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DNA markers in chicken for breed discrimination

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닭에서 품종 확인을 위한 DNA 마커에 관한 고찰

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Abstract : There is an emerging interest in using DNA markers for breed identification in animals. This article reviews the breed identification markers in chicken, mainly developed in Chungnam National University, with particular emphasis on the mitochondrial DNA markers and the nuclear DNA markers including the SNPs in MHC region and the plumage color related MC1R markers. This information would be very useful for an appropriate conservation breeding program as well as for the establishment of molecular markers for chicken breed identifications.

Key words **:** DNA marker, MC1R gene, Microsatellite, Mitochondrial DNA

I. Introduction

Identification of local breeds is an important concern for the conservation of domestic animal genetic diversity (FAO, 2007). Recently, the native species in livestock genetic resources become more important and large efforts have been concentrated on maintaining minimum number of animals for each native species (http://www.fao.org/dad-is/). Selective breeding program has established in many breeds, especially achieving high productivities and because of this intensive breeding program, the number of breeds is now decreasing because of their low productivities as well as pressure from the commercial farms that need high productive strains. In order to develop conservation strategies, recently United nations called for the identification of all native livestock breeds in the world (http://dad.fao. org).

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Chicken breeding has a very long history that dated back to 8,000 BC. From archaeological discoveries in the Indus Valley and Hebei Province, China, it is known that the chickens were probably domesticated from the red jungle fowl (*Gallus gallus*) as early as 5400 BC. As genetic variation is of vital importance to all animals living on Earth, DNA technology is rapidly developing for genetic traceability of domestic and wild populations. The effective tool to check and authenticated advanced identification system to assign animals to their claimed breed is the direct identification at DNA level. Moreover, the possibility to verify and assurance the origin of animals increases the value of quality certification, favoring the development of economically marginal areas through the revaluation of local and typical breeds and products leading to the conservation of local breeds and preserving biodiversity. In modern animal science with some high selected breeds and the survival of organisms, biodiversity is very important. In genetic study of

farm animals, polymorphic proteins and blood groups were the first markers. However, more often used to analyze genetic diversity is the microsatellite markers. Among the techniques developed with advance technology, the most important markers in recent years are single nucleotide polymorphism (SNP) markers and mitochondrial DNA analysis for in depth genome analysis and evaluation of genetic variation in different species.

Molecular characterization may help to maintain breed diversity and population structure for genetic management of small populations. Moreover, DNA marker for breed identification is required for designing more relevant breeding program and making conservation strategies in the Korean native chicken breeds. Therefore, this article review a variety of DNA markers that are implemented into breed identification or breed discrimination program in the Korean poultry industry.

II. Mitochondrial DNA markers

Mitochondrial DNA (mtDNA) is located within mitochondria which are organelles found in the cell cytoplasm and they are producing energy for the cells and body. This mtDNA has been considered as a powerful source of molecular information to trace the ancestry of the animals (Harpending et al., 1998). Therefore, polymorphisms in the mtDNA are widely used for phylogenetic and genetic analysis of diversity. Haploid mtDNA is characterized by maternal inheritance, a high mutation rate and lack of recombination (Coble et al., 2004; Vallone et al., 2004). These characteristics make it an ideal marker for reconstruction of evolutionary relationships between and within species. It may also enable rapid detection of hybridization between species and subspecies of farm animals (Nijman et al., 2003). Therefore, mtDNA has remained the marker of choice in many populations for biogeographic and phylogenetic studies. Its use has also been recommended in taxonomic studies, with the proposal that all described species are given an mtDNA sequence tag or bar-code (Hebert et al., 2003). The understanding of domestication history and basic genetic relationships of chicken breeds would help the conservation and improving the breeds.

