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Establishment of six homozygous MHC-*B* haplotype populations associated with susceptibility to Marek's disease in Chinese specific pathogen-free BWEL chickens

Caixia Gao [a], Lingxia Han [a,*], Jianlin Han [b], Jiasen Liu [a], Qian Jiang [a], Dongchun Guo [a], Liandong Qu [a,*]

[a] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Harbin 150001, China
[b] CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100193, China

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ABSTRACT

The highly polymorphic chicken major histocompatibility complex (MHC) is associated with different levels of immunologic responses to certain avian pathogens. MHC-*B* haplotype chickens are an important genetic resource for studying the genetic determination of pathogen resistance and susceptibility. The BWEL chicken population is the only specific pathogen-free (SPF) chickens bred and developed by the State Center of Poultry Genetic Resources of Laboratory Animals in China. In this study, we successfully established six homozygous MHC-*B* haplotype populations from the BWEL chickens using microsatellite marker technology, named as BW/G(1, 2, 3, 5, 6, 7) lines, and their molecular genotypes were matched to six serologically defined MHC-*B* haplotypes, B13, B15, B2, B5, B21 and B19, respectively. The sequences of *BF* genes exons 2 and 3 from four successive generations (F1–F4) of the BW/G(*n*) lines were completely consistent with those of serologically defined MHC-*B* haplotypes. Subsequently, six BW/G(*n*) line specific allo-antisera were prepared by immunization with red blood cells (RBCs) and hemagglutination tests results showed the BW/G(*n*) SPF chickens could be serologically differentiated. Additionally, susceptibility to Marek's disease (MD) in the BW/G3 (B2 haplotype) and BW/G7 (B19 haplotype) lines were determined by comparing mortality, macroscopic and histopathological lesions, and viral loads in feather pulp. The BW/G7 line showed greater genetic susceptibility to the very virulent MD virus (MDV) strain than the BW/G3 line. The establishment of MHC-*B* haplotype chicken populations associated with susceptibility to MD will be helpful for studying host immune responses and further developing the more effective vaccines in the context of MHC specificities, and they are also very useful for an understanding of MHC genes architecture and function.

1. Introduction

Chicken major histocompatibility complex (MHC) is the first non-mammalian MHC to be sequenced. It is compact, simple, considerably smaller than its mammalian counterpart, and strongly associated with resistance and susceptibility to certain avian diseases, including Marek's disease (MD) (Bacon et al., 2001), Rous sarcoma (Taylor, 2004), lymphoid leukosis (Bacon et al., 1981), infectious bronchitis (Banat et al., 2013), coccidiosis (Miller et al., 1994), and *salmonellosis* (Cotter et al., 1998). The chicken MHC comprises two regions, the MHC-*B* and the MHC-*Y* regions, both of which map to chromosome 16 (Miller et al., 2004). The MHC-*B* region includes three distinct loci, *BF*, *BLB* and *BG*, which encode MHC class I, class II and class IV molecules, respectively (Miller et al., 2004). It consists of at least 46 genes (Shiina et al., 2007), most of which are well known to encode a set of key proteins that regulate aspects of the host immune response system (Delany et al., 2009). Chicken MHC-*B* was first identified as a polymorphic blood group system based on the antigenic reaction of red blood cells (RBCs) (Briles et al., 1950), which led to the first serological method for identifying MHC-*B* haplotypes using a series of chicken allo-antisera. Eleven commonly encountered haplotypes (B2, B4, B5, B6, B7, B12, B13, B14, B15, B19 and B21), 16 less common

Abbreviations: MHC, major histocompatibility complex; SPF, specific pathogen-free; RBCs, red blood cells; MD, Marek's disease; MDV, MD virus; HVRI, Harbin Veterinary Research Institute; CAAS, Chinese Academy of Agricultural Sciences; HEPA, high-efficiency particulate air; PCR, polymerase chain reaction; PFU, plaque forming units; HVT, herpesvirus of turkey; WPC, weeks post challenge; DPC, days post challenge.

* Corresponding authors. Tel.: +86 18946066097; fax: +86 451 51997176 (L. Han). Tel.: +86 18946066123; fax: +86 451 51997176 (L. Qu).
E-mail addresses: hlx1993@126.com (L. Han), qld@hvri.ac.cn (L. Qu).

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haplotypes (B1, B3, B8, B9, B10, B11, B17, B18, B22, B23, B24, B25, B26, B27, B28 and B29) and several recombinant haplotypes have been serotyped by standard hemagglutination assays (Briles et al., 1982; Briles and Briles, 1982) and their serological reactivities were confirmed to be due to BF and BG antigens. However, cross-reactivity between different MHC-*B* haplotypes, the difficulty of typing outbred chicken populations (Kroemer et al., 1990) and the paucity of chicken MHC-*B* haplotype specific antisera (Fulton et al., 1996) have limited the use of the serological typing method. Therefore, in addition to the typical serological hemagglutination assay, other accurate and effective molecular biological methods are needed to identify chicken MHC-*B* haplotypes.

Microsatellite markers provide a very useful and practical genotyping method for large numbers of samples. Four microsatellite markers have been located within the chicken MHC-*B* region, LEI0258, MCW0371, MCW0312 and MHC-D (Buitenhuis et al., 2003; Crooijmans et al., 1997; Fulton et al., 2006; McConnell et al., 1999; Sironi et al., 2011), which can be effectively used to determine MHC-*B* haplotypes. Especially, LEI0258 marker genotypes could provide good indications of MHC-*B* haplotypes and can be used for preliminary screening in chicken populations (Chazara et al., 2013, 2011; Fulton et al., 2006; Nikbakht et al., 2013). It has been increasingly used in research and breeding for planning crosses and has become an important genetic factor to take into account in chicken breeding. Other molecular techniques for identifying MHC-*B* alleles rely directly on polymorphisms in the actual MHC *BF*, *BLB* or *BG* genes. This method can be used to confirm the characterization and homozygosis of MHC-*B* haplotypes.

