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Short communication

MHC class I *BFIV* gene polymorphisms in four Chinese native chicken breeds

Yin Dai [a, *, 1], Xue-Lan Liu [b, 1], Qing-Feng Tang [b], Xiao-Miao Hu [a], Xue-Huai Shen [a], Dan-Jun Zhang [a, **]

[a] *Institute of Animal Husbandry and Veterinary Science, Anhui Academy of Agricultural Science, Hefei, 230031, China*
[b] *Anhui Agricultural University, Hefei, 230036, China*

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ABSTRACT

The major histocompatibility complex (MHC) includes the most polymorphic genes in vertebrates, and balancing selection has been proposed as a main evolutionary force. Here we present one of the first data sets examining the genetic characteristics of chicken MHC I BFIV molecules in four Chinese native breeds, sourced from different regions in China. In all, 89 *BFIV* alleles were isolated from 102 individuals sampled, and 13 repeated alleles were observed. No significant correlation was found between genetic differentiation and geographical distance in the phylogenetic tree. *BFIV* genes exhibited a high level of nucleotide polymorphisms, and most of the polymorphic sites were located in the peptide-binding region (PBR) encoded in exons 2 and 3. A comparison of the three-dimensional structures of PBRs in chicken BFIV and human HLA-A molecules revealed evident structural and functional similarities. The results suggested that MHC I molecules had similar structural features in different species.

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1. Introduction

The major histocompatibility complex (MHC) is an extremely polymorphic locus in vertebrate genomes (Klein et al., 2007), but the underlying mechanism that helps maintain this remarkable polymorphism remains to be clarified. MHC molecules play an important role in the adaptive immune response by presenting antigens to T lymphocytes, so it is likely that the polymorphisms are maintained by pathogen-mediated selection. Indeed, many useful insights into the nature of balancing selection can be gained from the studies of MHC polymorphisms in vertebrates (Smith et al., 2011; Zhang and He, 2013; Hawley and Fleischer, 2012; Eizaguirre et al., 2012). A number of reports has also suggested that MHC polymorphisms are associated with environmental processes in animals. The high level of polymorphism indicates that MHC gene are under some form of balancing selection and are adaptive in natural populations (Campos et al., 2011). The obvious genetic differentiation among different finless porpoise (*Neophocaena phocaenoides*) populations in Chinese waters in a relatively short-term period were found, because of different selection environments (Xu et al., 2010a). The highly polymorphic MHC genes in many species are thought to reflect a long history of gene conversion events under strong balancing selection, most likely through pathogen-mediated selection, and the selection results in the birth-and-death evolution (Gu and Nei, 1999; Sin et al., 2012; Knafler et al., 2014). Moreover, MHC shows high levels of genetic variability and positive selection acting upon substitutions at peptide-binding region (PBR) sites (Bernatchez and Landry, 2003; Zhu et al., 2014) in addition to a large number of alleles maintained by balancing selection.

Avian systems have been suggested as particularly promising candidates for the study of the evolutionary ecology of MHC genes (Westerdahl, 2007). Chicken MHC has been characterized as small and relatively simple, "minimally essential" compared with that of mammals (Kaufman, 2000), and domestic chickens were among the first vertebrates to reveal a strong role for MHC genetics in pathogen resistance (Kaufman and Salomonsen, 1997). However, studies have been largely limited to the red jungle fowl (*Gallus gallus*), the ancestor of domestic chickens (Worley et al., 2008; Gillingham et al., 2009). Domestic chickens have been more artificially selected and bred, and subjected to intense pathogen pressure. To better understand the characteristics of MHC

* Corresponding author.
** Corresponding author.
E-mail addresses: daiyin2020@163.com (Y. Dai), 1693805400@qq.com (D.-J. Zhang).
[1] These authors contributed equally to this work.

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polymorphism in chickens and other species, we cloned and analyzed the genes encoding the MHC I BFIV molecule from four Chinese native chicken breeds, Qingyuan Partridge (QYP), Wenchang (WC), Huainai Partridge (HNP), and Huaibei Partridge (HBP), with diverse features and distributed in four different areas in China.

2. Materials and methods

2.1. Animals and blood sample collection

The individuals used in this study belonged to four native chicken breeds: QYP, WC, HNP, and HBP. The four breeds are selectively bred over hundreds of years in four different areas of China, extending thousands of miles apart. HBP and HNP originate from eastern China, Anhui Province, while QYP and WC original from southern China, Guangdong Province and Hainan Province, respectively. These breeds have different features, and they are considered separate, distinct local breeds. Blood was collected from the brachial vein of the chickens by venipuncture, using citrated syringes during a routine health inspection. Peripheral blood mononuclear cells (PBMCs) were isolated from the venous blood.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted using TRIZOL Reagent (Invitrogen) according to the Manufacturer's protocol. RNA integrity was verified by gel electrophoresis, and the purity was determined by ultraviolet absorbance at 260 and 280 nm. Next, using a cDNA synthesis kit (TaKaRa Biotechnology, Dalian, China), total RNA was reverse transcribed to cDNA with oligo (dT) primers as described in the manufacturer's guidelines, and the cDNA was stored at −20 °C until further use.

