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Chicken Quantitative Trait Loci for Growth and Body Composition Associated with Transforming Growth Factor-β Genes1

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Abstract

Transforming growth factor-β (TGF-β) belongs to a large family of multifunctional growth factors that regulate a broad spectrum of biological activities involved in morphogenesis, development, and differentiation. The current study was designed to investigate the effects of TGF-β genes on chicken growth and body composition traits. The Iowa Growth and Composition Resource Population was established by crossing broiler sires with dams from two unrelated highly inbred lines (Leghorn and Fayoumi). The F1 birds were intercrossed, within dam line, to produce two related F2 populations. Body weight and body composition traits were measured in the F 2 population. Primers for TGF-β2, TGF-β3, and TGF-β4 were designed from database chicken sequence. Polymorphisms between parental lines were detected by DNA sequencing, and PCR-RFLP methods were then developed to screen the F2 population. The TGF-β2 polymorphisms between broiler and Leghorn and the TGF-β4 polymorphism between broiler and Fayoumi were associated with traits of skeletal integrity, such as tibia length, bone mineral content, bone mineral density, and the percentage of each measure to BW. The TGF-β3 polymorphism between broilers and Leghorns was associated with traits of growth and body composition, such as BW, average daily gain, weight of breast muscle, abdominal fat pad and spleen, as well as the percentage of these organ weights to BW, and the percentage of shank weight and length to BW. The current research supports the broad effects of TGF-β genes on growth and development of chickens.

Keywords

Body composition, Chicken, Gene, Growth, Transforming growth factor-β

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Poultry or Avian Science

Comments

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Chicken Quantitative Trait Loci for Growth and Body Composition Associated with Transforming Growth Factor-β Genes[1]

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ABSTRACT Transforming growth factor-β (TGF-β) belongs to a large family of multifunctional growth factors that regulate a broad spectrum of biological activities involved in morphogenesis, development, and differentiation. The current study was designed to investigate the effects of TGF-β genes on chicken growth and body composition traits. The Iowa Growth and Composition Resource Population was established by crossing broiler sires with dams from two unrelated highly inbred lines (Leghorn and Fayoumi). The F_1 birds were intercrossed, within dam line, to produce two related F_2 populations. Body weight and body composition traits were measured in the F_2 population. Primers for TGF-β2, TGF-β3, and TGF-β4 were designed from database chicken sequence. Polymorphisms between parental lines were detected by DNA sequencing, and PCR-RFLP methods were then developed to screen the F_2 population. The TGF-β2 polymorphisms between broiler and Leghorn and the TGF-β4 polymorphism between broiler and Fayoumi were associated with traits of skeletal integrity, such as tibia length, bone mineral content, bone mineral density, and the percentage of each measure to BW. The TGF-β3 polymorphism between broilers and Leghorns was associated with traits of growth and body composition, such as BW, average daily gain, weight of breast muscle, abdominal fat pad and spleen, as well as the percentage of these organ weights to BW, and the percentage of shank weight and length to BW. The current research supports the broad effects of TGF-β genes on growth and development of chickens.

(*Key words*: transforming growth factor-β, gene, growth, body composition, chicken)

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INTRODUCTION

Although traditional selection for phenotypic values of broiler chickens has made significant improvements in growth rates and meat yields during the past half century, poultry geneticists face additional challenges today because of negative correlations between production and fitness traits. Accompanying selection for rapid growth, meat-type birds exhibit an increase in physiological disorders such as obesity, ascites, and leg problems, as well as a reduction in overall immunocompetence (Dunnington and Siegel 1996; Deeb and Lamont, 2002). Production performance and fitness traits are negatively correlated in the chicken (Martin et al., 1990; Pinard-van der Laan et al., 1998). Multitrait selection to simultaneously improve fitness traits and simultaneously increase production performance is, therefore, difficult to achieve by using only direct phenotype selection. Molecular marker-assisted selection may be required to increase selection efficiency and make further improvements in production performance. Genetic markers linked with QTL allow for direct selection on genotype (Lamont et al., 1996). The combination of traditional genetic selection and modern molecular methods may be preferred for breeding chickens in the future.

There are two basic methods of QTL identification: the candidate gene approach and whole-genome linkage-disequilibrium scanning (Rothschild and Soller, 1997; Dodgson and Cheng, 1999). The candidate gene approach is a powerful method for finding the QTL responsible for genetic variation in the traits of interest in agricultural animal species (Rothschild and Soller, 1997). Similar to other economically important traits, chicken growth and fitness traits are controlled by multiple genes (Deeb and Lamont, 2002). Understanding the genetic control of

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Abbreviation Key: ADG = average daily gain; AFW = abdominal fat pad weight; BMC = bone mineral content; BMD = bone mineral density; BMW = breast muscle weight; DEXA = dual-energy x-ray absorptiometry; LW = liver weight; SHW = shank weight; SHL = shank length; SHR = shank weight to length ratio; SNP = single nucleotide polymorphism; SW = spleen weight; TGF-β = transforming growth factor-β; TBL = tibia length.

growth in chickens will provide an opportunity for genetic enhancement of production performance and physiology.

