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Abstract

Molecular genetic selection on individual genes is a promising method to genetically improve economically important traits in chickens. A resource population was developed to study the genetics of growth, body composition, skeletal integrity, and metabolism traits. Broiler sires were crossed to dams of 2 diverse, highly inbred lines (Leghorn and Fayoumi), and the F1 birds were intermated by dam line to produce broiler-Leghorn and broiler-Fayoumi F2 offspring. Growth, body composition, skeletal integrity, and hormonal and metabolic factors were measured in 713 F2 individuals. Insulin-like growth factor-I (IGF1) was selected for study as a biological and positional candidate gene. A single nucleotide polymorphism (SNP) was identified between the founder lines in the IGF1 promoter region, and a PCR-RFLP assay was developed. A mixed model was used to statistically analyze associations of IGF1-SNP1 with phenotypic traits. The IGF1-SNP1 had significant associations with most recorded traits, except metabolic traits. Strong interactions between the IGF1 gene and genetic background on growth traits in the 2 F2 populations suggest that genetic interaction is an important aspect for consideration before using the IGF1-SNP1 in marker-assisted selection programs. Several beneficial effects (improved growth, increased breast muscle weight, decreased abdominal fat, and enhanced skeletal integrity) associated with 1 allele indicate the presence of 1 or more loci near IGF1-SNP1 controlling biologically diverse and economically important traits in chickens.

Keywords

insulin-like growth factor, single nucleotide polymorphism, growth, body composition, skeletal integrity

Comments

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Insulin-Like Growth Factor-I Gene Polymorphism Associations with Growth, Body Composition, Skeleton Integrity, and Metabolic Traits in Chickens[1]

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ABSTRACT Molecular genetic selection on individual genes is a promising method to genetically improve economically important traits in chickens. A resource population was developed to study the genetics of growth, body composition, skeletal integrity, and metabolism traits. Broiler sires were crossed to dams of 2 diverse, highly inbred lines (Leghorn and Fayoumi), and the F_1 birds were intermated by dam line to produce broiler-Leghorn and broiler-Fayoumi F_2 offspring. Growth, body composition, skeletal integrity, and hormonal and metabolic factors were measured in 713 F_2 individuals. Insulin-like growth factor-I (*IGF1*) was selected for study as a biological and positional candidate gene. A single nucleotide polymorphism (SNP) was identified between the founder lines in the *IGF1* promoter region, and a PCR-RFLP assay was developed. A mixed model was used to statistically analyze associations of *IGF1–SNP1* with phenotypic traits. The *IGF1–SNP1* had significant associations with most recorded traits, except metabolic traits. Strong interactions between the IGF1 gene and genetic background on growth traits in the 2 F_2 populations suggest that genetic interaction is an important aspect for consideration before using the *IGF1–SNP1* in marker-assisted selection programs. Several beneficial effects (improved growth, increased breast muscle weight, decreased abdominal fat, and enhanced skeletal integrity) associated with 1 allele indicate the presence of 1 or more loci near *IGF1–SNP1* controlling biologically diverse and economically important traits in chickens.

(*Key words*: insulin-like growth factor, single nucleotide polymorphism, growth, body composition, skeletal integrity)

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INTRODUCTION

Intense genetic selection of broilers has successfully increased growth rate and breast muscle percentage. However, physiological disorders are occurring, such as increased obesity and decreased skeletal integrity (Deeb and Lamont, 2002). To simultaneously improve production and fitness traits, molecular markers associated with one or both sets of traits may be useful.

Insulin-like growth factors (IGF) consist of a family of polypeptide hormones structurally associated with insulin with multiple metabolic and anabolic functions (McMurtry et al., 1997). The IGF-I and IGF-II stimulate the proliferation, differentiation, and metabolism of myogenic cell lines from different species (Florini et al., 1996). The IGFs have been shown to regulate body and muscle growth in chickens (Duclos et al., 1999). The *IGF1* gene may play important roles in growth of multiple tissues, including muscle cells (myocyte differentiation cell multiplication), cartilage (chondrocyte colony formation, alkaline phosphatase activity), and bone (osteoblast division and proliferation) (Zapf and Froesch, 1999). Several studies have shown that circulating IGF-I affects growth rate in poultry (Goddard et al., 1988; Scanes et al., 1989; Ballard et al., 1990). In chickens divergently selected for high or low growth rates, there were significantly higher *IGF1* mRNA levels in the high growth rate line than in the low growth rate line (Beccavin et al., 2001). Duclos (1998)

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Abbreviation Key: ADG = average daily gain; AFW = abdominal fat weight; BMC = bone mineral content; BMD = bone mineral density; BMW = breast muscle weight; DEXA = dual-energy x-ray absorptiometry; DSW = drumstick weight; FDR = false discovery rate; IGF = insulin-like growth factor; SHL = shank length; SHR = ratio of shank length by shank weight; SHW = shank weight; SNP = single nucleotide polymorphism; T3 = triiodothyronine, T4 = thyroxine; TBL = tibia length.

