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A Genome Wide Association Analysis Confirms a Complex, Polygenic Determinism of Resistance to *Salmonella* Carrier-State in Chicken

F. Calenge¹, A. Vignal², B. Bed'Hom¹, P. Kaiser³, P. Velge⁴, C. Beaumont⁵

¹INRA UMR1313 GABI, Jouy-en-Josas, France, ²INRA UMR GenPhySE Castanet-Tolosan, France, ³Roslin Institute, Edinburgh, UK, ⁴INRA UMR1282 ISP, Nouzilly, France and ⁵INRA UR83 URA, Nouzilly, France,

ABSTRACT: *Salmonella Enteritidis*, carried by apparently healthy chickens, is a threat to human health due to the consumption of contaminated meat or eggs. In this study, a genome wide association study was performed using a 60 K SNP chip to identify genomic regions associated with resistance to *Salmonella* carrier-state. We genotyped 209 chicks issued from an F8 advanced intercross line between two experimental White Leghorn inbred lines and experimentally infected with *S. Enteritidis*. Their carrier-state was assessed by measuring the fecal count of *S. Enteritidis* 4 weeks after infection. After quality controls, 22,557 markers could be used for a genome wide association analysis performed with the plink software. Half of the markers associated with the highest probabilities were located in genomic regions previously identified for their association with resistance to *S. Enteritidis* carrier-state or resistance to *S. Typhimurium* in chicken. This study therefore confirms and refines the location of several regions of interest.

Keywords:

Chicken
Salmonella Enteritidis
Carrier-state
GWAS

Introduction

Salmonellosis caused by the enterotype *Salmonella* Enteritidis does not harm adult chickens, which are usually able to tolerate these bacteria without displaying disease symptoms. But this carriage is responsible of a silent propagation of *S. E.* in poultry flock and of the contamination of meat and eggs, which are one of the main sources of human contamination. Reducing chicken carriage of *S. E.* through the selection of chicken lines more resistant to carrier-state would be a useful alternative to prophylactic measures, which are not always sufficient to control *S. E.* propagation in chicken flocks (for a review, see Calenge and Beaumont, 2012).

Previous studies have shown that resistance to *S. E.* carrier-state is under a complex, polygenic control. Several candidate genes and quantitative trait loci have been identified, each of them causing a weak effect varying according to animal's age, the parental lines studied and the QTL detection method used (Calenge et al, 2010; 2012). Several QTLs were identified in an F2 progeny derived from two White Leghorn experimental inbred layer lines using microsatellites (Tilquin et al, 2009). A more recent QTL analysis was performed using 384 informative SNP markers (Calenge et al, 2011, Tran et al, 2012). Among others, a dominant QTL with medium effect was observed

on chromosome 14 using regression analysis (Calenge et al, 2010). Beside, a genome-wide QTL on chromosome 2 already identified by Tilquin et al (2009) with a small set of microsatellites was confirmed by Tran et al (2012) using a maximum likelihood method with the panel of 384 informative SNP. In order to confirm these results and to refine the QTL locations, we developed a population of advanced intercross lines (AIL) from the F2 progeny previously studied, which we genotyped with a 60 K SNP chip.

Materials and Methods

Animals. 209 F8 AIL were produced from a F2 progeny (Calenge et al 2011) derived from the experimental White Leghorn inbred lines N and 6 (Bumstead and Barrow, 1988). At each generation, animals from distinct families were crossed, as specified in Darvasi and Soller (1995). Animals were reared at PEAT, Nouzilly (Pôle d'Expérimentation Avicole de Tours).

Experimental infections. Animals were experimentally infected at PFIE (Plate-forme d'Infectiologie Expérimentale), Nouzilly. One-week old birds were orally infected with 5×10^4 bacteria from the *S. E.* phage type 4 (PT 4) strain 1009, which is a spontaneous nalidixic acid and streptomycin resistant mutant strain. Feces were individually collected 4 weeks after infection by cloacal sampling to assess the number of colony forming units (cfu) per gram of feces. Data are expressed as log(cfu)/g feces.

GWAS analysis with plink. A 60k Illumina SNP chip (Groenen et al 2011) was used to genotype the 209 F8 Nx6 AIL. Genotypes were obtained at Labogena, Jouy-en-Josas. Markers with minor allelic frequencies (MAF)>0.05, or with a frequency of missing genotypes >0.2, or in Hardy-Weinberg disequilibrium were removed from the analysis. Individuals with more than 10% of missing genotypes were removed from the analysis. A simple association analysis was then performed using the `-assoc` command of plink.

Results and Discussion

Experimental infections. All animals were successfully infected, as attested by the presence of *S. E.* in the collected feces (Figure 1). The mean infection load was 5.00 ± 1.34 log(cfu)/ g feces with values ranging from 1.35 to 7.47 log(cfu)/g feces.

GWAS analysis. A total of 32,033 SNPs were removed from the analysis due to weak MAF. Most of them were homozygous markers. A high rate of homozygous markers was expected due to the inbred nature of the chicken lines studied on the one hand, and to the similar genetic origin of both lines (White Leghorn Layer lines) on the other hand. Only one individual showed more than 10% missing genotypes and 3045 markers displayed more than 20% missing data. Finally, 22,557 markers could be used for the genome-wide analysis, which results are shown on figures 2 and 3. The maximum probability of association of a marker with variations for the SE fecal load reached 2.5×10^{-5} (chromosome 4), *i.e.* not a very high value for such an analysis. Nevertheless, this result is concordant with previous results showing a very complex, polygenic control of resistance to S.E., with several candidate genes and QTL causing only weak and relatively unstable effects.

