Journal of Heredity 2005:96(6):698–703
doi:10.1093/jhered/esi114
Advance Access publication November 2, 2005

High Diversity of the Chicken Growth Hormone Gene and Effects on Growth and Carcass Traits

Q. NIE, B. SUN, D. ZHANG, C. LUO, N. A. ISHAG, M. LEI, G. YANG, AND X. ZHANG

From the Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou 510642, Guangdong, China.

Address correspondence to X. Zhang at the address above, or e-mail: xqzhang@scau.edu.cn.

Abstract

The chicken growth hormone (*cGH*) gene plays a crucial role in controlling growth and metabolism, leading to potential correlations between *cGH* polymorphisms and economic traits. In this study, DNA from four divergent chicken breeds were screened for single nucleotide polymorphisms (SNPs) in the *cGH* gene using denaturing high-performance liquid chromatography and sequencing. A total of 46 SNPs were identified, of which 4 were in the 5′ untranslated region, 1 in the 3′ untranslated region, 5 in exons (two of which are nonsynonymous), with the remaining 36 in introns. The nucleotide diversity in the *cGH* gene ($\theta = 2.7 \times 10^{-3}$) was higher than that reported for other chicken genes, even within the same breeds. The associations of five of these SNPs and their haplotypes with chicken growth and carcass traits were determined using polymerase chain reaction–restriction fragment length polymorphism analysis in a F_2 resource population cross of two of the four chicken breeds (White Recessive Rock and Xinghua). This analysis shows that, among other correlations, G+1705A was significantly associated with body weight at all ages measured, shank length at three of four ages measured, and average daily gain within weeks 0 to 4. Thus, this *cGH* polymorphism, or another polymorphism that is in linkage disequilibrium with G+1705A, appears to correspond to a significant growth-related quantitative trait locus difference between the two breeds used to construct the resource population.

The chicken growth hormone (*cGH*) gene is considered one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism (Byatt et al. 1993; Copras et al. 1993; Vasilatos-Younken et al. 2000). First isolated and sequenced by Lamb et al. (1988), polymorphisms in the *cGH* gene were widely studied by restriction fragment length polymorphisms (RFLPs) or sequencing. The gene encodes a 191–amino acid mature growth hormone protein and a 25–amino acid signal peptide. The *cGH* gene has 4,101 base pairs and consists of five exons and four introns, differing in this regard from its mammalian counterpart (Mou et al. 1995; Tanaka et al. 1992). A 50 bp deletion in intron 4 of the *cGH* gene was found in Chinese native Taihe Silkies chickens (Nie et al. 2002). The *cGH* gene in another native breed, Yellow Wai Chow, was found to have one silent substitution, 31 insertions, and other substitutions spread among the introns (Ip et al. 2001). A novel *Msp*I site in the first intron (Ip et al. 2001) and a *Sac*I and three *Msp*I polymorphic restriction sites were also detected in the *cGH* gene (Fotouhi et al., 1993). A *Sac*I polymorphism in the fourth intron of the gene was reported to be associated with the number of tissues with tumors in Marek's disease virus-infected White Leghorn chickens (Liu et al. 2001). Selection for abdominal fat appears to affect allele frequencies, and some alleles of these RFLPs were associated with juvenile body weight, egg weight, and egg-specific gravity (Feng et al. 1997; Fotouhi et al. 1993; Kuhnlein et al. 1997). Considerable diversity in the *cGH* gene existed between Chinese native breeds and commercial breeds such as Avian Parental, Arbor Acre broilers, and Hy-Line layers (Ip et al. 2001; Nie et al. 2002). Chinese native chickens are genetically diverse (Zhang et al. 2002) and have distinctive characteristics, including differences in feather color, growth rate, meat characteristics, and reproductive performance.

