

Contents lists available at ScienceDirect

Poultry Science



journal homepage: www.elsevier.com/locate/psj

Full-Length Article

Genetic markers associated with bone strength and density in Rhode Island Red laying hens

Qiaoxian Yue^a, Martin Johnsson^b, Peter W Wilson^c, Björn Andersson^d, Matthias Schmutz^d, Cristina Benavides^e, Nazaret Dominguez-Gasca^e, Estefania Sanchez-Rodriguez^e, Alejandro B Rodriguez-Navarro^e, Ian C Dunn^c, Dirk-Jan de Koning^{b,*}

^a Shanxi Agricultural University, Shanxi 030801, China

^b Department of Animal Biosciences, Swedish University of Agricultural Sciences, Box 7023 750 07, Uppsala 756 51, Sweden

^c The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK

^d Lohmann Breeders, Cuxhaven 27472, Germany

^e Departamento de Mineralogia y Petrologia, Universidad de Granada, Granada 18002, Spain

ARTICLE INFO

Keywords: Bone strength Genome-wide association study Laying hen Rhode Island Red

ABSTRACT

Damage to the keel bone in commercial laying hens represent one of the greatest welfare issues in laying hens. This study aims to identify the DNA markers and candidate genes for bone strength and density traits in a Rhode Island Red laying hen population. We conducted genome-wide association studies (**GWAS**) on bone quality traits using a sample of 925 Rhode Island Red laying hens genotyped with a genotyping array consisting of 60 000 DNA markers. With a univariate linear mixed model, we identified 52 suggestive genetic markers located within 28 candidate genes that are associated with the humerus, keel, and tibia strength and density. We also found overlaps between the GWAS results for medullary bone score and tibia strength and density with published quantitative trait loci (**QTL**) for eggshell effective layer thickness and abdominal fat weight, respectively. Heritability estimates for the humerus stiffness, tibia stiffness, medullary bone score and minor bone diameter ranged from 0.21 to 0.34. Annotation term enrichment analysis of genes within 2 Megabases of suggestive markers found that mTOR signalling pathway, tryptophan metabolism, TGF- β signalling pathway, and apoptosis were significantly enriched. These loci do not overlap previously published associations, and thus appear to be novel.

Introduction

High egg production makes laying hens prone to problems such as keel bone deformation and damage during the egg-laying period. Bone damage represents a considerable welfare and economic problems in commercial laying hens (Candelotto et al., 2017; Habig et al., 2021; Harlander-Matauschek et al., 2015; Webster, 2004). As the age increases, the decrease of mineralized structural bone mass during the laying period leads to declining the health status and egg quality of laying hens. Currently, bone health is one of the main factors limiting the performance of extra-long laying hens (Alfonso-Carrillo et al., 2021).

To date, a large number of QTLs and candidate genes that significantly affect chicken skeletal traits have been identified (Podisi et al., 2012; Schreiweis et al., 2005). A QTL region significantly associated with tibia and humerus breaking strength was identified on chromosome 1 in a hybrid F2 population of commercial purebred White Leghorn hen, which was recently fine-mapped and identified around the cystathionine β synthase (CBS) gene areas associated with osteoporosis (De Koning et al., 2020; Dunn et al., 2007). A GWAS analysis of hybrid F2 laying hens between a Chinese indigenous breed and a White Leghorn flock identified several candidate genes mapped to narrow regions related to bone development (Guo et al., 2017). GWAS combined selection signature analysis to analyze the genetic basis in an F2 population constructed by broiler and layer, and identified 21 candidate genes in 3 genomic regions significantly related to bone growth and development (Li et al., 2021).

The Rhode Island Red breed is one of the most common breeds in the world and is often used as a cross parent for many commercial layers (Kumar et al., 2002). Estimates of the heritability of bone quality traits in the Rhode Island Red population were recently reported, suggesting

https://doi.org/10.1016/j.psj.2025.105246

Received 18 March 2025; Accepted 1 May 2025 Available online 2 May 2025

0032-5791/© 2025 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

^{*} Corresponding author. *E-mail address*: dj.de-koning@slu.se (D.-J. de Koning).

Table 1

Descriptive statistics for bone traits.

Traits/unit	Ν	Min	Max	Mean	SD	CV/%	Number of SNPs
Thickness/mm	902	0.254	1.144	0.598	0.107	17.934	30,753
Tibia density/mm_Al_equiv	892	1.687	3.089	2.357	0.241	10.237	30,750
Tibia breaking strain/N	889	98.700	387.000	226.600	50.083	22.103	30,746
Tibia stiffness/N_mE	881	14.803	57.091	33.912	7.341	21.645	30,743
Minor diameter/mm	883	5.857	8.160	6.993	0.437	6.246	30,754
Major diameter/mm	884	6.953	10.023	8.527	0.576	6.750	30,740
Keel density/mm_Al_equiv	866	0.573	1.088	0.825	0.097	11.740	30,749
Humerus density/mm_Al_equiv	825	0.947	2.361	1.432	0.304	21.255	30,756
Humerus breaking strain/N	861	74.400	292.100	157.500	45.539	28.911	30,406
Humerus stiffness/N_mE	862	8.822	42.069	23.461	6.091	25.963	30,744
Medullary bone score	879	-	-	-	-	-	30,759

that bone quality could be alleviated through genetic selection (Dunn et al., 2021). The objective of this work was to identify associations and candidate genes for bone strength and density traits in the Rhode Island Red population, including the tibia breaking strength, tibia density, tibia stiffness, cortical thickness, the major and minor diameter of tibia, the humerus breaking strength, humerus density, humerus stiffness, medullary bone score, and the keel bone density using Illumina 60 K SNP array data.

Materials and methods

Animals and phenotypes

We used data from 925 Rhode Island Red hens previously studied by (Dunn et al., 2021; Sallam et al., 2023). Hens were housed with a companion hen in cages that were equipped with a perch. All hens had free access to feed and water and were managed in the same environment. Samples were collected at 68 weeks of age from four hatches as previously described. The breaking strength of the humerus and tibia was evaluated by a three-point bending test, and the density test of the humerus, tibia and keel was collected by an X-ray scanner as the previously described (Dunn et al., 2021). Descriptive statistics for body weight and bone traits are listed in Table 1. Covariates for genome-wide association studies were identified by fitting body weight, housing system, and hatch of week in a multiple regression model with bone traits as response variable, and birds that had high leverages (greater than three times the standard deviation) were removed.

Genotypes

The hens were genotyped with the Illumina 60 K chicken SNP array which contained 57,636 SNPs across 33 autosomes and two sex chromosomes (Groenen et al., 2011). We first discarded SNPs with unknown physical position and repeated genomic coordinates. The PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/; Purcell et al., 2007) was then used to control the quality of individuals by removing those with a missing genotype frequency higher than 0.05 and omitting SNPs with a minor allele frequency lower than 0.01 or a Hardy-Weinberg equilibrium *P* value lower than 1×10^{-4} . The number of variants located on autosomes eligible for the following analysis is shown in Table 1.

Genome-wide association analysis

Association between each genetic locus and bone quality trait, and heritability of each trait were evaluated under a univariate linear mixed model in the GEMMA (Zhou and Stephens, 2012) program with the command "-lmm 1". The statistical model is shown as follows:

 $y = \mathbf{W}\alpha + \mathbf{X}\beta + \mathbf{u} + \varepsilon$; $\mathbf{u} \sim MVN_n$ (0, $\lambda \tau^{-1}\mathbf{K}$); $\varepsilon \sim MNVn$ (0, $\tau^{-1}\mathbf{I}_n$)

Where y is a vector of phenotypic values. W is a matrix of covariates

(hatch, house and body weight) including a column of 1 s, α is a vector of the corresponding coefficients including the intercept. **X** is a vector of locus genotypes, β is the effect size of the locus. **u** is a vector of additive genetic effects, λ is the ratio of the random effect variance to the residual error variance, τ^{-1} represents the variance of the residual errors and **K** is a kinship matrix calculated from a pruned set of total SNP loci obtained by the command "-gk 2" in GEMMA. ε is a vector of residual errors, \mathbf{I}_n is an identity matrix.

Body weight, hatch and house were included as fixed effects in the genome-wide association study because of significant associations with traits when analyzed in a linear model (Supplementary Table 1).

In this study, a $1.6 \times 10^{-6} P$ value was used as the genome-wide significance level using Bonferroni correction (0.05/31000) and the suggestive significance threshold for *P*-value was arbitrarily set to 1×10^{-4} to provide a less conservative presentation of the results. The "QQman" package in the R software (Turner, 2018) was used to draw Manhattan and quantile—quantile plots.

SNP identification, candidate gene annotation and QTL detection

The GWAS peak SNPs were physically localized on the Galgal 6.0 reference genome assembly by the Ensembl Genes database (https://www.ensembl.org/index.html) and candidate genes closest to each tag SNP were identified. Based on the GWAS results, we used the location of the significant SNPs to search for candidate genes with GALLO R package (Fonseca et al., 2020) by entering the position of a SNP and \pm 500 kb. Furthermore, the chicken QTLdb (https://www.animalgeno me.org/cgi-bin/QTLdb/GG/index) (Hu et al., 2013) was used to find previously detected associations perform QTL enrichment testing with P_{adj} <0.05 and N_QTLS>2.

To identify a large set of suggestive SNPs for annotation term enrichment, we used a threshold to 0.0004, and a 2-Mb region was defined around each suggestive SNP. To investigate the biological associations among the candidate genes within these regions of all the GWAS lead SNPs, we performed GO and KEGG enrichment analysis by the DAVID database (https://david.ncifcrf.gov) (Huang da et al., 2009; Sherman et al., 2022).

