MOLECULAR PROFILING OF THE PERIPHERAL BLOOD RESPONSE TO ALLERGEN INHALATION CHALLENGE IN ASTHMATICS

by

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ABSTRACT

Allergen inhalation challenge (AIC) triggers biphasic responses in allergic asthmatic individuals. Airway narrowing represents the early phase response, which typically occurs within 30 minutes of allergen inhalation. In 50-60% of allergic asthmatic adults, the early response is followed by the late phase response, usually starting around 3 hours after AIC, and characterized by cellular inflammation of the airway, increased lung tissue permeability, and mucus secretion. The pathways leading to the late response are not completely understood. The purpose of this thesis is to investigate the mechanisms behind the allergic asthmatic response profiles using peripheral blood samples obtained from asthmatics prior to and 2 hours following AIC. Subjects exhibited either an isolated early response of ≥20% fall in FEV_1 (isolated early responder – ER), or an early response followed by a late phase response of ≥15% fall in FEV_1 (dual responder – DR). Genome-wide transcriptional profiling using microarrays indicated significant perturbations in the Nrf2 (NF-E2-related factor 2)-mediated oxidative stress response pathway following allergen inhalation. Notably, the *ABCC1* (ATP-binding cassette, sub-family C (CFTR/MRP), member 1) gene within the pathway showed a decreased expression post-challenge, as validated through RT-qPCR. Furthermore, a significant decrease in the level of plasma chemokine (C-C motif) ligand 2 (CCL2) was evident, which was replicated using immunoassays in additional cohorts of allergic rhinitis and individuals with occupational asthma. However, this may be attributable to inherent fluctuations, based on similar results from control subjects. The comparison of transcriptomic response profiles between ERs and DRs undergoing cat allergen inhalation challenge revealed linoleic acid metabolism as the most significant

pathway. Separation of whole-blood gene expression profiles into cell-specific signals using the csSAM algorithm suggested that key transcriptomic differences lie in eosinophils and lymphocytes when comparing between ERs and DRs at the post-challenge time point. These findings are in support of the current model of asthma pathophysiology and provide valuable insights into molecular changes occurring as early as 2 hours after allergen inhalation. Further study into the underlying mechanisms leading to the different response patterns may expose new therapeutic targets effective in minimizing the late response, which is associated with chronic asthma.

PREFACE

Subject recruitment, allergen challenges, and peripheral blood samples collection were performed by the following collaborators:

- Dr. GM Gauvreau and Dr. PM O'Byrne – McMaster University
- Dr. JM FitzGerald – University of British Columbia
- Dr. L-P Boulet – Université Laval
- Dr. A Ellis – Queen's University
- Dr. C Carlsten – University of British Columbia

I was responsible for conducting the experiments and analyses using the collected samples and clinical information.

The assessment of oxidative and inflammatory molecules in Chapter 2 and the multiplex cytokine assay described in Chapter 3 were conducted by Dr. D Radzioch and G Wojewodka, collaborators at McGill University. I was responsible for data analysis and all subsequent interpretations of results.

A portion of Chapter 2 has been modified and published. Kam SHY, Singh A, He J-Q, Ruan J, Gauvreau GM, O'Byrne PM, FitzGerald JM, Tebbutt SJ. Peripheral blood gene expression changes during allergen inhalation challenge in atopic asthmatic individuals. The Journal of Asthma. 2012 Feb 9;(5):219–26. I participated in data analysis, conducted interpretations of results, and wrote most of the manuscript.

This study has been approved by the Research Ethics Board of Providence Health Care Research Institute, UBC, with the ethics approval number of H09-02114.

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DEDICATION

To my family and friends,

who have always believed in me.

CHAPTER 1: INTRODUCTION

1.1 Asthma Overview and Pathogenesis

Asthma is a chronic inflammatory airway disorder characterized by airway hyperresponsiveness and reversible airflow obstruction, which manifests clinically as shortness of breath, chest tightness, wheezing, and coughing (1,2). Asthma affects up to 300 million people of all ages world-wide, with approximately 250,000 dying of the disease each year (1). In parallel with allergy, the prevalence of asthma is high in urbanized countries such as Canada. While the related mortality rate is low in North America, the direct medical costs (*e.g.*, pharmaceuticals) and indirect medical costs (*e.g.*, time lost from work) can be substantial (1).

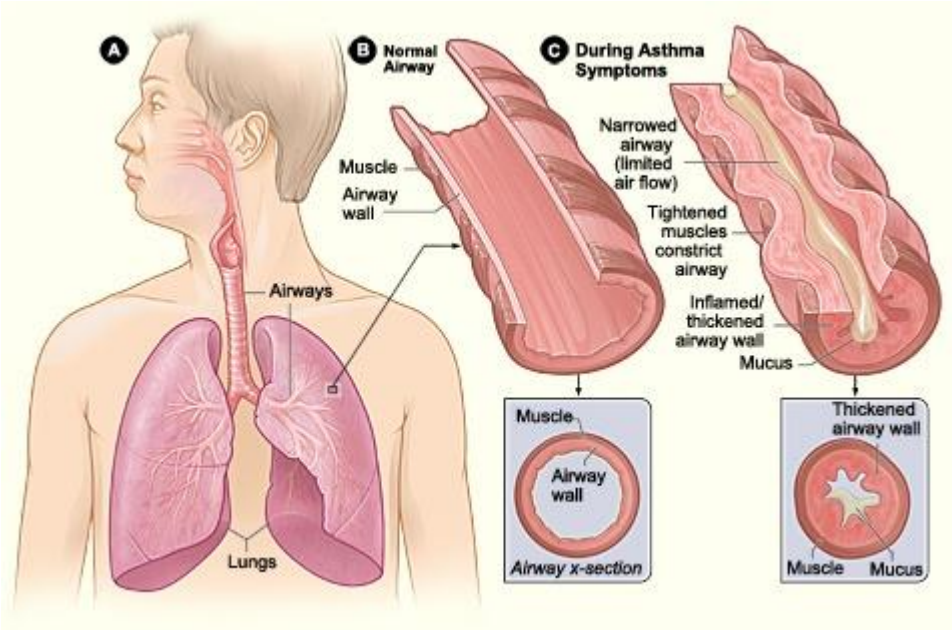
As a complex disease, asthma is believed to be caused by a combination of genetic and environmental factors. Various asthma susceptibility genes have been identified through genome-wide association studies (GWAS), linkage studies, and candidate gene approaches. An important locus lies on chromosome 17q21, where genes such as *ORMDL3* have been identified and successfully validated to be associated with asthma (3,4). The exact roles of many of these genes remain under active investigation.

Environmental exposures are also crucial in the onset of asthma. Multiple studies have suggested that maternal tobacco smoking, air pollution, and high ozone levels may lead to an increased risk of developing the disease (5). Past the stage of initial sensitization, environmental factors continue to play a role in provoking asthmatic responses, also known as asthma exacerbations or asthma attacks. Indeed, airborne

allergens inhaled by sensitized allergic asthmatic individuals act as the main trigger for an asthmatic response. Some of these stimulants include pollen of plants, house dust mites, and animal dander (6). Hence, people diagnosed with allergic asthma may be affected seasonally or year-round, depending on their allergen of sensitization. Because environmental factors are so important in evoking asthma, avoidance of such stimulants (if and when possible) is fundamental to reducing asthmatic responses (6).

Airflow obstruction experienced during an asthmatic response can be attributed to three main biological events (7) (Figure 1.1). First, there is bronchoconstriction, referring to contractions of the airway smooth muscles. Second, excessive mucus production results in the formation of mucus “plugs”, leading to airway congestion. Third, the airways are thickened due to inflammation and swelling, decreasing the overall lumen size. Chronic inflammation can further lead to airway remodeling, a process describing the alteration of normal airway structure, as often observed in patients with chronic severe asthma (8).

Figure 1.1 — Asthma Pathophysiology.



During an asthmatic response, the airways of a sensitized individual display three distinct characteristics: the tightening of airway smooth muscles, the production of excessive mucus, and the swelling and thickening of the airways. All of these contribute to the outcome of decreased airflow in the airways.

Source: National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services.
http://www.nhlbi.nih.gov/health/health-topics/topics/asthma/

1.2 Allergen Inhalation Challenge Model

One method of studying the asthmatic response is by using the allergen inhalation challenge (AIC) model. As the name implies, this involves "challenging" subjects with aerosolized allergens, administered through the route of inhalation. The overall goal is to elicit a response in allergic asthmatic individuals, thereby providing a platform on which to carry out clinical and research investigations. Some of these inquiries include the determination of allergic status, exploration into disease mechanisms, and testing of therapy efficacies (9). Although there are other biological models available for asthma research (such as the use of cell lines and animal models), AIC provides a unique human-based approach. This way, the findings are directly applicable to the human population, bypassing the need to demonstrate a human relevance, as is often required for *in vitro* and animal studies. As a well-established model, AIC has been employed since the 1970s (10) and continues to be an important tool in asthma research today.

However, as with any asthmatic responses, AIC-induced allergic response can be dangerous, potentially resulting in anaphylaxis and severe bronchoconstriction (9). Therefore, the administration of allergen must be well controlled in order to avoid over-dosage. One way of determining a safe starting allergen concentration is to rely on data obtained from a prior methacholine inhalation challenge and skin prick test. Methacholine inhalation challenge allows for the direct assessment of bronchial hyperresponsiveness, which can be represented by methacholine PC_{20} (defined as the provocative concentration of methacholine required to cause a 20% fall in lung function, as measured by forced expiratory volume in one second – FEV_1). Using the

methacholine PC_{20} value, along with the lowest allergen concentration needed to cause a 2 mm wheal during the skin prick test, the concentration of allergen extract to be used in AIC can be calculated with the Cockcroft formula (11).

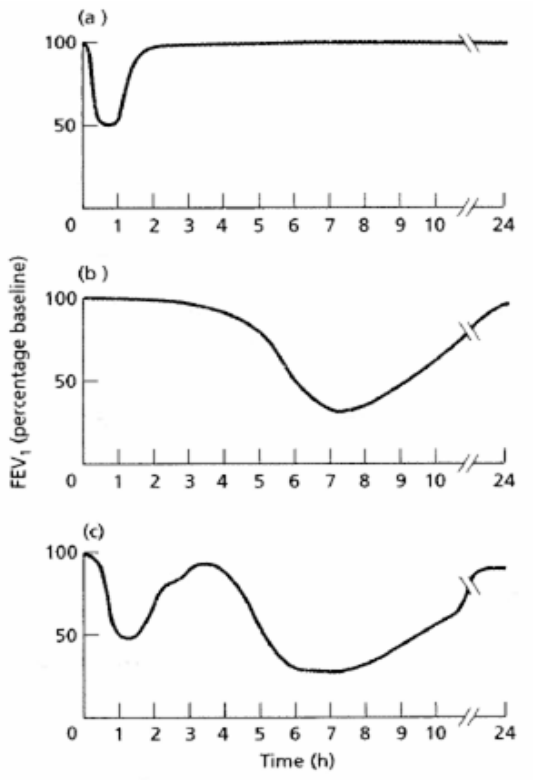
The resulting asthmatic response is measured using spirometry. In AIC, the goal is to stimulate a ≥20% decrease in FEV_1 as compared to baseline (pre-challenge) lung function. Beginning with an initial concentration of allergen extract as calculated above, the concentrations are gradually doubled until the ≥20% drop is reached, typically within 3 doubling concentrations (12). The lung function of the subject is then monitored for 7 hours post-allergen challenge.

Due to the many variabilities involved in human studies, the asthmatic responses observed during AIC can be heterogeneous. However, all response profiles of mild atopic asthmatics generally follow one of three patterns (Figure 1.2). Within ten minutes of allergen inhalation, bronchoconstriction is initiated and reaches a maximum within thirty minutes, finally resolving within 3 hours (13). This early asthmatic response (EAR) is detected as a drop in FEV_1, with a minimum decrease of 20% as compared to baseline. Individuals with this single response profile are accordingly known as isolated early responders (ERs). In 50-60% of allergic asthmatic adults, however, the early response is followed by a late asthmatic response (LAR) (13). These individuals are termed dual responders (DRs). The late response starts around 3 to 4 hours after allergen inhalation and is measured as a secondary FEV_1 drop of at least 15%. Compared to the EAR, the LAR is more sustained, lasting from a few hours to a few days (13). In addition to these two response profiles, which account for >90% of

asthmatic responses, <10% of asthmatic adults display an isolated late phase response (LR).

The asthmatic response pattern of either an isolated early or a dual response is generally reproducible in a given individual. Even so, studies have shown that the concentration of allergen administered during AIC may be influential to some extent. In individuals normally displaying an isolated early response profile, an increased allergen dosage appears to be effective in inducing the development of a LAR (14,15). A possible explanation is that when the concentration of allergen exceeds a threshold, additional immune cells (such as dendritic cells and T lymphocytes) are activated, leading to the downstream event of the late response (9). In addition to allergen dosage, the type of allergen used has also been shown to affect the response outcome. Hatzivlassiou *et al*. demonstrated that asthmatics sensitized to both grass pollen and house dust mite (HDM) displayed an isolated early response during grass pollen challenge, but a dual response to HDM challenge (16). This suggests that different allergens may trigger varying response pathways in the body.

Figure 1.2 — Typical Response Profiles after Allergen Inhalation Challenge.



After AIC, subjects typically display one of three lung function profiles, as assessed by FEV_1 measurements. (a) An isolated early response, (b) an isolated late response, and (c) a dual response.

1.3 Molecular Mechanisms of Early and Late Responses

1.3.1 Early Asthmatic Response

Despite decades of research, the exact mechanisms of the early and late asthmatic responses have not been fully elucidated. Nevertheless, a sequence of events has been proposed to explain the biology behind these responses (Figure 1.3). An asthmatic individual with prior sensitization to an allergen carries circulating IgE antibodies against that specific allergen. Upon re-exposure (during AIC, for example), the epitopes of the inhaled allergen molecules are recognized by IgE antibodies bound to mast cells and basophils. Multiple interactions between allergens and antibodies result in cross-linking of the FcεRI, the high affinity IgE receptor on cell surfaces (9). This leads to activation of downstream events, mainly cellular degranulation and the release of preformed molecules, including histamine, proteolytic enzymes, and proteoglycans (17). Furthermore, newly-synthesized mediators such as leukotrienes, prostaglandins, cytokines, and chemokines are also discharged.

Histamine is a potent mediator of acute inflammation, acting directly on airway smooth muscle cells and the endothelium to cause bronchoconstriction, vasodilation, mucus secretion, and edema (18). Because all of these events contribute to the immediate symptoms observed after allergen inhalation, histamine is considered to be a key player in the onset of the EAR. In addition to histamine, cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) released by mast cells and basophils are thought to be responsible for the majority of bronchoconstriction occurring immediately after allergen challenge (19). Indeed, cysteinyl leukotrienes receptor antagonists are commonly

prescribed by physicians for relief of asthmatic symptoms. Even without any medication, however, the early phase response typically resolves within 2 hours.

1.3.2 Late Asthmatic Response

While the EAR is predominantly a result of bronchoconstriction, the LAR is characterized by cellular inflammation of the airways, increased bronchovascular permeability, and heavy mucus secretion. Being a downstream event triggered by allergen inhalation, the biological mechanisms leading to the LAR are still unclear. Morphologically, the airways are infiltrated by a mixture of leukocytes, consisting of eosinophils, T lymphocytes, neutrophils, basophils, and mast cells (20–23). The attraction and activation of these inflammatory cells have been attributed to various mediators released by local mast cells, basophils, and airway epithelial cells (24).

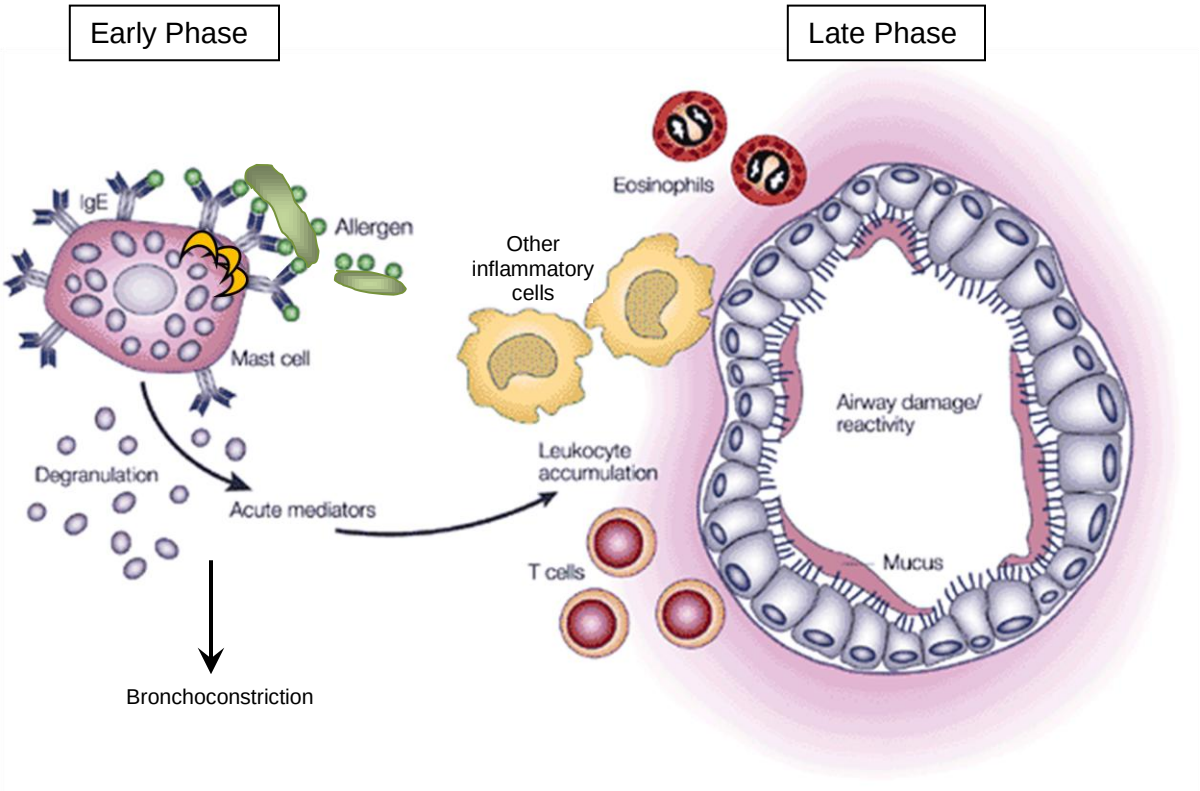
Each of the infiltrating cell types mentioned above plays a role in the inflammatory state of the late phase response. Dente *et al*. demonstrated that sputum eosinophilia was directly related to the severity of the LAR (25). Activated eosinophils release cytotoxic products, such as eosinophil cationic protein (ECP), which damages bronchial walls, adding to its hypersensitivity and worsening the response outcome (26). The action of eosinophils is further propagated by T lymphocytes, which have been shown to expand clonally (with allergen specificity) in response to allergen challenge (22). In the airways, activated CD4+ T cells with a T helper 2 (Th2) phenotype release pro-inflammatory cytokines such as IL-4 and IL-5, inducing B-cell class switching to IgE and recruiting additional eosinophils (19). Neutrophils have also been suggested to contribute to the inflammatory process, through release of mediators including IL-8 and

reactive oxygen species (27). Finally, mast cells and basophils have both been shown to be increased during the late phase response and may correlate with LAR severity (21). Their involvement in the inflammatory state of the airways includes the release of histamine, cysteinyl leukotrienes, and various cytokines which help stimulate other immune cells (9).

The cellular infiltrate is believed to originate from a heightened production of progenitor cells from the bone marrow (28). Previous studies have demonstrated an increase in the numbers of eosinophil/basophil colony-forming units in the bone marrow and in circulation at 24 hours post-allergen inhalation in DR but not ER subjects (29,30). Furthermore, when bone marrow cells were assessed for cytokine responsiveness through analysis of their receptors, IL-3-responsive progenitors were identified as early as 5 hours post-allergen inhalation, and IL-5-responsive progenitors were identified starting at 12 hours after AIC (29,31). IL-3 is a cytokine that promotes the differentiation of hematopoietic stem cells to the myeloid lineage and stimulates the proliferation of myeloid cells, whilst IL-5 is a key mediator in eosinophil activation and survival (32). Both cytokines may be secreted by activated T lymphocytes that are either resident in or trafficked to the bone marrow upon stimulation by allergen challenge (33). Indeed, Wood *et al*. have demonstrated an increased production of IL-5 by such T lymphocytes (33). Taken together, and considering that these observations were exclusive to DR individuals only, it appears that eosinophilic late phase response may be partially due to T lymphocytes in the bone marrow releasing cytokines to stimulate progenitor cell development.

The development of the LAR is associated with the hallmarks of chronic asthma, featuring bronchial hyperresponsiveness, constant inflammation of the airways, and long-lasting bronchoconstriction which is difficult to reverse. This is in contrast to the EAR, which is easily reversible with bronchodilators and involves minimal inflammation. In chronic asthma, cellular inflammation can further lead to tissue remodeling, causing permanent damage to the airways (8). Therefore, ongoing efforts have been made to identify the critical pathways leading to the LAR, in order to find ways to effectively inhibit it and prevent the worsening progression to chronic asthma.

Figure 1.3 — Mechanisms of the Early and Late Response.



The early phase response is initiated when allergen molecules bind to IgE antibodies on mast cells, causing the receptors (FceRI) to cross-link. This triggers degranulation and the release of mediators such as histamine, which causes acute bronchoconstriction. Other mediators are believed to cause subsequent progression to the late phase response, during which leukocytes such as eosinophils and T cells are recruited. The cellular inflammation leads to increased airway reactivity and damage.

1.4 Transcriptional Profiling of Peripheral Blood

Traditionally, quantification of a single mRNA molecule is performed using northern blots and reverse transcription quantitative polymerase chain reaction (RT-qPCR). With advancement in technology, and a desire to study multiple genes simultaneously, genome-wide expression profiling (transcriptomics) was developed. This technique allows for the assessment of every gene in the human genome, all measured on a single microarray chip. The result is an overview of cellular functions and biological processes, as observed from the samples studied. Nowadays, genome-wide transcriptional profiling has become one of the more powerful approaches to explore disease mechanisms, to assist diagnosis and to determine better treatment strategies. Indeed, there are over 17,000 articles related to microarray analysis listed in PubMed between 1995 to 2005 (34), testifying to the effectiveness of this technique. Even though analysis of microarray data can be difficult due to the problem of multiple hypothesis testing, genome-wide gene expression profiling remains a useful tool for initial investigations, the results of which can then be validated by other techniques, such as RT-qPCR.

Target tissues and cells are the ideal biological materials for gene expression studies, but they are usually difficult to obtain. In recent years, peripheral blood mononuclear cells (PBMCs) have been widely used for gene expression studies in biomedical research as well as in clinical services. Since isolation of blood cell-type fractions is time-consuming, expensive, and likely to introduce additional technical variation, peripheral whole-blood material, which includes granulocytes, has become increasingly popular among researchers conducting gene expression analyses. There

are several advantages to using peripheral whole-blood cells in genome-wide expression profile analysis. To start, they are very easy to obtain and are the most commonly used biological material in the clinical setting. In addition, peripheral whole-blood contains various cell types, many of which participate in the immune response and transport systems that contribute heavily to various biochemical conditions and disorders. Indeed, many of these cells play a role in the inflammation of the airways during the LAR, as discussed previously. Accumulating evidence has documented the usefulness of whole-blood material over PBMCs in gene expression profile research in large-scale studies (35,36).

1.5 Overview of Experimental Goals and Research Focus

Driven by the incomplete understanding of the biology behind the asthmatic response patterns, and the need for an effective treatment option to minimize the LAR, my research aimed to explore the underlying mechanisms behind why some allergic asthmatic individuals develop an isolated early response, while others develop a dual response. This was achieved through the use of the allergen inhalation challenge model in order to stimulate an asthmatic response in a controlled environment. Peripheral blood samples, collected prior to and after allergen inhalation, acted as the biological source of investigation. The longitudinal design allowed for direct comparisons between each individual's pre- and post-status, enabling a more accurate investigation into the exact changes occurring during an asthmatic response.

This project was carried out with three main objectives, as described in more details in the following chapters. The first step was a pilot study to determine whether

the chosen time point of 2 hours post-allergen challenge was sufficient and reasonable to detect genome-wide transcriptomic differences in the peripheral blood, as compared to pre-challenge levels. The plasma content of oxidative and inflammatory molecules was also assessed for additional support. Next, the focus was shifted to investigate pre- versus post-challenge changes in plasma cytokine levels. Through this study, chemokine (C-C motif) ligand 2 (CCL2) was identified and further examined with regards to any possible associations with subject demographics. The cohort was also expanded to include normal controls, as well as subjects with occupational asthma and allergic rhinitis. Finally, the differences in genome-wide transcriptomic profiles between ERs and DRs were explored, taking into account both the pre-challenge and post-challenge data. A concluding chapter summarizes the overall findings, future directions, and implications of the results.

CHAPTER 2: PRE VERSUS POST ALLERGEN INHALATION CHALLENGE

2.1 Introduction

The interlude between the early and the late phase response, as measured by FEV_1 in dual responders, typically occurs around 2 to 3 hours after an asthmatic individual undergoes AIC. Therefore, this may be a reasonable time to assess any deviations in pathway activation patterns between the isolated early response and the dual response. Previous studies of asthmatic AIC have concentrated on uncovering the disease mechanisms by obtaining and testing blood samples around 24 hours after the inhalation of allergen (37,38). However, reports on the possible biological changes that may occur as early as 2 hours after allergen challenge have been limited.

Given the importance of this time frame and the key role of the peripheral blood in relaying biological signals, it is of great interest to test the hypothesis that changes in the peripheral blood of asthmatic individuals can be detected within 2 to 3 hours after AIC. The determination of a valid time point to observe changes is a crucial first step before attempts to differentiate pathways between the isolated early and the dual responses can be taken. Furthermore, if molecular alterations are detectable within such a short time span, this may be helpful in identifying novel targets for early therapeutic interventions.

This chapter aims to assess the molecular changes in the peripheral blood 2 hours after AIC. This will be accomplished mainly through genome-wide transcriptional

profiling using microarrays. Also, perturbations in the levels of inflammatory and oxidative lipid molecules in the peripheral blood will be examined for additional support.

2.2 Methods

2.2.1 Subjects and Allergen Inhalation Challenge

Sixteen adult subjects (age 20-60) with stable, mild atopic asthma were recruited from McMaster University (10 subjects) and Vancouver General Hospital (VGH) - UBC (6 subjects), following informed consent. Diagnosis of asthma was based on the Global Initiative for Asthma (GINA) criteria. All subjects were non-smokers, free of other lung diseases, and not pregnant. All had a baseline forced expiratory volume in one second (FEV_1) ≥70% of predicted, and the provocative concentration of methacholine required to produce a 20% decrease in FEV_1 (PC_{20}) was ≤16 mg/ml in all except for one individual (39). Subject demographics are listed in Table 2.1.

A skin prick test and a methacholine inhalation challenge, as described by Cockcroft (40,41), were performed on each subject. Based on these procedures, the concentration of allergen required to achieve a 20% decrease in FEV_1 was predicted using the Cockcroft formula (11). One day after methacholine challenge, all participants underwent allergen inhalation challenges as described by O'Byrne *et al*. (42), using extracts of house dust mite (HDM; *Dermatophagoides pteronyssinus*), cat dander, or ragweed and grass (Timothy or Orchard) pollen, as indicated by the skin prick test. The FEV_1 lung function of subjects was monitored for 7 hours post-challenge. All subjects developed an EAR of ≥20% drop in FEV_1 from baseline between 0 and 3 hours.

Table 2.1 — Subject Demographics (Pre versus Post).

Cohort	Subject	Site	Age (yr)	Sex	Mch PC_{20}[a] (mg/ml)	Allergen[b]	% Fall in FEV_1 (EAR)	% Fall in FEV_1 (LAR)
PAXgene (4 Subjects)	1	VGH	36	M	3.2	Timothy Grass	-61	-38
	2	VGH	35	F	0.64	Timothy Grass	-27	-5
	3	VGH	47	M	0.28	Orchard Grass	-23	-17
	4	McM	21	F	1.09	HDMDP	-32.1	-12.5
EDTA (5 Subjects)	5	McM	20	F	9.62	Cat	-37.7	-17.3
	6	McM	27	F	1.45	HDMDP	-43.3	-16.7
	7	McM	23	M	10.9	Cat	-31.4	-15.1
	8	McM	60	F	2.19	Cat	-25.5	-6.7
	9	McM	22	M	4.28	HDMDP	-22.7	-19.9
PAXgene Validation (7 Subjects)	10	VGH	42	M	0.13	Cat	-23	-9
	11	VGH	41	M	1	Grass Mix	-21	-7
	11R[c]	VGH	41	M	0.69	Grass Mix	-42	-31
	12	VGH	52	F	N/A	Cat	-33	-27
	13	McM	21	F	6.96	Ragweed	-26.9	0
	14	McM	33	M	3.17	HDMDP	-29.5	-19.7
	15	McM	18	F	27.86	HDMDP	-46.4	-25.9
	16	McM	21	F	4.72	Cat	-20	-20
Lipids and Oxidative Molecules (8 Subjects)	1	VGH	36	M	3.2	Timothy Grass	-61	-38
	2	VGH	35	F	0.64	Timothy Grass	-27	-5
	3	VGH	47	M	0.28	Orchard Grass	-23	-17
	10	VGH	42	M	0.13	Cat	-23	-9
	13	McM	21	F	6.96	Ragweed	-26.9	0
	14	McM	33	M	3.17	HDMDP	-29.5	-19.7
	15	McM	18	F	27.86	HDMDP	-46.4	-25.9
	16	McM	21	F	4.72	Cat	-20	-20

[a] Methacholine PC_{20}.

[b] HDMDP=House dust mite (*Dermatophagoides pteronyssinus*).

[c] Subject 11 received a repeated allergen inhalation challenge one month later.

2.2.2 Blood Sample Collection and RNA Extraction

Peripheral venous blood samples were drawn at two time points, immediately prior to AIC and at 2 hours after the last inhaled allergen dose. The blood samples from 11 subjects were collected into PAXgene Blood RNA tubes (PreAnalytiX – Qiagen / BD, Valencia, CA, USA), with one subject (subject 11 in Table 2.1) undergoing a repeated AIC one month later, to make a total of 12 sets of PAXgene blood samples. For each of these PAXgene samples, a corresponding blood sample was drawn using standard EDTA tubes. Peripheral blood was also collected from 5 additional subjects into EDTA tubes only, without the use of PAXgene tubes. All of the samples collected pre- and post-allergen challenge were frozen and transported to the laboratory on dry ice, where they were stored at –80°C until RNA extraction.

From thawed PAXgene tube samples, intracellular RNA (excluding small RNA) was purified from 2.5 ml of whole blood according to manufacturer protocols using the PAXgene Blood RNA Kit (Qiagen, Chatsworth, CA, USA). RNA (excluding small RNA) was isolated from EDTA tube samples (3.0 ml of whole-blood) following a modified TRIzol-based extraction method that uses a combination of the TRIzol® LS Reagent (Invitrogen, Carlsbad, CA, USA) and the RNeasy mini kit (Qiagen, Chatsworth, CA, USA). The yield and quality of RNA were assessed by NanoDrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer following the RNA 6000 Nano Kit protocol (Agilent Technologies, Santa Clara, CA, USA).

2.2.3 Microarray Analysis

Microarray analysis was performed on a cohort of 4 PAXgene blood sample pairs and 5 EDTA blood sample pairs (different subjects). Genome-wide RNA labeling and array hybridization were carried out by the staff at the Centre for Translational and Applied Genomics at the BC Cancer Agency (Vancouver, BC), an Affymetrix-certified service provider. Affymetrix Human Gene 1.0 ST arrays were used (Affymetrix, Santa Clara, CA, USA). The pre- and post-challenge RNA samples from each subject were processed at the same time to avoid possible confounding batch effects.

2.2.4 RT-qPCR Validation

RT-qPCR of 9 selected genes was performed on an additional 8 PAXgene blood sample pairs. RNA was converted into cDNA using SuperScript RNase H- Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Quantitative PCR was carried out in duplicate. 20 ng cDNA was reacted with 2× Agilent Brilliant qPCR master mix with high ROX (internal reference dye), in a 12 µl reaction including appropriate forward and reverse primers (10 µM) and qPCR probe (5 µM) (Integrated DNA Technologies, Coralville, IA, USA). The sequences of primers and probes are provided in Appendix 1. Thermal cycling was carried out using an ABI 7900 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA) with the following profile: 95 °C for 10 min, 92 °C for 15 s and 60 °C for 1 min for 45 cycles. Three housekeeping genes, β-Glucuronidase (GUSB), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Phosphoglycerokinase (PGK1), were selected based on their low coefficient of variation (CV) values across all samples in the

microarray data. Efficiency standard curves were generated by plotting a serial dilution of the cDNA amount (derived from a pooled RNA sample) against the threshold cycle (Ct) value for each gene. The relative quantitative value of each sample was normalized using a factor calculated from the expression levels of the three housekeeping genes using the geNorm algorithm (43).

2.2.5 Oxidative and Inflammatory Molecules Analysis

Peripheral blood samples collected with EDTA-coated tubes were centrifuged at 500g for 10 minutes at room temperature to separate the layers of plasma, buffy coat, and erythrocytes. The plasma was isolated and sent to collaborators at McGill University, where several markers of oxidative stress response and inflammation were analyzed. In detail, plasma was first suspended in 1 mM butylated hydroxyanisole (BHA) in chloroform and methanol (2:1 vol) in order to protect the integrity of the samples. Following a previously described method (44), lipids were extracted from these plasma samples using chloroform/methanol (2:1). Thin layer chromatography was performed to identify the phospholipids present. Finally, the fatty acids were esterified using diazomethane and the esters were quantified using gas chromatography/mass spectrometry (Hewlett Packard5880A, WCOT capillary column (Supelco-10, 35 m×0.5 mm, 1 μm thick)) using commercial standards (Sigma-Aldrich, Oakville, ON, Canada). Arachidonic acid (AA) and docosahexaenoic acid (DHA) were extracted and analyzed as previously described (45). The AA/DHA ratio was calculated by dividing the concentration of AA by the concentration of DHA for individual samples. Initially, analysis was performed for a subset of 8 subjects (Table 2.1). This was

subsequently expanded to include another 21 individuals who were not previously analyzed (demographics not shown).

2.2.6 Statistical Analysis

CEL files containing the probe intensity data from the Affymetrix microarray analysis were imported into Partek Genomics Suite 6.5 (Partek Inc, St Louis, MO, USA). Background correction, quantile normalization and probe summarization were performed using robust multi-chip average (RMA) (46). Repeated measures ANOVA (paired analysis) and paired t-tests were used to detect differentially expressed probe sets (DEPs) or differentially expressed genes (DEGs) post- versus pre-allergen challenge. DEPs or DEGs were declared significant for initial discovery purposes at the $p \leq 0.01$ level (without multiple testing corrections) with a fold change ≥ 1.1 (based on $\log_2$ intensity values).

Gene set enrichment analysis (GSEA) was performed, using the gene sets databases c2.all.v2.5.symbols.gmt [Curated] and c5.all.v2.5.symbols.gmt [Gene ontology] (47,48). Sample pairing was achieved by baselining the raw intensity values (pre- subtracted from each corresponding post-value) prior to data input. The number of permutations was set to 1000 and the gene sets were considered significant at a false discovery rate (FDR) ≤0.25. Pathway analyses were carried out using Ingenuity Pathway Analysis (IPA – Ingenuity Systems, Redwood City, CA, USA). IPA organizes differentially expressed genes into related pathways and reports a p value that is representative of the probability that a specific set of genes has a significant number of members in a pathway. Analyses were restricted to consider genes related to immune

cells and lungs only. One-tailed paired t-tests were used to analyze the genes that were selected for validation using RT-qPCR (based on the hypothesis test that the direction of change would replicate). Significance was declared at the $p \leq 0.05$ level when comparing post- versus pre-allergen challenge samples. For lipids analysis, two-tailed paired t-tests were used, with the significance set at $p \leq 0.05$.

2.3 Results

2.3.1 Cohort Characteristics

The mean age of the 16 participants (7 males and 9 females) in the study was 32.4 ± 3.2 years (Table 2.1). The geometric mean for methacholine PC_{20} was 2.28 mg/ml, far below the guidelines of ≤16 mg/ml that is indicative of airway hyperresponsiveness (49). The type of allergens used were evenly distributed among the challenges, 5 of which were administered using HDM extracts, 6 with cat allergen, and 6 with either grass or ragweed pollen extracts. All subjects exhibited an EAR, with the mean drop in FEV_1 being 32.1 ± 2.7% between 0 and 3 hours. Of the 17 allergen inhalation challenges, 11 also produced a LAR of ≥15% FEV_1 drop.

2.3.2 RNA Quality

RNA samples to be analyzed using microarrays were first assessed for quality. An initial cohort of 4 PAXgene whole-blood sample pairs was of excellent quality with a mean RNA integrity number (RIN) of 8.68 ± 0.24, while RNA from EDTA whole-blood (5 sample pairs) was of moderate quality with a mean RIN of 6.21 ± 0.34. NanoDrop analysis showed that for PAXgene sample, the 260/280 ratio was 2.12 ± 0.01, while for

EDTA samples, it was 1.99 ± 0.02. The above values are expressed as mean ± standard error.

