Association study in naturally infected helminth layers shows evidence for influence of interferon-gamma gene variants on *Ascaridia galli* worm burden

Gesine Lühken¹*, Matthias Gauly², Falko Kaufmann² and Georg Erhardt¹

**Abstract**

Single nucleotide polymorphisms (SNPs) in the genes for interleukin-4, -13 and interferon-gamma, and 21 additional SNPs which previously had been significantly associated with immune traits in the chicken, were genotyped in white and brown layer hens and analyzed for their association with helminth burden following natural infections. A nucleotide substitution located upstream of the promoter of the interferon-gamma gene was significantly associated with the log transformed number of *Ascaridia galli* in the brown layer line (genotype CC: 6.4 ± 1.0 worms; genotype CT: 11.7 ± 2.2 worms). Therefore, *IFNG* seems to be a promising candidate gene for further studies on helminth resistance in the chicken.

**Introduction, Methods, and Results**

In the European Community, animal welfare issues and changes in consumer demands have resulted in a ban of conventional cages for laying hens from 2012 on (Council Directive 1999/74). This has resulted in an increased importance of floor husbandry systems and consequently in a renewed relevance of helminthoses [1]. The development of drug resistance in nematodes [2,3] and restrictions for the use of anthelmintics in food producing animals are two important aspects urging scientists to find alternative strategies for the control of gastrointestinal infections in laying hens. Estimated heritabilities and breed or line differences for immunological characteristics were not only shown in mammals but also in poultry [4,5]. Moreover, heritabilities estimated for parameters of susceptibility to helminthic infections, as mean worm or larvae counts [6-10], suggest that it is possible to select for helminth resistance in poultry.

Although immunity in birds is not as well understood as in mammals, it has been shown that as in mammals [11], helminth infection in chickens results in polarization towards a type 2 immune reaction, including augmented expression of interleukin-4 and interleukin-13 and diminished interferon-gamma expression [12]. In a single nucleotide polymorphism (SNP) study concerning innate and adaptive immune response across white and brown layer lines, 59 significant associations between immune traits and SNPs in immunological relevant genes were detected [13]; however, variants of interleukin-4 (*IL4*), interleukin-13 (*IL13*) and interferon-gamma (*IFNG*) genes were not included.

The aim of the present study was to determine genotypes of SNPs in the *IL4*, *IL13* and *IFNG* genes and of 21 additional SNPs significantly associated with immune traits in white and brown commercial layer lines and to analyze their association with worm numbers resulting from a natural helminth infection in order to identify gene regions as promising candidates for further studies on parasite resistance in chickens.

Whole blood samples, numbers of adult worms of *Ascaridia galli, Heterakis gallinarum, Capillaria* spp. and tapeworms of 197 Lohmann Brown (LB) and 246 Lohmann Selected Leghorn (LSL) hens and pedigree data (sires) were available from a recent study conducted by Kaufmann et al. [6]. Briefly, in their experiment LB and LSL hens were reared under helminth-free conditions.

*Correspondence: Gesine.Luehken@agrar.uni-giessen.de*  
¹Department of Animal Breeding and Genetics, Justus-Liebig University of Gießen, Ludwigsstrasse 21B, 35390 Gießen, Germany  
Full list of author information is available at the end of the article  

© 2011 Lühken et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
and kept afterwards together in a free range system. At the end of the laying period, hens were slaughtered and worms were counted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. Whereas LB hens showed a significantly ($P < 0.05$) higher mean number of adult *H. gallinarum*, *Capillaria* spp. and tapeworms compared to LSL animals, the latter had a tendency towards a higher number of adult *A. galli* worms. The estimated heritabilities for worm burdens of the different helminths and of the total worm burden ranged from 0.11 to 0.69 in LB and from 0.01 to 0.30 in LSL. Further details are given by Kaufmann et al. [6]. DNA was extracted from whole blood samples of these 443 hens using the Invisorb Blood Mini HTS 96 Kit (Invitek, Berlin, Germany). Quality and quantity of DNA were checked after extraction using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA).

