

Targeting Transcriptional Dysregulation for Atopic Dermatitis

Amy Eapen, MD

Mentors:

Leah Kottyan, PhD

Matthew Weirauch, PhD

David Bernstein, MD

Background

- In numerous nations across the globe, 20% of children develop atopic dermatitis (AD), contributing to a significant social and financial burden¹.
- 10-30% AD children have persistent disease as adults².
- Immunologically, **CD4⁺ T** cells produce inflammatory cytokines and contribute to dysfunction of the **skin barrier**³.
- **Gap: Why do some people develop allergic disease?**
 - Genetics
 - Environmental factors
 - Immunological pathways

1. Flohr, C. & Mann, J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy* **69**, 3-16, doi:10.1111/all.12270 (2014).
2. Perkin, M. R. *et al.* Natural history of atopic dermatitis and its relationship to serum total immunoglobulin E in a population-based birth cohort study. *Pediatr Allergy Immunol* **15**, (2004).
3. Werfel T, *et al.* Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2016.

Genetics of AD

- Genome-wide association studies have identified 29 independent genetic risk loci associated with AD ($p < 5 \times 10^{-8}$)⁴
 - 95% of these genetic risk variants are non-coding.
- Gene dysregulation is implicated as the mechanistic key to allergic disease initiation.
- **Transcription factors (TF)** are the primary regulatory molecules that control gene expression.

4. Paternoster, L. *et al.* Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* **47**, (2015).

Hypothesis

- AD genetic risk variants are bound in a genotype-dependent manner by particular TFs, resulting in increased disease risk through genotype-dependent dysregulation of gene expression
- We will identify TFs that bind numerous independent AD risk loci

Can we find TFs whose binding is enriched for disease-risk variants?

Step 1: Input chromosomal positions

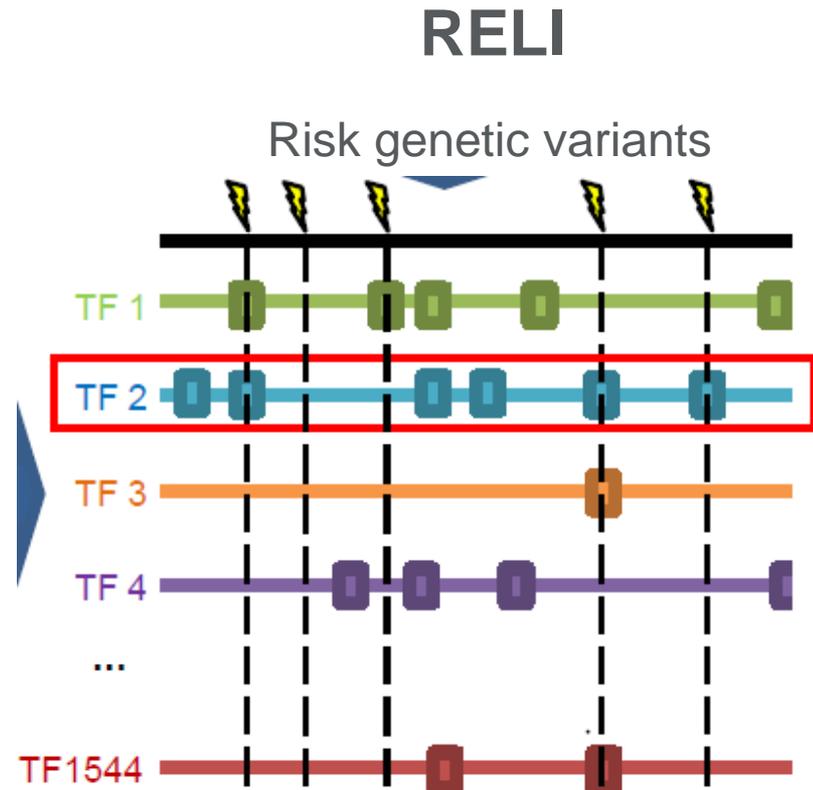
- Disease risk variants
- ChIP-seq reads