2.3.3 Differentially Expressed Probe Sets

Analyzing an initial 4 pairs of PAXgene samples using an ANOVA model, 524 significant probe sets (unadjusted p≤0.01 and fold change ≥1.1) were found to be differentially expressed between pre- and post-AIC (Appendix 2). Although the selection of a fold change threshold ≥1.1 seems lenient compared to other microarray-based studies that often use a 1.5 fold change threshold, it is important to take into account both the nature of the whole-blood material used for RNA extraction, and the small time interval (2 hours) between pre- and post-sampling. Considering the minimal effects of the fold change cut-off, it can be regarded that the p value alone was used to determine significance.

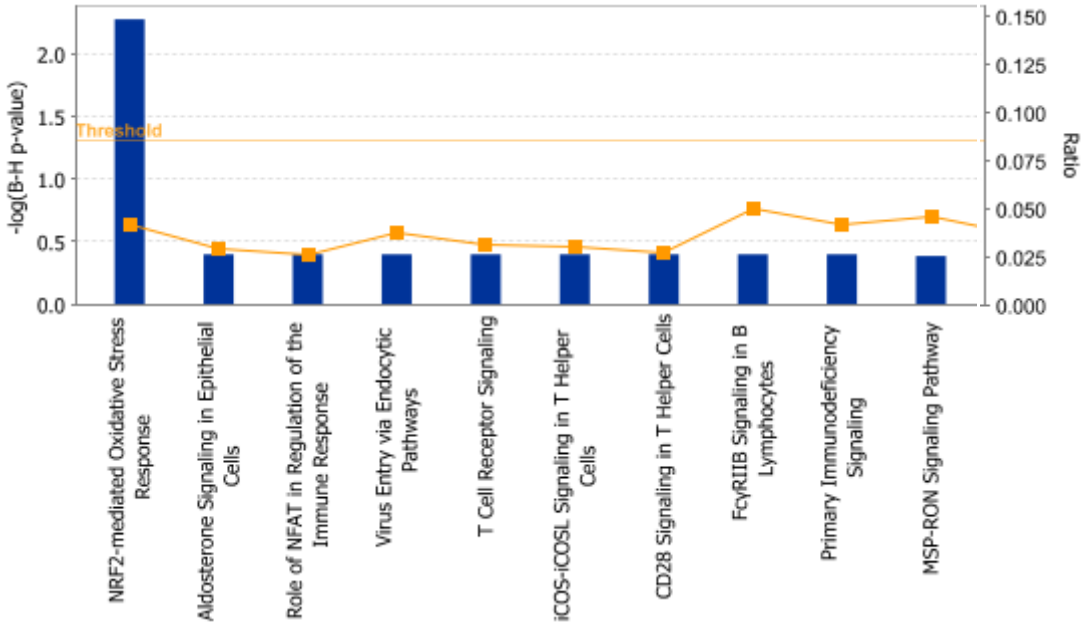
2.3.4 Gene Set Enrichment and Pathway Analysis

The complete dataset of normalized intensity values was entered into GSEA in order to explore the biological context of the perturbed genes. At the default cut-off of FDR≤0.25, 51 gene sets were found to be significantly down-regulated post-challenge, with the most significant gene set identified to be the Nakajima eosinophil gene set at FDR=3.82E-2 (Appendix 3). Using the same significance cut-off, 53 gene sets were found to be up-regulated post-challenge (Appendix 4).

Pathway analysis was performed based on the top ~100 probe sets using Ingenuity Pathway Analysis (IPA). This was done in order to reduce the false positive findings that are often associated with single gene level analysis. Of the 94 probe sets

initially entered into the pathway analysis, 82 met the criteria for inclusion. The biological functions identified failed to reach statistical significance after multiple testing corrections. For canonical pathways, the Nrf2 (NF-E2-related factor 2)-mediated oxidative stress response pathway was the sole significant canonical pathway at adjusted p=5.31E-3 (Figure 2.1). Nine genes were identified to be differentially expressed in this pathway: *CUL3, DNAJA2, DNAJC1, DNAJC19, DNAJC21, KRAS, SOD1,* and *PTPLAD1* were found to have an increased expression level post-challenge, while *ABCC1* displayed a decreased level of expression (Table 2.2).

Figure 2.1 — Canonical Pathways Identified by Ingenuity Pathway Analysis.



The top 10 canonical pathways and their significance values are presented. The horizontal threshold line (meeting the left y-axis at 1.30) denotes statistical significance at p≤0.05 after Benjamini and Hochberg multiple testing corrections. The square points represent the ratio, which was calculated as the number of genes in a given pathway that meet significance, divided by the total number of genes that make up that pathway. Nrf2-mediated oxidative stress response was the only canonical pathway to reach significance.

Table 2.2 — Nine Differentially Expressed Genes in Nrf2-Mediated Oxidative Stress Response Pathway.

Probe Set ID	Gene Symbol	4 PAXgene Sample Pairs (Microarray)		5 EDTA Sample Pairs (Microarray)		4 PAXgene Sample Pairs (RT-qPCR)		8 Independent Sample Pairs (RT-qPCR)	
		P Value[a] (2-Tailed)	Fold Change	P Value[a] (1-Tailed)	Fold Change	P Value (1-Tailed)	Fold Change	P Value (1-Tailed)	Fold Change
7993478	*ABCC1*	5.00E-04	-1.17	3.30E-03	-1.11	2.10E-02	-1.09	3.90E-02	-1.16
8059393	*CUL3*	4.72E-02	1.26	1.60E-03	1.11	8.90E-02	1.80	1.72E-01	2.78
8001185	*DNAJA2*	3.20E-03	1.17	2.82E-02	1.10	6.30E-02	1.07	2.02E-01	1.06
7932512	*DNAJC1*	9.00E-04	1.15	1.91E-02	1.11	1.88E-01	1.03	2.42E-01	-1.06
8092314	*DNAJC19*	2.89E-02	1.29	1.24E-02	1.10	1.38E-01	1.35	1.48E-01	-1.09
8104838	*DNAJC21*	5.40E-03	1.14	4.86E-02	1.11	3.50E-02	1.11	1.72E-01	-1.15
7961865	*KRAS*	8.40E-02	1.16	1.10E-03	1.16	3.12E-01	-1.02	1.99E-01	1.05
8068168	*SOD1*	7.60E-03	1.15	9.90E-03	1.09	9.10E-02	5.86	4.56E-01	1.04
7984263	*PTPLAD1*	3.62E-02	1.15	5.10E-03	1.15	2.80E-02	2.88	3.32E-01	-1.60

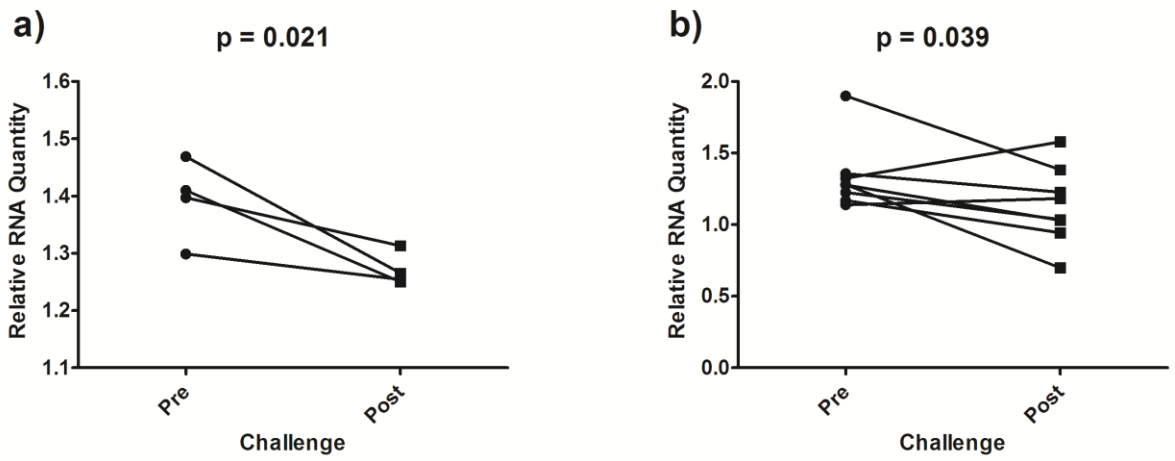
[a] P values were not corrected for multiple testing.

2.3.5 Validation of Selected Genes

Using microarray analysis, the 9 DEGs within the Nrf2-mediated oxidative stress response pathway were tested in a new cohort consisting of 5 independent subjects whose blood samples were collected into EDTA tubes. All of the 9 DEGs reached significant levels of differential expression post-allergen challenge with a one-tailed p value cut-off of 0.05 (Table 2.2).

In order to technically validate the 9 DEGs identified from the microarray results, RT-qPCR was performed on the RNA obtained from the same samples that were used for the initial microarray analysis (4 subjects). Three genes (*ABCC1, DNAJC21* and *PTPLAD1*) were replicated with a one-tailed p value cut-off of 0.05 (Table 2.2). Subsequently, RT-qPCR was carried out using 8 new sets of PAXgene tube samples as hypothesis-testing of the 9 genes. *ABCC1* was the only gene that replicated with a one-tailed p value cut-off of 0.05 (Table 2.2). Pre- and post-challenge *ABCC1* gene expression levels as detected by RT-qPCR are shown in Figure 2.2.

Figure 2.2 — Quantitative Expression of *ABCC1* Pre- and Post-Allergen Inhalation Challenge by RT-qPCR.



Relative mRNA levels of *ABCC1*, as detected in PAXgene peripheral blood samples, were determined using RT-qPCR on a) the 4 original sample pairs with the microarray data, and b) 8 additional sample pairs. Samples were run in triplicates. Values were normalized with a normalized factor calculated using three housekeeping genes: *GUSB, PGK1* and *GAPDH*. P values are based on a one-tailed paired t-test.

2.3.6 Oxidative and Inflammatory Molecules

Additional support for a perturbed oxidative stress response as implicated by the gene expression results was evident when examining plasma levels of oxidative and inflammatory molecules as assessed by collaborators at McGill University. The pre- and post-allergen challenge samples from a subset of 8 out of the 16 individuals (Table 2.1) were tested initially. The levels of free docosahexaenoic acid (DHA) and arachidonic acid (AA) phospholipid in the plasma were found to be significantly decreased following AIC ($p=6.1E-3$ and $p=1.8E-2$, respectively). The AA/DHA ratio was also significantly decreased post-challenge at $p=3.3E-2$. When an additional 21 subjects were included to form a cohort of 29 individuals with paired samples, the analysis showed an up-regulation of nitrotyrosine ($p=5.5E-3$) and a down-regulation of ceramide ($p=3.0E-2$) after challenge.

2.4 Discussion

2.4.1 Gene Sets

In this study, the levels of 9 gene transcripts in the Nrf2 pathway were found to be significantly higher (*CUL3, DNAJA2, DNAJC1, DNAJC19, DNAJC21, KRAS, SOD1*, and *PTPLAD1*) or lower (*ABCC1*) post-AIC. Three validation studies were conducted, including microarray analysis using a cohort of 5 independent subjects, RT-qPCR on the original PAXgene samples used to generate the initial data, and RT-qPCR on 8 new pairs of samples. *ABCC1* was the only gene displaying a consistently significant result, with lowered expression levels post-challenge in all sets of data.

Coupled with previous studies showing that 2 hours post-exposure to diesel could cause lung function decline with increased biomarkers of neutrophilic inflammation in sputum (50), and that peripheral blood can be used to decipher gene expression patterns in asthmatics (51), the data indicates that 2 hours post-AIC is a reasonable time point to observe changes in the peripheral blood gene expression profiles, even when using a small sample size.

GSEA identified the Nakajima eosinophil gene set (renamed from NAKAJIMA_MCSMBP_EOS) (52) as the most significantly down-regulated gene set post-challenge using an initial cohort of 4 pairs of PAXgene blood samples (Appendix 3). Because of the role of eosinophils in allergic diseases (26), a significant change post-challenge in this gene set is of particular interest in our study of asthmatics undergoing allergen inhalation challenge. Specifically, the discovery of perturbations in eosinophil-specific transcripts may lead to a better understanding of the biological processes occurring in eosinophils during an asthmatic response. A limitation in this study, however, was that the frequencies of the various cell types in the peripheral whole blood were not measured for all of the samples. Therefore, possible confounding effects of dynamic changes in cell type numbers on gene expression measurements could not be taken into account.

2.4.2 Nrf2-Mediated Oxidative Stress Response Pathway

The Nrf2-mediated oxidative stress response pathway was the only differentially expressed canonical pathway following AIC in asthmatics. To reduce the possibility that this was a false positive finding, the hypothesis was tested in an independent cohort

consisting of 5 subjects whose pre- and post-allergen challenge blood samples were collected into EDTA tubes. (These subjects were recruited and challenged prior to implementing the use of PAXgene tubes.) Additionally, the results were tested through RT-qPCR on 9 selected genes in the Nrf2-mediated oxidative stress response pathway. This was performed on the original 4 subjects previously used for the microarray analysis, as well as on 8 new sample pairs from additional subjects. *ABCC1* was the only gene showing a consistently lowered expression level post-challenge ($p \leq 0.05$).

Oxidative stress plays an important role in allergic reactions, as triggered by allergen challenges (53,54). In oxidative stress, Nrf2 detaches from its inhibitor Keap1 in the cytoplasm and translocates to the nucleus, leading to transcription of genes encoding a group of stress-induced proteins by binding to the antioxidant response elements (AREs) in the promoter regions (55–57). Thus, Nrf2 activates cellular rescue pathways against oxidative injury and inflammation/immunity (58). The present study has demonstrated that peripheral blood cell transcripts from a gene identified to be in the Nrf2 pathway – *ABCC1* – was expressed at a significantly lowered level in asthmatic subjects 2 hours post-AIC, as compared to pre-challenge.

2.4.3 *ABCC1* Gene

ABCC1/MRP1 (ATP-binding cassette, sub-family C (CFTR/MRP), member 1) was the single gene that retained significance through both the microarray and the RT-qPCR analyses. This gene codes for a protein belonging to the family of ATP-binding cassette transporters, which transports various molecules across cellular membranes, both intra-cellularly and extra-cellularly. Specifically, the protein product of *ABCC1*

mediates the ATP-dependent transport of various organic anions, using molecules such as oxidized glutathione and cysteinyl leukotrienes as substrates (59,60). Interestingly, oxidized glutathione forms a link back to the topic of oxidative stress; firstly because the molecule is generated by the activity of antioxidant enzymes (61). Secondly, an increase in the ratio of oxidized glutathione to its naturally reduced form is indicative of oxidative stress (62). Further relevance for the clinical presentation of asthma lies within cysteinyl leukotrienes, a type of fatty signaling molecule that can cause bronchial smooth muscle contraction and stimulate pro-inflammatory activities, including the recruitment of eosinophils to the asthmatic airways (63,64). Finally, a previous pharmacogenetics study has identified a single-nucleotide polymorphism (SNP) in the *ABCC1* gene that was associated with an increase in % predicted FEV_1 in asthma patients who had undergone treatment with the leukotriene receptor antagonist montelukast (65).

In the current study, the gene expression of *ABCC1* was shown to have decreased 2 hours post-AIC. This finding suggests that there may also be a corresponding reduction in the activity of the membrane transport system as facilitated by the gene product of *ABCC1*. However, such a conclusion, along with its biological implications, cannot be so readily drawn, given that peripheral blood samples rather than lung tissues were used as the source of mRNA. Peripheral blood contains a heterogeneous mixture of immune cells, all of which could contribute to the gene expression signals. Hence, it is difficult to decipher between the various cell types to understand the biological mechanisms, which also may not be reflective of the processes occurring directly in the lungs.

2.4.4 Oxidative and Inflammatory Molecules Support

Building on the finding of a differentially expressed oxidative stress response pathway, additional support was provided through the work of collaborators, who assessed molecular indicators of oxidative stress in the plasma. Using a subset of samples consisting of 8 pre- and post-challenge pairs, and a subsequent expanded cohort, the data indicated changes in the signals of oxidative stress and inflammation, through perturbed levels of DHA, AA, nitrotyrosine, and ceramide following AIC.

DHA, an omega-3 (ω-3) fatty acid, and AA, an omega-6 (ω-6) fatty acid, are both essential fatty acids in the human body involved in the inflammatory response. In the biological pathway, these two families of fatty acids are in close interactions, competing with each other for the same enzymes. However, the health benefits of these lipids are vastly different. DHA has been shown by numerous studies to be anti-inflammatory (66,67), lowering inflammatory markers and cytokines. AA, on the other hand, has been shown to contain pro-inflammatory properties and increase the risk of various diseases, especially when the levels of ω-6 fatty acids greatly exceed the levels of ω-3 (68–71) Hence, the ratio of AA/DHA is essential to assess the overall degree of inflammation. In the present data, a significant decrease post-allergen challenge was noted in the levels of DHA, AA, and AA/DHA ratio, indicating a perturbed state of inflammation in the peripheral blood after challenge.

Nitrotyrosine, which was found to be up-regulated, is a molecule that is indicative of the presence of reactive nitrogen species (RNS) (72). Because RNS are often associated with reactive oxygen species (ROS) in causing oxidative/nitrosative stress

resulting in cellular damage, this molecule was worth assessing within the context of a perturbed oxidative stress response, as already shown in my data.

Ceramide is a lipid that can act as a signaling molecule to regulate a wide range of cellular processes, including differentiation, proliferation, and apoptosis (73). Previous studies have suggested that oxidative stress and ceramide are closely linked, especially in the context of cell death induction (74). Evidence for this includes common apoptotic triggers for the production of ROS and ceramide, and extensive cross-talk between the two signaling pathways that are mutually influential to each other (74). Hence, a significant decrease in plasma ceramide level post-allergen challenge may be a reflection of an altered oxidative stress response, as was suggested through the microarray analysis.

2.5 Summary

This preliminary study has demonstrated that significant changes can be detected in the peripheral blood genomic profile at 2 hours after asthmatic individuals undergo allergen inhalation challenge. The Nrf2-mediated oxidative stress response pathway was identified as a perturbed pathway, and the *ABCC1* gene was validated by both microarray analysis and RT-qPCR to be expressing at a significantly lower level post-challenge. These findings were further supported by collaborators' work, which showed corresponding changes in the levels of various oxidative and inflammatory molecules in the plasma. All of these results are valuable contributions to an initial step in exploring the biology behind the asthmatic response at 2 hours post-allergen inhalation challenge.

CHAPTER 3: CHEMOKINE (C-C MOTIF) LIGAND 2

3.1 Introduction

In asthmatics undergoing AIC, cell signaling plays a vital role in determining the final outcome and severity of response. Cytokines represent one such means of intercellular communication that is extensively used, especially by cells in the immune system. Numerous studies have established their importance in the pathogenesis of asthma, demonstrating their effects on inflammation and airway remodeling (75,76). In particular, cytokines produced by T helper (Th) 2 cells are believed to be major contributors in mild asthma through stimulation of immune cell differentiation, and have been studied as potential therapeutic targets (77–80). On the other hand, cytokines with anti-inflammatory effects have also been suggested to have a role in helping to contain excessive allergic inflammation (81).

Given this and the ability of cytokines to travel through the blood stream to exert effects on distant cells, it is hypothesized that changes in cytokine levels can be detected in the peripheral blood 2 hours after AIC, as compared to pre-challenge levels. A positive finding would help reinforce the current model of asthma pathophysiology, in which the secretion of cytokines shortly after allergen inhalation is believed to be a major factor leading to the inflammatory state of the airways in the LAR. If shown to be true, this would also provide strong additional support for the results of the previous chapter – that molecular changes are indeed present in the peripheral blood at 2 hours post-challenge.

Furthermore, it is worthwhile to investigate any differences in cytokine profiles that may be distinguishable between the isolated early responders and the dual responders, especially at the time point of 2 hours post-challenge when these two response profiles start to diverge. Based on the proposed mechanism that the recruitment of additional inflammatory cells to the lungs is the main driving force for the LAR, it would be of great interest to observe a corresponding cytokine profile in DRs that is absent from ERs. Such discoveries can easily translate into potential therapeutic targets, by way of cytokines and their receptors, to minimize the late phase response.

The aim of this chapter is to explore the cytokine profiles as found in the peripheral blood plasma, pre- and 2 hours post-allergen inhalation challenge. This will be performed by testing the samples against a panel of cytokines/chemokines, with follow-up validation studies using plasma from various other cohorts, including control subjects.

One major finding, and the focus of a large portion of the chapter, is chemokine (C-C motif) ligand 2 (CCL2), also called monocyte chemotactic protein-1 (MCP-1). This cytokine is secreted by various cells, including monocytes, macrophages, lymphocytes, fibroblasts, and keratinocytes (7,82). It binds to the cell surface receptors CCR2 and CCR4 (83). As its name implies, this cytokine displays chemotactic abilities, attracting monocytes, basophils, and lymphocytes but not neutrophils or eosinophils (7,82,84). Accordingly, CCL2 has been implicated in many disorders involving monocyte infiltration, such as psoriasis, rheumatoid arthritis, and atherosclerosis (85). This cytokine also activates macrophages, stimulates histamine release from basophils, and promotes Th2 immunity (7). Because of its involvement in immune processes and its

chemotactic properties, it is suspected that CCL2 may have a role in the recruitment and differentiation of inflammatory cells in the airways of asthmatic individuals after AIC. This will be discussed in more detail later on in the chapter.

3.2 Methods

3.2.1 Cohorts

Four cohorts of adult subjects (age 18-60), as summarized in Table 3.1, were examined for the cytokine study. The groups represent individuals with atopic asthma (n=39), occupational asthma (n=7), allergic rhinitis (n=32), as well as control subjects (n=6), to form a total of 84 subjects.

The atopic asthmatic cohort consists of individuals recruited from Université Laval (24 subjects), McMaster University (9 subjects) and Vancouver General Hospital - UBC (6 subjects). Informed consents were obtained prior to the study. The inclusion and exclusion criteria were as described in section 2.2.1. Within this group, an initial discovery cohort was assembled using 32 subjects of mixed allergen sensitivity. Subgroups were also formed based on allergen sensitivity (cat dander - 12 subjects, grass pollen - 10 subjects, and house dust mite - 6 subjects). All participants within these subgroups were part of the initial cohort, with the exception of 7 new individuals within the grass pollen-sensitized group.

Seven individuals with occupational asthma who were sensitized to Western red cedar (WRC), or plicatic acid found within WRC dust, were also included in this study. A diagnosis of WRCA is based on both an occupational history and objective evidence that inhalation challenge test using extracts of plicatic acid results in acute respiratory

symptoms and lung function changes. All subjects were recruited from Vancouver General Hospital – UBC.

In addition, 39 individuals exhibiting seasonal allergic rhinitis symptoms to short ragweed pollen were also studied. They were recruited from Queen's University, with informed consent. Allergic rhinitis was determined by medical history, physician diagnosis and a positive response to skin prick test using short ragweed pollen.

Finally, six non-asthmatic healthy control subjects from St. Paul's Hospital were included in the investigation to enable comparison of data between allergic and non-allergic individuals. These three males and three females were age-matched as closely as possible with the subjects in the atopic asthmatic cohort.

Table 3.1 — Subject Demographics (CCL2).

Cohort	Sub-groups	# Subjects	# Challenges	Site (# Challenges)	Mean Age	Sex (M:F)	Allergen (# Challenges)	Responses[e] (# Challenges)
Atopic Asthma (n=39)	Discovery	32	35[a]	Laval (26), VGH (7), McM (2)	33.3 ± 1.8	13:19	Cat (19), Grass (7), HDM[b] (8), Other (1)	ER (13), DR (18), Undetermined (4)
	Cat Allergen	12	12	Laval (12)	30.8 ± 2.2	3:9	Cat (12)	ER (7), DR (5)
	Grass Allergen	10	11	Laval (2), VGH (1), McM (8)	26.1 ± 2.7	7:3	Grass[c] (11)	ER (5), DR (6)
	HDM Allergen	6	6	Laval (6)	31.2 ± 4.6	2:4	*D. pteronyssinus* (3), *D. farinae* (3)	ER (1), DR (5)
Occupational Asthma		7	8	VGH (8)	43.3 ± 5.4	7:0	Methacholine & Plicatic acid (8)	DR (3), LR (2), NR (3)
Allergic Rhinitis		32	32	Queens (32)	34.3 ± 1.6	15:17	Short Ragweed (32)	ER (9), DR (6), PER (17)
Control		6	6[d]	St. Paul's (6)	33.7 ± 4.3	3:3	N/A	N/A

[a] The number of challenges are different from the number of subjects because some subjects received a repeated allergen challenge.

[b] HDM=House dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina*).

[c] Grass allergen includes pollen from Timothy grass, Orchard grass, Grass mix, and Ragweed.

[d] Although control subjects did not undergo allergen challenge, 6 pre/post samples pairs were still collected.

[e] ER=Early responder, DR=Dual responder, LR=Late responder, NR=Non-responder, PER=Protracted early responder.

3.2.2 Allergen Challenges and Sample Processing

Allergen inhalation challenge was administered to atopic asthmatic individuals as described in the previous chapter. Peripheral venous blood samples were collected prior to allergen challenge and 2 hours after the last inhaled allergen dose using EDTA tubes. Four of the 39 individuals were given a second AIC, bringing the total pre/post sample pairs to 43. The FEV_1 lung function was monitored for 7 hours post-challenge and the response was categorized as either an isolated early response (FEV_1 drop ≥20% from baseline between 0 and 2 hours) or a dual response (FEV_1 drop ≥15% between 3 and 7 hours, in addition to the early response).

For individuals with occupational asthma, the allergen challenge procedure was similar to the above, with the exception that all subjects were challenged with plicatic acid extracts. In addition to the blood samples collected pre- and 2 hours post-AIC, peripheral blood was also drawn before and 2 hours after methacholine inhalation challenge on the previous day. Out of the 7 subjects, one individual received a second methacholine and allergen challenge. Subjects were categorized as ER, DR, LR (late response, defined by a single FEV_1 drop ≥15% between 3 and 7 hours), or NR (non-responder).

Allergic rhinitis subjects were given a skin prick test of ragweed pollen and 13 other allergens in order to confirm history of seasonal allergic rhinitis as part of the screening process. Eligible individuals returned for the allergen challenge in the Environmental Exposure Unit (EEU), where ragweed pollen was circulated at a controlled and consistent level, enabling all subjects to simultaneously receive an equal

amount of pollen exposure. Participants were required to record their rhinoconjunctivitis symptoms on score cards at 30 minute intervals throughout the 3 hour pollen exposure, and every hour from 6 to 12 hours after the start of the allergen challenge. Peripheral blood samples were collected into EDTA tubes immediately prior to and after the 3 hour ragweed pollen exposure in the EEU. Upon compilation of the score cards, subjects were categorized into either early responders (marked symptom scores increase, then drop ≥50% by 7 hours), dual responders (early response plus an increase in symptom scores after 2 hours of decreased scores), or protracted early responders (an initial increase in symptom scores, then decrease by <50% by 7 hours).

The 6 non-asthmatic control subjects did not undergo allergen challenge. However, venous blood samples were still drawn into EDTA tubes at two time points – 9 am and 12 noon – to parallel the blood sampling of the asthmatic subjects occurring pre- and 2 hours post- the last inhaled dose of allergen.

All of the samples collected pre- and post-allergen challenge were immediately centrifuged at 500 x g for 10 minutes at room temperature to separate the layers of plasma, buffy coat, and erythrocytes. These three blood fractions were isolated and aliquoted into microcentrifuge tubes, and were frozen and transported to the laboratory on dry ice to be stored at –80°C.

3.2.3 Multiplex Cytokine Assay

From the initial discovery cohort of atopic asthmatic challenge subjects, 35 pre/post pairs of thawed plasma samples were analyzed on a panel of 42 cytokines at our collaborator's laboratory at McGill University. For this procedure, the MILLIPLEX

MAP Human Cytokine/Chemokine Premixed 42-Plex Panel assay (Millipore, Billerica, MA, USA) was used, following the manufacturer's protocol. The signals were detected with the Luminex 100 system (Luminex, Austin, TX, USA), using xMAP technology, which allows for rapid and precise detection of fluorescent intensities in multiplexed assays.

3.2.4 MSD Immunoassay Validation

Follow-up study of a selected cytokine, CCL2/MCP-1, was performed using the Human MCP-1 Ultra-Sensitive Kit from MULTI-ARRAY Assay System (Meso Scale Discovery, Gaithersburg, MD, USA). This single-plex assay employs a sandwich immunoassay format in conjunction with an electrochemiluminescent label. Signals are generated and read when voltage is applied to the plate electrodes in the MSD SECTOR Imager 6000 instrument (Meso Scale Discovery, Gaithersburg, MD, USA). The cytokine concentrations in the samples were calculated based on a standard curve generated by plotting a known serial dilution of CCL2 (included in the kit) against the signal output. CCL2 validation studies by MSD technology was carried out on all of the allergen-specific subgroups (cat, grass, and HDM allergens) within the atopic asthma cohort, as well as on the occupational asthma cohort, the allergic rhinitis cohort, and the control subjects. Samples were run in duplicates and triplicates with high reproducibility of results (median CV=1.87%).

3.2.5 Statistical Analysis

For the initial discovery cohort of 35 sample pairs that were analyzed on the MILLIPLEX 42 cytokines panel, pre- versus post-allergen challenge results were

evaluated using two-tailed paired t-tests and declared significant at p≤0.05. Subsequent studies of the CCL2 cytokine using MSD technology employed the one-tailed t-test, assuming that the directionality of change would replicate. Significance cut-off remained at p≤0.05. Baseline CCL2 levels were evaluated with respect to allergen, age, and sex using a multiple linear regression model as performed in R (statistical computing program) version 2.12.0 (86). Significance was set at p≤0.05.

3.3 Results

3.3.1 Cohort Characteristics

A total of 84 subjects were recruited across the different cohorts for this study (Table 3.1). Among these participants, 97 pre/post sample pairs were collected from 83 allergen challenges, 8 methacholine challenges, and 6 healthy controls without challenge. The overall mean age was 33.86 ± 1.1, with the youngest cohort being the grass allergic asthma group (26.1 ± 2.7), and the oldest cohort being the plicatic acid occupational asthma group (43.3 ± 5.4). An equal number of males and females were enrolled, with exactly 42 individuals in each group. However, within cohorts and subgroups, the breakdown of the sexes was not so evenly distributed. Of particular note is the Western red cedar/plicatic acid occupational asthma cohort, which is comprised entirely of male participants.

Since different models of allergen challenges were used for the different allergic diseases, a universal indicator of positive response was not feasible. Nonetheless, in all subjects within the atopic asthma and the allergic rhinitis cohorts, a positive early phase response was detected within 3 hours, either by a FEV_1 drop ≥20% from

baseline, or by a marked increase in total nasal/non-nasal rhinitis symptom score. The late phase response was observed in 23 asthmatic challenges and 6 allergic rhinitis challenges. The remaining 17 allergic rhinitis subjects were categorized as protracted early responders (Table 3.1). In occupational asthma subjects undergoing plicatic acid challenge, none of the participants displayed an isolated early response. However, FEV_1 profiles of an isolated late responder and a non-responder were detected in two and three of the allergen challenges, respectively. Methacholine challenge of the same individuals resulted in an isolated early phase response in all subjects.

3.3.2 Multiplex Cytokine Assay Analysis

At McGill University, plasma samples from an initial discovery cohort of 32 atopic asthmatics undergoing 35 allergen challenges were analyzed against a panel of 42 cytokines. The results for pre- versus post-challenge analysis are presented in Appendix 5. Out of these cytokines, CCL2 (also known as MCP-1) and CCL5 (also known as RANTES – Regulated upon Activation, Normal T-cell Expressed, and Secreted) were the only cytokines identified by paired t-tests to have been significantly perturbed post-challenge, at p=5.7E-4 and p=2.2E-2, respectively. CCL2 showed a significant decrease while CCL5 showed a significant increase after AIC.

3.3.3 CCL2 Validations

Focusing on the most significant finding of a decreased level of CCL2 after AIC, hypothesis-testing experiments using MSD's electrochemiluminescence technology were performed. The results are summarized in Figure 3.1. Using the same samples that were analyzed in the initial discovery phase of the study, significance was

replicated when examining samples from a subgroup of 12 cat allergen-sensitized individuals ($p=5.5E-4$, using a one-tailed t-test). However, testing samples from a cohort of 6 HDM-sensitized subjects who were also part of the initial cohort failed to yield statistically significant results. When evaluating the subgroup of allergic asthmatics sensitized to grass allergen, in which 7 new individuals were included, the findings were once again significant ($p=2.4E-4$).

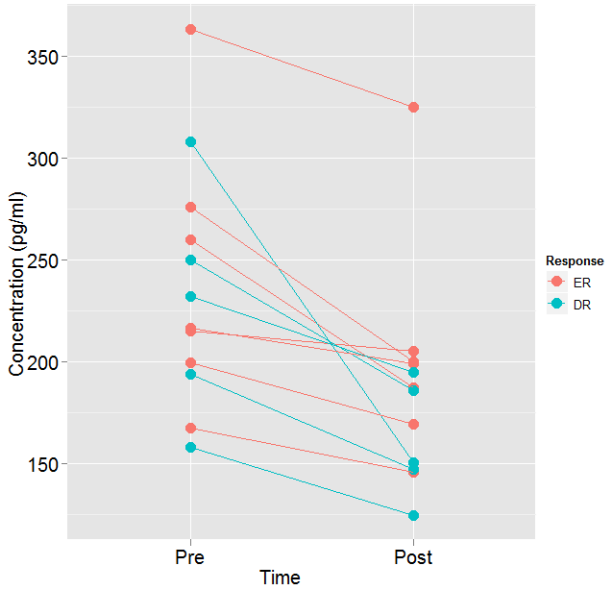
The investigation of perturbed plasma CCL2 levels post-challenge was extended to a cohort of subjects with occupational asthma. Although analysis of the samples taken before and after methacholine challenge were insignificant, pre- versus post-plicatic acid challenge examinations revealed significant results ($p=6.8E-3$). Broadening the study to include a cohort of allergic rhinitis subjects, the levels of CCL2 was still significantly decreased after ragweed pollen allergen exposure ($p=2.2E-10$). Finally, the samples from 6 control subjects who had no history of asthma or allergic rhinitis and who did not undergo allergen challenge were tested. When analyzed with a 2-tailed paired t-test, the CCL2 levels were found to be significantly decreased ($p=1.3E-2$).

In addition to evaluating changes in CCL2 levels between pre- and post-challenge, attempts were also made to examine any differential patterns of CCL2 perturbations between isolated early and dual responders. However, there did not appear to be any differences between the two response types, based on the analyses of data from either the atopic asthmatic or the allergic rhinitis cohorts (results not shown).