In a previous work, we sequenced the 5'-flanking and all coding regions of *IFNG*, *IL4* and *IL13* in 20 chickens, 10 each from the white and brown layer White Leghorn and New Hampshire breeds (unpublished). Among the identified SNPs, only those which were polymorphic in at least one of the breeds were selected for genotyping. Preferably, they were located in or near functional gene regions. Three of the selected SNPs had not been listed in the database of genetic variation [14] and therefore sequence information for those was sent to GenBank (GenBank:HQ888866-HQ888868). Genotyping of two *IFNG* and three *IL4* SNPs was done by PCR restriction-flanking-length-polymorphism (RFLP) analysis. For this purpose forward and reverse primers for *IFNG* (SNP in 5'-flanking region: 5'-tgaccttttaaaccataggt-3' and 5'-tttcgaagcttgctgagaa-3', 194 bp; SNP in exon 4: 5'-gctgtaacgctggagt-3' and 5'-ctctggctatggtctgtggc-3', 462 bp) and for *IL4* (SNPs in exon 1 and intron 1: 5'-actttacaggggagagagat-3' and 5'-tcgtgactgtctttctcta-3', 554 bp; SNP in intron 3: 5'-tgctgttctaatccactcaaga-3' and 5'-aagctgtctcatcacttttt-3', 725 bp) were used to amplify DNA fragments that were digested with appropriate restriction enzymes (Table 1) according to the manufacturers’ (MBI Fermentas, St. Leon-Rot, Germany; New England Biolabs, Frankfurt, Germany) recommendations. The last nucleotide of the forward primer for the *IFNG* 5'-flanking region was a mismatch in order to enable RFLP analysis by an amplification created restriction site [15].

SNP genotypes were discriminated after electrophoresis of the digested PCR products on agarose gels and ethidium bromide staining.

All other SNPs ($n = 22$) were genotyped with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) by Eurofins Medigenomix GmbH, Martinsried, Germany, using the Sequenom Massarray iPLEX Gold System (Sequenom, San Diego, USA). Twenty-one SNPs were chosen from the study of Biscarini et al. [13]. All of these SNPs were significantly associated with at least one of the analyzed immune traits (production of natural antibodies against exo- and endo-antigens and of acquired antibodies, activation of classical and alternative complement pathways) with a $P$-value < 0.01 [13]. Furthermore, a nucleotide substitution in intron 1 of *IL13*, already recorded in the db SNP database (rs15709642), was included in the MALDI-TOF MS multiplex assay.

For all genotyped SNPs, the rs number or a GenBank accession number, the chromosomal and gene location and the genotyping method used are given in Table 1.

SNP allele frequencies were calculated from the genotypes obtained. The significance of differences between allele frequencies of the genotyped SNPs in the two lines were analyzed with a chi square test, or with a Fisher exact test if the smallest cell contained less than six cases. Worm numbers were log transformed [log(worm number + 10)] to get approximately normally distributed data, as done before by Kaufmann et al. [6]. Association studies were performed for each SNP - showing a minor allele frequency $\geq 5\%$ per line - with each of the observed parasitological traits, using the following statistical model: $y_{ij} = \mu + SNP + e_{ij}$, where $y_{ij}$ represents the observation for the animal $j$, with SNP genotype $i$; $\mu$ is the overall mean of the trait; $SNP_i$ is the effect of the SNP genotype, either AA, AB or BB; and $e_{ij}$ is the random residual effect. Association analysis was done within each line for all SNPs and additionally across lines for SNPs whose allele frequencies were not significantly ($P < 0.05$) different between the two lines.

Allele frequencies of the 27 genotyped SNPs are shown in Figure 1 for each line. A total of 14 SNPs was monomorphic and 1 additional SNP had a minor allele frequency < 0.05 in LSL, whereas 3 SNPs were fixed and 3 showed a minor allele frequency < 0.05 in LB. SNP 26, located in the *BLB1* region of the major histocompatibility complex (MHC), was fixed in both lines. SNP 12 showed a minor allele frequency < 0.05 in LB and was monomorphic in LSL. Therefore SNPs 12 and 26 were not included in association analyses in any of the two lines.