Among the genes that are involved with the development of growth and fitness traits, the transforming growth factor β subfamily (TGF-β) is one of the most important groups (Barnard et al., 1990; Jakowlew et al., 1992, 1994; Burt and Law, 1994). The TGF-β subfamily molecules are cytokines in the TGF-β superfamily. The TGF-β superfamily members are multifunctional cell-cell signaling proteins that play pivotal roles in tissue homeostasis and development (Piek et al., 1999). Biological effects of TGF-β isoforms are broad, including effects on cell differentiation, cell proliferation, cell growth, extracellular matrix formation, and immune function (Barnard et al., 1990; Quere and Thorbecke, 1990; Lawrence, 1996; Sanders and Wride, 1997; Lechleider and Roberts, 1999). In general, the fundamental role of TGF-β is to regulate basic biological processes such as growth and development (Burt and Law, 1994; Lawrence, 1996). The TGF-β genes influence the growth and differentiation of many different cell types and play an important role in processes such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell differentiation, and adipogenesis (Massague et al., 1986; Roberts and Sporn, 1990; Burt and Law, 1994; Roark and Greer, 1994; Wall and Hogan, 1994). The TGF-β genes are, therefore, logical candidates for investigating effects on chicken growth and development.

The chicken TGF-β subfamily consists of four currently identified members: TGF-β1, TGF-β2, TGF-β3, and TGF-β4 (Burt and Paton, 1991, 1992; Roberts et al., 1991; Burt and Law, 1994). Chicken TGF-β1 maps to linkage group E25C31, TGF-β2 maps to chromosome 3, TGF-β3 maps to chromosome 5, and TGF-β4 has not yet been mapped (Groenen et al., 2000). The biological activities of chicken TGF-β isoforms appear to be similar to those of mammals (Cogburn et al., 2000).

The objectives of the present study were to identify polymorphisms of TGF-β genes, develop PCR-RFLP methods to detect those DNA polymorphisms in F_2 resource populations, and evaluate associations between TGF-β polymorphisms and traits of growth and body composition.

MATERIALS AND METHODS

Experimental Populations and Management

The Iowa Growth and Composition Resource Population (IGCRP), a unique resource family, was used (Deeb and Lamont, 2002). The population was established by crossing broiler sires with dams from two unrelated highly inbred lines (Leghorn and Fayoumi). These two lines are >99% inbred (Zhou and Lamont, 1999). The F_1 birds were intercrossed, within dam line, to produce two related F_2 populations. Males (n = 172 for the broiler by Leghorn cross, n = 148 for the broiler by Fayoumi cross) of the two F_2 populations were analyzed, with each population representing progeny from one grandsire and one F_1 sire of each cross. Birds were raised in floor pens on wood shavings and had access to feed and water ad libitum. Birds were fed commercial corn-soybean-based diets that met all NRC requirements (National Research Council, 1994). From hatch to 4 wk, birds received starter feed with 20% protein and 3% fat content (Purina Mills Meat Builder[4]). From 4 to 8 wk, birds were fed a grower ration[5] with 18% protein and 4.1% fat.

Trait Measurements

Body weight was measured at hatch and in 2-wk intervals up to 8 wk of age. Average daily gain (ADG) was calculated for each interval as the average daily change in BW between two consecutive BW measurements. Body composition traits were recorded at 8 wk of age. These measurements included breast muscle weight (BMW), drumstick weight, shank weight (SHW), shank length (SHL), tibia length (TBL), abdominal fat pad weight (AFW), spleen weight (SW), liver weight (LW), and heart weight. Tibias were analyzed for bone mineral characteristics using the dual-energy X-ray absorptiometry (DEXA) technique (Haarbo et al., 1991; Slosman et al., 1992; Svendsen et al., 1993; Mitchell et al., 1997). Measurements were carried out using a total-body DEXA scanner.[6] 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. Multiple tibias were scanned simultaneously, and subsequent 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). All traits were also expressed as percentage of BW at 8 wk of age. The SHW to SHL ratio (SHR) was calculated.

Development of PCR-RFLP Assays

Genomic DNA was isolated from venous blood collected in EDTA. A PCR was carried out with 50 ng genomic DNA from the one grandsire and four granddams (two from each inbred line) to investigate sequence polymorphisms of various regions of TGF-β genes. The PCR products were purified by a Microcon Centrifugal Filter.[7] Purified PCR products were sequenced by the DNA Sequence and Synthesis Facility.[8] Sequences were analyzed using Sequencher 3.1.[9] Restriction enzyme sites in these sequences were detected by the MBCR (Molecular Biology Computational Resource) package.[10]

[4]St. Louis, MO.
[5]Whiton Feeds, Perry, IA.
[6]Lunar DPX-L, Lunar Corporation, Madison, WI.
[7]Millipore Corporation, Bedford, MA.
[8]Iowa State University, Ames, IA.
[9]Gene Codes Corporation, Ann Arbor, MI.
[10]Biomedical Computing Facility, Baylor College of Medicine, Houston, TX.

