New Variations in Intron 4 of Growth Hormone Gene in Chinese Native Chickens


Polymorphism in intron 4 of chicken growth hormone (cGH) gene was studied in 20 Chinese native chicken populations and broiler or layer populations. A total of eight restriction digestion profiles were identified in intron 4 and confirmed by sequencing. Among 20 populations, there were distinctively different allele numbers and frequencies of intron 4 restriction fragment length polymorphisms (RFLPs) between Chinese native chickens and broilers or layers. Two new alleles, allele D and allele E, were identified in Taihe Silkees. Allele D was also identified in other Chinese native breeds and a 50 bp fragment deletion was identified in allele E.

Growth hormone (GH), a polypeptide hormone synthesized in and secreted by the pituitary gland, affects a wide variety of physiological parameters such as growth, egg production, body composition, appetite control, aging, and reproduction (Byatt et al. 1993; Copras et al. 1993; Vasilatos-Youken et al. 1997). GH gene polymorphisms have been studied in various animals. Recent studies on dairy cattle have shown that an MspI polymorphism at intron 3 of the bovine growth hormone (bGH) gene is linked to milk protein content (Lagziel et al. 1999). Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) studies on artificially inseminated (AI) bulls have also revealed an association between GH polymorphism and AI bull reproductive performance (Lechniak et al. 1999). Genomic DNA samples were collected from four types of chicken populations: Chinese native chickens, hybrids from Chinese native breeds and imported broilers, and layers (Table 1). ShekKai Native, YWC strain, Huiyang Bearded, Xinghua, Qingyuan, Taihe Silkees, Gushiu, Beijing Fatty, and Wenchung belong to Chinese native chickens. Taihe Silkees is a Silkees breed, Huiyang Bearded is characterized by a beard, and Beijing Fatty is characterized by a feathered shank and feathered comb. The YWC strain has been inbred from Huiyang Bearded over 20 years. All ShekKai Pure, ShekKai, ShekKai B, ShekKai AFD, Beijing ShekKai, ShekKai Hybrid, Partridge Line, and Yellow Line are hybrids of Chinese native chickens and broilers. The Kabir line and Avian Parental are broilers. The Hy-Line is a pure line from Leghorn.

Intron 4 was amplified by PCR and the two primers (Kuhnlein et al. 1997) were 5'-CTG GCT CTT CAA GAA TGA GGG-3' (PMPS1 forward) and 5'-AAC TTG TCG CTA AAG GAC CTG GAA GAA GGG-3' (PMPS1 reverse). The PCR products were then digested by restriction enzyme MspI and the digested DNA was analyzed on a 1.5% agarose gel. The DNA fragments were stained with ethidium bromide and photographed using an ultraviolet (UV) transilluminator to visualize the bands. Allele frequencies of all populations are presented in Table 1.

Polymorphism in the chicken growth hormone (cGH) gene has been reported. RFLPs have been identified at three MspI sites (PM1, PM2, and PM3) and one SacI site (PS1) in the intron region. It was suggested that these alleles in White Leghorn were associated with egg production traits, resistance to Marek’s disease, and avian leukosis (ALV) (Kuhnlein et al. 1997). The resistance-associated GH alleles were also dominant for the onset of ovulation and recessive for the persistence of egg production. However, no significant effect of the GH genotype was observed on juvenile body weight, egg weight, or egg specific gravity (Kuhnlein et al. 1997). Recently a novel MspI site in the first intron of the cGH gene was reported (Ip et al. 2001).

Chinese native chickens are genetically diverse. Unlike commercial broilers and layers, Chinese native chickens have distinguishing characteristics: colored appearance, slow growth rate, flavorful meat, and low reproductive performance (due to broodiness). Thus in the present study, polymorphism in intron 4 of the cGH gene will be studied and the relationship between RFLPs and characteristics of Chinese native chickens will be investigated. These studies will contribute to the understanding of the genetic diversity of Chinese native chicken breeds.

Table 1. Allele frequencies of different populations in intron 4 polymorphism

<table>
<thead>
<tr>
<th>Breed</th>
<th>Allele</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Breed</th>
<th>Allele</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<td>0.065</td>
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<td>0</td>
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<td>0.784</td>
<td>0.151</td>
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RFLP in each population were then calculated.

A PCR product of expected size, 1170 bp, was observed using primer PMPS1. A total of eight restriction digestion profiles were identified in intron 4 (Figure 1a) and confirmed by sequencing, and three polymorphic MspI sites were identified (Figure 1b). The eight profiles were determined by alleles A, B, C, D, and E. Allele A had no MspI site. Allele B had one MspI site only, producing two fragments of 578 and 586 bp, which appeared as one band. Allele C had one MspI site only, in which fragments of 682 and 482 bp were generated. Allele D had MspI sites a and b. In addition to MspI site c, a 50 bp fragment deletion was identified in allele E. Profile 1 was a homozygote determined by allele A, profile 2 was a homozygote determined by allele B, and profile 3 was a homozygote determined by allele C. Profiles 4–6 were heterozygotes consisting of alleles A, B, and C. Deletion of the 50 bp fragment, together with the formation of the MspI cut site c, at 560 nt, generated a new restriction fragment of size 460 bp. Profile 7 was formed by alleles A and E (Figure 1b). PCR using primer PMPS1 generated two PCR fragments of 50 bp for the heterozygote AE, as shown in Figure 1c. Profile 8 was formed by alleles A and D. The sequence of the band with the smaller size was analyzed with an ABI 377 sequence analyzer, which located the 50 bp deletion at 421 to 470 nt (Figure 1b).

