

ORIGINAL ARTICLE

Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens

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Abstract

Chicken insulin-like factor 1 gene (*IGF1*) is a biological candidate gene for the investigation of growth, body composition, and metabolic and skeletal traits, and is also a positional candidate gene for growth and fat deposition in chickens. Two broiler populations Ross 308 and Cobb 500 were used to study the relationship between *IGF1* gene polymorphism and phenotypic traits. A single nucleotide polymorphism (SNP) was identified in 132 individuals using the PCR-RFLP technique. Genotypical frequencies were, for genotype AA: 0.83–0.86, and for AC: 0.14–0.17. Associations between *IGF1* promotor polymorphism and liver weight ($P \leq 0.05$) and liver weight as a percentage of the weight of the poultry carcass with the giblets ($P \leq 0.05$), were found in the AC genotype in a comparison of broiler homozygous chickens AA in the Cobb 500 line. In these broilers, the breast muscle and leg muscle weight in the AC genotype were higher, and abdominal fat weight lower compared with AA genotype chickens, but these differences were not significant.

Key words: insulin-like growth factor; single nucleotide polymorphism; chicken; body weight; liver

INTRODUCTION

The intensive application of selection methods in poultry farming has resulted in an increased

growth rate and carcass yield (Nicholson 1998), but there are negative consequences to this process, including health problems, especially obesity and increased incidence of sudden death syndrome, immunosuppression, and diseases of the legs (Deeb and Lamont 2002). To improve production traits and health simultaneously it is appropriate to use molecular markers associated with one or two characteristics. The insulin-like growth factor gene (*IGF1*) is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou et

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al. 2005). Insulin-like growth factors belong to the family of polypeptide hormones, they are structural homologues of insulin and also have a similar function.

Hormones such as the growth hormone, IGF, thyroid hormones and insulin, play important and diverse roles in animal growth (Zhou et al. 2005). Most of the functions of the growth hormone in chickens are mediated by insulin-like growth factors (IGF) (Lei et al. 2005) which stimulate amino acid uptake, glucose metabolism, DNA synthesis (McMurtry 1998), protein synthesis, and the proliferation of different cell types, and is also involved in the regulation of growth (McMurtry et al. 1997). Growth rate stimulation by IGF is known in many species of animals. Studies have found that there is no direct dependence between the levels of growth hormone (GH) and the growth rate in chickens, and therefore it might be useful to study insulin-like growth factors (Beccavin et al. 2001), as mediators of the functions of the growth hormone (Lei et al. 2005).

Chicken *IGF1* mRNA is synthesized, as with humans, in many tissues, such as liver, brain, muscle and heart (Kajimoto and Rotwein 1989). First and foremost, the insulin-like growth factor I is produced in the liver in response to the action of the growth hormone in the pituitary (Zhou et al. 2007). The demonstration of *IGF1* gene expression can be detected already in the early stages of ontogeny. During embryonic development *IGF1* mRNA can be detected in tissues such as the eye, skeletal muscle and brain, but it is interesting that, in chickens, it is not detectable in the liver and heart until hatching (Kikuchi et al. 1991). This suggests that in the avian embryo the IGF-I level is of extrahepatic origin (McMurtry 1998). After chicken hatching, the IGF-I content in blood serum increases from 10 ng ml⁻¹ in the 1st week, to 35–40 ng ml⁻¹ from the 3rd to the 6th week, and the *IGF1* mRNA level in the liver increases during the 1st to the 7th week by almost 5 times. The increase from the 1st to the 3rd week corresponds with the increased content of the growth hormone (GH) in the serum: from 21 to 37 ng ml⁻¹ (Kikuchi et al. 1991).

The GH-IGF axis plays a major role in the control of skeletal muscle growth and differentiation. Circulating growth hormone acts on the liver to stimulate expression of the *IGF1* and *IGFBP3* genes, increasing the levels of these proteins in the blood circulation. GH may stimulate the expression of the *IGF1* gene in skeletal muscle, although in many cases it describes the overexpression of *IGF1* in the muscle in the absence of GH (Florini et al. 1996).

The objective of this study was to examine associations between genotypes of the *IGF1* gene and selected growth and body composition parameters in two broiler populations.

