylation mediated activation to prevent post natal asthma and in-utero atopycity, respectively (Lovinsky-Desir and Miller, 2012). In the promoter region and other cis-acting element of two important Th2 cytokines like IL4 and IL13 demethylation causes recruitment of STAT6 and GATA3 thereby enhancing their expression (Miller and Ho, 2008). In addition to that small non-coding RNA plays a crucial role in fine epigenetic tuning of genes which are key factors in asthma pathophysiology (Durham et al., 2011). These include let-7, miR-9, miR-21, miR-125, miR-146a, miR-147, and miR-155. For example let-7 families of micro RNA and mi R-155 are found to inhibit expression of IL 13. This miRNA was found to block the IL13 R alpha 1 and ultimately lower the expression of STAT 6 thus controlling the Th2/Th1 balance in macrophages (Kumar et al., 2011 and Martinez-Nunez et al., 2011). An overexpression of miR21 and an underexpression of miR1 were demonstrated in IL-13 induced transgenic mice. This miR-21 was also found to control expression of IL12, a molecule responsible for Th2 mediated cellular response (Lu et al., 2009). A G/C polymorphism in miRNA146a gene locus resulted in a functional variant that in turn can significantly modulate expression of genes such as TNF-α, IL-6, Cox-2, iNOS, and RANTES that are closely linked with asthma pathophysiology (Jiménez-Morales et al., 2012). This polymorphism was found to have statistically significant association with a pediatric Mexican cohort.

**Integrated approaches**

We have compiled the asthma biomarkers from different approaches including genomics, proteomics and epigenetics and have found little overlap amongst them as shown in Figure 1A. Detailed molecular information of all asthma related biomarkers are stored in DAAB. All the genes compiled from the high-throughput experiments have significant value (p = 0.05) of fold change, validated further by low-throughput techniques such as PCR, blotting and hold significantly close association with asthma pathophysiology.

Furthermore, we have listed fifteen genes in Table 1, which have been cited for two or more times in DAAB database. Asthma is dependent on many factors and thus it develops as a consequence of crosstalk among different pathways. Thus, we analyzed all the genes in our dataset compiled from several literatures in order to identify the pathways containing these biomarkers. Figure 1B shows cytokine pathways, ROS metabolism, NO metabolism and certain other metabolic pathways were significantly enriched (Detailed information of Figure 1B is shown in Table A1). In addition, Gene ontology of the biomarkers is shown in Figures 1C,D (Detailed information of GO terms are shown in Tables A2, A3). Cytokine activity, growth factor activity and Arginase activity were found to be significantly enriched in molecular function analysis. With respect to biological process inflammatory response, immune response and cell proliferation were found to be considerably predominating.

The most significant pathway triggering asthma has been the adipocyte signaling pathway. A few significant genes such as ACSL3, IL13, IL9, IL4, IL2, IL10, IFNA1, SOCS1, PON1, APOB, SOCS3, SCD, and NR1D1 were found to be the component of this pathway and associated with asthma pathogenesis (Tilg and Moschen, 2006; Diego et al., 2012). Adipokine or adipocytokine are cytokines secreted by the adipose tissues. These include Th2 cytokines and chemokines such as MCP1, RANTES, which are potent attractants of mast cells. There are also several clinical observations suggesting the role of obesity with asthma and one of the major conclusions so far has been the action of adipocies derived cytokines which inhibit the activity of Tregs thus decreasing the tolerance (Theoharides et al., 2008). Cytokines such as TNFa, IL6 secreted by the adipocytes are important mediators of asthma. These molecules also affect vascular function by modulating nitric oxide and superoxide release. Some molecules such as leptin, adiponectin are the most abundantly expressed adipocytokines and are involved in classical cytokine pathway thus showing an asthmatic phenotype (Guzik et al., 2006).

Another significant pathway has been the ROS signaling pathway which is characterized by production of free radicals from molecular oxygen due to recruitment of activated inflammatory cells and associated with mitochondrial dysfunction that result in variety of physiological changes including increased airway reactivity, tissue injury and mucus production (Zuo and Clanton, 2005). Presently certain metabolites such as malondialdehyde, 8-isoprostane, exhaled NO, thiobarbituric acid are used as markers to measure the disease severity in sputum or exhaled air (Zuo et al., 2013). Several genes including MPO, PRDX6, SOD1, and CYBB as molecules involved in asthmatic responses and linked to ROS generation and hold the potential of using as biomarkers.