TGF-β 2. The PCR primers (5′GCC ATA GGT TCA GTG CAA G 3′; 5′ TGA CAG AAG CTC TCA AGC C 3′) were designed to amplify a 284-bp promoter-region fragment by Oligo 5[11] according to chicken genomic sequence in the GenBank database (accession no. X58071). The reaction conditions were 94°C for 3 min; 35 cycles of 94°C, 1 min; 52°C, 1 min; 72°C, 1 min, and an extension at 72°C for 10 min. The 25-μL reaction volume included 50 ng of template, 1 × reaction buffer, 5 pmol of each primer, 0.16 m*M* dNTP, 1.5 m*M* $MgCl_2$, and 1 U *Taq* polymerase.[12]

TGF-β 3. Primers (5′ TCA GGG CAG GTA GAG GGT GT 3′; 5′ GCC ACT GGC AGG ATT CTC AC 3′) were designed from chicken genomic sequence (accession no. X60091) to amplify a 294-bp intron fragment with Oligo 5. The PCR reaction conditions were the same as for the TGF-β2 gene promoter, except that the annealing temperature was 58°C.

TGF-β 4. Primers (5′ GGG GTC TTC AAG CTG AGC GT 3′; 5′ TTG GCA ATG CTC TGC ATG TC 3′) were designed to amplify a 240-bp exon fragment by using Oligo 5 and chicken genomic sequence (accession no. M31160). The PCR reaction conditions were the same as for TGF-β 3, except that 5% dimethyl sulfoxide was added.

Screening of the F_2 Population for Restriction-Enzyme-Detectable Single Nucleotide Polymorphisms

A PCR of DNA from each F_2 bird was performed according to the conditions described above. For TGF-β2, the PCR product was digested using 6 U *Rsa*I[13] at 37°C overnight. The restriction digests were electrophoresed for 1.5 h at 100 V on a 2.0% agarose gel. For TGF-β3, 10 U *Bsl*I was used to digest at 55°C overnight, and the digested products were run for 1.5 h at 105 V on a 2.4% agarose gel. For the TGF-β4 gene, 5 U *Mbo*II[13] was used to digest at 37°C overnight, and digested products were electrophoresed for 1.5 h at 116 V on a 2.5% agarose gel. Individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern under ultraviolet light. The grandsire (broiler) allele was designated as "B" and granddam alleles as "L" or "F" for Leghorn and Fayoumi, respectively.

Statistical Analysis

Data were subjected to a three-way ANOVA using JMP (SAS Institute, 2000), with the genotype (G) as a fixed effect, and dam (D) and hatch (H) as random effects, according to the following model:

$$Y = \mu + G + D + H + e$$

where Y is the dependent variable, μ is population mean, and e is the random error. The interaction of G by H was not significant for all traits and, therefore, was not included in the model. The interactions of D by G and D by H were not included due to missing data for combinations of dams with a low number of progeny. Significant differences between least-squares means of the different genotypes were calculated using a contrast test. Significance was determined as $P < 0.05$, unless otherwise specified.

The percentage contribution of each gene to the total phenotypic variance was calculated as 100 times the ratio of the genotype sum of squares (SS) divided by the SS of all other components as were specified in the following model:

$$\frac{SS_G}{SS_G + SS_D + SS_H + SS_e} \times 100.$$

RESULTS

Sequence Variation and PCR-RFLP Analysis

For TGF-β2, the amplified 284-bp product from the promoter region was sequenced for multiple individuals of each parent line. There was a T/C single nucleotide polymorphisms (SNP) between broilers and Leghorns at Base −640 (accession #: X58071), and no SNP were detected between broiler and Fayoumi. The restriction enzyme *Rsa*I-digested PCR products had fragment sizes of 184 and 100 bp for the broiler line and 284 bp for Leghorn (Figure 1a).

For TGF-β3, a 294-bp product from the fourth intron was obtained from broiler sire and Leghorn and Fayoumi dams and was sequenced. A C/A SNP at base 2,833 (accession no. X60091) was found between broiler and Leghorn, and no SNP were found between broiler and Fayoumi. The restriction enzyme *Bsl*I-digested PCR products had fragments of 124, 75, 74, and 20 bp for the broiler line and fragments of 145, 75, and 74 bp for the Leghorn line (Figure 1b).

For TGF-β4, a 240-bp exon fragment was obtained for all three lines. A C/A SNP at base 632 (accession no. M31160) was found between broiler and Fayoumi. This SNP caused an amino acid change (Glu/Asp) of the protein. There were no SNP between the broiler and Leghorn lines. The restriction enzyme *Mbo*II-digested PCR products had fragments of 173 and 67 bp for the broiler line and 240 bp for the Fayoumi line (Figure 1c).

Association of the Three TGF-β Gene SNP with Growth, Skeletal, and Body Composition Traits

The TGF-β2 and TGF-β4 polymorphisms were mainly related to skeletal traits, and the TGF-β3 polymorphism

[11]National Bioscience, Inc., Plymouth, MN.
[12]Promega Corporation, Madison, WI.
[13]New England Biolabs, Inc., Beverly, MA.