Step 2: Identify and count overlaps at independent risk loci

Step 3: Identify the distribution of expected overlaps

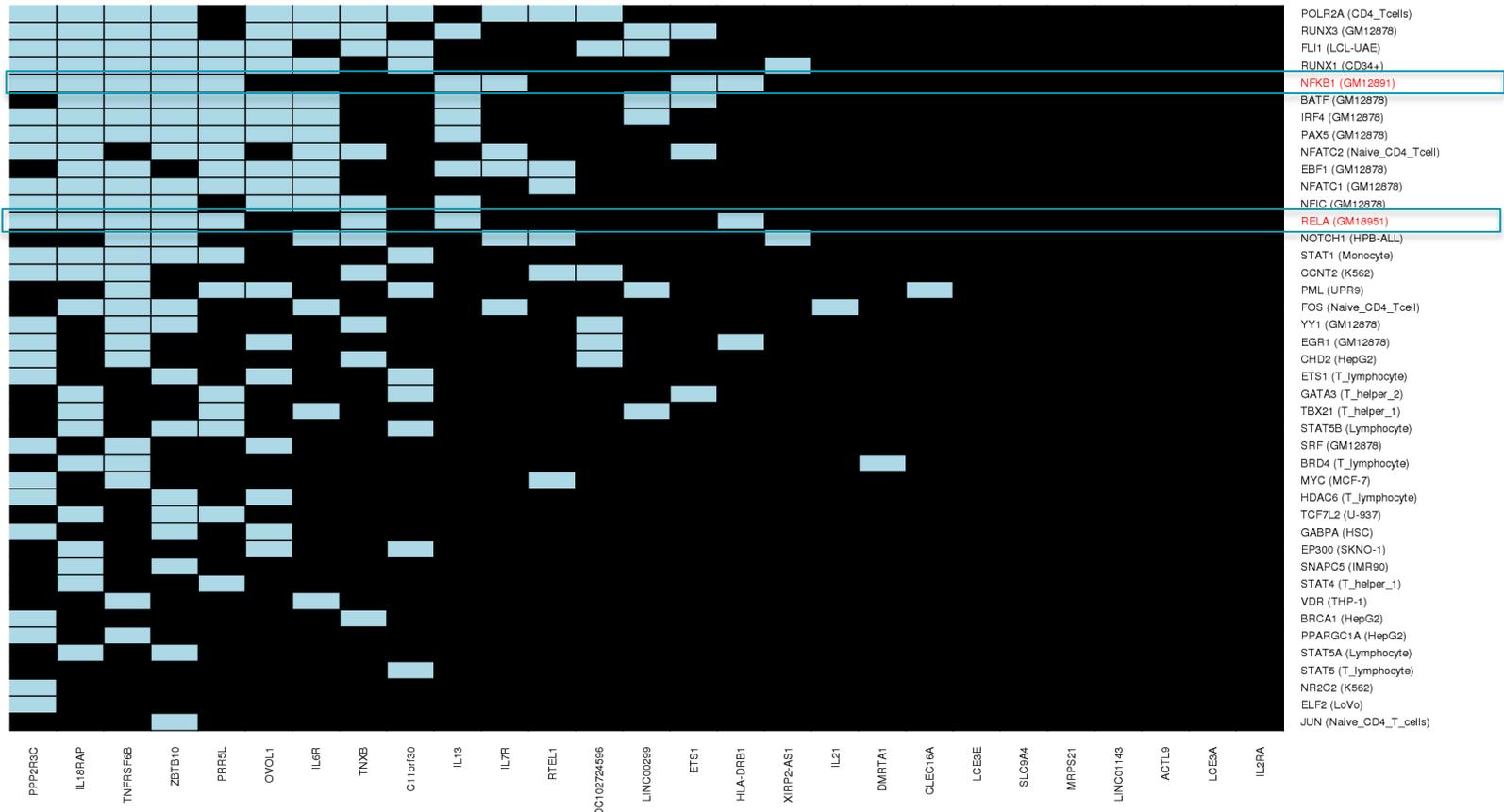
- 2000 null models of disease risk variants
- Allele frequency of lead variant, number of variants in LD block, LD block structure

