

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

Introduction

Over the past decades, poultry breeders have intensively selected animals with emphasis on increasing growth rate and meat production. However, rapid increased body size in commercial chicken has been accompanied by unintended changes in correlated traits such as increases in fat deposition, which reduces production efficiency and product quality (Doney et al. (2002); Zhang et al. (2006)). The better understanding about genes involved in chicken lipid metabolism and muscle development could provide ways to solve this unfavorable correlated response to selection in poultry breeding.

An interesting candidate gene for carcass traits in chicken and specifically fat deposition and muscle development is the *ADIPOR2* (*adiponectin receptor 2*). This gene and its counterpart *ADIPOR1* (*adiponectin receptor 1*) are the receptors of the adiponectin (ADIPOQ), an adipokine hormone that influences glucose utilization, insulin sensitivity, and energy homeostasis in several mammalian species (Kubota et al. (2002); Yamauchi et al. (2002)). Recently, the expression of adiponectin and its receptors was detected in multiple tissues in the chicken (Maddineni et al. (2005); Ramachandran et al. (2007)).

The objective of this study was to investigate the association of a SNP in the *ADIPOR2* gene with broiler carcass traits as a potential genetic marker to be used in poultry selection.

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, thigh and drumstick, 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 percent-age.

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' tcgcagccataatcgctccacagt 3', reverse primer: 5' caacaggaggcaaacccagtaga 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 ($1.6 \pm 0.62\text{g}$), wings weight ($2.28 \pm 0.97\text{g}$), drumstick muscle weight ($2.34 \pm 0.99\text{g}$), drumstick muscle yield ($0.07 \pm 0.03\%$), thigh muscle yield ($0.15 \pm 0.05\%$), thigh and drumstick muscle weight ($6.47 \pm 2.68\text{g}$), and thigh and drumstick muscle yield ($0.20 \pm 0.06\%$). On the other hand, the presence of the T allele at *ADIPOR2* C242T decreased breast skin weight ($1.17 \pm 0.46\text{g}$), breast skin yield ($0.08 \pm 0.02\%$) and drumstick yield ($0.12 \pm 0.05\%$) 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.

Traits /Models	P	Sex	Effects			
			a	SE	d	SE
a (sex)						
WSW	0.02	F	-1.6	0.62	-	-
WW	0.04	F	-2.28	0.97	-	-
DMW	0.04	F	-2.34	0.99	-	-
DMY	0.02	F	-0.07	0.03	-	-
TMY	0.03	F	-0.15	0.05	-	-
TDMW	0.04	F	-6.47	2.68	-	-
TDMY	0.005	F	-0.20	0.06	-	-
BSW	0.03	M	1.17	0.46	-	-
ad (sex)						
DY	0.006	M	0.12	0.05	0.17	0.06
BSY	0.006	M	0.08	0.02	0.05	0.03

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 Teimouri (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/GG/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.

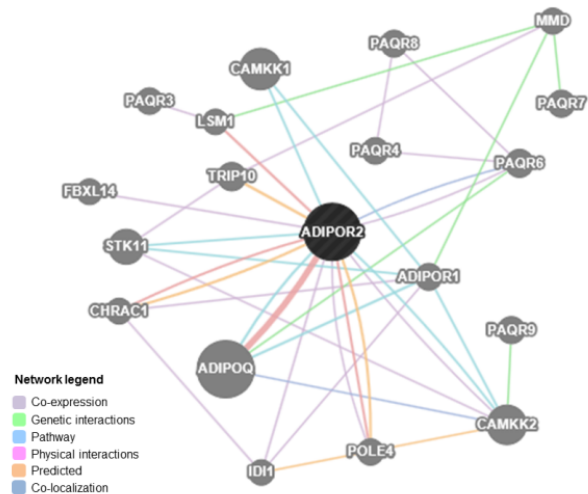


Figure 1. *ADIPOR2* gene network. Circles represent genes and connecting lines represent interactions between genes. Black circles represent the set of genes provided to the GeneMania software. Gray circles are the extra genes added to the network by the program that are strongly connected to query genes.

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.

(2010)). Other genes appear co-expressing with *ADIPOR2*, 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 adipoQ 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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