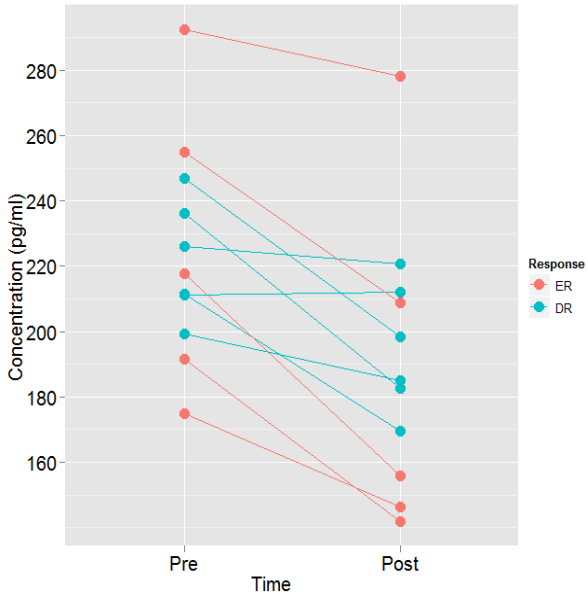
Figure 3.1 — Plasma CCL2 Levels at Pre- and Post-Allergen Challenge.

a) Dot Plot Representations

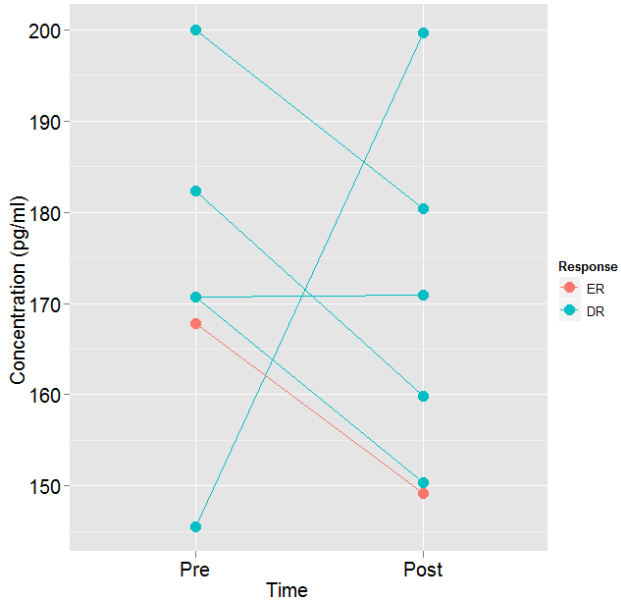
Cat Allergen*



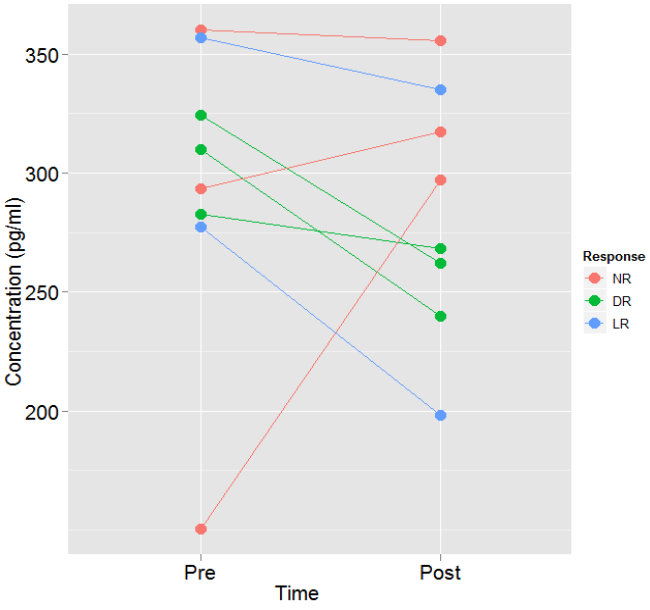
Grass Allergen*



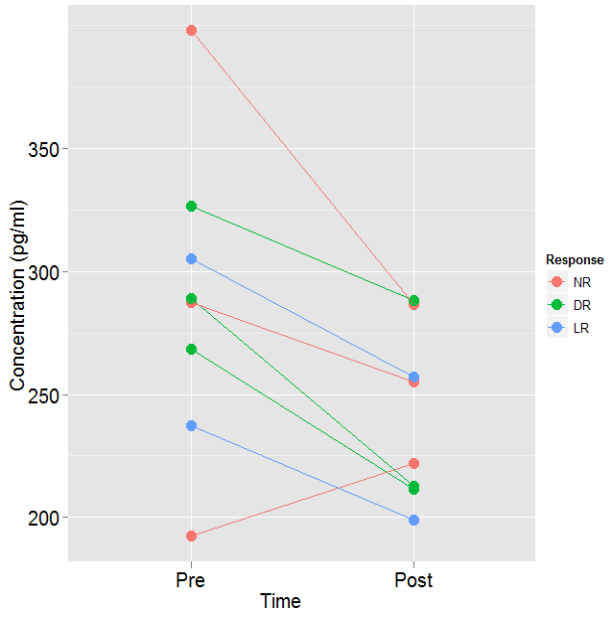
HDM Allergen



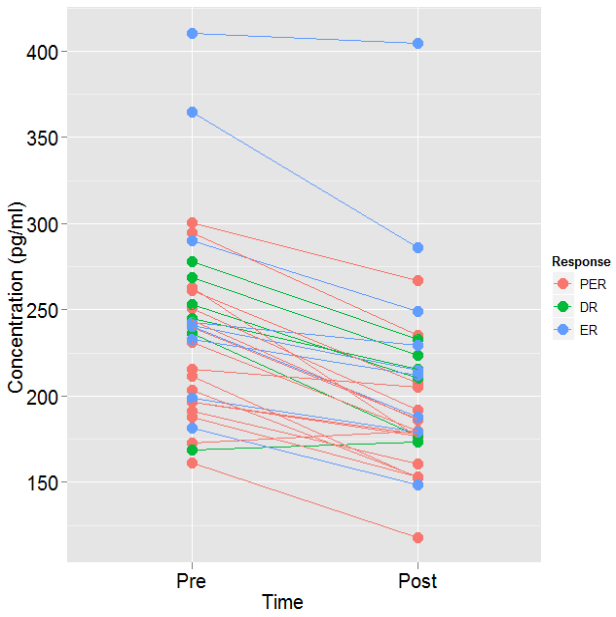
Methacholine



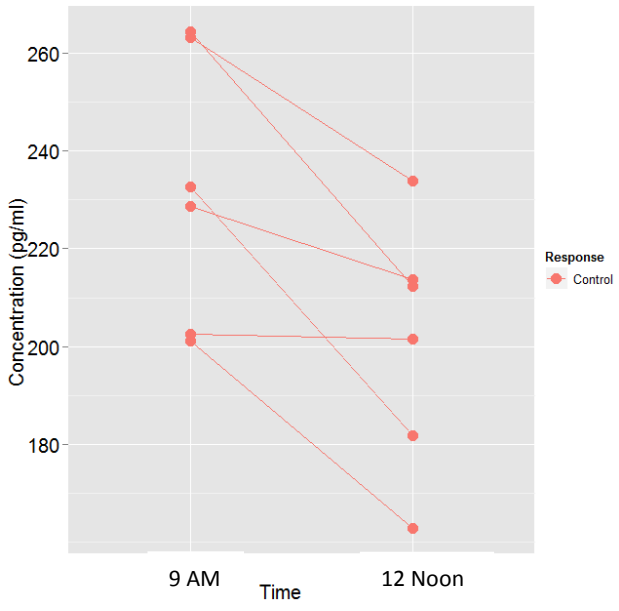
Plicatic Acid*



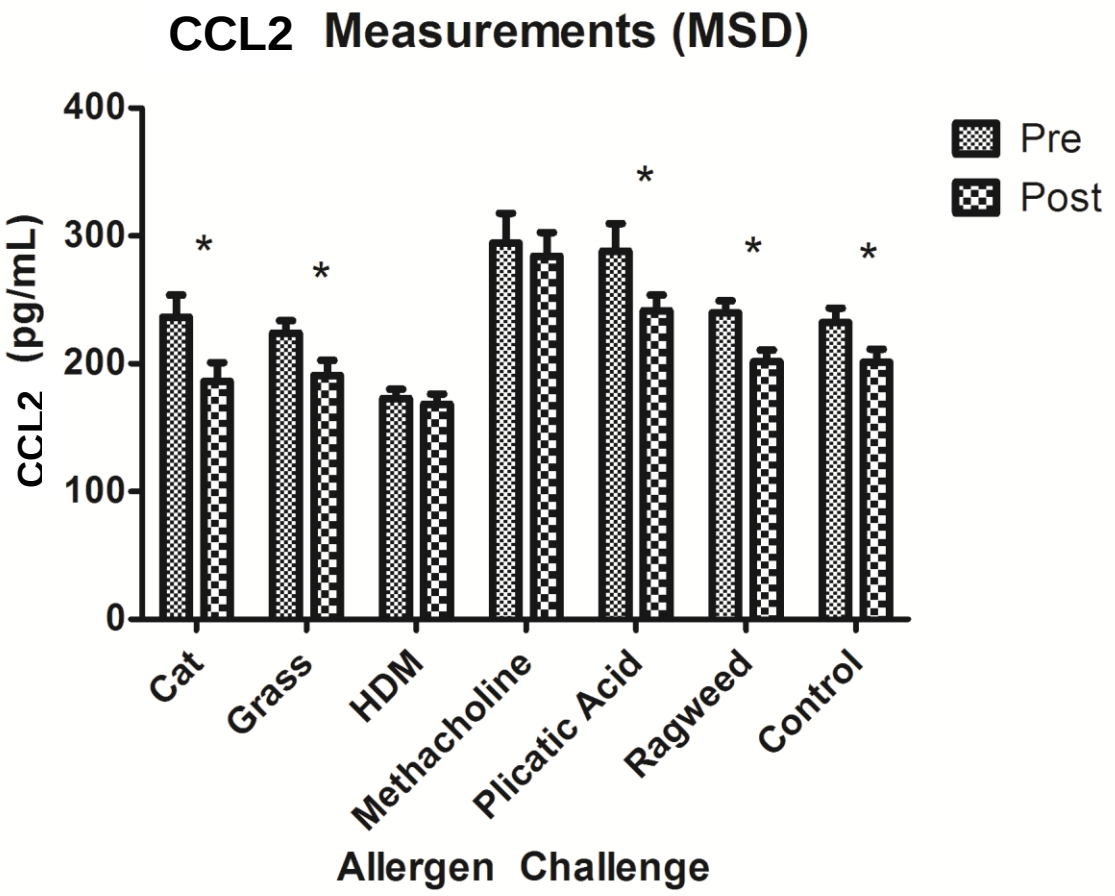
Ragweed Allergen*



Controls*



b) Histogram Representation



Pre- and post- plasma CCL2 levels (measured by MSD) are presented for each cohort of subjects grouped by allergens. Asterisks denote statistically significant results ($p \leq 0.05$) when comparing between pre- and post-challenge samples, as calculated by a one-tailed paired t-test. In the case of the control cohort, consisting of subjects who were not exposed to allergens, a two-tailed paired t-test was used. In dot plot representations (a), ER=Early responder, DR=Dual responder, PER=Protracted early responder, NR=Non-responder, LR=Late responder. In histogram representation (b), the mean and SEM are shown.

3.3.4 Baseline Level Correlations

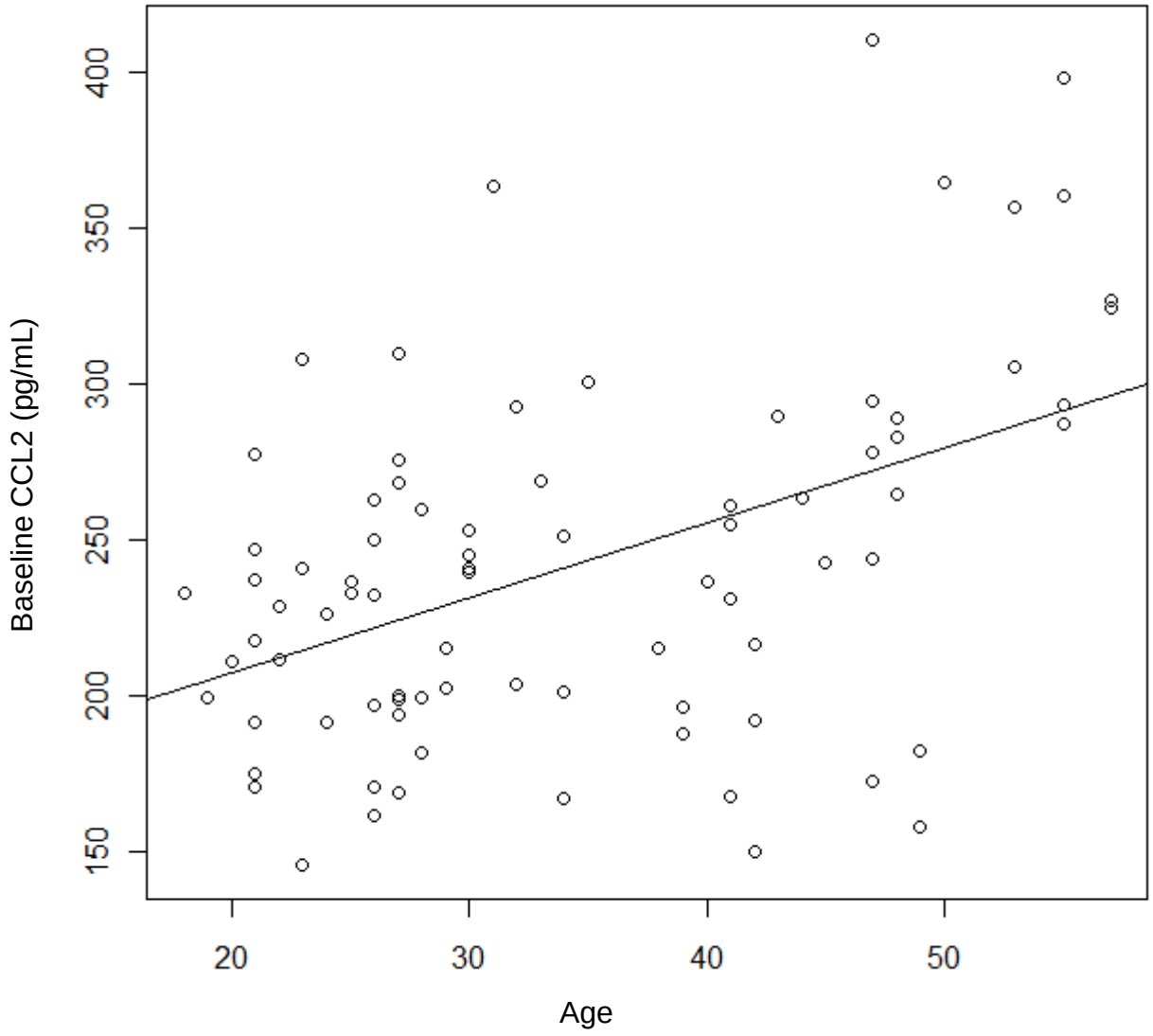
Pre-allergen challenge CCL2 levels were examined in conjunction with subject demographical and clinical data in order to explore whether baseline CCL2 levels may be influenced by any of these factors. Considering the disease categories of atopic asthma, occupational asthma, allergic rhinitis, and normal controls, the only significant difference was found between the occupational asthma group and the atopic asthma group. Specifically, baseline CCL2 levels were higher in subjects with occupational asthmatic as opposed to subjects with atopic asthma. CCL2 levels were not found to be different between control subjects and allergic subjects as a whole. When the groups were further broken down according to specific allergens, pre-challenge CCL2 levels were revealed to be different only on two occasions: 1) between the HDM-sensitized group (within the atopic asthmatic cohort) and the plicatic acid-sensitized group (occupational asthma cohort), and 2) between the HDM-sensitized group and the ragweed pollen-sensitized group (allergic rhinitis cohort). In both cases, lower CCL2 levels were noted in the HDM-sensitized individuals.

Subject demographics, specifically the age and sex of participants, were analyzed in conjunction with baseline levels of CCL2 using a linear model. CCL2 was found to be positively correlated with age in a simple regression analysis ($p=7.3E-6$, $r^2=0.2114$). This relationship is illustrated in Figure 3.2. When sex and the interaction between age and sex were added to the model, however, the results failed to reach significance. Next, the data were separated and plotted based on cohorts (Figure 3.3). This revealed a sampling bias in which atopic asthmatic subjects clustered in the younger age range, while participants in the occupational asthma cohort clustered in the

older age range. In order to minimize possible confounding effects related to allergen sensitivity and age, the previously described analysis was repeated using only the cohort of the most even age distribution – the allergic rhinitis group. The findings remained supportive of an increase in CCL2 with age, in both the single-covariate analysis (age only, p=4.0E-3), and the multi-covariate analysis (age and sex, p=6.1E-3). The results from the sex-based analysis did not reach significance.

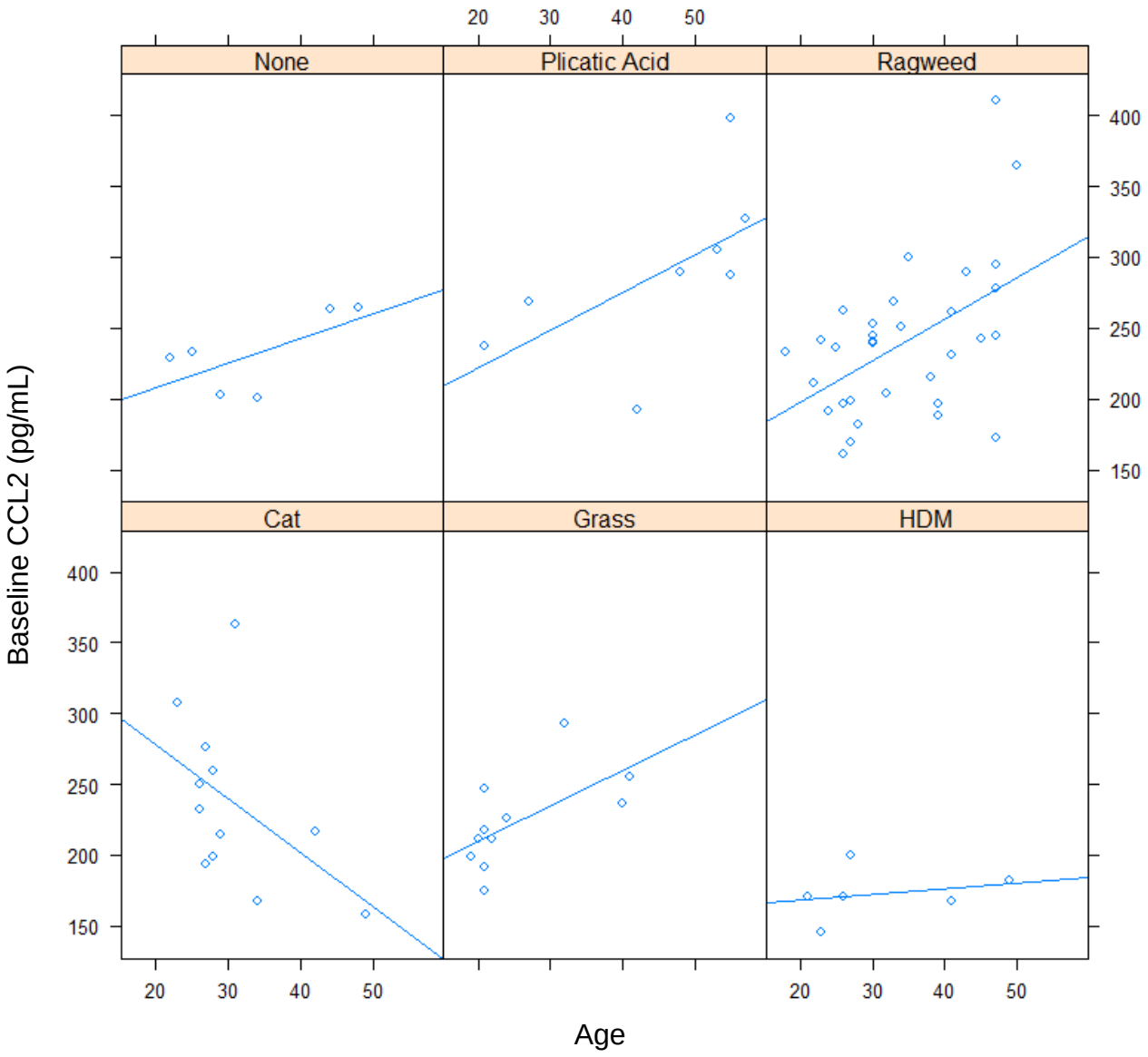
In addition to the age and sex of subjects, several clinical parameters were also assessed for possible associations with baseline CCL2 levels. The % FEV_1 drops in the EAR and the LAR, as measured during allergen challenges in the atopic asthma cohort, were analyzed and found to have no relationship with baseline CCL2. Similarly, no significant association was observed when the cytokine level was tested against the response phenotype (ER or DR) in either the atopic asthmatic cohort or the allergic rhinitis cohort. Peripheral blood monocyte counts were also assessed because of their role as the main target cell type of CCL2. The analysis of the monocyte counts (both absolute and relative counts) and CCL2 data (obtained from the allergic rhinitis cohort and the cat allergen cohort) did not reveal any significant associations between the baseline cytokine level and either the baseline monocyte level or the change in monocyte counts after allergen challenge.

Figure 3.2 — Baseline CCL2 Level and Age Association.



Subject age was correlated with baseline levels of CCL2. Older age is associated with an increase in baseline CCL2 level ($p=7.3E-6$, $r^2=0.2114$).

Figure 3.3 — Baseline CCL2 Level with Age in Allergen Cohorts.



Baseline CCL2 data was separated according to allergen cohorts. This revealed a sampling bias towards younger participants in the atopic asthmatic groups (cat, grass, HDM), but older participants in the occupational asthma group (plicatic acid). The only cohort to show an even distribution of age was the allergic rhinitis group (ragweed).

3.4 Discussion

3.4.1 CCL2 and CCL5 in Allergic Diseases

In this study of plasma cytokine profiles associated with AIC, CCL2 and CCL5 were identified to be cytokines of interest, displaying a significant decrease and a significant increase, respectively, 2 hours post-AIC. This was observed from a cohort of atopic asthmatic subjects with mixed allergen sensitivity, through the use of a multiplex assay on a cytokine panel. Subsequently, the CCL2 results were replicated in cohorts of various allergic diseases, using a more sensitive single-plex CCL2 immunoassay.

CCL5/RANTES is a chemokine that is produced by various cells including T lymphocytes, endothelial cells, fibroblasts, and eosinophils (87). It has the ability to attract Th cells, eosinophils, and basophils to the airways during an asthmatic response (88). Many studies have inquired into possible associations between CCL5 polymorphisms and asthma; however, results have been mixed (89–91). On the protein level, an increase in the cytokine has been demonstrated in the BAL fluid and serum of asthmatics, with a positive correlation to disease severity (87,92). Following neutralization of CCL5, decreased eosinophil recruitment to the allergic airways has also been observed in a mouse model, providing further support for a mechanistic role of the cytokine in asthma (93). In the present study, CCL5 was found to be increased in the plasma after AIC. This is in direct agreement with the work of Chihara *et al.*, who showed that plasma CCL5 levels were significantly higher in subjects undergoing an asthmatic response, as compared to the same subjects during the asymptomatic state, or to control subjects (94). The elevated level of CCL5 post-AIC suggests that it may

play an important role in asthma pathophysiology, perhaps through eosinophil recruitment and activation (95).

The involvement of the chemokine CCL2/MCP-1 in allergic diseases has previously been explored. Starting at the genetic level, there have been reports of polymorphisms found to be associated with increased asthma risk (96,97). For the protein, numerous studies have shown a significant increase in CCL2 levels in asthmatic BAL fluid and serum at baseline and after allergen challenge (92,98,99), a pattern that was also observed from subjects with allergic rhinitis (100). Additionally, many *in vitro* and *in vivo* studies have investigated further into the cytokine's possible roles in allergic asthma (101). As mentioned in the introduction of this chapter, CCL2 has the ability to attract inflammatory cells, such as monocytes and lymphocytes, to the airways (102,103). These cells can in turn release cytokines and attract additional immune cells, thereby amplifying the inflammatory response in an asthmatic reaction (101). Moreover, there is evidence that CCL2 may induce histamine and leukotriene release, which would exert a direct effect on bronchial hyperreactivity (104). Finally, CCL2 has been suggested to enhance Th2 activity, perhaps by encouraging the polarization of CD4 cells to the Th2 phenotype (105,106). Since Th2 cytokines are important in the development of asthma and allergic rhinitis, this provides additional support that CCL2 may play a pivotal role in allergic diseases (107).

Contradictory to the reports discussed above, the level of plasma CCL2 was observed to have decreased following allergen challenge in the current study. This may be explained by variations in experimental design, such as differences in the specimens tested and the nature of the allergen challenges. The sampling time point represents

another factor of consideration. In the present study, post-challenge samples were taken at 2 hours after allergen inhalation; whereas previously, the time point was set as 4 hours or more. Because the infiltration of inflammatory cells is a hallmark of the late phase response (which also occurs in allergic rhinitis subjects) and CCL2 can contribute to this event through binding to its receptor CCR2 on target cells (101), it is possible that the reduction in plasma CCL2 shortly after allergen inhalation challenge represents the uptake of the cytokine in such a process. Indeed, Maus *et al*. have demonstrated that an increase in monocyte accumulation is accompanied by a consumption of CCL2 (108). Although their *in vitro* study differs from the current investigation, it nonetheless offers a possible explanation for the decrease in CCL2 levels post-challenge.

3.4.2 Normal CCL2 Fluctuation

Despite all this, however, it is unlikely that the drop in plasma CCL2 is a result of biological processes triggered by allergen inhalation. The reason is that even in non-atopic control subjects who have not undergone allergen challenge, a significant decrease in plasma CCL2 levels remained evident when comparing between samples drawn at 9am and at noon. This suggests that there may be a physiological, and perhaps diurnal, fluctuation of CCL2 levels in the peripheral blood in all individuals, whether challenged or not.

To date, there have been no reports describing a CCL2 fluctuation in healthy individuals. Some studies have investigated the effects of stress, in the form of exercise, on circulating CCL2 levels in normal individuals. For instance, Fatouros *et al*. reported that serum CCL2 decreased after 30 minutes of exercise (109). However,

results have been conflicting, as other studies have found an increase in the cytokine level at 1 hour and 6 hours after exercise (110,111).

Despite these potential explanations, direct support of an inherent decrease in circulating CCL2 levels is still lacking from existing literature. Therefore, future time-course studies involving additional healthy subjects should be carried out in order to confirm the current findings. If shown to be true, this would have implications for other studies involving CCL2. For example, in the area of cardiovascular diseases, there have been reports of a decrease in serum CCL2 levels immediately after primary percutaneous coronary intervention as compared to prior to treatment (112). Garcia-Alonso *et al*. also described a lowered circulating CCL2 level at various time points (within 24 hours) after a morning ingestion of anthocyanin, a substance believed to be protective against coronary heart disease (113). Interestingly, the decrease was significant only at the 3 hour time point. Since the timing of the experimental procedure was similar to that of the AIC (in terms of morning initiation and ~3-hour post sampling), the CCL2 decrease reported by Garcia-Alonso *et al*. may possibly reflect a physiological fluctuation, as opposed to a biological outcome of anthocyanin ingestion. Hence, caution should be taken when interpreting such results.

3.4.3 Baseline CCL2 Associations

Baseline plasma CCL2 levels were analyzed in the context of subject demographics. Results indicated that age was a major factor influencing the cytokine level, with older subjects having higher baseline CCL2. Numerous studies have reported a positive relationship between aging and increasing CCL2 levels. Three

studies have investigated the level of circulating CCL2 in healthy subjects of various ages; the findings were unanimously in support of the current results (114–116). Using animal models, elevated CCL2 levels were also evident in different tissues of aging mice. Examples of these include heart tissue and vascular smooth muscle cells, attesting to the higher risk of cardiovascular diseases (as well as other inflammatory diseases) with age (117,118).

Different reasons have been proposed to explain this association. First, the increase in CCL2 has been suggested to be a reflection of the development of atherosclerosis, perhaps sub-clinically (114). This stems from many human and animal studies demonstrating a link between CCL2 and atherosclerosis (119). Another explanation is that the increase in CCL2 may be indicative of a shift towards the Th2 phenotype as the immune system ages (115). While this idea is supported by some studies (120,121), others have provided evidence for the opposing argument of an enhanced TH1 response with aging (122,123). In the current investigation, potential reasons for the correlation between CCL2 and age cannot be easily speculated upon without further work assessing subject history and other molecular signals of the immune system.

3.5 Summary

In this chapter, CCL2/MCP-1 was identified to be a cytokine that is significantly decreased in plasma post-allergen inhalation challenge. This finding was replicated in various cohorts of allergic diseases, including asthma and allergic rhinitis. A down-regulation was also observed in healthy control subjects, indicating that the decrease in

CCL2 level may reflect a physiological fluctuation of the cytokine, as opposed to an outcome of allergen inhalation. Finally, baseline CCL2 levels were found to be associated with age, with higher cytokine levels observed in older subjects.

CHAPTER 4: ISOLATED EARLY RESPONSE VERSUS DUAL RESPONSE

4.1 Introduction

Having shown in Chapters 2 and 3 that it is possible to observe peripheral blood molecular perturbations, especially transcriptomic changes, at 2 hours after AIC, the next step was to compare and contrast the isolated early and the dual responses. As discussed previously, these two response patterns are hypothesized to be the result of varying immunological processes, with the LAR believed to involve the infiltration of inflammatory cells to the airways, leading to a more detrimental clinical outcome as compared to the isolated early response (9). In order to address this experimental aim, the focus was on a cohort of allergic asthmatic adults challenged with cat allergens. The study was performed through the analysis of transcriptomic changes between pre- and post-AIC, as assessed by microarrays.

By attempting to distinguish between these two response phenotypes through the study of genome-wide gene expression changes, it was anticipated that the biological mechanisms underlying these response patterns could be further unraveled. In turn, this may aid in the phenotyping of asthma. Currently, ER and DR are separated based on FEV_1 profiles, which employ strict FEV_1 cut-off values for response categorization. Due to the heterogeneity of the asthmatic response, this method may easily lead to the miscategorization of subjects, especially those who are approaching the set FEV_1 cut-off. Therefore, the identification of crucial pathways unique to the development of a late phase response may give rise to better markers for the LAR. In

addition, a better understanding of disease mechanisms may lead to the identification of potential therapeutic targets to minimize the LAR.

4.2 Methods

4.2.1 Subjects, Allergen Inhalation Challenge, and Microarray Analysis

Subjects with mild allergic asthma who were sensitized to cat allergens were recruited for this study. The choice of allergen was based on a preliminary analysis demonstrating an even distribution of ERs and DRs following cat AIC (data not shown). In contrast, HDM allergen challenges produced mostly DRs, which was consistent with previous findings (16). Grass allergen was not chosen due to limited number of sensitized individuals.

The inclusion and exclusion criteria were as described in Chapter 2. Fourteen participants were included in total, which consisted of the 12 who were in the cat allergen group within the CCL2 study (as mentioned in Chapter 3, Table 3.1), and an additional 2 new subjects. The 12 participants were recruited from Université Laval, while the 2 new individuals were recruited from Vancouver General Hospital (VGH) – UBC. Subject demographics are summarized in Table 4.1.

Following the administration of a skin prick test and methacholine PC_{20} test, AIC using extracts of cat dander was performed as previously described. Methacholine inhalation challenge was also administered a day later to most subjects, in order to assess the airway hyperresponsiveness post-challenge. All participants were required to exhibit an FEV_1 drop ≥20% during the EAR, and DRs were required to demonstrate a LAR of ≥15% drop in FEV_1. For individuals who experienced a LAR FEV_1 drop that was

approaching the 15% cut-off, a determination of a dual response could be made if the methacholine PC_{20} value measured 24 hours post-AIC was decreased by at least half as compared to the original value recorded prior to allergen challenge (21), or if the subject had historically demonstrated a consistent dual response.

Following previous procedures, peripheral blood samples were collected in PAXgene Blood RNA tubes and EDTA tubes prior to and 2 hours after allergen challenge. A complete blood cell count with differential was taken from each EDTA blood sample using the Cell-Dyn® System (Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, USA). Total RNA was extracted from PAXgene blood samples and analyzed on the Affymetrix Human Gene 1.0 ST microarrays as detailed in Chapter 2.

Table 4.1 — Subject Demographics (ER versus DR).

Response	Subject	Site	Age (yr)	Sex	Pre Mch PC_{20}[a,b] (mg/ml)	Post Mch PC_{20}[a,b] (mg/ml)	Allergen	% Fall in FEV_1 (EAR)	% Fall in FEV_1 (LAR)
Isolated Early Responders (n=8)	1	VGH	42	M	0.13	N/A	Cat	-23	-9
	2	Laval	28	F	12.8	N/A	Cat	-20.3	-4.8
	3	Laval	29	F	0.35	N/A	Cat	-44.3	0
	4	Laval	34	F	2.69	6.11	Cat	-21	-1.5
	5	Laval	27	M	4.54	1.76	Cat	-34.4	0
	6	Laval	42	F	5.34	8.6	Cat	-42.1	-11.1
	7	Laval	31	M	11.75	16	Cat	-24.2	-7.5
	8	Laval	28	F	9.39	16	Cat	-27.1	-7.1
Mean ± SEM			**32.6 ± 2.2**	**3:5**	**2.82**	**7.50**		**29.6 ± 3.4**	**5.1 ± 1.5**
Dual Responders (n=6)	9	VGH	52	F	N/A	N/A	Cat	-33	-27
	10	Laval	23	F	0.3	0.18	Cat	-38.9	-31.8
	11	Laval	26	F	5.13	1.53	Cat	-31.4	-14.9
	12	Laval	49	F	3.61	0.99	Cat	-25.3	-12.6
	13	Laval	26	M	0.93	1.02	Cat	-31.5	-15.6
	14	Laval	27	F	0.63	0.12	Cat	-48.3	-25.8
Mean ± SEM			**33.8 ± 5.3**	**1:5**	**1.27**	**0.51**		**34.7 ± 3.2**	**21.3 ± 3.2**

[a] Methacholine PC_{20} was taken one day pre- and one day post-allergen inhalation challenge.
[b] Geometric means were calculated separately for the ER and the DR groups.

4.2.2 Statistical Analysis

Subject demographics and clinical FEV_1 measurements were compared between the ER and DR groups using 2-tailed t-tests, assuming unequal variances. Analysis of complete blood cell count and differential (comparing pre- versus post-AIC) were conducted using the 2-tailed paired t-test on each cell type. To investigate whether the cell count changes deviate between the two response types, the data was first baselined (pre- subtracted from each corresponding post-value), and the results were analyzed by comparing between ER and DR using the 2-tailed t-test, assuming unequal variances. For all of the above, significance cut-off was set at $p \leq 0.05$.

The raw probe intensity data from the microarray procedure, summarized in CEL files, were analyzed using an algorithm called significance analysis of microarrays (SAM), performed within the R-package (86). SAM employs a moderated t-test approach that uses permutations in order to estimate the variance of the genes and the resulting false discovery rate (124); thus it does not rely on the assumption of a parametric genes distribution. Data processing begins with RMA normalization, followed by the removal of non-annotated genes, and finally the application of the SAM algorithm. During the analysis, genes are ranked by a score that is assigned based on the change in gene expression relative to the standard deviation.

The analysis was conducted using an interaction model, which is intended for situations where two factors exert a combined, but not additive, effect on an outcome. Briefly, when there is interaction, the result of one variable is dependent on the level of the other. In the current study, the two main factors of interest were the status of the

challenge (pre and post) and the response type (ER and DR). Hence, the DEPs identified using the SAM interaction analysis were those that significantly increase post-challenge for ER but decrease for DR, and vice versa. In context of the SAM algorithm, the interaction analysis was carried out by comparing the pre-to-post change (achieved by baselining the data through subtracting pre-values from post-values) of ER versus DR. The significance threshold was set at FDR≤0.30, following previous report of a similar method (125).

A derivative of the SAM algorithm called cell-specific significance analysis of microarrays (csSAM) was employed for further data analysis. This method was developed to address the concern that the overall gene expression measured from a sample is a combination of the individual signals from multiple cell types; thus, the total gene expression may overlook many differentially expressed genes within each cell type (125). In response, csSAM statistically deconvolutes the total signal into cell type-specific gene expression profiles, using the SAM algorithm in conjunction with the measured relative cell type frequencies. This enables the identification of genes previously not found to be significant in the analysis of the whole sample gene expression.

CsSAM, conducted using the R-package, was employed for four analyses: a) pre- versus post-challenge for ERs only, b) pre- versus post-challenge for DRs only, c) ER versus DR at pre-challenge, and d) ER versus DR at post-challenge. The whole blood gene expression profile was separated based on the five types of peripheral blood leukocytes – neutrophils, lymphocytes, monocytes, eosinophils, and basophils – a process achieved through inclusion of the relative counts of each cell type into csSAM.

The analyses were performed using an unpaired approach, following the original statistical design of the algorithm (125). Although a paired analysis is likely to be more powerful in detecting differentially expressed genes, especially when comparing pre- and post-allergen challenge data, this was not carried out due to the lack of a validated csSAM pairing method. Correspondingly, unpaired SAM analyses were performed in order to generate reference data to which the results of the csSAM analyses could be compared. Genes were considered significant at FDR≤0.30, following the methods outlined in the original manuscript (125).

Pathway analyses were performed using IPA, as described in Chapter 2. Analyses were restricted to consider genes related to immune cells, lungs, and the nervous system, based on a study reporting sensory nerves as having a role in the LAR (126). Gene lists created using the SAM interaction analysis and csSAM analyses were used. Although a lenient significance cut-off of FDR≤0.30 was employed, pathway analyses helps to reduce the possible false positive errors associated with single-gene level analyses, by taking into account multiple genes in order to provide relevant biological information through networks and pathways (127).

