ASSOCIATION OF THE A211G POLYMORPHISM IN THE BONE SIALOPROTEIN GENE WITH SKELETAL STRUCTURE IN A PATERNAL BROILER LINE

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ABSTRACT

The improvement in growth rate and carcass yield in the last decades led more evident the need to select chickens with better skeletal structure to support the musculature of the modern broiler. Bone sialoprotein (IBSP) is one of the major noncollagenous proteins in bones, and has been suggested as a potential nucleator of the hydroxyapatite. In this work, a single nucleotide polymorphism (SNP) in the bone sialoprotein gene was genotyped by PCR-RFLP in 510 chickens from a paternal broiler line. Association analyses were carried out with QxPaK software for 38 phenotypic traits. The SNP additive, dominant and additive + dominant effects were tested, including their interaction with sex. The SNP IBSP A211G showed significant association with body weight at 41 days (p=0.03), wings weight (p=0.01), breast bone weight (p=0.008) and width of the tibia (p=0.009). Most of the results are explained by the additive effect of the SNP with the influence of sex. The A allele in the genotype has a favorable effect for most traits. These results show that the IBSP SNP is a potential genetic marker to improve chicken skeletal structure and other correlated traits.

KEYWORDS: candidate gene, single nucleotide polymorphism, PCR-RFLP, *Gallus gallus*

INTRODUCTION

The poultry breeding programs have enabled the improvement of important traits, such as growth rate, carcass yield and feed efficiency (LEDUR *et al.*, 2007). However, the increase in bone problems led more evident the need to select chickens with better skeletal structure to support the musculature and the high growth rate of the modern broiler (ZHOU *et al.*, 2007; ZHANG *et al.*, 2010). Skeletal problems in poultry production leads to economic losses, such as an increase in mortality and carcass condemnation, a decline in carcass yield and performance (COOK, 2000), as well as problems for animal welfare (ZHANG *et al.*, 2010). Genetic composition plays a

considerable role in the development of skeletal system (COOK, 2000). Therefore, the investigation of polymorphisms for marker-assisted selection (MAS) could be an alternative to reduce this problem (ZHANG *et al.*, 2010). Bone sialoprotein (IBSP) is a noncollagenous protein, especially present in areas where the bone is remodeled or synthesized (WUTTKE *et al.*, 2001). The association of IBSP with mineralization is still not completely understood, but it seems to act as an important nucleator of the hydroxyapatite (YANG *et al.*, 2010). In this paper, we investigate the association of the IBSP A211G SNP, identified in the intron 4 of the IBSP gene, with skeletal structure-related traits in a paternal broiler line.

MATERIALS AND METHODS

A total of 510 chickens from the TT Reference Population (PEIXOTO *et al.*, 2010), developed for marker validation and gene discovery by Embrapa Swine and Poultry National Research Center, were analyzed. Thirty eight phenotypic traits, including carcass yields, performance and skeletal traits were evaluated, such as: length, width, weight, percentage of dry matter, ash and resistance to bending of the femur and tibia, as well as weights of carcass, wings and breast bone were evaluated.

Genomic DNA extraction from blood was performed with DNAzol (*Invitrogen*). The animals were genotyped for the SNP A211G by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphisms (RFLP), using the primers forward and reverse (5'–agggacttgcatctgaaccatcct–3' and 5'–acatggatggcaatactcaggcca–3') based on the chicken sequence deposited in GenBank under accession number U67889. The amplicon of 809 bp was obtained by the PCR reaction, followed by cleavage reaction with *Hpa II* restriction endonuclease (New England Biolabs *Inc.*). The genotypes of the SNP A211G were classified in normal homozygotes (AA), heterozygotes (AG) and mutant homozygotes (GG) according to the size of the DNA fragments.

The association analysis of the SNP and the traits measured were carried out with QxPaK (PEREZ-ENCISO and MISZTAL, 2004) with a mixed model including the fixed effects of hatch, sex and SNP, and the infinitesimal and residuals as random effects. The additive (*a*), additive + dominant (*ad*) and dominant (*d*) effects of the SNP were evaluated as well as its interaction with sex.

RESULTS AND DISCUSSION

The digestion of the PCR product and subsequent electrophoresis revealed the presence of 298 chickens with the homozygous genotype for the normal allele (AA), 195 had the heterozygous genotype (AG) and 17 were mutants homozygous (GG) for the polymorphism. Association analysis were significant with the *a* model for body weight at 41 days (p=0.03), wings weight (p=0.01) and highly significant for width of the tibia (p=0.009) in females. For all of those traits, the addition of each A allele in the genotype of the SNP A211G in females, leads to an increase of 52.62g, 4.58g and 0.27mm, respectively, when compared to the GG genotype. The breast bone weight was highly significant (p=0.008) for the *ad* model in interaction with sex. Female broilers with the AG genotype had a lower weight (89.81g) compared to the average estimated weight of females with the AA (91.95g) and GG (92.21g) genotypes. The allele A also showed favorable effects for carcass yields and performance traits, which are directly related to general growth.

A possible explanation is that the A211G SNP is directly responsible for the observed variations. Even located on a non-expressed region of the gene, it is possible that this SNP could interfere in gene expression. Another possibility would be that the SNP is in linkage disequilibrium with another polymorphism. The investigation of polymorphisms in the IBSP gene is essentially described in human health researches. Nevertheless, chicken quantitative trait loci (QTL) for skeletal structure traits were identified in the region where the IBSP gene is located, including a QTL for tibia marrow diameter (SHARMAN *et al.*, 2007) and tibia width (SCHREIWEIS *et al.*, 2005).

All together, these results suggest that this gene has an important influence for the skeletal structure and other correlated traits in broilers, and might be used in MAS.

CONCLUSION

Our study demonstrates the potential of the bone sialoprotein as a candidate gene for skeletal structural traits, suggesting that the A211G SNP should be tested in other populations for its possible application as a marker in poultry breeding programs by marker-assisted selection.

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