An additional significant pathway uncovered has been the Urea cycle and arginine metabolism. iNOS, ARG1, and ARG2 belong to this pathway and have also been found to be induced significantly in several genomic, proteomic, and epigenetic studies (North et al., 2009; Breton et al., 2011; Cloots et al., 2013). In asthmatic airway inducible NOS in inflammatory cells catalyses the production of NO from L-arginine, which results in the
formation of reactive nitrogen species (RNS) that alters protein function by nitration of tyrosine residues thereby mediating inflammation and injury. In asthmatics upregulation of Arginase limits the availability of L-arg to iNOS thus generating peroxynitrite and concomitant nitration of proteins. It also enhances the level of L-ornithine which promotes airway remodeling by collagen deposition and excess cell proliferation (Ghosh and Erzurum, 2011).

CONCLUSION
In the last few decades efforts to understand the pathophysiology of allergic asthma has been intensified to a great extent because of increased mortality and morbidity. The aim of the present review is to focus on genes or their products which can be used as biomarker for allergic asthma. Occurrence of allergic asthma involves multiple genes, environmental factors and epigenetic mechanisms. Presently the potential difficulties to diagnose this disease are due to (i) remarkable overlap in symptoms of other pulmonary diseases, (ii) high interindividual and interpopulation variation at genetic level leads to changes in the uniformity of molecular marker, and (iii) absence of discriminative molecular markers, specific to atopic asthma, since most of the biomarkers currently used or in clinical trial are indicative of asthmatic inflammation irrespective of atopic background. Some of the common features of asthma exacerbation are eosinophilic inflammation, collagenitis, mucus deposition and extracellular matrix formation. However, these are common characteristics of other lung inflammations such as Chronic Obstructive Pulmonary Disease (COPD) or, non-allergic asthma. Therefore, the genes involved in these phenotypes may also be induced in all kinds of lung inflammations. To develop diagnostic markers exclusively for “allergic asthma” it is necessary to identify upstream components of the molecular pathways initiated immediately after allergen sensitization. Researchers can use these biomarkers for screening and risk assessment before the disease assumes severity by (i) identifying polymorphisms in wide population and (ii) correlating them with the alteration of signaling pathways that ultimately lead to allergic asthma. Since application

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**FIGURE 1**

(A) Venn diagram showing asthma biomarkers identified in three different approaches of Genomics [G], Proteomics [P] and Epigenetic [E] studies with overlaps among the intersects. (B) Significant pathways, (C) enriched gene ontology molecular functions, and (D) biological function terms are listed which are linked with asthma biomarkers (Pathway and Gene Ontology analyses were done using Pathway studio 7.12, Ariadane Genomics, Rockville, MD, USA). Pathways are significant where (−log_{10}P) ≥ 1.3 (0.05% significance).
of single biomarker approach to asthma research may not be realistic, newly identified biomarkers can be integrated in a multi-dimensional way to strengthen the treatment. Our mini review is focused on biomarker discovery by systemic approach using high-throughput “OMICS” platforms including genomics, proteomics and epigenetics and further some of them are well-studied in low-throughput experiments. Application of systems biology as a discipline provides a way to investigate the pathophysiology of asthma by giving a closer look to the system components, its dynamics and response to any kind of perturbation in the population level. Systemic approaches may emerge as a promising strategy to zoom into the global mechanism and identify features specific to asthma for developing better diagnostics and therapeutics.