1. D-loop region

Typically, coding genes of the mitochondrial genome are used for phylogenetic studies to distinguish species (Moore and Defilippis, 1997), whereas the control region or D (displacement)-loop is considered more suitable for inter-specific population studies (Baker and Marshall, 1997). Herein, this region is non-coding and evolves much faster than other regions of the mtDNA genome. This makes that the D-loop region is particularly useful for phylogenetic analysis (Avise, 1994). The first molecular genetics study was conducted by D-loop region in mtDNA sequences of Gallus species and domestic chickens had suggested that, domestic chicken have a monophyletic origin, the continental population of the red jungle fowl subspecies (*Gallus gallus gallus* in Southeast Asia) was the sole ancestor of all domestic chickens, and all the domestic breeds might have originated from a single domestication event that occurred in Thailand and adjacent areas (Fumihito et al., 1994; Fumihito et al., 1996). After that, Niu et al. (2002), examined the mtDNA of Chinese native chicken, confirm that their most likely origin is from Thailand and adjacent geographic areas. However, the polymorphism of the sequences of mtDNA D-loop hypervariable regions was analyzed to identify the wild ancestors of domestic animals, the geographic patterns of genetic diversity and the process of farm animal domestication (Bruford et al., 2003). Number of polymorphic sites were reported of indigenous chickens in several country of D-loop region in mtDNA have successfully been traced the biodiversity and origin of chicken breeds (Table 1). Polymorphism of the region was in-

Table 1. Number of polymorphic sites reported in the mtDNA D-loop region.

No. of Breed/Population	Country	No. of Sample	No. of polymorphic site	Reference
9	Korea	718	32	Hoque et al., 2011
4	Japan	556	5	Harumi et al., 2011
2	Vanatu, Marianas	48	25	Dancause et al., 2011
11	China	222	43	Cuc et al., 2011
23	Africa	512	37	Mwacharo et al., 2011
44	China, Vietnam	556	33	Berthouly-Salazar et al., 2010
25	China	328	59	Liu et al., 2010
9	Hungary	74	17	Revay et al., 2010
4	Korea	41	23	Hoque et al., 2009
5	Sri Lanka	140	44	Silva et al., 2008
5	Zimbabwe	283	32	Muchadeyi et al., 2008
23	Japan, Indonesia	166	45	Oka et al., 2007

vestigated by analysing 640 birds for the assessment of relationship between the populations of Asian (17 populations), south eastern European (20 populations) and north central European chickens (25 populations). The analysis of mtDNA polymorphisms of the chicken populations indicated that most European populations belong to a single mtDNA group, which may have originated from India (Weigend et al., 2010). Analysis of sequences of mtDNA control regions revealed that the variability of genetic diversity of native chicken breeds, which is high in Sri Lankan chickens (Silva et al., 2009), medium level in Zimbabwean and Indian chickens (Muchadeyi et al., 2008; Pirany et al., 2007) and low in Chinese, Japanese and several native African chickens (Niu et al., 2002; Oka et al., 2007; Muchadeyi et al., 2008). However, D-loop region in mtDNA may play a vital role in measurement of diversity and in describing and evaluating the population structure.

III. Nuclear DNA markers

Chicken genome is about 1,050 million base pair (Mb), which are about 20,000 genes scattered over 39 chromosome pairs including one pair of sex chromosome. Chicken genome contains fewer repetitive sequences compared with human genome and this largely affects the genome size differences. In 2004, a high quality assembly of the chicken genome covering the major part of chicken genome was published (International Chicken Genome Sequencing Consortium, 2004). Sex chromosomes and the MHC region on chromosome 16 are still not clearly identified. However before the chicken genome sequencing was published, Kaufman et al. (1999) investigated the chicken MHC region. As the results, large number of alleles located in the MHC region was identified. Also, the consistent association results were observed between LEI0258 microsatellite marker alleles and serologically defined MHC haplotypes (Fulton et al., 2006). In order to study the mechanism of evolution, nuclear DNA polymorphisms that exist widely in eukaryotic organisms providing virtually unlimited opportunities. Recently, chicken SNP chips were developed and give more opportunity for using the nuclear DNA markers (Zhang et al., 2003).