indicated that IGF-I stimulated glucose uptake, amino acid uptake, and protein synthesis and inhibited protein degradation by satellite cell derived myotubes. In another experiment, a quality line selected for increased breast yield and decreased fatness had significantly higher circulating IGF-I concentration than the unselected control line (Tesseraud et al., 2003). Tomas et al. (1998) showed that recombinant human IGF-I infusion in chickens enhanced growth and decreased carcass fat content. Associations of an *IGF1* promoter polymorphism with average daily gain (ADG) and feed efficiency were found in 2 genetically diverse Black Penedesenca chicken strains (Amills et al., 2003). The *IGF1* gene, therefore, was selected as a biological candidate gene to investigate growth, body composition, metabolic, and skeletal traits in chickens.

The chicken IGF1 gene maps to 165.95 cM on chromosome 1. In a broiler-layer F_2 population used to map BW QTL by a genome scan, a QTL affecting BW at 6 wk has been found at 160 cM (confidence interval 114 to 180 cM) on chromosome 1 (Sewalem et al., 2002). A QTL at 150 cM (confidence interval 100 to 182 cM) on chromosome 1 affecting abdominal fat weight (AFW) has been detected in the same F_2 cross (Ikeobi et al., 2002). Therefore, *IGF1* is also a positional candidate gene for growth and fat deposition.

A unique F_2 cross of an outbred meat-type line by 2 inbred lines provides an opportunity to investigate QTL affecting diverse traits in chickens (Deeb and Lamont, 2002). The objective of this study was to examine associations of an *IGF1* promoter polymorphism with growth, body composition, skeleton integrity, and metabolic traits in chickens.

MATERIALS AND METHODS

Experimental Populations

The Iowa Growth and Composition Resource Population (IGCRP) was established by crossing sires from a broiler breeder male line with dams from genetically distinct, highly inbred (>99%) chicken lines, the Leghorn G-B2 and Fayoumi M15.2 (Zhou and Lamont, 1999; Deeb and Lamont, 2002). The F_1 birds were intercrossed, within dam line, to produce 2 related F_2 populations. Birds (n = 392 in broiler by Leghorn cross, n = 321 in broiler by Fayoumi cross) of the 2 F_2 populations were analyzed, with each population representing progeny from one broiler grandsire and one F_1 sire of each cross.

Phenotypic Measurements

Body weight was measured at hatch and in 2-wk intervals up to 8 wk of age. The ADG was calculated as the average daily change in BW between 2 consecutive BW measurements. Body composition traits were recorded at 8 wk of age. These measurements included breast muscle weight (BMW), drumstick weight (DSW), shank weight (SHW), shank length (SHL), tibia length (TBL), AFW, spleen weight, liver weight, and heart weight. Tibias were analyzed for bone mineral characteristics using a dual-energy x-ray absorptiometry (DEXA) technique (Haarbo et al., 1991; Slosman et al., 1992; Mitchell et al., 1997) and a total-body DEXA scanner.[5] The differential attenuation of low (38 keV) and high energy (70 keV) x-rays were measured using the small animal total body research software package in high resolution scan mode. Image analysis was used to accurately measure the bone mineral content (BMC) of each tibia and the axial cross-sectional area for determination of bone mineral density (BMD) (BMD = BMC/area). Blood samples were collected in EDTA-treated tubes from 8-wk-old birds before euthanizing, and plasma was transferred into tubes containing 1,000 IU trasylol as a preservative. Plasma insulin, glucagon, triiodothyronine (T3), and thyroxine (T4) were measured (Ashwell et al., 2002). Double antibody radioimmunoassays were used to determine plasma concentrations of IGF-I with an intraassay CV of 2.6% (McMurtry et al., 1994) and chicken IGF-II with intraassay CV of 3.6% (McMurtry et al., 1998). All traits were also expressed as a percentage of BW at 8 wk of age. Sex was determined by macroscopic inspection of the gonads.

Development of PCR-RFLP Assay and F_2 Genotype

The PCR primers (forward: 5′-CATTGCGCAGGCTCTATCTG-3′; reverse: 5′-TCAAGAGAAGCCCTTCAAGC-3′) for chicken *IGF1* gene were used (Moody et al., 2003). The PCR was performed in a total volume of 10 uL, containing 20 ng of genomic DNA, 0.4 μM of each oligonucleotide primer, 0.09 m*M* $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, and 0.8 U of Taq DNA polymerase.[6] Cycle parameters were 94°C for 5 min then 35 cycles of 94°C for 1 min, 56°C for 45 s, and 72°C for 1 min, with a final extension step for 10 min at 72°C. The PCR was conducted with genomic DNA from grandsire and 2 birds from each inbred line (Leghorn G-B1 and Fayoumi M15.2) to detect potential sequence polymorphisms. Nucleotide sequencing was performed by the Iowa State University DNA Sequencing and Synthesis Facility. Sequences were analyzed using Sequencher 3.1 software.[7] The restriction enzyme sites on these sequences were detected by Webcutter 2.0.[8]

A PCR of DNA of each individual F_2 bird was performed according to the conditions described above. The PCR products were digested at 37°C overnight with 1 U of *Hinf* I.[9] Restriction digests were electrophoresed for 1 h at 100 V on a 2% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes in each sample were determined, based on standard DNA molecular weight markers for each gene, by viewing the banding pattern under UV light.

