Genetic markers associated with bone quality in Rhode Island Red laying hens

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Abstract
Bone weakness is a key challenge in laying hens. Detailed measurements on layers' bone combined with genotypes via GWAS analysis would identify QTLs affecting bone health. Infrared spectroscopy and thermogravimetry measurements on tibia and humerus medullary and cortical bones of 924 Rhode Island Red laying hens, were performed and combined with genotypes (57k SNP markers) for GWAS analysis. The results showed 13 SNP markers significantly ($P < 10^{-5}$ to $< 10^{-7}$) associated with tibia cortical lipid (chromosomes 2 and 3), humerus medullary score (chromosome 9), and tibia cortical mineral content (chromosome Z). Further analysis is needed to determine the potential correlations (LD) between these significant markers and other QTL throughout the Rhode Island Red genome.

Introduction
Most laying hens display a high tendency to suffer from bone damage (deviations or fractures), which is a major welfare challenge in the egg industry. Gregory & Wilkins (1989) reported ~30% of commercial layers with at least one bone fracture. Another recent study reported ~70% of keel bone fractures in Danish laying farms (Thøfner et al., 2021), this may be due to an increase of alternative housing systems. Housing, nutrition and genetics all contribute to bone health; the genetic component is particularly relevant in White Leghorn and Rhode Island Red breeds as they both represent the main grandparents of commercial layers.

To better understand bone health in these breeds, a detailed bone phenotyping (~55 traits) study was performed, including measurements for medullary and cortical bone separately in addition to whole bone strengths, egg production, and body weights (Dunn et al., 2021). The medullary measurements showed 0.18-0.41 heritability and 0.6-0.9 genetic correlations with tibia strengths traits in the Rhode Island Red breed. These moderate to strong heritabilities and strong genetic correlations suggest that genome-wide association (GWAS) could reveal genomic regions, which contribute to multiple aspects of bone health in laying hens. In this paper, we present preliminary results from a GWAS to find genetic marker associations with 47 bone measurements in a cohort of 924 Rhode Island Red laying hens.

Materials & Methods
Animals and phenotyping. A group of 924 RIR hens were investigated, which are bred for Lohmann Brown commercial layers (Lohmann Breeders GmbH, Germany). Hens from four hatches were assigned into two houses equipped with cages. Each cage had a perch and was occupied by two birds: one RIR bird and a companion from another breed. Hens were

euthanized at 68 weeks of age, weighed, and bone samples were collected including humerus, tibia, and keel bone for further measurements as described previously (Dunn et al., 2021).
The measurements included: a bone index, (0·27*KeelDensity + 0·37*HumerusStrength + 0·61*TibiaStrength – 0·25*BodyWeight) following (Bishop et al., 2000) to jointly map correlated effects on all three bone sites, the mineral content in tibia cortical bone (determined by thermogravimetric analysis, represents carbonate apatite; calcium phosphate with some CO_3 substitution of the PO_4), lipid content in tibia cortical bone (determined by infrared spectroscopy at main peak 1710 cm^{-1}) and humerus medullary bone score (on a scale of 0-3 where 0 represents no medullary bone and 3 a diaphyseal medullary cavity filled with bone).

Genotyping. For genome-wide association studies, the hens were genotyped on 57,636 single nucleotide polymorphisms using the Illumina Infinium assay. The genotyping was performed by the SNP&SEQ Technology Platform, Uppsala University, Sweden. We aligned the sequences flanking the markers against the GRCg6a chicken reference genome to find physical marker positions. 188 SNPs were removed because of high missingness and 21,230 were monomorphic in the sample, leaving 36,218 markers for GWAS.