The SNPs in exon 1 and intron 1 of *IL4* were genotyped by amplifying a single PCR product and digested with a single enzyme, enabling the demonstration of a total of 3 haplotypes (Figure 2). Only two of these haplotypes were identified in LB but all three in LSL (haplotype frequencies not shown).

Besides the fixed SNP 26, the allele frequencies of only two SNPs (7 and 21) were not significantly different between LB and LSL (Table 1). Therefore only those two SNPs were also analyzed for association with worm numbers across lines and not only within lines.
Table 1 Numbers, locations, genotyping methods and P-values for allele frequency differences between lines of analyzed SNPs

| SNP no. | rs or GenBank accession no. | GGA¹ | gene symbols (gene region) | genotyping method² | P (allele frequencies between lines) |
|---------|----------------------------|------|---------------------------|--------------------|-------------------------------------|
| 1       | HQ888866                   | 1    | IFNG (5′-flanking region) | PCR-RFLP (HinfI)   | < 0.001                             |
| 2       | HQ888867                   | 1    | IFNG (exon 4, synonymous) | PCR-RFLP (Mbol)    | < 0.001                             |
| 3       | rs13526054                 | 3    | IL17F (exon, synonymous)  | MALDI-TOF MS       | < 0.001                             |
| 4       | rs14082130                 | 3    | MAL (intron)              | MALDI-TOF MS       | < 0.001                             |
| 5       | rs15458146                 | 3    | IL17F (exon, nonsynonymous)| MALDI-TOF MS      | < 0.001                             |
| 6       | rs13520872                 | 4    | SHROOM3 (intron)          | MALDI-TOF MS       | < 0.001                             |
| 7       | rs13520980                 | 4    | NUP54 (intron)            | MALDI-TOF MS       | 0.834                               |
| 8       | rs13521841                 | 4    | no gene                   | MALDI-TOF MS       | < 0.001                             |
| 9       | rs15475503                 | 4    | HTR2C (intron)            | MALDI-TOF MS       | < 0.001                             |
| 10      | rs13586560                 | 5    | ENTPDS (intron)           | MALDI-TOF MS       | < 0.001                             |
| 11      | rs13586776                 | 5    | FLVCR2 (intron)           | MALDI-TOF MS       | < 0.001                             |
| 12      | rs13755931                 | 5    | SPTBN5 (intron)           | MALDI-TOF MS       | 0.007                               |
| 13      | rs15669480                 | 5    | TOLLIP (exon, synonymous) | MALDI-TOF MS       | < 0.001                             |
| 14      | rs14580491                 | 6    | CXCL12 (intron)           | MALDI-TOF MS       | < 0.001                             |
| 15      | rs13596817                 | 7    | no gene                   | MALDI-TOF MS       | < 0.001                             |
| 16      | rs13596877                 | 7    | no gene                   | MALDI-TOF MS       | < 0.001                             |
| 17      | rs13599559                 | 7    | SPOPL (intron)            | MALDI-TOF MS       | < 0.001                             |
| 18      | HQ888868                   | 13   | IL4 (exon 1, synonymous)  | PCR-RFLP (TaqI)    | < 0.001                             |
| 19      | rs13505561                 | 13   | IL4 (intron 1)            | PCR-RFLP (TaqI)    | 0.012                               |
| 20      | rs15709667                 | 13   | IL4 (intron 3)            | PCR-RFLP (BclI)    | < 0.001                             |
| 21      | rs14064765                 | 13   | GMCSF (5′-flanking region)| MALDI-TOF MS       | 0.248                               |
| 22      | rs14064896                 | 13   | IFR1 (3′-flanking region) | MALDI-TOF MS       | < 0.001                             |
| 23      | rs15677371                 | 13   | no gene                   | MALDI-TOF MS       | < 0.001                             |
| 24      | rs15677377                 | 13   | no gene                   | MALDI-TOF MS       | < 0.001                             |
| 25      | rs15709642                 | 13   | IL13 (intron)             | MALDI-TOF MS       | < 0.001                             |
| 26      | rs15788216                 | 16   | MHC, BLB1 (exon, nonsynonymous)| MALDI-TOF MS | 1.000                               |
| 27      | rs14119843                 | 19   | HSPB1 (3′-flanking region)| MALDI-TOF MS       | < 0.001                             |