[5]Lunar DPX-L, Lunar Corporation, Madison, WI.
[6]Promega Corporation, Madison, WI.
[7]Gene Codes Corporation, Ann Arbor, MI.
[8]www.firstmarket.com/cutter/cut2.html; Yale University, Date accessed: March 2, 2003.
[9]New England Biolabs, Inc., Beverly, MA.

Statistical Analysis

In general, data were analyzed with combined 2 F_2 subpopulations. The general linear mixed model tests for associations between genotype and phenotypic traits were conducted by using the JMP program (Sall and Lehman, 1996), according to the following model:

$$Y = \mu + G + L + S + D(L) + H + (G \times L) + (G \times H) + e$$

where Y is the dependent variable, with μ as population mean, genotype (G) of *IGF1-SNP1*, line cross (L), and sex (S) as fixed effects, dam nested in line cross [D(L)] and hatch (H) as random effects, interactions between genotype and line cross (G × L), and between genotype and hatch (G × H); and e as the random error. The interaction between genotype and sex was not included in the model, because there were only 2 significant interactions detected of 46 tested. Significant differences between least squares means of the 3 genotypes were analyzed using a contrast test. For growth traits, 4 out of 8 interactions between genotype and line cross were significant; therefore, data were analyzed separately for the 2 F_2 subpopulations, and L and G × L were then dropped from the model used to analyze growth traits.

RESULTS

Sequence Variation and PCR-RFLP Analysis

The amplified 813-bp product in chicken IGF1 includes 636 bp of the promoter region and 177 bp 5′ untranslated region. An A → C SNP between the broiler grandsire and 2 inbred lines at base 570 (accession number M74176) in the promoter region was identified, which was the same SNP previously detected in another study (Amills et al., 2003). The restriction enzyme *Hinf* I produced fragment sizes of 622 and 191 bp for the 2 inbred lines, whereas the broiler line had fragment sizes of 378, 244, and 191 bp.

Associations of the IGF1 Polymorphism with Phenotypic Traits

The probability values of main effects of the *IGF1* gene SNP on chicken growth, metabolic, composition, and skeletal integrity traits are presented in Table 1.

There were significant associations between the *IGF1–SNP1* and all growth traits (BW and ADG) except ADG at 6 to 8 wk, which approaches the 5% significance level.

There were significant effects of the *IGF1–SNP1* on T3, T3/T4, IGF-I, and percentages of insulin, T3, T4, and T3/T4.

For the absolute (not BW adjusted) measurements, there were significant associations between *IGF1–SNP1* and BMW and DSW. For the derived value from absolute measurements (percentages of BW), there were significant effects of the *IGF1–SNP1* on all traits except percentage of spleen weight.

There were significant associations between the *IGF1–SNP1* and all skeletal measurements (length, weight, BMC, and BMD) except percentage of ratio of shank length by shank weight (SHR).

Allelic Effect of the IGF1 Gene on Growth, Metabolic, Body Composition, and Skeletal Traits

The allelic effects of the *IGF1–SNP1* on growth, metabolic, body composition, and skeletal traits are presented in Table 2. For comparison purposes, the effect of the candidate gene alleles in all traits is presented, even though there were no significant differences in some traits. For the growth traits, the *IGF1–SNP1* broiler homozygote had significantly greater BW and ADG than the heterozygotes, and the heterozygotes were significantly greater than the inbred homozygotes except for ADG from 6 to 8 wk. The allelic effect of the growth traits acted as an additive mode.

For metabolic factors with significant differences between genotypes, the allelic effect differed by traits (Table 2). For T3/T4 and percentages of insulin, T4, IGF-I, and T3/T4, birds that were inbred homozygous for the *IGF1* polymorphism had significantly greater values than broiler homozygous birds, except for percentage of IGF-I circulating hormone levels. Birds inheriting both broiler *IGF1–SNP1* alleles had significantly higher circulating percentages of IGF-I than the inbred-allele homozygous birds. The allelic effect on these traits acted in a dominant fashion. For IGF-I and percentage of T3, the allelic effects of these 2 traits acted additively. The broiler homozygote had significantly greater IGF-I circulation levels than the heterozygotes, and the heterozygotes were significantly greater than the inbred homozygotes, whereas the opposite allelic effect for percentage of T3 was observed.

