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Identification of SNPs in Highly Variable Lysozyme Gene in Korean Native Chicken Populations

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한국 재래닭의 고변이 Lysozyme 유전자의 SNP 확인

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ABSTRACT

Single nucleotide polymorphisms (SNPs) in chicken lysozyme (LYZ) gene were investigated in this study. The identification of SNPs in both exon and intron in LYZ gene has led to understanding of evolution for the domestic chicken populations. A total of 24 samples from two Korean native commercial chicken populations (CCPs) were used for the initial identification of SNPs by mixing three DNA samples for sequencing experiments. By comparing with red jungle fowl (RJF), two commercial chicken populations have 18 common polymorphisms. Between two commercial chicken populations, 15 polymorphisms were identified. Of the 33 polymorphisms identified, two indels (21 and 4 bp) were found. Whereas, only one polymorphism in exon 2 at the bp position 1426 was a non-synonymous substitution (p.Ala49Val), indicating the amino acid changes. The identified non-synonymous substitution (p.Ala49Val) is located close to the catalytic sites of the enzyme, which might affect its activity. In our investigation, the polymorphisms in LYZ gene can provide broad ideas for the variation of Korean native chicken populations from the ancestor of chicken breeds as well as the some biological functions of the LYZ gene.

Key words: Lysozyme gene, SNPs, Indel, Korean native chicken

Ⅰ. Introduction

Lysozymes are extensive enzymes play a role in the body defense against infection occurring in many tissues and secretions with hydrolyses peptidoglycan and chitodextrin (Bachali et al., 2002; Holler et al., 1975a, b). The chicken lysozyme gene, one of the major egg white protein genes, is expressed in the oviduct and is also expressed in the myeloid lineage of the hematopoietic system. Expression is gradually switched on during macrophage differentiation (Theisen et al., 1986; Sippel et al., 1988). The transcription of lysozyme gene is regulated by a complex set of cis-regulatory DNA elements consisting of several tissue specific enhancers, a silencer and promoter elements (Sippel et al., 1988; Grewal et al., 1992; Bonifer et al., 1991; Theisen et al., 1986). Widespread studies on lysozyme have been committed to their structure, catalytic mechanism, relationship between structure and activity, phylogeny, immunology, and genetics (Jolles, 1996).

In Korea, two commercial Korean native chicken populations (CCP1 and CCP2) are recently developed. Basically, CCP1 has been developed at the National Institute of Animal Science (NIAS) and CCP2 is from a commercial company in Korea. Scientists believe that chicken has been domesticated from a single ancestor, mainly contributed by red jungle fowl (*Gallus gallus*), which originated in Southeast Asia (Akishinonomiya et al., 1994, 1996). The mtDNA sequences have successfully used to determine genetic diversity in Asian chicken (Niu et al., 2002; Liu et al., 2004) and African chicken (Mobegi et al., 2005). In recent development of mtDNA sequence tag or bar-code can give the guideline for selection of animals for the breeding purpose (Hebert et al., 2003). Recently, conservation of farm animal genetic resources has been focused of maintaining minimum number of animals for each breeds/species and there is some progress for this (http://www.fao.org/dad-is/). The MHC molecule play important roles in the regulation of

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the immune response by communicating among different cellular components of the immune system: T cells, B cells, and antigen-presenting cells (Lamont, 1998). The identification of specific alleles and this genotypes can give valuable information for the relationships with disease resistance and establishment of breeding strategies for the Korean native chicken, as well as to establish guidelines for breed discrimination markers. Certain immune gene that determine susceptibility to infection have been shown to be subject to selective forces in the chicken, such as Mx (Li et al., 2006; Hou et al., 2007; Berlin et al., 2008), MHC-B (Worley et al., 2008), IL1B (Downing et al., 2009b) and IL-4Ra (Downing et al., 2009c). The signature of high allelic diversity is evocative of the previous work on variation at chicken mtDNA (Liu et al., 2006), MHC-B (Worley et al., 2008; O'Neill et al., 2009), Mx (Berlin et al., 2008), IL1B (Downing et al., 2009b) and IL-4Ra (Downing et al., 2009c), suggesting that this may be the result of the complex population history of the chicken during domestication. Although the main source of chicken genetic variation is the RJF (Fumihito et al., 1994; International Chicken Genome Sequencing Consortium, 2004), multiple chicken domestications (Fumihito et al., 1996; Liu et al., 2006) and genetic introgressions of other JF into chicken populations (Nishibori et al., 2005; Eriksson et al., 2008; Silva et al., 2008) suggest that diverse alleles may have been introduced during domestication.