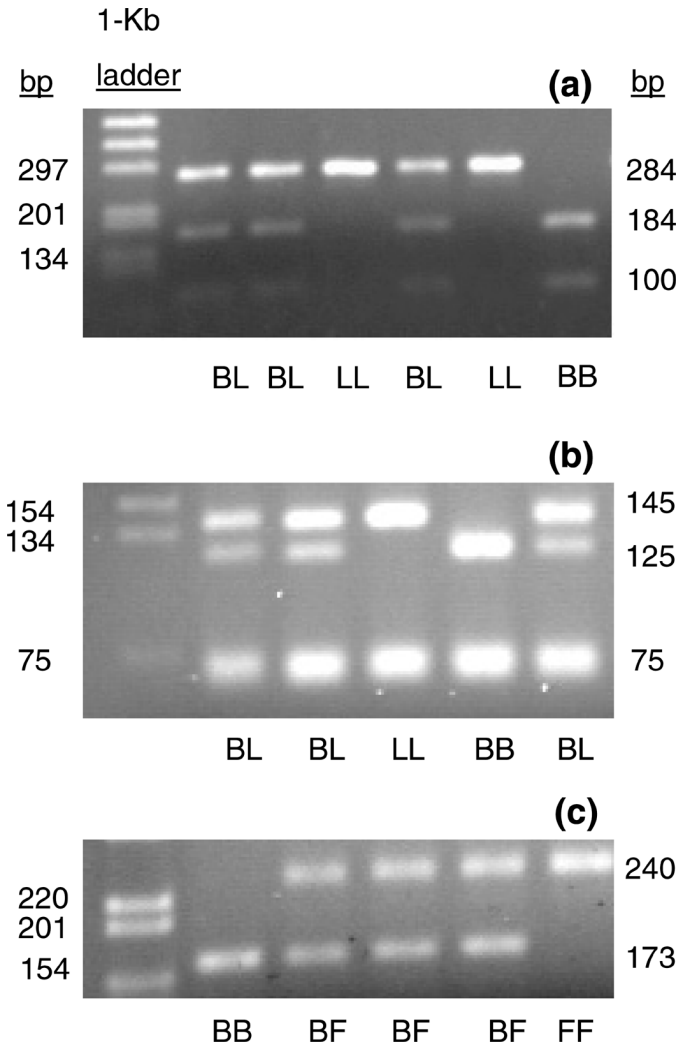

FIGURE 1. PCR-RFLP pattern for transforming growth factor-β (TGF-β) genes. (a) TGF-β2 promoter region with *Rsa*I digestion. (b) TGF-β3 intron 4 with *Bsl*I digestion. (c) TGF-β4 exon region with *Mbo*II digestion. B = grandsire (broiler) allele; L = granddam (Leghorn) allele; F = granddam (Fayoumi) allele. The numbers listed on the right of the figure are fragment sizes (bp).

were mainly related to growth and body composition traits (Table 1). Because of the specific line crosses in which the evaluated SNP existed, the effects of TGF-β2 and TGF-β3 were analyzed only in the broiler-Leghorn F_2 population; those of TGF-β4 were analyzed only in the broiler-Fayoumi F_2 population.

There were significant associations between the TGF-β2 SNP and bone quality traits (BMC, BMD, and %BMC) and bone length traits (TBL and %TBL) in F_2 progeny of the broiler by Leghorn cross (Table 2). There were significant associations between the TGF-β3 polymorphism and BW at 8 wk of age, ADG between 6 to 8 wk of age, bone traits (%SHL, %SHR), and body composition traits (BMW, AFW, %AFW, and %SW) in F_2 progeny of the broiler by Leghorn cross (Table 3). For F_2 progeny of the broiler by Fayoumi cross, there were significant associations between the TGF-β4 polymorphism and bone quality traits (BMC, BMD, and %BMD), bone length traits (TBL, %TBL, and SHL), and body composition traits (SW and %SW) (Table 4).

Effects of TGF-β Genotype on Growth, Skeletal, and Body Composition Traits

For TGF-β2, there were significantly higher BMC, BMD, and percentage BMC, but significantly lower TBL, in F_2 birds that were homozygous for the broiler alleles (TGF-β2-BB) than in those homozygous for the Leghorn alleles (TGF-β2-LL birds) (Table 2). There was no significant difference in percentage BMD and percentage TBL between F_2 birds with TGF-β2-BB and TGF-β2-LL genotype.

For TGF-β3, there were significantly higher BW at 6 wk, BW at 8 wk, ADG at 4 to 6 wk, and ADG at 6 to 8 wk in F_2 birds that were homozygous for the broiler B alleles (TGF-β3-BB) than those homozygous for the Leghorn alleles (TGF-β3-LL) (Table 3). With skeletal and body composition traits, there were significantly lower percentages of SHW, SHL, and SHR in F_2 birds with TGF-β3-BB genotype than in birds with TGF-β3-LL genotype. There were significantly higher BMW, AFW, SW, percentage AFW, and percentage SW in those F_2 birds with TGF-β3-BB genotype. There was no significant difference in percentage BMW between F_2 birds with TGF-β3-BB and TGF-β3-LL genotypes.

For TGF-β4, there were significantly higher percentage BMD, percentage TBL, SW, and percentage SW in F_2 birds that were homozygous for broiler B alleles (TGF-β4-BB) than in those homozygous for Fayoumi alleles (TGF-β4-FF) (Table 4). There was no significant difference in BMC, BMD, TBL, and SHL between F_2 birds with TGF-β4-BB and TGF-β4-FF genotypes.