Most of the variations in a gene are single nucleotide polymorphisms (SNPs) arising from substitution, deletion, or insertion of a single nucleotide. A single SNP can greatly affect performance traits. For example, the sex-linked dwarf allele in chickens is a single nucleotide mutation at an exon-intron junction of the GH receptor gene (*GHR*; Huang et al. 1993). Recently, significant progress has been made in associating quantitative trait loci (QTL) with SNPs in domestic animals. Van Laere et al. (2003) showed that a QTL for muscle growth

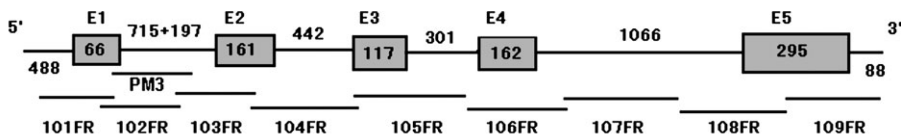

Figure 1. Location of primers 101–109, PM3 in the chicken growth hormone gene.

in pigs was caused by a nucleotide substitution in intron 3 of the insulin-like growth factor 2 gene (*IGF2*). Amills et al. (2003) identified three SNPs in chicken *IGF1* and *IGF2* that were associated with growth and feeding traits. SNPs can be genotyped with many techniques (Vignal et al. 2002). Denaturing high-performance liquid chromatography (DHPLC) is a highly sensitive and automated method based on the capability of ion-pair reverse-phase liquid chromatography to resolve homoduplex from heteroduplex molecules under conditions of partial denaturation, and it has proven to be an efficient method for discovering and genotyping SNPs (Abbas et al. 2004; Han et al. 2004; Nie et al. 2004). In the present study, the *cGH* gene was scanned for SNPs in 40 individuals of four different chicken breeds. Associations of these SNPs and their haplotypes with growth and carcass traits were analyzed in a F_2 resource population derived from a cross of a fast-growing line, White Recessive Rock (WRR), and a slow-growing line, Xinghua (X).

Materials and Methods

Chicken Populations

Leghorn (L), WRR, Taihe Silkies (TS) and X chickens with different growth rates and morphological characteristics were used to screen for SNPs in the *cGH* gene. Genomic DNA of 10 individuals in each breed was extracted from EDTA-anticoagulated blood. Both TS and X are Chinese native breeds with slow growth rates. A F_2 resource population was constructed by crossing the WRR and X breeds to analyze the association between *cGH* SNPs and chicken growth and carcass traits. Nine WRR males were crossed to nine X females, and six WRR females were crossed to six X males, producing 17 F_1 families and 454 F_2 full-sib individuals. F_2 chickens were raised in floor pens and fed with commercial corn- and soybean-based diets that met all National Research Council requirements. Body weight (BW) and shank length (SL) at different ages were recorded, along with hatch weight (HW) and average daily gain from 0 to 4 weeks of age (ADG0-4). All chickens were slaughtered at 90 days of age, and carcass traits were measured—including abdominal fat weight (AFW), small intestine length (SIL), cross-sectional area of leg muscle fiber (LA), and fat content of leg muscle (LFC). LFC was determined by the Soxtec system HT 1043 extraction unit (Tecator, Sweden).

Polymerase Chain Reaction Amplification, SNP Detection by DHPLC, and Sequencing Confirmation

Primers 101–109 of Nie et al. (2005) were used to amplify the full length of the *cGH* gene, and primer PM3, as described by Kuhnlein et al. (1997), was used to detect their reported polymorphisms in the *cGH* gene (Figure 1). Polymerase chain reaction (PCR) reactions and DHPLC analysis were performed and analyzed as described previously (Nie et al. 2005). According to the DHPLC profiles, representative PCR products with different mutations were purified and sequenced by BioAsia Biotechnology (Shanghai, China). For each PCR product, both forward and reverse sequencing were conducted. The sequencing results were analyzed with BLAST implemented in the DNASTAR program (http://www.biologysoft.com).

PCR-RFLP Analysis of F_2 Individuals in the Resource Population

Among the 46 SNPs found in the *cGH* gene, C-121T, G+119A, G+1705A, and G+3037T were in restriction sites for *Pag*I, *Msp*I, *Eco*RV and *Bsh1236*I, respectively. Another SNP, C+385T, reported by Fotouhi et al. (1993) and Kuhnlein et al. (1997) and confirmed in the draft sequence of the chicken genome (http://www.genome.wustl.esu/projects/chicken/; nt 144843 of chromosome 27), was also assayed in this study by *Msp*I digestion. After amplification with primer pairs 101 (for C-121T), 151 (for G+119A and C+385T), 105 (for G+1705A), and 108 (for G+3037T), PCR products were digested at 37°C overnight with *Pag*I, *Msp*I, *Eco*RV, and *Bsh1236*I, respectively. The digestion mixture contained 8 μL PCR products, 1× digestion buffer, and 3.0 units of enzyme. All 454 F_2, 34 F_1 and 30 F_0 individuals in the full-sib resource population were genotyped.