Power analysis

To explore the power characteristics of the study, we used the genpwr R package (Moore et al., 2019), assuming a linear model, additive genetics and a significance threshold like in this study. We estimated the power to detect loci explaining 0.1 %, 1 %, 5 %, 10 % of the variance at different sample sizes, as well as the effect size that could be detected with 80 % power assuming a sample size of 850 individuals. The results are shown in Supplementary Fig. 1.



Fig. 1. Manhattan and QQ plots for the association analyses of humerus and keel traits. In the Manhattan plots, $-\log_10(P-value)$ of the filtered high-quality SNPs (y-axis) is plotted against their genomic positions (x-axis). In the Q-Q plots, $-\log_10(p)$ of observed association statistics on the Y-axis were compared to those of the association statistics expected under the hypothesis of no association on the X-axis. The solid line represents concordance between observed and expected values. Genomic inflation factor, λ , is shown for each dataset.

Results

Genome-wide association studies

Thirty-eight SNPs were associated with humerus traits. The Q-Q plots were used to illustrate the level of potential P-value inflation. The average genomic inflation factor (λ) is 0.962 (Fig. 1, Table 2), which

indicated the absence of any obvious population stratification and the reliability of GWAS results. For keel density and humerus stiffness, there was only one suggestive SNP associated, with GGA 26 and 4, respectively. Fourteen significant SNPs associated with medullary bone score were clustered in two chromosomal regions, on GGA1 and 9. A total of twenty-two suggestive loci distributed on GGA 5, 9, 13, and 25 were associated with humerus density. Furthermore, eight suggestive SNPs

Table 2

Description of SNPs significantly associated with bone quality traits in Rhode Island Red hens.

