Genome-wide association study for femur-related traits in broilers

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Summary

Due to the intense selection for heavier and faster growing broilers, metabolic disorders such as skeletal problems became a worldwide concern. Advances in genome-wide association study (GWAS) methodologies increased the possibility of elucidating the genetic architecture controlling bone integrity traits. Therefore, the aim of this study was to perform a GWAS to identify potential genetic markers and candidate genes associated with femur traits in a paternal broiler line developed by Embrapa. To this, three femur bone-related traits were evaluated in 1,433 chickens: dry matter (FDM), ash content (FAC) and breaking strength (FBS). Chickens were genotyped using the 600K Affymetrix® Axiom® HD panel. A total of 16 regions associated to FAC, being a significant SNP in the GGA19 (rs317696422) and 15 suggestive SNPs in the GGA1, GGA2, GGA3, GGA5, GGA8, GGA13, GGA19 and GGA24. For FDM, only one SNP (GGA1) was significantly associated and was located in the *DSCAM* gene. For the FBS, two suggestive regions (GGA12 and GGA15) were found and no QTLs were described for this trait in these regions. According to the results, new candidate genes and miRNAs related to ossification, such as *TPVR2*, gga-mir-146a and *PCP4* were associated to important femur traits in the broiler line under study.

Keywords: bone integrity, leg problems, QTLs

Introduction

Poultry breeding companies have produced lines with more precocity and greater muscle development. However, this intense selection pressure has led to an increase of metabolic disorders, which includes the locomotor problems, resulting in significant economic losses and reduced animal welfare (Cook, 2000). It has been a consensus that the skeleton does not mature rapidly enough to support the maximum growth potential of modern broilers (Kestin et al., 2001). According to González-Cerón et al. (2015) the size and weight of bones do not necessarily reflect quality and bone strength, since these traits are determined by several bone components. One of the most important ways to measure bone quality is bone mineral density, which can be measured using bone mineral composition and breaking strength (Almeida Paz et al., 2008). Several ways to diagnose bone integrity problems are being developed and applied to improve

the quality of the skeleton system (Fornari et al., 2013).

Recently, advances in the genomic area have made possible to investigate ways to improve the skeletal structure in chickens without affecting animal performance (Zhou et al., 2007). The genome-wide association study (GWAS) with high density panels such as the 600 K Chicken genotyping panel greatly improve the precision of mapping quantitative trait loci (QTL) and the accuracy of effect estimates for small and medium size QTL. In addition, advances in GWAS methodologies increase the possibility of elucidating the genetic architecture controlling bone integrity traits. Also, it creates new opportunities for genetic breeding programs strategies to produce broilers with more desirable bone quality traits, while maintaining the efficiency in production. Therefore, the aim of this study was to perform a GWAS to identify potential genetic markers and candidate genes associated with femur traits in a paternal broiler line.

Material and Methods

Animals and data collection

This study was performed with the approval of the Embrapa Swine and Poultry Ethical Committee of Animal Use (CEUA) under protocol number 011/2011. This study used 1,433 chickens originated from the expansion of a paternal broiler line called TT, which was develop by the Poultry Breeding Program of the Embrapa Swine and Poultry National Research Center, in Concórdia, SC, Brazil. This line has been under multi-trait selection, with emphasis on body weight at 42 days of age (BW42). Chickens were kept in collective pens until 35 days of age and then housed in individual cages for feed conversion evaluation. Three femur bone-related traits were evaluated: dry matter (FDM), ash content (FAC) and breaking strength (FBS), as described by Grupioni et al. (2015).

Genotyping and association analysis

DNA extraction from total blood was performed with DNAzol (Invitrogen) reagent, following the manufacturer's protocol. All animals were genotyped using the 600K Affymetrix® Axiom® HD panel (Kranis et al., 2013). The quality control (QC) analysis was conducted with PLINK (Purcell et al. 2007) and samples with call rate less than 90% and/or heterozygosity rate $\pm$ 3 standard deviation from the mean were removed. The SNPs were excluded if they had call rate < 98%, minor allele frequency less than 0.02 and/or deviated from Hardy-Weinberg equilibrium (HWE) (p<1E−06). Finally, a total of 375,776 SNPs and 1408 samples remained for analysis.