¹number of chicken chromosome (Gallus gallus); ²for PCR-RFLP, used restriction enzymes are given in parentheses; CXCL12, chemokine (C-X-C motif) ligand 12; ENTPDS, ectonucleoside triphosphate diphosphohydrolase 5; FLVCR2, feline leukemia virus subgroup C cellular receptor family, member 2; GMCSF, granulocyte-macrophage colony-stimulating factor; HSPB1, heat shock 27kDa protein 1; HTR2C, 5-hydroxytryptamine (serotonin) receptor 2C; IFNG, interferon gamma; IL4, interleukin 4; IL17F, interleukin 17F; IFR1, interferon regulatory factor 1; MAL, mal, T-cell differentiation protein; MHC (BLB1), major histocompatibility complex class II antigen B-F minor heavy chain; NUP54, nucleoporin 54kDa; SHROOM3, shroom family member 3; SPOPL, speckle-type POZ protein-like; SPTBN5, spectrin, beta, non-erythrocytic 5; TOLLIP, toll-interacting protein.

P-values resulting from analysis of the association of SNP genotypes with worm numbers of A. galli, H. gallinarum, Capillaria spp., tapeworms and total worm burden are given in Table 2. Three SNPs (1 and 21, located in the 5′-flanking region of IFNG and GMCSF, respectively, and SNP 4, an intronic nucleotide substitution in MAL) were significantly associated with one of the traits analyzed; whereas 7 SNPs showed a tendency towards significance for association with one or more of the traits (Table 2). Although only SNPs with a minor allele frequency < 5% were tested for association with the different parasitological traits, for some of the SNPs studied, the statistical significance or tendency towards significance of an association was obviously connected with a genotype only present in a small portion of hens (< 10%). This was also the case for the significant associations of SNPs 4 and 21 with parasitological traits.

For SNP 1, showing significant association with the log transformed worm number of A. galli in LB, genotype CC was very frequent (83%), whereas genotype CT occurred in a lower frequency (17%). The average A. galli worm number was 6.4 ± 1.0 in LB hens with the genotype CC, whereas it was 11.7 ± 2.2 in hens with the genotype CT. As 10 of the 19 LB sires had only progeny with the CC genotype, the association analysis for SNP 1 regarding the number of A. galli in LB was repeated only with hens (n = 90) from the 9 other sires, resulting in a P-value of 0.011.
Discussion

The higher number of monomorphic SNPs in the white layer line (52% of the SNPs analyzed) compared to the brown (11% of the SNPs analyzed) was in accordance with other studies [13,16] and can be attributed to the smaller number of incorporated breeds in white lines [16,17]. Nevertheless, we did not expect such a large difference since Biscarini et al. [13] reported only 6% more fixed loci in 5 white layer lines compared to 4 in the brown lines. The larger difference observed in the present study could be due to the smaller number of analyzed SNPs or a higher homozygosity of LSL and/or lower homozygosity of LB compared to the average of the white and brown layer lines analyzed by Biscarini [13]. Interestingly, the lower homozygosity in LB is - excluding the *A. galli* worm number - accompanied by higher heritabilities for worm numbers and at the same time significantly higher worm numbers, compared to LSL [6]. Among the SNPs which showed significant associations with parasitological traits, SNP 1 is the only one where this was not obviously linked to a very rare genotype. Genotypes *CC* and *CT* of SNP 1, a nucleotide substitution we previously identified in the *IFNG* 5' flanking region of New Hampshire and White Leghorn, were significantly associated with the number of *A. galli* worms in LB. In sheep, where nematode resistance has been a breeding goal much longer than in poultry, *IFNG* variants and markers located in the same chromosomal region as *IFNG* have already been associated with nematode resistance [18-20]. However, any of these polymorphisms were considered to directly influence the investigated trait. The chicken *IFNG* SNP analyzed here is located outside and upstream of the gene promoter [21]. Together with the monomorphic status of this SNP in LSL hens, showing a variance in *A. galli* worm numbers as in LB, it is more likely that its association with the *A. galli* number in LB is due to a linkage with a causal SNP in *IFNG* than influencing the worm number itself. Repeating the association analyses with other hens and with additional neighboring SNPs will be necessary to confirm the genetic influence of *IFNG* on susceptibility to *A. galli* in chickens that is supposed here. However, additional *IFNG* SNPs will be mainly located in non-coding gene regions, since the chicken

