Sex-specific Association of a SNP in the ADIPOR2 Gene with Carcass Traits in a Paternal Broiler Line

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ABSTRACT: This study reports the association of a polymorphism in the ADIPOR2 gene with broiler carcass traits. Chickens (n= 1352) were genotyped by PCR-RFLP. The association was performed with QxPak software using a mixed model including sex, hatch and SNP as fixed effects, and the infinitesimal and residual as random effects. The additive and additive + dominant effects of the SNP were tested, including their interaction with sex. The SNP additive effect was significant for wing sticks weight, wings weight, drumstick muscle weight and yield, thigh muscle yield, thigh and drumstick muscle weight and yield only in females, and breast skin weight in males. The SNP additive + dominant effect was significant for breast skin and drumstick yields only in males. This study indicates great potential of the ADIPOR2 gene in increasing muscle weight and yield and reducing subcutaneous fat, which are relevant issues in poultry breeding.

Keywords: ADIPOR2; chicken; subcutaneous fat; muscle development

Material and Methods

Samples and Phenotypic Traits. A total of 1,352 42-day-old chickens from the TT Reference Population (Ledur et al. (2012)) was used in this study. TT is a paternal broiler line developed by the EMBRAPA Poultry Breeding Program, which has been under multi-trait selection since 1992. Animals were evaluated for 38 carcass traits (19 weights and 19 yields). Traits measured were: weights of carcass, abdominal fat, wing sticks, middle joint wings, wing tips, wings, drumstick muscle, drumstick skin, drumstick, thigh muscle, thigh skin, thigh, and drumstick muscle, breast, breast muscle, breast fillet, breast skin and back. The yield for every weight-related trait was calculated based on body weight at 42 days of age and expressed as percentage.

Polymorphism Genotyping. The chicken ADIPOR2 gene spans 20,393 bp and it is mapped on chromosome 1, between positions 61,087,371 and 61,107,763 (Gallus gallus-4.0, NCBI). The amplified fragment had 1,216 bp (forward primer: 5′-tggcagctaatgctgacagc-3′, reverse primer: 5′-caacaggagggaaaccctgatag-3′). The SNP selected for the current study was previously identified by our research group (data not shown) and is located in the intron 5 at the position C242T in the amplicon. The PCR-RFLP assay was performed for individual genotyping using the restriction enzyme BsrGI.

Statistical Analysis. The genotype frequencies of the SNP were calculated using the FREQ procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). With the QxPak program (Pérez-Enciso and Misztal (2004)), which uses the maximum likelihood methodology, the association of the SNP with chicken carcass traits was analyzed using a mixed model including the fixed effects of sex, hatch and SNP, and the infinitesimal and residual random effects. The additive (a) and additive + dominant (a+d) effects of the SNP were tested, including their interaction with sex. Significance was considered if p<0.05.

Results and Discussion

The frequencies of the CC, CT and TT genotypes were 58.36%, 37.65% and 3.99%, respectively.

The additive model fitted within sex and the additive plus dominant model fitted within sex were the ones that best explained the association between the C242T SNP and the evaluated carcass traits (Table 1). In females, the C→T substitution at ADIPOR2 C242T significantly
increased wing sticks weight (1.6 ± 0.62g), wings weight (2.28 ± 0.97g), drumstick muscle weight (2.34 ± 0.99g), drumstick muscle yield (0.07 ± 0.03%), thigh muscle yield (0.15 ± 0.05%), thigh and drumstick muscle weight (6.47 ± 2.68g), and thigh and drumstick muscle yield (0.20 ± 0.06%). On the other hand, the presence of the T allele at ADIPOR2 C242T decreased breast skin weight (1.17 ± 0.46g), breast skin yield (0.08 ± 0.02%) and drumstick yield (0.12 ± 0.05%) only in males, with a dominant effect in the last two traits. The results indicate that the effect of this SNP in the ADIPOR2 was influenced by sex.

Table 1. Carcass traits significantly associated with the ADIPOR2 C242T SNP in broiler chickens.

<table>
<thead>
<tr>
<th>Traits /Models</th>
<th>P</th>
<th>Sex</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (sex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WSW</td>
<td>0.02</td>
<td>F</td>
<td>-1.6</td>
</tr>
<tr>
<td>WW</td>
<td>0.04</td>
<td>F</td>
<td>-2.28</td>
</tr>
<tr>
<td>DMW</td>
<td>0.04</td>
<td>F</td>
<td>-2.34</td>
</tr>
<tr>
<td>DMY</td>
<td>0.02</td>
<td>F</td>
<td>-0.07</td>
</tr>
<tr>
<td>TMY</td>
<td>0.03</td>
<td>F</td>
<td>-0.15</td>
</tr>
<tr>
<td>TDMW</td>
<td>0.04</td>
<td>F</td>
<td>-6.47</td>
</tr>
<tr>
<td>TDMY</td>
<td>0.005</td>
<td>F</td>
<td>-0.20</td>
</tr>
<tr>
<td>BSW</td>
<td>0.03</td>
<td>M</td>
<td>1.17</td>
</tr>
<tr>
<td>ad (sex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DY</td>
<td>0.006</td>
<td>M</td>
<td>0.12</td>
</tr>
<tr>
<td>BSY</td>
<td>0.006</td>
<td>M</td>
<td>0.08</td>
</tr>
</tbody>
</table>

