

Recent Progress of the Poultry Genome Research Based on the Experience in CNU, Korea

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ABSTRACT

With the implementation of the restoration program for Korean Native Chicken (KNC), by the Korean government in the 1990s, the research interests on KNC were greatly increased. Accordingly, the Chungnam National University also conducted and continues many researches for the molecular genetics studies on KNC, including diversity analysis, QTL mapping, genome-wide association studies, selection signature studies, development of marker combinations for breed discriminations, and disease resistance and its related gene studies, particularly, the Major Histocompatibility Complex (MHC) gene diversity studies for KNC. Therefore, this paper briefly describes the progress of such studies obtained over the last decade.

Keywords: KNC, Diversity, SNP, QTL, GWAS, MHC

1. INTRODUCTION

As the world is hustling on achieving sustainable and healthy food supply goals to feed the increasing population worldwide, animal production has also been considered, with high priority, as a major source of proteins. Accordingly, the new USDA blueprint for Animal Genome Research implemented four main goals on animal health, production, and well-being improvement. They include; Providing nutritious food for a growing human population, improving the sustainability of animal agriculture, increasing animal fitness and improving animal welfare, Meeting consumer needs and choices [1].

Admiring the importance of these goals for the upliftment of animal production, especially chicken production, the intense molecular genetics-based approaches were initiated in Korea too. Consequently, the attention was directed towards the Korean Native Chicken (KNC) that are native to the Korean peninsula. Since it has obtained a higher

preference of Koreans to consume KNC over commercial broiler chicken concerning their superior tastes over commercial lines, with the national level intervention powered by the National Institute of Animal Science (NIAS) in 1992, advanced and pioneering studies were initiated [2].

By adhering to the emerging trend, the Chungnam National University (CNU) also inclined towards poultry genetics researches with special reference to KNC, adapting the various molecular biological technologies and fields including Genome-wide QTL mapping, Genome-wide Association Studies (GWAS), Discovering Single Nucleotide Polymorphism (SNP) marker combinations for breed classification and Major Histocompatibility Complex (MHC) diversity studies of chicken.

As they obtained splendid progress over these all years, it is believed that the dissemination of the knowledge generated with these studies might strengthen future researches on the relatively same

disciplines. Hence, the objective of this report is to summarize the results of the aforementioned studies.

2. DIVERSITY ANALYSIS IN KOREAN NATIVE CHICKEN

Aligned with the objective of diversity analysis of KNC, mtDNA D-loop variations, microsatellite markers, and SNP diversity analyses were conducted mainly. Based on the mtDNA studies, KNCs have multiple origins.

Moreover, corresponding to the mtDNA D-loop variations, the proprietary Hyunin chicken, which contained indigenous Korean Native Chicken, were analyzed for their diversity. As seventeen SNPs could be identified for the particular region, a total number of 6 haplotypes were determined (H2, H9, H10, H11, H12, H15) for the particular population, in which two haplotypes; H10, H15, were not previously known. Accordingly, the study enriched the knowledge on another aspect of KNC genetic resources based on the Hyunin chicken and presented indicative conservation measures [3].

Application of Microsatellite (MS) markers for diversity analysis and discrimination of five KNC lines was performed using 86 individuals. The study suggested 15 highly polymorphic MS markers from a pool of 150 markers of the Ark database that can effectively be utilized in line discriminations, developing conservation strategies, and planning breeding programs [4].

The SNP markers on KNC diversity with special reference to Linkage Disequilibrium (LD) decay was assessed using the 600K high-density SNP panel in which the results signified that the purebred KNC bear higher calculated LD ($r^2 = 0.24-0.37$) than its commercial Hanhyup line ($r^2 = 0.13-0.26$). These pieces of information are worthy, in assessing the effective population sizes, conservation statuses, and trait selections for breed developments [5]. As more chicken breeds are now available with high-density SNP chip data, such as the populations included in the "SYNBRED" project, it is very useful to identify the diversity and genetic relationship of local chicken in comparison with commercial and other local breeds. Local KNC included in this study agrees with previous findings for their origin where the KNC has closely clustered with brown egg layer breeds (not published).

3. QUANTITATIVE TRAIT LOCUS (QTL) MAPPING AND GWAS IN KOREAN NATIVE CHICKEN

As the chicken is considered a valuable model organism, and as the demand for native chicken

meat and its quality aspects have increased, the requirement for assessing genes that are responsible for economically important traits was raised. Hence, as an initial step, the development of its genetic linkage map is undoubtedly beneficial.

Accordingly, such a genetic linkage map was developed for KNC, adopting 131 MS markers and 8 SNP markers that resulted, a map with a total length of 2729.4 cM in which the 19.64 cM of the average distance between markers have reported. Further, the study revealed that the orders and the distances of markers perfectly align with the consensus linkage map with only three exceptions; *ADL0278* and *MCW0351* in *GGA8*. Moreover, the expected heterozygosities with an average number of alleles in addition to polymorphic information contents were calculated, hugely facilitating the QTL studies of KNC [6].