Allele frequencies of 20 populations of Chinese native chickens and broiler or layer populations in intron 4 polymorphism are shown in Table 1. Among these populations there were distinctively different numbers and frequencies of intron 4 RFLPs between Chinese native chickens and broilers or layers. A new allele, allele D, was identified at a high frequency in Taihe Silkies and Beijing Fatty, which originated from east China and north China, respectively.

Similar to GH genes identified in other mammals, the cGH gene consists of five exons and four introns. It has been reported that the size of the cGH gene is about 3.5 kb (Ip et al. 2001; Mou et al. 1995; Tanaka et al. 1992). RFLPs have been characterized in the introns of cGH gene of White Leghorn and it has been suggested that the alleles identified were linked to egg production traits, resistance to Marek's disease, and avian leukosis (ALV) (Kuhnlein et al. 1997). PCR-RFLPs were also studied in various populations of Chinese native chickens and it was suggested that an allele present in intron 1 might be linked to laying performance (Ip et al. 2001). Growth hormone gene polymorphisms have also been observed in other animals; for example, a polymorphism observed in intron 3 of bovine growth hormone (bGH) gene was found to be linked to milk protein content (Lagziel et al. 1999). In fact, regulatory elements have been identified in the intron region of the...
GH gene in various animals. A glucocorticoid regulatory element (GRE), which may be responsible for the transcriptional control of the human GH (hGH) gene, has been located in the first intron of the hGH gene (Moore et al. 1985; Slater et al. 1985). A pituitary-specific transcription factor, GFI1, which was suggested to be involved in the tissue-specific expression of the GH gene has also been identified in intron 3 of the rainbow trout GH gene (Bernardini et al. 1999). These investigations suggest that introns in the GH gene might play a crucial role in the regulation of GH gene expression.

Previous studies comparing the genomic cGH sequence in White Leghorn to the Chinese Yellow Wai Chow strain demonstrated a total of 32 substitutions and additions (Ip et al. 2001). Although most substitutions were identified in introns 1 and 4, a silent substitution was observed in exon 2. Compared to results of intron 1 polymorphism, the results on intron 4 polymorphism also demonstrated that Chinese native chickens display a much wider variety of alleles. Previous studies on allele frequencies of intron I polymorphism at MspI sites showed that there were significant differences between Chinese chickens and layer chickens (Ip et al. 2001). Although no difference has been observed in the allele frequencies at MspI sites among Chinese native chickens, broilers, and layers, two more alleles were identified in Chinese native chickens and its hybrids, but not in broilers and layers. Of these alleles, allele D was found in Taihe Silkies and Beijing Fatty, and other native breeds only. Taihe Silkies, a silkie breed, has silkie feathers and black skin, while Beijing Fatty has a feathered shank and comb (Qiu et al. 1988). Of interest is that both are slow growing. However, whether allele D is associated with slow growth rate or silkies feathers requires further study.

Allele E, which was characterized by a deletion of 50 bp from position 421 to 470, was identified in the Taihe Silkies population only. Deletion of the 50 bp fragment not only reduces the size of the PCR fragment (Figure 1b), but also, in addition to the introduction of an MspI site at 510 nt, forms a new fragment of 460 bp. Previous studies based on Northern and Southern blotting and PCR analysis revealed RFLP patterns of a 1.7 kb deletion of the intracellular domain of the GH receptor gene in dwarf broiler chickens (Agarwal et al. 1994). Deletion in the GH receptor gene also leads to decreasing muscle cell proliferation in chickens (Goddard et al. 1996). Recent studies on the genomic structure of the gilthead seabream GH gene also identified a length polymorphism in the first intron, which is due to differences in the number of repeat monomers (Almuly et al. 2000). As this 50 bp deletion was observed only in the Taihe Silkies population, whether the 50 bp deletion allele was associated with other phenotypes remains to be clarified in the future.

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References


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Spermatogonial and metaphase I chromosomes of the lumbricid earthworm Octodrilus complanatus (Annelida: Oligochaeta) were examined using fluorescent in situ hybridization (FISH) with three repetitive DNA probes—5S rDNA, 18S–26S rDNA, and (TTAGGG). Single-color FISH consistently mapped one chromosome pair per spread using either 5S rDNA or 18S–26S rDNA as probes. Simultaneous (18S–26S)-5S and (18S–26S)-(TTAGGG)-FISH demonstrated that repeated units of the two ribosomal families were overlapped and closely associated with telomeric sequences.

The genes coding for rRNA in eukaryotes are arranged into two different rDNA multicyclic families, that is, major (18S, 5.8S, and 28S rDNA) and minor (5S rDNA) ribosomal genes. The two rDNAs, detectable as chromosomal hybridized labels, were found to be located at different chro-