Humens density Gap. 15/97490 5 17.756.573 7 C 0.34 -0.776 0.189 0.105500 C CND1 Gap. 6.727555 5 17.756.573 5 17.756.573 5 0.759.0 0.189 0.0189 0.1056 CND1 Gap. 15.7505 5 17.816.774 5 0.323 -0.075 0.190 0.3166 ENSGALGO000049369 Gap. 15.877490 5 18.91.927 5 0.937 0.074 0.190 0.276.60 ENSGALGO000049369 Gap. 15.877490 5 19.827.50 A C 0.32 0.072 0.171 4.786.60 MIMA3C Gap. 15.878789 9 2.241.697 A C 0.32 0.072 0.173 5.56.60 ESRC1 Gap. 14.68278 9 2.247.924 A C 0.32 0.072 0.173 5.56.60 ESRC1 Gap. 14.68278 9 2.278.926 A C 0.32 0.072 0.171 5.356.60 <th>Trait</th> <th>Peak SNP</th> <th>CHR</th> <th>Pos (bp)</th> <th>A1</th> <th>A0</th> <th>MAF</th> <th>Beta (s.e)</th> <th>Var (%)</th> <th>P-value</th> <th>Nearest gene or transcript</th>	Trait	Peak SNP	CHR	Pos (bp)	A1	A0	MAF	Beta (s.e)	Var (%)	P-value	Nearest gene or transcript
gag, ni-14519800 5 17,735,200 A 6 0.734 -0.707 0.0189 6.408-05 CND11 Ggu, ni-507503 5 17,815,207 A 6 0.334 -0.075 0.0189 9.051-60 CND10 GGu, DATTP1 5 18,892,320 T C 0.323 -0.075 0.0190 9.047-60 DESCALCO000009390 GGu, DATTP157 5 18,897,320 T C 0.323 -0.075 0.0190 9.047-60 DESCALCO000009390 GGu, DATSPARS 9 2,237,150 T C 0.323 -0.075 0.0174 5.056-00 RSCALCO0000094930 GGu, DATAMARY 9 2,271,552 T C 0.323 0.072 0.0174 S.556-00 RSCALCO000004 GGu, DATAMARY 9 2,294,407 T C 0.382 0.072 0.0173 S.556-00 RSCALCO00004 GGu, DATAMARY 9 2,294,407 T C 0.382 0.072 0.0173 S.556	Humerus density	Gga_rs15674900	5	17,748,696	Т	С	0.341	-0.076	0.0189	6.105E-05	CCND1
GGaluG27753 5 17,93,200 A 6 0.342 -0.077 0.189 4.601 CND1 GGaluG427713 5 17,816,747 7 C 0.323 -0.075 0.109 9.180-L6 ENGALG0000004939 GGaluG427783 5 18,01,927 7 C 0.323 -0.074 0.109 9.747-L6 ENGALG0000004939 GGaluG427783 5 18,01,927 A C 0.321 -0.074 0.1019 9.747-L6 ENGALG0000004939 Gga,1559708 9 2,24,61,677 A C 0.382 0.072 0.0173 5.354-06 RSRC1 Gga,11462747 9 2,24,61,677 A C 0.382 0.072 0.0173 5.354-06 RSRC1 Gga,11462749 9 2,74,94,27 A C 0.382 0.072 0.0173 5.354-06 RSRC1 Gga,11462746 13 2,749,274 A C 0.382 0.072 0.0173 5.354-06 RSRC1		Gga_rs14519806	5	17,756,573	Α	G	0.341	-0.076	0.0189	6.449E-05	CCND1
gap.s1557503 5 17.816,747 A 6 0.343 -0.075 0.0190 9.051-60 PRSCALC0000009369 GGLUGA284775 5 18.967,370 7 C 0.323 -0.075 0.190 9.187-65 PRSCALC0000009369 GGLUGA28477 5 18.967,370 7 C 0.323 -0.075 0.190 9.472-65 PRSCALC0000009369 GGLUGA28477 5 3.897,370 7 C 0.325 -0.085 0.101 4.978-66 RENCL GGLUGA28477 9 2.25,71,599 7 C 0.382 0.072 0.107 5.38-66 RSCL GGLUGA384038 9 2.27,55,06 7 C 0.382 0.072 0.107 5.38-66 RSCL GGLUGA08408 9 2.275,506 A G 0.382 0.072 0.017 5.38-66 RSCL GGLUGA00840 13 2.613,738 A G 0.382 0.072 0.017 0.315 0.58-65 <t< td=""><td></td><td>GGaluGA277535</td><td>5</td><td>17,793,200</td><td>Α</td><td>G</td><td>0.342</td><td>-0.077</td><td>0.0189</td><td>4.601E-05</td><td>CCND1</td></t<>		GGaluGA277535	5	17,793,200	Α	G	0.342	-0.077	0.0189	4.601E-05	CCND1
Galui 6.27771SBis Bis Bis Bis Bis Bis Bis Bis Bis Bis		Gga_rs15675036	5	17,816,747	Α	G	0.343	-0.075	0.0189	9.051E-05	FGF19
Gap rs1495723 S 18,611,927 T C 0.774 0.1010 9.787-05 ELES GGaluGA2847785 S 18,967.795 T C 0.253 -0.075 0.1010 5.701-05 ELES Gga rs1680828 9 2.557,196 T C 0.328 0.072 0.173 5.586-06 RSRC1 GaluGA343947 9 2.267,196 T C 0.382 0.072 0.173 5.586-06 RSRC1 GGaluGA3443975 9 2.2716,989 T C 0.382 0.072 0.173 5.586-06 RSRC1 GGartS1462782 9 2.2751,989 T C 0.382 0.072 0.173 5.586-06 RSRC1 Gga rs1465746 13 2.413,785 T C 0.382 0.073 0.0175 2.586-05 PCDEGC3 GGaluGA040609 13 2.415,755 T C 0.382 0.073 0.0172 2.481-05 PCDEGC3 GGaluGA04040718<		GGaluGA277719	5	18,592,354	Т	С	0.323	-0.075	0.0190	9.180E-05	ENSGALG00000049369
Geala		Gga_rs14957234	5	18,611,927	Т	С	0.323	-0.075	0.0190	9.047E-05	ENSGALG00000049369
GalactA284.73GGGCDCDDD <t< td=""><td></td><td>GGaluGA277835</td><td>5</td><td>18,967,730</td><td>Α</td><td>G</td><td>0.317</td><td>-0.074</td><td>0.0190</td><td>9.270E-05</td><td>ELF5</td></t<>		GGaluGA277835	5	18,967,730	Α	G	0.317	-0.074	0.0190	9.270E-05	ELF5
GenGe		GGaluGA284473	5	39,327,053	Т	С	0.255	-0.085	0.0211	6.100E-05	TMEM63C
Gas Gas <td></td> <td>Gga_rs15987089</td> <td>9</td> <td>22,571,590</td> <td>Α</td> <td>G</td> <td>0.408</td> <td>0.073</td> <td>0.0171</td> <td>4.978E-06</td> <td>MFSD1</td>		Gga_rs15987089	9	22,571,590	Α	G	0.408	0.073	0.0171	4.978E-06	MFSD1
Genulocal-seqGenulo		Gga_rs16680382	9	22,641,697	Т	С	0.382	0.072	0.0173	5.536E-06	RSRC1
gga, rs1462747 9 22,716,989 7 C 0.322 0.072 0.173 5.368-06 RNC1 Gga, rs1373680 9 22,749,245 R G 0.322 0.072 0.173 5.368-06 RNC1 Gga, rs1376807 9 22,749,245 R G 0.322 0.072 0.173 5.368-06 RNC1 Gga, rs1468728 9 22,808,170 A G 0.383 0.0673 0.0173 2.536-05 PCDHGC3 Gga, rs1405766 13 2,617,385 A G 0.383 0.073 0.173 2.536-05 PCDHGC3 Gga, rs1405746 13 2,723,555 T C 0.382 0.073 0.172 2.481-05 PCDHGC3 Gga, rs1405746 13 2,723,555 T C 0.323 1.032 0.012 9.006-05 NEGL Huments stiffnes Gga, rs1367491 13 2,638,40 C 0.323 1.023 0.012 9.00-05 NEGL </td <td></td> <td>GGaluGA343964</td> <td>9</td> <td>22,694,487</td> <td>Α</td> <td>G</td> <td>0.381</td> <td>0.072</td> <td>0.0174</td> <td>8.577E-06</td> <td>RSRC1</td>		GGaluGA343964	9	22,694,487	Α	G	0.381	0.072	0.0174	8.577E-06	RSRC1
GalarGA4397 9 2,27,8,522 A G 0.322 0.072 0.173 5.368-06 RSRC1 Gga,rs1468752 9 2,278,016 A G 0.322 0.072 0.173 5.368-06 RSRC1 Gga,rs1463760 13 2,289,170 A G 0.323 0.069 0.0173 3.568-05 RSRC1 GalarGA034028 13 2,617,085 A G 0.390 0.073 0.171 2.998-05 PCDHGC3 GalarGA19603 13 2,216,085 A G 0.390 0.073 0.172 2.418-05 PCDHGC3 GalarGA19478 13 2,286,077 A G 0.390 0.072 0.172 2.418-05 PCDHGC3 GalarGA19478 13 2,786,047 A G 0.370 0.012 9.008-05 PCGZ Keel density Gga,rs1405740 14 63,729,936 A G 0.360 0.370 0.0173 5.1596-05 REGGL		Gga_rs14682747	9	22,716,989	Т	С	0.382	0.072	0.0173	5.536E-06	RSRC1
Gga rs13/29650 9 22,749,245 T 6 0.328 0.072 0.0173 5.536+06 RSRC1 GalanGA34028 9 22,808,170 A 6 0.383 0.072 0.0173 5.536+06 RSRC1 GalanGA00000 13 2,617,085 A 6 0.383 0.0173 0.0174 2,61405 PCDHCG3 GalanGA000000 13 2,728,507 T C 0.382 0.073 0.0172 2,445265 PCDHCG3 GalanGA009064 13 2,728,507 T C 0.324 0.073 0.0172 2,445265 PCDH1 GalanGA104718 2 30,500 T C 0.244 0.075 0.0122 9,8006-05 SNX27 Humerus stiffnes GalanGA104718 2 85,58,30 A G 0.234 0.0754 1.0276-05 ENGLG0000031405 Keel density GalanGA22677 1 6,35,370 T C 0.178 0.379 0.031 5.159-05		GGaluGA343975	9	22,718,522	Α	G	0.382	0.072	0.0173	5.536E-06	RSRC1
Gga ra1462722 9 22,755,016 A 6 0.328 0.072 0.173 5.536.00 RSRC1 Gga ladioGA344028 13 2.613,738 A 6 0.378 0.0173 1.516.05 PCDHGC3 Gga ladioGA00809 13 2.613,738 A 6 0.390 0.073 0.0175 2.641.60 PCDHGC3 Gga ladioGA008064 13 2.723,555 A 6 0.390 0.073 0.0172 2.445.60 PCDHGC3 Gga ladioGA07466 13 2.723,555 A 6 0.390 0.073 0.0192 9.8006.05 PN2A GalaioGA19478 2 4.1559 A 6 0.320 0.073 0.0192 9.8006.05 PN2A Medulary bone score Gga ra1587267 1 4 6.8 0.20 0.203 0.031 5.1596.05 RRGL Medulary bone score Gga ra1587267 1 6.324.90 0.379 0.931 5.1596.05 RRGL GalaioA24267		Gga_rs13736850	9	22,749,245	Т	G	0.382	0.072	0.0173	5.536E-06	RSRC1
Gradind 244028922,808,170AG0.3830.0790.01731.51E-05NINO2Gradind 2000809132,617,388AG0.3900.0730.01712,099E-05PCDHGC3Gradind 2000809132,723,555TC0.3280.0730.01722,445E-05PCDHGC3Gradind 19468325363,950TC0.2440.0750.01222,445E-05PCDH1Gradind 19468325363,950TC0.233-0.03200.01722,445E-05NSX27Humens stiffnesGradind 270428488,558,830AG0.0330.00751.207E-05NSX04.0000031807Keel densityGradind 2477163,701,762AG0.1800.0731.207E-05NSX04.0000031807Medullary bone scorGradind 24277163,723,930AG0.1800.0731.307E-05NSR04.0000031807Gradind 02257717163,896,924AG0.3680.0730.09315.159E-05RERGLGradind 02257717163,896,924AG0.3680.0720.09315.159E-05RERGLGradind 022577172,472,75AG0.3680.3790.09315.159E-05RERGLGradind 34391922,471,85AG0.3680.3240.07095.536E-06RSRC1Gradind 343975922,71,590AG0.324<		Gga_rs14682782	9	22,755,016	Α	G	0.382	0.072	0.0173	5.536E-06	RSRC1
Ga ga rs1090001132,613,738A CG0.7370.01753.565±05PCDHCG3GaluGA009004132,723,555TC0.3820.0730.01712,095±06PCDHCG3Ga Ga ga rs1090766132,723,555TC0.3820.0730.01722,445±05PCDHC1Ga GaluGA1946825363,50TC0.3240.0750.01929,800±05PCG2GaluGA1946825320,370C0.0330.00751.208±05PTPRAKedIdensityGa,rs1524910265,100,286TC0.3040.03915,159±05RERGLKedIdensityGaluGA20425716,373,96924AG0.3070.09315,159±05RERGLGaluGA02257717,347,934AG0.3790.09315,159±05RERGLGaluGA02257717,347,934AG0.3720.09315,159±05RERGLGaluGA02257717,947,934AG0.3720.09315,159±05RERGLGaluGA02257717,947,934AG0.3850.3720.09315,159±05RERGLGaluGA3496192,257,159AG0.3640.0703,35±06RERGLGaluGA3497592,271,592AG0.3850.3240.0705,35±06RSRC1GaluGA3496492,271,592AG0.3860.324 <td< td=""><td></td><td>GGaluGA344028</td><td>9</td><td>22,808,170</td><td>А</td><td>G</td><td>0.383</td><td>0.069</td><td>0.0173</td><td>1.351E-05</td><td>SHOX2</td></td<>		GGaluGA344028	9	22,808,170	А	G	0.383	0.069	0.0173	1.351E-05	SHOX2
GediabconoseGediabconoseJJJ <t< td=""><td></td><td>Gga_rs14057603</td><td>13</td><td>2,613,738</td><td>Α</td><td>G</td><td>0.378</td><td>0.073</td><td>0.0175</td><td>3.565E-05</td><td>PCDHGC3</td></t<>		Gga_rs14057603	13	2,613,738	Α	G	0.378	0.073	0.0175	3.565E-05	PCDHGC3
GaluckoneGaluckone1227C0.3820.0740.01752.6416.05ENSCALGO000034005Galucka1946825365,950TC0.3900.0720.01722.4456.05PCDH1Galucka1947825365,950TC0.2340.0750.10129.8006.05SNX27Galucka1947828421,590R.C0.233-1255.59030.019.646.05PTRAKeel densityGagars180720163,707.72RC0.3040.00751.2076.05ENSCALGO000031807Medullary bone scoreGagars18378420163,723,936AC0.1800.3790.09315.1596.05RERGLGalucA022457163,859,324AC0.1800.3790.09315.1596.05RERGLGalucA02679163,859,324AC0.1800.3790.09315.1596.05RERGLGalucA02679717.9.347.934AC0.3240.0705.366.06RSCLRSCLGalucA02679712.2.474.285AG0.3240.0705.366.06RSCLRSCLGalucA3196892.2.641.487AG0.3860.3240.0705.366.06RSCLGalucA3196492.2.641.487AG0.3860.3240.07095.366.06RSCLGalucA3196592.2.714.520AG0.3860.3240.07095.366.06		GGaluGA000809	13	2,617,085	Α	G	0.390	0.073	0.0171	2.099E-05	PCDHGC3
