ORIGINAL RESEARCH

# Integrative Genetic Analysis of Allergic Inflammation in the Murine Lung

Samir N. P. Kelada[1,2,3], Danielle E. Carpenter[1], David L. Aylor[4], Peter Chines[1], Holly Rutledge[2], Elissa J. Chesler[5], Gary A. Churchill[5], Fernando Pardo-Manuel de Villena[2,6], David A. Schwartz[7], and Francis S. Collins[1]

[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland; [2]Department of Genetics, [3]Marsico Lung Institute, and [6]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina; [4]Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina; [5]The Jackson Laboratory, Bar Harbor, Maine; and [7]Department of Medicine, University of Colorado, Denver, Colorado

## Abstract

Airway allergen exposure induces inflammation among individuals with atopy that is characterized by altered airway gene expression, elevated levels of T helper type 2 cytokines, mucus hypersecretion, and airflow obstruction. To identify the genetic determinants of the airway allergen response, we employed a systems genetics approach. We applied a house dust mite mouse model of allergic airway disease to 151 incipient lines of the Collaborative Cross, a new mouse genetic reference population, and measured serum IgE, airway eosinophilia, and gene expression in the lung. Allergen-induced serum IgE and airway eosinophilia were not correlated. We detected quantitative trait loci (QTL) for airway eosinophilia on chromosome (Chr) 11 (71.802–87.098 megabases [Mb]) and allergen-induced IgE on Chr 4 (13.950–31.660 Mb). More than 4,500 genes expressed in the lung had gene expression QTL (eQTL), the majority of which were located near the gene itself. However, we also detected approximately 1,700 *trans*-eQTL, and many of these *trans*-eQTL clustered into two regions on Chr 2. We show that one of these loci (at 147.6 Mb) is associated with the expression of more than 100 genes, and, using bioinformatics resources, fine-map this locus to a 53 kb-long interval. We also use the gene expression and eQTL data to identify a candidate gene, *Tlcd2*, for the eosinophil QTL. Our results demonstrate that hallmark allergic airway disease phenotypes are associated with distinct genetic loci on Chrs 4 and 11, and that gene expression in the allergically inflamed lung is controlled by both *cis* and *trans* regulatory factors.

**Keywords:** allergic airway disease; systems genetics; gene expression quantitative trait loci

### Clinical Relevance

Using a systems genetics approach and a new mouse genetics reference population, we examined correlations between key features of allergic inflammation, namely IgE and airway eosinophilia, and identified distinct genetic loci associated with each trait. We show that the expression of many genes (> 4,500) in the lung after allergen challenge is also under genetic control, in part by distal regulatory factors. These gene expression data provide insight about gene regulation in the context of allergen challenge, and also point to a novel candidate gene for allergic inflammation.

The prevalence of allergic airway diseases (AADs), including asthma and allergic rhinitis, has increased dramatically over the last 50 years, especially in more developed countries (1). These diseases affect some 8–16% of the population in the United States, and cause substantial decrements in quality of life and productivity (2–4). Hallmark phenotypes of AAD include elevated serum IgE, airway eosinophilia, and mucus hypersecretion. The etiology of AAD remains enigmatic and complex, and likely involves complex interactions between genes and environment (5–8). Genome-wide association studies in humans have identified several loci associated with asthma in multiple study populations (9–14), and a genome-wide

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Correspondence and requests for reprints should be addressed to Samir N. P. Kelada, Ph.D., M.P.H., Department of Genetics, University of North Carolina, 120 Mason Farm Road, Room 5072, Chapel Hill, NC 27510. E-mail: samir_kelada@med.unc.edu

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association study of allergic rhinitis also identified multiple loci, some of which overlap with asthma loci (e.g., *LRRC32*, *HLA-G*, and *TSLP*) (15). Several studies have also identified gene expression profiles that differentiate normal versus AAD in affected tissue (16–21).

We set out to expand on existing knowledge of the genetics and transcriptomics of AAD using a systems genetics approach. To accomplish this, we applied a house dust mite (HDM) mouse model of AAD in the lung (22) to incipient lines of the Collaborative Cross (CC). The CC is composed of a panel of recombinant inbred lines derived from eight-way crosses using five classical inbred strains (C57BL/6J, 129S1/SvImJ, A/J, NOD/ShiLtJ, and NZO/HlLtJ) and three wild-derived inbred strains (WSB/EiJ, PWK/PhJ, and CAST/EiJ) (23), the genomes of which have recently been sequenced (24, 25). Quantitative trait loci (QTL) for many traits have been identified using these incipient CC lines (26–29), which we refer to as "preCC" mice.

In the present work, we report on four types of correlations: between AAD phenotypes, between genotype and AAD phenotypes (i.e., QTL), between gene expression and AAD phenotype, and between genotype and gene expression (expression QTL or "eQTL"). We focused much of our work on eQTL discovery in the allergen-challenged mouse lung, because genetically determined variation in gene expression is thought to contribute to asthma (31, 32), and this has not yet been examined in a mouse model of AAD.

## Materials and Methods

Additional details about methods are provided in the online supplement.

### Mice

We obtained 151 male preCC mice from Oak Ridge National Laboratory (Oak Ridge, TN). Each mouse was from a distinct CC line that had undergone 5–14 generations of inbreeding. All mice were singly housed with α-dri bedding and fed NIH-31 Open Formula mouse sterilized diet in an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility at the National Institutes of Health (Bethesda, MD) under normal 12-hour light/dark cycles.