4.3 Results

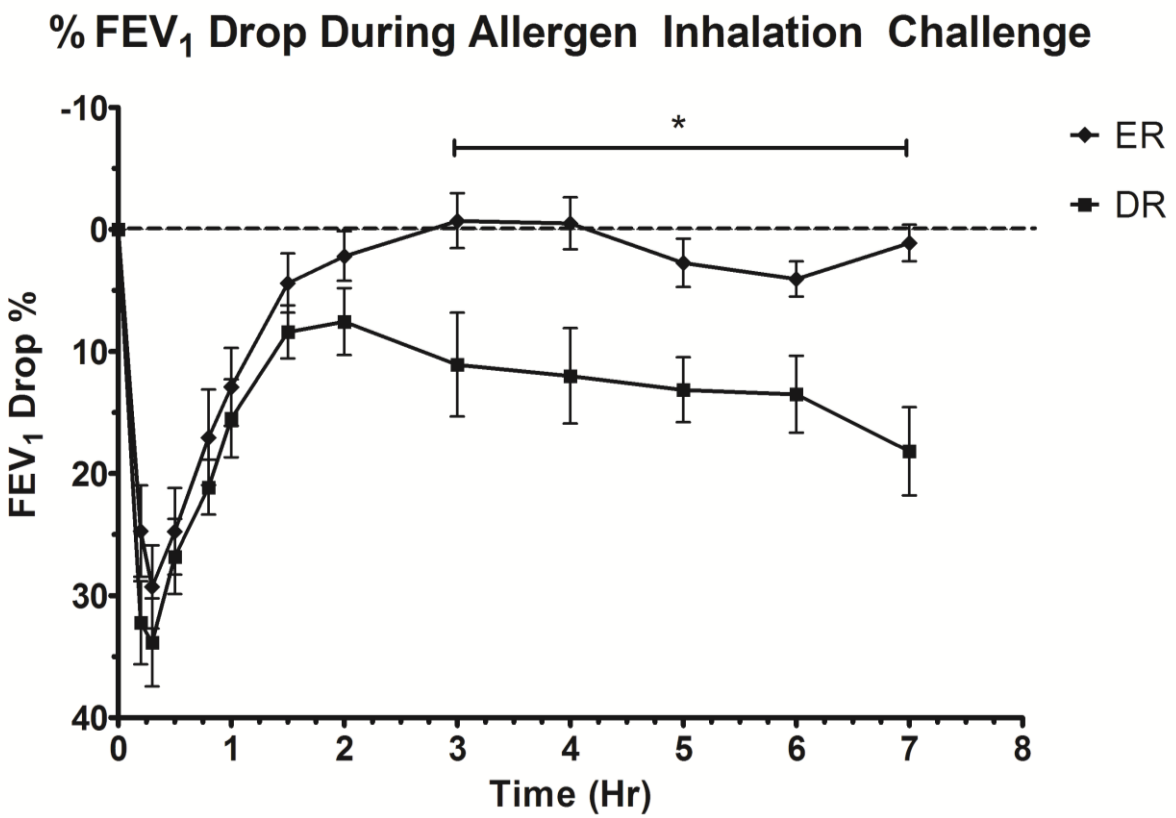
4.3.1 Cohort Characteristics

The study cohort was comprised of 8 ERs and 6 DRs (Table 4.1). The mean ages of the ER group and the DR group were not statistically different, at 32.6 ± 2.2 and 33.8 ± 5.3 respectively. There was an equal number of female participants within the two cohorts (5 each), but the ER group contained 2 additional male subjects as

compared to the DR group. The presence of a hyperresponsive airway was evident in both cohorts, as indicated by a pre-allergen challenge methacholine PC_{20} <16 mg/ml (39). The geometric means for pre-allergen challenge methacholine PC_{20} were 2.82 mg/ml and 1.57 mg/ml for the ER and DR groups, respectively. Post-allergen challenge, the methacholine PC_{20} values were significantly different between the two cohorts, with the geometric means being 7.50 mg/ml (ER) and 0.51 mg/ml (DR). Hence, a more hyperresponsive airway was detected for dual responders following AIC, which was consistent with literature findings (21).

All 14 subjects underwent AIC using extracts of cat dander. An EAR was detected in all participants, as shown by a drop in FEV_1 ≥20% between 0 and 3 hours. The mean FEV_1 drop for the ER group was 29.6 ± 3.4% and that for the DR group was 34.7 ± 3.2%; these values were not statistically different. Conversely, the two groups were found to be significantly different (p=2.5E-3) for the LAR, with the mean FEV_1 drop being 5.1 ± 1.5% for ER and 21.3 ± 3.2% for DR. Figure 4.1 presents the lung function profiles of isolated early responders and dual responders, through measurements of the FEV_1 drop from baseline after AIC. Comparing between the two responses at each data collection time point, the % drop in FEV_1 was not significantly different at any stage from the start of the allergen challenge until, and inclusive of, 2 hours into the challenge. Starting at 3 hours after allergen inhalation, however, the difference in % FEV_1 drop between ERs and DRs became increasingly significant with each passing hour. The only exception to this was at 6 hours after AIC, at which point the significance was decreased as compared to the two previous measurements taken at 4 and 5 hours (Figure 4.1).

Figure 4.1 — Lung Function Profiles for ER and DR.



Lung function profiles for the ER group and the DR group after allergen inhalation challenge were measured by spirometry and presented as % drop in FEV_1 from baseline. The data points represent the mean and the error bars represent the SEM for each response cohort. Comparing between the two response types at each measurement time point, the profiles significantly diverge starting at 3 hours after the challenge, with p values of 0.041, 0.023, 0.011, 0.029, and 0.004 for each point from 3 hours to 7 hours.

4.3.2 Complete Blood Cell Count and Differential Analysis

Complete blood cell count and differential in peripheral blood were measured from all 14 subjects using EDTA tube samples drawn prior to and following AIC. Comparing between pre- versus post-challenge (while disregarding ER and DR), total leukocyte counts in absolute terms were found to be significantly increased after allergen inhalation (p=6.1E-3). From the analysis of specific cell types, neutrophils and eosinophils were identified to be increased (p=1.2E-3) and decreased (p=4.1E-2), respectively post-challenge. The other leukocytes (lymphocytes, monocytes, and basophils) were not significantly changed with regards to cell counts.

Next, the ER and DR data were compared to assess whether each cell type was affected in a similar manner between the two responses. The results indicated no significant differences for any of the cell types, suggesting that differential cell count perturbations occurred (or did not occur) regardless of response. Another analysis was performed to compare the pre-allergen challenge cell counts of ER samples against those of DR samples. The baseline leukocyte counts for the different cell types were not found to be significantly different between ERs and DRs.

4.3.3 RNA Quality

To ensure that the RNA samples to be used for microarray analysis were not degraded, the quality of all 14 pre/post sample pairs was assessed. Electropherograms showed very distinct 18s and 28s peaks for all samples. Similar to the PAXgene blood samples collected for the experiments described in Chapter 2, the RIN indicated

excellent quality (range of 8.3 – 8.9). NanoDrop analysis provided further support for the purity of these samples, demonstrating the 260/280 ratio to be 2.09 ± 4.5E-3.

4.3.4 Differentially Expressed Probe Sets

The SAM interaction analysis, which takes into account both the response type and the pre/post time points of the samples as covariates, identified 501 DEPs as being perturbed differently between ER and DR (FDR≤0.30). All demonstrated an increase in gene expression post-allergen challenge for ER, but a decreased expression for DR. Out of this list, 251 probe sets remained significant at FDR≤0.25. The list of genes can be found in Appendix 6. Among the top genes of significance included a few with potential relevance to asthma, such as the genes for Interleukin 10 (*IL10*) and Kallikrein-1 (*KLK1*), both at FDR=0.14.

4.3.5 Pathway Analysis

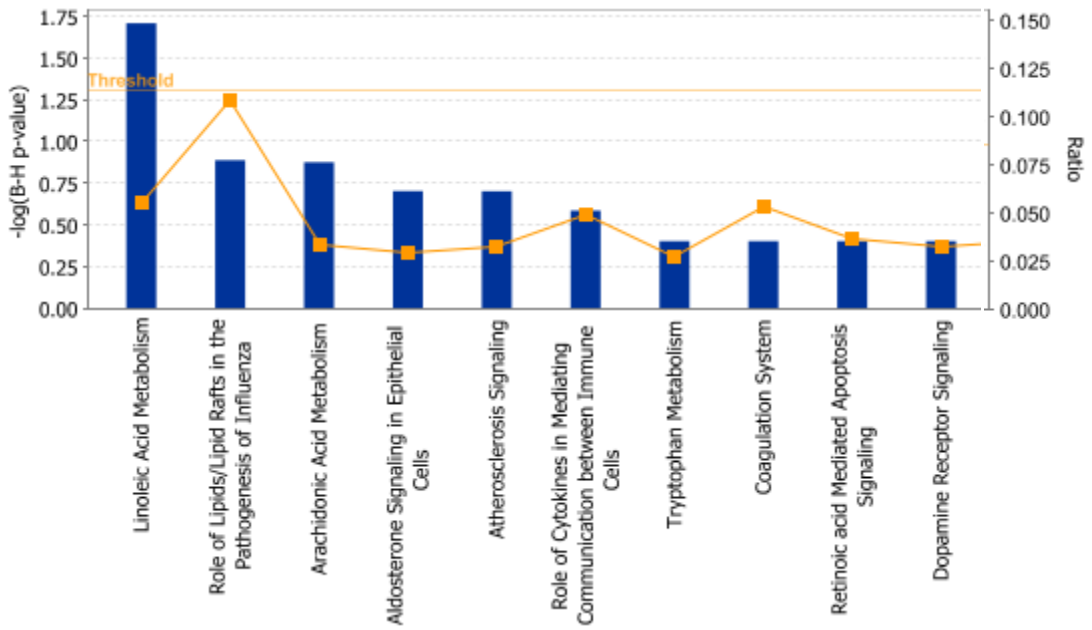
The list of DEPs (FDR≤0.30) identified in the interaction analysis was entered into IPA for pathway analysis. Of the 501 probe sets initially inputted, 328 met the criteria for inclusion. The significant biological functions are listed in Table 4.2, with the highest-ranked functions being cell-to-cell signaling and interaction, hematological system development and function, immune cell trafficking, and inflammatory response. The only canonical pathway perturbed differently between ER and DR was identified to be linoleic acid metabolism (adjusted p=1.96E-2). Other pathways such as arachidonic acid metabolism and the role of cytokines in mediating communication between immune cells were significant at unadjusted p values (Figure 4.2).

Table 4.2 — Biological Functions Identified by Interaction Analysis.

Rank	Biological Function	P Value[a]
1	Cell-To-Cell Signaling and Interaction	2.27E-02 – 1.62E-01
2	Hematological System Development and Function	2.27E-02 – 1.62E-01
3	Immune Cell Trafficking	2.27E-02 – 1.62E-01
4	Inflammatory Response	2.27E-02 – 1.62E-01
5	Cellular Growth and Proliferation	3.31E-02 – 1.42E-01
6	Cell-mediated Immune Response	3.31E-02 – 1.42E-01
7	Cellular Function and Maintenance	3.31E-02 – 1.52E-01
8	Hematopoiesis	3.31E-02 – 1.53E-01
9	Cellular Development	3.31E-02 – 1.53E-01
10	Cellular Movement	3.31E-02 – 1.61E-01
11	Tissue Development	3.31E-02 – 1.61E-01

[a] The p value represents how likely the association between a set of genes in the dataset and a related function is due to random chance. The range is based on the individual p values of the subcategories within that biological function. The results have been adjusted based on Benjamini and Hochberg multiple testing corrections.

Figure 4.2 — Canonical Pathways Identified by Interaction Analysis.



The top 10 canonical pathways and their significance values are presented. The horizontal threshold line (meeting the left y-axis at 1.30) denotes statistical significance at p≤0.05 after Benjamini and Hochberg multiple testing corrections. The square points represent the ratio, which was calculated as the number of genes in a given pathway that meet significance, divided by the total number of genes that make up that pathway. Linoleic acid metabolism was the only canonical pathway to reach significance.

4.3.6 Cell-Specific Significance Analysis of Microarrays

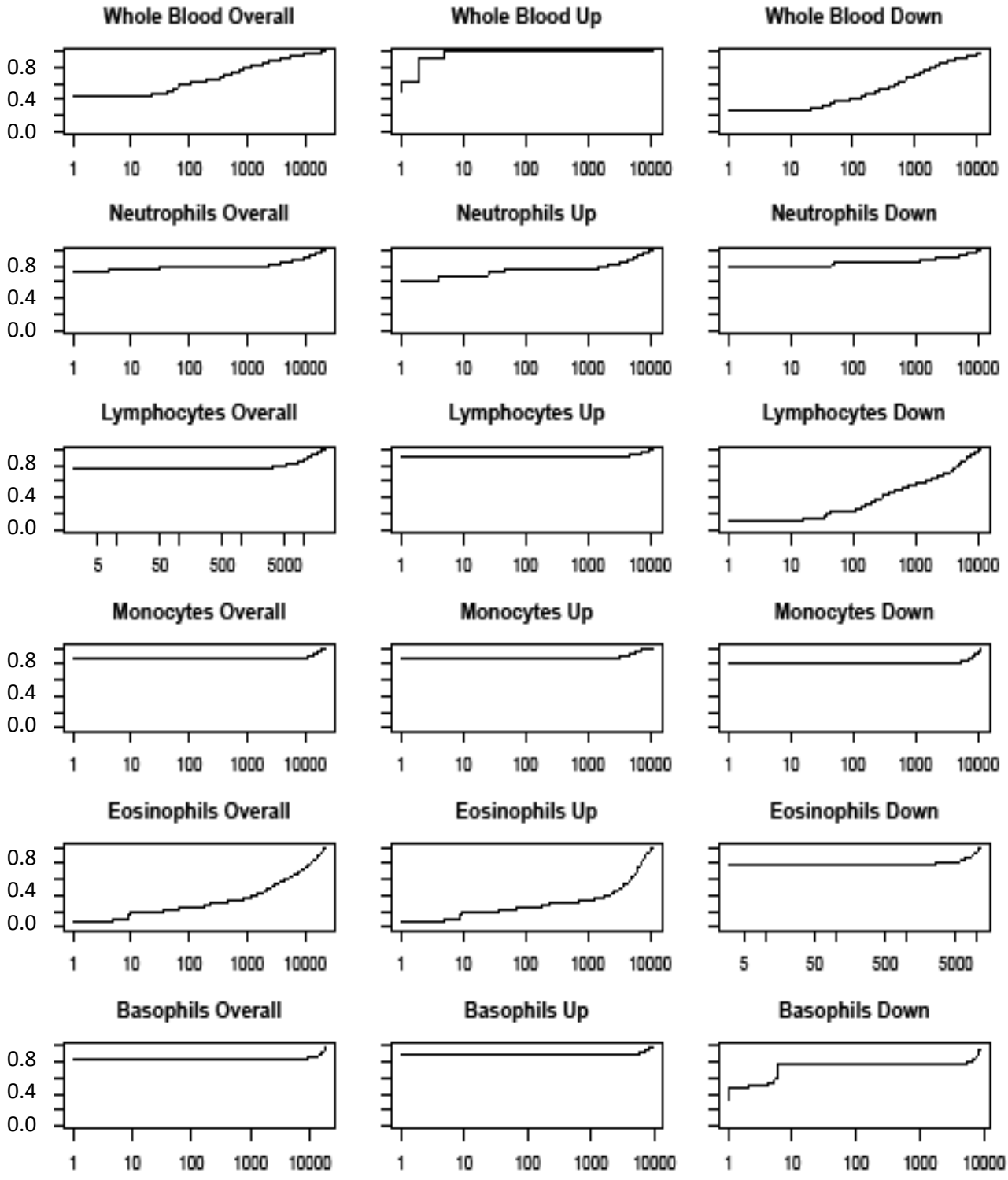
To further explore the gene expression profiles of isolated early and dual responders, the csSAM algorithm was applied in conjunction with the complete blood cell count and differentials, with the purpose of separating the whole blood gene expression signals into their respective peripheral blood leukocyte sources. As additional support for this method, the results generated using csSAM were compared against those created using parallel SAM algorithms. It is hypothesized that csSAM would be able to identify differential genes that may have been missed by the SAM analysis. Alternatively, csSAM may generate an adjusted FDR value for DEPs found in the whole blood analysis, such that confounding effects due to differences in complete blood cell count and differentials would decrease the number of DEPs.

The analyses of pre- versus post-allergen challenge in both the ER cohort and the DR cohort did not reveal any significant genes (FDR≤0.30) within the five cell types. However, when comparing ER and DR gene expression at the pre-allergen challenge (baseline) level, 10 genes correlating to basophils were found to exhibit significantly lower expression levels in DR as compared to ER. Similarly, two genes correlating to lymphocytes reached statistical significance as being expressed at a lower level in DR than in ER subjects. These results generated by csSAM were in contrast to those produced using the corresponding SAM algorithms (whole blood analyses), in which all of the genes demonstrated an FDR≥0.90.

The final analysis of ER versus DR at the post-allergen challenge time point yielded the most significant results (Figure 4.3). Whole blood SAM analysis initially identified the overall significance to be around FDR=0.45 for the top genes in the list.

Separating by directionality, genes that showed a lower expression for DR as compared to ER (“down”) were found to reach a significance level as low as FDR=0.25, while genes showing a higher expression for DR (“up”) barely reached FDR=0.50. CsSAM deconvolution revealed that lymphocytes and eosinophils were the main cell types of interest with regards to differential gene expression between ER and DR. Specifically, 140 genes correlating to lymphocytes were observed to be significantly (FDR≤0.30) lower expressed in DR than in ER (Appendix 7), and 262 genes correlating to eosinophils were detected to be expressed at a higher level in DR as compared to ER (Appendix 8). Overall, the largest gene expression discrepancies between ER and DR at 2 hours after allergen challenge seem to be associated with eosinophils.

Figure 4.3 — SAM and csSAM Results for the Analysis of ER versus DR at Post-Allergen Challenge.



Each of these graphs depicts the number of genes identified (x-axis, on a log scale) against the FDR significance values (y-axis). The “up” column displays the genes that were found to be higher expressed in DR than ER, while the “down” column shows genes that were lower expressed in DR than ER. The top row presents the whole blood results obtained using the unpaired SAM statistical approach. Applying the csSAM algorithm, additional significance of genes were detectable in association with specific cell types, mainly lymphocytes and eosinophils.

4.3.7 Pathway Analysis of csSAM Results

The two gene lists as identified to be associated with eosinophils and lymphocytes by the csSAM algorithm were entered into pathway analyses in IPA. Of the 262 genes found to be increased in expression in association with DR eosinophils, 58 were eligible to be included into the analysis. For the lymphocyte gene list, 39 out of 140 genes met the inclusion criteria. The biological functions identified by both lists failed to reach statistical significance after multiple testing corrections. For canonical pathways, mitotic roles of polo-like kinase was significant for both eosinophils and lymphocytes, at adjusted p=6.6E-5 and p=3.8E-2, respectively. Cell cycle: G2/M DNA damage checkpoint regulation was also significant for eosinophils (adjusted p=6.6E-5). Correspondingly, the protein products of the top genes found to be associated with both cell types included many that were known to be involved in DNA replication, spindle formation, and mitosis (data not shown).

4.4 Discussion

4.4.1 Interaction of Response and Time

In this chapter, the differences in gene expression profiles between asthmatic isolated early and dual responders were explored. This was performed through an interaction analysis, which incorporates both the response type and the time points into a single model. Doing so enables the comparison between ER and DR on the basis of the change in gene expression from pre- to post-AIC. The biological functions identified by IPA revealed many that are in agreement with the current pathophysiological model of the early and the late response. For instance, a major feature of the late asthmatic

response is the recruitment and influx of inflammatory cells to the airways. The top three biological functions listed in Table 4.2 are in direct support of this: “cell-to-cell signaling and interaction” are necessary to initiate the inflammatory cell recruitment process, “hematological system development and function” lead to the production and release of additional leukocytes from the bone marrow, and “immune cell trafficking” governs the overall movement of immune cells from the site of production, through the peripheral blood, and into the airways. Because these processes may be more subdued in isolated early responders, it is not surprising that results as such were found to be significantly different between ER and DR using the interaction model.

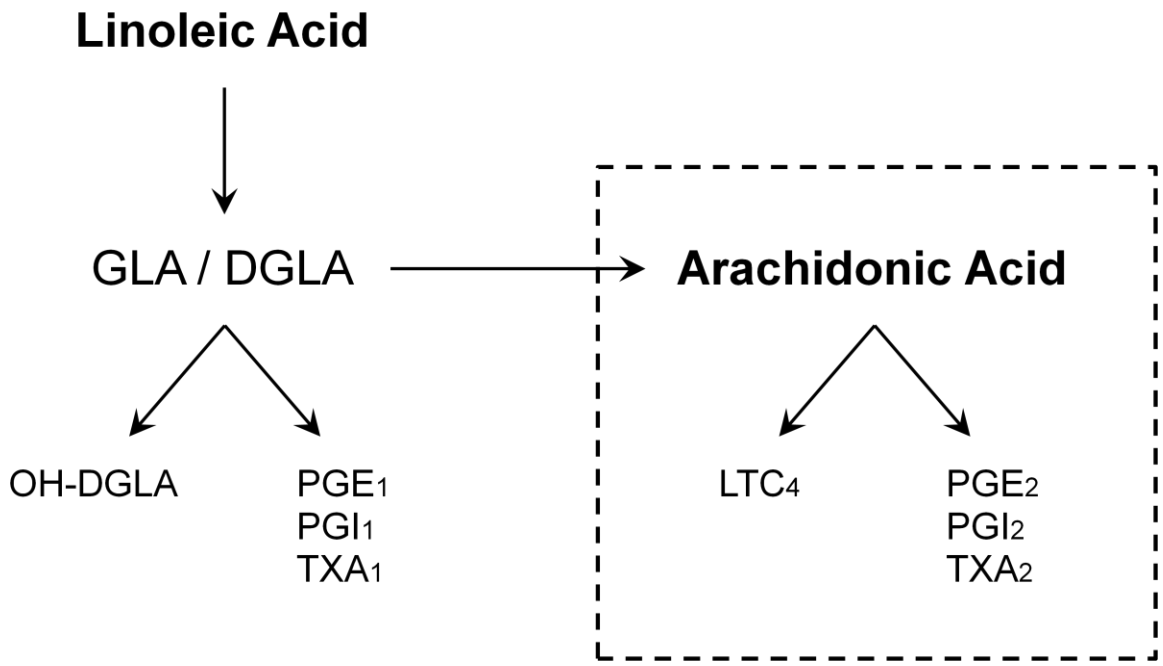
4.4.2 Linoleic Acid Metabolism

Linoleic acid (LA) metabolism was the single canonical pathway that reached statistical significance. Another related pathway, arachidonic acid (AA) metabolism, was also identified, but only at an unadjusted p value. These two polyunsaturated omega-6 fatty acids share a close relationship, as summarized in Figure 4.4 (128). Briefly, LA is an essential fatty acid that must be ingested (129). Through metabolism, it is converted to AA, from which many pro-inflammatory eicosanoids are produced, including prostaglandins, thromboxanes, and leukotrienes. Of special interest are the signaling molecules leukotrienes, which have been heavily implicated in the pathophysiology of asthma, particularly with respect to inflammation (130). Numerous reports have demonstrated their key roles in causing various asthmatic symptoms, including airway hyperresponsiveness, bronchoconstriction, inflammatory cell infiltration, mucus production, and airway remodeling (64,131–133), many of which are hallmarks of the LAR. Furthermore, several leukotriene receptor antagonists have been

successfully developed as a line of treatment for asthma (134), of which montelukast has shown promising results in significantly inhibiting the LAR (135). Additional evidence of LAR relevance is provided by studies that showed a suppression in the LAR due to the inhibition of AA-derived eicosanoid synthesis and release (136,137).

In the present study, the eicosanoid signaling canonical pathway failed to reach statistical significance. This does not detract from the points mentioned above, however, considering the time frame of sampling. Two hours after allergen inhalation challenge is a brief time span for the body to fully react to allergens. Hence, it is possible that the observed perturbations in the LA (and AA) metabolism pathways are occurring in preparation for the synthesis of downstream eicosanoid metabolites. These molecules will subsequently contribute to the inflammatory state of the LAR as exhibited in DRs. Indeed, in Chapter 2, a significant decrease in the level of AA phospholipid in the plasma following allergen challenge was demonstrated, which may be explained by such a mechanism. There are also literature reports in support of the importance of these fatty acids at the upstream level of LA and AA, suggesting a connection between dietary LA intake and the prevalence of wheeze and asthma (138,139). However, not all studies were consistent in identifying such a positive association (140,141). Hence, further work is needed before a conclusion can be drawn regarding the relationships of LA, AA, and eicosanoids with the pathophysiology of asthma, especially with respect to the early and late response.

Figure 4.4 — Linoleic Acid Metabolism.



Linoleic acid is metabolized to form arachidonic acid through intermediates of gamma linolenic acid (GLA) and dihomo-gamma linolenic acid (DGLA). Prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX), and leukotrienes (LT) – collectively known as eicosanoids – are subsequently produced. Those downstream of arachidonic acid, which accounts for the majority of eicosanoids, are pro-inflammatory. With respect to asthma, leukotrienes synthesized from arachidonic acid have been shown to play an active role in asthma pathophysiology.

4.4.3 CsSAM for ER versus DR at Post-Challenge

Using the csSAM algorithm, many significant genes were uncovered in correlation with lymphocytes and eosinophils in the analysis of ER versus DR at post-AIC. Both of these cell types have been suggested to be active contributors to the LAR (142), with eosinophils being a central effector cell (143) and Th2 lymphocytes producing cytokines to promote airway inflammation (144). In general, csSAM results indicated that eosinophils were associated with the largest gene expression discrepancies between ER and DR post-challenge (Figure 4.3). Considering the key role of eosinophils in the LAR, this finding provides support for the usefulness of the csSAM algorithm in uncovering cell-specific differentially expressed genes of true biological relevance.

Cell cycle regulation appears to be a major theme that differs between ER and DR post-allergen challenge, as demonstrated by the canonical pathways and the top genes associated with both the lymphocytes and the eosinophils. In accordance with the proposed model of asthma pathogenesis, this suggests that eosinophils and lymphocytes may be undergoing active cellular growth and division, contributing to the rising number of inflammatory cells as seen in the late response. Indeed, previous reports have described the release of eosinophil haematopoietic progenitors from the bone marrow into the peripheral blood after allergen challenge, as well as an increase in post-challenge blood eosinophil counts in dual responders but not in isolated early responders (28,145,146). Based on this, it may be deduced that DR eosinophils are undergoing cellular differentiation in the peripheral blood, a process seemingly absent in

ER samples. Further studies are needed to elucidate the exact biological processes occurring in eosinophils post-allergen challenge.

Research into lymphocytes during an asthmatic response has been more limited. Most of the work has focused on T lymphocytes (particularly Th2 cells), describing an increase in T cell activation post-challenge (147). However, reports have been conflicting with regards to T cell recruitment and an actual increase in the number of T cells in the airways (147–149). In the present study, csSAM has identified lymphocytes as expressing significantly different genes – with a cell cycle focus – between ER and DR samples at 2 hours post-challenge. A limitation in the study design, however, was that the lymphocyte sub-populations of natural killer cells, B cells, and various types of T cells were not distinguished. Such knowledge would have been useful in better understanding the disease mechanisms as relevant to each population of lymphocytes. For instance, CD4+ T cells have been found to have a Th2 phenotype, producing cytokines which promote a more severe asthmatic response (150). On the other hand, CD8+ T cells may help regulate these responses, as supported by the finding that an increase in these cells seems to suppress the development of the LAR (151). Without information on the composition of lymphocyte subsets, it is not feasible to attribute the csSAM findings, which showed a down-regulation of genes associated with DR lymphocytes, to certain cell types. Likewise, the identification of cell cycle regulation canonical pathways cannot be easily interpreted without further validation studies focusing on specific lymphocyte sub-populations.

4.5 Summary

The results presented in this chapter demonstrate that changes in gene expression from pre- to post-allergen challenge follow different patterns between isolated early and dual asthmatic responders. In whole blood analysis, linoleic acid metabolism was identified as a significant canonical pathway, with implications for the development of the LAR. In addition, through the use of the csSAM algorithm, eosinophils and lymphocytes were identified to be associated with the largest discrepancies in gene expression profiles between ER and DR at the post-challenge time point. In particular, cell cycle regulation processes were noted, which is in support of the current model of asthma pathophysiology demonstrating a key role of these cell types in cellular inflammation.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Overall Summary

The purpose of this research was to investigate the molecular profiles of the allergic asthmatic response, ultimately focusing on differences between isolated early responders and dual responders. To achieve this, peripheral blood samples drawn prior to and 2 hours after allergen inhalation challenge were examined. A preliminary study was undertaken in order to validate the usefulness of the chosen time point (2 hours) in detecting gene expression changes in the peripheral blood. Through genome-wide microarray analysis and pathway tools, it was demonstrated that the Nrf2-mediated oxidative stress response pathway was perturbed. A gene within this pathway, *ABCC1*, was further confirmed to be decreased in expression level post-allergen inhalation, as shown by RT-qPCR results on additional sample pairs. Differential levels of oxidative and inflammatory molecules in the plasma, such as DHA, AA, and nitrotyrosine, provided additional support for an altered oxidative stress response after AIC. Next, plasma samples were tested against a cytokine panel, which identified CCL2 as being reduced post-challenge. Extending the atopic asthmatic cohort to include subjects with allergic rhinitis and occupational asthma, comparable results were observed. Despite the highly significant findings and the known effects of CCL2 in allergic diseases (101), this decrease may be irrelevant of allergen exposure. This is because control subjects who did not undergo AIC were also shown to demonstrate a significant decrease in CCL2 levels, suggesting an inherent (perhaps diurnal) fluctuation in the cytokine. Additional analysis of baseline CCL2 revealed a positive association between aging and increasing CCL2 levels in plasma. Having shown that 2 hours after AIC is sufficient to

detect molecular changes in peripheral blood, samples from isolated early responders and dual responders were compared. Genome-wide transcriptomic profiles indicated that linoleic acid metabolism was perturbed differently between the two responses after cat allergen inhalation challenge. When the whole blood gene expression signals were deconvoluted in accordance with differential leukocyte numbers using the csSAM algorithm, eosinophils and lymphocytes were identified as the cell types associated with the most divergent transcriptomic profiles between ER and DR at the post-challenge time point.

5.2 Limitations and Future Directions

Much of the findings reported here are based on data generated by microarrays and pathway analyses (*e.g.*, GSEA, IPA). While these tools offer a useful means to conduct a general assessment of biological events, there are limitations. In addition to the errors associated with multiple hypothesis testing, a lack of a standard procedure for microarray data processing may lead to variations in significant findings (152). For pathway analysis software, which are driven by the current knowledge base, research trends and inaccurate or missing information may create a bias for certain pathways over others (153). Due to the exploratory nature of my study, limited follow-up analyses were conducted to confirm the microarray results and the pathways identified. Future directions include validation of ER and DR gene expression signals using RT-qPCR, as was performed for the preliminary analysis in Chapter 2. RT-qPCR is highly sensitive and specific, allowing for more accurate quantification of gene expression signals (154,155). In addition, the protein products of significant genes can be tested by enzyme-linked immunosorbent assays (ELISA) or other protein detection methods.

Because gene expression levels are not necessarily proportional to the level of proteins produced (due to variable rates of mRNA translation, for instance), it is important to assess whether changes in the transcriptome after AIC is reflected in protein output (152).

The results produced by the csSAM algorithm will also require further validation. Because this gene expression deconvolution method is relatively new, it would be ideal to have additional evidence as support. One possible experiment is to isolate the leukocyte subsets prior to measuring their transcriptomes, and then compare these results with that of csSAM. It must be noted, however, that the separation process may introduce alterations in gene expression profiles. Therefore, perhaps the immediate usefulness of csSAM is to aid in hypothesis formulation, as suggested by the authors of the algorithm (125). Having identified a cell type of interest – in this case, the eosinophils and lymphocytes – a next step may be to carry out assays targeted toward those cell types in order to assess their importance in the real biological context.

A future direction stemming from the cytokine study described in Chapter 3 is to follow up on CCL5, which was identified to be significantly increased after AIC. In the same way that CCL2 was validated, CCL5 immunoassays can be performed to test for a consistent pattern of perturbation in cytokine levels post-challenge, using different cohorts of allergic diseases and control subjects. Having observed a very high reproducibility of results using the MSD immunoassay system in the present study, it may be fitting to also assess CCL5 levels using this technology.

To further explore the mechanisms of the asthmatic response, additional molecular targets may be studied. For instance, microRNAs (miRNAs) are known to play important roles in regulating the mRNA translation process, through binding to complementary sections of mRNAs (156). Examining the miRNA profiles between pre- and post-AIC may reveal underlying factors contributing to altered gene expression, especially if complementary base pairs can be demonstrated between specific mRNAs and miRNAs. Metabolites can also be investigated. Through identification of molecules perturbed after AIC, the biological processes occurring during an asthmatic response can be better understood.

Because the current research is based around the use of peripheral blood samples, it may not fully reflect the situation of the airways. Therefore, future investigations may include the use of airway tissues taken directly from the site of injury, although obtaining these samples may be difficult. In conjunction with ongoing subject recruitment, research into these various areas as discussed above can add valuable insight into the pathophysiology of asthma, especially if additional sampling time points are included.

5.3 Relevance of Research

This study demonstrates the usefulness of the 2 hour time point in detecting molecular changes after allergen inhalation challenge, providing a model for future studies to also employ an early time point of investigation. Through transcriptome profiling, a list of new genes were suggested to be involved in the asthmatic responses, in both the isolated early responders and the dual responders. Follow-up of these

targets may confirm novel genes crucial to the disease, which can be further investigated in genetic studies. In addition, biomarkers may be revealed through these molecular analyses to enable new methods of disease detection and monitoring. If measurable at 2 hours after allergen inhalation, such biomarkers may also be useful in the prediction of the LAR before its clinical onset. Finally, this study enables a better insight into the mechanisms and pathways behind the asthmatic response. This may lead to possible pharmacologic targets to improve the treatment of asthma, especially with respect to minimizing the late phase response and the associated outcome of chronic asthma.