R-ga r.s14057460 13 2,786,047 A G 0.303 0.073 0.0172 2,445:05 PCH1 GaluGA194683 25 363,950 T C 0.244 0.075 0.0192 9.800E-05 SNX27 Humerus stiffness GaluGA194718 25 421,590 A C 0.320 -12535.90 3200,471 9.684E-05 PTPRA Keel density Gag r.s13287420 1 6.3723,932 A C 0.303 0.0073 5.159E-05 RERGL Medullary bone score Gag r.s1387420 1 6.3723,932 A C 0.180 0.379 0.0931 5.159E-05 RERGL GaluGA022507 1 6.3853,470 A C 0.370 0.0931 5.159E-05 RERGL GaluGA034708 9 2.2,471,285 A C 0.370 0.0931 5.159E-05 RERGL GaluGA343801 9 2.2,471,285 A C 0.340 0.222 0.0700 5.36E-06		GGaluGA090604	13	2,723,555	Т	С	0.382	0.074	0.0175	2.641E-05	ENSGALG0000034005
Galach19468325363,95TC0,2440.0750.01929.800E.05POCZHumerus stiffnessGalach21947182488,558,80AG0.233-1253.5903.200.47109.804E.05PTPRAKeel densityGga,r15204910265,100,262TC0.303-0.0330.00751.207E.05RERGI.Medullary bone scoreGga,r13878420163,701,762C0.1780.3790.09315.159E.05RERGI.GGalach202507163,853,470AG0.1800.3790.09315.159E.05RERGI.GGalach202507163,859,793AG0.324-0.2550.07285.604E.05HAO2GGalach202507179,3479,34AG0.3400.3790.09315.159E.05RERGI.GGalach202607179,3479,34AG0.3400.3790.0718.504E.06MESDI.GGalach2034961922,472,85AG0.3400.3790.0718.504E.06MESDI.GGalach203497922,472,85AG0.3660.3140.07095.536E.06RSRC1GGalach203498922,715,92AG0.3660.3240.07095.536E.06RSRC1GGalach203497922,715,92AG0.3660.3240.07095.536E.06RSRC1GGalach203497922,715,92AG0.3660.3240		Gga_rs14057466	13	2,786,047	А	G	0.390	0.073	0.0172	2.445E-05	PCDH1
Humenus Humenus GaluGA1947182541,590AG0.233-0.0750.01209.000-55NX272Humenus GaluGA270428Ga88,558,300AG0.323-1253.590300.47109.684.650PIPRAMedullary bone scoreGa,rs1620491266,100.286TC0.3030.00751.207E-05RKRGLMedullary bone scoreGa,rs138782016,372.396AG0.1800.3790.09315.159E-05RKRGLGaluGA02270716,385.3470TC0.1800.3790.09315.159E-05RKRGLGaluGA022707179.347.934AG0.3240.2550.07285.604E-05HAO2GaluGA023707179.347.934AG0.3240.07045.36E-06RKRGLGaluGA02370712,247.285AG0.3240.07095.36E-06RKRCLGaluGA3437692,247.285AG0.3860.3240.07095.36E-06RKRCLGaluGA3437592,271.852AG0.3860.3240.07095.36E-06RKRCLGaluGA34397592,275.950AG0.3860.3240.07095.36E-06RKRCLGaluGA3440892,247.852AG0.3860.3240.07095.36E-06RKRCLGaluGA3440792,275.950AG0.3860.3240.07095.36E-06RKRCL <td></td> <td>GGaluGA194683</td> <td>25</td> <td>363,950</td> <td>Т</td> <td>С</td> <td>0.244</td> <td>0.075</td> <td>0.0192</td> <td>9.800E-05</td> <td>POGZ</td>		GGaluGA194683	25	363,950	Т	С	0.244	0.075	0.0192	9.800E-05	POGZ
Humens stiffness GGalu GA270428 4 88,558,830 A C 0.323 -12535.990 3200.471 9.684-05 PTRA Keel density Gga, ris1387620 1 63,701,762 A G 0.703 0.0075 1.207E05 RRGL Medullary bone score Gga, ris13878420 1 63,701,752 A G 0.178 0.0730 0.0931 5.159E-05 RRGL GGaluGA022507 1 63,853,470 T C 0.180 0.379 0.0931 5.159E-05 RRGL GGaluGA022507 1 73,84,934 A G 0.372 -0.295 0.0720 5.179E-05 RRGL GGaluGA026797 1 73,47,934 A G 0.340 0.379 0.0720 5.36E-06 RRGL RRGL GGaluGA026797 1 73,47,934 A G 0.342 0.0709 5.35E-06 RRGL GGaluGA026797 9 22,671,590 A G 0.356 0.324 <		GGaluGA194718	25	421,590	А	G	0.244	0.075	0.0192	9.800E-05	SNX27
Keel density Gga_rs1a204910 26 5,100,286 T C 0.30 -0.033 0.075 1.207E-05 NSCALG0000031807 Medullary bone score Gga_rs13878250 1 63,73,7072 A G 0.178 0.370 0.0931 5.159-05 RERGL Gealur, GAU2457 1 63,853,470 T C 0.180 0.379 0.0931 5.159-05 RERGL Gealur, GAU24577 1 63,858,470 T C 0.180 0.379 0.0931 5.159-05 RERGL Gealur, GAU24577 1 63,858,470 A G 0.372 -0.295 0.0728 5.064-05 HAC1 Gealur, GAU243781 9 2.247,285 A G 0.322 0.0701 4.978-06 MSC1 Gealur, GAU343961 9 2.247,1285 A G 0.386 0.324 0.0701 5.356-06 RSRC1 Gaus 16452747 9 2.271,852 A G 0.386 0.324 0.0709	Humerus stiffness	GGaluGA270428	4	88,558,830	А	G	0.323	-12535.990	3200.4710	9.684E-05	PTPRA
Medullary bone score Ga_rs13878250 1 63,701,762 A G 0.178 0.370 0.0934 7.198-05 RERCL GaluGA022457 1 63,723,936 A G 0.180 0.379 0.0931 5.159-05 RERCL GaluGA022507 1 63,853,724 A G 0.180 0.379 0.0931 5.159-05 RERCL GaluGA022507 1 63,853,724 A G 0.180 0.379 0.0931 5.159-05 RERCL GaluGA026797 1 79,347,934 A G 0.360 0.2285 0.0720 8.173-055 ENSCALG00000039468 Gaga_r515987089 9 22,671,598 A G 0.386 0.324 0.0709 5.536-06 RSRC1 Gaga_r514682747 9 22,716,989 A G 0.386 0.324 0.0709 5.536-06 RSRC1 Gaga_r514682747 9 22,716,989 A G 0.386 0.324 0.0709 5.53	Keel density	Gga_rs16204910	26	5,100,286	Т	С	0.360	-0.033	0.0075	1.207E-05	ENSGALG00000031807
GGaluGA022457 1 63,723,936 A G 0.180 0.379 0.0931 5.159E-05 RERGL Ggars13878420 1 63,853,470 T C 0.180 0.379 0.0931 5.159E-05 RERGL GGaluGA022577 1 79,347,934 A G 0.372 -0.295 0.0728 5.604E-05 HAO2 GGaluGA343801 9 22,427,255 A G 0.340 0.285 0.0720 8.178E-06 RSGL ENGALGO000039468 Ggars16680382 9 22,564,487 A G 0.386 0.324 0.0709 5.36E-06 RSRC1 Ggars1458247 9 22,718,522 A G 0.386 0.324 0.0709 5.36E-06 RSRC1 Ggars1376850 9 22,718,522 A G 0.386 0.324 0.0709 5.36E-06 RSRC1 Ggars1376850 9 22,749,245 A G 0.386 0.324 0.0709 5.36E-06 <td< td=""><td>Medullary bone score</td><td>Gga_rs13878250</td><td>1</td><td>63,701,762</td><td>А</td><td>G</td><td>0.178</td><td>0.370</td><td>0.0934</td><td>7.918E-05</td><td>RERGL</td></td<>	Medullary bone score	Gga_rs13878250	1	63,701,762	А	G	0.178	0.370	0.0934	7.918E-05	RERGL
Rega Gga S13878420 1 63,853,470 T C 0.180 0.379 0.0931 5.159E.05 RERGL GGaluGA022507 1 63,896,924 A G 0.180 0.379 0.0931 5.159E.05 RERGL GGaluGA02507 1 79,347,934 A G 0.322 -0.295 0.0720 8.173E.06 ENSGLG00000039468 Gga,r51660032 9 22,671,590 A G 0.322 0.0700 4.978E.06 RSRC1 Gga,r51660032 9 22,691,487 A G 0.386 0.324 0.0709 5.536E.06 RSRC1 GGaluGA343975 9 22,718,522 A G 0.386 0.324 0.0709 5.536E.06 RSRC1 Gga,r514682782 9 22,755,154 A G 0.386 0.324 0.0709 5.536E.06 RSRC1 Gga,r514682782 9 22,058,170 A G 0.386 0.324 0.0709 5.536E.06	-	GGaluGA022457	1	63,723,936	А	G	0.180	0.379	0.0931	5.159E-05	RERGL
GaluGA022507 1 63,896,924 A G 0.180 0.379 0.0931 5.159E.05 RERGL GGaluGA025797 1 79,347,934 A G 0.372 -0.295 0.0728 5.004E.05 HAO2 GGaluGA343001 9 22,472,285 A G 0.340 0.285 0.0700 4.978E.06 MFSD1 Gga_rs16680382 9 22,641,697 A G 0.386 0.324 0.0709 5.36E.06 RSRC1 GGaluGA343964 9 22,641,697 A G 0.386 0.324 0.0709 5.36E.06 RSRC1 Gga_rs14682747 9 22,716,989 A G 0.386 0.324 0.0709 5.36E.06 RSRC1 Gga_rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.36E.06 RSRC1 Gga_rs14682782 9 22,755,016 A G 0.386 0.310 0.0709 5.36E.06 RSRC1 <t< td=""><td></td><td>Gga_rs13878420</td><td>1</td><td>63,853,470</td><td>Т</td><td>С</td><td>0.180</td><td>0.379</td><td>0.0931</td><td>5.159E-05</td><td>RERGL</td></t<>		Gga_rs13878420	1	63,853,470	Т	С	0.180	0.379	0.0931	5.159E-05	RERGL
Image: bit of the sector of the sec		GGaluGA022507	1	63,896,924	А	G	0.180	0.379	0.0931	5.159E-05	RERGL
Image: bit of the sector of the sec		GGaluGA026797	1	79,347,934	А	G	0.372	-0.295	0.0728	5.604E-05	HAO2
Image: bit is the second of the sec		GGaluGA343801	9	22,427,285	А	G	0.340	0.285	0.0720	8.173E-05	ENSGALG0000039468
Gga rs16680382 9 22,641,697 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 GGaluGA343954 9 22,694,487 A G 0.385 0.318 0.0711 8.577E-06 RSRC1 Gga rs14682747 9 22,716,522 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga rs14682747 9 22,718,522 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga rs14682782 9 22,808,170 A G 0.386 0.310 0.0709 1.51E-05 SHOX2 Gga rs14502791 17 3,353,238 A G 0.483 -11.001 2.5930 2.41E-05 KCNQ1 Gga rs14517040 5 13,645,074 A G 0.444 -0.052 0.0124 3.38E-05 KCNQ1 <td></td> <td>Gga_rs15987089</td> <td>9</td> <td>22,571,590</td> <td>А</td> <td>G</td> <td>0.412</td> <td>0.322</td> <td>0.0700</td> <td>4.978E-06</td> <td>MFSD1</td>		Gga_rs15987089	9	22,571,590	А	G	0.412	0.322	0.0700	4.978E-06	MFSD1
GGaluGA343964 9 22,694,487 A G 0.385 0.318 0.0711 8.577E-06 RSRC1 Gga,rs14682747 9 22,716,989 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 GGaluGA343975 9 22,749,282 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga,rs13736850 9 22,749,245 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga,rs14682782 9 22,795,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga,rs14145402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIPA Tibia breaking strength Gga,rs14517040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Tibia density Gga,rs1418617 2 2.0,486,067 T G 0.443 -0.052 0.0124 3.38E-05 CBK14 Gga,rs14150254 2 2.1,792,323<		Gga_rs16680382	9	22,641,697	А	G	0.386	0.324	0.0709	5.536E-06	RSRC1
Gga_rs14682747 9 22,716,989 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 GGaluGA343975 9 22,718,522 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs13736850 9 22,749,245 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs14682782 9 22,808,170 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs14145402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Tibia density Gga_rs1417040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Tibia stiffness Gga_rs1418617 2 20,486,067 T G 0.463 1553.890 3969.8630 9.805E-05 FAM171A1 Gga_rs14180107 2 21,774,23		GGaluGA343964	9	22,694,487	А	G	0.385	0.318	0.0711	8.577E-06	RSRC1
GGaluGA343975 9 22,718,522 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs13736850 9 22,749,245 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 GGaluGA344028 9 22,808,170 A G 0.386 0.310 0.0709 1.351E-05 SHOX2 Tibia breaking strength Gga_rs1415402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Gga_rs1415402 2 17,645,348 T C 0.443 -0.052 0.0124 3.138E-05 KCNQ1 Tibia density Gga_rs14154017 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga_rs14150107 2 21,774,232 T C 0.480 -16256.6		Gga_rs14682747	9	22,716,989	А	G	0.386	0.324	0.0709	5.536E-06	RSRC1