### Phenotyping Protocol

We adapted our model of HDM-induced AAD (22) to a longitudinal study design in which preCC mice were phenotyped at two different time points, baseline and final, as follows. On Day 9 of the study, we took serum samples from 8- to 12-week-old preCC mice for baseline measurements of serum IgE. We then sensitized mice with 10 μg of the immunodominant allergen from the *Dermatophagoides pteronyssinus* species of HDM, Der p 1, by intraperitoneal injection on Days 22 and 30, followed by challenge with 50 μg of Der p 1, administered by oropharyngeal aspiration, on Day 37. On Day 40, mice were killed, followed by collection of blood and whole-lung lavage fluid. After lavage, lung tissue was snap frozen. Cytokines in lavage fluid were measured using a multiplex bead assay from Millipore (Billerica, MA).

### Gene Expression Analysis and eQTL Mapping

Total lung RNA was isolated from lung tissue and then processed and hybridized to Illumina WG-6v2 arrays (San Diego, CA). Normalization was conducted using robust multi-average normalization with quantile normalization and log2 transformation. Array data have been deposited to Gene Expression Omnibus (GSE51768). eQTL mapping was performed in essentially the same fashion as QTL mapping, with some modifications, as described in the online supplement.

### Quantitative RT-PCR

We measured gene expression of a long, noncoding RNA (9030622O22Rik) in preCC lung RNA samples using quantitative RT-PCR with locked nucleic acid primers designed by Exiqon (Woburn, MA) that amplify one isoform of 9030622O22Rik, ENSMUST00000136555. For normalization, we measured the expression of *Rpl22* and calculated expression using the delta Ct method (33).

### Conditional Correlation Analysis

To identify eQTL that may underlie the QTL for eosinophils, we used conditional correlation. Specifically, we ran chromosome (Chr) 11 QTL scans for eosinophils or gene expression, with covariates, either gene expression or eosinophils, as follows:

Model 1 (eosinophils): eosinophils = QTL + ε

Model 2 (eosinophils|gene expression): eosinophils = QTL + gene expression + ε

Model 3 (gene expression): gene expression = QTL + ε

Model 4 (gene expression|eosinophils): gene expression = QTL + eosinophils + ε

A large drop in the logarithm of the odds (LOD) score ("delta LOD") between models 1 and 2 provides evidence to support a causal relationship (QTL→gene expression→eosinophils) (34). Conversely, a large LOD drop between models 3 and 4 is indicative of gene expression that is reactive to the phenotype (i.e., QTL→eosinophils→gene expression) (34). For these analyses, we had complete data from 132 mice, and we permuted the gene expression data (n = 1000 permutations) data to assess the statistical significance of the LOD drops that we observed.

## Results

### Phenotypes

We employed longitudinal study design in which preCC mice were phenotyped for serum IgE before and after sensitization and challenge with the HDM allergen Der p 1 at two different time points ("baseline" and "final"). Total IgE increased over the course of the experiment (Figure 1A), with a mean increase ("delta IgE") of 347 ng/ml ($P = 2.0 \times 10^{-4}$), demonstrating expected effects of allergen sensitization and challenge across the population. Interestingly, having high baseline IgE generally resulted in little or no increase in antibody concentrations with sensitization and challenge (Figure E1), suggesting that IgE concentration has a plateau value. Eosinophil counts in lung lavage fluid spanned four orders of magnitude (Figure 1B; range, $3.00 \times 10^2 - 3.10 \times 10^6$; mean = $8.00 \times 10^4$), demonstrating a wide range of responses among the preCC population. Eosinophil counts and final IgE were weakly correlated (Figure 1C; Pearson's $r = 0.26$, $P = 0.004$), but there was no correlation