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REFERENCES
Balzar, S., Fajt, M. L., Comhhair, S. A., Erzurum, S. C., Bleecker, E., Busse, W. W., et al. (2011). Mast cell phenotype, location and activation in severe and moderate asthma: data from the severe asthma research program. Am. J. Respir. Crit. Care Med. 183, 299–309. doi: 10.1164/rccm.201002-0295OC
Barnes, F. A., Bingle, L., and Bingle, C. D. (2008). Pulmonary genomics, proteomics, and PLUNCs. Am. J. Respir. Cell Mol. Biol. 38, 377–379. doi: 10.1165/rcmb.2007-0388TR
Bierbaum, S., Nickel, R., Koch, A., Lau, S., Deichmann, K. A., Wahn, U., et al. (2011). Mast cell phenotype, location, and activation in severe asthma but not with systemic lupus erythematosus and juvenile rheumatoid arthritis in Mexican patients. Tissue Antigens 73, 139–145. doi: 10.1111/j.1399-0039.2012.01929.x
Ito, K., Caramori, G., Lim, S., Oates, T., Chung, K. F., Barnes, P. J., et al. (2002). Expression and activity of histone deacetylases in human asthmatic airways. Am. J. Respir. Crit. Care Med. 3, 392–396. doi: 10.1164/rcrm.2110060
Jeong, H. C., Lee, S. Y., Lee, J. I., Jung, K. H., Kang, E. H., Lee, S. Y., et al. (2007). Proteomic analysis of peripheral T-lymphocytes in patients with asthma. Chest 132, 489–496. doi: 10.1378/chest.06-2980
Jiménez-Morales, S., Gamboa-Becerra, R., Baca, V., Río-Navarro, D., López-Ley, D. Y., Velázquez-Cruz, R., et al. (2012). MiR-146a polymorphism is associated with asthma but not with systemic lupus erythematosus and juvenile rheumatoid arthritis in Mexican patients. Tissue Antigens 4, 317–321. doi: 10.1111/j.1399-0039.2012.01929.x
Kanoh, S., Hasebe, T., and Rubin, B. K. (2011). IL-13-induced MUC5AC production is regulated by STAT6-dependent acute airway eosinophilia in asthmatics. J. Immunol. 183, 2999–3008. doi: 10.4049/jimmunol.1101632
Kumar, M., Ahmad, T., Sharma, A., Mabalirajan, U., Kulshrestha, A., Agrawal, A., et al. (2011). Let-7 microRNA-mediated regulation of IL-13 and allergic airway inflammation. J. Allergy Clin. Immunol. 5, 1077–1085. doi: 10.1016/j.jaci.2011.04.034
Laitinen, L. A., Heino, M., Laitinen, A., Kava, T., and Haathtela, T. (1985). Damage to lung epithelium and the effect of promoter variants on asthma and gene function. Am. J. Physiol. Lung Cell. Mol. Physiol. 211, 195–206. doi: 10.1152/ajplung.1985.211.2.195
Laird, R. H., Dhanasekaran, S., De Theije, C. P., Poynter, M. E., Terwindt, E., Van Dijk, P., et al. (2013). Ablation of Arg1 in hematopoietic cells improves respiratory function of lung parenchyma, but not that of larger airways or epithelium and the effect of promoter variants on asthma and gene function. Respir. Med. 107, 368–379. doi: 10.1016/j.rmed.2012.11.003
Lei, T. H., Hwang, J. S., Kim, T. H., Choi, Y. S., Shin, H. R., et al. (2013). Early elevation of S100 calcium binding protein A9 in sputum of neutrophilic inflammation in severe uncontrolled asthma. Ann. Allergy Asthma Immunol. 111, 268–275. doi: 10.1016/j.anai.2013.06.028
Li, P., Li, X., and Liu, J. (2011). Systems biology and the role of systems biology in severe asthma. Biochim. Biophys. Acta 1812, 620–621. doi: 10.1016/j.bbagen.2011.03.006
Linsenmayer, T. L., Kao, Y., and Erzurum, S. C. (2011). Nitric oxide metabolism in asthma pathophysiology. Biochim. Biophys. Acta 11, 1008–1016. doi: 10.1016/j.bbamem.2011.06.009
Greenlee, K. J., Corry, D. B., Engler, D. A., Matsunami, R. K., Tessier, P., Cook, R. G., et al. (2006). Proteomic identification of in vivo substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation. J. Immunol. 10, 7312–7321.
Asthma biomarkers