In this study, SNPs in lysozyme gene were investigated for the initial step of developing breed identification markers and deducing the biological roles.

II. Materials and Methods

1. Samples and genomic DNA extraction

A total of 24 individuals of two Korean native commercial chicken populations were considered for this experiment. Fresh liver and blood samples were collected from the chicken for the extraction of genomic DNA. Genomic DNA was extracted using PrimePrepTM Genomic DNA Isolation Kit (GeNet Bio, Korea) according to the manufacturer's instruction.

2. Primer design and PCR amplification

Primers were designed from red jungle fowl lysozyme gene sequence data (NCBI, Gene ID: 396218). A total of 3,688 bp linear DNA sequences were used for designing six primer sets. One of the primers did not amplify well and exclude the analysis. The rest five primer sets were successfully amplified with expected product sizes (Table 1). The PCR reaction mixture included approximately 100 ng of genomic DNA, 2.5 μL 10X PCR Gold Buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 1.5 μL 25 mM $MgCl_2$, 2 μL of each dNTPs (2.5 mM), 1 μL of each primer (10 pM) and 1 U *Taq* polymerase (AmpliTaq Gold^{TM}, Applied Biosystems, USA) in a 20 μL reaction volume. The PCR reaction was performed in a GeneAmp 2700 thermocycler (Applied Biosystems, USA) with an initial denaturation step at 94°C for 10 min followed by 35 cycles of 30 sec at 94°C, 30 sec at a specific annealing temperature at 60°C for each primer set, 30 sec at 72°C and a final step of extension at 72°C for 10 min. All the PCR products were run on 1.5% agarose gels stained with ethidium bromide and DNA bands were visualized under UV light (Fig. 1).

Table 1. Primer sequences used for SNP identification.

Primer No.	Sequence (5 - 3)	PCR product size (bp)
1	F: GAGGGCGTTTTTGACAACTG R: GCTTTAAAGTGGCCCCTCAC	770
2	F: CCAGAAAGAAAGTGGGCTGA R: GATGCGCTCTCCATCTCTTC	718
3	F: CGTTGCACGTGTTTTTCACT R: GTTCACCTCTCCTCCCCTTC	714
4	F: GAACCGTGTTCCCCTGTCTA R: ATCTGCAGCCAAGCTGTTTT	763
5	F: CTGTAACCGCAGGCTTTCTC R: CACCATGGGCTTCCAGATAC	771

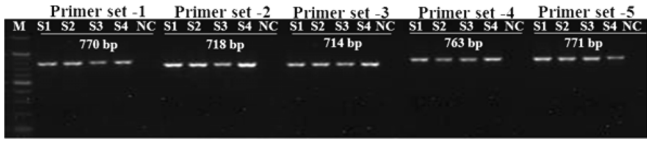

Fig. 1. Amplified PCR product size of primer sets for LYZ gene in Korean native commercial chicken populations. NC means negative control and M is the 100 bp molecular size marker (ELPIS, Korea).

3. Sequence analysis

SNPs were detected from the sequence data using Chromas program and reference sequences were extracted from the

NCBI database (http://www.ncbi.nlm.nih.gov). ClustalW program (http://www.ebi.ac.uk) was performed by alignment of multiple sequences to detected mismatch from other nucleotides. Exon-intron boundaries were identified by comparison of amino acids sequences from the genomic sequences and verified with the Genscan program (http://genes.mit.edu/GENSCAN.html). Conservation of amino acid sequences were shading of the aligned sequences achieved using the GeneDoc program version 2.7 (http://www.pse.edu/biomed/genedoc/).

