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SNPs in Region of NF-Kappa-B Gene Associated with Expression of Immune-Related Genes

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Summary and Implications
Selection to enhance immune response is difficult. Gene expression was assessed in two advanced intercross lines of chickens for various immune-related genes. Single nucleotide polymorphisms, SNPs, were identified in regions local to each gene and around a distant transcription factor, NF-Kappa-B, NFKB. These SNPs were used to identify expression QTL, eQTL, to aid in selection of immune gene expression. These eQTL may provide effective resources to which marker assisted selection may be applied.

Introduction
Differences in expression of immune-related genes are important for identifying variation in disease resistance. Expression QTL studies investigate associations between DNA sequence variation markers, e.g. SNPs, and varying levels of gene expression. SNPs from local regions of a gene, or cis-acting, were chosen along with distant, or trans-acting, SNPs surrounding the NFKB region. NFKB is a known factor in regulating many genes, including some responsible for an immune response.

Materials and Methods
Advanced intercross lines (AIL) of chickens were established at ISU from mating one broiler male to Leghorn and Fayoumi hens. Cecum and spleen tissues were taken from AIL F8 chicks of both lines one week after challenge with Salmonella enteritidis. Genomic DNA of each chick was genotyped using the chicken 3K SNP panel. Expression data were measured by quantitative polymerase chain reaction, Q-PCR, for 26 immune-related genes. Expression levels were corrected for starting RNA concentration and efficiency.

A consensus location for each gene was found using a variety of genomic tools. In order to find SNPs with cis-acting associations, six SNPs were located around each gene, with an attempt made to flank three SNPs on each side of the gene. Because of the known regulatory role of NFKB, six SNPs located around this region were also assessed for their trans-acting associations.

Each SNP genotype was valued as 0, 1, or 2. The statistical program JMP was used for each line to determine if a SNP was significantly associated with gene expression by the following model.
Gene expression = SNP + Sex + Necropsy Period + Sex*Necropsy Period + Sire + Plate + Room

The SNP, sex and necropsy period effects were considered fixed while sire, plate and room were considered random effects. The interaction of sex and necropsy period was excluded when the p-value was greater than 0.10.

Results and Discussion
Significant eQTL SNPs were found for both lines and tissues, using cis- and trans-acting SNPs (Figure 1). Several of the significant SNPs were unique to one line or tissue. In total 18 genes had significantly associated SNPs. Significant eQTL findings for the trans-acting SNPs located around the NFKB region suggest presence of regulatory element(s) in this region, which may be good locations to search for causal eQTL mutations.

Knowledge about eQTL for these genes will help to generate more refined eQTL searches and apply them to breeding programs. The significant associations between SNP and eQTL were found across different genetic background with differences in segregating SNP populations. This demonstrates that other populations have the potential to locate and utilize these eQTL SNPs successfully.

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Figure 1. Number of significant SNPs, both cis and trans-acting, found for each tissue and line.