

Genetic Variability of mtDNA D-loop Region in Korean Native Chickens

Md. Rashedul Hoque¹, Kie Chul Jung², Byung Kwon Park³, Kang Duk Choi⁴ and Jun Heon Lee^{1,†}

¹Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea

²Poultry Science Division, National Institute of Animal Science, RDA, Cheonan 331-801, Korea

³Department of Companion & Laboratory Animal Science, Kongju National University, Yesan 340-702, Korea

⁴The Graduate School of Bio & Information Technology, Hankyong National University, Ansung 456-749, Korea

ABSTRACT In order to determine the origin and genetic diversity among chicken breeds, mitochondrial (mt) DNA D-loop sequences have been widely used. In this study, 41 individuals from four breeds (Korean native chicken (Black and Brown) and two imported breeds, Rhode Island Red and Cornish) were used for identifying genetic relationships with other chicken breeds. We obtained ten haplotypes and the highest number of haplotype was represented by eight individuals each from haplotype 1 and haplotype 2. Neighbor-joining phylogenetic tree indicates that the black and brown Korean native chicken breeds were mixed in haplotype 2 and they were closely related with the red jungle fowl (*Gallus gallus*). We also investigated whether the D-loop hypervariable region in chicken mtDNA can be used for the breed identification marker. The results indicated that the combination of the SNPs in the D-loop region can be possibly used for the breed discriminating markers. The results obtained in this study can be used for designing proper breeding and conservation strategies for Korean native chicken, as well as development of breed identification markers.

(Key words : breed identification marker, Korean native chickens, phylogenetic analysis, SNP)

INTRODUCTION

Chicken is known to be domesticated from a single ancestor, mainly contributed by red jungle fowl (*Gallus gallus*), which originated in Southeast Asia (Akishinonmiya et al., 1994, 1996). However, the current chicken is formed from several *Gallus* subspecies (Crawford, 1990) and the number of subspecies which has contributed to the origin of chicken is still controversial and remains uncertainty. The total number of breeds is now decreasing because of low productivities of native chicken breeds and the concentrated demands for the high productive strains. Recently, conservation of farm animal genetic resources has been focused on maintaining minimum number of animals for each breeds/species and there is some progress for this (<http://www.fao.org/dad-is/>). 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).

Mitochondrial DNA has been continuously used for the population, biogeographic and phylogenetic studies. Chicken mtDNA is 16,775 base pair long (Desjardins and Morais, 1990) and the mitochondrial genome is maternally inherited in most species

and does not undergo recombination (Hayashi et al., 1985). The D (displacement)-loop is the major control region for mtDNA expression and highly polymorphic compared with the nuclear DNA. The evolutionary rate is five to ten times higher than that of the nuclear genome (Brown et al., 1982). This makes D-loop in mtDNA use for phylogeographic analysis (Avice, 1994) which has been largely applied to understand phylogenetic relationships in many animal species, including cattle (Loftus et al., 1994; Bradley et al., 1996; Mannen et al., 1998; Troy et al., 2001; Mannen et al., 2004; Bhuiyan et al., 2007), water buffalos (Lei et al., 2007), pig (Giuffra et al., 2000), sheep (Hiendleder et al., 1998, 2002), horse (Vila et al., 2001), goat (Luikart et al., 2001; Mannen et al., 2001; Sultana et al., 2003; Joshi et al., 2004; Sultana et al., 2004; Chen et al., 2005; Odahara et al., 2006) and chicken (Niu et al., 2002; Lui et al., 2004; Lui et al., 2006). Therefore, this hypervariable D-loop region of mtDNA can be used to detect ancient population structures, interspecies variability, archaeological inference about the origins and relationships between populations or species, identification of maternal lineages and post natal growth (Bradley et al., 1998; Troy et al., 2001; Lui et al., 2004; Malau-Aduli et al., 2004; Yoon et al., 2005;

[†] To whom correspondence should be addressed : junheon@cnu.ac.kr

Odahara et al., 2006; Lei et al., 2007; Lee et al., 2007). Along with hypervariable D-loop nucleotide substitutions in mtDNA, breed specific markers were also investigated in chicken for delineating the breed structures and phylogenetic relationships with other breeds for the conservation perspectives (Hillel et al., 2003).

In our study, the D-loop hypervariable region of mtDNA has been used for determining the relationships between Korean native chicken and other chicken breeds. Also, the possibility of using mtDNA markers has been investigated for the breed identification.