The Contribution of Individual QTL to F_2 Phenotypic Variance

The percentage of F_2 population phenotypic variance that was determined by each TGF-β gene was calculated for all traits (Figure 2). The contribution of TGF-β2 to bone traits (BMC, BMD, TBL, %BMC, and %TBL) ranged from 2.5 to 4.4%. The contribution of TGF-β3 to whole-body growth rate traits (BW8 and ADG6–8) ranged from 2.7 to 3.7%, to bone traits (%SHW, %SHL, and %SHR) ranged from 3.1 to 3.4%, and to body composition traits (BMW, AFW, SW, %BMW, %AFW, and %SW) ranged from 3.2 to 6.4%. The contribution of TGF-β4 to bone traits (BMC, BMD, TBL, SHL, %BMD, and %TBL) ranged from 3.0 to 5.8% and to body composition traits (SW and %SW) ranged from 4.2 to 4.6%.

DISCUSSION

Associations Between TGF-β Gene Polymorphisms and Traits

The study of candidate genes is one of the primary methods to determine whether specific genes are related to economic traits in farm animals. The TGF-β superfam-

TABLE 1. Effects (probabilities) of transforming growth factor (TGF)-β2, 3, and 4 polymorphisms on chicken growth, skeletal, and body composition traits

		Genes		
Traits (units)[1]	Age (wk)	TGF-β2	TGF-β3	TGF-β4
Growth measurements				
BW (g)	2	NS[2]	0.074	NS
BW (g)	4	NS	0.056	NS
BW (g)	6	NS	0.105	NS
BW (g)	8	NS	0.031	NS
ADG (g/d)	0–2	NS	0.058	NS
ADG (g/d)	2–4	NS	0.073	NS
ADG (g/d)	4–6	NS	0.095	NS
ADG (g/d)	6–8	NS	0.044	NS
Skeletal measurements				
BMC (g)	8	0.024	NS	0.041
BMD (g/cm^2)	8	0.048	NS	0.037
TBL (mm)	8	0.048	NS	0.010
SHL (cm)	8	NS	NS	0.040
SHW (g)	8	NS	NS	NS
SHR (g)	8	NS	NS	NS
%BMC (g/100 g BW)	8	0.047	NS	NS
%BMD (g/cm^2 per 100 g BW)	8	0.101	NS	0.026
%TBL (mm/100 g BW)	8	0.032	NS	0.026
%SHW (g/100 g BW)	8	NS	0.051	NS
%SHL (cm/100 g BW)	8	NS	0.033	NS
%SHR (g/cm per 100 g BW)	8	NS	0.047	NS
Body composition				
BMW (g)	8	NS	0.029	NS
AFW (g)	8	NS	0.002	NS
SW (g)	8	NS	0.053	0.036
LW (g)	8	NS	NS	NS
HW (g)	8	NS	NS	NS
%BMW (g/100 g BW)	8	NS	0.054	NS
%AFW (g/100 g BW)	8	NS	0.002	NS
%SW (g/100 g BW)	8	NS	0.044	0.032
%LW (g/100 g BW)	8	NS	NS	NS
%HW (g/100 g BW)	8	NS	NS	NS

[1]Percentage (%) indicates that traits are expressed as percentage of BW at 8 wk of age.

[2]Not significant at $P > 0.2$.

[3]ADG = average daily gain; BMC = bone (tibia) mineral content; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length; SHW = shank weight; SHR = shank weight to length ratio; BMW = breast muscle weight; AFW = abdominal fat weight; SW = spleen weight; LW = liver weight; HW = heart weight.

ily comprises many multifunctional cytokines, including myostatin (Lee and McPherron, 1999). In myostatin knockout mice, skeletal muscle mass is significantly increased, up to three times the normal size (Lee and McPherron, 2001). Myostatin mutation resulting in functional loss of the protein has been linked to double-muscled cattle breeds (Grobert et al., 1998). Another member of the TGF-β superfamily, insulin-like growth factor-1, has been shown to have serum protein levels associated with growth traits in beef cattle (Ge et al., 2000). Given the role that TGF-β superfamily genes play in growth and development, they are logical targets for investigation as candidate genes for economically important traits in chickens. Chicken TGF-β2, 3, and 4 mRNA were detected in cells of all three germ layers at Stage 10 of embryo development (Jakowlew et al., 1992, 1994), suggesting

TABLE 2. Effects of transforming growth factor-β2 genotype on skeletal traits (least-square means)

	Genotype[2]		
Traits[1]	BB	BL	LL
BMC (g)	1.7670^a	1.7032^a	1.4998^b
BMD (g/cm^2)	0.2450^a	0.2481^a	0.2416^b
TBL (mm)	113.27^b	115.26^{ab}	116.59^a
%BMC (g/100 g BW)	0.1017^a	0.1024^a	0.0933^b
%BMD (g/cm^2 per 100 g BW)	0.0145^b	0.0151^a	0.0146^{ab}
%TBL (mm/100 g BW)	6.6311^b	7.0301^a	6.8921^{ab}

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

[1]BMC = bone (tibia) mineral content; BMD = bone (tibia) mineral density; TBL = tibia length.