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APPENDICES

Appendix 1 — Sequences of Probes and Primers used in RT-qPCR

Gene	Component	No. of Bases	Sequence[a]
ABCC1	Forward primer 5′-3′	20	CTC AGG AGC ACA CGA AAG TC
	Reverse primer 5′-3′	19	GGC CAT GGA GTA GCC AAA C
	Probe 5′-3′	23	/56-FAM/CTG GGC ATT /ZEN/TCA CAA GGG ATC GC/3IABkFQ/
CUL3	Forward primer 5′-3′	20	CAA CAC TTG GCA AGG AGA CT
	Reverse primer 5′-3′	23	TGC TCA TAT CCC TAA ACA TTC CT
	Probe 5′-3′	30	/56-FAM/AGT TTT GAC /ZEN/GTG AAC TGA CAT CCA CAT TCA /3IABkFQ/
DNAJA2	Forward primer 5′-3′	21	GAT CAA CCC AGA CAA GCT TTC
	Reverse primer 5′-3′	22	CTC TAC CTC CTC TGT TTC TCC A
	Probe 5′-3′	25	/56-FAM/TCT GCC ATC /ZEN/TAG ACC GGA AGT TCC T/3IABkFQ/
DNAJC1	Forward primer 5′-3′	23	ACA CTA AAA GCA TTA CCT CAC CT
	Reverse primer 5′-3′	23	TCA GTT CTA GTC AGT GCA TCT TC
	Probe 5′-3′	28	/56-FAM/ATT TAG CAT /ZEN/AAA ACT GCC CAG CAT CCT G/3IABkFQ/
DNAJC19	Forward primer 5′-3′	21	AGT GAA GAT GAC AGT CCT TGC
	Reverse primer 5′-3′	23	CAC CGA ATA AGA AGA GTC CCT AC
	Probe 5′-3′	33	/56-FAM/TCA CAG TCT /ZEN/AAT TAC CAG TTT ATC AGT CTC CCA /3IABkFQ/
DNAJC21	Forward primer 5′-3′	21	AGT TTG GAG ATG GAT CGG ATG
	Reverse primer 5′-3′	20	GTC ATC ATA GAG CTC AGC GT
	Probe 5′-3′	28	/56-FAM/AGG AGG ATG /ZEN/GTA AAG ACA GTG ATG AGG C/3IABkFQ/
PTPLAD1	Forward primer 5′-3′	22	GGA TTC TCC TGG ATC TTT GTC A
	Reverse primer 5′-3′	21	ATA CAT CAT GTC AGC CAC AGT
	Probe 5′-3′	28	/56-FAM/TTC CCA AGA /ZEN/TAC AGA ATC GCA CAG TCA G/3IABkFQ/

Gene	Component	No. of Bases	Sequence*
KRAS	Forward primer 5′-3′	23	TGC CCT ACA TCT TAT TTC CTC AG
	Reverse primer 5′-3′	22	CCT ACT GTC GCT AAT GGA TTG G
	Probe 5′-3′	24	/56-FAM/AGG TGG TGG /ZEN/CTG ATG CTT TGA ACA /3IABkFQ/
SOD1	Forward primer 5′-3′	22	CGA GCA GAA GGA AAG TAA TGG A
	Reverse primer 5′-3′	25	CTG GAT AGA GGA TTA AAG TGA GGA C
	Probe 5′-3′	26	/56-FAM/TGA AGG TGT /ZEN/GGG GAA GCA TTA AAG GA/3IABkFQ/
GAPDH	Forward primer 5′-3′	21	CAG CCT CAA GAT CAT CAG CAA
	Reverse primer 5′-3′	19	GGC CAT CCA CAG TCT TCT G
	Probe 5′-3′	24	/56-FAM/ATG ACC ACA /ZEN/GTC CAT GCC ATC ACT /3IABkFQ/
PGK1	Forward primer 5′-3′	21	GTA GGA GTC AAT CTG CCA CAG
	Reverse primer 5′-3′	23	GAT CTT GTC TGC AAC TTT AGC TC
	Probe 5′-3′	26	/56-FAM/CCT TCT TCA /ZEN/TCA AAA ACC CAC CAG CC/3IABkFQ/
GUSB	Forward primer 5′-3′	22	AAG AGC CAG TTC CTC ATC AAT G
	Reverse primer 5′-3′	19	AGC GAA GCA GGT TGA AGT C
	Probe 5′-3′	22	/56-FAM/CGA AGC CCT /ZEN/TCC CTC GGA TGT C/3IABkFQ/

[a] ZEN = Internal ZEN quencher

Appendix 2 — Differentially Expressed Probe Sets, Post- versus Pre-Allergen Inhalation Challenge (p≤0.01 and fold change≥1.1)

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8112310	---	---	---	1.03E-05	-1.26567
8150592	CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	NM_005195	1.72E-05	-1.20166
7936968	ADAM12	ADAM metallopeptidase domain 12	NM_003474	2.96E-05	-1.14475
8030804	CD33	CD33 molecule	NM_001772	4.37E-05	-1.27568
7989193	---	---	---	7.21E-05	1.51098
8068305	ITSN1	Intersectin 1 (SH3 domain protein)	NM_003024	9.00E-05	-1.17278
8079060	VIPR1	Vasoactive intestinal peptide receptor 1	NM_004624	1.50E-04	-1.13714
8151281	TRAM1	Translocation associated membrane protein 1	NM_014294	1.77E-04	1.19815
8032275	MBD3	Methyl-cpg binding domain protein 3	NM_003926	1.97E-04	-1.10751
8070714	---	---	---	2.06E-04	1.28628
8029136	CD79A	CD79a molecule, immunoglobulin-associated alpha	NM_001783	2.42E-04	1.12324
7923442	SYT2	Synaptotagmin II	NM_177402	3.16E-04	-1.18247
7902512	DNAJB4	Dnaj (Hsp40) homolog, subfamily B, member 4	NM_007034	3.42E-04	1.36707
8120579	C6orf57	Chromosome 6 open reading frame 57	NM_145267	3.55E-04	1.13744
8157686	OR1L4	Olfactory receptor, family 1, subfamily L, member 4	NM_001005235	3.63E-04	-1.15665
8038126	CA11	Carbonic anhydrase XI	NM_001217	3.69E-04	-1.14335
8076169	NPTXR	Neuronal pentraxin receptor	NM_014293	3.70E-04	-1.11711
8000779	TBX6	T-box 6	NM_004608	3.87E-04	-1.12436
7966517	C12orf51	Chromosome 12 open reading frame 51	NM_001109662	3.95E-04	-1.11606
8036072	KRTDAP	Keratinocyte differentiation-associated protein	NM_207392	4.10E-04	-1.30462
8140967	SAMD9	Sterile alpha motif domain containing 9	NM_017654	4.29E-04	1.48976
7968926	---	---	---	4.69E-04	-1.3093
8070953	C21orf56	Chromosome 21 open reading frame 56	NM_001142854	4.79E-04	-1.12526
8108330	KDM3B	Lysine (K)-specific demethylase 3B	NM_016604	4.98E-04	-1.19477
8165486	TMEM203	Transmembrane protein 203	NM_053045	5.09E-04	1.13055

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8092541	LIPH	Lipase, member H	NM_139248	5.17E-04	-1.21411
7975713	FCF1	FCF1 small subunit (SSU) processome component homolog	NM_015962	5.25E-04	1.25408
7997726	FOXF1	Forkhead box F1	NM_001451	5.40E-04	-1.246
7993478	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	NM_004996	5.41E-04	-1.16902
8022711	DSC2	Desmocollin 2	NM_024422	5.43E-04	-1.11312
8043840	LIPT1	Lipoyltransferase 1	NM_145197	5.79E-04	1.31002
7963689	NPFF	Neuropeptide FF-amide peptide precursor	NM_003717	5.80E-04	-1.11916
7978838	C14orf104	Chromosome 14 open reading frame 104	NM_018139	6.09E-04	1.16651
7978343	CMA1	Chymase 1, mast cell	NM_001836	6.34E-04	-1.25589
8157141	ACTL7A	Actin-like 7A	NM_006687	6.93E-04	-1.17691
8106743	VCAN	Versican	NM_004385	7.30E-04	-1.44049
8065596	PDRG1	P53 and DNA-damage regulated 1	NM_030815	7.34E-04	1.2478
7921955	RXRG	Retinoid X receptor, gamma	NM_006917	7.35E-04	-1.16363
7941269	LOC100291851	Similar to Putative ubiquitin-like protein FU	ENST00000309775	7.38E-04	-1.13759
7929288	EXOC6	Exocyst complex component 6	NM_019053	7.57E-04	1.1611
7934133	PPA1	Pyrophosphatase (inorganic) 1	NM_021129	7.65E-04	1.23236
7911343	UIMC1	Ubiquitin interaction motif containing 1	AF284753	7.79E-04	-1.16777
8165703	UIMC1	Ubiquitin interaction motif containing 1	AF284753	7.79E-04	-1.16777
8145470	DPYSL2	Dihydropyrimidinase-like 2	NM_001386	7.90E-04	-1.24796
7925720	OR2C3	Olfactory receptor, family 2, subfamily C, member 3	NM_198074	8.01E-04	-1.21979
7936307	SMNDC1	Survival motor neuron domain containing 1	NM_005871	8.20E-04	1.12645
8012539	PIK3R6	Phosphoinositide-3-kinase, regulatory subunit 6	NM_001010855	8.38E-04	-1.26548
7932512	DNAJC1	Dnaj (Hsp40) homolog, subfamily C, member 1	NM_022365	8.60E-04	1.15399
8122182	TBPL1	TBP-like 1	NM_004865	8.83E-04	1.17923
8160805	C9orf25	Chromosome 9 open reading frame 25	NM_147202	9.11E-04	-1.24083
8097513	MGST2	Microsomal glutathione S-transferase 2	NM_002413	9.26E-04	-1.1378
7949146	SF1	Splicing factor 1	NM_004630	9.32E-04	-1.10772

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8157233	HSDL2	Hydroxysteroid dehydrogenase like 2	NM_032303	9.91E-04	1.13678
8083000	FAIM	Fas apoptotic inhibitory molecule	NM_001033030	1.01E-03	1.15125
7930487	TECTB	Tectorin beta	NM_058222	1.04E-03	-1.12094
8049952	C2orf85	Chromosome 2 open reading frame 85	NM_173821	1.05E-03	-1.18451
7940688	POLR2G	Polymerase (RNA) II (DNA directed) polypeptide G	NM_002696	1.06E-03	1.10543
8030871	ZNF613	Zinc finger protein 613	NM_001031721	1.08E-03	1.3041
8036865	---	---	---	1.12E-03	1.69279
8076403	NAGA	N-acetylgalactosaminidase, alpha	NM_000262	1.13E-03	-1.23033
8137979	ACTB	Actin, beta	NM_001101	1.14E-03	-1.13277
8025984	ZNF844	Zinc finger protein 844	NM_001136501	1.14E-03	1.4582
8144586	MTMR9	Myotubularin related protein 9	NM_015458	1.16E-03	1.23267
8113073	ARRDC3	Arrestin domain containing 3	NM_020801	1.17E-03	1.49373
8081333	---	---	---	1.18E-03	1.11655
7953428	CD4	CD4 molecule	NM_000616	1.20E-03	-1.22191
8111216	LOC391769	Histone cluster 2, H3c pseudogene	ENST00000426411	1.24E-03	-1.16401
8038347	TEAD2	TEA domain family member 2	NM_003598	1.27E-03	-1.14317
7899377	PPP1R8	Protein phosphatase 1, regulatory (inhibitor) subunit 8	NM_138558	1.29E-03	1.14782
8010426	RNF213	Ring finger protein 213	NM_020914	1.30E-03	-1.14256
8066776	TP53RK	TP53 regulating kinase	NM_033550	1.36E-03	1.18965
7978628	PPP2R3C	Protein phosphatase 2 (formerly 2A), regulatory subunit	NM_017917	1.38E-03	1.24676
8016452	HOXB4	Homeobox B4	NM_024015	1.40E-03	1.12138
8138363	SOSTDC1	Sclerostin domain containing 1	NM_015464	1.41E-03	-1.17249
8170013	---	---	---	1.41E-03	-1.3876
8175317	---	---	---	1.41E-03	-1.3876
7984112	RAB8B	RAB8B, member RAS oncogene family	NM_016530	1.42E-03	1.15603
8171533	---	---	---	1.44E-03	1.15697
8125341	AGER	Advanced glycosylation end product-specific receptor	NM_001136	1.46E-03	-1.1612
7952737	---	---	---	1.46E-03	-1.16748
8016546	ZNF652	Zinc finger protein 652	NM_014897	1.47E-03	-1.18

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
7899173	DHDDS	Dehydrodolichyl diphosphate synthase	NM_024887	1.47E-03	1.11766
8151525	PMP2	Peripheral myelin protein 2	NM_002677	1.57E-03	-1.18956
7901895	ATG4C	ATG4 autophagy related 4 homolog C (S. Cerevisiae)	NM_032852	1.58E-03	1.32368
8118571	PSMB9	Proteasome (prosome, macropain) subunit, beta type, 9	NM_002800	1.62E-03	1.13513
8178211	PSMB9	Proteasome (prosome, macropain) subunit, beta type, 9	NM_002800	1.62E-03	1.13513
8179495	PSMB9	Proteasome (prosome, macropain) subunit, beta type, 9	NM_002800	1.62E-03	1.13513
8153920	ZNF250	Zinc finger protein 250	NM_021061	1.62E-03	1.12214
7964745	TMBIM4	Transmembrane BAX inhibitor motif containing 4	NM_016056	1.66E-03	1.15701
8163948	RBM18	RNA binding motif protein 18	NM_033117	1.66E-03	1.24803
8151254	NCOA2	Nuclear receptor coactivator 2	NM_006540	1.70E-03	-1.10391
8146669	TRIM55	Tripartite motif-containing 55	NM_033058	1.70E-03	-1.12018
7946569	RNF141	Ring finger protein 141	NM_016422	1.72E-03	1.2994
7930537	TCF7L2	Transcription factor 7-like 2 (T-cell specific)	NM_001146274	1.72E-03	-1.3209
8169949	MST4	Serine/threonine protein kinase MST4	NM_016542	1.79E-03	1.10567
8061685	TM9SF4	Transmembrane 9 superfamily protein member 4	NM_014742	1.81E-03	-1.11646
8041000	GPN1	GPN-loop gtpase 1	NM_007266	1.90E-03	1.12161
7972428	OXGR1	Oxoglutarate (alpha-ketoglutarate) receptor 1	NM_080818	1.90E-03	-1.24016
7935425	RRP12	Ribosomal RNA processing 12 homolog (S. Cerevisiae)	NM_015179	1.91E-03	-1.17084
7987180	C15orf29	Chromosome 15 open reading frame 29	NM_024713	1.91E-03	1.20996
7961453	---	---	---	1.94E-03	-1.18735
7995258	ZNF267	Zinc finger protein 267	NM_003414	2.00E-03	1.36448
7948364	MPEG1	Macrophage expressed 1	NM_001039396	2.03E-03	-1.20873
8104760	TARS	Threonyl-trna synthetase	NM_152295	2.04E-03	1.14868
8075390	SEC14L4	SEC14-like 4 (S. Cerevisiae)	NM_174977	2.06E-03	-1.17798
7913776	IL28RA	Interleukin 28 receptor, alpha (interferon, lambda receptor)	NM_170743	2.22E-03	-1.1591
7967810	GOLGA3	Golgin A3	NM_005895	2.25E-03	-1.12119
8106429	AGGF1	Angiogenic factor with G patch and FHA domains 1	NM_018046	2.27E-03	1.17784
8156519	MIRLET7A1	Microrna let-7a-1	NR_029476	2.27E-03	1.46682
8047505	FLJ39061	Hypothetical protein FLJ39061	BC118982	2.29E-03	1.30762

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8081953	GTF2E1	General transcription factor IIE, polypeptide 1, alpha 56	NM_005513	2.31E-03	1.25709
8042993	CTNNA2	Catenin (cadherin-associated protein), alpha 2	NM_004389	2.31E-03	-1.20376
7967082	---	---	---	2.31E-03	-1.28529
8028213	ZNF568	Zinc finger protein 568	NM_198539	2.33E-03	1.29579
7929768	CUTC	Cutc copper transporter homolog (E. Coli)	NM_015960	2.33E-03	1.1695
7981335	HSP90AA1	Heat shock protein 90kda alpha (cytosolic), class A	NM_001017963	2.33E-03	1.25236
8003075	---	---	---	2.35E-03	-1.18137
8022892	ZNF396	Zinc finger protein 396	NM_145756	2.36E-03	1.21188
7918487	DENND2D	Denn	NM_024901	2.37E-03	1.11399
8154283	---	---	---	2.37E-03	1.18639
8055909	---	---	---	2.37E-03	-1.15294
8126147	C6orf64	Chromosome 6 open reading frame 64	BC022007	2.39E-03	1.27922
7985695	AKAP13	A kinase (PRKA) anchor protein 13	NM_006738	2.42E-03	-1.17961
8139706	SEC61G	Sec61 gamma subunit	NM_014302	2.45E-03	1.28001
8025998	ZNF136	Zinc finger protein 136	NM_003437	2.47E-03	1.34469
8081343	RG9MTD1	RNA (guanine-9-) methyltransferase domain containing 1	NM_017819	2.47E-03	1.19456
8169701	MCTS1	Malignant T cell amplified sequence 1	NM_014060	2.49E-03	1.31653
8147461	SDC2	Syndecan 2	NM_002998	2.50E-03	-1.17103
7981290	WARS	Tryptophanyl-trna synthetase	NM_004184	2.51E-03	-1.12132
7983763	MAPK6	Mitogen-activated protein kinase 6	NM_002748	2.52E-03	1.3585
8035793	ZNF737	Zinc finger protein 737	NM_001159293	2.57E-03	1.64101
7960553	MRPL51	Mitochondrial ribosomal protein L51	NM_016497	2.58E-03	1.14919
7912622	LRRC38	Leucine rich repeat containing 38	ENST00000376085	2.59E-03	-1.11591
8087985	GLT8D1	Glycosyltransferase 8 domain containing 1	NM_001010983	2.65E-03	1.1045
8130032	FBXO30	F-box protein 30	NM_032145	2.65E-03	1.21992
8162294	SPTLC1	Serine palmitoyltransferase, long chain base subunit 1	NM_006415	2.65E-03	1.10764
7977820	PRMT5	Protein arginine methyltransferase 5	NM_001039619	2.65E-03	-1.1109
8158976	CEL	Carboxyl ester lipase (bile salt-stimulated lipase)	NM_001807	2.67E-03	-1.12395
8101844	ADH5	Alcohol dehydrogenase 5 (class III), chi polypeptide	NM_000671	2.68E-03	1.21997

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8116867	TMEM14B	Transmembrane protein 14B	NM_030969	2.70E-03	1.17658
8005785	KSR1	Kinase suppressor of ras 1	NM_014238	2.72E-03	-1.31962
8143247	KIAA1549	Kiaa1549	NM_020910	2.74E-03	-1.17018
8135214	---	---	---	2.75E-03	1.10153
8134470	TRRAP	Transformation/transcription domain-associated protein	NM_003496	2.76E-03	-1.17021
8111552	C5orf33	Chromosome 5 open reading frame 33	NM_001085411	2.78E-03	1.26942
7955179	TUBA1C	Tubulin, alpha 1c	NM_032704	2.80E-03	-1.17772
8040552	NCOA1	Nuclear receptor coactivator 1	NM_147223	2.82E-03	-1.1738
8149942	CCDC25	Coiled-coil domain containing 25	NM_018246	2.84E-03	1.14967
7998952	TIGD7	Tigger transposable element derived 7	NM_033208	2.87E-03	1.43642
8106999	C5orf27	Chromosome 5 open reading frame 27	NR_026936	2.89E-03	-1.20616
8176576	---	---	---	2.91E-03	1.31481
7900426	SMAP2	Small arfgap2	NM_022733	2.91E-03	-1.24032
7956470	MBD6	Methyl-cpg binding domain protein 6	NM_052897	2.93E-03	-1.243
8104746	NPR3	Natriuretic peptide receptor C/guanylate cyclase C (atriona)	NM_000908	2.95E-03	-1.19369
8096361	HERC5	Hect domain and RLD 5	NM_016323	3.08E-03	1.2598
8008870	TMEM49	Transmembrane protein 49	NM_030938	3.09E-03	1.13606
8102162	INTS12	Integrator complex subunit 12	NM_020395	3.10E-03	1.22349
8028991	CYP2S1	Cytochrome P450, family 2, subfamily S, polypeptide 1	NM_030622	3.12E-03	-1.1428
7961083	CLEC2B	C-type lectin domain family 2, member B	NM_005127	3.13E-03	1.43407
7983606	EID1	EP300 interacting inhibitor of differentiation 1	NM_014335	3.17E-03	1.207
8130674	PDE10A	Phosphodiesterase 10A	NM_006661	3.20E-03	1.15849
8006298	RAB11FIP4	RAB11 family interacting protein 4 (class II)	NM_032932	3.20E-03	-1.10213
8001185	DNAJA2	Dnaj (Hsp40) homolog, subfamily A, member 2	NM_005880	3.21E-03	1.17375
8068593	ETS2	V-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	NM_005239	3.21E-03	-1.17137
8003861	SPNS3	Spinster homolog 3 (Drosophila)	NM_182538	3.23E-03	-1.14045
8150757	RB1CC1	RB1-inducible coiled-coil 1	NM_014781	3.23E-03	1.2585
8012416	C17orf44	Chromosome 17 open reading frame 44	NR_026951	3.23E-03	1.15166
7907090	LOC100128751	Inm04	AY194294	3.24E-03	-1.13747

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8095360	---	---	---	3.26E-03	1.21952
8119974	SLC29A1	Solute carrier family 29 (nucleoside transporters), m	NM_001078175	3.28E-03	-1.21406
8000746	---	---	---	3.29E-03	-1.72316
8103859	DCTD	Dcmp deaminase	NM_001012732	3.31E-03	1.16184
8071107	SLC25A18	Solute carrier family 25 (mitochondrial carrier), membe	NM_031481	3.34E-03	-1.23484
8009713	OTOP3	Otopetrin 3	NM_178233	3.34E-03	-1.17842
8175169	RAP2C	RAP2C, member of RAS oncogene family	NM_021183	3.37E-03	1.10334
8109350	SLC36A1	Solute carrier family 36 (proton/amino acid symporter)	NM_078483	3.37E-03	-1.18422
7951652	---	---	---	3.39E-03	-1.30607
7916130	KTI12	KTI12 homolog, chromatin associated (S. Cerevisiae)	NM_138417	3.48E-03	1.23518
8013331	B9D1	B9 protein domain 1	NM_015681	3.51E-03	-1.17347
8135544	FOXP2	Forkhead box P2	NM_148898	3.51E-03	1.25093
7999171	---	---	---	3.52E-03	-1.23007
8152133	RRM2B	Ribonucleotide reductase M2 B (TP53 inducible)	NM_015713	3.53E-03	1.26378
8175558	SPANXE	SPANX family, member E	NM_145665	3.58E-03	1.11203
8038839	SIGLEC8	Sialic acid binding Ig-like lectin 8	NM_014442	3.58E-03	-1.4403
8162669	ZNF322A	Zinc finger protein 322A	NM_024639	3.58E-03	1.29728
8038305	NTF4	Neurotrophin 4	NM_006179	3.60E-03	-1.23748
8081055	CHMP2B	Chromatin modifying protein 2B	NM_014043	3.61E-03	1.27483
8102817	ELF2	E74-like factor 2 (ets domain transcription factor)	NM_201999	3.61E-03	1.13295
8151906	GDF6	Growth differentiation factor 6	NM_001001557	3.65E-03	-1.23203
7944359	---	---	---	3.69E-03	1.20255
8130765	FAM103A1	Family with sequence similarity 103, member A1	BC112329	3.69E-03	1.12894
7972055	KCTD12	Potassium channel tetramerisation domain containing 12	NM_138444	3.71E-03	-1.23263
7940000	OR5AK2	Olfactory receptor, family 5, subfamily AK, member 2	NM_001005323	3.75E-03	-1.50096
8171747	EIF1AX	Eukaryotic translation initiation factor 1A, X-linked	NM_001412	3.79E-03	1.30772
7972711	---	---	---	3.81E-03	1.18899
8001496	NUDT21	Nudix (nucleoside diphosphate linked moiety X)-type motif	NM_007006	3.83E-03	1.12702
8169294	COL4A5	Collagen, type IV, alpha 5	NM_000495	3.84E-03	-1.11804

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
7960666	ZNF384	Zinc finger protein 384	NM_133476	3.84E-03	-1.10853
8030978	ZNF845	Zinc finger protein 845	NM_138374	3.85E-03	1.88412
7999936	UMOD	Uromodulin	NM_003361	3.85E-03	-1.11571
8113591	PGGT1B	Protein geranylgeranyltransferase type I, beta subunit	NM_005023	3.87E-03	1.24213
7925511	PLD5	Phospholipase D family, member 5	NM_152666	3.90E-03	-1.32485
8043480	---	---	---	3.92E-03	1.10136
8059578	---	---	---	3.93E-03	-1.7047
8025058	TRIP10	Thyroid hormone receptor interactor 10	NM_004240	3.94E-03	-1.10619
8040843	CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase	NM_004341	3.94E-03	-1.16946
8115476	MED7	Mediator complex subunit 7	NM_004270	3.95E-03	1.32722
8080853	---	---	---	3.95E-03	-1.15156
8175302	FAM127B	Family with sequence similarity 127, member B	NM_001078172	3.96E-03	-1.15817
8033789	ZNF121	Zinc finger protein 121	NM_001008727	3.96E-03	1.23886
7929072	IFIT5	Interferon-induced protein with tetratricopeptide repeats	NM_012420	3.98E-03	1.42628
8096753	HADH	Hydroxyacyl-coa dehydrogenase	NM_005327	3.99E-03	1.1301
8039484	IL11	Interleukin 11	NM_000641	3.99E-03	-1.13164
7978570	SNX6	Sorting nexin 6	NM_021249	3.99E-03	1.2031
7908694	NAV1	Neuron navigator 1	NM_020443	4.00E-03	-1.12593
8137517	HTR5A	5-hydroxytryptamine (serotonin) receptor 5A	NM_024012	4.13E-03	-1.10157
8017010	HSF5	Heat shock transcription factor family member 5	NM_001080439	4.16E-03	1.18222
8023591	---	---	---	4.17E-03	-1.13458
8102362	TIFA	TRAF-interacting protein with forkhead-associated domain	NM_052864	4.21E-03	1.24717
8150689		---	---	4.23E-03	-1.58565
8117580	HIST1H2AI	Histone cluster 1, h2ai	NM_003509	4.24E-03	1.19217
8160870	CCL27	Chemokine (C-C motif) ligand 27	NM_006664	4.27E-03	-1.20421
7925741	OR2T33	Olfactory receptor, family 2, subfamily T, member 33	NM_001004695	4.36E-03	-1.14785
7932881	LOC390414	Hypothetical LOC390414	AK094743	4.37E-03	1.12186
7897293	---	---	---	4.37E-03	-1.19685
8102936	---	---	---	4.38E-03	-1.27925

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8088979	VGLL3	Vestigial like 3 (Drosophila)	NM_016206	4.46E-03	1.14327
7964250	PTGES3	Prostaglandin E synthase 3 (cytosolic)	NM_006601	4.47E-03	1.27373
8071877	POM121L9P	POM121 membrane glycoprotein-like 9 (rat) pseudogene	NR_003714	4.52E-03	1.17563
7931348	FOXI2	Forkhead box I2	NM_207426	4.54E-03	-1.36326
7986010	IQGAP1	IQ motif containing gtpase activating protein 1	NM_003870	4.56E-03	-1.1765
8018972	TIMP2	TIMP metallopeptidase inhibitor 2	AK057217	4.58E-03	-1.29485
8042086	VRK2	Vaccinia related kinase 2	NM_006296	4.60E-03	1.37976
8121861	NCOA7	Nuclear receptor coactivator 7	NM_181782	4.62E-03	1.23347
8102775	---	---	---	4.68E-03	-1.17632
8041122	PPP1CB	Protein phosphatase 1, catalytic subunit, beta isozyme	NM_002709	4.69E-03	1.19299
8176698	CYorf15A	Chromosome Y open reading frame 15A	NM_001005852	4.69E-03	1.30982
8023703	C18orf20	Chromosome 18 open reading frame 20	BC029565	4.71E-03	-1.34055
8094609	FAM114A1	Family with sequence similarity 114, member A1	NM_138389	4.73E-03	-1.24832
8126428	TRERF1	Transcriptional regulating factor 1	NM_033502	4.75E-03	-1.11786
7951807	CADM1	Cell adhesion molecule 1	NM_014333	4.76E-03	1.2536
7943552	AASDHPPT	Aminoadipate-semialdehyde dehydrogenase-phosphopantethe	NM_015423	4.77E-03	1.23434
7908147	TSEN15	Trna splicing endonuclease 15 homolog (S. Cerevisiae)	NM_052965	4.82E-03	1.19128
8177044	---	---	---	4.84E-03	-1.24861
8115756	KCNMB1	Potassium large conductance calcium-activated channel	NM_004137	4.87E-03	-1.1305
8127364	GUSBL2	Glucuronidase, beta-like 2	NR_003660	4.89E-03	1.18323
8174494	---	---	---	4.92E-03	-1.21063
8031857	ZNF135	Zinc finger protein 135	NM_003436	4.93E-03	1.11346
8146198	POLB	Polymerase (DNA directed), beta	NM_002690	4.96E-03	1.18361
7938561	---	---	---	4.96E-03	-1.11715
7959016	NCRNA00173	Non-protein coding RNA 173	NR_027345	4.96E-03	-1.3502
8160405	KLHL9	Kelch-like 9 (Drosophila)	NM_018847	5.00E-03	1.21661
7904421	HSD3B1	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroi	NM_000862	5.00E-03	-1.18104
7899849	---	---	---	5.01E-03	-1.11461
7957850	GAS2L3	Growth arrest-specific 2 like 3	NM_174942	5.05E-03	-1.19668

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8101648	HSD17B11	Hydroxysteroid (17-beta) dehydrogenase 11	NM_016245	5.06E-03	1.17003
8127425	LMBRD1	LMBR1 domain containing 1	NM_018368	5.07E-03	1.32028
7910146	PSEN2	Presenilin 2 (Alzheimer disease 4)	NM_000447	5.10E-03	-1.14841
8117522	ABT1	Activator of basal transcription 1	NM_013375	5.12E-03	1.13459
8120194	TFAP2B	Transcription factor AP-2 beta (activating enhancer binding	NM_003221	5.13E-03	-1.29097
8013529	---	---	---	5.16E-03	1.56883
8137081	---	---	---	5.16E-03	1.17944
7998367	RPUSD1	RNA pseudouridylate synthase domain containing 1	NM_058192	5.17E-03	-1.16696
8104449	CCT5	Chaperonin containing TCP1, subunit 5 (epsilon)	NM_012073	5.17E-03	1.11649
8178771	AGER	Advanced glycosylation end product-specific receptor	NM_001136	5.18E-03	-1.17383
8011131	RILP	Rab interacting lysosomal protein	NM_031430	5.20E-03	-1.2329
8094974	OCIAD1	OCIA domain containing 1	NM_017830	5.20E-03	1.14929
8093943	LOC93622	Hypothetical LOC93622	NR_015433	5.22E-03	1.20837
8016366	MRPL10	Mitochondrial ribosomal protein L10	NM_145255	5.23E-03	1.21708
8120378	KIAA1586	Kiaa1586	NM_020931	5.27E-03	1.37772
8116996	---	---	---	5.31E-03	-1.28381
8024584	NCLN	Nicalin homolog (zebrafish)	NM_020170	5.33E-03	-1.13934
8099912	C4orf34	Chromosome 4 open reading frame 34	BC008502	5.37E-03	1.18735
8032094	LPPR3	Lipid phosphate phosphatase-related protein type 3	NM_024888	5.42E-03	-1.167
8104838	DNAJC21	Dnaj (Hsp40) homolog, subfamily C, member 21	NM_194283	5.43E-03	1.14285
7995252	ZNF720	Zinc finger protein 720	NM_001130913	5.45E-03	1.32296
8116113	FAM193B	Family with sequence similarity 193, member B	NR_024019	5.47E-03	-1.16049
8098414	SPCS3	Signal peptidase complex subunit 3 homolog (S. Cerevisiae)	NM_021928	5.47E-03	1.26024
7918857	TSPAN2	Tetraspanin 2	NM_005725	5.48E-03	1.14187
7979260	GMFB	Glia maturation factor, beta	NM_004124	5.51E-03	1.49342
8105146	MGC42105	Serine/threonine-protein kinase NIM1	NM_153361	5.51E-03	1.15908
8172252	---	---	---	5.51E-03	-1.37094
7902308	FPGT	Fucose-1-phosphate guanylyltransferase	NM_003838	5.54E-03	1.35375
8153959	DOCK8	Dedicator of cytokinesis 8	NM_203447	5.56E-03	-1.18992

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7911767	MMEL1	Membrane metallo-endopeptidase-like 1	NM_033467	5.59E-03	-1.10775
7936529	KIAA1598	Kiaa1598	NM_001127211	5.63E-03	-1.40202
8020382	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	NM_005406	5.65E-03	-1.24697
8108217	TGFBI	Transforming growth factor, beta-induced, 68kda	NM_000358	5.70E-03	-1.3237
8058182	FAM126B	Family with sequence similarity 126, member B	NM_173822	5.71E-03	1.19851
8073309	LOC100288034	Similar to FKSG62	XM_002346783	5.71E-03	-1.29482
8083808	LRRIQ4	Leucine-rich repeats and IQ motif containing 4	NM_001080460	5.73E-03	-1.21301
8090448	RUVBL1	Ruvb-like 1 (E. Coli)	NM_003707	5.76E-03	1.10492
8174527	CAPN6	Calpain 6	NM_014289	5.78E-03	-1.24495
8087250	MIR425	Microrna 425	NR_029948	5.78E-03	-1.1211
8096635	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer	NM_003998	5.81E-03	-1.16142
7899898	HMGB4	High-mobility group box 4	NM_145205	5.81E-03	-1.13529
8074748	PI4KAP2	Phosphatidylinositol 4-kinase, catalytic, alpha pseudoge	NR_003700	5.83E-03	-1.12807
7942553	SPCS2	Signal peptidase complex subunit 2 homolog (S. Cerevisiae)	NM_014752	5.83E-03	1.25436
7964033	ANKRD52	Ankyrin repeat domain 52	NM_173595	5.86E-03	-1.15954
7929247	5-Mar	Membrane-associated ring finger (C3HC4) 5	NM_017824	5.89E-03	1.15729
8092083	SLC2A2	Solute carrier family 2 (facilitated glucose transporter)	NM_000340	5.89E-03	1.30066
7959251	P2RX7	Purinergic receptor P2X, ligand-gated ion channel, 7	NM_002562	5.90E-03	-1.36582
7960794	CD163	CD163 molecule	NM_004244	5.91E-03	-1.53933
8151136	COPS5	COP9 constitutive photomorphogenic homolog subunit 5	NM_006837	5.93E-03	1.26846
8035156	CHERP	Calcium homeostasis endoplasmic reticulum protein	NM_006387	5.94E-03	-1.18384
7913547	WNT4	Wingless-type MMTV integration site family, member 4	NM_030761	5.97E-03	-1.12117
8148796	SCXA	Scleraxis homolog A (mouse)	NM_001008271	6.02E-03	-1.22332
8148821	SCXA	Scleraxis homolog A (mouse)	NM_001008271	6.02E-03	-1.22332
8121193	KLHL32	Kelch-like 32 (Drosophila)	NM_052904	6.07E-03	1.12749
8152656	ZHX1	Zinc fingers and homeoboxes 1	NM_001017926	6.09E-03	1.1922
7965112	PAWR	PRKC, apoptosis, WT1, regulator	NM_002583	6.14E-03	1.23685
7932552	PRO3077	Hypothetical protein PRO3077	ENST00000451889	6.17E-03	-1.15013

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8157608	---	---	---	6.20E-03	-1.15644
8039680	ZNF671	Zinc finger protein 671	NM_024833	6.21E-03	1.15837
8061426	---	---	---	6.21E-03	-1.20461
8049888	ATG4B	ATG4 autophagy related 4 homolog B (S. Cerevisiae)	NM_013325	6.23E-03	-1.11258
8054092	TMEM131	Transmembrane protein 131	NM_015348	6.27E-03	-1.12901
7942409	P2RY6	Pyrimidinergic receptor P2Y, G-protein coupled, 6	NM_176796	6.27E-03	-1.27005
7949916	CHKA	Choline kinase alpha	NM_001277	6.30E-03	-1.12516
8156450	---	---	---	6.32E-03	1.168
7917904	LOC100286918	Similar to NADH dehydrogenase [ubiquinone] iron	XM_002342095	6.34E-03	1.17976
7999317	TMEM186	Transmembrane protein 186	NM_015421	6.38E-03	1.26018
7911376	HES4	Hairy and enhancer of split 4 (Drosophila)	NM_001142467	6.41E-03	-1.16515
8042830	MTHFD2	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent)	NR_027405	6.41E-03	1.2007
7935746	BLOC1S2	Biogenesis of lysosomal organelles complex-1, subunit	NM_001001342	6.41E-03	1.14561
8064868	GPCPD1	Glycerophosphocholine phosphodiesterase GDE1 homolog	NM_019593	6.42E-03	1.20813
7998978	ZNF597	Zinc finger protein 597	NM_152457	6.42E-03	1.14856
8031640	ZNF583	Zinc finger protein 583	NM_152478	6.45E-03	1.3487
7960381	EFCAB4B	EF-hand calcium binding domain 4B	CR627161	6.45E-03	1.21593
7997832	FLJ40448	Hypothetical protein FLJ40448	ENST00000333666	6.49E-03	-1.10069
7988077	LCMT2	Leucine carboxyl methyltransferase 2	NM_014793	6.51E-03	1.21419
8165642	TMEM203	Transmembrane protein 203	NM_053045	6.51E-03	1.17362
7974303	TMX1	Thioredoxin-related transmembrane protein 1	NM_030755	6.52E-03	1.30075
7920487	C1orf189	Chromosome 1 open reading frame 189	BC127710	6.55E-03	1.16694
7903586	TMEM167B	Transmembrane protein 167B	NM_020141	6.56E-03	1.19438
8039905	TMEM167B	Transmembrane protein 167B	NM_020141	6.56E-03	1.19438
7916372	TMEM59	Transmembrane protein 59	NM_004872	6.56E-03	1.16785
8124459	ZNF322A	Zinc finger protein 322A	NM_024639	6.56E-03	1.30038
8073578	C22orf32	Chromosome 22 open reading frame 32	BC024237	6.59E-03	1.15679
7989887	MEGF11	Multiple EGF-like-domains 11	NM_032445	6.60E-03	-1.13047

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8053666	---	---	---	6.61E-03	-1.16697
8084092	NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16k	NM_002492	6.61E-03	1.19391
7978666	MBIP	MAP3K12 binding inhibitory protein 1	NM_016586	6.64E-03	1.24038
7948303	---	---	---	6.66E-03	-1.10451
7955119	C12orf54	Chromosome 12 open reading frame 54	NM_152319	6.66E-03	-1.15551
7968370	B3GALTL	Beta 1,3-galactosyltransferase-like	NM_194318	6.67E-03	1.14704
8151795	CDH17	Cadherin 17, LI cadherin (liver-intestine)	NM_004063	6.67E-03	-1.20232
8121066	SPACA1	Sperm acrosome associated 1	NM_030960	6.70E-03	-1.1173
7961067	---	---	---	6.71E-03	-1.11767
7981824	CYFIP1	Cytoplasmic FMR1 interacting protein 1	NM_014608	6.71E-03	-1.33191
8011141	PRPF8	PRP8 pre-mrna processing factor 8 homolog (S. Cerevisiae)	NM_006445	6.72E-03	-1.17393
8113064	LYSMD3	Lysm, putative peptidoglycan-binding, domain containing 3	NM_198273	6.75E-03	1.39831
7931455	LRRC27	Leucine rich repeat containing 27	NM_001143757	6.77E-03	-1.1114
8151250	---	---	---	6.77E-03	-1.24611
8088846	---	---	---	6.78E-03	1.13931
8051226	TRMT61B	Trna methyltransferase 61 homolog B (S. Cerevisiae)	NM_017910	6.89E-03	1.39182
8029340	ZNF155	Zinc finger protein 155	NM_003445	6.90E-03	1.29108
7964064	CS	Citrate synthase	NM_004077	6.91E-03	-1.20299
7902493	---	---	---	6.91E-03	-1.18574
8110018	RPL26L1	Ribosomal protein L26-like 1	NM_016093	6.92E-03	1.23744
8096957	---	---	---	6.92E-03	1.26108
8167998	AR	Androgen receptor	NM_000044	6.97E-03	-1.20819
8126279	TREM2	Triggering receptor expressed on myeloid cells 2	NM_018965	7.00E-03	-1.12687
8050548	LAPTM4A	Lysosomal protein transmembrane 4 alpha	NM_014713	7.01E-03	1.12742
8027510	C19orf40	Chromosome 19 open reading frame 40	NM_152266	7.05E-03	1.11488
8022426	LOC646359	Similar to telomeric repeat binding factor (NIMA	ENST00000342224	7.07E-03	-1.33448
7944162	---	---	---	7.08E-03	-1.13436

