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Characterization of *Akirin 2* gene in Langshan chicken

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Akirin, with 180–204 amino acid residues and a predicted molecular weight about 20–25 kDa (Macqueen *et al.* 2010), is a nuclear factor required for innate immune responses (Goto *et al.* 2008). It also plays an important role in skeletal myogenesis (Davies 2006, Kelly 2006, Marshall *et al.* 2008, Salerno *et al.* 2009, Macqueen *et al.* 2010, Macqueen *et al.* 2010) and is negatively regulated by myostatin in skeletal muscle (Marshall *et al.* 2008). The *Akirin* gene was first isolated from *Drosophila melanogaster* (Goto *et al.* 2008). *Akirin 1* and *Akirin 2* are found in most vertebrates (Macqueen and Johnston 2009). In chicken genome the *Akirin 1* gene is absent and it only has *Akirin 2* gene (Macqueen and Johnston 2009). Chicken's *Akirin 2* gene, including six exons and five introns, is mapped to the long arm of the 3rd chromosome. The single-nucleotide polymorphism (SNP) c.*188G>A in the 3' untranslated region of the *Akirin 2* gene is not only significantly associated with longissimus muscle area and marbling score in Korean native cattle (Kim *et al.* 2013), but is also associated with marbling in Japanese black beef (Sasaki *et al.* 2009). No report is available, however, on the *Akirin 2* gene sequence variations in chicken. Thus, we detected the coding sequences of the *Akirin 2* gene in the well-known Chinese indigenous chicken (Langshan chicken) population to investigate the possibility of using this gene for marker-assisted selection.

Blood samples were obtained from 300 female Langshan chickens. They had access to feed (commercial corn-soybean diets meeting the National Research Council's [NRC] requirements) and water *ad lib*. These Langshan chickens were reared on the same farm from Nantong, a medium-sized city in the Jiangsu province of China. Two milliliters of blood were collected aseptically in a tube containing anticoagulant ACD (citric acid: sodium citrate: dextrose-10: 27: 38) from the winged vein. All samples were brought back to the laboratory in an ice box. The genomic DNA was extracted from whole blood according to Mullenbach *et al.* (1989). The DNA samples were dissolved in TE buffer made from 10 mM Tris-Cl (pH 7.5) and 1 mM

Table 1. Characteristics of primers for the *Akirin* 2 gene

Gene	Primer sets	Primer sequences (from 52 to 32)	Annealing temperature (°)	Product length (bp)	Notes (locations)
Akirin 2	Ak1	F: CGTCTGCCCGTCCCCTCT R: CCTTTTCAAAGTGGCGCAC	55.8	85	exon 1
	Ak2	F: CTGTCCTCCTGTGACCTT R: CATACTGGAGCACAATAAGAAG	50.6	249	partial intron 1, exon 2 and partial intron 2
	Ak3	F: GTCGTCTATGTTGGATGTTC R: AGAATGCTGTGGCTACCT	50.1	255	partial intron 2, exon 3 and partial intron 3
	Ak4	F: GGCTCTTCTCCAAATAGT R: AGTTTTATCTGCCCTGTC	59	419	partial intron 3, exon 4 and partial intron 4
	Ak5	F: CTCACACTTGTAATGCTTCA R: CTCACACTTGTAATGCTTCA	49	152	partial intron 4, exon 5 and partial intron 5
	Ak6	F: ACTTGACATTGACTGTATGG R: TTGAAGTAACCTGTGTTGAC	48.8	326	partial intron 5, coding sequence 6 and partial exon 6