For body composition traits with significant differences between genotypes, the allelic effect of BMW, spleen weight, and percentages of BMW, AFW, liver weight, and heart weight acted in a dominant mode. The broiler homozygote had significantly greater value for the first 3 traits than the inbred homozygotes, whereas the opposite allelic effect was observed for the last 3 traits. For drumstick traits, whether an absolute or BW adjusted trait, the mean of the *IGF1* broiler allele homozygous birds was significantly higher than the heterozygotes, and the heterozygotes were significantly greater than the inbred homozygotes. The allelic effect of DSW and percentage of DSW acted in an additive mode.

For the skeletal measurements, all traits show significant effects between *IGF1–SNP1* genotypes except percentage of SHR (Table 2). The allelic effect of these traits exhibited a dominant mode. The broiler-allele homozygous birds were significantly greater than the inbred-allele homozygous, except for percentages of BMD, TBL, and SHL, which shared an opposite effect.

TABLE 1. Effects (*P*-value) of polymorphism of insulin-like growth factor-I promoter on chicken growth, skeletal, body composition, and metabolic traits

Trait[1]	Genotype[2]	Genotype × line[3]	Trait	Genotype	Genotype × line
Growth Measurement			Body composition		
BW (g) 2 wk	<0.0001	NS	BMW (g)	<0.0001	NS
BW (g) 4 wk	<0.0001	0.0012	AFW (g)	NS	NS
BW (g) 6 wk	<0.0001	0.0183	SW (g)	0.077	NS
BW (g) 8 wk	<0.0001	0.0495	LW (g)	NS	NS
ADG (g/d) 0–2 wk	<0.0001	NS	HW (g)	NS	0.0021
ADG (g/d) 2–4 wk	<0.0001	<0.0001	DS (g)	<0.0001	0.12
ADG (g/d) 4–6 wk	<0.0001	NS	%BMW (g/100 g)	0.022	NS
ADG (g/d) 6–8 wk	0.068	NS	%AFW (g/100 g)	0.0018	NS
			%SW (g/100 g)	NS	NS
			%LW (g/100 g)	0.015	NS
			%HW (g/100 g)	0.0041	NS
			%DS (g/100 g)	<0.0001	NS
Metabolic trait					
IGR	NS	NS			
Insulin (ng/mL)	NS	0.045	Skeletal measurement		
T3 (ng/mL)	0.003	NS	BMC (g)	<0.0001	0.14
T4 (ng/mL)	NS	NS	BMD (g)	0.019	0.08
T3/T4	0.017	NS	TBL (mm)	0.0005	NS
IGF1 (ng/mL)	<0.0001	0.19	SHL (cm)	0.0014	NS
IGF II (ng/mL)	NS	NS	SHW (g)	<0.0001	NS
%IGR (/100 per g)	0.069	NS	SHR (g/cm)	<0.0001	NS
%Insulin (ng/mL per 100 g)	0.047	0.0142	%BMC (g/100 g)	0.0002	NS
%T3 (ng/mL per 100 g)	<0.0001	NS	%BMD (g/cm^2/100 g)	0.0021	NS
%T4 (ng/mL per 100 g)	0.02	NS	%TBL (mm/100 g)	0.0082	0.043
%T3/T4 (/100 g)	0.0019	NS	%SHL (cm/100 g)	0.0034	0.053
%IGF1 (ng/mL per 100 g)	0.14	0.017	%SHW (g/100 g)	0.0053	NS
%IGF-II (ng/mL per 100 g)	0.13	NS	%SHR (g/100 g)	NS	0.18

[1]Traits expressed as percentage of BW at 8 wk of age are indicated by a percentage sign (%); NS = $P > 0.20$; ADG = average daily gain; IGR = insulin/glucagon ratio; T3 = triiodothyronine, T4 = thyroxine; IGF = insulin-like growth factor; BMW = breast muscle weight; AFW = abdominal fat weight; SW = spleen weight; LW = liver weight; HW = heart weight; DSW = drumstick weight; BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = ratio of shank length by shank weight.

[2]Genotype = IGF1-SNP1 genotype.

[3]Line = line cross.

Interaction Between the IGF1 Gene and Genetic Background of Two Inbred Lines

Four out of 8 interactions of *IGF1–SNP1* and line cross (genetic background) in growth traits were significant (Table 1). The probability values of main effects of the *IGF1* gene SNP on chicken growth traits in 2 line crosses are presented in Table 3. There were significant associations between *IGF1–SNP1* and all growth traits in the broiler-Leghorn cross, whereas there were significant associations of only 2 traits in the broiler-Fayoumi cross. There were similar allelic effects of *IGF1–SNP1* on growth traits in each of the 2 F_2 line crosses. Homozygotes of the broiler *IGF1–SNP1* had greater values than the heterozygotes, and the heterozygotes were greater than the inbred homozygotes. The significant line cross by *IGF1–SNP1* genotype interaction arose because of differences in magnitude, not in direction, of allelic effect (Table 4).