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8018731	RHBDF2	Rhomboid 5 homolog 2 (Drosophila)	NM_024599	7.08E-03	-1.14046
8103520	TRIM61	Tripartite motif-containing 61	NM_001012414	7.13E-03	1.18905
7964701	GNS	Glucosamine (N-acetyl)-6-sulfatase	NM_002076	7.14E-03	-1.27976
7902911	---	---	---	7.15E-03	-1.2657
8177068	---	---	---	7.21E-03	-1.25224
7903203	SNX7	Sorting nexin 7	NM_015976	7.22E-03	-1.17834
7999304	FAM86A	Family with sequence similarity 86, member A	NM_201400	7.25E-03	-1.11058
8131000	HEATR2	HEAT repeat containing 2	NM_017802	7.28E-03	-1.1045
7916562	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	NM_002136	7.35E-03	1.13724
8110618	ARPP19	Camp-regulated phosphoprotein, 19kda	NM_006628	7.36E-03	1.10296
8112807	ARSB	Arylsulfatase B	NM_000046	7.37E-03	-1.1574
7907171	BLZF1	Basic leucine zipper nuclear factor 1	NM_003666	7.40E-03	1.2842
8155550	LOC554249	Hypothetical LOC554249	AK292642	7.42E-03	1.1044
7954029	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	NM_004064	7.49E-03	1.19399
8163257	LPAR1	Lysophosphatidic acid receptor 1	NM_057159	7.50E-03	-1.18531
8124484	HIST1H2BJ	Histone cluster 1, h2bj	NM_021058	7.50E-03	-1.1749
8081820	---	---	---	7.58E-03	-1.24684
8086607	LTF	Lactotransferrin	NM_002343	7.58E-03	-1.26088
8131867	---	---	---	7.60E-03	-1.23084
8106107	PTCD2	Pentatricopeptide repeat domain 2	NM_024754	7.61E-03	1.28064
8106702	ZCCHC9	Zinc finger, CCHC domain containing 9	NM_032280	7.62E-03	1.16093
8068168	SOD1	Superoxide dismutase 1, soluble	NM_000454	7.63E-03	1.14687
8001385	---	---	---	7.64E-03	-1.15954
8084630	---	---	---	7.65E-03	-1.17809
8096081	ENOPH1	Enolase-phosphatase 1	NM_021204	7.69E-03	1.15722
7970655	MTMR6	Myotubularin related protein 6	NM_004685	7.69E-03	1.24128
8178095	C2	Complement component 2	NM_000063	7.70E-03	-1.16351
8179331	C2	Complement component 2	NM_000063	7.70E-03	-1.16351
7938263	EIF3F	Eukaryotic translation initiation factor 3, subunit F	NM_003754	7.73E-03	1.14948

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7979455	RTN1	Reticulon 1	NM_021136	7.75E-03	-1.37064
7933084	NAMPT	Nicotinamide phosphoribosyltransferase	NM_005746	7.76E-03	1.19403
8096251	NUDT9	Nudix (nucleoside diphosphate linked moiety X)-type motif	NM_024047	7.77E-03	1.17498
8077528	SETD5	SET domain containing 5	NM_001080517	7.79E-03	-1.12273
7945859	MRGPRE	MAS-related GPR, member E	NM_001039165	7.80E-03	-1.2183
8151234	SLCO5A1	Solute carrier organic anion transporter family, member 5	AF205075	7.80E-03	-1.28021
8119034	BRPF3	Bromodomain and PHD finger containing, 3	NM_015695	7.84E-03	-1.1626
7943519	---	---	---	7.90E-03	1.1165
8100756	---	---	---	7.91E-03	-1.26327
7899604	ZCCHC17	Zinc finger, CCHC domain containing 17	NM_016505	7.93E-03	1.15967
8016232	SH3D20	SH3 domain containing 20	NM_174919	7.94E-03	-1.19651
8173745	CYSLTR1	Cysteinyl leukotriene receptor 1	NM_006639	7.95E-03	1.21873
8102037	---	---	---	7.98E-03	1.27825
7971950	DACH1	Dachshund homolog 1 (Drosophila)	NM_080759	7.98E-03	-1.17053
8069644	APP	Amyloid beta (A4) precursor protein	NM_000484	7.99E-03	-1.3248
8049534	LRRFIP1	Leucine rich repeat (in FLII) interacting protein 1	NM_001137550	7.99E-03	-1.2279
7920165	FLG	Filaggrin	NM_002016	7.99E-03	-1.11366
7977435	---	---	---	8.00E-03	1.27651
7989953	AAGAB	Alpha- and gamma-adaptin binding protein	NM_024666	8.02E-03	1.15777
8155453	LOC100289385	Hypothetical protein LOC100289385	XM_002342912	8.08E-03	1.31562
8161375	LOC100289385	Hypothetical protein LOC100289385	XM_002342912	8.08E-03	1.31562
7900413	ZMPSTE24	Zinc metallopeptidase (STE24 homolog, S. Cerevisiae)	NM_005857	8.08E-03	1.2369
8108873	ARHGAP26	Rho gtpase activating protein 26	NM_015071	8.09E-03	-1.15711
8141708	CLDN15	Claudin 15	NM_014343	8.13E-03	-1.15809
7973754	---	---	---	8.14E-03	-1.14992
8146894	---	---	---	8.14E-03	1.13833
8029399	ZNF226	Zinc finger protein 226	NM_001032372	8.17E-03	1.40976
8038989	ZNF600	Zinc finger protein 600	NM_198457	8.20E-03	1.2762
8089128	TOMM70A	Translocase of outer mitochondrial membrane 70 homolog A	NM_014820	8.22E-03	1.10493

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8006345	RHOT1	Ras homolog gene family, member T1	NM_001033568	8.23E-03	1.24848
8037103	GRIK5	Glutamate receptor, ionotropic, kainate 5	NM_002088	8.23E-03	-1.2738
8099897	UGDH	UDP-glucose 6-dehydrogenase	NM_003359	8.24E-03	1.27072
7925128	---	---	---	8.28E-03	-1.20397
8061445	---	---	---	8.32E-03	-1.15966
8091385	CP	Ceruloplasmin (ferroxidase)	NM_000096	8.32E-03	1.33097
7990848	TMC3	Transmembrane channel-like 3	NM_001080532	8.38E-03	-1.10521
7925691	ZNF124	Zinc finger protein 124	NM_003431	8.39E-03	1.29967
8046997	ASNSD1	Asparagine synthetase domain containing 1	NM_019048	8.40E-03	1.25924
7907353	METTL13	Methyltransferase like 13	NM_015935	8.41E-03	1.12419
7911199	C1orf150	Chromosome 1 open reading frame 150	NM_145278	8.45E-03	1.16755
7950644	NDUFC2	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2	NM_004549	8.47E-03	1.22001
8003991	MINK1	Misshapen-like kinase 1 (zebrafish)	NM_153827	8.47E-03	-1.19442
7906435	DARC	Duffy blood group, chemokine receptor	NM_002036	8.51E-03	-1.22871
8126784	PLA2G7	Phospholipase A2, group VII (platelet-activating factor	NM_001168357	8.52E-03	-1.10783
7956005	OR2AP1	Olfactory receptor, family 2, subfamily AP, member	ENST00000321688	8.54E-03	1.27053
8039013	ZNF321	Zinc finger protein 321	NM_203307	8.54E-03	1.35424
7991630	TM2D3	TM2 domain containing 3	NM_078474	8.56E-03	1.17958
8044049	IL18RAP	Interleukin 18 receptor accessory protein	NM_003853	8.61E-03	1.2754
7903703	GNAI3	Guanine nucleotide binding protein (G protein), alpha inhibitor	NM_006496	8.62E-03	1.2552
7909689	SMYD2	SET and MYND domain containing 2	NM_020197	8.62E-03	1.18907
7968297	POMP	Proteasome maturation protein	NM_015932	8.65E-03	1.16822
7964733	RPSAP52	Ribosomal protein SA pseudogene 52	NR_026825	8.68E-03	1.32673
8119722	CUL9	Cullin 9	NM_015089	8.68E-03	-1.11298
7972737	LIG4	Ligase IV, DNA, ATP-dependent	NM_002312	8.71E-03	1.28653
8093601	FAM193A	Family with sequence similarity 193, member A	NM_003704	8.72E-03	-1.1764
8162191	LOC392364	Chromosome 15 open reading frame 2 pseudogene	BC086877	8.72E-03	-1.24198

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8152867	ASAP1	Arfgap with SH3 domain, ankyrin repeat and PH domain 1	NM_018482	8.80E-03	-1.32645
8035304	BST2	Bone marrow stromal cell antigen 2	NM_004335	8.82E-03	1.16059
7901867	USP1	Ubiquitin specific peptidase 1	NM_003368	8.82E-03	1.39398
8047286	---	---	---	8.83E-03	-1.3013
7935968	LDB1	LIM domain binding 1	NM_003893	8.84E-03	-1.14475
8102006	MANBA	Mannosidase, beta A, lysosomal	NM_005908	8.88E-03	-1.19819
8094378	PI4K2B	Phosphatidylinositol 4-kinase type 2 beta	NM_018323	8.91E-03	1.19533
7911676	---	---	---	8.92E-03	-1.29941
8096461	ATOH1	Atonal homolog 1 (Drosophila)	NM_005172	8.92E-03	-1.11833
8055862	ARL5A	ADP-ribosylation factor-like 5A	NM_012097	8.96E-03	1.22757
8011542	ZZEF1	Zinc finger, ZZ-type with EF-hand domain 1	NM_015113	8.96E-03	-1.17171
7996377	CES8	Carboxylesterase 8 (putative)	NM_173815	8.97E-03	-1.13189
8046003	GCA	Grancalcin, EF-hand calcium binding protein	NM_012198	8.98E-03	1.26908
8026007	ZNF791	Zinc finger protein 791	NM_153358	8.98E-03	1.22662
8025103	EMR1	Egf-like module containing, mucin-like, hormone receptor-li	NM_001974	9.01E-03	-1.47335
8128626	PDSS2	Prenyl (decaprenyl) diphosphate synthase, subunit 2	NM_020381	9.03E-03	1.27126
8024019	PTBP1	Polypyrimidine tract binding protein 1	NM_002819	9.04E-03	-1.11968
7989315	GTF2A2	General transcription factor IIA, 2, 12kda	NM_004492	9.06E-03	1.26504
8137264	TMEM176A	Transmembrane protein 176A	NM_018487	9.06E-03	-1.35027
8135909	LEP	Leptin	NM_000230	9.08E-03	-1.15852
7945420	RNH1	Ribonuclease/angiogenin inhibitor 1	NM_002939	9.08E-03	-1.11763
8091698	SHOX2	Short stature homeobox 2	NM_003030	9.08E-03	-1.18357
8076339	PHF5A	PHD finger protein 5A	NM_032758	9.08E-03	1.21107
7964832	---	---	---	9.08E-03	1.1847
7963988	SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator	NM_003075	9.14E-03	-1.14109
7985268	FAH	Fumarylacetoacetate hydrolase (fumarylacetoacetase)	NM_000137	9.16E-03	-1.21621
7930921	BAG3	BCL2-associated athanogene 3	NM_004281	9.17E-03	-1.21844
8102210	---	---	---	9.18E-03	-1.19381
8037535	---	---	---	9.20E-03	1.10952

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
7927505	C10orf53	Chromosome 10 open reading frame 53	NM_182554	9.21E-03	-1.13661
8122637	SASH1	SAM and SH3 domain containing 1	NM_015278	9.25E-03	-1.16624
8003156	---	---	---	9.27E-03	1.23751
8148265	RNF139	Ring finger protein 139	NM_007218	9.27E-03	1.20819
8104443	---	---	---	9.28E-03	-1.207
7924817	PRO2012	Hypothetical protein PRO2012	BC019830	9.28E-03	1.27355
7985587	SCAND2	SCAN domain containing 2 pseudogene	NR_004859	9.30E-03	1.24522
7959995	EP400	E1A binding protein p400	NM_015409	9.38E-03	-1.18418
8151788	RBM12B	RNA binding motif protein 12B	NM_203390	9.40E-03	1.4164
8043487	FKSG73	ARP3 actin-related protein 3 homolog B pseudogene	NR_027714	9.41E-03	-1.13056
7904965	PDE4DIP	Phosphodiesterase 4D interacting protein	AB042555	9.42E-03	1.31278
8045919	7-Mar	Membrane-associated ring finger (C3HC4) 7	NM_022826	9.45E-03	1.26964
7945182	APLP2	Amyloid beta (A4) precursor-like protein 2	NM_001642	9.48E-03	-1.12202
8022295	FAM38B	Family with sequence similarity 38, member B	NM_022068	9.48E-03	1.17219
8104625	---	---	---	9.49E-03	1.24254
8131600	TSPAN13	Tetraspanin 13	NM_014399	9.50E-03	1.20646
8054075	UBTFL1	Upstream binding transcription factor, RNA polymerase	NM_001143975	9.50E-03	-1.15066
7976876	DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1	NM_001376	9.53E-03	-1.21384
8059648	---	---	---	9.53E-03	1.31455
7978558	EAPP	E2F-associated phosphoprotein	NM_018453	9.54E-03	1.37411
7961363	---	---	---	9.58E-03	-1.21749
7932796	SVIL	Supervillin	NM_021738	9.59E-03	-1.13936
8022118	EPB41L3	Erythrocyte membrane protein band 4.1-like 3	NM_012307	9.60E-03	-1.23694
8163729	MIR147	Microrna 147	NR_029604	9.60E-03	-1.18801
8036956	C19orf54	Chromosome 19 open reading frame 54	NM_198476	9.61E-03	-1.13823
8066567	---	---	---	9.63E-03	1.19606
8019559	B3GNTL1	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase	AK126018	9.64E-03	-1.15365
8079074	SS18L2	Synovial sarcoma translocation gene on chromosome 18-like	NM_016305	9.69E-03	1.15093

Probe Set ID	Gene Symbol	Gene Name	RefSeq	P Value	Fold Change
8130211	SYNE1	Spectrin repeat containing, nuclear envelope 1	NM_182961	9.75E-03	-1.13687
7901982	UBE2U	Ubiquitin-conjugating enzyme E2U (putative)	NM_152489	9.77E-03	-1.20212
8054943	---	---	---	9.80E-03	-1.22989
7957245	GLIPR1L1	GLI pathogenesis-related 1 like 1	NM_152779	9.84E-03	1.20256
8019149	SLC38A10	Solute carrier family 38, member 10	NM_001037984	9.85E-03	-1.16543
8080973	PPP4R2	Protein phosphatase 4, regulatory subunit 2	NM_174907	9.85E-03	1.26828
7992692	SRRM2	Serine/arginine repetitive matrix 2	NM_016333	9.85E-03	-1.22791
8014241	SLFN12	Schlafen family member 12	NM_018042	9.85E-03	1.28066
8120943	CYB5R4	Cytochrome b5 reductase 4	NM_016230	9.91E-03	1.20619
7897288	ESPN	Espin	NM_031475	9.91E-03	-1.2696
7913814	SYF2	SYF2 homolog, RNA splicing factor (S. Cerevisiae)	NM_015484	9.92E-03	1.18457
8030044	KCNJ14	Potassium inwardly-rectifying channel, subfamily J, member 14	NM_170720	9.94E-03	-1.19379
8035842	ZNF91	Zinc finger protein 91	NM_003430	9.97E-03	1.37192
8030993	ZNF761	Zinc finger protein 761	NM_001008401	9.99E-03	1.44775
8173609	---	---	---	9.99E-03	1.28596

Appendix 3 — Down-Regulated Gene Sets (FDR≤0.25)

Gene Set	Size	FDR
NAKAJIMA_MCSMBP_EOS	28	3.82E-02
PASSERINI_INFLAMMATION	24	6.44E-02
ACTIN_CYTOSKELETON_ORGANIZATION_AND_ BIOGENESIS	100	8.59E-02
ACTIN_FILAMENT_BINDING	23	8.62E-02
ROSS_MLL_FUSION	70	8.95E-02
RAS_GUANYL_NUCLEOTIDE_EXCHANGE_FACTOR_ ACTIVITY	17	8.97E-02
ROSS_CBF_MYH	46	9.01E-02
INTEGRIN_MEDIATED_CELL_ADHESION_KEGG	83	9.11E-02
SCHURINGA_STAT5A_UP	16	9.15E-02
HBX_HCC_DN	22	9.23E-02
REGULATION_OF_SMALL_GTPASE_MEDIATED_SIGNAL_ TRANSDUCTION	22	9.29E-02
HSA04670_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	98	9.57E-02
STEMCELL_COMMON_DN	55	9.68E-02
ACTIN_FILAMENT_BASED_PROCESS	109	9.71E-02
REGULATION_OF_RAS_PROTEIN_SIGNAL_ TRANSDUCTION	18	9.92E-02
YAGI_AML_PROGNOSIS	29	1.04E-01
GLUTATHIONE_METABOLISM	28	1.07E-01
HSA04330_NOTCH_SIGNALING_PATHWAY	38	1.13E-01
HEARTFAILURE_ATRIA_UP	23	1.14E-01
TGF_BETA_SIGNALING_PATHWAY	47	1.17E-01
INTEGRIN_COMPLEX	18	1.42E-01
JECHLINGER_EMT_DN	38	1.77E-01
ETSPATHWAY	17	1.80E-01
HEMATOPOESIS_RELATED_TRANSCRIPTION_FACTORS	78	1.81E-01
HSA01510_NEURODEGENERATIVE_DISEASES	36	1.81E-01
CORTICAL_CYTOSKELETON	19	1.82E-01
CELL_ADHESION_RECEPTOR_ACTIVITY	32	1.82E-01

Gene Set	Size	FDR
ATPASE_ACTIVITY__COUPLED_TO_TRANSMEMBRANE_ MOVEMENT_OF_IONS__PHOSPHORYLATIVE_MECHANISM	19	1.83E-01
RUFFLE	28	1.84E-01
HSA00480_GLUTATHIONE_METABOLISM	34	1.85E-01
PROTEASE_INHIBITOR_ACTIVITY	41	1.86E-01
CARDIACEGFPATHWAY	16	1.86E-01
GUANYL_NUCLEOTIDE_EXCHANGE_FACTOR_ACTIVITY	41	1.87E-01
CELL_CORTEX_PART	22	1.87E-01
BRG1_ALAB_UP	39	1.88E-01
AGED_MOUSE_CEREBELLUM_UP	57	1.89E-01
ABBUD_LIF_DN	23	1.89E-01
HSA04520_ADHERENS_JUNCTION	74	1.94E-01
CYTOSKELETAL_PROTEIN_BINDING	145	1.95E-01
CELL_JUNCTION	75	1.97E-01
CELL_CORTEX	35	1.99E-01
HSA04810_REGULATION_OF_ACTIN_CYTOSKELETON	186	2.00E-01
BRENTANI_CYTOSKELETON	19	2.01E-01
ACTIN_BINDING	68	2.03E-01
HSA04630_JAK_STAT_SIGNALING_PATHWAY	140	2.04E-01
PROTEIN_DOMAIN_SPECIFIC_BINDING	60	2.04E-01
TGFBETA_ALL_UP	77	2.14E-01
ACTIN_CYTOSKELETON	119	2.15E-01
HSA01032_GLYCAN_STRUCTURES_DEGRADATION	28	2.37E-01
UCALPAINPATHWAY	15	2.41E-01
EMT_DN	51	2.49E-01

Appendix 4 — Up-Regulated Gene Sets (FDR≤0.25)

Gene Set	Size	FDR
PROTEIN_AMINO_ACID_LIPIDATION	22	1.20E-02
LIPOPROTEIN_BIOSYNTHETIC_PROCESS	24	1.20E-02
TRANSCRIPTION_FROM_RNA_POLYMERASE_III_ PROMOTER	16	1.64E-02
HSA03022_BASAL_TRANSCRIPTION_FACTORS	29	1.75E-02
LIPOPROTEIN_METABOLIC_PROCESS	31	1.93E-02
IFN_BETA_UP	61	4.04E-02
CELLULAR_RESPIRATION	18	4.47E-02
NF90_UP	22	5.21E-02
HSA03050_PROTEASOME	22	7.09E-02
ZHAN_MM_CD1_VS_CD2_UP	69	7.18E-02
NOUZOVA_CPG_H4_UP	91	7.26E-02
NUCLEOTIDE_BIOSYNTHETIC_PROCESS	17	7.41E-02
MITOCHONDRIAL_RIBOSOME	17	7.66E-02
CASPASEPATHWAY	21	7.80E-02
TAKEDA_NUP8_HOXA9_10D_UP	155	7.87E-02
RIBOSOMAL_SUBUNIT	15	7.95E-02
DNA_DEPENDENT_DNA_REPLICATION	51	8.05E-02
CHEN_HOXA5_TARGETS_UP	187	8.06E-02
ORGANELLAR_RIBOSOME	17	8.21E-02
ERM_KO_TESTES_DN	18	9.41E-02
AGUIRRE_PANCREAS_CHR6	25	1.19E-01
DNA_HELICASE_ACTIVITY	22	1.22E-01
MITOCHONDRIAL_LUMEN	40	1.40E-01
HSA00670_ONE_CARBON_POOL_BY_FOLATE	15	1.41E-01
PEROXISOME	42	1.42E-01
CHROMATIN	34	1.43E-01
IFN_ANY_UP	76	1.44E-01
MITOCHONDRIAL_RESPIRATORY_CHAIN	21	1.44E-01
MITOCHONDRIAL_MATRIX	40	1.45E-01
MICROBODY	42	1.47E-01

Gene Set	Size	FDR
S_ADENOSYLMETHIONINE_DEPENDENT_ METHYLTRANSFERASE_ ACTIVITY	22	1.49E-01
DNA_POLYMERASE_ACTIVITY	17	1.51E-01
MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS	42	1.65E-01
DER_IFNB_UP	85	1.71E-01
PROTEASOME	16	1.71E-01
CMV_HCMV_6HRS_UP	21	1.74E-01
REOVIRUS_HEK293_UP	200	1.75E-01
RNA_TRANSCRIPTION_REACTOME	31	1.75E-01
PHOSPHATASE_REGULATOR_ACTIVITY	25	1.76E-01
MOREAUX_TACI_HI_VS_LOW_DN	138	1.78E-01
BLEO_MOUSE_LYMPH_LOW_24HRS_DN	24	1.79E-01
PROPANOATE_METABOLISM	29	1.81E-01
KIM_TH_CELLS_UP	41	1.82E-01
MITOCHONDRIAL_PART	124	1.90E-01
G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE	25	1.93E-01
RIBONUCLEOPROTEIN_COMPLEX	117	2.03E-01
PROTEASOMEPATHWAY	19	2.05E-01
MITOCHONDRION	298	2.05E-01
IFNA_HCMV_6HRS_UP	47	2.05E-01
DNA_DIRECTED_RNA_POLYMERASE_II__HOLOENZYME	61	2.06E-01
ZHAN_MMPC_LATEVS	41	2.23E-01
RIBOSOME	33	2.24E-01
BRCA_BRCA1_POS	92	2.24E-01

Appendix 5 — Cytokine Level Changes after Allergen Inhalation Challenge

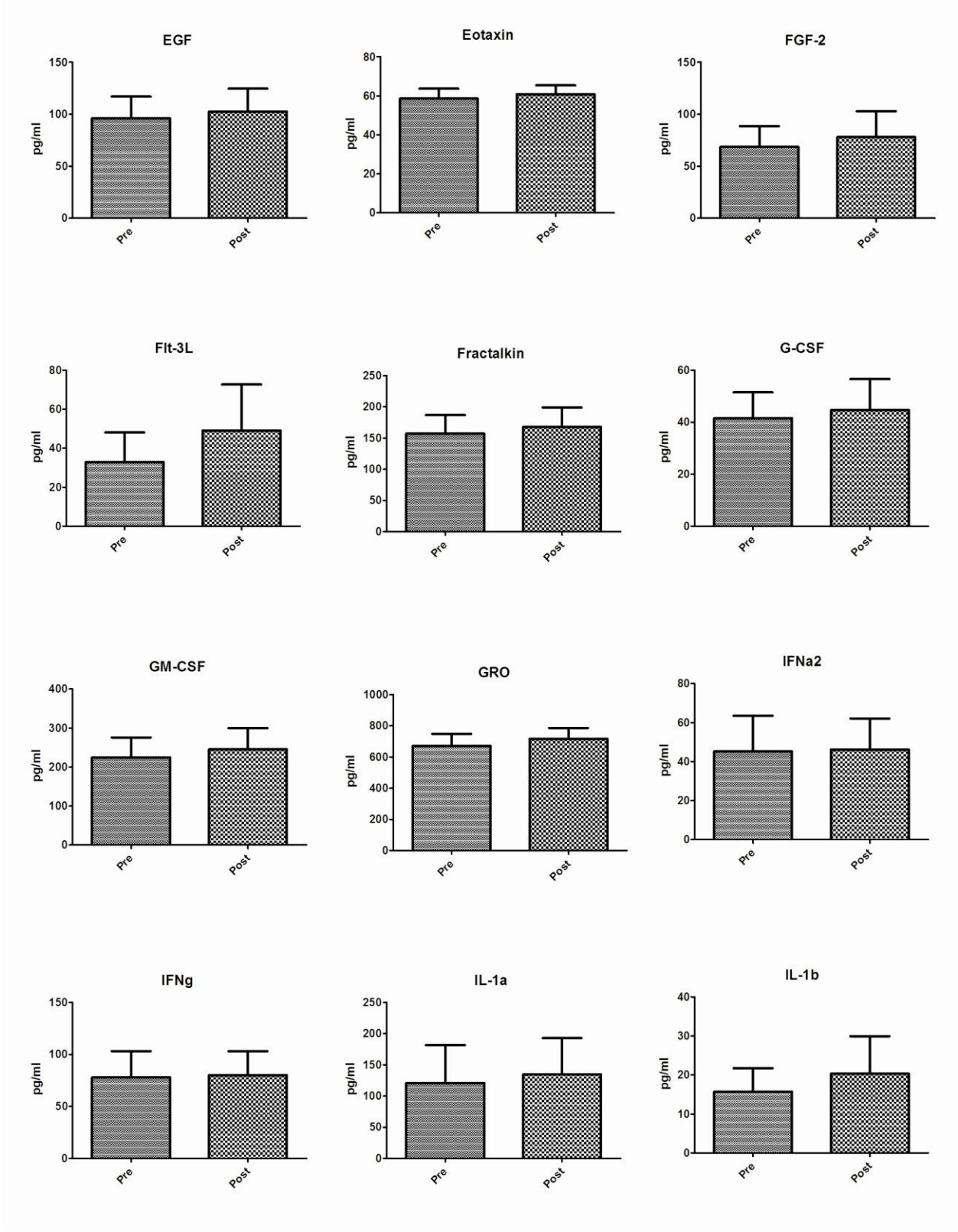
a) P Values and Fold Changes

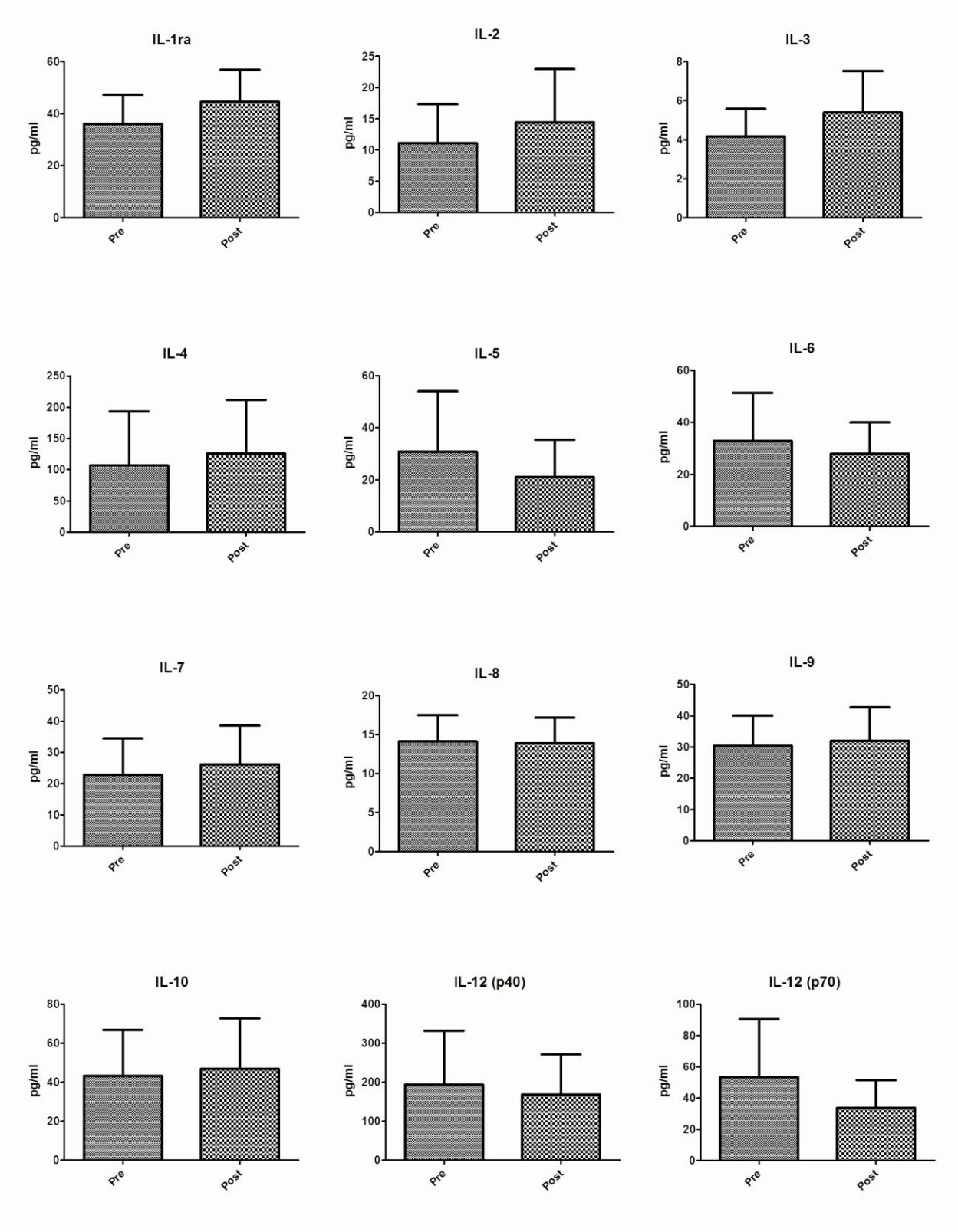
Cytokines	P Value[a]	Fold Change[b]
EGF	7.01E-01	1.067
Eotaxin	4.93E-01	1.038
FGF-2	1.79E-01	1.141
Flt-3 ligand	4.62E-01	1.489
Fractalkine	2.18E-01	1.069
G-CSF	4.01E-01	1.076
GM-CSF	3.47E-01	1.093
GRO	3.73E-01	1.065
IFN-α2	9.57E-01	1.021
IFN-γ	7.05E-01	1.028
IL-1α	6.83E-01	1.117
IL-1β	2.68E-01	1.290
IL-1Rα	2.11E-01	1.237
IL-2	1.91E-01	1.297
IL-3	2.19E-01	1.293
IL-4	7.43E-01	1.182
IL-5	4.26E-01	-1.464
IL-6	5.77E-01	-1.174
IL-7	6.39E-01	1.150
IL-8	8.42E-01	-1.017
IL-9	2.75E-01	1.055
IL-10	7.33E-01	1.081
IL-12 (p40)	4.21E-01	-1.153
IL-12 (p70)	3.83E-01	-1.581
IL-13	3.94E-01	-1.711

Cytokines	P Value[a]	Fold Change[b]
IL-15	5.14E-01	1.006
IL-17	7.50E-01	1.004
IP-10	6.68E-01	-1.033
MCP-1	5.74E-04	-1.227
MCP-3	9.07E-01	-1.034
MDC (CCL22)	9.45E-01	1.003
MIP-1α	4.74E-01	1.038
MIP-1β	9.16E-01	-1.005
PDGF-AA	1.60E-01	1.162
PDGF-AB/BB	3.92E-01	1.057
RANTES	2.24E-02	1.204
sCD40L	9.87E-01	1.002
sIL-2Rα	2.34E-01	1.801
TGF-α	4.57E-01	-2.218
TNF-α	9.52E-01	-1.024
TNF-β	5.01E-01	-1.089
VEGF	8.31E-01	-1.044

[a] Based on two-tailed paired t-test.
[b] Fold change was calculated from averaged pre- and post-values, without accounting for subject pairs.

b) Histogram Representations

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IL-3
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8
6
4
2
0
Pre
Post
IL-4
pg/ml
250
200
150
100
50
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Post
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IL-9
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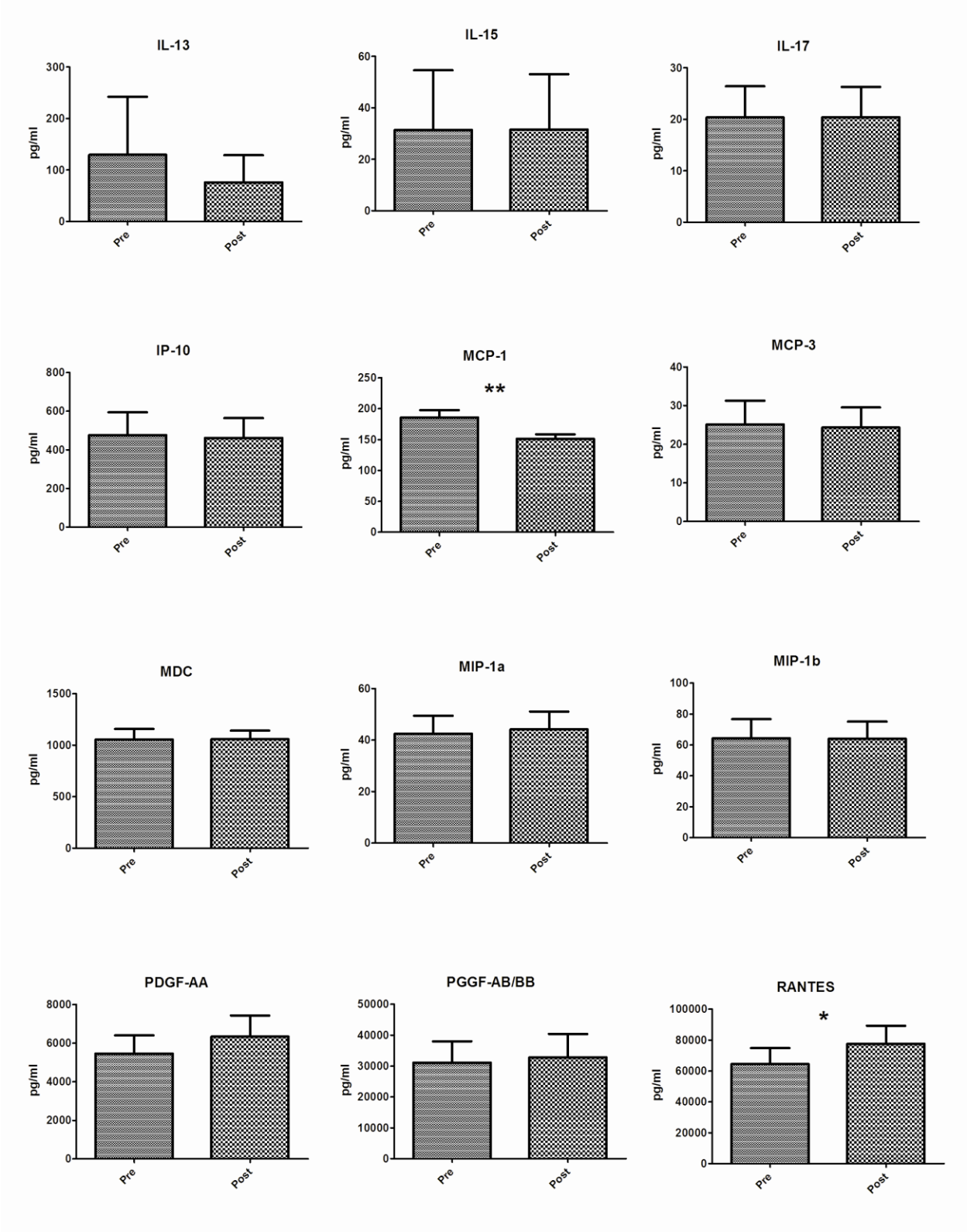
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Pre
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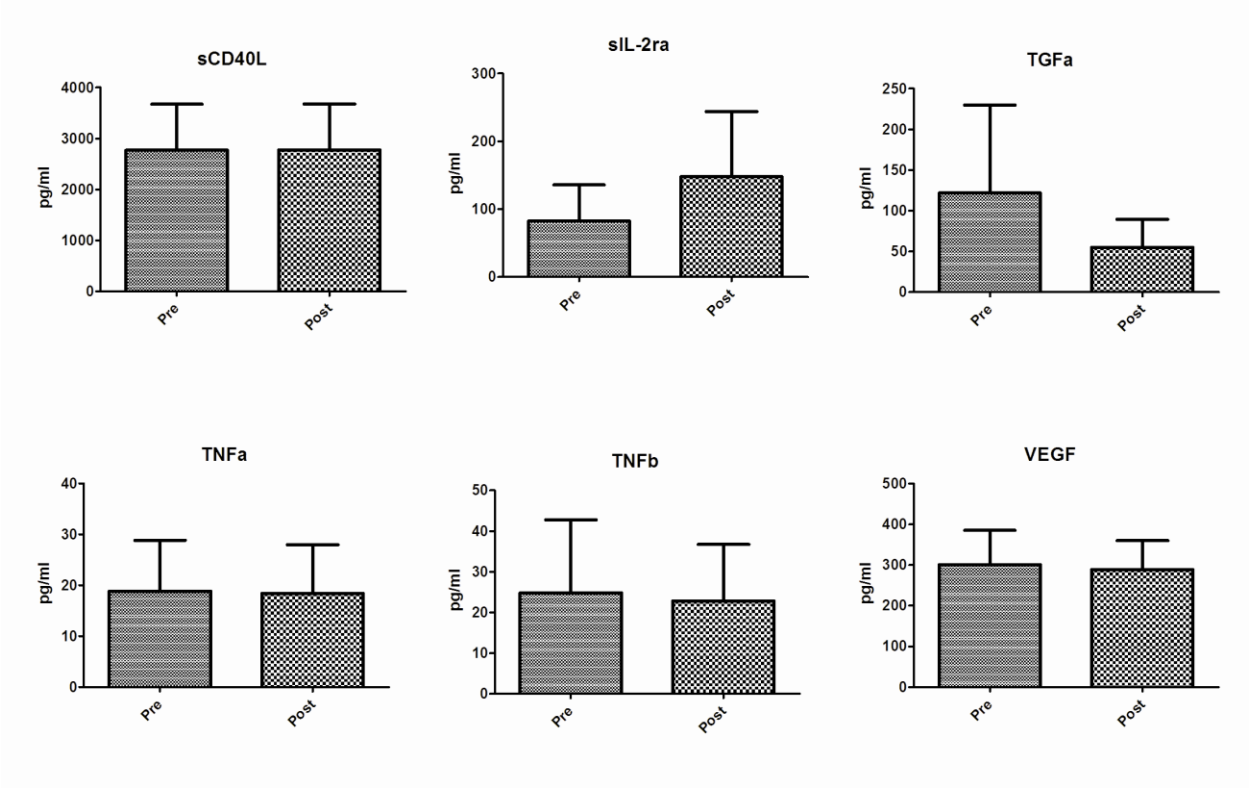
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sIL-2ra
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TGFa
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250
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50
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Pre
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TNFa
pg/ml
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TNFb
pg/ml
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VEGF
pg/ml
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100
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Pre
Post