López, E., Zafra, M. P., Sastre, B., Gámez, C., Lahoz, C., and del Pozo, V. (2011). Gene expression profiling in lungs of chronic asthmatic mice treated with galecin-3: downregulation of inflammatory and regulatory genes. *Mediat. Inflamm.* 2011, 823279. doi: 10.1155/2011/823279

Lourenço, J., Mattsson, J. D., Malinao, M. C., Li, Y., Emson, C., Vega, F., et al. (2012). Biomarkers of disease and treatment in murine and cynomolgus models of chronic asthma. *Biomark. Insights* 7, 87. doi: 10.4137/BML.S9776

Lovinsky-Desir, S., and Miller, R. L. (2012). Epigenetics, asthma, and allergic diseases: a review of the latest advancements. *Carr. Allergy Asthma Rep.* 12, 211–220. doi: 10.1186/s11882-012-0257-4

Lu, T. X., Munitz, A., and Rothenberg, M. E. (2009). MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. *J. Immunol.* 183, 4994–5002. doi: 10.4049/jimmunol.0803560

Lukaca, N. W. (2001). Role of chemokines in the pathogenesis of asthma. *Nat. Rev. Immunol.* 2, 108–116. doi: 10.1038/35010503

Lyons, H., Lange, C., Lake, S., Silverman, E. K., Randolph, A. G., Kwiatkowski, D., et al. (2004). IL10 gene polymorphisms are associated with asthma phenotypes in children. *Genet. Epidemiol.* 26, 155–165. doi: 10.1002/gepi.10298

Martinez-Nunez, R. T., Louafi, F., and Sanchez-Elsner, T. (2011). The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor α1 (IL13Rα1). *J. Biol. Chem.* 286, 1786–1794. doi: 10.1074/jbc.M110.169367

Michel, O., Doyen, V., Leroy, B., Bopp, B., Dinh, D. H., Corazza, F., et al. (2013). Expression of calgranulin A/B heterodimer after acute inhalation of endotoxin: proteomic approach and validation. *BMC Pulm. Med.* 13:65. doi: 10.1186/1471-2466-13-65

Miller, R. L., and Ho, S.-M. (2008). Environmental epigenetics and asthma: current concepts and call for studies. *Am. J. Respir. Crit. Care Med.* 177, 567. doi: 10.1164/rcrm.200710-1511PP

Murugan, A., Prys-Picard, C., and Calhoun, W. J. (2009). Biomarkers in asthma. *Carr. Opin. Pulm. Med.* 1, 12–18. doi: 10.1097/MCPB013e32831dc235

North, M. L., Khanna, N., Marsden, P. A., Grasseman, H., and Scott, J. A. (2009). Functionally important role for arginase 1 in the airway hyperresponsive-ness of asthma. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 296, L911–1920. doi: 10.1152/ajplung.00255.2009

Ordóñez, C. L., Khashayar, R., Wong, H. H., Ferrando, R., Wu, R., Hyde, D. M., et al. (2001). Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am. J. Respir. Crit. Care Med.* 163, 517–523. doi: 10.1164/ajcc.163.2.2004039

Park, S. G., Choi, J.-W., Kim, H. J., Roh, G. S., Jeong, B., Min, J. G., et al. (2008). Genome-wide profiling of antigen-induced time course expression using murine models for acute and chronic asthma. *Int. Arch. Allergy Immunol.* 146, 44–56. doi: 10.1159/000112502

Pascal, M., Sanz, C., Isidoro-Garcia, M., Davila, I., Moreno, E., Laffond, E., et al. (2008). (CCTTT)n polymorphism of NOS2A in nasal polyposis and asthma: a case-control study. *J. Investig. Allergol. Clin. Immunol.* 18, 239.