GWAS. For testing each SNP marker association with the trait of interest, we used the following linear mixed model implemented in GEMMA (Zhou & Stephens, 2012):

$$\mathbf{y} = \mathbf{X}\,\mathbf{b} + g\,\mathbf{snp} + \mathbf{Z}\,\mathbf{hen} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the standardized trait measurement, $\mathbf{X}$ and $\mathbf{Z}$ are design matrices that relate the measurement $\mathbf{y}$ to the corresponding fixed and random effect, g is the marker effect, and **hen** the genomic breeding value for each hen based on genomic relationship matrix. We included hatch and house as fixed effects, and body weight as a covariate.

Results

Three traits showed associations with p-value < 10-5: tibia cortical lipid (on chromosomes 2 and 3), humerus medullary score (on chromosome 9), and tibia cortical mineral content (on chromosome Z). Figure 1 shows Manhattan plots of these three traits. Table 1 shows position, estimated marker effect and p-value for the most significant associations. In addition, we report the result of bone index (Figure 1) as a joint measure of bone quality, previously used in selection and genetic mapping studies.

Table 1. The most significant SNP markers with their positions, estimated marker effect and p-value.

	SNP position			
Trait	Chr.	Base pair	Effect size[1]	P-value[2]
Tibia cortical lipid	2	2585350	-0.27	8.64E-06
	3	27204115	0.30	7.58E-08
	3	27351346	0.29	1.95E-07
	3	27434588	0.31	4.54E-08
	3	27548492	0.29	1.03E-06
	3	27648733	0.29	1.70E-06
Humerus medullary score	9	22641697	0.26	5.09E-06
	9	22694487	0.25	8.26E-06
	9	22716989	0.26	5.20E-06
	9	22718522	0.26	5.20E-06
	9	22749245	0.26	5.20E-06

	9	22755016	0.26	5.20E-06
Tibia cortical mineral%	Z	41701480	-0.63	1.32E-06

[1] All traits were standardized (with zero mean and one standard deviation) to facilitate effect size interpretation.
[2] Wald test P-value.