MATERIALS AND METHODS

1. Experimental Animals and Sampling

A total of 41 blood samples were collected from Poultry Science Division, National Institute of Animal Science (NIAS) in Korea, which were composed of seven black Korean native chickens, fifteen brown Korean native chickens, five Rhode Island Red and fourteen Cornish breeds.

2. DNA Purification, PCR Amplification and Sequencing

DNAs were extracted by using the procedures described by the manufacturer's standard procedure of G-Dex™ Genomic DNA Extraction Kit (iNtRON Biotechnology, Inc). Polymerase chain reaction (PCR) was used for amplifying the chicken mtDNA D-loop region. The primer pair (Forward: 5'-AGGACTACGGCTTGAAAAGC-3' and Reverse: 5'-ATGTGCCTGACCGAGGAACCAG-3') was used to amplify about 600 bp of the D-loop hypervariable region from the mtDNA. The PCR reactions were included approximately 100 ng of genomic DNA, 2.5 μ L of 10 \times buffer [contains Tris-HCl (pH 9.0), PCR enhancers, (NH₄)₂ SO₄, 20 mM MgCl₂], 2.0 μ L of 10 mM dNTPs mixture (2.5 mM each of dATP, dCTP, dGTP and dTTP), 1 μ L of 10 pM of each primer and 1 U *HS Prime Taq* (GeNet Bio, Korea) in a 25 μ L reaction volume. The PCR reaction was performed in a My-Genie96 Thermal Block (Bioneer, Korea) with an initial denaturation step at 94°C for 10 min followed by 35 cycles of 30 sec at 94°C, 30 sec at 61°C, 40 sec at 72°C and a final extension step at 72°C for 10 min. Purification of PCR products was per-

formed using Accuprep® PCR purification kit (Bioneer, Korea) according to the manufacturer's instructions. All the PCR products were run on 1.5% agarose gels stained with ethidium bromide and DNA bands were visualized under UV light. Purified PCR products were sequenced by Genotech (www.genotech.co.kr).

3. Data Analyses

The chicken mtDNA D-loop nucleotide sequences obtained in this study were aligned using the ClustalW program (Thompson et al., 1994) and saved as bioedit format. Nucleotide replacement export data (Fig. 1) were carried out in haplotype sequences

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R-1  CATTATATATT CCAATCCCAC TTC
R-2  .....
R-3  .....
R-4  .....
R-5  .....
Kbl-1 TT...C.CC TTG.CT.GT ...
Kbl-2 TT...C.CC TTG.CT.GT ...
Kbl-3 T.CC.C.CC T...T... ..T
Kbl-5 ...C.....
Kbl-6 TT...C.CC TTG.CT.GT ...
Kbl-7 ...C.....
C-1  T..C..C.CC T...T... ..T
C-2  .....G...
C-3  T.CC.C.CC T...T... ..T
C-4  .....G...
C-5  .....G...
C-6  T.CC.C.CC T...T... ..T
C-7  T..C..C.CC T...T... ..T
C-8  .....G...
C-9  .....G...
C-10 T.CC.C.CC T...T... ..T
C-11 T.CC.C.CC T...T... ..T
C-12 .....G...
C-13 T..C..C.CC T...T... ..T
C-14 .....G...
Kbr-1 .....
Kbr-2 .....
Kbr-3 .....
Kbr-4 T..C..C.CC T...T... ..T
Kbr-5 .....G...T... CC.
Kbr-6 T..C..C.CC T...T... ..T
Kbr-7 T..C..CC.CC T...T... ..T
Kbr-8 TT...C.CC TTG.CT.GT ...
Kbr-9 ..C.....G...T... CC.
Kbr-10 T.CC.C.CC T...T... ..T
Kbr-11 TT...C.CC TTG.CT.GT ...
Kbr-12 T.CC.C.CC T...T... ..T
Kbr-13 TT...C.CC TTG.CT.GT ...
Kbr-14 TT...C.CC TTG.CT.GT ...
Kbr-15 T..C..C.CC T...T... ..T

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Fig. 1. Sequence variations observed from Rhode Island Red (R), black Korean native chicken (Kbl), Cornish (C) and brown Korean native chicken (Kbr) for the D-loop hypervariable region in mtDNA.

and identical sequences were considered as the same haplotypes by using MEGA software version 4.0.2 (Kumar et al., 2008). The published complete and partial mtDNA sequence data of domestic chicken populations from National Center for Biotechnology Information (NCBI) were also included in the analysis (GenBank accession numbers- AP003318, AP003580, AY235571, NC_007235, NC_007236, NC_007237, AB086102-AB007723, AB007719, AB098692, AB098697-AB009441, AB009443, AY704710, AY644966, AY465988, AF512273, AF512075). Neighbor-joining phylogenetic tree was estimated by 1,000 random bootstrap resampling of the data (Kumar et al., 2008).