Interaction Between the IGF1 Gene and Sex

There were no significant interactions between *IGF1–SNP1* and sex for growth, metabolic traits, or skeletal traits (data not shown). For body composition traits, there were significant interactions between *IGF1–SNP1* and sex for percentages of BMW and DSW (data not shown). For percentage of BMW, the heterozygotes were greater than the inbred homozygotes but not the broiler homozygotes in females, whereas the opposite effect was observed in males. For percentage of DSW, the heterozygotes were significantly greater than the inbred homozygotes but not the broiler homozygotes in females, whereas the heterozygotes were greater than the broiler homozygotes but not the inbred homozygotes in males (data not shown).

DISCUSSION

The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals (Rothschild and Soller, 1997). Many studies have examined growth, skeletal, and immune function traits using the candidate gene approach in chickens (e.g., Zhou et al., 2001; Amills et al., 2003; Li et al., 2003). The *IGF1* gene was selected as a candidate gene to investigate associations of gene polymorphisms with growth, body composition, skeletal integrity, and metabolic factors in F_2 broiler-inbred line crosses.

TABLE 2. Least square mean growth, metabolic traits, body composition, and skeletal traits, by genotype, of chicken insulin-like growth factor-I (*IGF1*) genes in F_2 Leghorn cross and Fayoumi cross

		IGF1-SNP1 Genotype (nucleotide at 570-bp position)		
Trait[1] (U)	Age (wk)	AA	AC	CC
Growth measurement				
BW (g)	2	208.74 ± 1.86[a]	203.04 ± 1.39[b]	197.27 ± 1.87[c]
BW (g)	4	622.33 ± 6.05[a]	599.34 ± 4.50[b]	581.80 ± 6.06[c]
BW (g)	6	1,062.00 ± 10.81[c]	1,013.52 ± 8.04[b]	981.07 ± 10.83[c]
BW (g)	8	1,573.62 ± 16.48[a]	1,515.50 ± 12.26[b]	1,469.37 ± 6.51[c]
ADG (g/d)	0–2	12.38 ± 0.13[a]	11.96 ± 0.10[b]	11.53 ± 0.13[c]
ADG (g/d)	2–4	29.54 ± 0.33[a]	28.31 ± 0.25[b]	27.47 ± 0.33[c]
ADG (g/d)	4–6	31.41 ± 0.43[a]	29.58 ± 0.32[b]	28.52 ± 0.43[c]
ADG (g/d)	6–8	36.54 ± 0.52[a]	35.86 ± 0.39[ab]	34.88 ± 0.52[b]
Metabolic measurement				
IGR	8	11.21 ± 0.70[a]	11.03 ± 0.53[a]	12.15 ± 0.72[a]
Insulin (ng/mL)	8	2.84 ± 0.088[a]	2.80 ± 0.067[a]	2.92 ± 0.091[a]
T3 (ng/mL)	8	1.91 ± 0.050[a]	2.00 ± 0.038[a]	2.5 ± 0.052[a]
T4 (ng/mL)	8	9.16 ± 0.14[a]	9.17 ± 0.10[a]	9.10 ± 0.14[a]
T3/T4	8	22.30 ± 0.84[a]	23.45 ± 0.64[a]	25.63 ± 0.87[b]
IGF-I (ng/mL)	8	50.15 ± 0.77[a]	46.57 ± 0.59[b]	44.00 ± 0.80[c]
IGF-II (ng/mL)	8	51.35 ± 1.97[a]	53.98 ± 1.50[a]	52.85 ± 2.04[a]
%IGR (/100 g)	8	0.74 ± 0.049[a]	0.74 ± 0.037[a]	0.87 ± 0.051[a]
%Insulin (ng/mL per 100 g)	8	0.187 ± 0.0066[a]	0.190 ± 0.005[a]	0.208 ± 0.0068[b]
%T3 (ng/mL per 100 g)	8	0.126 ± 0.004[a]	0.137 ± 0.003[b]	0.151 ± 0.004[c]
%T4 (ng/mL per 100 g)	8	0.597 ± 0.011[a]	0.617 ± 0.008[ab]	0.639 ± 0.011[b]
%T3/T4 (/100 g)	8	1.47 ± 0.067[a]	1.61 ± 0.051[a]	1.81 ± 0.069[b]
%IGF-I (ng/mL per 100 g)	8	3.28 ± 0.065[a]	3.14 ± 0.050[b]	3.11 ± 0.067[b]
%IGF-II (ng/mL per 100 g)	8	3.39 ± 0.16[a]	3.70 ± 0.12[a]	3.81 ± 0.16[a]
Body composition				
BMW (g)	8	205.12 ± 2.48[a]	193.81 ± 1.86[b]	189.87 ± 2.52[b]
AFW (g)	8	49.57 ± 1.20[a]	50.95 ± 0.90[a]	51.72 ± 1.22[a]
SW (g)	8	2.56 ± 0.042[a]	2.46 ± 0.032[ab]	2.43 ± 0.043[b]
LW (g)	8	37.43 ± 0.62[a]	37.73 ± 0.46[a]	37.10 ± 0.63[a]
HW (g)	8	6.57 ± 0.088[a]	6.54 ± 0.066[a]	6.52 ± 0.089[a]
DS (g)	8	72.28 ± 0.82[a]	68.54 ± 0.62[b]	65.24 ± 0.83[c]
%BMW (g/100 g)	8	12.72 ± 0.074[a]	12.48 ± 0.055[b]	12.48 ± 0.075[b]
%AFW (g/100 g)	8	3.10 ± 0.069[a]	3.28 ± 0.052[b]	3.44 ± 0.07[b]
%SW (g/100 g	8	0.159 ± 0.0024[a]	0.158 ± 0.0018[a]	0.160 ± 0.0024[a]
%LW (g/100 g)	8	2.33 ± 0.031[a]	2.43 ± 0.024[b]	2.45 ± 0.032[b]
%HW (g/100 g)	8	0.406 ± 0.0044[a]	0.419 ± 0.0033[b]	0.426 ± 0.0045[b]
%DS (g/100 g)	8	4.46 ± 0.023[a]	4.39 ± 0.017[b]	4.27 ± 0.023[c]
Skeletal Measurement				
BMC (g)	8	1.659 ± 0.023[a]	1.511 ± 0.022[b]	1.459 ± 0.030[b]
BMD (g/cm^2)	8	0.246 ± 0.0011[a]	0.243 ± 0.0008[ab]	0.241 ± 0.0011[b]
TBL (mm)	8	111.92 ± 0.45[a]	110.44 ± 0.34[b]	109.48 ± 0.45[b]
SHL (cm)	8	8.76 ± 0.033[a]	8.65 ± 0.025[b]	8.60 ± 0.034[b]
SHW (g)	8	31.58 ± 0.40[a]	29.68 ± 0.30[b]	28.26 ± 0.41[b]
SHR (g)	8	3.56 ± 0.036[a]	3.40 ± 0.027[b]	3.31 ± 0.037[b]
%BMC (g/100 g)	8	0.1042 ± 0.0013[a]	0.0992 ± 0.0010[b]	0.0967 ± 0.0014[b]
%BMD (g/cm^2 per 100 g)	8	0.0160 ± 0.00015[a]	0.0164 ± 0.00011[b]	0.0168 ± 0.00015[b]
%TBL (mm/100 g)	8	7.32 ± 0.071[a]	7.47 ± 0.053[ab]	7.62 ± 0.072[b]
%SHW (g/100 g)	8	1.94 ± 0.014[a]	1.90 ± 0.011[b]	1.88 ± 0.014[b]
%SHL (cm/100 g)	8	0.555 ± 0.0049[a]	0.567 ± 0.0037[b]	0.578 ± 0.0050[b]
%SHR (g/cm per 100 g)	8	0.221 ± 0.0015[a]	0.220 ± 0.0011[a]	0.218 ± 0.0015[a]