Statistical Analysis

To estimate the nucleotide diversity of the *cGH* gene, the normalized numbers of variant sites (θ) was calculated as the number of observed nucleotide changes (*K*) divided by the total sequence length in base pairs (*L*), corrected for sample size (*n*), as described by Cargill et al. (1999).

$$\theta = K / \sum_{i=1}^{n-1} i^{-1} L$$

Five SNPs—C-121T, G+119A, C+385T, G+1705A, G+3037T—were used to reconstruct haplotypes with PHASE 2.0 software (Stephens et al. 2001). Marker-trait linkage analysis was performed by the SAS GLM procedure (SAS Institute 1996), and the genetic effects were analyzed using the following mixed model:

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

Table 1. Single nucleotide polymorphisms (SNPs) detected by denaturing high-performance liquid chromatography and sequencing in the chicken growth hormone gene

	SNP[a]	Location	Change of amino acid	Restriction fragment length polymorphism enzyme
1	G-360A	5′UTR		
2	T-359G	5′UTR		
3	G-334A	5′UTR		
4	C-121T	5′UTR		*Pag* I
5	G+119A	Intron 1		*Msp* I
6	C+219A	Intron 1		
7	A+262C	Intron 1		
8	C+263A	Intron 1		
9	G+552C	Intron 1		
10	C+621T	Intron 1		
11	A+638C	Intron 1		
12	G+647C	Intron 1		
13	A+766G	Intron 1		
14	T+836C	Intron 1		
15	G+837A	Intron 1		
16	G+951A	Exon 1	A13T	
17	G+1227A	Intron 2		
18	G+1396A	Intron 2		
19	T+1419C	Intron 2		
20	G+1478A	Intron 2		
21	G+1498A	Intron 2		
22	G+1505A	Intron 2		*Ava III*
23	G+1527A	Intron 2		
24	G+1532A	Exon 2	R59H	
25	G+1705A	Intron 3		*EcoR* V
26	C+1715T	Intron 3		
27	A+1811G	Intron 3		
28	G+1819A	Intron 3		
29	G+1823A	Intron 3		
30	C+1993G	Exon 4	Synonymous	
31	C+1996T	Exon 4	Synonymous	
32	A+2118G	Intron 4		
33	C+2187T	Intron 4		
34	C+2264T	Intron 4		
35	G+2362A	Intron 4		
36	T+2551C	Intron 4		*Msp* I
37	T+2656C	Intron 4		*Msp* I
38	G+2725A	Intron 4		
39	A+2938G	Intron 4		
40	G+2978A	Intron 4		
41	G+3037T	Intron 4		*Bsh1236* I
42	C+3045T	Intron 4		
43	T+3098C	Intron 4		
44	A+3172G	Intron 4		
45	G+3313A	Exon 5	Synonymous	
46	T+3382C	3′UTR		

[a] The first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being −1.

where Y is a trait observation, μ is the overall population mean, G is the fixed effect of genotype, D is the random effect of dam, H is the fixed effect of hatch, S is the fixed effect of sex (male or female), and e is the residual random error.

Table 2. Nucleotide diversity of the chicken growth hormone gene and others reported in chicken

Genes	No. of SNPs	Base pairs	Individuals (*n*)	Adjusted θ ($\times 10^{-3}$)	References
BMPR2	14	2,788	4	2.4	Cisar et al. 2003[a]
cGH	46	3,948	40	2.7	Present study
GHR	33	4,007	40	1.9	Nie et al. 2005
Ghrelin	19	2,536	40	1.8	Nie et al. 2004
GHSR	27	3,628	40	1.7	Nie et al. 2005
IGF-I	15	2,578	40	1.4	Nie et al. 2005
IGF-II	4	1,681	40	0.6	Nie et al. 2005
IGFBP-2	35	4,311	40	1.9	Nie et al. 2005
LEPR	9	1,070	40	2.0	Nie et al. 2005
PEPCK-C	19	3,792	64	1.1	Parsanejad et al. 2002
PIT-1	23	2,400	40	2.2	Nie et al. 2005

[a] Three clones were sequenced, and their sequences were compared with the wild-type *BMPR2* mRNA sequence, which gave rise to four individuals in total.