Gga_rs13736850 9 22,749,245 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Gga_rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Tibia breaking strength Gga_rs1445402 2 17,645,348 T C 0.483 -11.001 2.5936 2.441E-05 PIP4K2A Gga_rs1415402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Gga_rs1417040 5 13,545,074 A G 0.097 -17.884 4.5094 7.903E-05 FCNQ1 Gga_rs1416807 2 13,66,911 A G 0.445 -0.052 0.0124 3.138E-05 KCNQ1 Tibia stiffness Gga_rs1418617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FCM171A1 Gga_rs14150107 2 21,774,232 T C 0.480 -1		GGaluGA343975	9	22,718,522	А	G	0.386	0.324	0.0709	5.536E-06	RSRC1
Gga_rs14682782 9 22,755,016 A G 0.386 0.324 0.0709 5.536E-06 RSRC1 Tibia breaking strength Gga_rs1445402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Tibia breaking strength Gga_rs1445402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Gga_rs145107 17 3,353,238 A G 0.044 -0.052 0.0124 3.38-05 KCNQ1 Tibia density Gga_rs14464648 5 13,646,971 A G 0.444 -0.052 0.0124 3.247E-05 KCNQ1 Tibia stiffness Gga_rs14148617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga_rs14150107 2 21,774,232 T C 0.480 -15952.890 4046.8040 8.723E-05 CDK14 Gga_rs14620154 7 26,367,786 </td <td></td> <td>Gga_rs13736850</td> <td>9</td> <td>22,749,245</td> <td>Α</td> <td>G</td> <td>0.386</td> <td>0.324</td> <td>0.0709</td> <td>5.536E-06</td> <td>RSRC1</td>		Gga_rs13736850	9	22,749,245	Α	G	0.386	0.324	0.0709	5.536E-06	RSRC1
GGaluGA344028 9 22,808,170 A G 0.386 0.310 0.0709 1.351E-05 SHOX2 Tibia breaking strength Gga_rs14145402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Gga_rs15032791 17 3,353,238 A G 0.097 -17.884 4.5094 7.903E-05 PAPA Tibia density Gga_rs14517040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Tibia stiffness Gga_rs1418617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga_rs14150107 2 21,774,232 T C 0.463 -15952.890 4046.8040 8.722.05 CDK14 Gga_rs14150254 2 21,912,734 A G 0.480 -16256.690 4038.7280 6.186E-05 CDK14 Gga_rs14620154 7 26,637,786 A <td< td=""><td></td><td>Gga_rs14682782</td><td>9</td><td>22,755,016</td><td>Α</td><td>G</td><td>0.386</td><td>0.324</td><td>0.0709</td><td>5.536E-06</td><td>RSRC1</td></td<>		Gga_rs14682782	9	22,755,016	Α	G	0.386	0.324	0.0709	5.536E-06	RSRC1
Tibia breaking strength Gga rs14145402 2 17,645,348 T C 0.483 -11.001 2.5930 2.441E-05 PIP4K2A Gga rs15032791 17 3,353,238 A G 0.097 -17.884 4.5094 7.903E-05 PAPPA Tibia density Gga rs14517040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Gga rs16468468 5 13,606,911 A G 0.445 -0.052 0.0124 3.247E-05 KCNQ1 Tibia stiffness Gga rs14148617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga rs14150107 2 21,774,232 T C 0.480 -16256.690 4038.7280 6.186-05 CDK14 Gga rs1450254 2 21,912,734 A G 0.429 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Gga rs14620154 7 26,637,786 A<		GGaluGA344028	9	22,808,170	А	G	0.386	0.310	0.0709	1.351E-05	SHOX2
Gga_rs15032791 17 3,353,238 A G 0.097 -17.884 4.5094 7.903E-05 PAPPA Tibia density Gga_rs14517040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Tibia stiffness Gga_rs1448617 2 20,486,067 T G 0.445 -0.052 0.0124 3.247E-05 KCNQ1 Tibia stiffness Gga_rs1418617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga_rs14150170 2 21,774,232 T C 0.460 -15952.890 4046.8040 8.723E-05 CDK14 Gga_rs14150170 2 21,974,232 T C 0.480 -16256.609 4038.7280 6.186E-05 CDK14 Gga_rs14620139 7 26,263.298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Gga_rs14620154 7 26,363,786 A	Tibia breaking strength	Gga_rs14145402	2	17,645,348	Т	С	0.483	-11.001	2.5930	2.441E-05	PIP4K2A
Tibia density Gars14517040 5 13,545,074 A G 0.444 -0.052 0.0124 3.138E-05 KCNQ1 Gars16468468 5 13,606,911 A G 0.445 -0.052 0.0124 3.247E-05 KCNQ1 Tibia stiffness Ggars14148617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Ggars14150107 2 21,774,232 T C 0.480 -16256.690 4046.8040 8.723E-05 CDK14 Ggars14150024 2 21,912,734 A G 0.480 -16256.690 4038.7280 6.186E-05 CDK14 Ggars14620039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Ggars14620154 7 26,367,786 A G 0.229 -18485.500 4671.5250 9.275E-05 ENSGALG00000026460 Ggars14620121 7 26,364,936 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 <		Gga_rs15032791	17	3,353,238	Α	G	0.097	-17.884	4.5094	7.903E-05	PAPPA
Gga rs16468468 5 13,606,911 A G 0.445 -0.052 0.0124 3.247E-05 KCNQ1 Tibia stiffness Gga rs14148617 2 20,486,067 T G 0.445 -0.052 0.0124 3.247E-05 KCNQ1 Gga rs14148617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAM171A1 Gga rs14150107 2 21,774,232 T C 0.480 -15952.890 4046.8040 8.723E-05 CDK14 Gga rs14502039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Gga rs14620154 7 26,367,786 A G 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Gga rs14620154 7 26,367,786 A G 0.229 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga rs14620121 7 26,367,786 A G 0.229 <td>Tibia density</td> <td>Gga_rs14517040</td> <td>5</td> <td>13,545,074</td> <td>А</td> <td>G</td> <td>0.444</td> <td>-0.052</td> <td>0.0124</td> <td>3.138E-05</td> <td>KCNQ1</td>	Tibia density	Gga_rs14517040	5	13,545,074	А	G	0.444	-0.052	0.0124	3.138E-05	KCNQ1
Tibia stiffness Gga rs14148617 2 20,486,067 T G 0.463 15535.890 3969.8630 9.805E-05 FAMI71A1 Gga rs14150107 2 21,774,232 T C 0.463 15535.890 4046.8040 8.723E-05 CDK14 Gga rs14150107 2 21,774,232 T C 0.480 -15952.890 4046.8040 8.723E-05 CDK14 Gga rs14150254 2 21,912,734 A G 0.480 -16256.690 4038.7280 6.186E-05 CDK14 Gga rs14620039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG00000026460 Gga rs14620154 7 26,367,786 A G 0.229 -18468.990 4537.1030 5.113E-05 SLC15A2 Gga rs14620196 7 26,384,936 T C 0.216 -18476.380 4676.4900 8.411E-05 SLC15A2 Gga rs14620121 7 26,411,766 T C 0.230 </td <td>-</td> <td>Gga_rs16468468</td> <td>5</td> <td>13,606,911</td> <td>А</td> <td>G</td> <td>0.445</td> <td>-0.052</td> <td>0.0124</td> <td>3.247E-05</td> <td>KCNQ1</td>	-	Gga_rs16468468	5	13,606,911	А	G	0.445	-0.052	0.0124	3.247E-05	KCNQ1
Gga_rs14150107 2 21,774,232 T C 0.480 -15952.890 4046.8040 8.723E-05 CDK14 Gga_rs14150254 2 21,912,734 A G 0.480 -16256.690 4038.7280 6.186E-05 CDK14 Gga_rs14620039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG0000026460 Gga_rs14620154 7 26,367,786 A G 0.229 -18468.990 4537.1030 5.113E-05 SLC15A2 Gga_rs14620196 7 26,364,786 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga_rs14620211 7 26,411,766 T C 0.220 -19411.710 4536.9180 2.088E-05 SLC15A2 Gga_rs15008991 14 7,144,668 T C 0.221 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15008918 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Gga_rs16407339 5	Tibia stiffness	Gga_rs14148617	2	20,486,067	Т	G	0.463	15535.890	3969.8630	9.805E-05	FAM171A1
Gga_rs14150254 2 21,912,734 A G 0.480 -16256.690 4038.7280 6.186E-05 CDK14 Gga_rs14620039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG0000026460 Gga_rs14620154 7 26,367,786 A G 0.229 -18468.990 4537.1030 5.113E-05 SLC15A2 Gga_rs14620196 7 26,384,936 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga_rs14620211 7 26,411,766 T C 0.201 54588.740 13897.9700 9.247E-05 SLC15A2 Gga_rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15008918 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga rs16470339 5 15,066.389 A C		Gga_rs14150107	2	21,774,232	Т	С	0.480	-15952.890	4046.8040	8.723E-05	CDK14
Gga rs14620039 7 26,263,298 T C 0.215 -18345.500 4671.5250 9.275E-05 ENSGALG0000026460 Gga rs14620154 7 26,367,786 A G 0.229 -18468.990 4537.1030 5.113E-05 SLC15A2 Gga rs14620196 7 26,384,936 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga rs1462021 7 26,411,766 T C 0.230 -19411.710 4536.9180 2.088E-05 SLC15A2 Gga rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga rs15008991 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga rs16470339 5 15,066,389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs14150254	2	21,912,734	А	G	0.480	-16256.690	4038.7280	6.186E-05	CDK14
Gga_rs14620154 7 26,367,786 A G 0.229 -18468.990 4537.1030 5.113E-05 SLC15A2 Gga_rs14620196 7 26,384,936 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga_rs14620221 7 26,411,766 T C 0.230 -19411.710 4536.9180 2.088E-05 SLC15A2 Gga_rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15009148 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga rs16470339 5 15,066.389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs14620039	7	26,263,298	Т	С	0.215	-18345.500	4671.5250	9.275E-05	ENSGALG00000026460
Gga_rs14620196 7 26,384,936 T C 0.216 -18476.380 4676.4090 8.411E-05 SLC15A2 Gga_rs14620221 7 26,411,766 T C 0.230 -19411.710 4536.9180 2.088E-05 SLC15A2 Gga_rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15009148 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga rs16470339 5 15,066.389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs14620154	7	26,367,786	А	G	0.229	-18468.990	4537.1030	5.113E-05	SLC15A2
Gga_rs14620221 7 26,411,766 T C 0.230 -19411.710 4536.9180 2.088E-05 SLC15A2 Gga_rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15009148 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga rs16470339 5 15,066.389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs14620196	7	26,384,936	Т	С	0.216	-18476.380	4676.4090	8.411E-05	SLC15A2
Gga_rs15008991 14 7,144,668 T C 0.021 54588.740 13897.9700 9.247E-05 CACNG3 Gga_rs15009148 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG00000006140 Bone diameter major Gga_rs16470339 5 15,066.389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs14620221	7	26,411,766	Т	С	0.230	-19411.710	4536.9180	2.088E-05	SLC15A2
Gga_rs15009148 14 7,330,887 T C 0.026 48740.250 12382.3700 8.934E-05 ENSGALG0000006140 Bone diameter major Gga_rs16470339 5 15,066.389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs15008991	14	7,144,668	Т	С	0.021	54588.740	13897.9700	9.247E-05	CACNG3
Bone diameter major Gga rs16470339 5 15,066,389 A C 0.215 0.141 0.0357 7.961E-05 MUC5B		Gga_rs15009148	14	7,330,887	Т	С	0.026	48740.250	12382.3700	8.934E-05	ENSGALG0000006140
	Bone diameter major	Gga_rs16470339	5	15,066,389	Α	С	0.215	0.141	0.0357	7.961E-05	MUC5B