2.3. PCR amplification of the BFIV gene

The primers for PCR were designed using Oligo6.0 software, according to the complete cDNA sequence of a known chicken MHC class I BFIV glycoprotein (GenBank accession no. S78682), to amplify overlapping conserved regions from the 5′UTR to the 3′UTR. The sense primer sequence was 5′-GAGAGTGCAGCGGTGCGAGGCGAT-3′. The antisense primer sequence was 5′-AATGCTGGTGTGGACTGTTGGCTC-3′. PCR amplifications were carried out in a 50-μl reaction volume containing 50 ng cDNA, 20 pmol of each primer, 0.25 mM of each dNTP, 1.5 units of *Pyrobest*™ DNA polymerase (Takara), and 5 μl of 10 × *Pyrobest* Buffer II. The PCR was performed by an initial denaturation at 95 °C for 5 min, 30 amplification cycles of 1 min denaturation at 95 °C, 1 min annealing at 67 °C, and 2 min extension at 72 °C, and terminated by a final extension step at 72 °C for 10 min. The PCR fragments were cloned in a vector (TA-cloning Kit, Takara). Nucleotide sequencing was performed by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). All sequences were obtained from randomly chosen clones, and the nucleotide data were obtained by sequencing the forward and reverse strands.

2.4. Analysis of chicken BFIV gene

Multiple nucleotide or amino acid sequence alignments were performed using Clustal X 1.83 (Thompson et al., 1997). A phylogenetic tree from the predicted amino acid sequences was constructed using the neighbor-joining method in MEGA 4.1 (Tamura et al., 2007). The rates of nonsynonymous (dN) and synonymous (dS) substitutions were estimated using MEGA 4.1, and positive selection was inferred by a modified version of the Nei-Gojobori method (Nei and Gojobori, 1986) with the P-distance parameter. The sequence variability per site was computed using two different metrics: the Shannon diversity index (Shannon, 1948) and the Wu-Kabat variability coefficient (Wu and Kabat, 1970). The amino acid residues interacted with peptides were based on these methods, described by Shannon and Wu-Kabat (Reche and Reinherz, 2003).

2.5. Three-dimensional (3-D) structure modeling of PBR in MHC I molecules

The 3-D protein structures of MHC I molecules were modeled using the SWISS-MODEL web server (http://www.expasy.ch/swissmod/SWISS-MODEL.html), which allows a search for similar templates of available structures from the Protein Data Bank (PDB). The MHC I molecules of chicken and human were based on the X-ray structure of BFIV (PDB ID 3bev.1) (Koch et al., 2007) and HLA-A*0301 (PDB ID 3rl2.1) (Zhang et al., 2011), respectively. SWISS-Pdb viewer was used to generate a three-dimensional image.

3. Results and discussion

3.1. Characterization of BFIV genes of the four chicken breeds

According to the unique amino acid residues of chicken MHC class I molecule (Shaw et al., 2007; Livant et al., 2004), 102 nucleotide sequences of *BFIV* genes were obtained from individuals of the QYP, WC, HBP, and HNP breeds of chicken. *BFIV* genes included a complete coding sequence (CDS) of 1068 bp or 1035 bp and were composed of 7 or 8 exons. Some sequences were 33 bp shorter than the others and lacked exon 7. The signal peptide (SIG), α1, α2, α3, and TM/CY regions are encoded by exon 1, exon 2, exon 3, exon 4, and exon 5–8, respectively.

We found 89 *BFIV* alleles in the nucleotide sequences, and 13 repeated alleles were observed in the remaining sequences of *BFIV* genes. The GenBank accession numbers for a representative sequence from each of the QYP, HBP, WC and HNP breeds were HQ435242-HQ435264, HQ435265-HQ435289, HQ435290-HQ435309, and KM014703-KM014730, respectively (Fig. 1). A phylogram was created based on the deduced amino acid sequences of *BFIV* alleles. The result indicated that the alleles from the four breeds converged into five major groups, which we designated as clusters A, B, C, D, and E. Clusters A, B, C, D, and E included 25, 21, 15, 3, and 25 alleles, respectively, accounting for 28.1%, 23.6%, 16.9%, 3.4%, and 28.1% of the total alleles. Moreover, the phylogram showed clearly that the alleles did not cluster together, on the basis of geographical distances. Some alleles of the same breed were spread throughout the phylogenetic tree. There was no striking correlation between geographical distance and genotype cluster among the four breeds. Similar results have been reported from other studies. Even with the high level of MHC genetic diversity in natural populations of European and Asian brown hares (*Lepus europaeus*), a strong phylogeographic signal in the full-length exon 2 *eeu-DQA* alleles was not found (Koutsogiannouli et al., 2009). However, three MHC alleles (*DQB*, *DRA*, and *MHC-I*) of cetaceans also showed the similarities in their phylogenies (Xu et al., 2010a).

3.2. Most polymorphic sites in PBRs

The *BFIV* gene exhibited a high level of polymorphism, and most of these mutations were nonsynonymous. The dN values in the PBRs were 2.4–4.7 times higher than the values in the non-PBRs from the respective breeds (Table 1). In addition, the PBRs and full-length sequences showed high dN/dS ratios that were greater than 1 in four chicken breeds. As predicted under a paradigm of pathogen-mediated selection, a comparison of dN/dS ratios found

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