Results

SNPs and Nucleotide Diversity of the *cGH* Gene

PCR amplification of the *cGH* gene surveyed a region of 3,948 bp in four chicken breeds, 10 individuals from each breed. Forty-six SNPs were found (Table 1), or one SNP per 86 bp on average. Most of these SNPs (36 of 46) were located in introns, with four in the 5′UTR, one in the 3′UTR, and five in coding exons. Two of the five coding SNPs led to amino acid changes. All 46 SNPs were nucleotide substitutions, and transitions (38) occurred more frequently than transversions (8). One of two nonsynonymous coding SNPs (G+951A) altered an amino acid in the *cGH* precursor (A13T), and the other (G+1532A) changed an amino acid in the mature *cGH* (R59H).

The adjusted nucleotide (θ) diversity of the total *cGH* gene was 2.7×10^{-3} overall, whereas it was 3.1×10^{-3} within introns. When compared with some other chicken genes—such as *GHR*, *ghrelin*, the growth hormone secretagogue receptor (*GHSR*), *IGF1* and *IGF2*, the insulin-like growth factor binding protein 2 (*IGFBP-2*), the leptin receptor (*LEPR*), the pituitary-specific transcription factor-1 (*PIT-1*), the bone morphogenetic protein receptor type II (*BMPR2*), and the phosphoenolpyruvate carboxykinase-C (*PEPCK-C*) gene—the nucleotide diversity of the *cGH* gene was somewhat higher (Table 2), even within a similar base populations (Nie et al. 2005).

Table 3. The probability of associations (*P* value) of polymorphisms in five single SNPs (single nucleotide polymorphisms) and their haplotypes with growth and carcass traits

Traits[a]	Single SNP					Haplotypes	
	C-121T	G+119A	C+385T	G+1705A	G+3037T	≥ 5%	In total
HW (g)	0.11	0.21	0.40	0.24	0.046*	0.22	0.18
BW14 (g)	0.61	0.20	0.86	0.012*	0.18	0.15	0.026*
BW21 (g)	0.44	0.41	0.82	0.007**	0.31	0.23	0.12
BW28 (g)	0.75	0.49	0.67	0.004**	0.34	0.36	0.48
BW35 (g)	0.34	0.87	0.97	0.044*	0.25	0.59	0.46
BW42 (g)	0.37	0.30	0.95	0.041*	0.14	0.33	0.41
BW49 (g)	0.27	0.31	0.67	0.026*	0.20	0.68	0.51
BW63 (g)	0.85	0.55	0.98	0.030*	0.52	0.84	0.65
BW70 (g)	0.54	0.50	0.47	0.010**	0.09	0.61	0.27
BW77 (g)	0.23	0.15	0.24	0.017*	0.13	0.50	0.26
BW84 (g)	0.39	0.20	0.58	0.004**	0.60	0.34	0.48
SL49 (mm)	0.69	0.54	0.48	0.024*	0.64	0.65	0.57
SL56 (mm)	0.12	0.10	0.31	0.043*	0.08	0.14	0.08
SL70 (mm)	0.015*	0.89	0.21	0.20	0.001**	0.16	0.11
SL84 (mm)	0.016*	0.85	0.17	0.03*	0.003**	0.015*	0.05
ADG0-4	0.74	0.43	0.56	0.006**	0.36	0.43	0.54
AFW (g)	0.80	0.033*	0.86	0.30	0.41	0.86	0.44
SIL (cm)	0.22	0.008**	0.30	0.87	0.46	0.40	0.39
LA (μm^2)	0.020*	0.90	0.06	0.67	0.09	0.004**	0.06
LFC (%)	0.040*	0.24	0.55	0.57	0.20	0.28	0.24

[a] HW = hatch weight; BW14 = body weight at 14 days of age; SL49 = shank length at 49 days of age; ADW0-4 = average daily gain during 0–4 weeks of growth; AFW = abdominal fat weight; SIL = length of small intestine; LA = cross-sectional area of leg muscle fiber; LFC = fat content of leg muscle.
* $P < .05$, ** $P < .01$.