III. Results and Discussion

1. Identification of polymorphisms

The lysozyme gene has 3688 bp linear DNA with four exons and three introns and this gene is highly polymorphic in nucleotide sequences. Previous study reported that 59 SNPs were identified among domestic chicken sequences in this gene and 3 SNPs are located in the coding regions (Downing et al., 2009a; Hou et al., 2010). In Korean native commercial chicken population the current investigation of, 33 SNPs were identified in the LYZ gene. Among them, 32 SNPs were located in intron and only one SNP was identified from the exonic sequences (Table 2). In our results, 16 SNPs were located in intron 1 of the chicken LYZ gene and 8 of them did not show any polymorphism between the two Korean native commercial chicken populations by comparing with RJF. However, 8 of them gave the polymorphisms between the two commercial chicken populations. Also intron 2 has 14 SNPs and 2 indels in this gene. Out of 14 SNPs, 7 SNPs are monomorphic and 7 of them are polymorphic SNPs

Table 2. Identified of polymorphi는 and indels between Korean native commercial chicken populations compared with RJF.

Genomic position	Position	CCP1	CCP2	Mutation type
Intron 1	267	C/T	C/T	SNP
	361	C/T	C/T	
	382	C/T	C/T	
	419	A/G	A/G	
	436	C/T	C/T	
	487	-	A/G	
	520	A/C	A/C	
	521	C/T	-	
	593	A/G	-	
	650	A/C	-	
	814	A/G	-	
	926	C/T	-	
	1206	C/T	C/T	
	1226	-	C/T	
	1287	A/G	A/G	
	1299	-	C/G	
Exon 2	1426	C/T	C/T	SNP
Intron 2	1635	A/G	-	SNP
	1684	C/T	C/T	
	1959	T/G	T/G	
	1975	C/T	-	
	1996	A/G	A/G	
	2013	C/G	-	
	2192	A/G	A/G	
Intron 2	2370-2390 2405-2408	ATAGCACAGGGCTTATGCTGC GGAT	ATAGCACAGGGCTTATGCTGC GGAT	Indel
Intron 2	2411	-	C/T	SNP
	2641	C/G	-	
	2668	C/T	C/T	
	2766	-	T/G	
	2847	A/G	A/G	
	2885	C/T	C/T	
	2958	C/T	-	

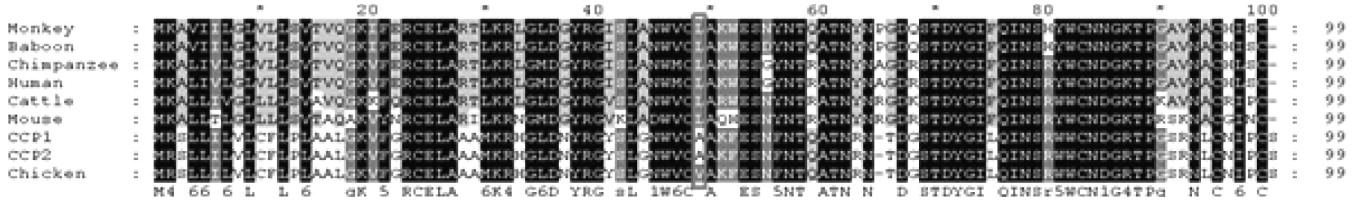

Fig. 2. Comparison of deduced lysozyme amino acids among different species. References from GenBank accession number in human (NP_000230) in shared by mammals Baboon (NP_001106112), Chimpanzee (NP_001009073), Monkey (NP_001095203), Mouse (NP_038618), Cattle (NP_001071297) and Chicken (RJF) (NP_990612).

between Korean native CCPs which are compared with RJF. Two indel variations in intron 2 were identified. They are "ATAGCACAGGGCTTATGCTGC" (21 bp) at the position of 2370-2390 bp and "GGAT" (4 bp) at the position of 2405-2408 bp, which are the common indels between Korean native commercial chicken populations that are different from the RJF. In general, intron structure is requiring RNA splicing for the generation of mRNA, however some intronic polymorphisms controlled by untranslated region (UTR) which has led to new ideas about evolution of genes. It was implied that the construction of new genes could be significantly assisted by recombinational events within intervening sequences (Doolittle et al., 1978; Darnell et al., 1978).