RESULTS AND DISCUSSION

1. mtDNA D-loop Sequence Variations in Chicken

Analysis of sequences from D-loop hypervariable region in chicken mtDNA identified a total of 23 nucleotide substitutions, which were classified as ten haplotypes (Table 1). The highest common haplotype group contains eight individuals for both haplotype 1 and haplotype 2. The 2nd common haplotype groups contain seven individuals in haplotype 3 and haplotype 4. Only one haplotype group comprised of four individuals in type 5, and the haplotype 6 and 7 contained 2 individuals, respectively. The remaining three haplotypes (haplotype 8, 9, 10) were repre-

sented by only one individual. No deletion or insertion was detected in our sequence analysis. Based on our study, brown Korean native chicken breed has 7 different haplotypes (haplotype 1, 2, 4, 5, 8, 9, 10), black Korean native chicken and Cornish breed have only 3 haplotypes each (haplotype 2, 4, 6 for black Korean native chicken) and (haplotypes 3, 4, 5 for Cornish). On the other hand, Rhode Island Red has only one haplotype (haplotype 1). Therefore, we can identify that the haplotype 2 is the common haplotype for the Korean native chicken and the unique haplotype 6 and 7 were identified in black Korean native chicken and brown Korean native chicken, respectively and haplotype 4 is the common haplotype for the three breeds investigated in this study.

2. Phylogenetic Analysis

Phylogenetic tree was constructed using neighbor-joining method with mtDNA D-loop sequences from the three chicken breeds and the published reference sequences obtained from NCBI database (Fig. 2). Eighteen published reference sequences were included in the analysis for making phylogenetic tree. The neighbor-joining tree indicated that brown Korean native chicken shared with several haplotypes and some haplotypes were not closely related with recognized reference breeds. In our observations, haplotype 9 was closely related with the New Hampshire Red and haplotype 5 was very closely related with White

Table 1. Mitochondrial D-loop sequence polymorphisms identified in four breeds of chickens

Haplotypes	Nucleotide position in mt DNA ¹																						
	167	177	199	217	220	222	225	239	243	256	261	265	281	291	306	310	313	330	342	363	367	370	446
Type 1 (8) ²	C	A	T	T	T	A	T	A	T	T	C	C	A	A	T	C	C	C	A	C	T	T	C
Type 2 (8)	T	T	-	-	-	-	C	-	C	C	T	T	G	-	C	T	-	-	G	T	-	-	-
Type 3 (7)	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Type 4 (7)	T	-	C	C	-	-	C	-	C	C	T	-	-	-	-	T	-	-	-	-	-	-	T
Type 5 (4)	T	-	-	C	-	-	C	-	C	C	T	-	-	-	-	T	-	-	-	-	-	-	T
Type 6 (2)	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Type 7 (2)	T	-	-	C	-	-	C	-	C	C	T	-	-	-	-	T	-	T	-	-	-	-	T
Type 8 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	T	-	-	-	C	C	-
Type 9 (1)	T	-	-	C	-	G	C	-	C	C	T	-	-	-	-	T	-	T	-	-	-	-	T
Type 10 (1)	-	-	C	-	-	-	-	-	-	-	-	-	-	G	-	-	T	-	-	-	C	C	-

¹Numbers indicate nucleotide base position in mitochondrial D-loop region and hyphen represents the identical nucleotide with the type 1 sequence.

²Numbers in parentheses indicate the observed number of chickens.

