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Single nucleotide polymorphism analysis in meat-production related genes in broiler chickens

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SUMMARY

In broiler chickens, the intensive selection for growth rate, feed efficiency, body composition (breast muscle weight) traits in the last decades was successful. To improve economically important characteristics, it is possible to use molecular markers associated with meat production traits. The aim of this study was to examine genotype polymorphisms in ROSS 308 broilers for thyroid hormone responsive Spot14α, insulin-like growth factor 1 (IGF1), IGF-binding protein 2 (IGFBP2), somatostatin (SST) and prolactin (PRL) genes. A further goal of this investigation was to study the relationship between the polymorphisms and phenotypic characteristics.

In the investigated broiler population, the frequency for CC homozygous genotype was 0.77 in Spot14α (AY568628), AA homozygous genotype was 0.80 in IGF1 (M74176), GG homozygous genotype was 0.85 in IGFBP2 (U15086), DD homozygous genotype was 0.60 in PRL (FJ663023 or FJ434669). Only the AA homozygous genotype was found in SST (X60191). Chickens with AC genotype in Spot14α, and with GG genotype in IGFBP2 had higher body weight (BW) and carcass weight (CW), compared to CC and GT genotypes. However, the differences were not significant (P>0.05). There was significant association (P<0.05) between PRL genotypes and body and carcass weight, where chicken with homozygous DD surpassed individuals with homozygous II genotypes.

Keywords*: broiler chicken, body composition trait, allele and genotype frequency, Spot14α, IGF1, IGFBP2, SST, PRL*

INTRODUCTION

The intensive application of selection methods in broiler chicken has successfully increased growth rate, feed efficiency, carcass yield, and breast muscle percentage. Allele frequency analyses are often used to identify potential candidate genes and polymorphisms, and are important for the selection of advantageous genotypes. Application of molecular markers associated with meat and egg production is a potential tool to improve production yields.

The thyroid-hormone responsive *Spot14* gene (also known as *THRSP*) was identified as duplicated polymorphic paralogs in chicken, namely *Spot14α* and *Spot14β*. *Spot14* is implicated as a transcription factor on lipogenic genes promoters, involved in control of lipogenic enzymes (Wang et al. 2004). Furthermore *Spot14α* is maintaining a connection between thyroid hormone concentrations and growth (Cao et al. 2007, d'André Hirwa et al. 2010). Expression of *Spot14α* mRNA is also regulated by thyroid hormone status (Wang et al. 2004).

The *insulin-like growth factor 1* (*IGF1*) is a candidate gene for growth, body composition, metabolic, fat deposition and skeletal traits in chicken (Zhou et al. 2005). Most of the functions of growth hormone (*GH*) in chickens are mediated by *IGF*s (Lei et al. 2005). There is a negative genetic correlation between breast muscle and fat deposition in chicken (Sato et al. 2012).

The *IGF-binding protein type 2* (*IGFBP2*) may play an important role in the modification of the growth supporting effect of circulating *IGF1* by producing the *IGFBP* complex in chicken (Kita et al. 2002), and by regulating *IGF* transport to tissues and IGF bioavailability to IGF receptors at cell membrane level (Silha and Murphy 2005). The association of the *IGFBP2* gene with body weights has been proved in Jinghai chickens (Zhao et al. 2015).

The *somatostatin* (*SST*) encodes for two biologically active cyclic peptide forms (*SST-14* and *SST-28*). The SST regulates the secretion of numerous hormones. Despite polymorphisms in *SST* gene have large effects on growth and body composition in mammals SST is characterized up to now in four chicken populations (Nie et al. 2005). Genotype-growth associations have not been performed.

Prolactin (*PRL*) hormone has a diverse spectrum of functions including growth, development, metabolism, reproduction, behaviour, and immune regulation in vertebrates, and plays main roles in several reproductive processes in avian species (Angelier and Chastel 2009). *PRL* takes part in several biological functions; however, only limited genotype-growth associations have been reported in chicken (Bhattacharya et al. 2011).