associated with the medullary bone score were also found to be associated with humerus density at a suggestive level.

Fourteen SNPs were associated with tibia strength and density traits on GGA 2, 5, 7, 14, and 17, and the average genomic inflation factor (λ) is 1.000 (Fig. 2, Table 2). Two suggestive SNPs for tibia density were located on GGA 5, respectively. Two suggestive SNPs for tibia breaking strength, on GGA 2 and 17, respectively. And only one suggestive SNP for major bone diameter was located on GGA 5. A total of nine suggestive SNPs distributed on GGA 2, 7and 14 were associated with tibia stiffness. No SNP was associated with cortical bone thickness and minor bone diameter at the GWAS significance level.

QTL and annotation term enrichment

We overlapped the regions from genome-wide association studies with published quantitative trait loci from ChickenQTLdb. The results showed that the abdominal fat weight QTL overlapped regions for five bone traits, while eggshell thickness QTL overlapped the medullary bone score regions. There were also overlaps with breast, carcass and body weight QTL (Figs. 3 and 4).

Annotation term enrichment analysis revealed 12 Gene Ontology terms with FDR<0.1, among them, keratin filament for the minor bone diameter, and adenylate cyclase-modulating G-protein coupled receptor signalling pathway for the tibia density. Seven terms were enriched for the medullary bone score including K-threo-aldose 1-dehydrogenase activity, bile acid transmembrane transporter activity, G-protein coupled purinergic nucleotide receptor activity, and humerus density, three terms for tibia breaking strength which are ATPase activity, coupled to transmembrane movement of substances and voltage-gated calcium channel activity (Table 3). According to the KEGG database, eight pathway annotations were significantly enriched, including mTOR signalling pathway, tryptophan metabolism, TGF- β signalling pathway, and apoptosis (Table 4).



Fig. 2. Manhattan and QQ plots for the association analyses of tibia traits. In the Manhattan plots, -log10(P-value) of the filtered high-quality SNPs (y-axis) is plotted against their genomic positions (x-axis). In the Q-Q plots, -log10(p) of observed association statistics on the Y-axis were compared to those of the association statistics expected under the hypothesis of no association on the X-axis. The solid line represents concordance between observed and expected values. Genomic inflation factor, λ , is shown for each dataset.



Fig. 3. Enrichment of QTL overlapping humerus traits quality associations.

Discussion

This study aimed to identify potential candidate genes associated with genetic variation in humerus and tibia bone strength and density using blood samples from 925 Rhode Island Red hens. There was a lack of major large-effect loci, but we identified suggestive genetic associations for humerus (38 SNPs) and tibia (14 SNPs) traits. These loci did not overlap previously published bone QTL, and thus appear to be novel (Johnsson et al., 2022; Raymond et al., 2018). There was also no overlap between suggestive associations for bone composition traits identified in the same population (Sallam et al., 2023).

The relative absence of major loci in the presence of substantial genomic heritability reaffirms the polygenicity of bone strength and density traits and suggests that genomic prediction of bone traits may be fruitful. Indeed, the potential for genomic prediction of tibial bone strength within the Rhode Island Red pure line has been demonstrated (Sallam et al., 2025).

Genetic association with the humerus traits

In the current study, the top SNP is located within the MFSD1 (major facilitator superfamily domain containing 1) gene on GGA9 and is the signal most strongly associated with humerus density and medullary bone score, referred to as pleiotropy, which is prevalent in the genetic architecture of chickens. A significant phenotypic correlation existed between humerus density and medullary bone score (P < 0.01). MFSD1 is an unglycosylated protein, and the immunofluorescence results showed that MFSD1 is present in the lysosomes of differentiated osteoclasts (Massa Lopez et al., 2016). The trabecular bone of vertebrae in MFSD1-deficient mice had decreased BMD and BV/TV ratio, increased osteoblast number and osteoclast activity, leading to the decline of bone mineral density (Lopez, 2018). Four medullary bone score associations on GGA1 are located within 166 kb upstream of the RERGL (Ras-like estrogen-regulated growth inhibitor-like) gene and overlap with QTLs for eggshell effective layer thickness and eggshell thickness in crossed F2 individuals (Duan et al., 2016; Liu et al., 2011). Mineralized medullary bone serves as a "calcium store" for eggshell formation, and the medullary bone undergoes turnover during a daily egg-laying cycle (Mueller et al., 1969).

Some markers that were found to be associated with humerus density on GGA5 overlap with the QTL for carcass weight in crosses between New Hampshire and White Leghorn chicken (Nassar et al., 2012), and QTL for breast muscle weight and abdominal fat weight that were detected in White Plymouth Rock (Atzmon et al., 2008). Among the markers, three suggestive markers are located within *CCND1* (Cyclin D1) gene, Let-7b targets *CCND1* to regulate osteoblast differentiation in mouse MC3T3-E1 cells (Wang and Cai, 2020). MiR-23b-3p functioned as a positive factor through regulating cell cycle, proliferation, apoptosis, and differentiation of MC3T3-E1 cells via targeting *CCND1* (Wang and Zhao, 2021). A suggestive marker overlaps with the *FGF19* (Fibroblast growth factor 19) gene on GGA5, which may inhibit osteoclastogenesis by regulating osteoprotegerin (OPG)/NF-kb ligand (RANKL) axis receptor activator (Guo et al., 2022) and enhance osteogenic differentiation via the Wnt/ β -linked protein pathway that is associated with the regulation of osteogenic differentiation and bone formation (Teufel and Hartmann, 2019).

