815. Effect of a *TLR1A* polymorphism on natural antibodies in poultry

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Abstract

The aim of the current study was to confirm and characterise the effect of a polymorphism in the Toll-like receptor 1 family member A (*TLR1A*) gene on levels of natural antibodies (NAb) in poultry. The study population consisted of a base population and 7 generations of birds from a High and Low selection line. Genotypes for a polymorphism in *TLR1A* were available for chickens in generations 5, 6 and 7. The TLR1A polymorphism had large and significant effects on total NAb levels (IgTotal) and IgM NAb, with full dominance of the *TLR1A* C allele. Significant (*TLR1A*×Generation) interactions were detected for IgM and IgTotal. The frequency of the *TLR1A* C allele was 0.45 in the base population (based on imputed genotypes), 0.66 and 0.04 in generation 7 of the High and Low line, respectively. Results confirm the major effect of this polymorphism in *TLR1A* on NAb levels suggest high sensitivity of the effect to environmental factors.

Introduction

Diseases pose a serious and increasing challenge to livestock production. In recent years, poultry layer production in several countries shifted from battery cages to free roaming housing systems. This increased the number of contacts between chickens, resulting in a higher risk of pathogen spreading. Another recent trend is the restricted use of antibiotics in order to reduce the risk of antibiotic resistance. These developments require other ways to limit the impact of diseases on poultry production and selective breeding might be part of an integrated animal health management approach.

Selective breeding for disease resistance is challenging. Preferably, selection should not be for resistance to one or a few diseases, but for enhanced immune competence resulting in a broad (general) disease resistance. Furthermore, traits that reflect general disease resistance should be easy and cheap to measure and ideally early in the animal's life. NAb could be of value in selection for general disease resistance. NAb show a broad specificity repertoire and act as a first line of defence against infections (Palma *et al.,* 2018). NAb can be measured relatively easily and at a young age in blood samples. We showed in previous studies that selection for high NAb against keyhole limpet hemocyanin (KLH) leads to a higher survival in chickens which are challenged with avian pathogenic *Escherichia coli* (Berghof *et al.,* 2019). Therefore, selection for high NAb levels might contribute to improved general disease resistance in poultry.

In a Genome-Wide Association Study (GWAS) we identified a genomic region on chromosome 4 with a major effect on IgM NAb. This chromosomal region contains the Toll-like receptor 1 family member A (*TLR1A*) gene and a polymorphism in this gene was identified as being the most likely causal variant affecting NAb levels (Berghof *et al.*, 2018). The aim of the current study was to confirm and characterise the effect of this polymorphism in the *TLR1A* gene.

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Materials & methods

Birds used in this study were from a commercial pure-bred elite white leghorn layer line provided by Hendrix Genetics (Boxmeer, the Netherlands). The study population consisted of a base population, which was unselected for NAb, and 7 subsequent generations of birds from a High and Low selection line. Birds were divergently selected for total KLH-binding NAb titers (IgTotal). Blood plasma samples were taken at 16 weeks of age. An ELISA was performed to determine IgTotal, and KLH-binding IgM and IgG isotypes. A detailed description of the procedure is given by Berghof *et al.* (2018).

The single nucleotide polymorphism (SNP) in *TLR1A* consists of a cytosine (C)/guanine (G) polymorphism, and results in a phenylalanine (F)/leucine (L) amino acid substitution in *TLR1A* at protein position 126. Imputed genotypes for this *TLR1A* SNP were available for the base population (Berghof *et al.,* 2018). These imputed genotypes were used to derive the allele frequency in the base population but were not used in the current study to estimate the effect of the *TLR1A* polymorphism. Genotypes for the *TLR1A* SNP of chickens in generation 5, 6 and 7 of the selection experiment were determined based on a TaqMan assay.

To estimate the effect of the *TLR1A* polymorphism, genotypic and phenotypic information from chickens in generation 5 (n=1105), 6 (n=681) and 7 (n=978) of the selection experiment were available. Genetic differences between selection lines were accounted for in the model by including a line effect. Genotypic effects were estimated using the following model:

$$y_{ijklmn} = \mu + Plate_i + Sex_j + Line_k + TLR1A_l + a_m + d_n + e_{ijklmn} \quad (1)$$

where y_{ijklmn} is IgTotal, IgM, or IgG titer, μ is the mean, $Plate_i$ is the fixed effect of ELISA plate effect (with i = 1 to 166), Sex_j is the fixed effect of sex (with j = 1,2; male or female), $line_k$ is the fixed effect of selection line (with k=1,2; High or Low), $TLR1A_l$ is the effect of the polymorphism in the *TLR1A* gene (with l=1,3; genotypes CC, CG, or GG), a_m is the random additive genetic effect of the m^{th} animal, which is assumed to be distributed as $N(0,A\sigma_A^2)$, d_n is the random effect of the n^{th} dam, which is assumed to be distributed as $N(0,I\sigma_m^2)$, and e_{ijklmn} is the random residual term, which is assumed to be distributed as $N(0,I\sigma_e^2)$. To build the A matrix, pedigree information of 12,443 individuals from 14 generations were used. Generation effects are confounded with ELISA plate effects and therefore 'Generation' was not included as a separate effect in the model.