Pesce, J. T., Ramalingam, T. R., Wilson, M. S., Mentink-Kane, M. M., Thompson, D., et al. (2004). IL10 gene polymorphisms are associated with asthma and with treatment response to corticosteroids. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15858–15863. doi: 10.1073/pnas.0707413104

Wu, J., Kobayashi, M., Sousa, E. A., Liu, W., Cai, J., Goldman, S. J., et al. (2005). Differential proteomic analysis of bronchoalveolar lavage fluid in asthmatics following segmental antigen challenge. *Mol. Cell. Proteomics* 4, 1251–1264. doi: 10.1074/mcp.M500041-MCP200

Zhao, J., Zhu, H., Wong, C. H., Leung, K. Y., and Wong, W. S. (2005). Increased lung kinase and chitinase levels in allergic airway inflammation: a proteomics approach. *Proteomics* 11, 2799–2807. doi: 10.1002/pmic.200401169

Zhou, Y., Dong, Q., Louahed, J., Dragwa, C., Savio, D., Huang, M., et al. (2001). Characterization of a calcium-activated chloride channel as a shared target of Th2 cytokine pathways and its potential involvement in asthma. *Am. J. Respir. Cell Mol. Biol.* 25, 486–491. doi: 10.1165/ajrccm.25.4.45578

Zhu, Z., Zheng, T., Horner, R. J., Kim, Y. K., Chen, N. Y., Cohn, L., et al. (2004). Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 307, 1678–1682. doi: 10.1126/science.1095336

Zimmermann, N., King, N. E., Laporte, J., Yang, M., Mishra, A., Pope, S. M., et al. (2003). Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. *J. Clin. Investig.* 112, 1863–1874. doi: 10.1172/JCI200317912

Zou, J., Young, S., Zhu, E., Gheyas, F., Skeans, S., Wan, Y., et al. (2002). Microarray profile of differentially expressed genes in a monkey model of asthmatic lung. *Genome Biol.* 5, 20. doi: 10.1186/gb-2002-3-5-research0020

Zuo, L., and Clanton, T. L. (2005). Reactive oxygen species formation in the transition to hypoxia in skeletal muscle. *Am. J. Physiol. Cell Physiol.* 1, C207–C216. doi: 10.1152/ajpcell.00449.2004

Zuo, L., Otenbaker, N. P., Rose, B. A., and Salisbury, K. S. (2013). Molecular mechanisms of reactive oxygen species-related pulmonary inflammation and asthma. *Mol. Immunol.* 1, 57–63. doi: 10.1016/j.molimm.2013.04.002

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## APPENDIX

Table A1 | The list of pathways that play key role in asthma pathogenesis, as evident from the biomarkers identified by genomics, proteomics and epigenomics approaches.