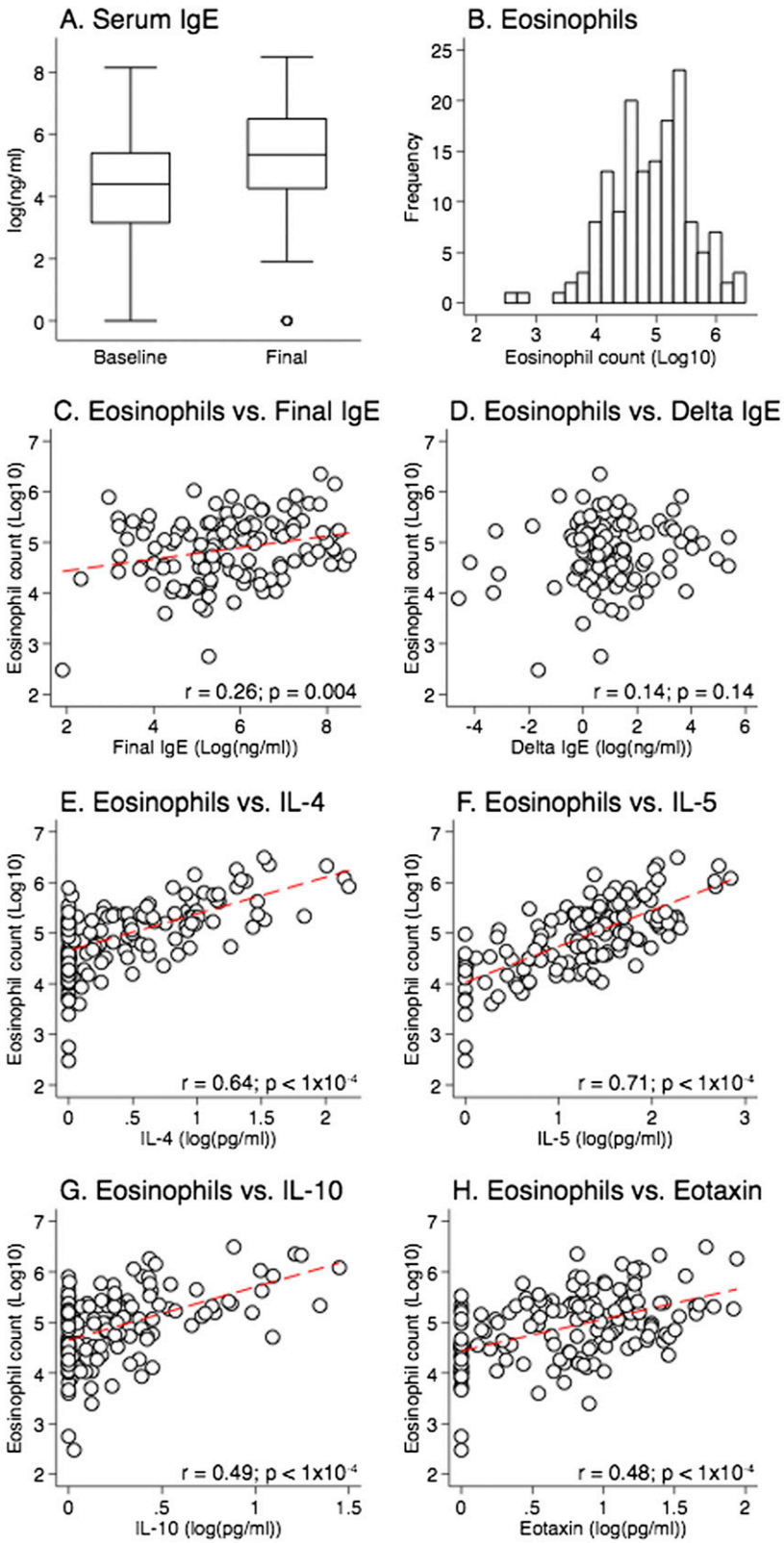


**Figure 1.** Allergic airway disease (AAD) phenotypes in pre–Collaborative Cross (preCC) mice. (*A*) Serum IgE before and after house dust mite (HDM) sensitization and challenge. A total of 12 mice had undetectable IgE levels at the final time point. Each data point represents one mouse. (*B*) Eosinophil counts in lung lavage fluid. (*C–H*) Pairwise correlations between traits. *Dashed red lines* represent linear best fit.

between eosinophil counts and delta IgE (Figure 1D). These results argue against a predominant role for IgE in inflammation in this model of AAD. We did, however, detect very strong correlations between eosinophils and IL-4, -5, and -10 and eotaxin in lavage fluid (Figures 1E and 1F), as expected, based on the known role of these cytokines in T helper type 2 inflammation.

### Genetic Analyses

We conducted genome scans using the preCC data and found a single QTL for eosinophil counts on Chr 11 (*Dpe1*, Der p 1–induced pulmonary eosinophils; 72.904–87.098 megabases [Mb], LOD = 7.0, $P < 0.05$), as shown in Figure 2A. This QTL accounted for 19% of total variation in phenotype. We found a suggestive peak ($P < 0.2$) for final IgE on Chr 11 (42.350–51.087 Mb, LOD = 6.2; Figure 2B) that is distinct from the eosinophil QTL. For delta IgE, we found one significant QTL on Chr 4 (*DsIgE*, 13.950–31.660 Mb, LOD = 7.4; Figure 2C), which explained 26% of variation in delta IgE, and one suggestive QTL ($P < 0.1$) on Chr 8 (119.086–122.313 Mb, LOD = 6.8). The lack of overlap between QTL for IgE and eosinophils further argues against a major role for IgE in this model of AAD. No QTL were detected for any of the cytokines in lavage fluid.

We focused our subsequent analysis on the eosinophil QTL with the goal of identifying candidate genes. The eosinophil QTL on Chr 11 spans 14.2 Mb (72.9–87.1 Mb), and contains 359 genes, 252 of which are protein coding and 107 of which are noncoding RNAs (*see* Table E1 in the online supplement). Among these genes, *Ccl11* (eotaxin) is the most obvious candidate gene based on its known role in eosinophil chemotaxis. We noted that there is no coding region variation in *Ccl11* in CC founder strains, as determined by sequencing data (24); thus, if variation in *Ccl11* underlies the eosinophil QTL, the effect must be mediated through mechanisms other than variation in CCL11 protein sequence variation.

We also asked whether syntenic regions in the human genome contain genes related to relevant phenotypes. The syntenic regions in human genome lie on Chr 17p