Additive genetic and maternal environmental variances in these analyses were fixed at the values obtained using all data from the selection experiment consisting of 10,878 birds; heritabilities were fixed at 0.30 for IgM, 0.12 for IgG and 0.12 for IgTotal, maternal environmental effects were fixed at 0.03 for IgM, 0.02 for IgG and 0.03 for IgTotal. Additional analyses were performed to test for possible interactions between *TLR1A* and Sex, TLR1A and Line and TLR1A and Generation. All statistical analyses were performed using ASREML 4 (Gilmour *et al.* 2015).

Theoretically expected changes in *TLR1A* frequencies due to mass selection for IgTotal were approximated using Falconer and Mackay (1996). The coefficient of selection was approximated as:

$$s = i_r\left(\frac{2a}{\sigma_p}\right) \quad (2)$$

where i_r is the standardized realized selection differential, a is the estimated additive genetic effect of the *TLR1A* polymorphism on IgTotal and σ_p is the phenotypic standard deviation of IgTotal. The estimated additive genetic effect of the *TLR1A* polymorphism on IgTotal and the phenotypic standard deviation were

obtained from analyses using model (1). The theoretically expected change in *TLR1A* G frequency was calculated as (Falconer and Mackay, 1996):

$$\Delta q = -sq^2(1 - q) \quad (3)$$

Changes in *TLR1A* G frequency were calculated separately for males and females and subsequently averaged. Approximate changes in *TLR1A* frequencies assume that effects are constant over generations and show complete dominance i.e. assuming that effects of CC and GC on IgTotal are identical.

Results

Table 1 shows that the *TLR1A* polymorphism had a highly significant effect on IgM and IgTotal NAb titers. The CC and CG genotypes had more than 1 titer point higher IgM NAb levels than the GG genotype. The difference between the CC and CG genotypes for NAb IgM titers was small and not significant, suggesting full dominance of the C allele. Similar as for IgM, CC and CG genotypes had higher IgTotal NAb titers than the GG genotypes and the difference between CG and GG genotypes was not significant.

For IgG no significant interactions between the *TLR1A* polymorphism and Sex, Line or Generation were detected.The *TLR1A* effect on IgM and IgTotal suggested full dominance of the C allele and therefore for the interaction analyses we only distinguished two genotype classes: CC/CG and GG. For IgTotal no significant (TLR1A×Sex) interaction was detected. For IgM a significant (TLR1A×Sex) interaction was detected (P=0.004); the effect of the *TLR1A* polymorphism (CC/CG versus GG) in males (1.15) was larger than in females (0.92). No significant (TLR1A×Line) interactions were detected indicating that effects do not differ significantly between the High and Low selection line. Highly significant (TLR1A×Generation) interactions were detected, both for IgM (P<0.001) and IgTotal (P=0.008). For IgTotal the difference between CC/CG and GG was 0.43 titer points in generation 5, 0.23 titer points in generation 6 and 0.71 titer points in generation 7. For IgM the difference between CC/CG and GG was 0.70 titer points in generation 5, 1.22 titer points in generation 6 and 1.28 titer points in generation 7. The frequency of the *TLR1A* C allele was 0.45 in the base population (based on imputed genotypes). The frequency of the C allele in the High line was 0.71 in generation 5, 0.64 in generation 6 and 0.66 in generation 7 (Figure 1). In the Low line the frequency of the C allele was 0.09 in generation 6, 0.06 in generation 6 and 0.04 in generation 7. In generation 5 of the Low line there were 5 chickens with genotype CC and in generation 6 and 7 of the Low line there were no chickens left with the CC genotype. In generation 7 the selection response for IgM due to *TLR1A* allele frequency changes was -0.65 titer points in the Low line and +0.21 titer points in the High line. The segregation of the TLR1A polymorphism therefore resulted in an asymmetrical selection response for IgM.