MATERIAL AND METHODS

Experimental population, sample and data collection at the abattoir

The analyzed hybrids were ROSS 308 (meat-type chicken). Five polymorphisms in growth or egg production-related genes (see below) were selected for genotyping in chicken populations. The experimental animals (n=40) were fattened under identical housing and feeding conditions. Feed was produced by the same plant. Feed and water were given *ad libitum*. The broilers involved in this investigation were randomly selected. The sex of animals was genetically not determined. Males were selected by means of visual discrimination. The chickens were slaughtered at 35 days of age. Legs of chickens were individually marked. Feathers samples were collected from the individuals for the identification of *Spot14α, IGF1,*

IGFBP2, SST, PRL genotypes. Samples were stored at -20 °C pending processing.

The body weight (*BW*) of broilers was measured before slaughtering, and carcass weight (*CW*) was measured before refrigerating. After refrigerating (2 hours at 4 °C), the breast muscle fillets with (*BMWS*) and without skin (*BMW*), the legs with skin and bone (*LW*) were measured, and their percentage of carcass and body weight were also calculated. Chickens were dissected by the slaughterhouse staff.

Isolation of DNA and PCR-RFLP

Extraction of DNA from feathers was done with Wizard Genomic DNA Isolation kits (Promega, USA), according to manufacturer's instructions. DNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, USA), both 260/280 and 260/230 ratios of samples exceeded 1.8. The integrity of DNA was controlled by agarose gel electrophoresis.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques with agarose gel electrophoresis were used to identify genotype. With slight amendments the genotyping methods for the A213C locus in *Spot14α* by Cao et al. (2007), the A570C locus in *IGF1* by Zhou et al. (2005), the 24 bp insertion in *PRL* by Jiang et al. (2005) were applied. Primers were designed and RFLP was constructed for non-synonymous SNPs, the G645T SNP in *IGFBP2* and A370G SNP in *SST* (*Table 1*). These SNPs were described by Nie et al. (2005).

Table 1

Polymorphisms, primers and restriction enzyme information

Polymorphism	Primer sequence (forward/reverse)	Ta (°C)	Length (bp)	RE
A213C exon 1 of Spot14α (AY568628)	CAGGAGGGAGCAGAGGGATAG/ GGTCGGTCAGAACCTGCTGC	60	419	*Bsa*HI
A570C promoter of IGF1 (M74176)	CATTGCGCAGGCTCTATCTG/ TCAAGAGAAGCCCTTCAAGC	57	813	*Hinf*I
G645T exon 2 of IGFBP2 (U15086)	AACAGGCATGAAGGAGATGG/ CTCGCCAGCACATCAAAGT	52	315	*Bse*GI
A370G exon 2 of SST (X60191)	CCTGTTTTCTCTCCCCTCAC/ AGTCTTCGCCTCTCGTGGT	55	330	*Bsr*BI
24 bp insertion promoter of PRL (FJ663023 or FJ434669)	GGTGGGTGAAGAGACAAGGA/ TGCTGAGTATGGCTGGATGT	56	201 and/or 177	-

Note: Ta – annealing temperature in °C; Length – length of PCR products (bp); RE – restriction endonuclease

DNA amplification (PCR) of each individual broiler was performed according to the following conditions: the PCR was performed in a total volume of 25 µl mixtures containing 1 µl (200 ng) of genomic DNA, 12.5 µl 2x PCR Mastermix (Promega, USA; Thermo Fisher Scientific, USA), 1 µl of appropriate oligonucleotide primer (0.4 µM; *Table 1*) and nuclease free water up to 25 µl final volume. The PCR parameters were: 4 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at annealing temperature (*Table 1*), 1 min at 72 °C and final extension of 4 min at 72 °C. The PCR products were digested in a total volume of 20 µl reaction containing 10 µl of the PCR products, 10 U of appropriate restriction enzyme (*Table 1*) according to manufacturers' (Promega and Thermo Fischer Scientific) recommendations, and nuclease-free water up to final volume. Restriction fragments were electrophoresed on 2% agarose gels (Agarose, LE, Analytical Grade; Promega) with ethidium-bromide (10 mg/ml stock concentration, 0.5 µg/ml final concentration; Promega), and individual PCR-RFLP fragment size in each samples were determined by FastRuler Low Range DNA Ladder (Thermo Scientific) under UV illumination. The 24 bp insertion of PRL genotyping was done by loading PCR products straight on 3% agarose gel.