[a,b]Means with no common superscripts differ significantly ($P < 0.05$).

[1]Traits expressed as a percentage of BW at 8 wk of age are indicated by a percentage sign (%); ADG = average daily gain; IGR = insulin/glucagon ratio; T3 = triiodothyronine, T4 = thyroxine; IGF = insulin-like growth factor; BMW = breast meat weight; AFW = abdominal fat weight; SW = spleen weight; LW = liver weight; HW = heart weight; DS = drumstick weight; BMC = bone mineral content; BMD = bone mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight by shank length.

Growth is a composite of complex developments that result from genetic, nutritional, and environmental factors (Scanes et al., 1984). Birds inheriting *IGF1–SNP1* broiler alleles had, in the present study, heavier BW at all ages to market weight. The birds inheriting the broiler allele had ($P < 0.05$) higher BW and ADG than birds with the Leghorn allele. In the F_2 broiler-Fayoumi cross, significant effects did not appear in all traits; however, the same allele effect trend occurred. In another study of the same mutation of *IGF1* in 2 genetically diverse maternal and paternal Black Penedesenca chicken strains, significant association of the *IGF1–SNP1* was found for ADG only to 107 d in one strain (Amills et al., 2003). The direction of effect, by SNP, differed between the 2 studies, which may be because the SNP identifies different alleles in these unrelated populations or because of the different ages evaluated or interactions with genetic background. The consistency of identifying significant associations of

TABLE 3. Effects (*P*-value) of polymorphism of insulin-like growth factor-I promoter on chicken growth traits

	F_2 cross	
Trait	Broiler-Leghorn	Broiler-Fayoumi
BW (g) 2 wk	0.0006	0.025
BW (g) 4 wk	<0.0001	NS[1]
BW (g) 6 wk	<0.0001	0.161
BW (g) 8 wk	<0.0001	NS
ADG (g/d) 0–2 wk	0.0003	0.020
ADG (g/d) 2–4 wk	<0.0001	NS
ADG (g/d) 4–6 wk	<0.0001	0.083
ADG (g/d) 6–8 wk	0.026	0.192

[1]NS = $P > 0.20$; ADG = average daily gain.

the *IGF1–SNP1* with growth and ADG in multiple independent studies suggests that use of *IGF1* variation may be valuable for efficient genetic selection for growth in broiler chickens.