[2]B = grandsire (broiler) allele; L = granddam (Leghorn) allele.

TABLE 3. Effects of transforming growth factor-β3 genotype on growth and body composition traits (least-square means)

Traits[1]	Genotype[2]		
	BB	BL	LL
BW2 (g)	215.4[ab]	217.2[a]	206.9[b]
BW4 (g)	638.4[ab]	655.3[a]	618.4[b]
BW6 (g)	1,137.4[a]	1,131.4[a]	1,077.7[b]
BW8 (g)	1,725.0[a]	1,729.3[a]	1,616.8[b]
ADG 0–2 (g)	12.8[ab]	12.9[a]	12.1[b]
ADG 2–4 (g)	30.5[ab]	31.3[a]	29.4[b]
ADG 4–6 (g)	35.5[a]	34.9[ab]	32.8[b]
ADG 6–8 (g)	43.0[a]	40.1[b]	40.4[b]
%SHR (g/cm per 100 g BW)	0.222[b]	0.229[a]	0.231[a]
%SHW (g/100 g BW)	2.022[b]	2.098[a]	2.120[a]
%SHL (cm/100 g BW)	0.526[b]	0.528[b]	0.553[a]
BMW (g)	217.2[a]	218.7[a]	202.2[b]
AFW (g)	60.3[a]	52.4[b]	48.6[b]
SW (g)	2.788[a]	2.703[a]	2.477[b]
%BMW (g/100 g BW)	12.2[ab]	12.4[a]	12.0[b]
%AFW (g/100 g BW)	3.408[a]	2.980[b]	2.799[b]
%SW (g/100 g BW)	0.158[a]	0.155[a]	0.144[b]

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

[1]BW2 = body weight at 2 wk; ADG 0–2 = average daily gain between 0 to 2 wk of age; %SHR = shank weight to shank length ratio expressed as percentage of BW at 8 wk of age; %SHW = shank weight expressed as percentage of BW at 8 wk of age; %SHL = shank length expressed as percentage of BW at 8 wk of age; BMW = breast muscle weight; AFW = abdominal fat weight; SW = spleen weight.

[2]B = grandsire (broiler) allele; L = granddam (Leghorn) allele.

important roles in the development of many tissues. The TGF-β genes are expressed in many locations in the early avian embryo and may be involved in the regulation of phenotypic transformation, matrix deposition, and cell proliferation (Sanders and Wride, 1997).

The detected mutations within TGF-β family genes were located in promoter (TGF-β2), intron (TGF-β3), and exon (TGF-β4) regions. In an F_2 cross population of divergent lines, however, the linkage disequilibrium is substantial. The examined SNP, therefore, may either be causal or linked with functional polymorphisms of any region of that TGF-β gene or other nearby genes. The SNP should only be interpreted as molecular markers of the parental alleles from the genomic region containing the gene with the identified SNP.

Effects of TGF-β Family Gene Genotypes on Traits

BW and Gain. Growth is a comprehensive reflection of development of various parts of a chicken body, and its final expression is the result of interaction among genetic, nutritional, and environmental factors (Scanes et al., 1984). Growth is under complex genetic control, and uncovering the molecular mechanisms of growth will contribute to more efficient selection for growth in broiler chickens. Birds with TGF-β3-BB (two broiler alleles) genotype had higher BW at 6 and 8 wk of age and greater ADG between 4 to 6 and 6 to 8 wk of age than birds with TGF-β3-LL (two Leghorn alleles) genotype in the F_2 population. Comparison of mean values for all three genotypes suggests that TGF-β3 generally acts in a dominant fashion on growth traits, with the broiler allele contributing to greater BW and ADG. This finding is consistent with the selection history of broilers, emphasizing growth rate to market age. The present study thus identifies TGF-β3 as a candidate gene of QTL for growth, which may be used to increase growth rate or market weight in molecular marker-assisted selection programs.

Skeleton. Leg problems in broilers may result from a lack of coordination of development and growth between whole body mass and the skeleton system (Julian, 1998). Increasing bone strength and keeping proper skeletal proportions are objectives in breeding of heavy-bodied poultry. In the current study, BMC, BMD, TBL, SHL, and SHW were measured as indicators of bone strength and leg growth. The BMD and BMC have been used to evaluate skeleton strength and to investigate and predict osteoporosis in humans and mice (Arden et al., 1996; Klein et al., 1998; Devoto et al., 2001; Li et al., 2001). Many investigations, both in vivo and in vitro (O'Keefe et al., 1988; Jakowlew et al., 1991; Thorp et al., 1992; Loveridge et al., 1993; Roark and Greer, 1994; Ren et al., 1997), have shown that TGF-β family genes are involved in chicken bone (including bone matrix) formation, growth, and metabolism.