MATERIAL AND METHODS

Experimental chickens and phenotypic measurements

Two broiler breeder lines – Ross 308 (n = 48) and Cobb 500 (n = 84) – were used. The sex ratio was 1:1, 1.05:1 respectively. Birds were individually marked by wing markers and reared on a deep litter of crushed straw. Feed and water were given *ad libitum*. They were fed by commercially produced feed mixtures designed for chicken fattening: a mixture of BR1 – NL / ME_N 22.9% / 12.6 MJ kg⁻¹ (from 1st to the 21st day of fattening), BR2 – NL / ME_N 19.7% / 13.2 MJ kg⁻¹ (from 22nd to the 36th day of fattening) and BR3 – NL / ME_N 19.5% / 13.4 MJ kg⁻¹ (from 37th to the 42nd day of fattening). The BR1 mixture was loose, mixtures BR2 and BR3 were granulated. During the experiment, the individual weight of chickens was measured after hatching, in the 14th, 21st, 28th, 35th and 42nd of days. The experiment was finished on the 42nd day of age of the chickens. Blood samples were collected in EDTA-treated tubes from the 6-wk-old chickens before slaughter for identification of the *IGF1* genotypes.

Slaughter analysis

After slaughter and processing, the sex of the chickens was determined, and the carcasses were stored in a refrigerator at 3 to 4 °C for 24 hours. After 24 hours, the weights of the poultry carcasses (WPC), the weight of the edible organs – liver, stomach, heart, spleen, and total giblets – and the WPC with giblets were measured. The dressing percentage, and the percentage weight of individual organs from the WPC with giblets were also calculated. The weight of the abdominal fat and its percentage share of the WPC with giblets and abdominal fat were also found. Other observed slaughter characteristics were: breast muscle and leg muscle weight with skin and without skin and their percentage share of the WPC.

100 g of pure integral muscle was taken from the right breast muscle for the determination of the drip loss. The sample was weighed exactly and placed in a plastic bag for 24 hours and stored at a temperature of 3 to 4 °C. After 24 hours, the

meat was dried, weighed and the percentage of the drip loss was calculated.

The texture of raw meat was also measured in the right breast muscle by the Warner-Bratzler shear force test. Ten samples 10 mm × 10 mm were cut perpendicular to the longitudinal orientation of muscle fibers using the Texture Analyzer instrument TA.XTPlus from Stable Micro Systems and evaluated using the Texture Exponent 32 software version 2.0.7.0. The cutter used was by Warner-Bratzler. The instrument settings included: Pre-Test Speed 5.00 mm s⁻¹; Speed Test 3.33 mm s⁻¹; Post-Test Speed 10.00 mm s⁻¹; Distance 30 mm Trigger Type – Auto (Force), and Trigger Force 5.0 G. The maximum force required for cutting was expressed in kilograms.

Establishment of a PCR-RFLP assay

Blood samples were collected in EDTA-treated tubes from the 6-wk-old birds before slaughter. The PCR primers for the chicken *IGF1* gene were used (forward: 5'-CATTGCGCAGGCTCTATCTG-3'; reverse: 5'-TCAAGAGAAGCCCTTCAAGC-3') (Moody et al. 2003). DNA amplification of each individual bird was performed according to the following conditions: the PCR was performed in a total volume of 20 µL, containing 2 µL of genomic DNA, 10 pmol of each oligonucleotide primer, 2 µL 25 mM MgCl₂, 2 µL of 1 mM deoxynucleotide triphosphate mixture, and 1 U of *Taq* DNA polymerase; cycle parameters were 94 °C for 5 min then 35 cycles of 94 °C for 1 min, 61 °C for 1 min, and 72 °C for 1 min, with a final extension step for 4 min at 72 °C; the PCR products with length 813 bp were digested at 37 °C overnight with 10 U of *Hinf* I. Restriction digests were electrophoresed for 4 h at 80 V on a 3.5% agarose gel with ethidium bromide, and individual PCR-RFLP fragment sizes in each sample were determined, based on a standard DNA molecular weight marker, by viewing the banding pattern under UV light on the transilluminator.

Statistical analysis

Data were processed using the analysis of variance in Statistica v. 8, and for simple classification, scattering analysis according to the following model:

$$x_{ij} = \mu + \alpha_j + e_{ij},$$

where: x_{ij} – the i^{th} trait value in the j^{th} selection; μ – the common part of the mean; α_j – expresses the influence of the j^{th} level of genotype factor (1 – AA, 2 – AC), and e_{ij} – the random error.

Assumptions for analysis of variance were tested by using the Bartlett test of homogeneity of variances.

For processing of data not assuming equal variances, we used the one-way analysis of means – Welch t-test for different variances using R statistical software.