WSW: Wing sticks weight (g), WW: Wings weight (g), DMW: Drumstick muscle weight (g), DMY: Drumstick muscle yield (%), TMY: Thigh muscle yield (%), TDMW: Thigh and drumstick muscle weight (g), TDMY: Thigh and drumstick muscle yield (%), BSW: Breast skin weight (g), DY: Drumstick yield (%), BSY: Breast skin yield (%), a (sex): additive within sex, ad (sex): additive + dominant within sex, P: P-value, a: additive model, SE: standard error, d: dominant effect.

The ADIPOQ gene, through its receptors ADIPOR1 and ADIPOR2, plays a fundamental role in lipid and carbohydrate metabolism by decreasing plasma triglycerides, stimulating fatty acid oxidation and improving glucose metabolism by the increase of insulin sensitivity (Yamauchi et al. 2002)). Results of this study indicate that selection pressure to increase the frequency of the T allele in males of this population might result in chickens with less breast skin weight and yield. These findings highlight the importance of this gene in chickens, since their main sites for fat deposition are the abdomen and the subcutaneous tissue (Tumova and Teimour (2010)).

In addition to the association found with breast skin, this report shows the association of the ADIPOR2 with skeletal muscle processes, expressed through the increase of the weight and yield of several muscle cuts, mainly in female chickens. It has been reported that this gene is ubiquitously expressed in chicken tissues, such as adipose tissue, skeletal muscle, liver, ovary, anterior pituitary gland, kidney and spleen (Ramachandran et al. 2007). Moreover, adiponectin stimulates skeletal muscle fatty acid oxidation via activation of AMP-dependent protein kinase (AMPK), and is also associated with improvements in insulin response (Yamauchi et al. 2002)).

Furthermore, in the ADIPOR2 region (Chr1: 61087371..61107763), several QTLs for carcass traits have been identified, such as for wing weight, abdominal fat weight, drumstick meat-to-bone ratio, thigh muscle percent, drumstick and thigh weight, drumstick and thigh muscle weight, subcutaneous fat thickness, thigh weight, drumstick weight, breast muscle weight and drumstick muscle weight (http://www.animalgenome.org/cgi-bin/QTLdb/CG/index). It is possible that the ADIPOR2 gene might explain, at least partially, some of these QTLs detected.

Despite the associations found, the production traits are complex, and therefore, controlled by several genes. To improve the discussion about the possible interaction of the ADIPOR2 with other genes and its role in carcass traits, a gene network was performed with GeneMania (http://www.genemania.org; Zuberi et al. (2013)).

The network was constructed considering gene and physical interactions, co-expression, co-localization and pathways, and was based on Homo sapiens genome (Figure 1). Several genes appeared enriching the network, which can be helpful to explain the association results obtained. For instance, calcium/calmodulin-dependent protein kinase kinase 1 alpha (CAMKK1) and beta (CAMKK2), and serine/threonine kinase 11 (STK11) genes participate in the ADIPOQ/ADIPOR1/ADIPOR2 pathway.

Figure 1. ADIPOR2 gene network. Circles represent genes and connecting lines represent interactions between genes. Black circles represent the set of genes provided to the GeneMania software. Gray circles are the extra genes added to the network by the program that are strongly connected to query genes.

Interestingly, the STK11 gene (also known as liver kinase B1; LKB1) is a tumor-suppressor that is involved in the regulation of muscle metabolism and growth by phosphorylating and activating AMP-activated protein kinase (AMPK) family members (Witczak et al. 2008). LKB1 knockout mice presented type II muscle fiber atrophy and loss of hindlimb muscle function (Thomson et al.
Other genes appear co-expressing with ADIPO2, as the isopentenyl-diphosphate delta isomerase 1 (IDI1), an isomerase involved on the cholesterol synthesis (Hahn et al. (1996)) and members of the progestin and adipQ receptor (PAQR) family. The PAQR group of genes mediates progesterone actions in the reproductive system (Thomas and Pang (2012)). The gender-related variations found in the present report might be due to the interaction of the PAQR genes with the ADIPOR2.

The consistent associations observed in a pure line, together with the ADIPOR2 gene location in the genome, and the pathways in which it is involved in humans, suggest that this gene might be directly responsible for the significant associations observed.

**Conclusion**

The results from the current study indicated that the associations found between the ADIPOR2 C242T SNP and carcass traits in broiler chickens were influenced by sex. Therefore, the ADIPOR2 SNP is a potential marker for use in marker-assisted selection programs, considering its sex-specific effect. Moreover, extensive study on chicken ADIPOR2 gene may help in the understanding of regulation of body fatness and muscle development in poultry.

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**Literature Cited**