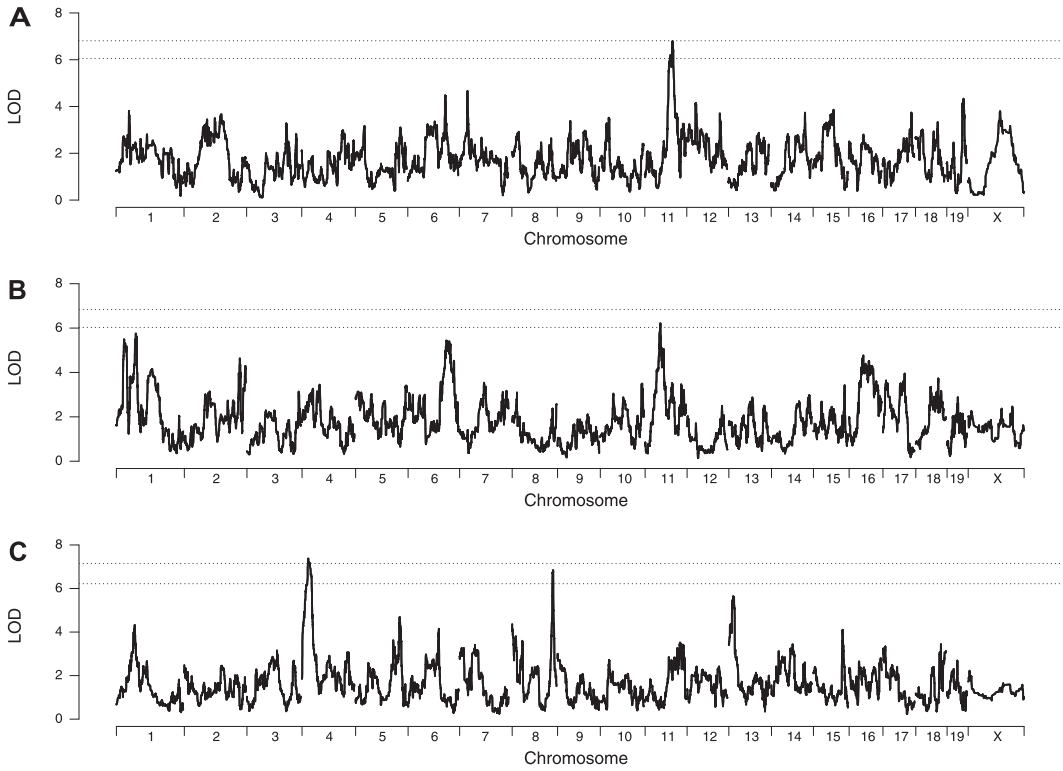


**Figure 2.** Genetic analysis of eosinophils and IgE. Genome scans for eosinophils (*A*), final IgE (*B*), and delta IgE (*C*). The *dashed lines* denote 95 and 80% significance thresholds determined by permutation. LOD, logarithm of the odds.

and 17q, specifically 0–16.5 Mb, 25.5–28.9 Mb, 29.1–36.2 Mb, and 45.6–60.3 Mb. A total of 94 genes in the mouse QTL have human orthologs in these regions (Table E2). Of these, CCL11 is the only gene with prior evidence of association with asthma or other allergic inflammation phenotypes.

### Gene Expression

We turned to gene expression as a way to identify candidate genes for the eosinophil QTL in an unbiased manner. We measured gene expression in whole-lung samples from 138 preCC mice and used these data in two ways. First, we identified expressed transcripts that were correlated with eosinophils, so called quantitative trait transcripts (QTTs) (30), by linear regression. We identified 2,852 genes that were correlated with eosinophil counts at a false discovery rate of 5% (Table E3). As expected, well known cytokines related to AAD are in this list (e.g., *Il4*, *Il10*, and *Il33*), as are genes previously associated with asthma (e.g., *Chi3l4*) and genes associated with mucous cell metaplasia (e.g., *Muc5ac*, *Spdef,* and *Foxa3*). On the whole, this set of QTTs is significantly enriched for a number of immune response pathways (Table E4). We also found that this gene set is enriched for genes associated with alternative activation of macrophages (35) (*Alox15*, *Arg1*, *Ccl17*, *Ccl22*, *Ccl24*, *Ccr5*, *Chi3l1*, *Clec7a*, *Col6a2*, *Fcer2a*, *Il10*, *Il1rn*, *Mertk*, *Mgl2*, *Mmp12*, *Mrc1*, *Retnla*, *Retnlg*, *Rgs1*, *Tgfb1*; $P = 6.47 \times 10^{-6}$), a pathway of renewed interest in AAD (36). Hence, it is apparent that this gene set reflects contributions from both resident lung cell types, such as airway epithelia and infiltrating leukocytes (e.g., macrophages).

We then used the gene expression data to identify eQTL. As described in the online supplement, we focused our eQTL analysis on genes for which the expression array probe did not contain single nucleotide polymorphisms (SNPs), as these can bias eQTL detection (37, 38). Using these data, we identified 6,457 eQTL associated with 4,556 genes (Figure 3A, Table E5). Of these eQTL, the large majority ($n$ = 4,759, 73.7%) were located on the same chromosome as the cognate gene, whereas 1,698 (26.3%) were located on other chromosomes. The latter are indicative of *trans*-regulatory variation (e.g., a polymorphic transcription factor located on one chromosome that binds to the promoter of a gene on another chromosome, and are referred to as *trans*-eQTL).

We characterized eQTL located on the same chromosome as the cognate gene in terms of location relative to the corresponding gene and effect size. In most cases, the eQTL confidence intervals contain the cognate gene ($n$ = 4,065 out of 4,759 [85.4%], which equates to 63% of all eQTL); hence, most eQTL act in *cis*. Among *cis*-eQTL, there is an intuitive relationship between LOD score and distance to the gene–eQTL with the highest LOD scores tending to be located close to the gene (Figure E2). A small number of eQTL ($n$ = 228 [4.81% of eQTL located on the same chromosome as cognate gene]) were located further than 10 Mb away, and we categorized these eQTL as *trans*-eQTL (*see* the online supplement). Overall, our results suggest that regulatory variation located close to genes is a major source of eQTL in the lung.