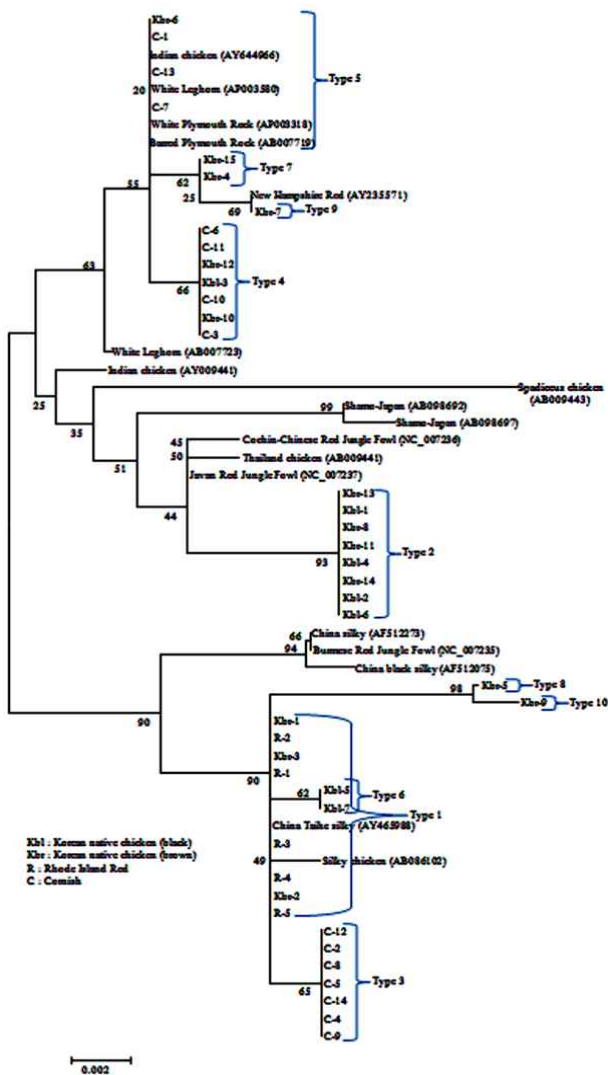


Fig. 2. Neighbor-joining phylogenetic tree for the Rhode Island Red, Cornish and Korean native chicken breeds. Haplotypes in Table 1 are also indicated.

Plymouth Rock, White Leghorn, Barred Plymouth Rock and Indian chicken breeds. Meanwhile, haplotype 1 contains all Rhode Island Red individuals which were very closely related with China Taihe silky and Silky chicken breeds. Whereas, haplotype 2 contains both black and brown Korean native chicken which have close relationship with Javan red jungle fowl, Cochinchinese red jungle fowl and Thailand chicken breeds. It has been widely understood that red jungle fowl was the main contributor in monophyletic origin theory (Crawford, 1990) and we can expect that the haplotype 2 is one of the ancient haplotypes. Cornish breed has three haplotypes based on the haplo-

type analysis. Of these, haplotype 4 and 5 were mixed with black Korean native chicken and brown Korean native chicken. On the other hand, haplotype 3 contained 7 Cornish individuals which were not closely related with the reference sequences in this study. In case of black Korean native chicken, three haplotypes (2, 4 and 6) were observed. From these, two individuals belong to the haplotype 6, which were closely related with haplotype 1. Another one individual (Kbl-3) belongs to the haplotype 4, which also contains brown Korean native chicken and Cornish breeds. Large numbers of Korean native chicken (both black and brown) individuals have the haplotype 2. These individuals are closely related with red jungle fowl and Thailand chicken breeds. This also indicates that the Korean native chicken is more closely related with the ancient chicken breeds compared with the other commercial breeds.

In conclusion, the phylogenetic analysis of four chicken breeds containing black Korean native chicken, brown Korean native chicken, Rhode Island Red and Cornish breeds using D-loop region in mtDNA indicated that Korean native chickens have relationship with the major lineage of Red jungle fowl. The results presented here can give the broad idea for the phylogenetic relationship of Korean native chicken and provide opportunities for the development of breed specific markers. In order to maintain valuable genetic materials, more detailed studies have to be conducted along with the appropriate breeding programs.

국문요약

닭의 품종 기원을 결정하거나 유전적 변이의 정도를 확인 하는데 미토콘드리아 DNA D-loop 염기서열을 이용하여 하고 있다. 본 연구는 한국재래계 갈색종과 흑색종, 로드아일랜드레드종, 코니쉬종의 4품종 41개체의 염기서열을 분석함으로써 품종간의 유전적 연관관계를 확인하였다. 그 결과 총 10개의 haplotype를 확인할 수 있었으며, haplotype 1과 2는 가장 많은 수인 8개체씩이 포함되었다. 계통도 분석을 통해 한국재래계 흑색종과 갈색종은 haplotype 2를 모두 가지고 있는 것으로 확인되었으며, 이 haplotype은 적색야계와 유전적으로 가깝게 위치한 것을 알 수 있었다. 본 연구를 통해 D-loop 염기서열 변이가 품종 판별 마커로 이용 가능성이 있는지 확인하였다. 그 결과 여러 단일염기다형 마커의 조합으

로 품종의 구분이 가능할 것으로 추정되며, 앞으로 더 많은 연구가 진행되어야 할 것으로 생각된다. 이 연구의 결과는 한국재래계의 보존 및 육종계획 수립과 더불어 품종판별 마커의 개발의 기초 자료를 제공할 것으로 생각된다.

(색인어: 계통도, 단일염기다형, 품종판별 마커, 한국재래계)

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