When fixing an arbitrary probability threshold of 10^{-3} , 12 regions are found associated with resistance to SE carrier-state: one on chromosome 4, one on chromosome 10, one on chromosome 12, five on chromosome 2, three on chromosome 1 and two on chromosome 6. Markers with the highest probabilities are located on chromosome 4 (at 88.1-88.2 Mb; $p=2.5 \times 10^{-5}$ to 4.69×10^{-5}). Interestingly, this region has already been identified in the F2 progeny from which the AIL are derived (89.7-94 Mb; Calenge et al 2011), using the fecal load 5 weeks p.i.. Nevertheless, it is possible that they are statistical artefacts, since they are isolated: there are no adjacent markers displaying high probabilities. Markers belonging to a second region located between 16.0 and 16.4 Mb on chromosome 10 are associated with the second highest set of probabilities, ranging between 1.05×10^{-4} and 3.36×10^{-4} . Intriguingly, this region has never been associated previously with resistance to S.E. carrier-state.

Although the probability threshold of 10^{-3} is arbitrary, it is interesting to note that six of the other regions associated with probabilities under this threshold are located close to already identified regions, as summarized in table 1. The two most striking co-localisations are two regions on chromosome 2 (Figure 3). One of these regions is located between 34 and 35 Mb on chromosome 2, that is close to or at the position of the only genome-wide QTL identified in the F2 progeny from which the AIL used in this study are originated (confidence interval: 31.8 – 38.3 Mb; Tran et al. 2012). In addition, several interesting candidate genes known for their involvement in immune processes are located in this interval (Tran et al, 2012). The second one is located at 21-22 Mb and is located close to a QTL for resistance to intestinal colonization by *S. Typhimurium* (with a peak at 20 Mb; Fife et al, 2010). Although Tran et al. (2012) discussed the possibility that both QTLs, the first one at 20 Mb and the second one between 31.8 and 38.3 Mb, are in fact the same QTLs, our data showing two distinct peaks (Figure 2) rather support the existence of two distinct QTLs.

At the opposite, several of the QTLs identified by Calenge et al (2011) with a regression method and by Tran et al (2012) with a maximum likelihood method in the F2 progeny from which the AIL used in this study were derived were not confirmed with this analysis. Many

reasons can be put forward to explain this lack of stability. First, the present study used only one of the traits previously used to assess carrier state: fecal load 4 weeks pi. Previous studies also used fecal and cecal loads 5 weeks pi. In addition, QTL causing small effects are often unstable, especially when using different methods, which is our case (regression in Calenge et al, 2011, maximum likelihood in Tran et al, 2012 and Wald test with plink). However the most significant QTL was observed again, showing that this region deserves further investigations.

Conclusion

These preliminary results confirm that the genetic control of resistance to carrier state in White Leghorn lines is complex, polygenic, with many regions causing weak effects. Nevertheless, co-localisations with previously identified regions, especially on chromosome 2, strengthen the interest of these regions for more refined analyses.

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Table 1: Positions on the genome (Chr: chromosome) of QTLs for resistance to *Salmonella* previously identified (SE: *Salmonella* Enteritidis; ST: *Salmonella* Typhimurium). Traits used for previous studies and references are also indicated.

Chr	Current study	Previous studies		
	Position (Mb)	Position (Mb)	Trait	References
1	35.9	33.57	Fecal load (CSW4)	Tilquin 2005; Calenge 2010
1	198	196.2-200.9	Fecal load (CSW5)	Calenge 2011
2	20.6	20	Cecal load	Fife 2010
2	34.6-35.8	31.8-38.3	Cecal load (CAEC)	Tran 2012
4	88.1-88.2	89.7-94	Fecal load (CSW5)	Calenge 2011
6	10.4	10.09	Vaccine response	Kaiser 2002

Figure 1: Distribution of individuals belonging to the Nx6 F8 AIL population according to their fecal load in *Salmonella* Enteritidis (expressed in Log(ufc)/g feces).

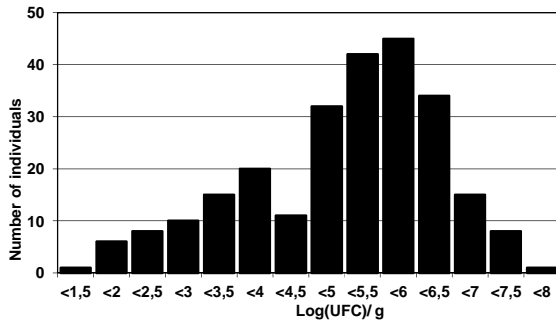


Figure 2: Results of the GWAS analysis performed in the Nx6 F8 AIL progeny using the fecal loads data and the 60k SNP chip.

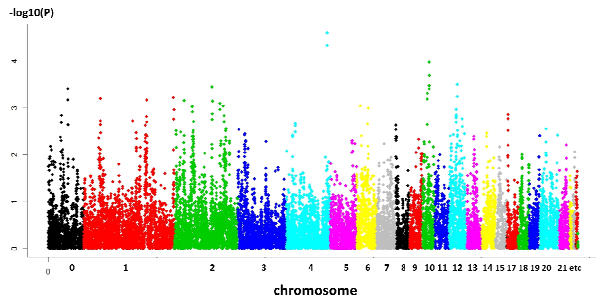


Figure 3: results of the GWAS analysis obtained for chromosome 2. The two circles show the markers located in previously identified regions.