In the F_2 population, TGF-β2-BB (broiler homozygous) birds had higher BMC, BMD, and percentage BMC but lower TBL compared with TGF-β2-LL (Leghorn homozygous) birds. The broiler allele appeared to be dominant for these three traits. The genotype effects were consistent with the observation in founder lines (unpublished data) that the broiler line had higher BMC, BMD, and percentage BMC than Leghorns, but TBL was contrary in that the broiler line had higher TBL than Leghorns. The TGF-β2 may act as a cryptic allele for TBL in the broiler population, being associated with an unexpected allelic effect, which is only detectable when outcrossed with other

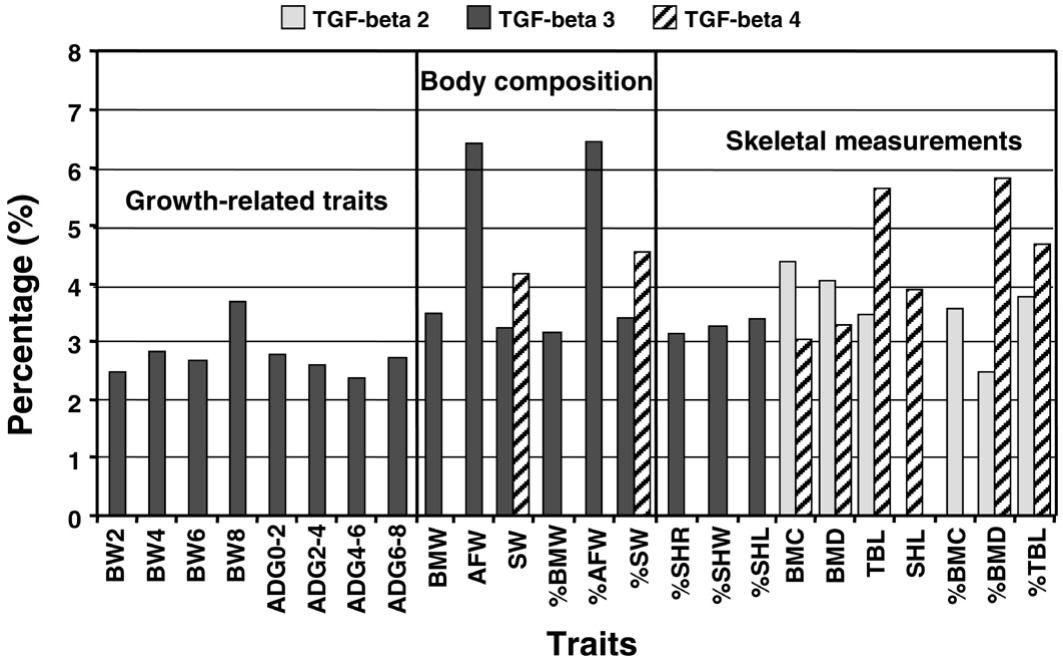

FIGURE 2. The contribution of individual QTL to F_2 population phenotypic variance. BW2 = body weight at 2 wk; ADG 0–2 = average daily gain between 0 to 2 wk of age; BMW = breast muscle weight; AFW = abdominal fat weight; SW = spleen weight; %BMW = breast muscle weight expressed as percentage of BW at 8 wk of age; %SHR = shank weight to shank length ratio expressed as percentage of BW at 8 wk of age; %SHW = shank weight expressed as percentage of BW at 8 wk of age; %SHL = shank length expressed as percentage of BW at 8 wk of age; BMC = bone (tibia) mineral content; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length. The percentages were calculated as described in the statistical analysis section.

lines. Considering the current results, TGF-β2 may be a good candidate gene of QTL to increase leg bone strength in chickens.

In the F_2 population, TGF-β4-BB (broiler homozygous) birds had higher percentages of BMD and TBL than TGF-β4-FF (Fayoumi homozygous) birds, which was opposite the observation in the founder lines (unpublished data), in that the broiler line had lower percentages of BMD and TBL than the Fayoumi line. There was no significant difference between TGF-β4-BB birds and TGF-β4-FF birds on BMC, BMD, TBL, and SHL in the F_2 population. Whether TGF-β4 can be used as a candidate gene of QTL useful for selecting for bone strength and length, therefore, needs further investigation.

The F_2 birds with TGF-β3-BB genotype had lower percentages of SHW, SHL, and SHR than birds with TGF-β3-LL genotype. Because birds with TGF-β3-BB genotype had higher BW at 8 wk of age, it is not unexpected that they had lower percentages of SHW, SHL, and SHR. This result was consistent with the measurements on broiler and Leghorn lines in which the broiler line was lower in the three shank percentage traits than the Leghorn line. The results, therefore, point to TGF-β3 as a candidate gene of QTL that could be used in selection of SHW, SHL, and SHR.

Much attention has been focused on tibial dyschondroplasia in broiler breeding and production (Sheridan et al., 1978; Wong-Valle et al., 1993; Kuhlers and McDaniel, 1996). Expression of TGF-β is reduced in the transitional chondrocytes of chicks with tibial dyschondroplasia, and where the lesion was being repaired, there was increased expression of TGF-β (Loveridge et al., 1993). Therefore, TGF-β may be an important element of the cascade of events that leads to chondrocyte differentiation, hypertrophy, and mineralization. Our results, showing TGF-β genes being associated with tibia BMC, BMD, and length, support the hypothesis that TGF-β genes are involved in chicken bone development and growth.