*Cis*-eQTL were distributed genome wide (Figure 3B), but *trans*-eQTL clustered primarily into two hotspots (often referred to as "*trans*-bands") on Chr 2 (Figure 3C). The existence of *trans*-bands on Chr 2 suggests that these loci harbor master

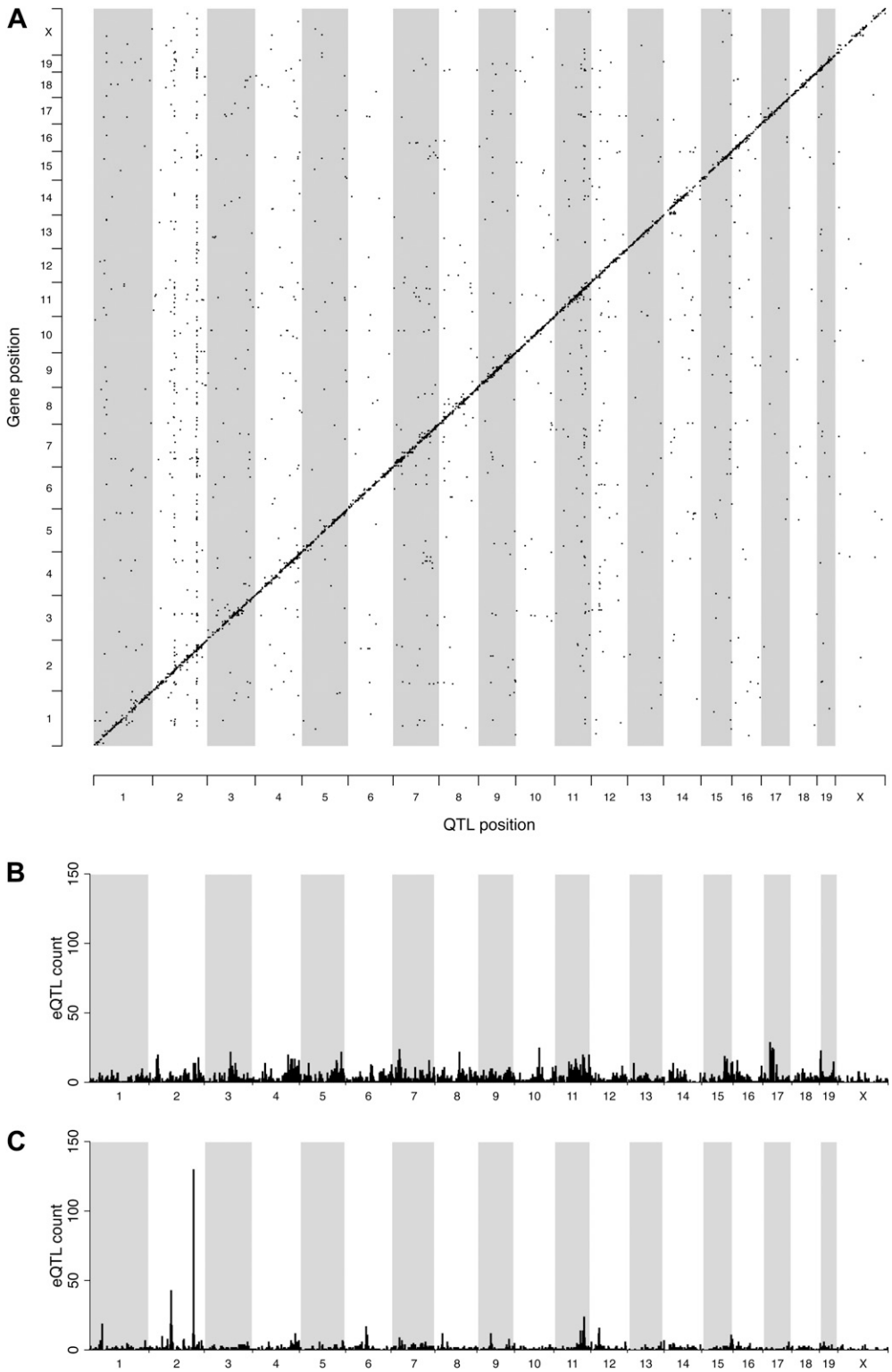


**Figure 3.** Expression quantitative trait locus (eQTL) identification. (*A*) Genome-wide plot of eQTL ($n$ = 6,457). (*B*) Distribution of *cis*-eQTL. (*C*) Distribution of *trans*-eQTL. The *y axis* represents eQTL counts per 2-megabase (Mb) window.

regulators of gene expression in the allergen-challenged lung. Closer examination of these two regions revealed that each was dominated by a single locus. Specifically, markers at 73.0 Mb and 147.6 Mb were associated with the expression of 22 and 41 genes, respectively. We also found that an additional 12 and 33 genes had *trans*-eQTL at a lower significance

threshold of $P$ less than 0.1 at these two loci, respectively. In addition, when we included probes that contain SNPs in this analysis, these numbers increased to 51 and 102, respectively. We asked whether genes with eQTL at these two loci on Chr 2 were enriched among the set of eosinophil QTTs, and found significant enrichment for genes with *trans*-eQTL at the distal locus (147.6 Mb, 1.5-fold enriched; $P = 0.003$), but not for the more proximal locus (73.0 Mb; $P > 0.1$). This focused our interest on the distal locus as a potential regulator of eosinophilic inflammation. We examined the allele effects for all *trans*-eQTL at the distal locus and found that, for the majority of genes (95 out of 102 [93%]), gene expression derived from A/J and C57BL/6J alleles was different than expression derived from the other six founder alleles. A representative gene is shown in Figure 4A. These eQTL allele effects suggested that A/J and C57BL/6J share an allele that is distinct from all other CC founder strains, and is associated with altered gene expression. We looked for a shared allele among these two strains in the *trans*-band confidence interval (Chr2: 146–150 Mb) using sequence data (24) and identified a 53-kb interval spanning 147,807,490–147,860,365 bp in which these two CC founder strains are 99.6% identical. Phylogenetic analysis of this interval shows that A/J and C57BL/6J share a haplotype that is distinct from all other CC founder strains (Figure 4B). A total of 68 SNPs in this region distinguish A/J and C57BL/6J from all other CC founder strains, and thus are potential causal variants for the *trans*-band (Table E6). All 68 SNPs lie within the putative long noncoding RNA (lncRNA) 9030622O22Rik.