Appendix 6 — Differentially Expressed Probe Sets Identified by Interaction Analysis (FDR≤0.30)

Probe Set ID	Gene Symbol	Gene Name	FDR
8065396	CST9L	Cystatin 9-like	0.00E+00
8168817	DRP2	Dystrophin related protein 2	0.00E+00
7937707	FAM99A	Family with sequence similarity 99, member A	0.00E+00
8075555	C22orf42	Chromosome 22 open reading frame 42	1.42E-01
7923907	IL10	Interleukin 10	1.42E-01
8152314	RSPO2	R-spondin 2 homolog (Xenopus laevis)	1.42E-01
8087547	MST1R	Macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	1.42E-01
7994074	SCNN1B	Sodium channel, nonvoltage-gated 1, beta	1.42E-01
7943521	DDI1	DNA-damage inducible 1 homolog 1 (S. Cerevisiae)	1.42E-01
8104163	LRRC14B	Leucine rich repeat containing 14B	1.42E-01
7986977	TJP1	Tight junction protein 1 (zona occludens 1)	1.42E-01
8066161	C20orf132	Chromosome 20 open reading frame 132	1.42E-01
8024676	GIPC3	GIPC PDZ domain containing family, member 3	1.42E-01
7977771	OR10G2	Olfactory receptor, family 10, subfamily G, member 2	1.42E-01
8038633	KLK1	Kallikrein 1	1.42E-01
7960529	SCNN1A	Sodium channel, nonvoltage-gated 1 alpha	1.42E-01
8145691	UBXN8	UBX domain protein 8	1.42E-01
8091515	GPR87	G protein-coupled receptor 87	1.42E-01
7917634	HFM1	HFM1, ATP-dependent DNA helicase homolog (S. Cerevisiae)	1.42E-01
8048749	KCNE4	Potassium voltage-gated channel, Isk-related family, member 4	1.42E-01
7912975	ALDH4A1	Aldehyde dehydrogenase 4 family, member A1	1.42E-01
8139640	DDC	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	1.42E-01
8166705	PRRG1	Proline rich Gla (G-carboxyglutamic acid) 1	1.42E-01
8124495	POM121L2	POM121 membrane glycoprotein-like 2	1.70E-01
8053872	ASTL	Astacin-like metallo-endopeptidase (M12 family)	1.70E-01
8071541	TMEM191A	Transmembrane protein 191A	1.70E-01
7970954	DCLK1	Doublecortin-like kinase 1	1.70E-01
8124129	NHLRC1	NHL repeat containing 1	1.70E-01
8118995	LHFPL5	Lipoma HMGIC fusion partner-like 5	1.70E-01
8173189	SPIN2B	Spindlin family, member 2B	1.70E-01
8113660	RPS25	Ribosomal protein S25	1.70E-01
7948630	FADS3	Fatty acid desaturase 3	1.70E-01
8094184	C1QTNF7	C1q and tumor necrosis factor related protein 7	1.70E-01
7995030	ZNF668	Zinc finger protein 668	1.70E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7929932	KAZALD1	Kazal-type serine peptidase inhibitor domain 1	1.70E-01
7985238	ANKRD34C	Ankyrin repeat domain 34C	1.70E-01
7938646	CALCB	Calcitonin-related polypeptide beta	1.70E-01
8007701	HIGD1B	HIG1 hypoxia inducible domain family, member 1B	1.73E-01
8162674	LOC286359	Hypothetical LOC286359	1.73E-01
8178291	OR11A1	Olfactory receptor, family 11, subfamily A, member 1	1.73E-01
8179591	OR11A1	Olfactory receptor, family 11, subfamily A, member 1	1.73E-01
8056151	PLA2R1	Phospholipase A2 receptor 1, 180kda	1.73E-01
8066542	SPINLW1	Serine peptidase inhibitor-like, with Kunitz and WAP domains 1 (eppin)	1.73E-01
8167758	GPR173	G protein-coupled receptor 173	1.81E-01
8060086	MYEOV2	Myeloma overexpressed 2	1.81E-01
7979529	KCNH5	Potassium voltage-gated channel, subfamily H (eag-related), member 5	1.81E-01
8139456	SNORA9	Small nucleolar RNA, H/ACA box 9	1.81E-01
7911233	OR2T8	Olfactory receptor, family 2, subfamily T, member 8	1.81E-01
8020411	SNRPD1	Small nuclear ribonucleoprotein D1 polypeptide 16kda	1.81E-01
8022856	NOL4	Nucleolar protein 4	1.81E-01
8045795	KCNJ3	Potassium inwardly-rectifying channel, subfamily J, member 3	1.81E-01
8061082	OTOR	Otoraplin	1.81E-01
8145570	ESCO2	Establishment of cohesion 1 homolog 2 (S. Cerevisiae)	1.81E-01
8071368	TMEM191A	Transmembrane protein 191A	1.81E-01
8131965	LOC441204	Hypothetical locus LOC441204	1.81E-01
8025452	MBD3L1	Methyl-cpg binding domain protein 3-like 1	1.81E-01
7906355	CD1E	CD1e molecule	1.81E-01
8160900	C9orf144	Transmembrane protein c9orf144b pseudogene	1.81E-01
8064976	DNAJC9	Dnaj (Hsp40) homolog, subfamily C, member 9	1.81E-01
8108912	SH3RF2	SH3 domain containing ring finger 2	1.96E-01
8088986	POU1F1	POU class 1 homeobox 1	1.96E-01
8096528	PDHA2	Pyruvate dehydrogenase (lipoamide) alpha 2	1.96E-01
8163678	ASTN2	Astrotactin 2	1.96E-01
8047829	CPO	Carboxypeptidase O	1.96E-01
7906163	RHBG	Rh family, B glycoprotein (gene/pseudogene)	1.96E-01
7919208	GNRHR2	Gonadotropin-releasing hormone (type 2) receptor 2	1.96E-01
8039593	ZNF667	Zinc finger protein 667	1.96E-01
8074856	PRAME	Preferentially expressed antigen in melanoma	1.96E-01
8125936	CLPS	Colipase, pancreatic	1.96E-01
7898002	PRAMEF22	PRAME family member 22	1.96E-01
8098214	TLL1	Tolloid-like 1	1.96E-01
7900340	BMP8A	Bone morphogenetic protein 8a	1.96E-01
8122127	TAAR9	Trace amine associated receptor 9 (gene/pseudogene)	1.96E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7927202	ZNF22	Zinc finger protein 22 (KOX 15)	1.96E-01
8098307	GALNTL6	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 6	1.96E-01
8071953	SGSM1	Small G protein signaling modulator 1	1.96E-01
8159850	VLDLR	Very low density lipoprotein receptor	1.96E-01
8007348	RAMP2	Receptor (G protein-coupled) activity modifying protein 2	1.96E-01
7955317	ACCN2	Amiloride-sensitive cation channel 2, neuronal	1.96E-01
8037283	PSG4	Pregnancy specific beta-1-glycoprotein 4	1.96E-01
7940108	OR6Q1	Olfactory receptor, family 6, subfamily Q, member 1	1.96E-01
8052940	PAIP2B	Poly(A) binding protein interacting protein 2B	1.96E-01
8149629	GFRA2	GDNF family receptor alpha 2	1.96E-01
8055239	CFC1	Cripto, FRL-1, cryptic family 1	1.96E-01
8150879	MOS	V-mos Moloney murine sarcoma viral oncogene homolog	1.96E-01
8038942	ZNF432	Zinc finger protein 432	1.96E-01
8068139	KRTAP15-1	Keratin associated protein 15-1	1.96E-01
7985233	RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	1.96E-01
8142452	TFEC	Transcription factor EC	1.96E-01
8036420	ZFP30	Zinc finger protein 30 homolog (mouse)	1.96E-01
8100007	PHOX2B	Paired-like homeobox 2b	1.96E-01
8039892	KIR2DS5	Killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5	1.96E-01
8024792	EBI3	Epstein-Barr virus induced 3	1.96E-01
8086908	PLXNB1	Plexin B1	2.01E-01
8013473	LOC339240	Keratin pseudogene	2.01E-01
7953181	PRMT8	Protein arginine methyltransferase 8	2.01E-01
8156393	SUSD3	Sushi domain containing 3	2.01E-01
8000028	DCUN1D3	DCN1, defective in cullin neddylation 1, domain containing 3 (S. Cerevisiae)	2.01E-01
8089082	DCBLD2	Discoidin, CUB and LCCL domain containing 2	2.01E-01
7969861	ITGBL1	Integrin, beta-like 1 (with EGF-like repeat domains)	2.01E-01
8174207	NXF3	Nuclear RNA export factor 3	2.01E-01
7976852	MIR377	Microrna 377	2.01E-01
7945660	FAM99A	Family with sequence similarity 99, member A	2.01E-01
8154563	ACER2	Alkaline ceramidase 2	2.01E-01
7909155	AVPR1B	Arginine vasopressin receptor 1B	2.01E-01
8076424	CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	2.01E-01
8153835	PPP1R16A	Protein phosphatase 1, regulatory (inhibitor) subunit 16A	2.01E-01
8024808	SHD	Src homology 2 domain containing transforming protein D	2.01E-01
7905490	LCE3C	Late cornified envelope 3C	2.01E-01
7907907	LOC100287948	Hypothetical LOC100287948	2.01E-01
8124645	OR12D3	Olfactory receptor, family 12, subfamily D, member 3	2.01E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8076113	LOC646851	Hypothetical LOC646851	2.01E-01
7980024	HEATR4	HEAT repeat containing 4	2.01E-01
8169115	NRK	Nik related kinase	2.01E-01
8174737	NKAP	NFKB activating protein	2.01E-01
8152062	SNX31	Sorting nexin 31	2.01E-01
8170704	ABCD1	ATP-binding cassette, sub-family D (ALD), member 1	2.01E-01
7976842	MIR382	Microrna 382	2.01E-01
8015884	PPY	Pancreatic polypeptide	2.01E-01
8028984	CYP2F1	Cytochrome P450, family 2, subfamily F, polypeptide 1	2.01E-01
7924842	C1orf35	Chromosome 1 open reading frame 35	2.01E-01
8135763	WNT16	Wingless-type MMTV integration site family, member 16	2.01E-01
8044804	DBI	Diazepam binding inhibitor (GABA receptor modulator, acyl-coa binding protein)	2.01E-01
7901048	SNORD46	Small nucleolar RNA, C/D box 46	2.01E-01
7901720	PRKAA2	Protein kinase, AMP-activated, alpha 2 catalytic subunit	2.01E-01
7987230	LPCAT4	Lysophosphatidylcholine acyltransferase 4	2.01E-01
7957140	LGR5	Leucine-rich repeat containing G protein-coupled receptor 5	2.16E-01
8027473	PDCD5	Programmed cell death 5	2.16E-01
8026568	C19orf44	Chromosome 19 open reading frame 44	2.16E-01
8010787	HEXDC	Hexosaminidase (glycosyl hydrolase family 20, catalytic domain) containing	2.16E-01
8097692	EDNRA	Endothelin receptor type A	2.16E-01
8068894	C21orf125	Chromosome 21 open reading frame 125	2.16E-01
8026496	LOC126536	Hypothetical LOC126536	2.16E-01
8108716	PCDHB16	Protocadherin beta 16	2.16E-01
7942328	FOLR3	Folate receptor 3 (gamma)	2.16E-01
8008914	C17orf64	Chromosome 17 open reading frame 64	2.16E-01
8146625	NKAIN3	Na+/K+ transporting atpase interacting 3	2.16E-01
7985253	C15orf37	Chromosome 15 open reading frame 37	2.16E-01
8178289	OR12D3	Olfactory receptor, family 12, subfamily D, member 3	2.16E-01
7896863	MIR429	Microrna 429	2.16E-01
8068496	SIM2	Single-minded homolog 2 (Drosophila)	2.16E-01
8021416	MIR122	Microrna 122	2.16E-01
7922229	SELE	Selectin E	2.16E-01
8070315	NCRNA00114	Non-protein coding RNA 114	2.16E-01
8022295	FAM38B	Family with sequence similarity 38, member B	2.16E-01
8175685	MAGEA5	Melanoma antigen family A, 5	2.16E-01
8180303	SAA2	Serum amyloid A2	2.16E-01
8033445	CD209	CD209 molecule	2.16E-01
7905483	LCE5A	Late cornified envelope 5A	2.16E-01
7985025	ODF3L1	Outer dense fiber of sperm tails 3-like 1	2.16E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8157632	MORN5	MORN repeat containing 5	2.16E-01
7942261	KRTAP5-9	Keratin associated protein 5-9	2.16E-01
8074880	RAB36	RAB36, member RAS oncogene family	2.16E-01
8003656	SERPINF2	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	2.16E-01
8086595	XCR1	Chemokine (C motif) receptor 1	2.16E-01
8046186	KLHL23	Kelch-like 23 (Drosophila)	2.16E-01
7924403	MIR194-1	Microrna 194-1	2.37E-01
7937483	SNORA52	Small nucleolar RNA, H/ACA box 52	2.37E-01
8113726	PPIC	Peptidylprolyl isomerase C (cyclophilin C)	2.37E-01
7948643	RAB3IL1	RAB3A interacting protein (rabin3)-like 1	2.37E-01
8037947	TPRX1	Tetra-peptide repeat homeobox 1	2.37E-01
7955993	OR6C75	Olfactory receptor, family 6, subfamily C, member 75	2.37E-01
8021461	GRP	Gastrin-releasing peptide	2.37E-01
8059868	ASB18	Ankyrin repeat and SOCS box containing 18	2.37E-01
8038505	SIGLEC11	Sialic acid binding Ig-like lectin 11	2.37E-01
8138799	TRIL	TLR4 interactor with leucine-rich repeats	2.37E-01
8095628	ALB	Albumin	2.37E-01
8036151	HSPB6	Heat shock protein, alpha-crystallin-related, B6	2.37E-01
8175808	TREX2	Three prime repair exonuclease 2	2.37E-01
7909422	MIR205	Microrna 205	2.37E-01
8067233	PMEPA1	Prostate transmembrane protein, androgen induced 1	2.37E-01
8180374	APOBEC3F	Apolipoprotein B mrna editing enzyme, catalytic polypeptide-like 3F	2.37E-01
7943749	LAYN	Layilin	2.37E-01
7958620	IFT81	Intraflagellar transport 81 homolog (Chlamydomonas)	2.37E-01
7946436	ASCL3	Achaete-scute complex homolog 3 (Drosophila)	2.37E-01
7939959	OR5AS1	Olfactory receptor, family 5, subfamily AS, member 1	2.37E-01
8144473	DEFB103B	Defensin, beta 103B	2.37E-01
8149172	DEFB103B	Defensin, beta 103B	2.37E-01
8082926	SOX14	SRY (sex determining region Y)-box 14	2.37E-01
8159211	FCN2	Ficolin (collagen/fibrinogen domain containing lectin) 2 (hucolin)	2.37E-01
8106722	ATP6AP1L	Atpase, H+ transporting, lysosomal accessory protein 1-like	2.37E-01
7905507	LCE2A	Late cornified envelope 2A	2.37E-01
8138930	RP9P	Retinitis pigmentosa 9 pseudogene	2.37E-01
8157090	TAL2	T-cell acute lymphocytic leukemia 2	2.37E-01
8014812	STAC2	SH3 and cysteine rich domain 2	2.37E-01
8116168	NHP2	NHP2 ribonucleoprotein homolog (yeast)	2.37E-01
8177130	NHEDC1	Na+/H+ exchanger domain containing 1	2.37E-01
8038695	KLK7	Kallikrein-related peptidase 7	2.37E-01
8166587	MAGEB10	Melanoma antigen family B, 10	2.37E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7997059	DDX19A	DEAD (Asp-Glu-Ala-As) box polypeptide 19A	2.37E-01
8173059	WNK3	WNK lysine deficient protein kinase 3	2.37E-01
8084832	PYDC2	Pyrin domain containing 2	2.37E-01
7902512	DNAJB4	Dnaj (Hsp40) homolog, subfamily B, member 4	2.37E-01
8002882	CHST6	Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6	2.37E-01
7952631	TP53AIP1	Tumor protein p53 regulated apoptosis inducing protein 1	2.37E-01
8152902	ADCY8	Adenylate cyclase 8 (brain)	2.37E-01
8173551	PHKA1	Phosphorylase kinase, alpha 1 (muscle)	2.47E-01
7991577	LOC440313	Hypothetical LOC440313	2.47E-01
8043040	FUNDC2P2	FUN14 domain containing 2 pseudogene 2	2.47E-01
7992409	RNF151	Ring finger protein 151	2.47E-01
7984862	CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	2.47E-01
8108900	HMHB1	Histocompatibility (minor) HB-1	2.47E-01
8108706	PCDHB17	Protocadherin beta 17 pseudogene	2.47E-01
8137707	MIR339	Microrna 339	2.47E-01
7989887	MEGF11	Multiple EGF-like-domains 11	2.47E-01
8096568	C4orf17	Chromosome 4 open reading frame 17	2.47E-01
8110821	SLC6A3	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	2.47E-01
8149955	PBK	PDZ binding kinase	2.47E-01
8138231	THSD7A	Thrombospondin, type I, domain containing 7A	2.47E-01
7993848	OTOA	Otoancorin	2.47E-01
7899562	PTPRU	Protein tyrosine phosphatase, receptor type, U	2.47E-01
8003939	TM4SF5	Transmembrane 4 L six family member 5	2.47E-01
8130837	FRMD1	FERM domain containing 1	2.47E-01
8126891	CRISP2	Cysteine-rich secretory protein 2	2.47E-01
7928126	KIAA1274	Kiaa1274	2.47E-01
8142663	NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kda	2.47E-01
8100768	UGT2B10	UDP glucuronosyltransferase 2 family, polypeptide B10	2.47E-01
7988350	DUOX2	Dual oxidase 2	2.47E-01
8004394	SPEM1	Spermatid maturation 1	2.47E-01
8016433	HOXB1	Homeobox B1	2.47E-01
7947599	CHST1	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	2.47E-01
8068157	KRTAP20-4	Keratin associated protein 20-4	2.47E-01
8144880	SH2D4A	SH2 domain containing 4A	2.47E-01
8109752	ODZ2	Odz, odd Oz/ten-m homolog 2 (Drosophila)	2.47E-01
8092520	C3orf70	Chromosome 3 open reading frame 70	2.47E-01
8019177	TMEM105	Transmembrane protein 105	2.47E-01
8150244	GOT1L1	Glutamic-oxaloacetic transaminase 1-like 1	2.47E-01
8111203	LOC285696	Hypothetical LOC285696	2.47E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7965206	SLC6A15	Solute carrier family 6 (neutral amino acid transporter), member 15	2.47E-01
8030299	CCDC155	Coiled-coil domain containing 155	2.47E-01
8026388	OR7C2	Olfactory receptor, family 7, subfamily C, member 2	2.47E-01
7961798	SOX5	SRY (sex determining region Y)-box 5	2.47E-01
8005586	RNF112	Ring finger protein 112	2.47E-01
7973510	CPNE6	Copine VI (neuronal)	2.47E-01
8160360	IFNB1	Interferon, beta 1, fibroblast	2.47E-01
8069146	KRTAP10-7	Keratin associated protein 10-7	2.47E-01
7930208	INA	Internexin neuronal intermediate filament protein, alpha	2.47E-01
8084838	HRASLS	HRAS-like suppressor	2.47E-01
8005446	LOC339240	Keratin pseudogene	2.47E-01
8165508	NRARP	NOTCH-regulated ankyrin repeat protein	2.47E-01
7911529	MXRA8	Matrix-remodelling associated 8	2.47E-01
7977478	OR4K13	Olfactory receptor, family 4, subfamily K, member 13	2.47E-01
7948129	OR5R1	Olfactory receptor, family 5, subfamily R, member 1	2.47E-01
8039389	PTPRH	Protein tyrosine phosphatase, receptor type, H	2.47E-01
8161482	ANKRD30BL	Ankyrin repeat domain 30B-like	2.47E-01
8027222	CILP2	Cartilage intermediate layer protein 2	2.47E-01
8175666	GABRE	Gamma-aminobutyric acid (GABA) A receptor, epsilon	2.47E-01
8150253	STAR	Steroidogenic acute regulatory protein	2.47E-01
8147516	MATN2	Matrilin 2	2.47E-01
8045198	CFC1B	Cripto, FRL-1, cryptic family 1B	2.47E-01
8061272	C20orf26	Chromosome 20 open reading frame 26	2.47E-01
7957503	C12orf37	Chromosome 12 open reading frame 37	2.69E-01
8011339	OR1E1	Olfactory receptor, family 1, subfamily E, member 1	2.69E-01
7942991	TYR	Tyrosinase (oculocutaneous albinism IA)	2.69E-01
8046824	FSIP2	Fibrous sheath interacting protein 2	2.69E-01
7917942	FLJ35409	FLJ35409 protein	2.69E-01
8087252	MIR191	Microrna 191	2.69E-01
8044212	SULT1C2	Sulfotransferase family, cytosolic, 1C, member 2	2.69E-01
8022986	SYT4	Synaptotagmin IV	2.69E-01
8034390	ZNF799	Zinc finger protein 799	2.69E-01
8110382	PRR7	Proline rich 7 (synaptic)	2.69E-01
8160392	IFNA16	Interferon, alpha 16	2.69E-01
8070757	TSPEAR	Thrombospondin-type laminin G domain and EAR repeats	2.69E-01
7986426	DNM1P46	DNM1 pseudogene 46	2.69E-01
8127646	FILIP1	Filamin A interacting protein 1	2.69E-01
8013035	ZNF624	Zinc finger protein 624	2.69E-01
8015115	KRT12	Keratin 12	2.69E-01
7905533	IVL	Involucrin	2.69E-01
7933194	CXCL12	Chemokine (C-X-C motif) ligand 12	2.69E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7912520	NPPB	Natriuretic peptide B	2.69E-01
7926708	THNSL1	Threonine synthase-like 1 (S. Cerevisiae)	2.69E-01
8018754	CYGB	Cytoglobin	2.69E-01
8062796	GDAP1L1	Ganglioside-induced differentiation-associated protein 1-like 1	2.69E-01
8058542	C2orf80	Chromosome 2 open reading frame 80	2.69E-01
7987554	DNAJC17	Dnaj (Hsp40) homolog, subfamily C, member 17	2.69E-01
7902353	LHX8	LIM homeobox 8	2.69E-01
8006865	PPP1R1B	Protein phosphatase 1, regulatory (inhibitor) subunit 1B	2.69E-01
7947274	MPPED2	Metallophosphoesterase domain containing 2	2.69E-01
8067662	PTK6	PTK6 protein tyrosine kinase 6	2.69E-01
7998427	TPSG1	Tryptase gamma 1	2.69E-01
7976681	CYP46A1	Cytochrome P450, family 46, subfamily A, polypeptide 1	2.69E-01
8180251	FLJ45508	Hypothetical protein LOC643721	2.69E-01
8045229	ARHGEF4	Rho guanine nucleotide exchange factor (GEF) 4	2.69E-01
7917875	F3	Coagulation factor III (thromboplastin, tissue factor)	2.69E-01
7901969	ROR1	Receptor tyrosine kinase-like orphan receptor 1	2.69E-01
8118963	FANCE	Fanconi anemia, complementation group E	2.69E-01
8123259	PLG	Plasminogen	2.69E-01
8090388	C3orf22	Chromosome 3 open reading frame 22	2.69E-01
8015242	KRTAP4-2	Keratin associated protein 4-2	2.69E-01
7961249	TAS2R10	Taste receptor, type 2, member 10	2.69E-01
8070789	KRTAP12-4	Keratin associated protein 12-4	2.69E-01
7901187	C1orf190	Chromosome 1 open reading frame 190	2.69E-01
7993713	IQCK	IQ motif containing K	2.69E-01
7905283	ANXA9	Annexin A9	2.69E-01
8017867	FAM20A	Family with sequence similarity 20, member A	2.69E-01
8164396	MIR199B	Microrna 199b	2.69E-01
7903358	VCAM1	Vascular cell adhesion molecule 1	2.69E-01
8095728	EREG	Epiregulin	2.69E-01
8025169	C19orf45	Chromosome 19 open reading frame 45	2.69E-01
8131705	RPL23P8	Ribosomal protein L23 pseudogene 8	2.69E-01
8091546	TMEM14E	Transmembrane protein 14E	2.69E-01
7906900	DDR2	Discoidin domain receptor tyrosine kinase 2	2.69E-01
8177120	LOC100132288	Hypothetical protein LOC100132288	2.69E-01
8162006	GKAP1	G kinase anchoring protein 1	2.69E-01
7934178	PCBD1	Pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha	2.69E-01
8143708	ZNF425	Zinc finger protein 425	2.69E-01
8111821	HEATR7B2	HEAT repeat family member 7B2	2.69E-01
8130993	FAM20C	Family with sequence similarity 20, member C	2.69E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8088550	PRICKLE2	Prickle homolog 2 (Drosophila)	2.69E-01
7941917	CABP4	Calcium binding protein 4	2.69E-01
7963513	KRT76	Keratin 76	2.69E-01
8074153	ACR	Acrosin	2.69E-01
8098368	ADAM29	ADAM metallopeptidase domain 29	2.69E-01
8099302	MIR95	Microrna 95	2.69E-01
8102468	PRSS12	Protease, serine, 12 (neurotrypsin, motopsin)	2.69E-01
8031253	LILRP2	Leukocyte immunoglobulin-like receptor pseudogene 2	2.69E-01
8060461	TMC2	Transmembrane channel-like 2	2.69E-01
7931097	HTRA1	Htra serine peptidase 1	2.69E-01
8015293	KRT32	Keratin 32	2.69E-01
8175572	SPANXN3	SPANX family, member N3	2.69E-01
7906197	HAPLN2	Hyaluronan and proteoglycan link protein 2	2.69E-01
8075785	FOXRED2	FAD-dependent oxidoreductase domain containing 2	2.69E-01
7929388	PLCE1	Phospholipase C, epsilon 1	2.69E-01
7944351	FOXR1	Forkhead box R1	2.69E-01
8000346	ERN2	Endoplasmic reticulum to nucleus signaling 2	2.69E-01
7903884	PROK1	Prokineticin 1	2.69E-01
7928766	C10orf99	Chromosome 10 open reading frame 99	2.69E-01
8136863	TMEM139	Transmembrane protein 139	2.69E-01
8039577	ZSCAN5A	Zinc finger and SCAN domain containing 5A	2.69E-01
8037240	PSG1	Pregnancy specific beta-1-glycoprotein 1	2.69E-01
8173862	SATL1	Spermidine/spermine N1-acetyl transferase-like 1	2.69E-01
8179926	DOM3Z	Dom-3 homolog Z (C. Elegans)	2.69E-01
8001163	MYLK3	Myosin light chain kinase 3	2.69E-01
8137485	DPP6	Dipeptidyl-peptidase 6	2.69E-01
8155747	C9orf135	Chromosome 9 open reading frame 135	2.69E-01
7997374	DYNLRB2	Dynein, light chain, roadblock-type 2	2.69E-01
7964535	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1	2.69E-01
7944302	PHLDB1	Pleckstrin homology-like domain, family B, member 1	2.69E-01
8083576	LEKR1	Leucine, glutamate and lysine rich 1	2.69E-01
7960283	CACNA2D4	Calcium channel, voltage-dependent, alpha 2/delta subunit 4	2.69E-01
7947096	MRGPRX2	MAS-related GPR, member X2	2.69E-01
8092661	MASP1	Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	2.69E-01
8038314	C19orf73	Chromosome 19 open reading frame 73	2.69E-01
7964142	APOF	Apolipoprotein F	2.69E-01
7987310	GJD2	Gap junction protein, delta 2, 36kda	2.69E-01
8015221	KRTAP4-11	Keratin associated protein 4-11	2.69E-01
8053551	REEP1	Receptor accessory protein 1	2.69E-01
8166619	MAGEB1	Melanoma antigen family B, 1	2.69E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8117537	HIST1H4I	Histone cluster 1, h4i	2.69E-01
8091491	CLRN1	Clarin 1	2.69E-01
7905503	LCE2C	Late cornified envelope 2C	2.69E-01
8062490	SNORA60	Small nucleolar RNA, H/ACA box 60	2.69E-01
8160417	IFNA6	Interferon, alpha 6	2.69E-01
7934074	TACR2	Tachykinin receptor 2	2.69E-01
8039779	SLC27A5	Solute carrier family 27 (fatty acid transporter), member 5	2.69E-01
7993815	ANKS4B	Ankyrin repeat and sterile alpha motif domain containing 4B	2.69E-01
8091972	MECOM	MDS1 and EVI1 complex locus	2.69E-01
8015366	KRT14	Keratin 14	2.69E-01
8169836	XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	2.69E-01
8160024	GLDC	Glycine dehydrogenase (decarboxylating)	2.69E-01
7995242	KIAA0664L3	KIAA0664-like 3	2.69E-01
8015868	MPP2	Membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)	2.69E-01
7927723	C10orf107	Chromosome 10 open reading frame 107	2.69E-01
8026442	CYP4F8	Cytochrome P450, family 4, subfamily F, polypeptide 8	2.69E-01
8078435	TRIM71	Tripartite motif containing 71	2.69E-01
8160546	LINGO2	Leucine rich repeat and Ig domain containing 2	2.69E-01
7965231	MGAT4C	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme C (putative)	2.69E-01
7972231	SLITRK1	SLIT and NTRK-like family, member 1	2.69E-01
7973022	OR4L1	Olfactory receptor, family 4, subfamily L, member 1	2.69E-01
7931140	FLJ46361	Deleted in malignant brain tumors 1 pseudogene	2.69E-01
8132715	C7orf57	Chromosome 7 open reading frame 57	2.69E-01
8020762	DSG3	Desmoglein 3	2.69E-01
8090955	A4GNT	Alpha-1,4-N-acetylglucosaminyltransferase	2.69E-01
7996819	CDH3	Cadherin 3, type 1, P-cadherin (placental)	2.69E-01
7943787	HSPB2	Heat shock 27kda protein 2	2.69E-01
8100808	SULT1E1	Sulfotransferase family 1E, estrogen-preferring, member 1	2.69E-01
8109651	GABRA6	Gamma-aminobutyric acid (GABA) A receptor, alpha 6	2.69E-01
8011214	RTN4RL1	Reticulon 4 receptor-like 1	2.69E-01
8163618	TNFSF15	Tumor necrosis factor (ligand) superfamily, member 15	2.69E-01
8088315	DNAH12	Dynein, axonemal, heavy chain 12	2.69E-01
8064739	C20orf27	Chromosome 20 open reading frame 27	2.69E-01
8050844	LOC100131510	Hypothetical LOC100131510	2.69E-01
7982663	BUB1B	Budding uninhibited by benzimidazoles 1 homolog beta (yeast)	2.69E-01
7927529	MSMB	Microseminoprotein, beta	2.69E-01
7902754	CLCA3P	Chloride channel accessory 3 (pseudogene)	2.94E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7913990	GPATCH3	G patch domain containing 3	2.94E-01
8003844	GSG2	Germ cell associated 2 (haspin)	2.94E-01
7909503	SERTAD4	SERTA domain containing 4	2.94E-01
7937882	ART1	ADP-ribosyltransferase 1	2.94E-01
8109908	LOC257358	Hypothetical LOC257358	2.94E-01
7899615	SERINC2	Serine incorporator 2	2.94E-01
7922420	SERPINC1	Serpin peptidase inhibitor, clade C (antithrombin), member 1	2.94E-01
8066536	WFDC6	WAP four-disulfide core domain 6	2.94E-01
7920141	TCHH	Trichohyalin	2.94E-01
8154245	PDCD1LG2	Programmed cell death 1 ligand 2	2.94E-01
7965573	NTN4	Netrin 4	2.94E-01
8091991	MECOM	MDS1 and EVI1 complex locus	2.94E-01
8053139	C2orf81	Chromosome 2 open reading frame 81	2.94E-01
7937404	C11orf35	Chromosome 11 open reading frame 35	2.94E-01
7986822	GABRB3	Gamma-aminobutyric acid (GABA) A receptor, beta 3	2.94E-01
7931561	ZNF511	Zinc finger protein 511	2.94E-01
8107350	SRP19	Signal recognition particle 19kda	2.94E-01
7989985	ITGA11	Integrin, alpha 11	2.94E-01
8001387	SALL1	Sal-like 1 (Drosophila)	2.94E-01
8037970	PLA2G4C	Phospholipase A2, group IVC (cytosolic, calcium-independent)	2.94E-01
8161255	SHB	Src homology 2 domain containing adaptor protein B	2.94E-01
8153664	BOP1	Block of proliferation 1	2.94E-01
7978093	JPH4	Junctophilin 4	2.94E-01
8147721	FLJ45248	FLJ45248 protein	2.94E-01
7966229	MGC14436	Hypothetical LOC84983	2.94E-01
8135865	FSCN3	Fascin homolog 3, actin-bundling protein, testicular (Strongylocentrotus purpuratus)	2.94E-01
8074845	ZNF280B	Zinc finger protein 280B	2.94E-01
8126240	FLJ41649	Hypothetical LOC401260	2.94E-01
7975562	PAPLN	Papilin, proteoglycan-like sulfated glycoprotein	2.94E-01
8090938	DZIP1L	DAZ interacting protein 1-like	2.94E-01
7976571	C14orf129	Chromosome 14 open reading frame 129	2.94E-01
8118644	RPS18	Ribosomal protein S18	2.94E-01
8178253	RPS18	Ribosomal protein S18	2.94E-01
8179544	RPS18	Ribosomal protein S18	2.94E-01
8097938	NPY2R	Neuropeptide Y receptor Y2	2.94E-01
8071049	LOC51152	Melanoma antigen	2.94E-01
7951545	EXPH5	Exophilin 5	2.94E-01
8109333	GPX3	Glutathione peroxidase 3 (plasma)	2.94E-01
8127754	FLJ13744	Hypothetical FLJ13744	2.94E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8066489	WFDC12	WAP four-disulfide core domain 12	2.94E-01
8103005	ANAPC10	Anaphase promoting complex subunit 10	2.94E-01
8154449	CNTLN	Centlein, centrosomal protein	2.94E-01
8141169	MGC72080	MGC72080 pseudogene	2.94E-01
8160597	TAF1L	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 210kda-like	2.94E-01
8059955	RAB17	RAB17, member RAS oncogene family	2.94E-01
7996034	CCL17	Chemokine (C-C motif) ligand 17	2.94E-01
8086400	LYZL4	Lysozyme-like 4	2.94E-01
7978021	MYH7	Myosin, heavy chain 7, cardiac muscle, beta	2.94E-01
8069744	RWDD2B	RWD domain containing 2B	2.94E-01
8126486	CUL7	Cullin 7	2.94E-01
8170786	LCA10	Lung carcinoma-associated protein 10	2.94E-01
7933446	FRMPD2	FERM and PDZ domain containing 2	2.94E-01
8102321	PLA2G12A	Phospholipase A2, group XIIA	2.94E-01
8101934	DNAJB14	Dnaj (Hsp40) homolog, subfamily B, member 14	2.94E-01
8117334	HIST1H4A	Histone cluster 1, h4a	2.94E-01
7972428	OXGR1	Oxoglutarate (alpha-ketoglutarate) receptor 1	2.94E-01
7957790	FAM71C	Family with sequence similarity 71, member C	2.94E-01
8096070	BMP3	Bone morphogenetic protein 3	2.94E-01
8044204	SULT1C3	Sulfotransferase family, cytosolic, 1C, member 3	2.94E-01
7925741	OR2T33	Olfactory receptor, family 2, subfamily T, member 33	2.94E-01
8043782	CNGA3	Cyclic nucleotide gated channel alpha 3	2.94E-01
8115840	NKX2-5	NK2 transcription factor related, locus 5 (Drosophila)	2.94E-01
8083887	CLDN11	Claudin 11	2.94E-01
7977105	TRMT61A	Trna methyltransferase 61 homolog A (S. Cerevisiae)	2.94E-01
7949588	CD248	CD248 molecule, endosialin	2.94E-01
8084397	ECE2	Endothelin converting enzyme 2	2.94E-01
7969300	PRR20A	Proline rich 20A	2.94E-01
7969306	PRR20A	Proline rich 20A	2.94E-01
7969312	PRR20A	Proline rich 20A	2.94E-01
7969318	PRR20A	Proline rich 20A	2.94E-01
7969324	PRR20A	Proline rich 20A	2.94E-01
7896921	TAS1R3	Taste receptor, type 1, member 3	2.94E-01
7977273	ADSSL1	Adenylosuccinate synthase like 1	2.94E-01
8043682	FAHD2B	Fumarylacetoacetate hydrolase domain containing 2B	2.94E-01
8067985	NCAM2	Neural cell adhesion molecule 2	2.94E-01
8072328	SEC14L2	SEC14-like 2 (S. Cerevisiae)	2.94E-01
7929901	C10orf2	Chromosome 10 open reading frame 2	2.94E-01
8046048	CSRNP3	Cysteine-serine-rich nuclear protein 3	2.94E-01
8062944	SEMG2	Semenogelin II	2.94E-01
8011990	TEKT1	Tektin 1	2.94E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8136961	OR2F2	Olfactory receptor, family 2, subfamily F, member 2	2.94E-01
7980485	DIO2	Deiodinase, iodothyronine, type II	2.94E-01
7959921	LOC338797	Hypothetical LOC338797	2.94E-01
8056710	C2orf77	Chromosome 2 open reading frame 77	2.94E-01
8061799	BPIL3	Bactericidal/permeability-increasing protein-like 3	2.94E-01
8154848	PRSS3	Protease, serine, 3	2.94E-01
8175933	RENBP	Renin binding protein	2.94E-01
8096411	TIGD2	Tigger transposable element derived 2	2.94E-01
7979351	C14orf33	Chromosome 14 open reading frame 33	2.94E-01
7904761	ITGA10	Integrin, alpha 10	2.94E-01
8071206	MRPL40	Mitochondrial ribosomal protein L40	2.94E-01
8157696	OR5C1	Olfactory receptor, family 5, subfamily C, member 1	2.94E-01
7934959	MIR107	Microrna 107	2.94E-01
7924987	AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	2.94E-01
8105596	RGS7BP	Regulator of G-protein signaling 7 binding protein	2.94E-01
8015554	KCNH4	Potassium voltage-gated channel, subfamily H (eag-related), member 4	2.94E-01
8179011	MOG	Myelin oligodendrocyte glycoprotein	2.94E-01
8018114	SDK2	Sidekick homolog 2 (chicken)	2.94E-01
8055872	CACNB4	Calcium channel, voltage-dependent, beta 4 subunit	2.94E-01
8151423	JPH1	Junctophilin 1	2.94E-01
7990309	STRA6	Stimulated by retinoic acid gene 6 homolog (mouse)	2.94E-01
8125048	DDAH2	Dimethylarginine dimethylaminohydrolase 2	2.94E-01
7939971	OR5J2	Olfactory receptor, family 5, subfamily J, member 2	2.94E-01
8070563	C21orf128	Chromosome 21 open reading frame 128	2.94E-01
8068231	OLIG2	Oligodendrocyte lineage transcription factor 2	2.94E-01
8049825	HDLBP	High density lipoprotein binding protein	2.94E-01
7987145	FMN1	Formin 1	2.94E-01
8166899	NYX	Nyctalopin	2.94E-01
8026587	NWD1	NACHT and WD repeat domain containing 1	2.94E-01
8135031	MUC12	Mucin 12, cell surface associated	2.94E-01
8154491	ADAMTSL1	ADAMTS-like 1	2.94E-01
8148966	RPL23AP53	Ribosomal protein L23a pseudogene 53	2.94E-01
8040419	MYCN	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	2.94E-01
7987511	C15orf52	Chromosome 15 open reading frame 52	2.94E-01
8100664	TMPRSS11D	Transmembrane protease, serine 11D	2.94E-01
7930320	CCDC147	Coiled-coil domain containing 147	2.94E-01