| Name of the pathway                                      | Total entities | Expanded of entities | Overlap | Percent overlap | Overlapping entities                                                                 | p-value   | (−log_{10} p)-value |
|----------------------------------------------------------|----------------|----------------------|---------|----------------|--------------------------------------------------------------------------------------|-----------|---------------------|
| Adipocytokine signaling                                  | 52             | 780                  | 13      | 1              | ACSL3, IL13, IL9, IL4, IL2, IL10, IFNA1, SOCS1, PON1, APOB, SOCS3, SCD, NR1D1        | 0.001809  | 2.742605            |
| ROS metabolism                                           | 43             | 34                   | 3       | 4              | PRDX6, SOD1, CYBB                                                                     | 0.004493  | 2.347426            |
| ActivinR -> SMAD2/3 signaling                            | 23             | 23                   | 2       | 8              | INHBA, INHA                                                                          | 0.010913  | 1.962064            |
| Urea cycle and arginine metabolism                      | 86             | 110                  | 3       | 2              | NOS2A, ARG1, ARG2                                                                     | 0.013536  | 1.868503            |
| Translation control                                      | 86             | 984                  | 13      | 1              | CCL11, EGFR, IL13, IL9, IL4, IL2, IL10, CCL21, IFNA1, SOCS1, VEGFC, SOCS3, GNB2       | 0.013549  | 1.86809             |
| ActivinR/BMPR -> SMAD1/5/9 signaling                     | 27             | 27                   | 2       | 7              | INHBA, INHA                                                                          | 0.014876  | 1.827505            |
| Apoptosis regulation                                     | 69             | 613                  | 9       | 1              | IL13, IL9, IL4, TGFBI, IL2, IL10, IFNA1, INHBA, INHA                                  | 0.022869  | 1.640747            |
| Mast cell activation                                     | 64             | 529                  | 8       | 1              | PTGS2, IL13, IL9, IL4, IL2, IL10, IFNA1, ALOX15                                       | 0.027347  | 1.563092            |
| Skeletal myogenesis control                              | 70             | 569                  | 8       | 1              | EGFR, TGFBI, SOCS1, INHBA, CYBB, VEGFC, INHA, SOCS3                                   | 0.039923  | 1.39878             |
| NK Cell Activation                                       | 59             | 523                  | 7       | 1              | IL13, IL9, IL4, IL2, IL10, IFNA1, TYROBP                                             | 0.067085  | 1.173375            |
| EDG2 -> ELK-SRF signaling                                | 33             | 78                   | 2       | 2              | EGFR, GNB2                                                                          | 0.102033  | 0.991259            |
| T cell activation                                        | 81             | 1100                 | 11      | 1              | SGPP1, PTGS2, IL13, IL9, IL4, IL2, IL10, CTLA4, IFNA1, ALOX15, CNN1                   | 0.129288  | 0.888442            |
| GFR -> FOXO3A signaling                                  | 7              | 94                   | 2       | 2              | EGFR, VEGFC                                                                          | 0.138723  | 0.857852            |
| DopamineR2 -> AP-1/CREB/ELK-SRF signaling                | 47             | 95                   | 2       | 2              | EGFR, GNB2                                                                          | 0.141106  | 0.850455            |
| CholinergicRm -> CREB/ELK-SRF signaling                  | 41             | 107                  | 2       | 1              | EGFR, GNB2                                                                          | 0.170351  | 0.768655            |
| GRM1/5 -> CREB signaling                                 | 39             | 110                  | 2       | 1              | EGFR, GNB2                                                                          | 0.177823  | 0.750012            |
| Melanogenesis                                            | 51             | 682                  | 7       | 1              | INMT, CCL11, EGFR, CCL21, PRDX6, VEGFC, GNB2                                         | 0.190612  | 0.71985             |
| Adherens junction regulation                             | 41             | 692                  | 7       | 1              | EGFR, TGFBI, INHBA, VEGFC, CDH11, INHA, DSP                                          | 0.20054   | 0.697799            |
| GFR -> NCOR2 signaling                                   | 27             | 130                  | 2       | 1              | EGFR, VEGFC                                                                          | 0.228743  | 0.640652            |
| GFR -> AP-1/CREB/CREBBP/ELK-SRF/MYC signaling            | 50             | 156                  | 2       | 1              | EGFR, VEGFC                                                                          | 0.296227  | 0.528375            |

The data was generated using Pathway studio 7.1, Ariadane Genomics, Rockville, MD, USA. The column names are: Name of the pathway; Total entities; expanded entities; overlap; percent overlap; overlapping entities; p-value and (−log_{10} p)-value.
Table A2 | The list of Gene Ontology Molecular Function (GOMF) terms that are significant in asthma pathogenesis, as evident from the biomarkers identified by genomics, proteomics and epigenomics approaches.