Step 4: Identify enrichment, account for multiple testing



Xiaoting Chen, PhD

NF κ B CHIP-seq datasets overlap and are enriched for 48% AD risk loci



What about CD4+ T cells?

| Anti-NFKB1 ChIP-seq data set | Percentage of AD risk SNPs at independent loci overlapping RELA ChIP-seq peaks | Enriched binding | Corrected P-value |
|--|--|---------------------|----------------------|
| Naïve CD4+ T cells (crosslinked CD3/CD28) | 4/29 | 9.02 | 8.9×10^{-6} |

Artem Barski, PhD
Sreeja Parameswaran, PhD

Pathway analysis: comparison of genes in AD genetic risk loci by NF κ B

Overlapped by NF κ B

| Biological Process | P-value | Corrected P-value | Enrichment |
|----------------------------|----------|-------------------|------------|
| IL12 signaling by STAT4 | 1.79E-05 | 2.68E-04 | 29.33 |
| Cellular response to IL-18 | 6.9E-05 | 6.6E-03 | 27.17 |

Not overlapped by NF κ B

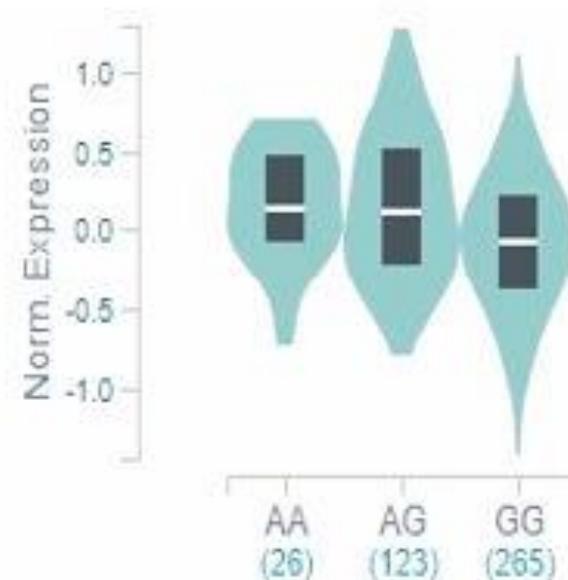
| Biological Process | P-value | Corrected P-value | Enrichment |
|--|----------|-------------------|------------|
| Cellular response to IL-2 | 1.24E-04 | 3.38E-03 | 23.12 |
| Positive regulation of tissue remodeling | 6.76E-05 | 3.38E-03 | 22.63 |

Does gene data relate to tissue?

- **eQTL: expression quantitative trait loci**
 - A locus that explains a fraction of the genetic variation of a gene expression phenotype
 - Looks at the specific cell line and expression of the gene at the cellular level
- AD genetic risk variants are eQTLs for several immunologic genes in **skin, esophagus and CD4+ T cells**

