Chicken *Gallinacin* Gene Cluster Associated with *Salmonella* Colonization in Two Advanced Intercross Lines

A.S. Leaflet R2215

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Summary and Implications

Two related advanced intercross lines were analyzed for association of *Gallinacin* genotypic variation with *Salmonella enteritidis* (SE) burden levels in the spleen and cecum. Four *Gallinacin* genes were associated with bacterial load in the cecal content (*GAL3, GAL11, GAL12,* and GAL13) and one gene with SE bacterial burden in the spleen (*GAL5*). These results strongly support a role of the Gallinacins as defense molecules against enteric pathogens.

Introduction

The young chicken's immune system is immature and unable to provide adequate protection against bacterial pathogens. The *Gallinacin* proteins, known as beta defensins in mammals, play a vital role in the innate immune response to bacterial infections. The *Gallinacin* genes in poultry are well suited for candidate gene analysis due to their genomic organization, tissue expression, and their roles in the immune response to bacterial pathogens.

Materials and Methods

Two related eight-generation advanced intercross lines of chickens were utilized. Birds from two hatches were inoculated with SE at one day of age, and spleens and ceca were collected at one week. Bacterial burden in the organs was quantified. Genomic DNA was amplified through PCR. SNP-specific extension primers were designed to amplify across one intronic SNP for each of the 13 *Gallinacin* genes. Gene products were multiplexed by altering lengths of the extension primers. Reactions were run using an ABI PRISM SNaPshot Multiplex Kit in three separate pools. Final genotypes were recorded utilizing an ABI PRISM 3100 Genetic Analyzer at the ISU DNA Sequencing and Synthesis Facility.

Association between each *Gallinacin* gene SNP in the advanced intercross line birds and the SE bacterial count was determined using the following linear mixed model:

$$Y_{ijklmnpq} = \mu + \text{Gene Allele}_i + \text{Sex}_j + \text{Necropsy Day}_k + \text{Body Weight}_l + \text{Sire}_m + \text{Dam(Sire)}_n + \text{Room}_p + \text{Hatch}_q + \text{Hatch*Room}_{pq} + \text{Gene Allele*Sex}_{ij} + \varepsilon_{ijklmnpq}$$

where Y is the response variable from each individual advanced intercross bird. Each line was analyzed separately.

Results and Discussion

Ninety-five SNPs were initially identified yielding a rate of 14.8 SNP/kilobase of DNA. Four *Gallinacin* gene SNPs were significantly associated with cecal SE bacterial burden (*GAL3, GAL11, GAL12,* and *GAL13*). One *Gallinacin* gene (*GAL5*) was associated with the spleen bacterial burden. The other *GAL* genes had no association with either spleen or cecum bacterial burden. The positive associations should be considered within the context of each line-by-tissue combination (Table 1). In this context, there is very strong evidence for *Gallinacin* SNP effect on cecal colonization in the Broiler * Leghorn (Br * Leg) advance intercross birds (4 of 10 tests significant at $P<0.05$).

To address rising concerns over the biosafety of food products, researchers are attempting to modify the immune response of food animals through genetic selection. Through host genetic modulation, it may be possible to enhance the innate immune system's response to SE infection. The Br * Leg birds had four *Gallinacin* genes associated with SE cecal burden, while the Broiler * Fayoumi (Br * Fay) birds had one gene associated with SE burden in the spleen (Figure 1).

The SNaPshot technology used in this study provides a scalable SNP typing system that can be modified to genotype anywhere from 2 to 14 genes in one reaction. SNaPshot is a highly flexible system and requires few addition primers in order to genotype SNPs of interest. This technology provides a larger number of usable SNPs for association studies than conventional PCR-RFLP candidate gene tests because SNaPshot is not limited to the availability of restriction endonuclease cut sites.

This study also demonstrated the usefulness of the advanced intercross mating strategy in disease association studies. This study identified two possible recombinational hot spots in a relatively small region of the chicken genome. Under either F_2 or backcross designs, these hotspots would not have been detected, due to large levels of linkage disequilibrium.

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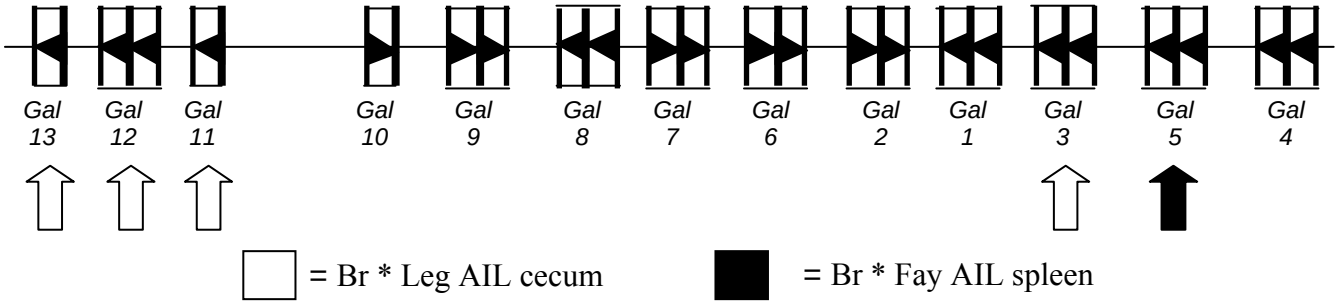
Figure 1. *Gallinacin* genome organization and *Salmonella enteritidis* bacterial burden associations by line and tissue.



The figure shows the *Gallinacin* gene placement and direction as on Chromosome 3 in the chicken genome. The arrows indicate genes with significant single nucleotide polymorphism associations with *Salmonella enteritidis* bacterial burdens ($P \leq 0.05$). White arrows represent genes within the Broiler * Leghorn (Br * Leg) line with significant associations with cecal bacterial burden. The solid black arrow indicates the gene that is associated with the cecal bacterial burden within the Broiler * Fayoumi (Br * Fay) line.

Table 1. Number of significant tests, by line and tissue studied, compared to number of possible tests.

	Cecum	Spleen
Br * Leg	4/10	0/10
Br * Fay	0/13	1/13