Appendix 7 — Lymphocyte-Correlated Probe Sets Significantly Lower-Expressed in DR than ER, Post-Allergen Inhalation Challenge (FDR≤0.30)

Probe Set ID	Gene Symbol	Gene Name	FDR
7937915	RRM1	Ribonucleotide reductase M1	9.86E-02
7982712	C15orf23	Chromosome 15 open reading frame 23	9.86E-02
8049297	SCARNA5	Small Cajal body-specific RNA 5	9.86E-02
8124521	HIST1H4K	Histone cluster 1, h4k	9.86E-02
8124531	HIST1H3I	Histone cluster 1, h3i	9.86E-02
8150962	TOX	Thymocyte selection-associated high mobility group box	9.86E-02
8180255	HIST2H4B	Histone cluster 2, h4b	9.86E-02
8180321	HIST2H4A	Histone cluster 2, h4a	9.86E-02
7909601	SNORA16B	Small nucleolar RNA, H/ACA box 16B	1.19E-01
7919589	HIST2H3D	Histone cluster 2, h3d	1.19E-01
7919606	HIST2H2BF	Histone cluster 2, h2bf	1.19E-01
7938348	WEE1	WEE1 homolog (S. Pombe)	1.19E-01
7952914	CCDC77	Coiled-coil domain containing 77	1.19E-01
7984263	PTPLAD1	Protein tyrosine phosphatase-like A domain containing 1	1.19E-01
8009417	KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	1.19E-01
8019737	KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	1.19E-01
8060813	MCM8	Minichromosome maintenance complex component 8	1.19E-01
8117598	HIST1H4J	Histone cluster 1, h4j	1.19E-01
7919612	HIST2H3D	Histone cluster 2, h3d	1.20E-01
8055426	MCM6	Minichromosome maintenance complex component 6	1.20E-01
7920244	S100A8	S100 calcium binding protein A8	1.39E-01
8047702	ICOS	Inducible T-cell co-stimulator	1.39E-01
8060503	SNORD57	Small nucleolar RNA, C/D box 57	1.39E-01
8105828	CCNB1	Cyclin B1	1.39E-01
8106006	SMN1	Survival of motor neuron 1, telomeric	1.39E-01
8124416	HIST1H3D	Histone cluster 1, h3d	1.39E-01
7913869	STMN1	Stathmin 1	1.41E-01
7919642	HIST2H2AB	Histone cluster 2, h2ab	1.41E-01
7967386	MPHOSPH9	M-phase phosphoprotein 9	1.41E-01
7979307	DLGAP5	Discs, large (Drosophila) homolog-associated protein 5	1.41E-01
7985829	FANCI	Fanconi anemia, complementation group I	1.41E-01
8049299	SCARNA6	Small Cajal body-specific RNA 6	1.41E-01
8053584	CD8A	CD8a molecule	1.41E-01
8068898	HIST1H2BK	Histone cluster 1, h2bk	1.41E-01
8083272	GYG1	Glycogenin 1	1.41E-01
8124397	HIST1H1C	Histone cluster 1, h1c	1.41E-01
8135576	TES	Testis derived transcript (3 LIM domains)	1.41E-01
7902398	SNORD45A	Small nucleolar RNA, C/D box 45A	1.74E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7952339	SNORD14C	Small nucleolar RNA, C/D box 14C	1.74E-01
8053366	SUCLG1	Succinate-coa ligase, alpha subunit	1.74E-01
8095139	SRD5A3	Steroid 5 alpha-reductase 3	1.74E-01
8124534	HIST1H4L	Histone cluster 1, h4l	1.74E-01
7905163	MRPS21	Mitochondrial ribosomal protein S21	2.01E-01
7926259	MCM10	Minichromosome maintenance complex component 10	2.01E-01
7947694	CKAP5	Cytoskeleton associated protein 5	2.01E-01
8066074	DSN1	DSN1, MIND kinetochore complex component, homolog (S. Cerevisiae)	2.01E-01
8147396	INTS8	Integrator complex subunit 8	2.01E-01
8160151	ZDHHC21	Zinc finger, DHHC-type containing 21	2.01E-01
7904465	HIST2H2BA	Histone cluster 2, h2ba	2.32E-01
7904853	GPR89A	G protein-coupled receptor 89A	2.32E-01
7905079	HIST2H2AA3	Histone cluster 2, h2aa3	2.32E-01
7905085	HIST2H3A	Histone cluster 2, h3a	2.32E-01
7906386	PYHIN1	Pyrin and HIN domain family, member 1	2.32E-01
7909568	DTL	Denticleless homolog (Drosophila)	2.32E-01
7910997	EXO1	Exonuclease 1	2.32E-01
7919243	CD160	CD160 molecule	2.32E-01
7919584	HIST2H2BF	Histone cluster 2, h2bf	2.32E-01
7919614	HIST2H3A	Histone cluster 2, h3a	2.32E-01
7919619	HIST2H2AA3	Histone cluster 2, h2aa3	2.32E-01
7921434	AIM2	Absent in melanoma 2	2.32E-01
7921900	SH2D1B	SH2 domain containing 1B	2.32E-01
7922174	F5	Coagulation factor V (proaccelerin, labile factor)	2.32E-01
7929438	HELLS	Helicase, lymphoid-specific	2.32E-01
7934026	DNA2	DNA replication helicase 2 homolog (yeast)	2.32E-01
7937020	MKI67	Antigen identified by monoclonal antibody Ki-67	2.32E-01
7948902	SNORD29	Small nucleolar RNA, C/D box 29	2.32E-01
7948904	SNORD28	Small nucleolar RNA, C/D box 28	2.32E-01
7953351	NCAPD2	Non-SMC condensin I complex, subunit D2	2.32E-01
7960728	SCARNA12	Small Cajal body-specific RNA 12	2.32E-01
7961198	KLRAP1	Killer cell lectin-like receptor subfamily A pseudogene 1	2.32E-01
7969243	CKAP2	Cytoskeleton associated protein 2	2.32E-01
7981181	SCARNA13	Small Cajal body-specific RNA 13	2.32E-01
7982333	LOC100499221	Hypothetical LOC100499221	2.32E-01
7982358	ARHGAP11A	Rho gtpase activating protein 11A	2.32E-01
7985480	SCARNA15	Small Cajal body-specific RNA 15	2.32E-01
7986068	BLM	Bloom syndrome, recq helicase-like	2.32E-01
7994109	PLK1	Polo-like kinase 1	2.32E-01
8005547	SNORD3A	Small nucleolar RNA, C/D box 3A	2.32E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8005553	SNORD3A	Small nucleolar RNA, C/D box 3A	2.32E-01
8013323	SNORD3A	Small nucleolar RNA, C/D box 3A	2.32E-01
8013325	SNORD3A	Small nucleolar RNA, C/D box 3A	2.32E-01
8013329	SNORD3A	Small nucleolar RNA, C/D box 3A	2.32E-01
8018849	TK1	Thymidine kinase 1, soluble	2.32E-01
8019842	TYMS	Thymidylate synthetase	2.32E-01
8022640	DHFR	Dihydrofolate reductase	2.32E-01
8033667	ZNF558	Zinc finger protein 558	2.32E-01
8040142	CPSF3	Cleavage and polyadenylation specific factor 3, 73kda	2.32E-01
8042211	B3GNT2	UDP-glcnac:betagal beta-1,3-N-acetylglucosaminyltransferase 2	2.32E-01
8043036	LOC1720	Dihydrofolate reductase pseudogene	2.32E-01
8054580	BUB1	Budding uninhibited by benzimidazoles 1 homolog (yeast)	2.32E-01
8058052	HSPD1	Heat shock 60kda protein 1 (chaperonin)	2.32E-01
8064844	PCNA	Proliferating cell nuclear antigen	2.32E-01
8074925	LOC91316	Glucuronidase, beta/immunoglobulin lambda-like polypeptide 1 pseudogene	2.32E-01
8093053	TFRC	Transferrin receptor (p90, CD71)	2.32E-01
8094240	CD38	CD38 molecule	2.32E-01
8095986	ANXA3	Annexin A3	2.32E-01
8102643	CCNA2	Cyclin A2	2.32E-01
8105949	SERF1A	Small EDRK-rich factor 1A (telomeric)	2.32E-01
8105958	SMN1	Survival of motor neuron 1, telomeric	2.32E-01
8105995	SMA5	Glucuronidase, beta pseudogene	2.32E-01
8105997	SERF1A	Small EDRK-rich factor 1A (telomeric)	2.32E-01
8107706	LMNB1	Lamin B1	2.32E-01
8109639	PTTG1	Pituitary tumor-transforming 1	2.32E-01
8111892	OXCT1	3-oxoacid coa transferase 1	2.32E-01
8117395	HIST1H2BF	Histone cluster 1, h2bf	2.32E-01
8117408	HIST1H2AE	Histone cluster 1, h2ae	2.32E-01
8117422	HIST1H4F	Histone cluster 1, h4f	2.32E-01
8117426	HIST1H2BH	Histone cluster 1, h2bh	2.32E-01
8124385	HIST1H4B	Histone cluster 1, h4b	2.32E-01
8124527	HIST1H1B	Histone cluster 1, h1b	2.32E-01
8127364	GUSBP4	Glucuronidase, beta pseudogene 4	2.32E-01
8144228	FLJ36840	Hypothetical LOC645524	2.32E-01
8146357	MCM4	Minichromosome maintenance complex component 4	2.32E-01
8151561	ZFAND1	Zinc finger, AN1-type domain 1	2.32E-01
8160238	PSIP1	PC4 and SFRS1 interacting protein 1	2.32E-01
8168470	COX7B	Cytochrome c oxidase subunit viib	2.32E-01
8177647	SMN1	Survival of motor neuron 1, telomeric	2.32E-01
8177658	SERF1A	Small EDRK-rich factor 1A (telomeric)	2.32E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7899134	CCDC21	Coiled-coil domain containing 21	2.41E-01
7906613	SLAMF7	SLAM family member 7	2.41E-01
7918657	PTPN22	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	2.41E-01
7921625	SLAMF6	SLAM family member 6	2.41E-01
7938366	WEE1	WEE1 homolog (S. Pombe)	2.41E-01
7950906	CTSC	Cathepsin C	2.41E-01
7970864	HSPH1	Heat shock 105kda/110kda protein 1	2.41E-01
7989128	CNOT6L	CCR4-NOT transcription complex, subunit 6-like	2.41E-01
7989132	RFX7	Regulatory factor X, 7	2.41E-01
7995128	ITGAX	Integrin, alpha X (complement component 3 receptor 4 subunit)	2.41E-01
8019802	RNU2-1	RNA, U2 small nuclear 1	2.41E-01
8081953	GTF2E1	General transcription factor IIE, polypeptide 1, alpha 56kda	2.41E-01
8112902	DHFR	Dihydrofolate reductase	2.41E-01
8117368	HIST1H4C	Histone cluster 1, h4c	2.41E-01
8117594	HIST1H2BM	Histone cluster 1, h2bm	2.41E-01
8123044	TULP4	Tubby like protein 4	2.41E-01
8124524	HIST1H2AK	Histone cluster 1, h2ak	2.41E-01
8124537	HIST1H3J	Histone cluster 1, h3j	2.41E-01
8131709	SP4	Sp4 transcription factor	2.41E-01
8144812	PCM1	Pericentriolar material 1	2.41E-01
8160033	SNRPE	Small nuclear ribonucleoprotein polypeptide E	2.41E-01
8166989	ZNF673	Zinc finger family member 673	2.41E-01

Appendix 8 — Eosinophil-Correlated Probe Sets Significantly Higher-Expressed in DR than ER, Post-Allergen Inhalation Challenge (FDR≤0.30)

Probe Set ID	Gene Symbol	Gene Name	FDR
7905085	HIST2H3A	Histone cluster 2, h3a	6.20E-02
7919589	HIST2H3D	Histone cluster 2, h3d	6.20E-02
7919612	HIST2H3D	Histone cluster 2, h3d	6.20E-02
7919614	HIST2H3A	Histone cluster 2, h3a	6.20E-02
7929258	KIF11	Kinesin family member 11	6.20E-02
8105828	CCNB1	Cyclin B1	8.60E-02
8061579	TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	8.83E-02
7929541	CC2D2B	Coiled-coil and C2 domain containing 2B	8.86E-02
8124527	HIST1H1B	Histone cluster 1, h1b	1.06E-01
7941505	CST6	Cystatin E/M	1.68E-01
7926259	MCM10	Minichromosome maintenance complex component 10	1.72E-01
7937020	MKI67	Antigen identified by monoclonal antibody Ki-67	1.72E-01
8001133	SHCBP1	SHC SH2-domain binding protein 1	1.72E-01
8124537	HIST1H3J	Histone cluster 1, h3j	1.72E-01
7900699	CDC20	Cell division cycle 20 homolog (S. Cerevisiae)	1.73E-01
7923086	ASPM	Asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	1.73E-01
7952914	CCDC77	Coiled-coil domain containing 77	1.73E-01
7983969	CCNB2	Cyclin B2	1.73E-01
7989647	KIAA0101	Kiaa0101	1.73E-01
7993267	TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	1.73E-01
8053584	CD8A	CD8a molecule	1.73E-01
8054580	BUB1	Budding uninhibited by benzimidazoles 1 homolog (yeast)	1.73E-01
8089694	ZNF80	Zinc finger protein 80	1.73E-01
8096528	PDHA2	Pyruvate dehydrogenase (lipoamide) alpha 2	1.73E-01
8127987	SNORD50A	Small nucleolar RNA, C/D box 50A	1.73E-01
8149109	DEFA4	Defensin, alpha 4, corticostatin	1.73E-01
8179564	KIFC1	Kinesin family member C1	1.73E-01
7913869	STMN1	Stathmin 1	1.89E-01
8068740	UMODL1	Uromodulin-like 1	1.89E-01
8103932	MLF1IP	MLF1 interacting protein	1.89E-01
8117594	HIST1H2BM	Histone cluster 1, h2bm	1.89E-01
8118669	KIFC1	Kinesin family member C1	1.89E-01
8124531	HIST1H3I	Histone cluster 1, h3i	1.89E-01
8124534	HIST1H4L	Histone cluster 1, h4l	1.89E-01
7919642	HIST2H2AB	Histone cluster 2, h2ab	1.90E-01
7969288	OLFM4	Olfactomedin 4	1.90E-01
8014974	TOP2A	Topoisomerase (DNA) II alpha 170kda	1.90E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8025450	OR2Z1	Olfactory receptor, family 2, subfamily Z, member 1	1.90E-01
8090972	TXNDC6	Thioredoxin domain containing 6	1.90E-01
8122058	ARG1	Arginase, liver	1.90E-01
7919606	HIST2H2BF	Histone cluster 2, h2bf	2.08E-01
7932221	C10orf111	Chromosome 10 open reading frame 111	2.08E-01
7940561	FEN1	Flap structure-specific endonuclease 1	2.08E-01
7952406	OR8B12	Olfactory receptor, family 8, subfamily B, member 12	2.08E-01
7954065	GPRC5A	G protein-coupled receptor, family C, group 5, member A	2.08E-01
7963020	DHH	Desert hedgehog	2.08E-01
7980425	ISM2	Isthmin 2 homolog (zebrafish)	2.08E-01
7994109	PLK1	Polo-like kinase 1	2.08E-01
8016494	TTLL6	Tubulin tyrosine ligase-like family, member 6	2.08E-01
8022640	DHFR	Dihydrofolate reductase	2.08E-01
8027748	FXYD3	FXYD domain containing ion transport regulator 3	2.08E-01
8029098	CEACAM6	Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	2.08E-01
8031031	MIR516B2	Microrna 516b-2	2.08E-01
8059864	GBX2	Gastrulation brain homeobox 2	2.08E-01
8087907	SEMA3G	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G	2.08E-01
8094278	NCAPG	Non-SMC condensin I complex, subunit G	2.08E-01
8108301	KIF20A	Kinesin family member 20A	2.08E-01
8123760	LY86-AS1	LY86 antisense RNA 1 (non-protein coding)	2.08E-01
8124391	HIST1H2AB	Histone cluster 1, h2ab	2.08E-01
8149955	PBK	PDZ binding kinase	2.08E-01
8157446	ORM1	Orosomucoid 1	2.08E-01
8160417	IFNA6	Interferon, alpha 6	2.08E-01
7914851	CLSPN	Claspin	2.21E-01
7933190	LOC100131195	Hypothetical protein LOC100131195	2.21E-01
7938366	WEE1	WEE1 homolog (S. Pombe)	2.21E-01
7945660	FAM99A	Family with sequence similarity 99, member A	2.21E-01
7951246	MMP8	Matrix metallopeptidase 8 (neutrophil collagenase)	2.21E-01
7991750	HBZ	Hemoglobin, zeta	2.21E-01
8007071	CDC6	Cell division cycle 6 homolog (S. Cerevisiae)	2.21E-01
8037222	CEACAM8	Carcinoembryonic antigen-related cell adhesion molecule 8	2.21E-01
8079370	CCR9	Chemokine (C-C motif) receptor 9	2.21E-01
8100758	UGT2B7	UDP glucuronosyltransferase 2 family, polypeptide B7	2.21E-01
8100971	PPBP	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	2.21E-01
8148548	PSCA	Prostate stem cell antigen	2.21E-01
8150962	TOX	Thymocyte selection-associated high mobility group box	2.21E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8166585	FLJ32742	Hypothetical locus FLJ32742	2.21E-01
7902808	LOC339524	Hypothetical LOC339524	2.34E-01
7903565	GPSM2	G-protein signaling modulator 2	2.34E-01
7908161	C1orf21	Chromosome 1 open reading frame 21	2.34E-01
7909568	DTL	Denticleless homolog (Drosophila)	2.34E-01
7909708	CENPF	Centromere protein F, 350/400kda (mitosin)	2.34E-01
7919243	CD160	CD160 molecule	2.34E-01
7928882	C10orf116	Chromosome 10 open reading frame 116	2.34E-01
7929334	CEP55	Centrosomal protein 55kda	2.34E-01
7943349	ARHGAP42	Rho gtpase activating protein 42	2.34E-01
7945774	SLC22A18AS	Solute carrier family 22 (organic cation transporter), member 18 antisense	2.34E-01
7945857	MRGPRG	MAS-related GPR, member G	2.34E-01
7958711	CCDC63	Coiled-coil domain containing 63	2.34E-01
7963817	GTSF1	Gametocyte specific factor 1	2.34E-01
7977732	SNORD8	Small nucleolar RNA, C/D box 8	2.34E-01
7981601	IGHV4-31	Immunoglobulin heavy variable 4-31	2.34E-01
7982889	NUSAP1	Nucleolar and spindle associated protein 1	2.34E-01
7984263	PTPLAD1	Protein tyrosine phosphatase-like A domain containing 1	2.34E-01
7985829	FANCI	Fanconi anemia, complementation group I	2.34E-01
7990683	ACSBG1	Acyl-coa synthetase bubblegum family member 1	2.34E-01
7991406	PRC1	Protein regulator of cytokinesis 1	2.34E-01
7998722	SNORD60	Small nucleolar RNA, C/D box 60	2.34E-01
8008201	NGFR	Nerve growth factor receptor	2.34E-01
8019842	TYMS	Thymidylate synthetase	2.34E-01
8030823	IGLON5	Iglon family member 5	2.34E-01
8034974	EPHX3	Epoxide hydrolase 3	2.34E-01
8040898	TRIM54	Tripartite motif containing 54	2.34E-01
8049297	SCARNA5	Small Cajal body-specific RNA 5	2.34E-01
8055941	RPRM	Reprimo, TP53 dependent G2 arrest mediator candidate	2.34E-01
8061471	GINS1	GINS complex subunit 1 (Psf1 homolog)	2.34E-01
8062766	MYBL2	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	2.34E-01
8063043	UBE2C	Ubiquitin-conjugating enzyme E2C	2.34E-01
8064939	TMX4	Thioredoxin-related transmembrane protein 4	2.34E-01
8066384	GTSF1L	Gametocyte specific factor 1-like	2.34E-01
8068898	HIST1H2BK	Histone cluster 1, h2bk	2.34E-01
8092640	RFC4	Replication factor C (activator 1) 4, 37kda	2.34E-01
8092750	FGF12	Fibroblast growth factor 12	2.34E-01
8096875	ENPEP	Glutamyl aminopeptidase (aminopeptidase A)	2.34E-01
8102643	CCNA2	Cyclin A2	2.34E-01
8113003	FLJ11292	Hypothetical protein FLJ11292	2.34E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8116707	KU-MEL-3	Ku-mel-3	2.34E-01
8117395	HIST1H2BF	Histone cluster 1, h2bf	2.34E-01
8117422	HIST1H4F	Histone cluster 1, h4f	2.34E-01
8121784	FABP7	Fatty acid binding protein 7, brain	2.34E-01
8124385	HIST1H4B	Histone cluster 1, h4b	2.34E-01
8126244	LRFN2	Leucine rich repeat and fibronectin type III domain containing 2	2.34E-01
8129880	PERP	PERP, TP53 apoptosis effector	2.34E-01
8132743	ABCA13	ATP-binding cassette, sub-family A (ABC1), member 13	2.34E-01
8132811	C7orf72	Chromosome 7 open reading frame 72	2.34E-01
8132860	EGFR	Epidermal growth factor receptor	2.34E-01
8139796	LOC441233	Hypothetical LOC441233	2.34E-01
8152962	LRRC6	Leucine rich repeat containing 6	2.34E-01
8160033	SNRPE	Small nuclear ribonucleoprotein polypeptide E	2.34E-01
8180273	PCDHA12	Protocadherin alpha 12	2.34E-01
7901913	FOXD3	Forkhead box D3	2.45E-01
7904429	HSD3BP4	Hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 4	2.45E-01
7904465	HIST2H2BA	Histone cluster 2, h2ba	2.45E-01
7912629	KAZN	Kazrin, periplakin interacting protein	2.45E-01
7921840	NR1I3	Nuclear receptor subfamily 1, group I, member 3	2.45E-01
7923562	CHIT1	Chitinase 1 (chitotriosidase)	2.45E-01
7926638	ARMC3	Armadillo repeat containing 3	2.45E-01
7927710	CDK1	Cyclin-dependent kinase 1	2.45E-01
7929674	C10orf62	Chromosome 10 open reading frame 62	2.45E-01
7934959	MIR107	Microrna 107	2.45E-01
7937868	C11orf36	Chromosome 11 open reading frame 36	2.45E-01
7938008	OR52D1	Olfactory receptor, family 52, subfamily D, member 1	2.45E-01
7938082	CNGA4	Cyclic nucleotide gated channel alpha 4	2.45E-01
7938348	WEE1	WEE1 homolog (S. Pombe)	2.45E-01
7944797	OR10G4	Olfactory receptor, family 10, subfamily G, member 4	2.45E-01
7947110	E2F8	E2F transcription factor 8	2.45E-01
7948107	OR5W2	Olfactory receptor, family 5, subfamily W, member 2	2.45E-01
7959761	FAM101A	Family with sequence similarity 101, member A	2.45E-01
7961111	CLEC1A	C-type lectin domain family 1, member A	2.45E-01
7964535	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1	2.45E-01
7967386	MPHOSPH9	M-phase phosphoprotein 9	2.45E-01
7968062	ATP12A	Atpase, H+/K+ transporting, nongastric, alpha polypeptide	2.45E-01
7969243	CKAP2	Cytoskeleton associated protein 2	2.45E-01
7972018	LOC100288208	Hypothetical protein LOC100288208	2.45E-01
7981020	ASB2	Ankyrin repeat and SOCS box containing 2	2.45E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
7982271	GOLGA8IP	Golgin A8 family, member I (pseudogene)	2.45E-01
7982287	ARHGAP11B	Rho gtpase activating protein 11B	2.45E-01
7983490	HMGN2P46	High-mobility group nucleosomal binding domain 2 pseudogene 46	2.45E-01
7989657	CSNK1G1	Casein kinase 1, gamma 1	2.45E-01
8001402	CHD9	Chromodomain helicase DNA binding protein 9	2.45E-01
8013671	SPAG5	Sperm associated antigen 5	2.45E-01
8018849	TK1	Thymidine kinase 1, soluble	2.45E-01
8025237	KIAA1543	Kiaa1543	2.45E-01
8025470	OR7D2	Olfactory receptor, family 7, subfamily D, member 2	2.45E-01
8026503	FLJ25328	Hypothetical LOC148231	2.45E-01
8026631	F2RL3	Coagulation factor II (thrombin) receptor-like 3	2.45E-01
8036270	THAP8	THAP domain containing 8	2.45E-01
8043890	NMS	Neuromedin S	2.45E-01
8053753	TEKT4	Tektin 4	2.45E-01
8055143	LOC440905	Hypothetical LOC440905	2.45E-01
8055606	GTDC1	Glycosyltransferase-like domain containing 1	2.45E-01
8060503	SNORD57	Small nucleolar RNA, C/D box 57	2.45E-01
8060813	MCM8	Minichromosome maintenance complex component 8	2.45E-01
8061303	INSM1	Insulinoma-associated 1	2.45E-01
8079590	CAMP	Cathelicidin antimicrobial peptide	2.45E-01
8089714	LSAMP	Limbic system-associated membrane protein	2.45E-01
8098576	SLC25A4	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	2.45E-01
8100827	IGJ	Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	2.45E-01
8108716	PCDHB16	Protocadherin beta 16	2.45E-01
8112260	DEPDC1B	DEP domain containing 1B	2.45E-01
8114470	LRRTM2	Leucine rich repeat transmembrane neuronal 2	2.45E-01
8114511	MZB1	Marginal zone B and B1 cell-specific protein	2.45E-01
8117334	HIST1H4A	Histone cluster 1, h4a	2.45E-01
8117408	HIST1H2AE	Histone cluster 1, h2ae	2.45E-01
8124437	HIST1H3F	Histone cluster 1, h3f	2.45E-01
8124521	HIST1H4K	Histone cluster 1, h4k	2.45E-01
8126095	C6orf129	Chromosome 6 open reading frame 129	2.45E-01
8127346	RAB23	RAB23, member RAS oncogene family	2.45E-01
8132642	PPIA	Peptidylprolyl isomerase A (cyclophilin A)	2.45E-01
8135224	NFE4	Transcription factor NF-E4	2.45E-01
8136837	OR6V1	Olfactory receptor, family 6, subfamily V, member 1	2.45E-01
8138765	HOXA11	Homeobox A11	2.45E-01
8141846	FBXL13	F-box and leucine-rich repeat protein 13	2.45E-01
8144982	NPM2	Nucleophosmin/nucleoplasmin 2	2.45E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8146649	MTFR1	Mitochondrial fission regulator 1	2.45E-01
8150433	NKX6-3	NK6 homeobox 3	2.45E-01
8151032	GGH	Gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)	2.45E-01
8155214	MELK	Maternal embryonic leucine zipper kinase	2.45E-01
8158081	C9orf117	Chromosome 9 open reading frame 117	2.45E-01
8158167	LCN2	Lipocalin 2	2.45E-01
8168976	GPRASP2	G protein-coupled receptor associated sorting protein 2	2.45E-01
8171867	ARX	Aristaless related homeobox	2.45E-01
8171885	DCAF8L1	DDB1 and CUL4 associated factor 8-like 1	2.45E-01
8175629	MAGEA11	Melanoma antigen family A, 11	2.45E-01
8180255	HIST2H4B	Histone cluster 2, h4b	2.45E-01
8180321	HIST2H4A	Histone cluster 2, h4a	2.45E-01
7896863	MIR429	Microrna 429	2.76E-01
7898616	PLA2G2F	Phospholipase A2, group IIF	2.76E-01
7905496	C1orf46	Chromosome 1 open reading frame 46	2.76E-01
7905505	LCE2B	Late cornified envelope 2B	2.76E-01
7919584	HIST2H2BF	Histone cluster 2, h2bf	2.76E-01
7921275	FCRL3	Fc receptor-like 3	2.76E-01
7922174	F5	Coagulation factor V (proaccelerin, labile factor)	2.76E-01
7924096	NEK2	NIMA (never in mitosis gene a)-related kinase 2	2.76E-01
7924821	ZNF847P	Zinc finger protein 847, pseudogene	2.76E-01
7937404	C11orf35	Chromosome 11 open reading frame 35	2.76E-01
7939897	FOLH1	Folate hydrolase (prostate-specific membrane antigen) 1	2.76E-01
7940626	SCGB2A1	Secretoglobin, family 2A, member 1	2.76E-01
7947694	CKAP5	Cytoskeleton associated protein 5	2.76E-01
7973105	RNASE3	Ribonuclease, rnase A family, 3	2.76E-01
7973797	COCH	Coagulation factor C homolog, cochlin (Limulus polyphemus)	2.76E-01
7979307	DLGAP5	Discs, large (Drosophila) homolog-associated protein 5	2.76E-01
7979963	DPF3	D4, zinc and double PHD fingers, family 3	2.76E-01
7981718	IGHM	Immunoglobulin heavy constant mu	2.76E-01
7982333	LOC100499221	Hypothetical LOC100499221	2.76E-01
8000779	TBX6	T-box 6	2.76E-01
8004784	ALOX15B	Arachidonate 15-lipoxygenase, type B	2.76E-01
8005733	C20orf191	Nuclear receptor co-repressor 1 pseudogene	2.76E-01
8011218	MIR132	Microrna 132	2.76E-01
8020419	MIR320C1	Microrna 320c-1	2.76E-01
8039078	BIRC8	Baculoviral IAP repeat containing 8	2.76E-01
8040469	LOC100131373	Hypothetical LOC100131373	2.76E-01
8047702	ICOS	Inducible T-cell co-stimulator	2.76E-01

Probe Set ID	Gene Symbol	Gene Name	FDR
8062444	BPI	Bactericidal/permeability-increasing protein	2.76E-01
8068496	SIM2	Single-minded homolog 2 (Drosophila)	2.76E-01
8070933	FTCD	Formiminotransferase cyclodeaminase	2.76E-01
8071086	CECR2	Cat eye syndrome chromosome region, candidate 2	2.76E-01
8083897	TMEM212	Transmembrane protein 212	2.76E-01
8085287	C3orf10	Chromosome 3 open reading frame 10	2.76E-01
8086689	MYL3	Myosin, light chain 3, alkali; ventricular, skeletal, slow	2.76E-01
8101031	CDKL2	Cyclin-dependent kinase-like 2 (CDC2-related kinase)	2.76E-01
8103728	HMGB2	High-mobility group box 2	2.76E-01
8112902	DHFR	Dihydrofolate reductase	2.76E-01
8124388	HIST1H3B	Histone cluster 1, h3b	2.76E-01
8130785	GPR31	G protein-coupled receptor 31	2.76E-01
8131949	CBX3	Chromobox homolog 3	2.76E-01
8133728	ZP3	Zona pellucida glycoprotein 3 (sperm receptor)	2.76E-01
8134263	COL1A2	Collagen, type I, alpha 2	2.76E-01
8138547	TOMM7	Translocase of outer mitochondrial membrane 7 homolog (yeast)	2.76E-01
8138977	DPY19L1	Dpy-19-like 1 (C. Elegans)	2.76E-01
8144625	BLK	B lymphoid tyrosine kinase	2.76E-01
8146357	MCM4	Minichromosome maintenance complex component 4	2.76E-01
8149774	LOXL2	Lysyl oxidase-like 2	2.76E-01
8151709	OSGIN2	Oxidative stress induced growth inhibitor family member 2	2.76E-01
8152715	KLHL38	Kelch-like 38 (Drosophila)	2.76E-01
8160284	HAUS6	HAUS augmin-like complex, subunit 6	2.76E-01
8163107	MIR32	Microrna 32	2.76E-01
8163892	C9orf31	Chromosome 9 open reading frame 31	2.76E-01
8164200	ANGPTL2	Angiopoietin-like 2	2.76E-01
8166665	FAM47B	Family with sequence similarity 47, member B	2.76E-01
8166705	PRRG1	Proline rich Gla (G-carboxyglutamic acid) 1	2.76E-01
8168416	USMG5	Up-regulated during skeletal muscle growth 5 homolog (mouse)	2.76E-01
8176336	ASMTL-AS1	ASMTL antisense RNA 1 (non-protein coding)	2.76E-01