![Figure 1 Allele frequencies (%) of SNPs in Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens. For rs or GenBank accession numbers of SNPs and their location on chromosomes and in genes see Table 1.](image-url)
Figure 2 Genotyping of chicken IL4 haplotypes (SNP exon 1 - SNP intron 1) by PCR-RFLP analysis. PCR products from hens with different genotypes (1-5) digested with Taq I, separated by agarose gel electrophoresis and stained with ethidium bromide. 1 = G/G/G, 2 = G/G/A, 3 = G/G/C, 4 = G/C/G, 5 = G/C/A (554 bp fragment usually not completely digested in genotype 5). M: 100 bp DNA size marker. Left numbers: marker sizes in bp. Right numbers: DNA fragment sizes in bp.

Table 2 P-values for association of SNPs with worm numbers in LB and LSL or both (all).

| SNP no. | line | A. galli | H. gallinarum | Capillaria ssp. | tapeworms | all helminths |
|--------|------|----------|---------------|-----------------|-----------|--------------|
| 1      | LB   | 0.017    | 0.175         | 0.356           | 0.289     | 0.287        |
| 2      | LB   | 0.994    | 0.487         | 0.695           | 0.163     | 0.754        |
| 3      | LB   | 0.375    | 0.977         | 0.129           | 0.408     | 0.842        |
| 4      | LB   | 0.479    | **0.052**     | **0.077**       | 0.215     | 0.133        |
|        | LSL  | 0.186    | 0.472         | 0.540           | 0.992     | 0.280        |
| 5      | LB   | 0.301    | 0.983         | 0.152           | 0.494     | 0.855        |
| 6      | LB   | 0.304    | 0.439         | 0.105           | 0.980     | 0.680        |
|        | LSL  | 0.068    | **0.082**     | 0.346           | 0.828     | **0.063**    |
| 7      | LB   | 0.838    | 0.892         | 0.913           | 0.757     | 0.916        |
|        | LSL  | 0.793    | 0.231         | 0.604           | 0.537     | 0.265        |
|        | all  | 0.984    | 0.673         | 0.659           | 0.886     | 0.716        |
| 8      | LB   | 0.493    | 0.203         | 0.722           | 0.479     | 0.153        |
| 9      | LB   | **0.086**| 0.515         | 0.154           | 0.693     | 0.316        |
| 10     | LB   | 0.488    | 0.951         | 0.750           | 0.137     | 0.841        |
|        | LSL  | 0.489    | 0.672         | 0.872           | 0.667     | 0.823        |
| 11     | LB   | 0.741    | 0.647         | 0.620           | 0.686     | 0.577        |
**IFNG** is known for its high degree of sequence conservation especially in coding regions [21,22]. The existence of only weak linkage to a causal SNP may also be the reason that some SNPs only tended to be associated with one or more of the traits and in only one of the two lines. Therefore, additionally to SNPs in **IFNG**, some of them may be worth studying in further experiments, especially variants of **IL13** as SNP 25 and other adjacent SNPs.

### Acknowledgements

The animals originated from Lohmann Tierzucht GmbH, Cuxhaven, Germany. The authors thank the Ministry of Nutrition, Agriculture, Consumer Protection and State Development of Lower Saxony, Germany, for financial support.