We then asked whether variation in expression of this putative lncRNA could underlie the *trans*-band. We measured the expression level of one isoform of the lncRNA (ENSMUST00000136555) using quantitative RT-PCR and found that it had an eQTL that overlapped with the region on Chr 2 where A/J and C57BL/6J share a haplotype (147,417,620–150,318,592 bp, LOD = 11.45). We found that alleles derived from A/J and C57BL/6J were also associated with lower lncRNA eQTL expression (Figure E3), but also that the CAST/EiJ allele was associated with relatively low lncRNA expression. Hence, the allele effects for lncRNA eQTL and genes in the *trans*-band are not fully congruent. In addition, we examined

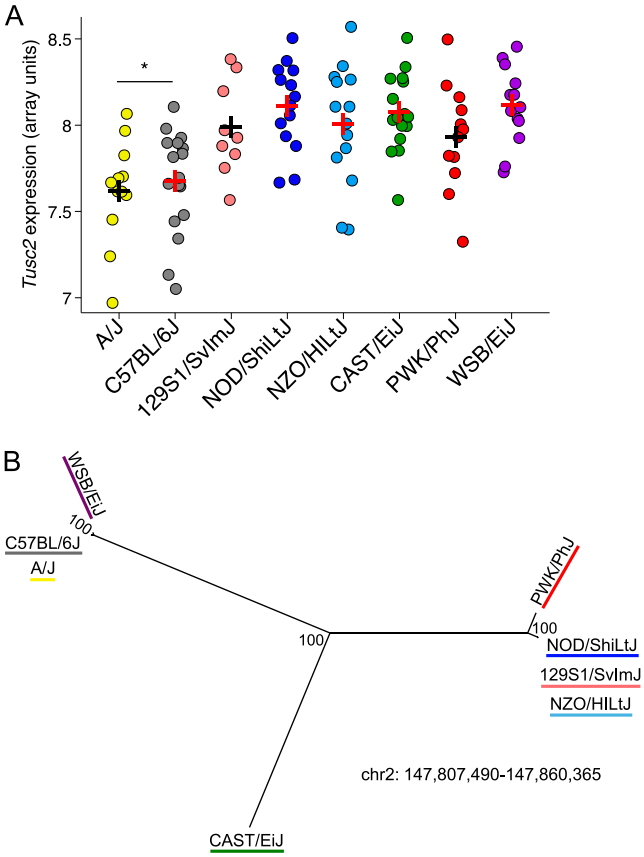


**Figure 4.** Fine mapping of a *trans*-band on Chr 2. (*A*) Allele effects plot for a representative gene in the *trans*-band, *Tusc2*, showing separation of A/J and C57BL/6J allele effects from the other CC founder strain alleles. Array-based measurements of *Tusc2* expression from preCC mice partitioned by their founder haplotype at the peak locus for the eQTL. For the sake of clarity, only homozygous mice are shown ($n = 99$). Each data point represents a preCC mouse and *plus symbols* (+) represent the means for each genotype. $P < 0.05$ for contrast between A/J. (*B*) Maximum likelihood phylogenetic tree for the region of shared ancestry within the *trans*-band confidence interval. The tree was constructed using single nucleotide polymorphism data from Sanger MGP Wellcome Trust Sanger Mouse Genomes Project (*see* the online supplement), and the numbers indicate bootstrap support estimates for each branch. Note that C57BL/6J and A/J haplotypes are separated by a short branch (with 100% bootstrap support) from WSB/EiJ. This indicates that the number of genetic variants that causes lower gene expression among CC mice with A/J or C57BL/6J haplotypes at this locus is limited to a small number ($n = 68$; *see* Table E6). chr, chromosome.

pairwise correlations between lncRNA expression and the expression of genes in the *trans*-band, and found no correlations. Collectively, these results indicate that, even though the lncRNA has a colocalized eQTL, it is unlikely to be the source of the *trans*-band, at least by means of a direct relationship between its expression and the expression of genes in the *trans*-band.

### Identification of Candidate Genes for the Eosinophil QTL (*Dpe1*) Using Gene Expression Data

Finally, we used our gene expression to identify candidate genes for the eosinophil QTL. Several studies have found that variants associated with disease risk in humans are often eQTL and imply that disease risk is a function of variation in gene expression, and one study used lung eQTL to identify gene networks associated with asthma (32). Therefore, we asked whether lung eQTL could explain eosinophil recruitment responses to allergen. First, we identified genes that met two criteria: (*1*) their expression was linearly correlated with eosinophils (i.e., the gene is a QTT); and (*2*) they had an eQTL within the eosinophil QTL confidence interval (Chr 11: 72.904–87.098 Mb). A total of 18 genes met these criteria: *2610507B11Rik*, *Atad5*, *Ccl11*,

*Ccl7*, *Coil*, *Inpp5k*, *Lig3*, *Mmp28*, *Poldip2*, *Ppm1d*, *Ppm1e*, *Rpa1*, *Slc6a4*, *Ssh2*, *Supt4h1*, *Tax1bp3*, *Tlcd1*, and *Tlcd2*.

We then used conditional correlation to evaluate whether the variation in expression in any of these genes could underlie the eosinophil QTL. Accounting for *Ccl11* expression did not reduce the LOD score as would be expected if there were a direct relationship between the QTL, *Ccl11* expression, and eosinophil recruitment. In fact, the LOD score increased from 5.6 to 8.2 ($P < 0.001$, Table 1), which is indicative of a more complex (indirect) relationship. We found the greatest drop in the LOD score was associated with the gene *Tlcd2* (LOD dropped from 5.6 to 3.1; $P < 0.001$; Table 1). Because *Tlcd2* expression was negatively correlated with eosinophil counts (Table 1), our results suggest that *Tlcd2* is a candidate negative regulator of eosinophil recruitment responses to allergen.