1. MHC region

The major histocompatibility complex (MHC), which is physically distributed across the genome located on the chromosome 16, is the dense region of immune-related genes with high levels of polymorphisms. Some MHC alleles show the trans species polymorphisms, which indicate the sharing alleles between highly

diverged species. Compared with other part of human genome, MHC genes are mainly associated with diseases. With the extensive knowledge of the structure and function of the domestic chicken MHC, the role of selection could be examined to other fowl populations as an ideal model. Furthermore, the 'minimal essential' in nature of the fowl MHC region allows the characterization of functional diversity at the locus-specific level (Kaufman et al., 1999). Studies are aiming to investigate the processes responsible for maintaining MHC diversity within populations which is essential for reliable methods of screening variation across individuals. All these methods have different advantages, such as analyzing of functional diversity can be inferred from sequence variation and relatively easy to perform with large scale SNP genotyping.

2. LEI0258 marker

The location of LEI0258 marker is between the BG and BF regions in the MHC B locus which turned out to be informative and will be used as a marker in MHC region. The considerable diversity in size is shown by the LEI0258 alleles which is not expected for typical microsatellite marker. Allele size is the sequence information of the repeat region in addition the two flanking regions surrounding the repeat. The entire LEI0258 allele was reported in chicken genetic resources following numbered from −78 to −1 for the region immediately upstream of the repeat, and numbered from 1 to 88 for the region including the last repeat and downstream through the reverse primer (Fulton et al., 2006). The two independent repeat elements were replicated from 1 to 28 times of "CTATGTCTTCTTT" and from 2 to 20 times of "CTTTCCTTCTTT" for the 13 and 12 repeat, respectively. The LEI0258 allele sizes with those repeat and polymorphic structure shared among several distinct B haplotypes which completely sequence the LEI0258 locus in Korean native chicken compared with genetic resources (Table 2).

Table 2. Allele sizes of MHC region for LEI0258 marker identified in Korean native chicken compared with genetic resources.

LEI0258 allele (bp)	B haplotype	Genbank accession no.	Line/Breed
193	15.1	DQ239512	GH15.1, Wisc3, Commercial, NIU, Korean native chicken (Brown)
	11	DQ239495	
	61	DQ239547	
	27	DQ239538	
194	BW3	DQ239561	13, Korean native chicken (Brown)
205	13	DQ239501	133, 15.P-13, GH13, UCD380, 11, Cornish
	13.2	DQ239505	
	17	DQ239514	
	BW11	DQ239560	
241	15.2	DQ239513	UCD253, Korean native chicken (Black)
	22	DQ239531	
	73	DQ239551	
249	15.2	DQ239513	M15.2, UNH105, Commercial, Korean native chicken (Black), Rhode Island Red
	22	DQ239531	
	73	DQ239551	
295	11.1	DQ239496	15.151-5, Commercial, Wisc3, Rhode Island Red, Cornish
	5	DQ239541	
309	10	DQ239494	Commercial, UCD312, UNH105, Korean native chicken (Black), Rhode Island Red, Cornish
	24	DQ239533	
	26	DQ239537	
	76	DQ239554	
381	13.1	DQ239504	Commercial, Cornish
443	6	DQ239544	36, GHs6, Korean native chicken (Brown)

These sequence data revealed distinguishing SNP differences for some of these haplotypes. Despite the many generation of separation, the allelic identity is carried by the sequence information. The historical recombination events have occurred in LEI0258 alleles and the serologically detected gene products of the chicken MHC. For the identification of LEI0258 alleles from serologically defined MHC sources to determine this marker could be used to discriminate haplotypes for chicken breeding purpose.