RESULTS AND DISCUSSION

Detection of *IGF-I* gene polymorphism and distribution of genotypic and allelic frequencies of *IGF1-SNP*

The electrophoresis pattern of SNP of chicken *IGF1* is shown in Fig. 1. Two of three genotypes were found: AA and AC; the CC homozygous genotype was not found. The *Hinf* I PCR-RFLP analysis showed fragments of 622, 378, 244 and 191, and 378, 244, and 191 bp. Allele A is characterized by three fragments of sizes 378, 244 and 191 bp and allele C is given by the length of fragments 622 and 191 bp (Moe et al. 2009). The genotypic and allelic frequencies of the *IGF1* gene in the two broiler populations are given in Table 1. In both populations, the observed genotype frequencies were in good agreement with Hardy-Weinberg expectations. In the broilers, the allelic frequency of A (0.915–0.93) was higher than that of C (0.07–0.085).

Average values of phenotypic traits and associations of the *IGF1* polymorphism with these characteristics

The average values of growth and body composition traits are presented in Tables 2 and 3. In the growth traits, there were no significant differences between AA, AC genotypes and body weight and average gain. In the body composition traits, there was a significant association between the *IGF1-SNP1* and liver weight, and the liver weight as a percentage of the weight of the poultry carcass with giblets at the significance level $P \leq 0.05$ in the Cobb 500 broiler population. The sex effect in genotype AC wasn't manifested by statistical significance. In this chicken line, the breast muscle and leg muscle weight in the AC genotype was higher, and the abdominal fat weight lower, than in birds with the AA genotype, but the difference was not significant.

The *IGF1* gene is essential for normal embryonic and postnatal growth in mammals (Bian et al. 2008). In the chicken, this is a functional candidate gene for growth, body composition and

metabolism (Zhou et al. 2005). The chicken *IGF1* polymorphism has been reported in previous studies (Nagaraja et al. 2000, Amills et al. 2003, Zhou et al. 2005, Bennett et al. 2006, Moe et al.

2009) but no report exists on the association of *IGF1*-SNP with body composition traits in the Ross 308 broiler line.

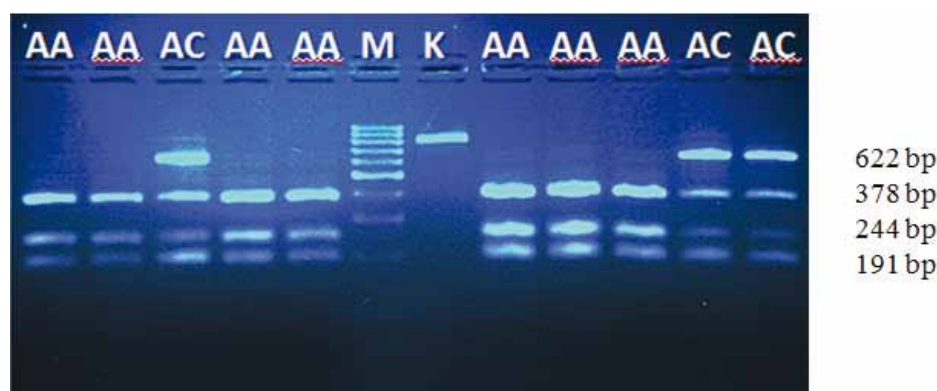


Fig. 1. PCR-RFLP based on the SNP of *IGF1* gene. AA and AC were PCR-RFLP types; M – molecular weight marker; K – PCR product.

Table 1. Genotypic and allelic frequencies of *IGF1* gene of broilers

Meat type	Allele		IGF1/Hinf I Genotype ¹⁾			χ^2 ²⁾	
	A	C	AA	AC	CC		
Ross 308			abs.	40 (41.51)	8 (6.25)	0	0.1268
				♂ 21, ♀ 19	♂ 3, ♀ 5		
	0.915	0.085	rel.	0.83	0.17	0.00	
Cobb 500			abs.	72 (70.33)	12 (13.07)	0	0.545
				♂ 40, ♀ 32	♂ 3, ♀ 9		
	0.93	0.07	rel.	0.86	0.14	0.00	

¹⁾ Numbers in parentheses are values from Hardy-Weinberg expectations

²⁾ P: Hardy-Weinberg equilibrium test

In this study, we investigated the genotypic and allelic frequencies of SNP within the chicken *IGF1* gene promoter region in two broiler populations. The allele frequencies of *IGF1*-SNP1 were in Hardy-Weinberg equilibrium, suggesting that the *IGF1* polymorphism does not affect the fitness of birds (Moe et al. 2009). In both commercial broiler lines, the allelic frequency of A was higher than that of the allele C. This result suggests that the broilers as chickens bred for high intensity growth, were high in distribution of allele A. This can be explained as an effect of selection on growth traits because native and layer chicken populations showed a higher frequency of allele C than that of allele A (Moe et al. 2009).