Statistical analysis

The Hardy–Weinberg Equilibrium (HWE) was tested by Chi-square analysis (SPSS 20.0 for Windows) for observed and expected genotype frequencies. HWE analyses for *SST* were not adaptable, since only one allele of the examined locus was present in the investigated populations. Genotype-trait associations were tested by independent samples t-tests or Mann–Whitney tests.

RESULTS AND DISCUSSION

The genotype and allele frequencies of *Spot14α*, *IGF1*, *IGFBP2*, *SST* and *PRL* are shown in *Table 2*. Chi-square test of HWE was not applicable for *SST* since only allele A was present in the population, whereas other genes were in HWE (P>0.05).

The A213C SNP in *Spot14α* results in aspartic (allele C) to glutamic (allele A) acid change. In broiler population only two of three genotypes were found in *Spot14α*: AC and CC; whereas AA genotype was not found. A higher frequency of allele C was represented (*Table 2*). Allele A was identified by a 419 bp fragment, allele C was demonstrated by 319 and 100 bp long fragments. The *Spot14α* genotype had no significant (P>0.05) effect on body weight or other measured traits in the investigating broiler population *(Table 3).*

Table 2

Genotype and allele frequencies in broilers

Locus	Allele frequency	Genotype frequency	HWE Chi-square values
A213C in *Spot14α*	A=0.11 C=0.89	AA=0.00 AC=0.23 CC=0.77	0.675
A570C in *IGF1*	A=0.90 C= 0.10	AA=0.80 AC=0.20 CC=0.00	0.494
G645T in *IGFBP2*	G=0.93 T=0.07	GG=0.85 GT=0.15 TT=0.00	0.263
A370G in *SST*	A=1.00 G=0.00	AA=1.00 AG=0.00 GG=0.00	n.a.
24 bp indel in *PRL*	I=0.22 D=0.78	DD=0.60 ID=0.35 II=0.05	0.001

Table 3

Genotype and body composition trait associations in broilers

Genotype		BW (g)	CW (g)	BMWS (g)	BMW (g)	LW (g)
Spot14α	AC	2059.01±238.46	1541.57±177.13	506.67±88.98	452.67±90.44	470.56±55.69
	CC	2010.30±206.11	1520.60±156.34	507.804±73.08	469.77±80.97	459.31±59.24
IGFBP2	GG	2025.53±216.33	1530.35±161.18	507.03±77.19	459.31±82.78	462.56±59.53
	GT	1991.17±176.05	1492.00±140.89	504.83±67.31	476.50±77.57	459.00±38.25
PRL	DD	2067.54±198.70[a]	1562.67±148.15[a]	524.50±70.33	476.55±75.72	479.08±49.67[a]
	ID	1978.86±207.48	1486.00±159.87	482.64±81.04	449.50±91.69	441.79±59.55[b]
	II	1745.00±91.92[b]	1338.00±29.00[b]	461.50±19.09	417.00±12.73	399.00±28.28[b]

Note: BW – body weight at 38th day; CW – carcass weight; BMWS – breast muscle weight with skin; BMW – breast muscle weight without skin; LW – leg weight with skin and bone