Hormones, including growth hormone, IGF, thyroid hormones, and insulin, play important and diverse roles in animal growth. The *IGF1–SNP1* had significant effects on half of the metabolic traits in this study. Birds inheriting both broiler *IGF1* alleles had greater circulating IGF-I levels, higher growth rates, and higher ADG than those inheriting both alleles from the inbred lines. The relationships previously reported in other studies between circulating IGF-I and growth in chickens have not been consistent. Significant associations between circulating IGF-I and growth occurred in some, but not all, genetic lines (Goddard et al., 1988; Scanes et al., 1989; McGuinness and Cogburn, 1990). The *IGF1–SNP1* broiler allele homozygotes in the present study had significantly higher circulating IGF-I protein levels than the inbred allele homozygous birds. Because the studied SNP was in the promoter region, it is hypothesized that the *IGF1–SNP1* polymorphism may exert its effect on growth via modulation of gene expression. The present study confirmed the *IGF1–SNP1* polymorphism was associated with differences in plasma IGF-I hormone levels (Table 2).

Breast muscle weight and percentage are the most economically valuable traits for broilers. Because of difficulties of collecting phenotypic data, selection for improving breast muscle percentage lags behind the growth traits. DSW is a measurement combining muscle, bone, and other tissues of the upper leg for which breeders usually have not been selected. The *IGF1–SNP1* showed significant effects on BMW, DSW, and percentages of BMW and DSW.

Abdominal fat has been recognized as an undesirable trait. Infusion of IGF-I into chickens can increase circulating IGF-I concentration, stimulate growth, decrease insulin levels, and lower consequent lipogenic activity, thereby reducing fatness (Huybrechts et al., 1992; Tomas et al., 1998). The birds with the broiler homozygous *IGF1–SNP1* had significantly lower percentages of AFW than *IGF1* inbred homozygous birds. The broiler line had higher percentages of AFW than the 2 inbred lines, however, based on observations of the founder lines (Deeb and Lamont, 2002). The *IGF1* allele from the broiler had generally beneficial effects on improving growth and BMW in the F_2. This specific gene SNP presents the opportunity to select at the molecular level, against the general tendency of broilers toward excess fat deposition and thereby overcoming a general negative correlation of growth and fat percentage.

The heavy weight of broilers and intensive egg-laying performance in modern layers are associated with leg problems and broken bones (Julian, 1998; Knowles and Wilkins, 1998). Bones play an important role in support of the body mass and protection of internal organs in chickens (Korver et al., 2004). Continued selection for growth rate of birds has resulted in potential skeletal problems, which might lead to premature death or culling in industry (Lilburn, 1994). Therefore, enhancing bone strength and keeping appropriate skeletal proportions are becoming a major breeding objective. Several skeletal parameters (BMC, BMD, TBL, SHL, and SHW) were measured as indicators of bone strength and leg growth in the present study. Both BMD and BMC have been used to investigate and predict osteoporosis in humans and mice (Klein et al., 1998; Devoto et al., 2001). The circulating IGF-I level in *IGF1* knock-out mice is associated with bone growth and density (Sjogren et al., 2002; Yakar et al., 2002). Kocamis et al. (2000) has implicated in ovo administration of IGF-I with increased BW and postnatal long bone (femur and tibia) concentrations of hydroxyproline

TABLE 4. Least square mean of growth traits by genotype of chicken insulin-like growth factor-I–single nucleotide polymorphism-I (*IGF1–SNP1*), in an F_2 Leghorn cross and Fayoumi cross

		Broiler × Leghorn cross			Broiler × Fayoumi cross		
Trait[1] (U)	Age (wk)	AA	AC	CC	AA	AC	CC
BW (g)	2	212.36 ± 2.33^{a}	205.8 ± 1.77^{b}	198.94 ± 2.41^{c}	205.34 ± 2.78^{a}	199.54 ± 2.11^{b}	194.08 ± 2.81^{c}
BW (g)	4	638.76 ± 7.48^{a}	603.71 ± 5.69^{b}	573.76 ± 5.73^{c}	605.67 ± 9.20^{a}	594.23 ± 7.12^{a}	591.03 ± 9.32^{a}
BW (g)	6	1,095.7 ± 13.7^{a}	1,024.4 ± 10.4^{b}	973.7 ± 14.1^{c}	1,032.2 ± 15.9^{a}	1,003.3 ± 12.1^{b}	990.72 ± 16.13^{b}
BW (g)	8	1,625.4 ± 21.5^{a}	1,525.4 ± 16.3^{b}	1,467.8 ± 21.2^{c}	1,528.7 ± 23.5^{a}	1,504.8 ± 17.8^{ab}	1,472.4 ± 23.7^{b}
ADG (g/d)	0–2	12.59 ± 0.16^{a}	12.13 ± 0.13^{b}	11.50 ± 0.17^{c}	12.23 ± 0.20^{a}	11.81 ± 0.15^{b}	11.39 ± 0.20^{c}
ADG (g/d)	2–4	30.69 ± 0.40^{a}	28.44 ± 0.31^{b}	26.63 ± 0.42^{c}	28.44 ± 0.51^{a}	28.09 ± 0.39^{a}	28.22 ± 0.51^{a}
ADG (g/d)	4–6	32.34 ± 0.45^{a}	29.94 ± 0.42^{b}	28.79 ± 0.56^{c}	30.46 ± 0.62^{a}	29.26 ± 0.47^{ab}	28.54 ± 0.63^{b}
ADG (g/d)	6–8	37.84 ± 0.60^{a}	35.81 ± 0.53^{b}	35.26 ± 0.73^{b}	35.54 ± 0.70^{a}	35.76 ± 0.54^{a}	34.39 ± 0.71^{a}