An increase in the intensity of growth enabled a reduction in the fattening period of chickens to 42 days. 18 years ago, broilers reached slaughter weight 10 days later (Deeb and Lamont 2002).

As regards genotypic frequencies, from the three known restriction patterns only two genotypes were detected: AA and AC. The CC homozygous genotype was not found at all in the broiler population. This confirms a study by Moe et al. (2009) in which also no CC genotype was found in Cobb broilers.

As shown in Table 1, both allelic and genotypic frequencies in both chicken populations were very consistent. These results suggest that both lines are probably genetically similar.

Table 2. Growth traits in broilers

Trait (U)	Age (days)	IGF1-SNP1 Genotype			
		Ross 308		Cobb 500	
		AA	AC	AA	AC
		$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
BW (g)	1	56.45±4.26	54.88±2.57	45.94±4.16	45.17±4.71
BW (g)	14	395.93±64.50	391.38±27.35	388.44±79.95	424.92±62.53
BW (g)	21	847.30±151.17	838.25±42.65	831.19±136.29	908.67±103.93
BW (g)	28	1472.75±227.08	1449.75±84.01	1441.04±193.74	1514.17±153.64
BW (g)	35	2158.90±306.51	2052.25±133.41	2121.06±245.30	2180.83±214.18
BW (g)	42	2741.40±359.41	2646.50±258.78	2641.19±289.79	2644.50±252.64
AG (g)	1–14	339.48±62.51	336.50±25.51	342.5±77.90	379.75±59.77
AG (g)	1–21	790.85±149.45	738.38±41.89	785.25±134.26	863.50±101.45
AG (g)	1–28	1416.30±226.01	1394.88±83.27	1395.10±191.78	1469.00±151.89
AG (g)	1–35	2102.45±305.83	1997.38±132.71	2075.11±243.44	2135.67±212.80
AG (g)	1–42	2684.95±358.77	2591.63±258.27	2595.25±288.23	2599.33±251.89
WPC (g)	42	1961.25±285.89	1913.13±163.93	1897.43±259.45	1892.42±193.01
WPCG (g)	42	2057.50±292.23	2005.51±170.51	1994.46±268.07	1996.32±200.46
WPCGF (g)	42	2128.49±297.23	2074.42±178.24	2048.42±271.64	2047.03±203.26
DP (%)	42	74.97±3.40	75.90±2.30	75.35±2.83	75.50±2.64

$\bar{x}$ – arithmetic mean; s_x – standard deviation

BW – body weight; AG – average gain; WPC – weight of poultry carcass; DP – dressing percentage

We compared the genotypes using a number of phenotypic traits. Beccavin et al. (2001) followed the levels of circulating IGF-I, IGF-II, IGF-BP and their mRNAs in chickens with a high and a low intensity of growth and found that the content of IGF-I is significantly influenced by genotype, and plays a key role in controlling the growth rate in young broilers. In chickens with a higher intensity of growth the amount of circulating IGF-I increases from 1–6 weeks of age; in chickens with a slower intensity of growth it increases over a period of from 1–12 weeks. Amills et al. (2003) found suggestive associations ($P \leq 0.05$) between *IGF1-SNP1*, average daily gain at 107 days and feed efficiency at 44, 73, and 107 days. In the present study, in the Ross 308 line, AA homozygous birds had a higher body weight and average gain on all the observed control days to market weight compared to those with AC genotypes. This confirms the results of Zhou et al. (2005), who demonstrated heavier body weight at all ages to 8wk-market weight in birds inheriting broiler alleles. In contrast, in the present study the same genotype effect trend in the second broiler population did not occur. In the Cobb

500, an opposite tendency was found (expected body weight on the 1st day) in the AA genotype. The observed dependences were not significant. Based on previous results (Moe et al. 2009) which indicate statistically significant differences ($P \leq 0.05$) between AA and CC genotypes in body weight and average daily gain, it can be assumed that significant differences could be found in the breeds with identification of all three genotypes, such as layer breeds. Zhou et al. (2005) also found in the F₂ generation of hybrids Leghorn × broiler Fayoum × broiler, a significant association (at 5% significance level) between *IGF1-SNP1* and body weight and average daily gains, with the exception of the increase from 6 to 8 weeks of age.