| Name of the GOMF terms                        | Total entities | Expanded number of entities | Overlap | Percent overlap | Overlapping entities                                                                 | p-value     | (−log10 p-value) |
|-----------------------------------------------|----------------|-----------------------------|---------|-----------------|--------------------------------------------------------------------------------------|------------|------------------|
| Cytokine activity                             | 217            | 217                         | 13      | 5               | Ccl8, Cxc15, CCL11, IL13, IL9, IL4, IL2, IL10, CCL21, IFNA1, INHBA, INHA, SCGB3A1  | 3.30E–13   | 12.48204        |
| Growth factor activity                        | 198            | 198                         | 8       | 4               | IL9, IL4, TGFBI, IL2, INHBA, VEGFC, INHA, TFF2                                       | 3.10E–07   | 6.509308        |
| Arginase activity                             | 2              | 2                           | 2       | 100             | ARG1, ARG2                                                                          | 1.08E–05   | 4.967132        |
| Hematopoietin-interferon-class (D200-domain) cytokine receptor binding | 47             | 47                          | 4       | 8               | IL13, IL9, IL4, IFNA1                                                               | 1.78E–05   | 4.750718        |
| Chemokine activity                            | 56             | 56                          | 4       | 7               | Muc5ac, Serpinb3c, Apoa1, ACSL3, Igh-6, IGHG1, PTGS2, EGFR, ORM1, IL13, IL4, TGFBI, IL2, IL10, CTLA4, NOS2A, SERPINA1, SOCS1, SOD1, ARG2, APOB, CYBB, TIMP4, FCGR2B, POSTN, FOXP3, CDH11, INHA, S100A9, SOCS3, TYROBP, VIM, TCF21, FBN1, C4BPA, AATF, SCNN1G, HSPA1B, ITIH1, LCN1, GNB2 | 3.57E–05   | 4.446842        |
| Protein binding                               | 7274           | 7274                        | 41      | 0               | EGFR, TGFBI, INHBA, APOB, CYBB, INHA                                               | 7.37E–05   | 4.132694        |
| Protein heterodimerization activity           | 268            | 268                         | 6       | 2               | EGFR, TGFBI, INHBA, APOB, CYBB, INHA                                               | 0.000265   | 3.576101        |
| Metallopeptidase activity                     | 178            | 178                         | 5       | 2               | CPA4, MMP12, ADAM33, ACE, ADAM8                                                    | 0.000314   | 3.502791        |
| Complement binding                            | 9              | 9                           | 2       | 22              | CFb, C4BPA                                                                         | 0.000383   | 3.417369        |
| Chitinase activity                            | 11             | 11                          | 2       | 18              | Chb3, ChiA                                                                          | 0.000582   | 3.235176        |
| High-density lipoprotein binding              | 11             | 11                          | 2       | 18              | Apoa1, PON1                                                                         | 0.000582   | 3.235176        |
| Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amidines | 11             | 11                          | 2       | 18              | ARG1, ARG2                                                                         | 0.000582   | 3.235176        |
| Endopeptidase inhibitor activity              | 118            | 118                         | 4       | 3               | SERPINA1, SERPINB2, CSTA, ITIH1                                                    | 0.000639   | 3.194323        |
| Protein binding, bridging                     | 60             | 60                          | 3       | 5               | DSP, CSTA, COL11A1                                                                 | 0.001039   | 2.983192        |
| Peptidase activity                            | 633            | 633                         | 8       | 1               | Slpi, CPA4, MMP12, SERPINA1, ADAM33, ACE, CFb, ADAM8                               | 0.001171   | 2.93128         |
| Phospholipid binding                          | 64             | 64                          | 3       | 4               | Apoa1, PON1, APOB                                                                  | 0.001254   | 2.901838        |
| Cholesterol transporter activity              | 16             | 16                          | 2       | 12              | Apoa1, APOB                                                                       | 0.001256   | 2.901021        |
| Serine-type endopeptidase inhibitor activity  | 150            | 150                         | 4       | 2               | Serpinb3c, SERPINA1, SERPINB2, ITIH1                                               | 0.001558   | 2.807388        |
| Antioxidant activity                          | 20             | 20                          | 2       | 10              | PRDX6, SOD1                                                                        | 0.001972   | 2.705175        |
| Antigen binding                               | 79             | 79                          | 3       | 3               | Igh-2, Igh-6, IGHG1                                                                | 0.002296   | 2.63899         |

The data was generated using Pathway studio 7.1, Ariadane Genomics, Rockville, MD, USA. The column names are: Name of the GOMF terms; Total entities; expanded entities; overlap; percent overlap; overlapping entities; p-value and −log10 p-value.
Table A3 | The list of Gene Ontology Biological Process (GOBP) terms that are significant in asthma pathogenesis, as evident from the biomarkers identified by genomics, proteomics and epigenomics approaches.