## Discussion

HDM sensitization and challenge produced a wide range eosinophil recruitment responses in preCC mice, and this diversity facilitated the identification of a QTL for eosinophil counts. It is interesting that despite the fact the distribution of eosinophil counts is suggestive of a polygenic basis of inheritance in which many loci contribute to the phenotype, we only found one QTL associated with eosinophil counts. Additional QTL may be detectable with greater power, and the availability of biological replicates for inbred CC lines will facilitate this. In addition, we detected significant changes in IgE over the course of the experiment. However, these changes were not correlated with eosinophilic inflammation. These results are similar to those of a human genome-wide association study that detected loci associated with asthma and loci associated with IgE, but the two were not the same (14).

We identified a small set of priority candidate genes for the eosinophil QTL by merging the phenotype, gene expression, and eQTL data. We tested whether the expression of any single gene could underlie eosinophil recruitment responses using a simple linear model. Unexpectedly, we did not find evidence for a direct relationship between the QTL, *Ccl11* (eotaxin) expression, and eosinophil counts. Rather, our results point to a more complex relationship that opposes the direct effect of *Tlcd2*, which we suggest is a novel candidate gene. *Tlcd2* is an intriguing candidate gene in that TLC domain–containing proteins are thought to act as sphingolipid sensors (39) that are involved in lung inflammation (40, 41), but little else is known about this gene at present. We did not observe changes in *Tlcd2* gene expression due to allergen sensitization and challenge in our previous study (22), nor is there evidence of differential expression in other lung disease models for which gene expression data is available in National Center for Biotechnology Information's Gene Expression Omnibus database. Thus the manner by which variation in *Tlcd2* gene expression affects allergic inflammation is an important question to address in future studies, as is the question of which lung cell type(s) express this gene.

We detected a large number of lung eQTL ($n$ = 6,457, corresponding to 4,556 genes). This indicates that the expression of many

**Table 1.** Results of Conditional Correlation Analyses to Identify Candidate Genes for the Eosinophil Quantitative Trait Locus (*Dpe1*)

| | | | | Eosinophil Correlation | | eQTL | | | Conditional Analysis† | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene Symbol | Entrez Gene ID | Transcript | Probe | Pearson's *r* | *P* Value | Peak (*Mb*) | LOD | EOS LOD* | EOS\|GEX | GEX\|EOS |
| *Tlcd2* | 380,712 | NM_027249 | ILMN_2814385 | −0.40 | 1.91E−06 | 73.353 | 35.90 | 5.60 | 3.12 | 32.13 |
| *Tlcd2* | 380,712 | NM_027249 | ILMN_1249828 | −0.41 | 8.23E−07 | 76.537 | 22.38 | 5.60 | 3.27 | 16.89 |
| *Tlcd2* | 380,712 | NM_027249 | ILMN_1213090 | −0.27 | 1.84E−03 | 75.073 | 27.72 | 5.60 | 3.88 | 23.89 |
| *Ppm1d* | 53,892 | NM_016910 | ILMN_1226504 | 0.33 | 1.18E−04 | 83.453 | 16.53 | 5.60 | 3.99 | 15.65 |
| *Mmp28* | 118,453 | NM_172797 | ILMN_1218211 | −0.24 | 5.00E−03 | 83.408 | 16.24 | 5.60 | 4.17 | 14.06 |
| *Ccl7* | 20,306 | NM_013654 | ILMN_2835117 | 0.32 | 1.65E−04 | 83.257 | 15.21 | 5.60 | 4.90 | 15.21 |
| *Tax1bp3* | 76,281 | NM_029564 | ILMN_2746086 | −0.26 | 2.89E−03 | 73.353 | 10.57 | 5.60 | 4.97 | 10.42 |
| *Slc6a4* | 15,567 | NM_010484 | ILMN_2713714 | −0.26 | 2.90E−03 | 75.265 | 17.94 | 5.60 | 4.99 | 17.27 |
| *Poldip2* | 67,811 | NM_026389 | ILMN_2543314 | −0.24 | 4.76E−03 | 76.453 | 10.63 | 5.60 | 5.16 | 10.60 |
| *Supt4h1* | 20,922 | NM_009296 | ILMN_1247505 | −0.31 | 3.73E−04 | 87.098 | 30.71 | 5.60 | 5.47 | 30.75 |
| *Ssh2* | 237,860 | NM_177710 | ILMN_1228331 | −0.24 | 4.78E−03 | 83.444 | 10.37 | 5.60 | 5.49 | 10.24 |
| *Lig3* | 16,882 | NM_010716 | ILMN_1256377 | 0.33 | 8.96E−05 | 82.222 | 6.87 | 5.60 | 5.52 | 8.60 |
| *Inpp5k* | 19,062 | NM_008916 | ILMN_2584558 | −0.33 | 1.41E−04 | 73.872 | 9.76 | 5.60 | 5.53 | 9.98 |
| *2610507B11Rik* | 72,503 | NM_001002004 | ILMN_3014965 | 0.30 | 4.74E−04 | 77.338 | 9.15 | 5.60 | 5.65 | 8.21 |
| *Coil* | 12,812 | NM_016706 | ILMN_2746210 | −0.25 | 3.89E−03 | 84.286 | 11.46 | 5.60 | 5.65 | 11.51 |
| *Ccl7* | 20,306 | NM_013654 | ILMN_2771176 | 0.31 | 2.67E−04 | 81.456 | 10.47 | 5.60 | 5.86 | 9.82 |
| *Tlcd1* | 68,385 | NM_026708 | ILMN_2781458 | −0.24 | 6.02E−03 | 78.007 | 11.01 | 5.60 | 5.87 | 11.73 |
| *Ppm1e* | 32,0472 | NM_177167 | ILMN_2702374 | 0.30 | 4.33E−04 | 83.474 | 10.67 | 5.60 | 5.99 | 11.44 |
| *Rpa1* | 68,275 | NM_026653 | ILMN_2750801 | 0.32 | 1.71E−04 | 75.265 | 9.63 | 5.60 | 6.51 | 11.68 |
| *Atad5* | 237,877 | NM_001029856 | ILMN_2534259 | 0.38 | 5.94E−06 | 81.302 | 18.06 | 5.60 | 7.21 | 19.12 |
| *Ccl11* | 20,292 | NM_011330 | ILMN_2647757 | 0.30 | 5.12E−04 | 82.000 | 17.07 | 5.60 | 8.21 | 19.33 |