Associations of Single SNP With Chicken Growth and Carcass Traits

Association analysis of *cGH* SNP with chicken growth and carcass traits in a F_2 reciprocal cross between the WRR and X breeds showed that genotypes at C-121T were significantly ($P < .05$) associated with LA and LFC and with SL at the ages of 70 and 84 days. SNP G+119A was significantly associated with AFW and highly significant ($P < .01$) with SIL. No significant associations between C+385T and any growth-related traits were observed. SNP G+1705A was significantly associated with BW at the ages of 14, 35, 42, 49, 63, 77 days and with SL at the ages of 49, 56, and 84 days and highly significant with BW21, 28, 70, 84, and ADG0-4. Finally, SNP C+3037T was significantly associated with HW and highly significant with SL70 and SL84 (Table 3). The effect of the G+1705A SNP genotype on the various BW, SL traits, and ADG0-4 appeared to be additive, although in some cases the heterozygous (AG) trait measure did not significantly differ from one or both homozygotes (Table 4). For all the traits listed, the AA homozygote differed from the GG homozygote at the significant or highly significant level.

Haplotype Reconstruction and Linkage Analysis

When considering these five *cGH* SNPs as a whole, we found 15 haplotypes of H1–H15 and 44 diplotypes in our F_2 reciprocal cross. Of these 15 haplotypes, H1 (C/G/T/G/G) and H2 (C/A/T/G/G) were the most common, at frequencies of 32% and 23%, respectively, whereas H8 (C/G/T/G/T), H9 (T/G/T/G/G), H10 (T/G/T/G/T), H11 (C/A/T/A/T), and H12 (C/G/T/A/G) were minor haplotypes, with frequencies of less than 5%; H13 (C/G/C/G/T), H14 (T/A/T/G/G), and H15 (C/G/C/A/G) were rare (1%). Linkage analysis showed that diplotypes based on all haplotypes were significantly associated with only BW14 ($P < .05$). However,

Table 4. Differences in growth and body composition traits between chickens with different genotypes of G+1705A

Traits[a]	Genotypes[b]		
	AA (18)	AG (85)	GG (348)
BW14 (g)*	131 ± 3.6	129 ± 2.1	122 ± 1.1
BW21 (g)**	225 ± 6.9	220 ± 4.0	206 ± 2.1
BW28 (g)**	339 ± 11	323 ± 6.4	304 ± 3.3
BW35 (g)*	471 ± 17	449 ± 10	428 ± 5.1
BW42 (g)*	620 ± 22	585 ± 13	561 ± 6.8
BW49 (g)*	770 ± 26	722 ± 15	694 ± 8.0
BW63 (g)*	1,130 ± 46	1,050 ± 29	997 ± 15
BW70 (g)**	1,270 ± 52	1,150 ± 28	1,100 ± 14
BW77 (g)*	1,470 ± 58	1,360 ± 32	1,290 ± 16
BW84 (g)**	1,670 ± 75	1,550 ± 40	1,430 ± 20
SL49 (mm)*	71.2 ± 1.4	68.5 ± 0.94	67.0 ± 0.47
SL56 (mm)*	73.9 ± 1.1	73.3 ± 0.66	71.5 ± 0.34
SL84 (mm)*	91.4 ± 1.7	90.2 ± 0.91	87.5 ± 0.45
ADG0-4 (g)**	11.0 ± 0.38	10.5 ± 0.23	9.78 ± 0.12

[a] BW14 = body weight at 14 days of age; SL49 = shank length at 49 days of age; ADG0-4 = average daily gain during weeks 0–4.
[b] Numbers in brackets show the numbers of tested individuals of each genotype.
* $P < .05$, ** $P < .01$.

significant associations of diplotypes with SL84 ($P < .05$) and LA ($P < .01$) were observed when considering the major haplotypes with frequencies more than 5% (Table 3).