| Name of GOBP terms                                      | Total entities | Expanded number of entities | Overlap | Percent overlap | Overlapping entities                                                                 | p-value | (−log10 p-value) |
|----------------------------------------------------------|----------------|----------------------------|---------|-----------------|---------------------------------------------------------------------------------------|---------|------------------|
| Inflammatory response                                    | 293            | 15                         | 5       | 5               | Ccl8, Cxcl15, CCL11, PTGS2, ORM1, IL13, IL9, IL4, TGFβ1, IL10, NOS2A, CCL21, CYBB, ALOX15, S100A9 | 4.54E–15 | 14.34325         |
| Immune response                                          | 604            | 16                         | 2       | 2               | LILRA6, Ccl8, Cxcl15, IGHG1, CCL11, IL13, IL9, IL4, IL2, IL10, CTLA4, CCL21, FCGR2B, CFB, C4BPA, CHIA | 1.24E–11 | 10.90786         |
| Negative regulation of immune response                  | 14             | 4                          | 28      | 28              | TGFβ1, CTLA4, FCGR2B, FOXP3                                                           | 5.90E–08 | 7229314          |
| Negative regulation of interferon-gamma biosynthetic process | 4             | 3                          | 75      | 75              | INHBA, FOXP3, INHA                                                                    | 8.79E–08 | 7055796          |
| Anti-apoptosis                                           | 198            | 8                          | 4       | 4               | IL2, IL10, SOD1, ALOX15, SOCS3, SERPINB2, AATF, HSPA1B                                | 9.69E–08 | 7013485          |
| Regulation of cell proliferation                         | 135            | 7                          | 5       | 5               | PTGS2, EGFR, TGFβ1, NOS2A, ADAM33, INHA, SCGB3A1                                     | 1.18E–07 | 6.929752         |
| Response to drug                                         | 295            | 9                          | 3       | 3               | Apoa1, PTGS2, MMP12, TGFβ1, SOCS1, SOD1, CYBB, TIMP4, SOCS3                         | 1.62E–07 | 6.791126         |
| Positive regulation of B cell proliferation              | 23             | 4                          | 17      | 17              | Igh-6, IL13, IL4, IL2                                                               | 5.12E–07 | 6.290996         |
| Negative regulation of T cell proliferation              | 26             | 4                          | 15      | 15              | TGFβ1, IL10, CTLA4, FOXP3                                                           | 8.58E–07 | 6.066353         |
| Response to cytokine stimulus                            | 77             | 5                          | 6       | 6               | PTGS2, SERPINA1, SOCS1, TIMP4, SOCS3                                                | 2.73E–06 | 5.563052         |
| Response to estradiol stimulus                           | 79             | 5                          | 6       | 6               | PTGS2, TGFβ1, ERPINA1, SOCS1, SOCS3                                                | 3.11E–06 | 5.50781          |
| Skeletal system development                              | 147            | 6                          | 4       | 4               | TGFβ1, INHBA, POSTN, CDH11, INHA, FBN1                                              | 3.98E–06 | 5.400395         |
| Positive regulation of epithelial cell proliferation     | 44             | 4                          | 9       | 9               | EGFR, MMP12, TGFβ1, VEGFC                                                         | 7.50E–06 | 5.125108         |
| Response to lipopolysaccharide                            | 99             | 5                          | 5       | 5               | PTGS2, SERPINA1, SOCS1, TIMP4, SOCS3                                               | 9.43E–06 | 5.025503         |
| Organ regeneration                                       | 49             | 4                          | 8       | 8               | Apoa1, TGFβ1, SOCS1, SOCS3                                                        | 1.16E–05 | 4.936449         |
| Cell–cell signaling                                     | 275            | 7                          | 2       | 2               | IL13, IL2, IL10, CCL21, INHBA, INHA, S100A9                                        | 1.35E–05 | 4.869351         |
| Response to hypoxia                                      | 184            | 6                          | 3       | 3               | TGFβ1, NOS2A, SERPINA1, ACE, SOCS3, SCNN1G                                        | 1.44E–05 | 4.842389         |
| Positive regulation of follicle-stimulating hormone secretion | 3             | 2                          | 66      | 66              | INHBA, INHA                                                                       | 2.38E–05 | 4.62283          |
| Positive regulation of regulatory T cell differentiation | 3             | 2                          | 66      | 66              | IL2, FOXP3                                                                         | 2.38E–05 | 4.62283          |
| Response to external stimulus                            | 23             | 3                          | 13      | 13              | INHBA, PON1, INHA                                                                     | 3.75E–05 | 4.426548         |

The data was generated using Pathway studio 7.1, Ariadane Genomics, Rockville, MD, USA. The column names are: Name of the GOBP terms; Total entities; expanded entities; overlap; percent overlap; overlapping entities; p-value and (−log10 p-value).