SKIN

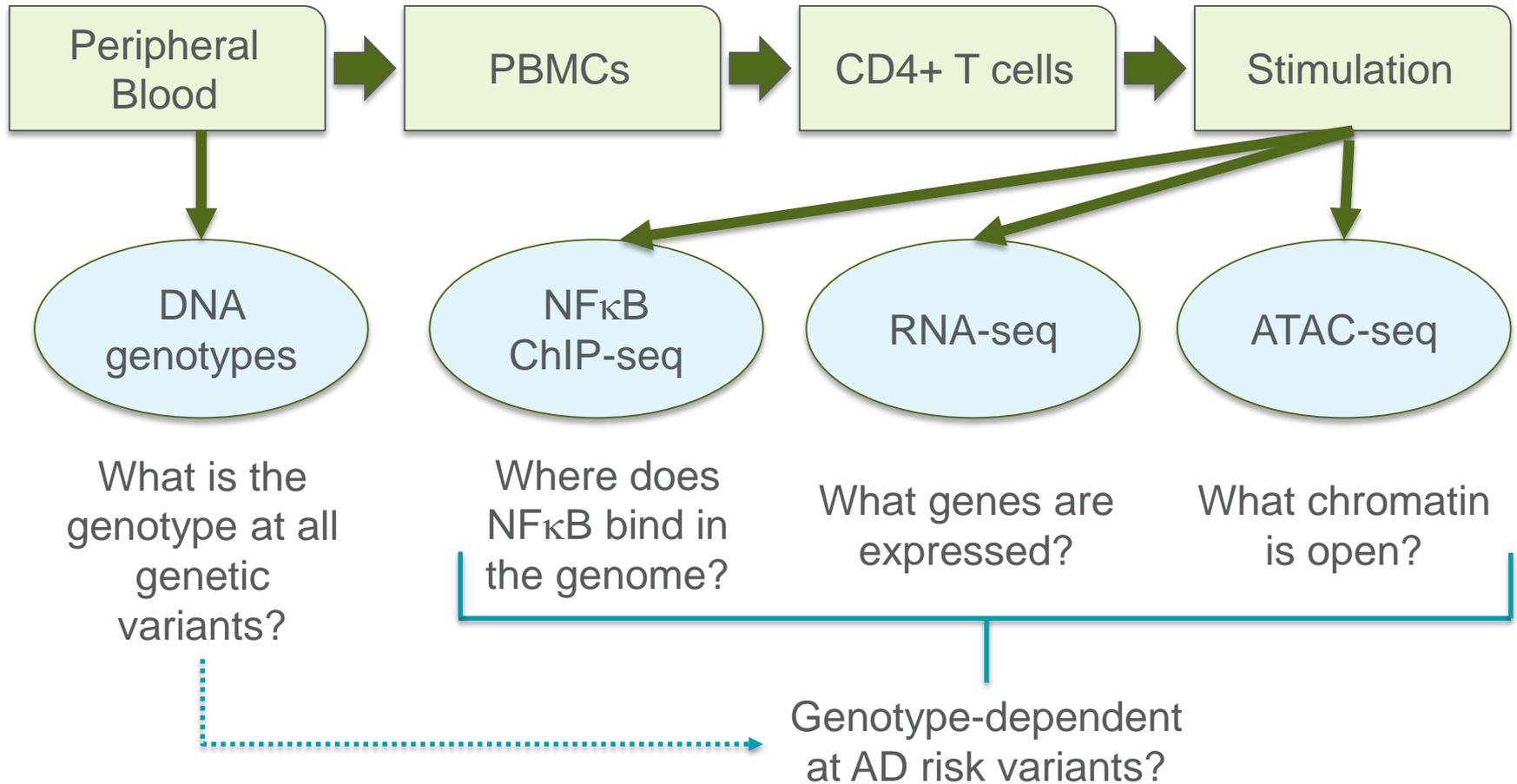
IL18R1



Do we see enriched NF κ B binding at AD risk loci in patient cells? Is it genotype-dependent?

- Subjects: 10 patients with moderate to severe AD and 10 controls – adults (n=5) and children (n=5)
- Inclusion criteria:
 - Moderate to severe AD:
 - Moderate to severe disease defined by EASI \geq 16 and IGA \geq 3⁵
 - Presence of atopy: positive SPT to aeroallergens +/- elevated total IgE
 - Controls
 - Negative SPT to aeroallergens +/- normal total IgE
 - No history of allergic rhinitis or atopic dermatitis
- Exclusion criteria
 - Moderate to severe AD:
 - On current monoclonal antibody, steroids or immunosuppression
 - Controls
 - On current monoclonal antibody, steroids or immunosuppression
 - Presence of allergic disease

Experimental design



Conclusions

- AD risk variants overlapped by NF κ B show enrichment for IL12 and IL18 signaling pathways
 - Th1 vs Th2 skewing
- Tissue specific eQTL enrichment for AD risk loci show enrichment for skin and CD4 T cells
- We are developing a datasets to test our hypothesis in patient and control-derived cells
- MPRAs will be a valuable tool that can be used as a drug screen to identify functional SNPs and determine genetic endotypes for which drugs would be best received for specific AD subsets

Thank you

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Allergy and Immunology: Senior Advisory Committee

David Bernstein, MD
Kim Risma, MD, PhD
Artem Barski, PhD

**Transcription Factor Genetics
Working Group**