Genetic association with the tibia traits

The marker on GGA2 associated with tibia breaking strength located within the PIP4K2A (phosphatidylinositol-5-phosphate 4-kinase type 2 alpha) gene, is actively involved in regulating intracellular cholesterol transport and negatively correlated with bone mineral density, mutations in this gene did not affect humerus mineral density of laying hens (Hu et al., 2018a). Two tibia stiffness associations on GGA2 (bp: 21,774, 232 and 21,912,734) are located downstream the CDK14 (Cyclin dependent kinase 14) gene, a novel cell cycle protein-dependent kinase, is a cell cycle regulator whose upregulation indicates increased cell proliferation during peri-implant bone healing (Davidson and Niehrs, 2010). SLC15A (Solute carrier family 15 member 2) associated with tibia stiffness located on GGA7 is a transmembrane transporter protein expressed in cell membranes and organelle membranes. Osteoclasts can be formed from tissue-specific macrophages in inflammatory and immunological environments (Sun et al., 2021). In mice, SLC15A is highly expressed in mature immune cells and macrophages in the bone marrow (Hu et al., 2018b). A marker association with the tibia density on GGA5 (bp: 13,606,911) is located within the KCNQ1 (Potassium voltage-gated channel subfamily Q member 1) gene. lnc-KCNQ1OT1 (KCNQ1 opposite strand/ antisense transcript 1), often regarded as an imprinted lincRNA, regulates osteogenic differentiation of mesenchymal stem cells by miR-214/BMP2 axis (Wang et al., 2019) and alleviates osteoclast differentiation (Zhang et al., 2021).

These markers that were found to be associated with tibia strength and density traits on GGA 2, GGA5, and GGA7 all overlap with a QTL for abdominal fat weight in a chicken interline cross with White Plymouth Rock background (Atzmon et al., 2008). A study on Korean men showed that abdominal obesity is a risk factor for osteoporosis whether in young or old men (Kim et al., 2019), the research on Chinese men showed that abdominal fat and visceral fat mass have negative effects on bone microstructure (Lv et al., 2016). On the contrary, excess fat increases the



Fig. 4. Enrichment of QTL overlapping tibia traits quality associations.

mechanical load on bones and results in higher bone mineral density (Rosen and Bouxsein, 2006). Therefore, the potential mechanism between abdominal fat and bone mass in laying hens deserves further investigation.

Table 3

_

GO term enrichment test involving the genes within 2 Mb of the SNPs with P < 0.0004.

Trait	ID	Term	Gene count	<i>P-</i> Value	FDR
Bone diameter minor	GO:0045095	Keratin filament	11	1.88E- 15	3.48E- 13
Medullary bone score	GO:0047834	D-threo-aldose 1-de- hydrogenase activity	5	1.98E- 05	4.96E- 03
Medullary bone score	GO:0015125	Bile acid transmembrane transporter activity	4	3.94E- 05	4.96E- 03
Medullary bone score	GO:0045028	G-protein coupled purinergic nucleotide receptor activity	4	6.25E- 05	5.25E- 03
Medullary bone score	GO:0004032	alditol:NADP+ 1- oxidoreductase activity	4	1.32E- 04	8.31E- 03
Medullary bone score	GO:0015347	Sodium- independent organic anion transmembrane transporter activity	4	1.80E- 04	9.07E- 03
Tibia breaking strength	GO:0042626	ATPase activity, coupled to transmembrane movement of substances	5	1.41E- 04	2.18E- 02
Tibia breaking strength	GO:0005245	Voltage-gated calcium channel activity	4	6.11E- 04	4.74E- 02
Medullary bone	GO:0015721	Bile acid and bile salt transport	4	1.74E- 04	6.60E- 02
Medullary bone	GO:0043252	Sodium- independent organic	4	2.38E- 04	6.60E- 02
Tibia breaking	GO:0005886	Plasma membrane	26	4.81E- 04	6.64E- 02
Tibia density	GO:0007188	Adenylate cyclase- modulating G- protein coupled receptor signaling pathway	9	4.71E- 05	7.31E- 02

Table 4

Gene set enrichment test involving the genes within 2 Mb of the SNPs with P < 0.0004.

Term	Gene count	P-value
mTOR signaling pathway	29	5.813E-03
Influenza A	26	1.487E-02
ABC transporters	11	1.992E-02
Apoptosis	24	2.027E-02
Tryptophan metabolism	10	2.704E-02
Fanconi anemia pathway	12	2.721E-02
Folate biosynthesis	8	3.541E-02
TGF-beta signaling pathway	18	4.755E-02

The annotation term enrichment for each bone trait

The candidate genes detected in this study are mainly related to skeletal development, mTORC1 and mTORC2 both implicated in regulating osteoblast differentiation and function (Chen and Long, 2015). Besides, mTORC1 and mTORC2 signaling play an important and minor role in endochondral skeletal development, respectively. TGF- α signaling pathway have diverse functions in osteoblast differentiation, skeletal development, and bone formation (Chen et al., 2012; Wu et al., 2016). The primary active metabolites of

tryptophan metabolism are serotonin, melatonin, and kynurenine, all of which play vital roles in bone biology (Al Saedi et al., 2020). Serotonin is divided into brain- and gut-derived, and both are functionally independent. Among them, brain-derived serotonin enhances bone formation and inhibits bone resorption (Ducy and Karsenty;, 2010). Gut-derived serotonin inhibits bone formation with no changes in bone resorption (de Vernejoul et al., 2012; Yadav et al., 2008). Increasing kynurenine levels results in accelerated skeletal aging by impairing osteoblastic differentiation and increasing osteoclastic resorption (Refaey et al., 2017).

Conclusion

In the current study, we conducted an association analysis based on the SNPs data and humerus, keel and tibia traits of the Rhode Island Red hens. We obtained 52 suggestive SNPs loci, and there was a total of 28 genes near these SNPs. These loci do not overlap previously published associations, and thus appear to be novel.

Supplementary Fig. 1. Power analysis for a GWAS design. The vertical dashed line indicates the size of the present study while the horizontal line corresponds to a statistical power of 80 %

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Two of the co-authors are employes by Lohmann Animal Breeding: Matthias Schmutz and Björn Andersson. Lohmann Animal Breeding provided the birds for this study but did not perform the analysis. All results are presented in full without any embargo or restrictions by Lohmann Animal Breeding. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by an ERANET grant to ICD (BBSRC BB/ M028291/1), DJdK (Svenska Forskningsrådet Formas, 2014-01840) and ARN (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, 291815), and the China National Scholarship Fund (202010230008). The genotyping was performed by the SNP&SEQ Technology Platform in Uppsala (www.genotyping.se). The facility is part of the National Genomics Infrastructure supported by the Swedish Research Council for Infrastructures and Science for Life Laboratory, Sweden.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105246.

References

- Al Saedi, A., Sharma, S., Summers, M.A., Nurgali, K., Duque, G., 2020. The multiple faces of tryptophan in bone biology. Exp. Gerontol. 129, 110778.
- Alfonso-Carrillo, C., Benavides-Reyes, C., de Los Mozos, J., Dominguez-Gasca, N., Sanchez-Rodríguez, E., Garcia-Ruiz, A.I., Rodriguez-Navarro, A.B., 2021. Relationship between bone quality, egg production and eggshell quality in laying hens at the end of an extended production cycle (105 weeks). Animals 11, 623. https://doi.org/10.3390/ani11030623.
- Atzmon, G., Blum, S., Feldman, M., Cahaner, A., Lavi, U., Hillel, J., 2008. QTLs detected in a multigenerational resource chicken population. J. Hered. 99, 528–538.
- Candelotto, L., Stratmann, A., Gebhardt-Henrich, S.G., Rufener, C., van de Braak, T., Toscano, M.J., 2017. Susceptibility to keel bone fractures in laying hens and the role of genetic variation. Poult. Sci. 96, 3517–3528.
- Chen, G., Deng, C., Li, Y.P., 2012. TGF- β and BMP signaling in osteoblast differentiation and bone formation. Int. J. Biol. Sci. 8, 272–288.

