Exploring Biomarkers for Early and Dual Asthmatic Response

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Introduction

- **Asthma** is a common chronic condition whereby patients experience airway constriction upon being exposed to an allergen. While not necessarily fatal, asthma has long-term implications on quality of life and symptom management costs.
- While most patients fully recover a few hours after the initial attack, a subset of asthmatic patients experience an additional onset of **airway inflammation**. We call the former group of patients **early responders (ER)** and the latter group, **late responders (DR)**. Visually, the difference between ER's and DR's is shown in **figure 1**, where the lung function, measured by FEV (forced expiratory volume), of DR's, shown in red, do not return to normal like those of the ER's, shown in blue.

Problem

Given our phenotype, or **two classes of subjects: ER & DR**,

1. **Find biometric features** highly correlated with the phenotype to discover potential biomarkers.
2. **Comprehend** how these biometric features contribute to the phenotype by exploring associations between features.

Data

- Subjects: n = 35 (18 ER, 17 DR)
  - Collected from UBC (1), McMaster (16), and Laval (18)
  - Homogenous demographics e.g. 33 Caucasians, 27 of whom are of ages 19-37
- Biometrics: (n x m) (subject x feature)
  - **Blood-based transcriptomics** sampled before challenge: RNA seq + Nanostring pancancer panel + Nanostring elements panel (m = 9323 ensemble genes + 600 filtered genes + 166 immune genes, from which [2] had found a set of genes, or panel, that can differentiate between ER & DR.
  - **Genotype**:
    - Affymetrix Axiom (m = 261,958 SNP's , single nucleotide polymorphisms).

Conclusion

Early and dual asthmatic responders are differentiated based on the presence of inflammation in the airways, but the cause of this difference involves a mixture of mechanisms e.g. fibrinogen production, and apostasis. Therefore, this projects' aim is to find possible explanatory biomarkers from transcriptomics and genotype data for this phenotype. We showed that by including multiple data sets, we are able to discover information that have otherwise remain hidden. We hope to further this research by validating our results on larger data sets and testing other probabilistic scenarios -- to solidify causal relationships between the genotype and our phenotype on top of the eQTL framework.

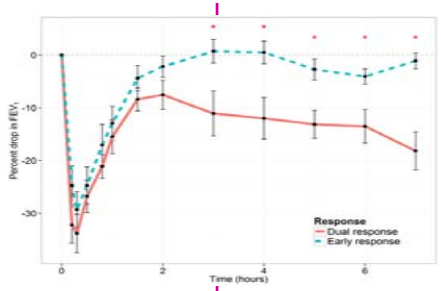

Figure 1 FEV (forced expiratory volume) of early and dual asthmatic responders over time (hours) after being exposed to an allergen at time = 0 [1]

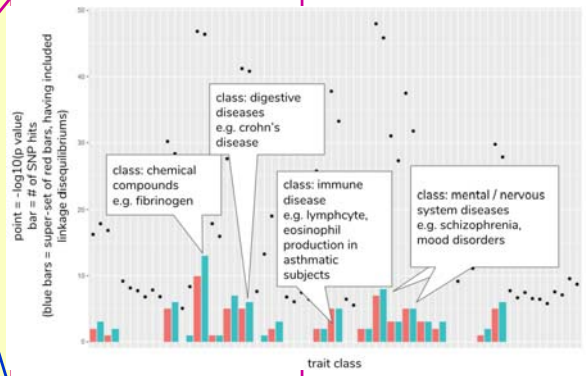

Figure 2 SNP enrichment analysis on specific trait classes.

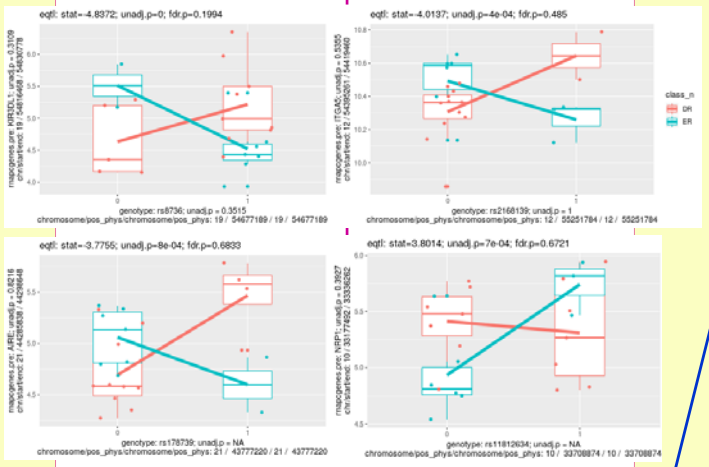

Figure 3 Sample of transcript counts (y-axis) as a linear function of interaction between phenotype and genotype (dominant model: 0 = common allele homozygous, 1 = rare allele hetero / homozygous).

Methods & Results

1. **Find biometric features**: Given our sample size, we take on an exploratory approach to find new hypotheses in this section.
   - **Blood-based transcriptomics:**
     - [2] presents genes that correlate with the phenotype.
   - **Genotype:**
     - GWAS: We did a GWAS (genome wide association study) by testing each SNP with our phenotype using the Chi-square significance test.
     - SNP enrichment: Using SNP's whose p values < 0.01, we calculated SNP enrichment p values (i.e. what other traits are our SNP's associated with significantly) using the binomial test* (see **figure 2**).
     - Results:
       - 939 significant SNP's are found, including CASP8AP2 corroborating with [1].
       - The highest enrichment p values are in the inflammation, immune system (specifically thyroid & lung illness), and mental illness trait classes.
       - Only the former two classes were hypothesized o be enriched, but the latter can also be supported e.g. [3].
2. **Comprehension**: To understand how the SNP's might be affecting our phenotype, we conduct an eQTL (expression quantitative trait loci) study, modelling the **transcript counts as a linear function of the interaction between phenotype and genotype** (dominant model). (see **figure 3**).
   - Results: several independently non-significant genes from the transcriptomics popped up as significant after incorporating genotype data including:
     - KIR3DL1 (Killer cell immunoglobulin-like receptor 3DL1; regulates immune response)
     - AIRE (autoimmune regulator; when AIRE is defective, self-recognizing T cells do not undergo apostasis and flows into the blood causing a variety of autoimmune illnesses)
     - FUT7 (oligosaccharide enzyme allows leukocytes to get to lymphoid tissues and inflammation sites)
     - NRP1 (interacts with growth factor; elevated in brain, prostate, breast, and lung cancer patients)
     - ITGA5 (contributes to production of fibronectin receptor involved in cell growth)

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References

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2. Singh, Amrit, et al. "Novel blood-based transcriptional biomarker panels predict the late-phase asthmatic response." American journal of respiratory and critical care medicine 197.4 (2018): 450-462.
3. Raji, Mukaila A. "The nervous system as potential targets for asthma treatments: lessons learned from a centennial history of asthma research." American journal of respiratory cell and molecular biology 33.2 (2005): 211-211.

*under the assumption that seeing a SNP being associated with a trait in literature is (# of SNP's in literature) / (# of all SNP's in the genome).