2. Functional variation

The protein coding region in chicken LYZ gene has affect the functional variation in chicken populations and therefore it affects growth traits (Downing et al., 2009a; Hou et al., 2010). The high conservations in coding regions, p.Ala49Val and p.Tyr71Ser, were possibly give some clues for the functional relevance for the divergence of chicken populations (Downing et al., 2009a). Also 3 polymorphisms, G111A within exon 1, T1426C and C1492T within exon 2, were reported for the effects on growth traits of chicken (Hou et al., 2010). Diversity among the chicken populations was distributed around amino acid substitution at position 49 that propose a result of latent admixture following domestication and selective processes (Downing et al., 2009a). Our results also supported by the distributed around amino acid substitution at position 49 for the domestication from the ancestor chicken breed and also correlated with growth traits in chicken. The multiple alignments with several species were sketched conservation of deduced lysozyme amino acid (Fig. 2). Even though, we could not identify any long conserve region of amino acid sequences, amino acid at position 49 was represented leucine (L) for human and other mammals and the other amino acids valine (V) for RJF. Whereas, comparison with our Korean native commercial chicken populations contained different amino acid alanine (A), which may be a result of concealed admixture through domestication. The identified non-synonymous substitution (p.Ala49Val) is located close to the catalytic sites of the enzyme, which might affect its activity.

The previous study of the signature of high allelic diversity indicated on the variation at chicken mtDNA (Liu et al., 2006), MHC-B (Worley et al., 2008; O'Neill et al., 2009), Mx (Berlin et al., 2008), IL1B (Downing et al., 2009b) and IL-4Ra (Downing et al., 2009c), suggesting that this may be the result of the complex population history of the chicken during domestication. Although the ancestors of chicken genetic variation is the RJF (Fumihito et al., 1994; International Chicken Genome Sequencing Consortium, 2004), multiple chicken domestications (Fumihito et al., 1996; Liu et al., 2006) and genetic relationships of other JF into chicken populations (Nishibori et al., 2005; Eriksson et al., 2008; Silva et al., 2008) suggest that diverse alleles may have been introduced during domestication. Though the promoted allele variation may be a relic of chicken domestication, this includes the proposal of disease related selective pressure, which might explain the continued resolution of the divergent alleles in modern chicken populations. Therefore, the LYZ gene is a potential marker for marker-assisted selection programs. In our investigation, the polymorphisms in LYZ gene can provide broad ideas for the variation of Korean native chicken populations from the ancestor of chicken breeds as well as the some biological functions of this gene.

IV. Summary

닭의 진화를 이해하기 위하여 변이가 많다고 알려진 LYZ 유전자의 엑손과 인트론에 존재하는 단일염기다형이 본 연

구를 통해 확인되었다. 2개의 한국 재래실용계에서 총 24개체의 DNA 샘플이 본 연구에서 이용되었으며 단일염기 다형의 확인을 위하여 3개체의 샘플을 혼합하여 염기서열 분석을 실시하였다. 적색야계와의 비교를 통하여 두 한국 재래실용계는 18개의 염기서열변이를 확인할 수 있었으며 한국 재래실용계 간에는 15개의 염기서열 변이를 확인할 수 있었다. 총 33개의 변이 중 두 개의 삽입변이(21 bp와 4 bp)가 확인되었다. 한편, 2번째 엑손의 1426 bp 위치에 존재하는 단일염기 다형(p.Ala49Val)은 아미노산의 변이를 나타내는 미스센스 돌연변이로 확인되었다. 이 돌연변이는 이 lysozyme 효소의 촉매작용을 하는 위치에 놓여 있어 효소의 활성과 밀접한 관계가 있을 것으로 추정된다. 본 연구에서 밝혀진 LYZ 유전자의 변이는 이 유전자의 기능뿐 아니라 한국 재래실용계 집단의 구조를 이해하는데 기초자료로 이용될 것으로 사료된다.

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