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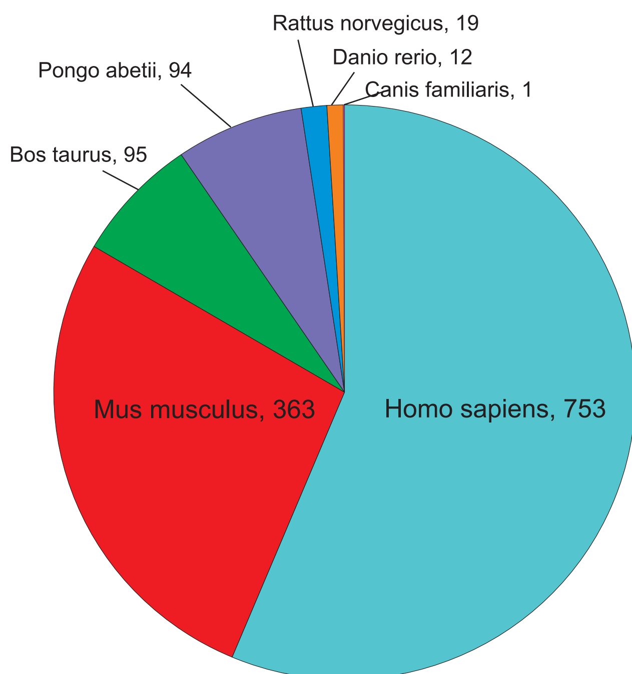

Fig. 1. The organism proportion of the *Akirin 2* gene SNP searching results in NCBI

EDTA (pH 8.0), and subsequently stored at –20°C.

Based on the Gallus gallus *Akirin* 2 gene sequence (GenBank accession number: NC_006090.3), the following 6 pairs of polymerase chain reaction (PCR) primers (Table 1) were designed by the Primer Premier 5 software to amplify the coding sequences. The 25uL reaction mixture contained 50ng genomic DNA, 1uM of each primer, 1.5mM MgCl2, 200uM dNTPs (dATP, dTTP, dCTP and dGTP), and 0.6 units of Taq DNA polymerase. The cycling protocol was 5 min at 94°, 35 cycles of denaturing at 94° for 45 s, annealing at X° (Table 1) for 1 min, extension at 72° for 45 s, with a final extension at 72° for 10 min.

Single-strand conformation polymorphism (SSCP) method was used to scan mutations within the amplified regions. Aliquots of 5 uL PCR products were mixed with 5 uL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene–cyanole and 0.025% bromophenol blue), heated for 10 min at 98° and chilled in ice immediately. Denatured DNA was subjected to 10% PAGE (polyacrylamide gel electrophoresis) in TBE buffer and constant voltage (130 V) for 15 h at a constant temperature of 4°, then gels were stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde). Then the PCR products were sequenced in both directions by a commercial laboratory in an ABI 377 DNA sequencer and the sequences were analyzed with DNAMAN software (version 5.2.2).

Six polymerase chain reaction products from the *Akirin 2* gene were amplified specifically. The SSCP analysis showed that there were no polymorphisms in the six amplified regions of the chicken's *Akirin 2* gene. The mixture samples of the DNA amplification fragments were sent to be sequenced, the result of which was consistent to the SSCP analysis. After searching the SNPs of the *Akirin 2* gene in NCBI, a total of 1337 SNPs in the different kinds of organisms were found (Figure 1). However, there was no SNP record about chicken. *Akirin* genes have been found to play an important role not only in innate immune responses but also in skeletal myogenesis (Chen *et al.* 2013). Thus, the loss of the *Akirin 1* gene in the evolution process may have made the chicken's *Akirin 2* gene more important and conserved. This may be the reason for the chicken's *Akirin 2* gene's lack of mutations. There is a need to study this gene in diverse populations to find SNPs which may then be associated with the meat traits.

SUMMARY

Akirin play an important role not only in innate immune response but also in skeletal myogenesis. The chicken's *Akirin* gene family only has *Akirin 2*. We detected the coding sequences of the *Akirin 2* gene in a Chinese indigenous chicken (Langshan chicken) population to investigate the possibility of using this gene for chicken marker-assisted selection. The results of PCR-SSCP and DNA sequence showed that there were no polymorphisms in the six amplified regions of the *Akirin 2* gene. In addition, there was no SNP record about the chicken's *Akirin 2* gene by searching in NCBI. Thus, the *Akirin 2* gene may not be suitable to be used in chicken as a gene marker for marker-assisted selection.

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