*Definition of abbreviations*: EOS, eosinophils; eQTL, expression quantitative trait locus; GEX, gene expression; LOD, logarithm of the odds; Mb, megabases.
*LOD score for EOS on chromosome 11. This analysis was based on complete data from 132 mice. In this population, the LOD score was 5.6 (versus 7.0 from the analysis of 151 mice with eosinophil data).
†*See* Materials and Methods for description of conditional models.

genes in the lung is controlled in part by genetic variation. Based on our study design, we cannot estimate what fraction of variation in gene expression is due to allergen challenge versus genetic variation or a combination thereof (i.e., gene-by-HDM interactions). In the future, it will be worthwhile to address this, as well as categorizing eQTL by whether they affect gene expression only in the naive/unchallenged lung, only in the allergen-challenged lung, or both ("dynamic" versus "static" eQTL [43]). It will be particularly interesting to investigate which type of eQTL are more important in determining response to allergen. Intuitively, we would expect that the eQTL that regulate the induction/repression of gene expression after allergen challenge are more important, but this hypothesis should be tested directly, as this does not appear to be the case for *Tlcd2* and eosinophilia.

Whole lung is, of course, a heterogeneous tissue, and contains numerous cell types, particularly in the context of AAD in which leukocytes are recruited. This has both positive and negative ramifications for eQTL identification and characterization. On the positive side, it is likely that we detected many eQTL that would not have been detected in naive or unchallenged lung, because they are expressed in infiltrating leukocytes. On the other hand, identifying which specific cell type(s) is the source of any particular eQTL remains a significant challenge, but is crucial to gaining further understanding of the biology related to AAD.

One of the most interesting features or our eQTL study was the identification of numerous *trans*-eQTL. The relative abundance of *trans*- versus *cis*-eQTL may be a function of the allergen model, as others have observed that *trans*-eQTL tend be more common in models where an environmental perturbation is applied (44). We also found that *trans*-eQTL clustered to specific genomic regions, most notably a locus on Chr 2 that was associated with expression of approximately 100 genes, many of which were correlated with eosinophil counts. We note, however, that the locus on Chr 2 was not itself QTL for eosinophil counts.

Some have expressed concern that *trans*-bands may be an artifact of hidden confounding in the data (45), but given that we employed randomization procedures to avoid such confounding, we do not think this is the case for this locus. We conducted *post hoc* analysis to test whether potential confounders or batch effects (RNA extraction date, hybridization date, or enrichment of a particular genotype and batch) could have resulted in spurious associations, and found no evidence of such biases. Based on the consistent allele effects that we observed, we narrowed the confidence interval for the *trans*-band to a 53-kb region containing a single gene, a putative lncRNA. lncRNAs are known to regulate gene expression, both in *cis* and in *trans* (46), and affect lung phenotypes (42); hence, we evaluated whether the lncRNA could underlie the *trans*-band. Although we found that this lncRNA also has an eQTL at the same locus, its expression did not correlate with genes in the *trans*-band, and thus it appears unlikely that the lncRNA explains the *trans*-band exclusively through variation in expression. It remains to be determined, however, whether the 68 SNPs in this putative lncRNA that distinguish A/J and C57BL/6J alleles from the other six CC founder alleles could give rise to structurally different RNAs that differentially control gene expression. Alternatively, this locus may contain other regulatory elements that control gene expression in *trans*.

It is also interesting to note that the *trans*-band on Chr 2 lies in a region that appears to be a QTL hotspot for other phenotypes. Previous studies have found QTL in this region for metabolism-related phenotypes, including blood glucose level (47), plasma levels of sterol (48), and growth and body fat parameters (49, 50). In addition, QTL for immune-related phenotypes, including natural killer T cell number in the thymus (51), experimentally induced induced colitis (52), and chemically induced bladder tumorigenesis (53), also map to this locus. It will be interesting to see whether the genes with *trans*-eQTL at this locus may be related to those phenotypes. A QTL for bronchial hyperresponsiveness also contained this locus (54). However, the bronchial hyperresponsiveness QTL was identified using C57BL/6J and A/J strains, the two strains that share a haplotype at the locus that we identified. Therefore, the *trans*-band locus cannot explain the bronchial hyperresponsiveness QTL.

In summary, we used a genetically diverse mouse population to quantify correlations between AAD phenotypes and identify genetic loci that control AAD phenotypes, including gene expression in the lung. We identified a candidate gene for eosinophilic inflammation caused by HDM using the gene expression data. Finally, our eQTL data also provide insights about gene regulation in the context of allergen challenge. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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