Discussion

The diversity of the *cGH* gene detected in this study was substantial. In the past, only a few *cGH* SNPs (i.e., G+119A, C+385T, T+2551C, and T+2556C) had been found (Fotouhi et al. 1993; Kuhnlein et al. 1997; Liu et al. 2001; Nie et al. 2002), and no variation had been reported in its 5′-regulatory region (Zhang et al. 1998). In this study, 46 point mutations were identified across the whole *cGH* gene within four divergent chicken breeds (Table 1). The nucleotide diversity of the *cGH* gene (corrected $\theta = 2.7 \times 10^{-3}$) was higher than those of several other chicken genes (Table 2), even when those genes were sampled in the same four breeds (Nie et al. 2005). Furthermore, after correcting for sample size, the θ value of the *cGH* gene was higher than those reported in chickens (Schmid et al. 2000; Vignal et al. 2000), humans (Cargill et al. 1999), pigs (Jungerius et al. 2003), and cattle (Heaton et al. 2001). The high diversity of the *cGH* gene appears to be confirmed in a preliminary genome-wide scan for chicken SNPs that reported 2.8 million SNPs across the whole chicken genome, with 23 SNPs located in the region (chr27: 141644–145748) of the *cGH* gene (International Chicken Polymorphism Map Consortium 2004).

It was interesting that G+1705A was significantly associated with almost all growth traits (Table 3), with the A allele exhibiting a generally positive effect on chicken growth (Table 4). This was consistent with the higher A frequencies in F_0 chickens of WRR than in those of X, even though G was still the dominant allele in both breeds and, therefore, fewer F_2 individuals with AA genotype (18) were generated. Previous studies on other polymorphisms in introns of the *cGH* gene indicated associations with chicken growth, fat deposition, and egg production (Feng et al. 1997; Fotouhi et al. 1993; Kuhnlein et al. 1997). A recent study showed that a single mutation in intron 3 of the *IGF2* gene encoded a major QTL affecting pig muscle growth (Van Laere et al. 2003). As in this case, the G+1705A in intron 3 of *cGH* could have a direct effect on chicken growth via an influence on *cGH* gene expression. On the other hand, the G+1705A *cGH* polymorphism may be in linkage disequilibrium with some other causative polymorphism that influences growth traits in our resource population. The other four *cGH* SNPs that we tested may fail to exhibit this level of disequilibrium with the growth trait QTL due to their different respective histories within the two parental breeds employed. Further analysis will be required to differentiate between these possibilities.

Acknowledgments

This work was funded by project under the Major State Basic Research Development Program, China, project G2000016102. Dr. Y. Da in the University of Minnesota, Minnisapolis, gave helpful suggestions on resource population construction.

References

Abbas A, Lepelley M, Lechevrel M, and Sichel F, 2004. Assessment of DHPLC usefulness in the genotyping of GSTP1 exon 5 SNP: comparison to the PCR-RFLP method. J Biochem Biophys Methods 59:121–126.

Amills M, Jimenez N, Villalba D, Tor M, Molina E, Cubilo D, Marcos C, Francesch A, Sanchez A, and Estany J, 2003. Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. Poult Sci 82:1485–1493.

Byatt JC, Staten NR, Salsgiver WJ, Kostele JC, and Collier RJ, 1993. Stimilation of food intaker and weight gain in mature female rats by bovine prolactin and bovine growth hormone. Am J Physiol 264:986–992.

Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, and Lander ES, 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 22:231–238.

Cisar CR, Balog JM, Anthony NB, and Donoghue AM, 2003. Sequence analysis of bone morphogenetic protein receptor type II mRNA from ascitic and nonascitic commercial broilers. Poult Sci 82:1494–1499.

Copras E, Harman SM, and Blackman MR, 1993. Human growth hormone and human aging. Endocr Rev 14:20–39.

Feng XP, Kuhnlein U, Aggrey SE, Gavora JS, and Zadworny D, 1997. Trait association of genetic markers in the growth hormone and growth hormone receptor gene in a white Leghorn strain. Poult Sci 76:1770–1775.

Fotouhi N, Karatzas CN, Kuhlein U, and Zadworny D, 1993. Identification of growth hormone DNA polymorphisms which response to divergent selection for abdominal in chickens. Theor Appl Genet 85:931–936.

Han W, Yip SP, Wang J, and Yap MK, 2004. Using denaturing HPLC for SNP discovery and genotyping, and establishing the linkage disequilibrium pattern for the all-trans-retinol dehydrogenase (RDH8) gene. J Hum Genet 49:16–23.

Heaton MP, Grosse WM, Kappes SM, Keele JW, Chitko-McKown CG, Cundiff LV, Braun A, Little DP, and Laegreid WW, 2001. Estimation of DNA sequence diversity in bovine cytokine genes. Mamm Genome 12:32–37.