### Author details

1. Department of Animal Breeding and Genetics, Justus-Liebig University of Giessen, Ludwigstrasse 218, 35390 Giessen, Germany.
2. Department of Animal Science, Georg-August University of Goettingen, Albrecht-Thaer-Weg 3, 37075 Goettingen, Germany.

### Authors’ contributions

GL conceived the study, designed and carried out the molecular genetic and association analyses and drafted the manuscript. MG conceived and designed the infection study, acquired funding and contributed to the interpretation of data. FK acquired, analyzed and interpreted the parasitological data. GE conceived the study, participated in its design and helped draft the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

**Received:** 4 March 2011  **Accepted:** 12 July 2011  **Published:** 12 July 2011

### References

1. Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J, Nansen P: Prevalence of gastrointestinal helminths in different poultry production systems. **Br Poult Sci** 1999, 40:439-443.
2. Jackson F, Miller J: Alternative approaches to control – quo vadit? **Vet Parasitol** 2006, 139:371-384.
3. Leignel V, Silvestre A, Humbert JF, Cabaret J: Alternation of anthelmintic treatments: a molecular evaluation for benzimidazole resistance in nematodes. **Vet Parasitol** 2010, 172:80-88.
4. Lamont SJ, Pinard MH, Cahaner A, van der Poel J, Parmentier HK: Selection for disease resistance: direct selection on the immune response. In *Poultry Genetics, Breeding and Biotechnology*. Edited by: Muir WM, Aggrey SE. Wallingford: CAB International; 2003:399-414.
5. Wijga S, Parmentier HK, Nieuwland MG, Bovenhuis H: Genetic parameters for levels of natural antibodies in chicken lines divergently selected for specific antibody response. **Poult Sci** 2009, 88:1805-1810.
6. Kaufmann F, Das G, Preisinger R, Schmutz M, König S, Gauly M: Genetic resistance to natural helmith infections in two chicken layer lines. **Vet Parasitol** 2011, 176:250-257.
7. Gauly M, Kanan A, Brandt H, Weigend S, Moors E, Erhardt G: Genetic resistance to Heterakis gallinarum in two chicken layer lines following a single dose infection. **Vet Parasitol** 2008, 155:74-79.
8. Gauly M, Bauer C, Preisinger R, Erhardt G: Genetic differences of Ascaridia galli egg output in laying hens following a single dose infection. **Vet Parasitol** 2002, 103:99-107.
9. Permin A, Ranvig H: Genetic resistance to *Ascaridia galli* infections in chickens. **Vet Parasitol** 2001, 102:101-111.

### Table 2 P-values for association of SNPs with worm numbers in LB and LSL or both (all). (Continued)

|   |   |   |   | P-value |
|---|---|---|---|--------|
| 13 | LB | 0.687 | 0.215 | 0.926 | 0.743 | 0.165 |
|   | LSL | 0.205 | 0.398 | 0.067 | 0.600 | 0.212 |
| 14 | LB | 0.535 | 0.306 | 0.365 | 0.734 | 0.487 |
| 15 | LB | 0.677 | 0.225 | 0.669 | 0.639 | 0.219 |
| 16 | LB | 0.359 | 0.609 | 0.827 | 0.604 | 0.796 |
|   | LSL | 0.187 | 0.692 | 0.547 | 0.150 | 0.938 |
| 17 | LB | 0.500 | 0.126 | 0.141 | 0.216 | 0.319 |
|   | LSL | 0.081 | 0.375 | 0.146 | 0.537 | 0.179 |
| 18 | LSL | 0.462 | 0.193 | 0.758 | 0.830 | 0.303 |
| 19 | LSL | 0.613 | 0.317 | 0.676 | 0.620 | 0.427 |
| 20 | LSL | 0.607 | 0.431 | 0.841 | 0.756 | 0.615 |
| 21 | LB | 0.960 | 0.322 | 0.875 | 0.056 | 0.529 |
|   | LSL | 0.090 | 0.678 | 0.061 | 0.540 | 0.296 |
| 22 | LB | 0.628 | 0.292 | 0.371 | 0.878 | 0.459 |
|   | LSL | 0.605 | 0.182 | 0.337 | 0.789 | 0.200 |
| 23 | LB | 0.067 | 0.213 | 0.138 | 0.411 | 0.122 |
| 24 | LB | 0.930 | 0.938 | 0.327 | 0.207 | 0.623 |
| 25 | LB | 0.495 | 0.886 | 0.260 | 0.625 | 0.632 |
|   | LSL | 0.337 | 0.074 | 0.067 | 0.413 | 0.113 |
| 27 | LB | 0.133 | 0.305 | 0.124 | 0.924 | 0.471 |