MD, caused by MD virus (MDV), is a highly cell-associated lymphotropic alphaherpesvirus that induces different clinical signs in chicken breeds with different genetic backgrounds, especially in relation to MHC genes (Bacon et al., 2001; Schat et al., 1994). For example, B21 chickens are highly resistant to MD while B19 chickens are highly susceptible. The severity of infection may thus depend partly on the genetic background of the infected chickens, including differences in immune responses (Dawes et al., 2014; Garcia-Camacho et al., 2003; Kaiser et al., 2003; Omar and Schat, 1997; Sarson et al., 2008). Furthermore, MHC-*B* haplotypes also played a very important role in vaccinal immunity against MD in both experimental and commercial chicken strains (Chang et al., 2010). B15 haplotype chickens developed intermediate protection against MD compared with B2 or B13 haplotype after vaccination with either serotype 1, 2 or 3. Further, B5 and B21 chickens developed variable protection after vaccination with different MD vaccines of serotypes 1, 2 and 3 (Bacon and Witter, 1993). Therefore, identification of the genetic factors affecting resistance/susceptibility to pathogens would improve selection schemes for the development of resistant flocks of chickens.

Various MHC-*B* haplotype chicken breed populations have been developed and used worldwide. For example, a series of MHC congenic lines with different MHC-*B* haplotypes had been developed at the Avian Disease and Oncology Lab in the late 1970s (Bacon et al., 2000). However, there has been no such genetic resource in China to date, which has greatly restricted the study of the structure and function of MHC genes. Therefore, in this study, we attempted to establish the homozygous MHC-*B* haplotype chicken lines from BWEL specific pathogen-free (SPF) chickens using microsatellite marker technology. Sequencing of *BF* genes polymorphic exons and hemagglutination tests using allo-antisera specific to the established chicken lines were subsequently performed to confirm the characterization of the MHC-*B* haplotypes. Finally, susceptibility of the BW/G3 (B2 haplotype) and BW/G7 (B19 haplotype) lines to MD were compared by examining mortality, macroscopic and histopathological lesions, and viral loads in feather pulp. The MHC-*B* haplotype SPF chicken lines associated with susceptibility to MD can be used as valuable animal models for clarifying the molecular basis of disease susceptibility and host immune responses.

2. Materials and methods

2.1. Chickens

BWEL chicken, originated from the Beijing White chicken which contains both Chinese native chicken breed and White Leghorn blood, is an important genetic resource of the Chinese State Resource Center of Poultry Laboratory Animal, which is affiliated with the Harbin Veterinary Research Institute (HVRI) of the Chinese Academy of Agricultural Sciences (CAAS). BWEL chickens have been free of 19 major avian diseases as well as their corresponding antibodies, including *Salmonella pullorum*, avian influenza (type A), infectious bronchitis, infectious bursal disease, infectious laryngotracheitis, Newcastle disease, fowlpox, MD, *Haemophilus paragallinarum*, *Pasteurella multocida*, avian adenovirus group III (EDS_{76}), *Mycoplasma Gallisepticum*, *Mycoplasma Synoviae*, avian encephalomyelitis, lymphoid leukosis, reticuloendotheliosis, avian reovirus, avian adenovirus group I and chicken infectious anemia. All the chickens were kept in positive air pressure-isolators with a high-efficiency particulate air (HEPA) filtration system from one day of age and throughout their lifespan. Generally, each isolator held three cocks and 24 hens. A circular mating pattern has been adopted among the original eight BWEL families from generations F1 to F14 (Fig. 1). Five of the alleles of 14 microsatellite loci (35.7%) randomly mapped in chromosomes had been fixed at single alleles or approached fixation with single predominant alleles (>0.9) according to a previous population genetic study of the F15 generation, with significant reductions in general allele variations within the population (Xiao et al., 2010).

2.2. Genotyping and restructuring of populations

Blood samples were obtained from a total of 498 BWEL SPF chickens from the F15 generation. Genomic DNA was extracted from whole blood samples using the conventional phenol–chloroform extraction method. The LEI0258 allele was genotyped by

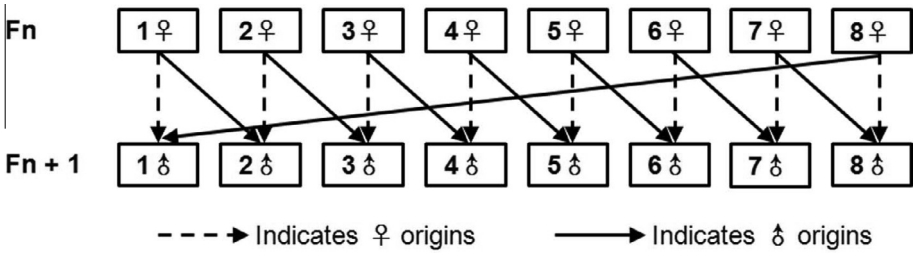

Fig. 1. Circular mating scheme for eight chicken families from F1 to F14 generations. Fn: the n^{th} progeny, F*n* + 1, the next progeny after the n^{th}. Arabic numbers indicate the different families.