Breast Muscle. Increased BMW and breast muscle yield are major goals in breeding of broiler chickens. Using established cell lines and primary cultures of chicken embryo myoblasts to investigate the effect of TGF-β on the differentiation of skeletal muscle myoblasts, Massague et al. (1986) found that TGF-β is a potent inhibitor of myogenesis. The TGF-β 2, 3, and 4 mRNA were detected in cultured chicken embryo cardiac myocytes and in heart and muscle tissues of developing chicken embryo (Jakowlew et al., 1991, 1994). All these results suggest that TGF-β family genes regulate muscle development in vivo. In the current study, there was a significant difference between the F_2 birds that were homozygous for the broiler TGF-β3 alleles (TGF-β3-BB) and those homozygous for the Leghorn L alleles (TGF-β3-LL) in BMW, and the TGF-β3-BB birds were heavier. The heterozygote genotype did not differ from the broiler homozygote, suggesting dominance of the broiler TGF-β3 on BMW, as was also found for total BW and ADG. There was no significant difference between F_2 TGF-β3-BB and TGF-β3-LL birds in percentage BMW. Measurements of the founder lines (Deeb and Lamont, 2002) demonstrated that broilers were, as expected, much heavier in BMW and much higher in percentage BMW than Leghorn. Results from the current

TABLE 4. Effects of transforming growth factor-β4 on skeletal and body composition traits (least-square means)

	Genotype[2]		
Traits[1]	BB	BF	FF
BMC (g)	2.051[a]	1.852[b]	1.936[ab]
BMD (g/cm^2)	0.258[a]	0.250[b]	0.253[ab]
TBL (mm)	114.69[a]	111.54[b]	113.63[a]
SHL (cm)	9.117[a]	8.890[b]	9.040[ab]
%BMD (g/cm^2 per 100 g BW)	0.0159[a]	0.0155[a]	0.0148[b]
%TBL (mm/100 g BW)	7.216[a]	6.909[b]	6.739[b]
SW (g)	3.032[a]	2.730[b]	2.740[b]
%SW (g/100 g BW)	0.178[a]	0.164[b]	0.161[b]

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

[1]BMC = bone (tibia) mineral content; BMD = bone (tibia) mineral density; TBL = tibia length; SHL = shank length; BMD = bone (tibia) mineral density expressed as percentage of BW at 8 wk of age; TBL = tibia length expressed as percentage of BW at 8 wk of age; SW = spleen weight; %SW = spleen weight expressed as percentage of BW at 8 wk of age.

[2]B = grandsire (broiler) allele; F = granddam (Fayoumi) allele.

study identified TGF-β3 as a potential candidate gene of a QTL useful for selection to increase BMW of the chicken.

Abdominal Fat. Excessive fat in chickens should be limited in order to enhance production efficiency and product quality. The TGF-β proteins act as autocrine and paracrine regulators of adipocyte precursor cell proliferation and differentiation, and influence the development of fat tissues of the chicken (Burt et al., 1992; Marie et al., 2000). In the current study, the F_2 birds homozygous for the broiler alleles (TGF-β3-BB birds) had higher AFW and percentage AFW than the other two genotypes. Likewise, the founder broilers had higher AFW and percentage AFW than the two inbred parental lines (Deeb and Lamont, 2002). Excessive fat is a typical feature of modern broiler chickens. On average, F_2 birds with TGF-β3-BB genotype grew faster and simultaneously deposited more fat in the body. The results point to the possible identification of TGF-β3 as a candidate gene of a QTL useful for altering growth rate or abdominal fat.

SW. As an organ that contains a very high number of T and B lymphocytes, the spleen plays a crucial role in immune response and disease resistance. Because the mRNA for TGF-β 2, 3, and 4 are expressed in the spleen of embryo, newly hatched chicks, and adults (Jakowlew et al., 1997), they may play a role in development of the spleen in the chicken. The F_2 birds that were homozygous for broiler TGF-β3 and TGF-β4 alleles had heavier SW and higher percentage SW than the birds that were homozygous for Leghorn TGF-β3 alleles and Fayoumi TGF-β4 alleles. Means of the heterozygous birds suggest that the TGF-β3 broiler allele is dominant, contributing to greater total SW. However, for TGF-β4, the Fayoumi allele appears dominant, contributing to lower SW. Observations in founder lines showed that broilers had heavier SW but lower percentage SW compared with the Leghorn line (Deeb and Lamont, 2002). Thus, the results regarding the effects of TGF-β3 and TGF-β4 genotype on SW indicated possible identification of these genes as QTL of SW.

In summary, commercial breeding programs of broiler chickens have become more complex and challenging because many objectives need to be considered to reduce production costs, maintain health, and improve product quality. Several objectives include increased growth rate, increased breast muscle yield, decreased abdominal fat, maintenance of good development and growth of the skeletal system, and increased overall fitness. The relationship of these traits is complex, and some of the traits are very difficult to measure. Therefore, molecular marker-assisted selection is needed. The results from the current study indicate that TGF-β genes may be important in growth rate, tibia and shank development and growth, breast muscle weight and yield, amount of abdominal fat, and spleen weight in chickens growing to market weight. The TGF-β genes are, therefore, potential markers for use in molecular marker-assisted selection programs.

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