Chicken with genotype AC had heavier body weight, nevertheless the effect of allele A was not significant (P>0.05). Cao et al. (2007) found in an F2 broiler-layer cross significant association between the polymorphism and body weight of 5 to 12-week-old chickens, individuals with CC genotype had significantly (P<0.05) higher body and carcass weight than with other genotypes. The SNP had no effect on the measured body composition traits. In an F2 population of White Recessive Rock and Xinghua breeds, SNPs located in the 5' flanking region of this gene were associated with body weight at younger ages [hatch and 28-day weight, Hirwa et al. (2010)]. Based on these contradictory results, a non-direct effect of A213C SNP was proposed, suggesting that the actual causative mutation was nearly linked to the investigated SNP, and was in inverse linkage in the different populations. Before using this SNP in marker assisted selection programs, attention must be given to survey the actual direction of the allele substitution effect in the particular breed or line.

Two of three *IGF1* genotypes (A570 C SNP) were found (AA, AC) in the investigated broiler population; the CC genotype was not found (*Table 2*). Allele A was characterized by three fragments of sizes 378, 244, and 191 bp, whereas allele C was represented by 622 and 191 bp long fragments. Similar to other authors, allele A frequency was higher in the broiler population. There was no *IGF1* genotype-phenotype comparison in this study, because a different set (n=40) of the ROSS 308 broiler population without production trait measurements were used to determine *IGF1* genotype frequencies. However, in several studies the role of *IGF1* in growth and breast muscle weight was confirmed (Lei et al. 2005, Zhou et al. 2005, Kadlec et al. 2011, Sato et al. 2012).

Two of three genotypes were observed in *IGFBP2* (GG, GT), the TT genotype was not found (*Table 2*). Allele G was demonstrated by 198 and 117 bp long products, allele T was represented by a 315 bp long fragment. In the examined broiler population, in which two genotypes in *IGFBP2* were found, homozygous G birds had heavier body and carcass weight, than chickens with heterozygous genotype (*Table 3*), but the difference was not significant (P>0.05). Association between *IGFBP2* and some growth traits (Nie et al. 2005, Li et al. 2006), furthermore carcass and body composition traits (Li et al. 2006, Zhao et al. 2015) were reported in several chicken populations. The G645T is non-synonymous and significant differences (P<0.05) were found between polymorphism and body weight in F2 designed population was made up of reciprocal cross between White Recessive Rock and Xinghua chickens (Lei et al. 2005).

Allele A at A370G locus in *SST* was fixed in the examined broiler population (*Table 2*). The fixed genotype was characterized by the uncut 330 bp long PCR product. This SNP was described by Nie et al. (2005), when Leghorn, White Recessive Rock, Taihe silkies and Xinghua breed were contrasted. Tempfli et al. (2015) also reported the fixed AA genotype in Hungarian Yellow chicken. Other reports have not been presented about genotype-trait association of this polymorphism. Therefore, further investigations involving other breeds are necessary to assess the effect and frequency of the polymorphism.

In the broiler population all the three genotypes (DD, ID, II) were found in *PRL*. Allele I was identified as a 201 bp long fragment, whereas allele D was characterized by a 177 bp long product. In the examined broiler population there was significant difference (P<0.05) in body, carcass, and leg weight (*Table 3*). All measured traits were greater in homozygous D chicken, compared to heterozygous animals. However, genotype differences regarding

body and carcass weight were not significant (P>0.05). Jiang et al. (2005) reported that the insertion frequency greatly differed among breeds, and was commonly higher in layer populations. Based on the highly polymorphic *PRL* promoter, also significant (P<0.05) *PRL* haplogroup associations were described in White Leghorn lines with body weight of chickens at sexual maturity and at 16 and 64 weeks of age (Bhattacharya et al. 2011).

CONCLUSION

There were no significant differences (P>0.05) between measured production traits of *Spot14α* and *IGFBP2* genotypes. However, some authors previously found differences among the genotypes and growth traits. The *SST* was fixed in the investigated population. In *PRL* there was significant difference (P<0.05) in some important production traits (live and carcass weight). Because of the low samples number, involvement of more samples is planned in further experiments. The analyzed polymorphic markers provide potentially useful information for breed improvement.

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