Table 1. Estimated effects of the *TLR1A* polymorphism on NAb titers based on chickens in generation 5 (n=1,105), 6 (n=681) and 7 (n=978) of a selection experiment.

	IgTotal	IgM	IgG
GG	0	0	0
GC	$0.49_{(0.09)}$	$1.01_{(0.07)}$	$0.22_{(0.10)}$
CC	$0.44_{(0.11)}$	$1.12_{(0.09)}$	$0.07_{(0.13)}$
P	<0.001	<0.001	0.048

Discussion

Highly significant effects of *TLR1A* on IgTotal and IgM reported by Berghof *et al.* (2018) were confirmed in the current study. Estimated genotypic effects of *TLR1A* on IgM and IgTotal were larger than those reported by Berghof *et al.* (2018). The current data allowed testing for the interaction between *TLR1A* and sex, line or generation. Surprisingly, we detected highly significant *TLR1A* by generation interactions for IgM and IgTotal. In the selection experiment, conditions were kept as stable as possible. However, despite this standardization generation-specific variation cannot be avoided. Each generation was raised as a single batch and therefore several factors might be responsible for the observed *TLR1A* by generation interactions. Despite high sanitary status of the research facility, immune status and presence of pathogens can be other major effectors of TLR expression. As far as we know such strong genotype by environment interaction effects have not been reported before for disease resistance-related traits. Possibly because such effects were either not present or not investigated, but it does highlight the importance of the test environment when selecting for KLH-binding NAb, and possibly for disease resistance-related traits in general.

Highly significant effects of *TLR1A* on IgTotal were also confirmed based on the observed allele frequency changes in the High and Low selection line. Results provided unique experimental verification of changes in allele frequencies at a major gene with dominant gene action affecting a quantitative trait that is subjected to mass selection. Theoretically, allele frequencies might not only change due to selection but also due to drift. Increase of inbreeding per generation is low; on average 0.26% in the Low line and 0.31% in the High line.

Therefore, allele frequency changes due to drift are expected to be small. If NAb play an important role in the immune defence of poultry one might expect that purifying (natural) selection would remove alleles with deleterious effects. However, if pure line individuals are kept under strict hygienic conditions in breeding nuclei, their immune response is not seriously challenged, and purifying (natural) selection will not be effective. Reciprocal recurrent selection also might not be effective in removing deleterious alleles

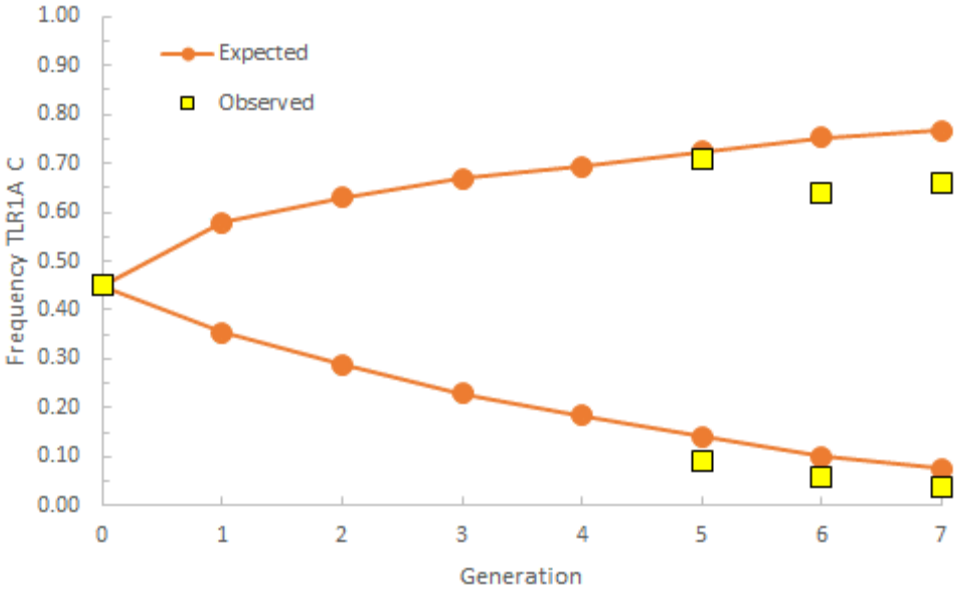

Figure 1. Realized and theoretically expected changes of *TLR1A* C allele frequencies in a High and Low selection line for NAb.

when the other parental line is homozygous for the favourable *TLR1A* allele (CC). Therefore, for situations with genotype by environment interaction and complete dominant mode of gene action, the crossbreeding system might be 'hacked' by deleterious mutations.

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