3. SNP markers

The chicken MHC region is a major locus comprising of multiple genes with very high level of polymorphisms which are located in the B region. It is one of the most difficult areas of the genome to study due to its inherent sequence characteristics. This gene-dense region spans to be about 0.5 Mb in MHC B region. This region has been widely studied and is proposed to contain genomic sequences that contribute to the majority of autoimmune and inflamatory disorders. In order to cover the entire B region, Bed'Hom's research group in INRA, France developed 96 SNP panel for increase the resolution of genotyping and understand the evolutive history of the different alleles. These SNPs were selected from a list of more than 4,500 SNPs identified by comparison of sequences available in databases, and by a resequencing approach of 24 PCR in 48 different chicken populations, including local breeds. Regarding this concepts, Korean native chicken breeds and other foreign breeds were performed a panel comprised of 96 SNPs using Illumina GoldenGate assay. Of these, 480 samples were genotyped from twelve chicken breeds resulted in phylogenetic relationships, breed structure and also discriminated allele frequencies among the chicken breeds. While, SNP markers are fixed DNA point mutations resulting from transition or transversion. Importance of identification SNP markers in MHC region is more informative for the breed discrimination.

4. MC1R gene

Modern domestic animal species display a bewildering diversity in coat color, and the melanocortin receptor 1 (MC1R) locus, which is most consistently polymorphic, has been previously documented well and gave known association with coat color variations in horse, cattle, fox, pig, sheep, dog and chicken (Kerje et al., 2003; Kijas et al., 1998; Kijas et al., 2001; Klungland et al., 1995; Marklund et al., 1996; Newton et al., 2000; Vage et al., 1997; Vage et al., 1999). The chicken MC1R gene was assigned to chromosome 11 and contains only one exon encoding a 314 amino acid protein sharing 64% identity with that of the mammalian gene (Kerje et al., 2003; Takeuchi et al., 1996). This gene involves the regulation of melanogenesis. Also, mutations in this gene were associated with eumelanin (black/brown), while loss of function mutations are

Table 3. The identified MC1R gene polymorphisms.

Nucleotide position	Amino acid Position	Type of mutation	Reference
69	23	Synonymous	Guo et al., 2010; Kerje et al., 2003
212	71	Nonsynonymous	Kerje et al., 2003; Guo et al. 2010; Tixier-Biochard, 2011
274	92	Nonsynonymous	Kerje et al., 2003; Guo et al., 2010; Tixier-Biochard, 2011
376	126	Nonsynonymous	Kerje et al., 2003; Guo et al., 2010; Tixier-Biochard, 2011
398	133	Nonsynonymous	Guo et al., 2010; Tixier-Biochard, 2011
427	143	Nonsynonymous	Kerje et al., 2003; Guo et al., 2010; Tixier-Biochard, 2011
636	212	Synonymous	Guo et al., 2010; Kerje et al., 2003; Tixier-Biochard, 2011
637	213	Nonsynonymous	Kerje et al., 2003; Guo et al., 2010; Tixier-Biochard, 2011

associated with pheomelanin (red/yellow) phenotype. The MC1R gene sequence data were identified 34 polymorphic sites on synonymous, nosynonymous and indel (insertion/deletion) mutations, which were previously reported (Guo et al., 2010; Kerje et al., 2003; Tixier-Biochard, 2011) (Table 3). The highly significant nonsynonymous mutations (Met71Thr, Glu92Lys, Ala126Ile, Thr143Ala, Cys213Arg and His215Pro) were associated with eumelanin and pheomelanin in chicken plumage colors (Guo et al., 2010; Kerje et al., 2003). Study in chicken breed discrimination has extended exiting knowledge on plumage color controlled by the MC1R gene locus might be a candidate marker.

IV. Conclusion

The study of chicken breed discrimination was mainly used D-loop sequences in mtDNA. As a genome sequencing technology has developed, the number of DNA markers are available in poultry industry. For example, the hypervariable microsatellite LEI0258, located within the MHC locus, and the SNP markers in MHC region were used for chicken breed discrimination or breed identification. Moreover, one of the causal gene controlling chicken coat colour, MC1R gene was also one of the best example used for the breed identification. The combination of molecular and pedigree information such as a weighted genotype relationship matrix (GRM) provide a better way to trace back to their pedigree and breed composition among the chicken breeds. Therefore, increase of the accuracy to estimate breed discrimination and composition using molecular markers would be very useful for an appropriate conservation breeding program as well as for the establishment of more accurate native chicken breeds.

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