Huang N, Cogburn LA, Agarwal SK, Marks HL, and Burnside J, 1993. Overexpression of a truncated growth hormone receptor in the sex-linked dwarf chicken: evidence for a splice mutation. Mol Endocrinol 7:1391–1398.

International Chicken Polymorphism Map Consortium, 2004. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature 432:717–722.

Ip SC, Zhang X, and Leung FC, 2001. Genomic growth hormone gene polymorphism in native Chinese chicken. Exp Biol Med 226:458–462.

Jungerius BJ, Rattink AP, Crooijmans RP, van der Poel JJ, van Oost BA, te Pas MF, and Groenen MA, 2003. Development of a single nucleotide polymorphism map of porcine chromosome 2. Anim Genet 34:429–437.

Kuhnlein U, Ni L, Weigend S, Gavora JS, Fairfull W, and Zadworny D, 1997. DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Anim Genet 28:116–123.

Lamb LC, Galehouse DM, and Foster DN, 1988. Chicken growth hormone cDNA sequence. Nucleic Acids Res 16:9339.

Liu HC, Kung HJ, Fulton JE, Morgan RW, and Cheng HH, 2001. Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. Proc Natl Acad Sci USA 98: 9203–9208.

Mou L, Liu N, Zadworny D, Chalifour L, and Kuhnlein U, 1995. Presence of an additional *Pst*I fragment in intron 1 of the chicken growth hormone-encoding gene. Gene 160:313–314.

Nie Q, Ip SCY, Zhang X, Leung FC, and Yang G, 2002. New variations in intron 4 of growth hormone gene in Chinese native chickens. J Hered 93:277–279.

Nie Q, Lei M, Ouyang J, Zeng H, Yang G, and Zhang X, 2005. Identification and characterization of single nucleotide polymorphisms in 12 chicken growth-correlated genes by denaturing high performance liquid chromatography. Genet Sel Evol 37:339–360.

Nie Q, Zeng H, Lei M, Ishag NA, Fang M, Sun B, Yang G, and Zhang X, 2004. Genomic organization of the chicken ghrelin gene and its single nucleotide polymorphisms detected by denaturing high-performance liquid chromatography. Br Poult Sci 45:611–618.

Parsanejad R, Zadworny D, and Kuhnlein U, 2002. Genetic variability of the cytosolic phosphoenolpyruvate carboxykinase gene in white leghorn chickens. Poult Sci 81:1668–1670.

SAS Institute, 1996. SAS user's guide. Statistics. Version 6.12. Cary, NC: SAS Institute.

Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn M, Schartl M, Haaf T, Weigend S, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke T, Groenen MA, Crooijmans RP, Vignal A, Fillon V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Maki-Tanila A, Cheng HH, Delany ME, Burside J, and Mizuno S, 2000. First report on chicken genes and chromosomes 2000. Cytogenet Cell Genet 90:169–218.

Stephens M, Smith NJ, and Donnelly P, 2001. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989.

Tanaka M, Hosokawa Y, Watahiki M, and Nakashima K, 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. Gene 112:235–239.

Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M, Andersson G, Georges M, and Andersson L, 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425:832–836.

Vasilatos-Younken R, Zhou Y, Wang X, McMurtry JP, Rosebrough W, Decuypere E, Buys N, Darras VM, Geyten V, and Tomas F, 2000. Altered chicken thyroid hormone metabolism with chronic GH enhancement in vivo: consequences for skeletal muscle growth. J Endocrinol 166:609–620.

Vignal A, Milan D, SanCristobal M, and Eggen A, 2002. A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol 34:275–305.

Vignal A, Monbrun C, Thomson P, Barre-Dirie A, Wimmers K, and Weigend S, 2000. Estimation of SNP frequencies in European chicken populations. In: Proceedings of the 27th International Conference on Animal Genetics. July 22–26, Minneapolis. International Society of Animal Genetics; 71.

Zhang X, Leung FC, Chan DK, Yang G, and Wu C, 2002. Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism. Poult Sci 81:1463–72.

Zhang Y, Li N, and Zhang Y, 1998. Molecular cloning and analysis of the partial 5′ flanking regulatory region of chicken growth hormone gene. Acta Genetica Sinica 25:427–432.

Received May 17, 2005
Accepted September 21, 2005

Corresponding Editor: Jerry Dodgson