- Chen, J., Long, F., 2015. mTORC1 signaling promotes osteoblast differentiation from preosteoblasts. PLoS One 10, e0130627.
- Davidson, G., Niehrs, C., 2010. Emerging links between CDK cell cycle regulators and wnt signaling. Trends Cell Biol. 20, 453–460.
- De Koning, D.J., Dominguez-Gasca, N., Fleming, R.H., Gill, A., Kurian, D., Law, A., McCormack, H.A., Morrice, D., Sanchez-Rodriguez, E., Rodriguez-Navarro, A.B., Preisinger, R., Schmutz, M., Smídová, V., Turner, F., Wilson, P.W., Zhou, R., Dunn, I. C., 2020. An eQTL in the cystathionine beta synthase gene is linked to osteoporosis in laying hens. Genet. Sel. Evol. 52, 13. https://doi.org/10.1186/s12711-020-00532-y.
- De Vernejoul, M.C., Collet, C., and Chabbi-Achengli Y., 2012. Serotonin: good or bad for bone. Bonekey Rep. 1:120. doi:10.1038/bonekey.2012.120.
- Duan, Z., Sun, C., Shen, M., Wang, K., Yang, N., Zheng, J., Xu, G., 2016. Genetic architecture dissection by genome-wide association analysis reveals avian eggshell ultrastructure traits. Sci. Rep. 6, 28836. https://doi.org/10.1038/srep28836.
- Ducy, P., Karsenty, G., 2010. The two faces of serotonin in bone biology. J. Cell Biol. 191, 7–13.
 Dunn, I.C., De Koning, D.J., McCormack, H.A., Fleming, R.H., Wilson, P.W.,
- Julin, LC., De Koling, D.J., McColinack, H.A., Fleining, K.H., Wilson, F.W., Andersson, B., Schmutz, M., Benavides, C., Dominguez-Gasca, N., Sanchez-Rodriguez, E., Rodriguez-Navarro, A.B., 2021. No evidence that selection for egg production persistency causes loss of bone quality in laying hens. Genet. Sel. Evol. 53, 11. https://doi.org/10.1186/s12711-021-00603-8.
- Dunn, I.C., Fleming, R.H., McCormack, H.A., Morrice, D., Burt, D.W., Preisinger, R., Whitehead, C.C., 2007. A QTL for osteoporosis detected in an F2 population derived from White Leghorn chicken lines divergently selected for bone index. Anim. Genet. 38, 45–49.
- Fonseca, P.A.S., Suárez-Vega, A., Marras, G., Cánovas, Á., 2020. GALLO: an R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci. Gigascience 9, giaa149. https://doi.org/10.1093/ gigascience/giaa149.
- Groenen, M.A., Megens, H.J., Zare, Y., et al., 2011. The development and characterization of a 60K SNP chip for chicken. BMC Genom. 12, 274.
- Guo, A., Li, K., Tian, H.C., Tao, B.L., Xiao, Q., Jiang, D.M., 2022. FGF19 protects against obesity-induced bone loss by promoting osteogenic differentiation. Biomed. PharmacOther 146, 112524. https://doi.org/10.1016/j.biopha.2021.112524.
- Guo, J., Sun, C., Qu, L., Shen, M., Dou, T., Ma, M., Wang, K., Yang, N., 2017. Genetic architecture of bone quality variation in layer chickens revealed by a genome-wide association study. Sci. Rep. 7, 45317.
- Habig, C., Henning, M., Baulain, U., Jansen, S., Scholz, A.M., Weigend, S., 2021. Keel bone damage in laying hens-its relation to bone mineral density, body growth rate and laying performance. Animals 11, 1546.
- Harlander-Matauschek, A., Rodenburg, T.B., Sandilands, V., Tobalske, B.W., Toscano, M. J., 2015. Causes of keel bone damage and their solutions in laying hens. World's Poult. Sci. J. 71, 461–472.
- Hu, A., Zhao, X.T., Tu, H., Xiao, T., Fu, T., Wang, Y., Liu, Y., Shi, X.J., Luo, J., Song, B.L., 2018a. PIP4K2A regulates intracellular cholesterol transport through modulating PI (4,5)P(2) homeostasis. J. Lipid Res. 59, 507–514.
- Hu, Y., Song, F., Jiang, H., Nuñez, G., Smith, D.E., 2018b. SLC15A2 and SLC15A4 mediate the transport of bacterially derived di/tripeptides to enhance the nucleotide-binding oligomerization domain-dependent immune response in mouse bone marrow-derived macrophages. J. Immunol. 201, 652–662.
- Hu, Z.L., Park, C.A., Wu, X.L., Reecy, J.M., 2013. Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. Nucleic Acids Res. 41, D871–D879.
- Huang, d.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57.
- Johnsson, M., Wall, H., Lopes Pinto, F.A., Fleming, R.H., McCormack, H.A., Benavides-Reyes, C., Dominguez-Gasca, N., Sanchez-Rodriguez, E., Dunn, I.C., Rodriguez-Navarro, A.B., Kindmark, A., Koning, D.J., 2022. Genetics of tibia bone properties of crossbred commercial laying hens in different housing systems. G3: Genes Genomes Genet 13 ikac302 https://doi.org/10.1093/c3iournal/ikac302
- Genet. 13, jkac302. https://doi.org/10.1093/g3journal/jkac302.
 Kim, M.H., Song, S.W., Kim, K.S., 2019. Abdominal obesity is associated with lower bone mineral density in non-weight-bearing site in Korean men. Am. J. Mens Health 13. https://doi.org/10.1177/1557988318813499.
- Kumar, A., Sharma, R.K., Singh, H., Singh, C.V., Singh, B., 2002. Genetic studies on some economic traits of Rhode Island Red. Indian J. Poult. Sci. 37 (1), 31–34.
- Li, Y.D., Liu, X., Li, Z.W., Wang, W.J., Li, Y.M., Cao, Z.P., Luan, P., Xiao, F., Gao, H.H, Guo, H.S., Wang, N., Li, H., 2021. A combination of genome-wide association study and selection signature analysis dissects the genetic architecture underlying bone traits in chickens. Animal 15, 100322. https://doi.org/10.1016/j. animal.2021.100322.
- Liu, W., Li, D., Liu, J., Chen, S., Qu, L., Zheng, J., Xu, G., Yang, N., 2011. A genome-wide SNP scan reveals novel loci for egg production and quality traits in white leghorn and brown-egg dwarf layers. PLoS One 6, e28600.
- Lopez, D.M., 2018. Characterisation of the major facilitator superfamily domain containing 1 protein (MFSD1) and study of its physiological role in the mouse. Christian-Albrechts-Universität zu Kiel. urn:nbn:de:gbv:8-diss-242968.
- Lv, S., Zhang, A., Di, W., Sheng, Y., Cheng, P., Qi, H., Liu, J., Yu, J., Ding, G., Cai, J., Lai, B., 2016. Assessment of fat distribution and bone quality with trabecular bone score (TBS) in healthy Chinese men. Sci. Rep. 6, 24935.
- Massa Lopez, D., Damme, M., Saftig, P., 2016. Characterising the role of the lysosomal membrane proteins MFSD1 and TMEM106b in osteoclasts. Bone Abstr. 5, 185. https://doi.org/10.1530/boneabs.5.P185.
- Moore, C.M., Jacobso, S.A., Fingerlin, T.E., 2019. Power and sample size calculations for genetic association studies in the presence of genetic model mis-specification. Hum. Hered. 84, 256–271.

Q. Yue et al.

Mueller, W.J., Brubaker, R.L., Caplan, M.D., 1969. Egg shell formation and bone resorption in laying hens. Federation Proc. 28, 1851–1856.

Nassar, M.K., Goraga, Z.S., Brockmann, G.A., 2012. Quantitative trait loci segregating in crosses between New Hampshire and White Leghorn chicken lines: II. Muscle weight and carcass composition. Anim. Genet. 43, 739–745.

Podisi, B.K., Knott, S.A., Dunn, I.C., Burt, D.W., Hocking, P.M., 2012. Bone mineral density QTL at sexual maturity and end of lay. Br. Poult. Sci. 53, 763–769.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. Am. J. Hum. Genet. 81, 559–575.

- Raymond, B., Johansson, A.M., McCormack, H.A., Fleming, R.H., Schmutz, M., Dunn, I. C., De Koning, D.J., 2018. Genome-wide association study for bone strength in laying hens. J. Anim. Sci. 96, 2525–2535.
- Refaey, M.E., McGee-Lawrence, M.E., Fulzele, S., Kennedy, E.J., Bollag, W.B., Elsalanty, M., Zhong, Q., Ding, K.H., Bendzunas, N.G., Shi, X.M., 2017. Kynurenine, a tryptophan metabolite that accumulates with age, induces bone loss. J. Bone Miner. Res. 32, 2182–2193.
- Rosen, C.J., Bouxsein, M.L., 2006. Mechanisms of disease: is osteoporosis the obesity of bone? Nat. Clin. Pract. Rheumatol. 2, 35–43.
- Sallam, M., Wilson, P.W., Andersson, B., Schmutz, M., Benavides, C., Dominguez Gasca, N., Sanchez Rodriguez, E., Rodriguez Navarro, A.B., Dunn, I.C., De Koning, D. J., Johnsson, M., 2023. Genetic markers associated with bone composition in Rhode Island Red laying hens. Genet. Sel. Evol. 55, 44.
- Sallam, M., Wall, H., Wilson, P.W., Andersson, B., Schmutz, M., Benavides, C., Checa, M., Sanchez-Rodriguez, E., Rodriguez-Navarro, A.B., Kindmark, A., Dunn, I.C., de Koning, D.J., Johnsson, M., 2025. Genomic prediction of bone strength in laying hens using different sources of information. Animal 19, 101452. https://doi.org/ 10.1016/j.animal.2025.101452.
- Schreiweis, M.A., Hester, P.Y., Moody, D.E., 2005. Identification of quantitative trait loci associated with bone traits and body weight in an F2 resource population of chickens. Genet. Sel. Evol. 37, 677–698.

- Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., Chang, W., 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 50, W216–W221.
- Sun, Y., Li, J., Xie, X., Gu, F., Sui, Z., Zhang, K., Yu, T., 2021. Macrophage-osteoclast associations: origin, polarization, and subgroups. Front. Immunol. 12, 778078.

Teufel, S., Hartmann, C., 2019. Wnt-signaling in skeletal development. Curr. Top. Dev. Biol. 133, 235–279.

- Turner, S.D., 2018. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. J. Open Source Softw. 3, 731.
- Wang, C.G., Liao, Z., Xiao, H., Liu, H., Hu, Y.H., Liao, Q.D., Zhong, D., 2019. LncRNA KCNQ10T1 promoted BMP2 expression to regulate osteogenic differentiation by sponging miRNA-214. Exp. Mol. Pathol. 107, 77–84.
- Wang, J.Z., Zhao, B.H., 2021. MiR-23b-3p functions as a positive factor for osteoporosis progression by targeting CCND1 in MC3T3-E1 cells. In Vitro Cell Dev. Biol. Anim. 57, 324–331.
- Wang, L.J., Cai, H.Q., 2020. Let-7b downgrades CCND1 to repress osteogenic proliferation and differentiation of MC3T3-E1 cells: an implication in osteoporosis. Kaohsiung J. Med. Sci. 36, 775–785.
- Webster, A.B., 2004. Welfare implications of avian osteoporosis. Poult. Sci. 83, 184–192. Wu, M., Chen, G., Li, Y.P., 2016. TGF- β and BMP signaling in osteoblast, skeletal
- development, and bone formation, homeostasis and disease. Bone Res. 4, 16009. Yadav, V.K., Ryu, J.H., Suda, N., Tanaka, K.F., Gingrich, J.A., Schütz, G., Glorieux, F.H., Chiang, C.Y., Zajac, J.D., Insogna, K.L., 2008. Lrp5 controls bone formation by
- inhibiting serotonin synthesis in the duodenum. Cell 135, 825–837.
 Zhang, K., Shi, Z., Ren, Y., Han, X., Wang, J., Hong, W., 2021. Kcnq1ot1 promotes osteogenic differentiation and suppresses osteoclast differentiation. J. South Med. Univ. 41, 31–38.
- Zhou, X., Stephens, M., 2012. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44, 821–824.