Massively Parallel Reporter Assays (MPRAs) Identify Allelic Transcriptional Dysregulation in Atopic Dermatitis

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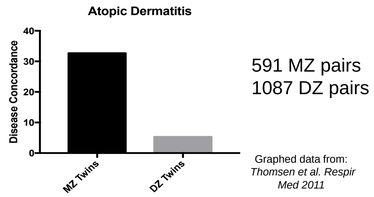
Background

- 20% of children develop atopic dermatitis (AD)
- 10-30% AD children have persistent disease as adults
- **CD4⁺ T cells** produce inflammatory cytokines and contribute to dysfunction of the **skin barrier**
- There is a strong genetic component to AD development as documented by twin concordance studies



591 MZ pairs
1087 DZ pairs

Graphed data from:
Thomsen et al. Respir Med 2011

- Genome-wide association studies have identified 29 independent genetic risk loci associated with AD ($p<5x10^{-8}$)
  - 95% of these genetic risk variants are non-coding

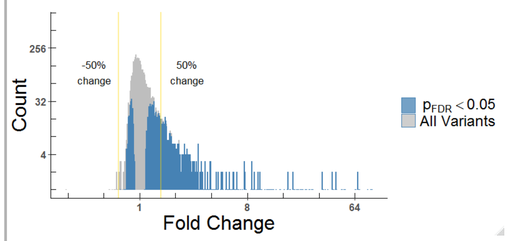
Experimental Design

- 3,143 SNPs and insertions/deletions accounting for 29 independent loci.
  - Loci were identified from 122 reported "tag" variants reaching genome-wide significance)
- 150 bp around each SNP
- Calibration oligos that are from genetic variants that have been previously shown to be allelic or not in previous MPRA assays
- 300-500 tags per allele (oligo)*
- Replicates – 5 transfections/condition
- Sequencing → 30-fold coverage

Massively Parallel Reporter Assays

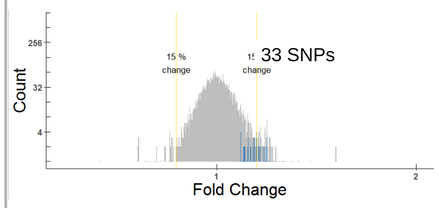
- MPRAs allow us to assess gene regulatory activity of DNA surrounding 1000's of genetic variants at the same time
  - Any cell type
  - Any condition (e.g. stimulation, therapeutics)
- A pool of reporter constructs is assembled
- DNA "tags" are used instead of luciferase as a reporter
  - Each reporter is made many times with many different tags
- Pool is transfected into cells
- Measures enhancer activity and genotype-dependent enhancer activity

Enhancer activity at AD risk variants

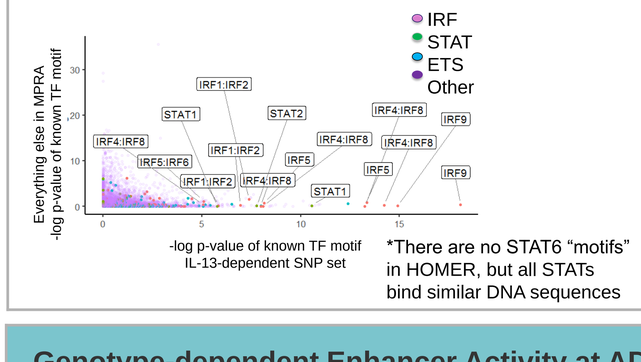


619 AD oligos → 302/3143 variants have enhancer activity ($p<0.05$, >50% change)

Effect of IL13 on enhancer activity at AD risk variants



The DNA sequence around variants with IL13 dependence were enriched for STAT* and IRF motifs



*There are no STAT6 "motifs" in HOMER, but all STATs bind similar DNA sequences

Ongoing work

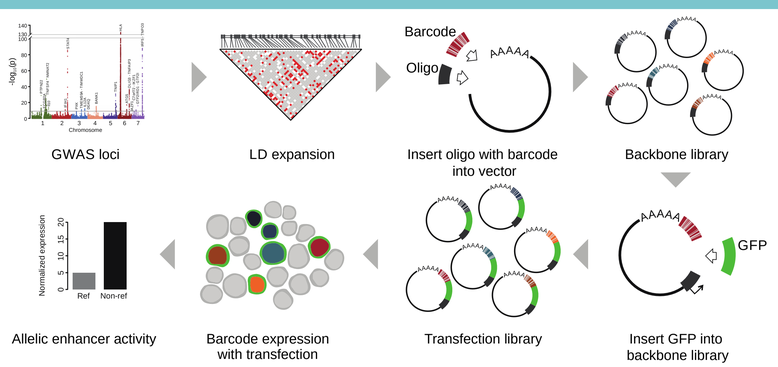
- Different cell lines + stimulation – e.g. HaCaT(immortalized human keratinocytes) and Jurkat (immortalized human T cells) +/- TNFα
- Integration with other functional genomic data to nominate causal allelic transcriptional regulatory mechanisms at AD disease risk variants
- Complement with eQTL data, chromatin conformation assay data, genome-editing to identify genes that are regulated by SNPs

Conclusions

Our results are consistent with a model in which AD genetic risk variants regulate gene expression in a genotype-dependent manner.

We nominate 108 plausibly causal variants for AD.

Schematic of MPRA design and library preparation

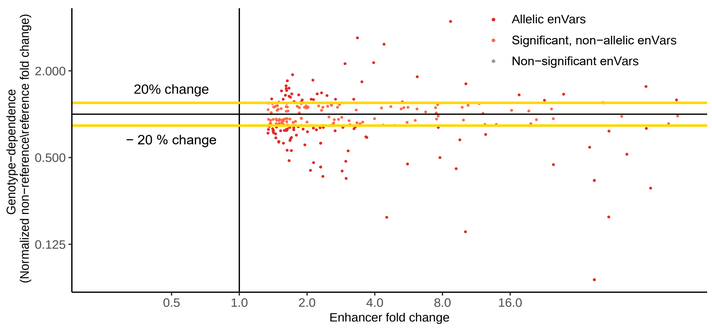


Sequence DNA and RNA of reporters

Genomic sequence-Tag RNA count / Plasmid Tag DNA count

See Lu *et al.* pre-print in BioRxiv

Genotype-dependent Enhancer Activity at AD-disease risk variants



108/387 AD risk variants have genotype-dependent enhancer activity ($p<0.05$, >20% change)

enVars: enhancer variants that increase minimal promoter activity in the MPRA as measured by increased RNA:DNA ratio of barcodes

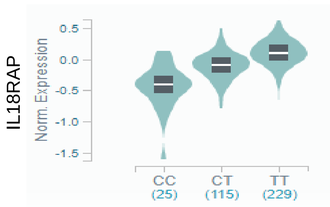
Allelic: Genotype-dependent

Genotype-dependent Enhancer Activity at AD-disease risk variants: Example locus

Genetic Variant ID	DNA	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Replicate #5	P value	P value (FDR adjusted)
rs6729638	0.85	2.06	1.82	1.85	1.97	1.96	$3.63x10^{-6}$	$4.54x10^{-5}$

Genetic variant in LD with disease risk SNPs:
Atopic dermatitis
Celiac disease
Cerebrospinal fluid biomarker levels

GTEx – skin biopsy



There are 98 variants in LD with rs6729638 (r2>0.8), and we know the single variant with allelic activity (MPRA) and the gene affects (eQTL)!