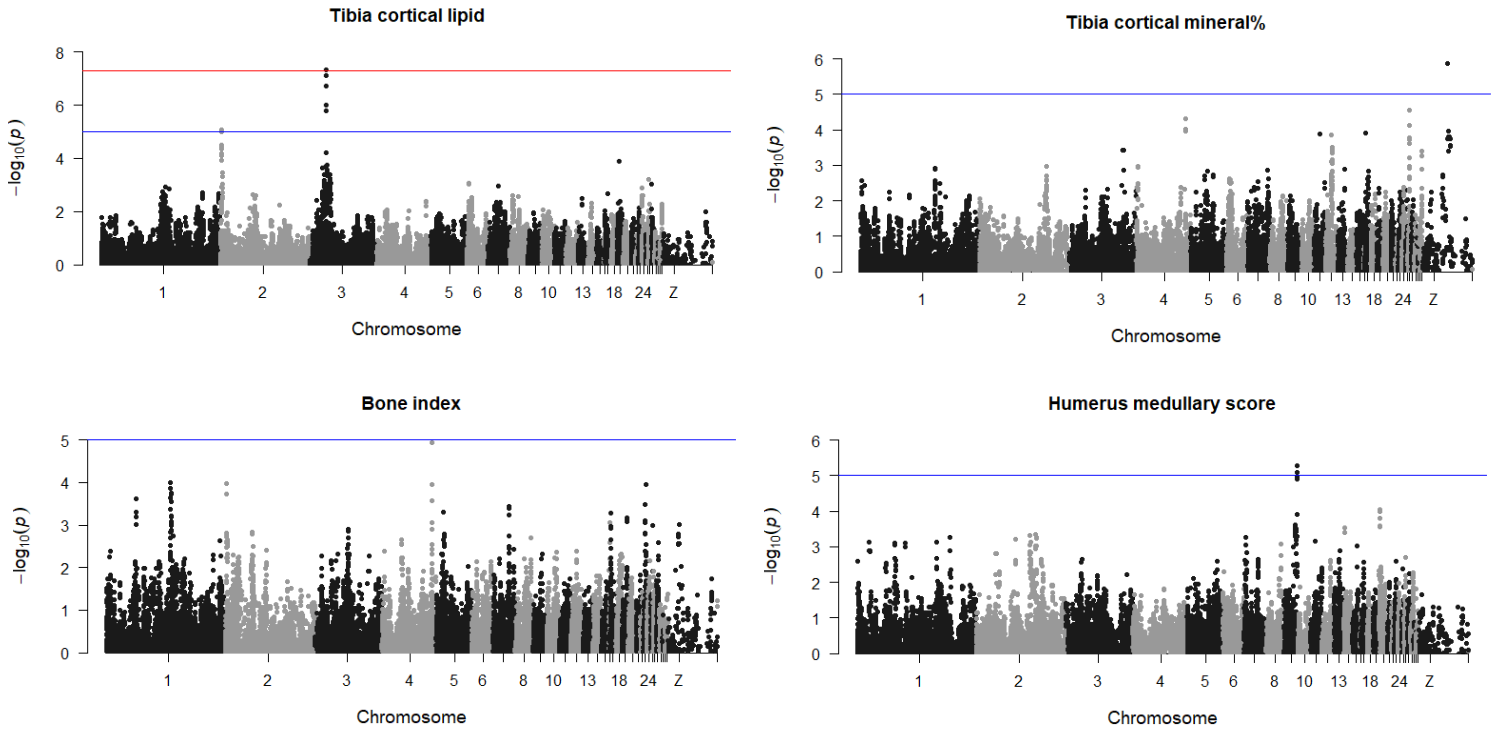

Figure 1. Manhattan plots showing –log$_{10}$(Wald P-value) for each SNP marker in Tibia cortical lipid/mineral%, humerus medullary score, and bone index. The red line is to the conventional significance threshold of 5*10^{-8} and the blue a suggestive threshold of 10^{-5}.

Discussion

In this study, we detected seven loci associated with bone traits in a Rhode Island Red population. Tibia cortical lipid showed the most significant associations. Tibia cortical lipid is phenotypically correlated with the degree of mineralization (mineral% and phosphate/organic ratio) of both medulla and cortex, tibia breaking strength and density. Varying levels of lipids may be caused by variation in the cell population of the cortex. It remains to be investigated whether lipid content may be used as an intermediary physiological phenotype for bone quality.

The regions represent novel associations for bone traits. None overlap the tibial breaking strength QTL detected by Raymond et al., (2018) in White Leghorn layers of the Lohmann breeding program. When comparing to QTL in Chicken QTLdb, overlaps included QTL for feed intake (Mignon-Grasteau et al., 2015) and comb, feather and wattle traits in a Chinese chicken breed (Sun et al., 2015). The lipid association on chromosome 2 overlaps a suggestive locus for cortical carbonate content from Johnsson et al. (2021).

In contrast, there were no strong associations for bone breaking strength and the bone index. Tibia and humerus breaking strength show high heritabilities (~ 0.5) in this population (Dunn et al., 2021). The genomic heritabilities estimated in the current GWAS were comparable:

0.47 for tibia breaking strength, 0.35 for humerus breaking strength and 0.48 for bone index. This suggests that the SNP chip tags most of the additive genetic variance in the trait, without detecting major QTL. Previously, a major QTL for tibial breaking strength has been detected and fine-mapped to the *cystathionine beta synthase* (*CBS*) gene in White Leghorn line (De Koning et al., 2020). This locus was not detected in the current Rhode Island Red line. This finding suggests a polygenic architecture for bone-breaking strength in Rhode Island Red.

This study demonstrates the importance of developing bone measurements that enable large-scale genetic mapping studies and genomic selection of bone traits. Ideally, recording should be possible on live birds and for the keel bone, which appears the most important for layer health yet hardest to accurately phenotype (Thøfner et al., 2021).

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