Another study was conducted to identify the QTL regions of KNC that affect the body weight and important growth traits using an approach called multipoint variance component linkage. Correspondingly growth-related two significant QTLs and body weight-related four QTLs (one significant and three suggestive) were identified. The discovery of these significant and suggestive QTLs for the traits concerned improves the understanding of the aforementioned traits in KNC and contributes to their trait selection practices [7].

Besides, quantitative measurements on KNC shank colors [8], fatty acid traits [9], and Serum chemical traits have been studied. In the case of the studies targetted on serum chemical traits, the following studies including the meat quality and clinical chemical traits based on amylase (*AMY1A*, *AMY2A*) genes [10], serum α -amylase level, cholesterol, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and creatinine levels [11], total protein, glucose, total cholesterol, etc. [12] can be listed and they largely contributed to enlarge the QTL knowledge base for KNC.

To increase the accuracy and power of detecting variation in complex traits by relating genotypes of large number of markers (SNP markers) to observed traits is the main goal of GWAS. Recently, we have been conducting the GWAS studies for the aforementioned traits using the Bayesian approach and conventional GWAS approach.

4. SELECTION SIGNATURE STUDIES IN KOREAN NATIVE CHICKEN

Aiming at comparing the selection signatures of KNC for the ultimate goal of their effective improvements, the purebred lines from NIAS and commercial Hanhyup line were investigated with

600K SNP chip data, determined the haplotypes, and directed the selection signals. The conclusion of the study suggested improving the traits related to egg and meat production in NL (KNC black line) and NW (KNC white line) lines respectively and multiple traits in other lines [13].

5. DISCOVERING SNP MAKER COMBINATIONS FOR BREED DISCRIMINATION

As the ability to accurately discriminate the chicken breed improves consumer confidence, the development of a genetics-based discrimination method with high accuracy seems important. The approach for discriminating the KNC from exotic breeds consisted of the application of machine learning models to the marker combinations from 600K SNP array. SNPs were selected based on the LD and GWAS results. The discrimination power of selected markers was analyzed using principal component analysis (PCA). However, 96 LD-pruned SNPs were recognized as the best combination for the discrimination (100% accuracy), whereas 8 SNPs from the Decision Tree (DT) model was obtained as the smallest possible combination with 97.9% accuracy [14]. Additionally, a similar approach is being applied for the five conserved pure lines of KNC at present (not published).

6. DIVERSITY STUDIES FOR MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) GENES

The chicken MHC region is a crucial and highly compacted region of genes that are responsible for the immunogenicity of chicken. The clear and exclusive information on this region largely influences the understanding of mechanisms and their molecular bases in addition to applying such knowledge in health care improvements including the development of vaccines, seriously upgrading their management practices, especially regarding disease traits. Accordingly, the MHC region was analyzed first for their MS and SNP marker diversities, followed by long-range PCR and NGS for more precise variant identifications.

Concerning the MS markers, 11 markers for the MHC-B region were investigated, including the LEI0258 marker, which was recognized, as the most polymorphic marker. Based on the results obtained, it indicated high MHC diversity in local chicken, though it is relatively lesser than the local chicken of Bangladesh, Sri Lanka, and Nigeria, exposing the possibilities of differential immune responses [15]. Alternatively, the SNP diversity analysis in the MHC-B region based on 96 MHC-B SNP panels

was evaluated and revealed that the MHC-B diversity is higher than that of commercial breeds, suggesting their immune capabilities over many pathogens [16, 17]. Moreover, the use of 600K SNP data, high-resolution long-range PCR, molecular cloning, and NGS sequence-based approaches in investigating the MHC diversity with special reference to KNC, is proceeding nowadays to widen the understanding of MHC diversities and their influences on the immune response of KNC.

As chicken MHC class I and II have two copies of genes, the expression of each copy is different where the class I-BF2 and class II-BL β 2 have higher expression while class I-BF1 and class II- BL β 1 have minor and tissue-specific expressions. Generating the full-length coding sequence from cDNA of these genes in novel haplotypes is necessary to understand the prevalence expression of genes that are related to immune responses and other biological functions controlled by MHC genes. Our recent studies on differentially expressed genes in the cecum junction in response to the Coccidiosis infection would enhance the current understanding of immunogenetics.

7. CONCLUSION

Many types of molecular genetics-based studies have been conducted on KNC as described and these studies have addressed the timely important objectives while adapting chronologically updating molecular biological tools and techniques that ultimately resulted in a strong molecular genetic resource for the development and conservation of local chicken populations.

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