[a–c]Means with no common superscripts differ significantly ($P < 0.05$).

[1]ADG = average daily gain.

in male broiler chickens. In the present study, the *IGF1* broiler homozygous birds had significantly higher BMC, BMD, TBL, SHL, SHW, SHR, and percentage of BMC but lower percentages of BMD, TBL, and SHL than the *IGF1-SNP1* inbred homozygous birds. These relationships were consistent with measurements of founder broiler and Leghorn lines (unpublished data). Based on strong significant associations between *IGF1–SNP1* and skeletal integrity measurements, the current study supports the hypothesis that the *IGF1* gene is involved in chicken bone development and growth.

In a multiple-test situation, false positives are an important statistical analysis issue. There are several ways to control false positives. Bonferroni adjustment can be used to control false positives with multiple independent tests (Weller, 2001). Benjamini and Hochberg (1995) proposed the false discovery rate (FDR) to control experiment-wise error rate for the general problem of multiple testing, especially with multiple dependent tests. They defined the FDR as "the expected proportion of true null hypothesis within the class of rejected null hypotheses." The current study is a case with multiple dependent traits; therefore, the FDR is the most appropriate way to control for false positives (Storey, 2003). The FDR value in the present study was 0.10 for $P = 0.047$, which means that 10% of significant associations might be false positives based on a cut-off value of $P \leq 0.047$. There were a total of 41 significant associations out of 92 tests in the current study, and FDR predicts that 4 out 41 might be false positives. The large number of significant tests, well above the predicted FDR, gives strong confidence in the true significance of most of the detected associations.

The unique population design in this study (a single broiler grandsire crossed with 2 distinct inbred dam lines: Leghorn and Fayoumi) provided an opportunity to detect the interaction between the *IGF1* gene and genetic background of 2 inbred lines on growth, body composition, metabolic traits, and skeletal integrity in chickens. Despite similar body mass value of the 2 inbred lines, they significantly differ for most of their other body measurements (Deeb and Lamont, 2002). These phenotypic differences between the 2 lines likely reflect their different genetic backgrounds. The Leghorn line was sampled in the 1950s from the commercial US layer population, whereas the Fayoumi line was derived from a native chicken population in Egypt (Zhou and Lamont, 1999). Strong interactions between the *IGF1* gene and genetic background were detected for the associations between the *IGF1–SNP1* and growth traits in the 2 F_2 populations, which illustrates the importance of defining gene effects in specific populations before future applications using marker-assisted selection programs. The gene by line-cross interaction is also in agreement with the estimate of a large number of genes influencing growth rate in this population (Deeb and Lamont, 2002).

The studied *IGF1–SNP1* is in the promoter region. Multiple alignments among human, mouse, pig, cattle, goat, and chicken *IGF1* promoter sequences have shown that the promoter sequence is very conserved around the SNP location studied. The substitution A → C in the promoter region is involved the suppression of one potential CdxA transcription factor binding site (Amills et al., 2003). Therefore, the studied mutation detected is hypothesized to affect the transcription rate of both alleles and, thus, the gene expression level of *IGF1*, as was confirmed by circulating IGF-I levels.

The population design of a divergent F_2 cross is powerful to detect QTL-linked markers because of the extensive linkage disequilibrium generated in the F_2 population. Therefore, the *IGF1–SNP1* might be the causative mutations or a linked marker for the actual QTL(s) of the measured biological effect.

In summary, this study presents strong evidence of significant and simultaneous beneficial effects of an *IGF1–SNP1* associated with chicken growth, body composition, and skeletal traits. Additionally, the same polymorphism of *IGF1* showed significant associations with many growth and body composition traits in other independent chicken resource populations (Amills et al., 2003; N. Li, China Agricultural University, personal communication). Therefore, this identified *IGF1–SNP1*, as a specific candidate gene or marker, lays the foundation for future marker-assisted selection to simultaneously modify several phenotypes of importance in poultry production efficiency and fitness and highlights this chromosomal region as warranting functional genomic anlaysis.

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