The GWAS was performed using the Qxpak software version 5.05 (Pérez-Enciso & Misztal, 2011) fitting a univariate linear mixed model, which included the fixed effect of sex, hatch and SNP (additive), and the infinitesimal and residual error as random effect. Genome-wide significance threshold was defined based on the number of independent SNPs, which was calculated via PLINK (Purcell et al. 2007) using the indep-pairwise option with a window size of 25 SNPs, a step of five SNPs, and a r^2 threshold of 0.2. This resulted in 25,658 independent SNPs, which yielded a threshold Bonferroni p-value of suggestive significance of 3.897E-05 (1/25658) and a genome-wide 5% significance level of 1.949E-06 (0.05/25658). Manhattan plots were created using R program v3.3 (R Core team, 2017). Using the UCSC genome browser (https://genome.ucsc.edu/) with chicken genome version galGal5 and the chicken QTLdb (Hu et

al., 2016), SNPs significantly associated were used to identify the candidate genes and QTL regions, respectively.

Results and Discussion

A total of 16 regions was found in the chicken genome related to FAC (*Figure 1*), being a significant SNP (p<1.949E-06) in the GGA19 (rs317696422) and 15 suggestive SNPs in the GGA1, GGA2, GGA3, GGA5, GGA8, GGA13, GGA19 and GGA24. It is known that about 70% of bone mass is genetically heritable (Eastell & Lambert, 2002), which reinforces the importance to understand the genetic architecture of bone metabolism for improve bone quality in broilers. Several QTLs have already been described in those chromosomes, including some related to bone, such as, bone index and tibia breaking strength and some related to growth, carcass and fat weight (Dunn et al., 2007; Livant et al., 2007). The SNP rs317696422, located in the *TRPV2* (transient receptor potential cation channel subfamily V member 2) gene, in GGA19, was associated with FAC. This gene has a functional role on late osteoclast differentiation by inducing the expression of *RANKL* and releasing intra and extracellular Ca^{2+} (Kajiya et al., 2010). Also, other new candidate genes found, such as *PPIL4* (Peptidylprolyl Isomerase Like 4) and *ADRA1B* (adrenergic receptor, alpha 1b), are both involved in ossification processes (Stelzer et al., 2017). In addition, several miRNAs and lincRNAs were located in the QTL regions identified, indicating a possible involvement of epigenetic mechanisms related to FAC, such as the miRNA gga-mir-146a, which has a role on osteogenesis (Xie et al., 2017).

One SNP (rs317287867, GGA1) was significantly associated to FDM (*Figure 1*) and was located in the *DSCAM* (DS Cell Adhesion Molecule) gene, a promoter of cell adhesion (Stelzer et al., 2017). No function related to ossification has been described for this gene. However, close to *DSCAM,* the *PCP4* (Purkinje Cell Protein 4) gene is located, a regulator of calmodulin signaling (Stelzer et al., 2017), that is upregulated during the osteoplastic differentiation and also involved with deposition of calcium, moduling bone morphogenetic pathways (Xiao et al., 2008). This gene might be directly involved with FDM in chicken carcass.

For the FBS, two suggestive regions were found in GGA12 and GGA15 (*Figure 1*). No QTL was described for FBS in these suggestive regions. However, the SNP in the GGA12 is located into the *FLNB* (filamin B) gene, which regulates bone structure and bone mineral density in humans (Wilson et al., 2009; Mullin et al., 2013).

Conclusions

Several regions involved with femur integrity traits were found in this study, highlighting the polygenic control of bone metabolism regulation. Also, new candidate genes and miRNAs related to ossification, such as *TRPV2*, gga-mir-146a and *PCP4* were associated to important femur traits in the broiler line under study.

Figure 1.Manhattan plot of genome-wide association study for femur ash content (A), dry matter (B) and breaking strength (C). The red line indicates genome-wide significance association (p<1.949E-06) and the blue line indicates genome-wide suggestive association (p<3.897E-05).

Acknowledgements

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