Significant associations (P < 0.05) and associations with a tendency towards significance (P < 0.1) are bold typed.
10. Schou T, Permin A, Roepstorff A, Sorensen P, Kjaer J. Comparative genetic resistance to Ascaridia galli infections of 4 different commercial layer-lines. Br Poult Sci 2003, 44:182-185.

11. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immuno Today 1996, 17:138-146.

12. Degen WG, Daal N, Rothwell L, Kaiser P, Schijns VE. Th1/Th2 polarization by viral and helminth infection in birds. Vet Microbiol 2005, 105:163-167.

13. Biscarini F, Bovenhuis H, van Arendonk JA, Parmentier HK, Jungerius AP, van der Poel JJ. Across-line SNP association study of innate and adaptive immune response in laying hens. Anim Genet 2010, 41:26-38.

14. Database of genetic variation (dbSNP). [http://www.ncbi.nlm.nih.gov/snp].

15. Haliassos A, Chomel JC, Tesson L, Baudis M, Kruh J, Kaplan JC, Kitzis A. Modification of enzymatically amplified DNA for the detection of point mutations. Nucleic Acids Res 1989, 17:3606.

16. Hillel J, Groenen MA, Tixier-Boichard M, Korol AB, David L, Kirzner WM, Burke T, Barre-Dine A, Crooijmans RP, Elo K, Feldman MW, Friedlin PI, Maki-Tanila A, Ortonwijn M, Thomson P, Vignal A, Wimmers K, Weigend S. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet Sel Evol 2003, 35:533-557.

17. Muir WM, Wang GQ, Zhang Y, Wang J, Groenen MA, Crooijmans RP, Megens HJ, Zhang H, Okimoto R, Vereijken A, Jungenerius A, Albers GA, Lavley CT, Delany ME, MacEachern S, Ching HH. Genome-wide assessment of worldwide chicken SNP genetic diversity indicates significant absence of rare alleles in commercial breeds. Proc Natl Acad Sci USA 2008, 105:17312-17317.

18. Coltman DW, Wilson K, Pilkington JG, Stear MJ, Pemberton JM. A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. Parasitology 2001, 122:571-582.

19. Davies G, Stear MJ, Benoistman M, Abuagob O, Kerr A, Mitchell S, Bishop SC. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. Heredity 2006, 96:252-258.

20. Sayers G, Good B, Hanrahan JP, Ryan M, Sweeney T. Intron 1 of the interferon gamma gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. Res Vet Sci 2003, 79:191-196.

21. Kaiser P, Wain HM, Rothwell L. Structure of the chicken interferon-gamma gene, and comparison to mammalian homologues. Gene 1998, 207:25-32.

22. Downing T, Lynn DJ, Connell S, Lloyd AT, Bhuian AK, Silva P, Naqvi AN, Sanfo R, Sow RS, Podisi B, O’Farrelly C, Hanotte O, Bradley DG. Contrasting evolution of diversity at two disease-associated chicken genes. Immunogenetics 2000, 61:303-314.

doi:10.1186/1297-9716-42-84

Cite this article as: Lühken et al. Association study in naturally infected helminth layers shows evidence for influence of interferon-gamma gene variants on Ascaridia galli worm burden. Veterinary Research 2011 42:84.