IGF-I plays a key role in the development of muscle tissue and positively affects the growth of muscle (Duclos 2005). IGF also regulates glucose, fat and muscle protein metabolism (Yun et al. 2005). Breast muscle weight and percentage are the most economically valuable traits for broilers. Contrary to the results of Zhou et al. (2005), in this study, the *IGF1-SNP1* did not show significant effects on breast muscle weight.

Table 3. Body composition traits in broilers

Trait (U)	<i>IGF1-SNP1 Genotype</i>			
	Ross 308		Cobb 500	
	AA	AC	AA	AC
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
LW (g)	55.59±7.35	52.35±9.56	54.61±8.54*	61.41±11.19*
LW percentage of WPCG (%)	2.74±0.42	2.61±0.38	2.74±0.30**	3.07±0.45**
SW (g)	26.21±3.39	25.31±2.45	26.90±5.92	25.84±3.21
SW percentage of WPCG (%)	1.29±0.16	1.27±0.14	1.37±0.32	1.31±0.23
HW (g)	10.64±1.97	10.75±2.60	11.77±2.46	12.08±2.06
HW percentage of WPCG (%)	0.52±0.09	0.53±0.11	0.59±0.11	0.60±0.07
SpW (g)	3.55±0.99	3.97±1.02	3.74±1.13	4.57±0.87
SpW percentage of WPCG (%)	0.17±0.04	0.20±0.05	0.19 ±0.05	0.23±0.05
TGW (g)	96.00±10.69	92.38±12.45	97.03±13.09	103.90±10.95
AFW (g)	70.99±17.93	68.92±9.20	53.96±14.98	50.71±13.33
AFW percentage of WPCGF (%)	3.37±0.81	3.31±0.26	2.65±0.72	2.48±0.62
BMW with skin (g)	596.84±112.72	558.95±101.37	566.83±92.84	574.26±51.45
BMW with skin percentage of WPC (%)	30.41±3.53	29.18±4.21	29.83±2.31	30.41±1.35
BMW without skin (g)	535.98±101.98	506.46±106.54	519.35±85.64	527.92±48.94
BMW without skin percentage of WPC (%)	27.30±3.16	26.39±4.44	27.34±2.23	27.95±1.21
LMW with skin (g)	433.07±70.25	439.56±39.23	396.21±62.93	407.84±61.65
LMW with skin percentage of WPC (%)	22.13±1.99	22.98±0.80	20.89±1.91	21.51±1.87
LMV without skin (g)	364.51±62.98	371.29±34.92	344.56±56.72	353.13±59.13
LMW without skin percentage of WPC (%)	18.58±1.60	19.40±0.68	18.16±1.64	18.60±1.80
Drip loss (%)	2.00±0.86	1.66±0.51	1.49±0.78	2.04±1.76
Texture (kg)	1.79±0.29	1.83±0.18	1.55±0.36	1.52±0.38

$\bar{x}$ – arithmetic mean; s_x – standard deviation

LW – liver weight; WPCG – weight of poultry carcass with giblets; SW – stomach weight; HW – hearth weight; SpW – spleen weight; TGW – total giblets; AFW – abdominal fat weight; WPCGF – weight of poultry carcass with giblets and abdominal fat; BMW – breast muscle weight; LMW – leg muscle weight; WPC – weight of poultry carcass

*,** Means differ significantly ($P \leq 0.05$): * analysis of variance (Bartlett test homogeneity of variances: $P=0.155$), ** Welch t-test for different variances

Chicken *IGF1* mRNA is synthesized in various tissues: in liver, brain, muscles and heart (Kajimoto and Rotwein, 1989), but it is interesting that until the chickens were hatched, IGF-I was not detected in the liver and heart (Kikuchi et al. 1991). Thus, in the avian embryo, the level of circulating IGF-I is of extrahepatic origin (McMurtry 1998), but after hatching, IGF-I is produced primarily in this organ (Zhou et al. 2007). A significant correlation between plasma IGF-I concentration and hepatic *IGF1* gene expression was found in chicks re-fed after 2 days of fasting, suggesting that the short-term increase in plasma IGF-I concentration may be partly regulated by an alteration in hepatic

IGF1 mRNA (Kita et al. 1998). In this study, in the Cobb 500 population, chickens with the AC genotype showed a significantly higher difference in liver weight and liver weight as a percentage of weight of poultry carcass with giblets ($P \leq 0.05$), compared to that with the AA genotype. In this study, genotype CC was not detected, thus confirming the results of other studies.

In contrast, this genotype does occur in native chicken breeds, and greater differences were found between these breeders and broilers. A finding of the absence of CC genotype in the hybrid lines we used may be interpreted as a probable result of association selection on growth rate.

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