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Research Article

Molecular Genotype Identification of Different Chickens: Major Histocompatibility Complex

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Abstract. Chicken is a main poultry in China. Molecular breeding for disease resistance plays an important role in the control of diseases, especially infectious diseases. Choice of genes for disease resistance is the key technology of molecular breeding. The major histocompatibility complex (MHC) is of great interest to poultry breeding scientists for its extraordinary polymorphism and close relation with traits of resistance against infectious diseases. The MHC-B haplotype plays an important role in the study of disease resistance in chicken. The traditional chicken MHC-B haplotype is commonly defined by serologic reactions of erythrocytes and the majority of studies have been conducted in Leghorn and broiler but study about other chicken breeds is little. In this study, firstly, the microsatellite marker LEI0258 which is located within the MHC was sequenced by using target sequence capture assay in different chicken breeds, and then according to the number of repeated structures and polymorphic sequences in microsatellite, sequence information for the region defined by LEI0258 was obtained for different haplotypes. Afterwards, we identified the relation between MHC-B haplotypes and disease resistance. Collectively, these observed results provided the reference data for disease-resistant breeding association with blood type and for further study of MHC gene function in poultry.

Keywords: Chicken, MHC, Microsatellite, blood type, resistant traits

1. Introduction

The chicken major histocompatibility complex (MHC) is known to have a very strong association with disease resistance and susceptibility to numerous pathogens including Marek's disease virus [1–3], Rous sarcoma tumor virus [4, 5], avian leukosis virus [6], fowl cholera [7], coccidiosis [8], and salmonella [9]. Thus, the chicken MHC gene is the preferred marker gene in immune genetics and breeding for disease resistance [10]. Therefore, there is considerable interest in accurate identification of MHC types in chickens.

The chicken MHC first was found as blood type group locus, also known as the B complex [11]. B blood type group locus has a relationship with control tissue compatibility [12]. It and the nucleolar organizer region (NOR) linked and highly polymorphic and contain the B locus and Rfp-Y locus; they are localized on chromosome 16, but the two loci are genetically unlinked [13–15]. The B complex is mainly composed of BF, BL, and BG loci in 3 different functions. BF and BL antigens were similar to those Class I and class II antigens of mammalian MHC in structure and function. BF antigen is present in all cells and the BL antigen only exists on the surface of some lymphocytes; they are mainly

factors deciding chicken rejection response. While the BG antigen is unique to birds, only at the surface of red blood cells, reflecting the different blood group, and irrelevant with allograft rejection response, BG (major) and BF (secondary) antigens are common code for the same antigen existing on red cell surface that is the basis for distinguishing B haplotypes using agglutination reaction.

The chicken MHC is commonly identified with polyclonal antisera produced by immunizations between birds having different haplotypes [16, 17]. The MHC haplotype nomenclature was standardized initially using serologic reagents [18]. But traditional serology for MHC identification has several limitations, including subjectivity in interpretation of serological reactions and expertise required for production of new reagents. In addition, the cost and time involved in serological reagent development are huge. With the advent of molecular biology tools, some methods can be used to determine B haplotypes, such as two-dimensional (2D) gels, restriction fragment length polymorphism (RFLP), DNA sequence, single strand conformation polymorphism (SSCP), and sequence-specific polymerase chain reaction (SS-PCR). These methods identify either protein or DNA differences within much defined regions producing very consistent and repeatable results among labs. Unfortunately, these techniques are not always practical for large numbers of samples [19].

Fulton (2006) [19] used two microsatellite (LEI0258 and MCW0371) markers in the B region for MHC polymorphism typing, and had found that these two markers have association with blood type and genetic resistance. Compared with microsatellite MCW0371, LEI0258 has more genetic variation, so it is our main research object. For LEI0258, two internal repeats whose lengths were 13 and 12 base pair (bp), respectively, are the primary basis for allelic variability. Allele size variation ranges from 182 to 552 bp. Four indels in all kinds of chicken and five single nucleotide polymorphisms (SNPs) in the surrounding sequence provide additional means for distinguishing alleles. The association between LEI0258 allele and serologically defined MHC haplotype was very consistent for the same haplotype from multiple sources. Fulton (2006) focused on examining LEI0258 alleles from serologically defined MHC sources and commercial egg-laying chickens to discriminate haplotypes. Chinese local chicken species show abundant genetic variation, but the study of blood type and resistance has not been reported. In this study, we sequenced microsatellite marker LEI0258 of 26 chicken breeds by NimbleGen Sequence Capture technology (Roche, Switzerland). Here, the chicken breeds we used include red jungle fowl (from wild), some broilers, Leghorn (specific pathogen free, SPF), and many Chinese indigenous breeds. Subsequently, MHC genetic polymorphism was analyzed using microsatellite technology and different haplotypes were obtained for different chicken breeds. Lastly, we discuss the relation between MHC-B haplotypes and disease resistance. Finally we provided the reference data for disease-resistant breeding association with blood type and for further study of MHC gene function in poultry.

2. Materials and Methods

In this study, we selected 26 chicken breeds for target capture sequencing, including Xianju chicken (XJ), Chahua chicken (CH), Luyuan chicken (Lu), Baier chicken (BE), Tibetan chicken (ZJ), Gushi chicken (GS), Dagu chicken (DG), Gamecock (DJ), Langshan chicken (LS), Silky fowl (WG), Xiaoshan chicken (XS), and Beijing fatty chicken (BY) that were from national local bird resources gene pool of Poultry Science Institute in Chinese Research Institute of Agricultural Science. Red jungle fowl (HY) collected from wildlife rescue center in Yunnan Province and the other 13 chicken breeds, Ross chicken (Ross), AA chicken (AA), Recessive white feather chicken (YB), Anka chicken (AK), Leghorn (LH), Rucao chicken (RC), Xueshan grass chicken (XC), Wenchang chicken (WC), Taihu chicken (TH), Liyang chicken (LY), Green shelled egg chicken (LD), Guangxi yellow chicken (GH), and Partridge shank chicken (QM), were from the avian species gene pool in our lab (Genetic Resources Laboratory from College of Animal Science and Technology in Yangzhou University). For each chicken breed, we collected 10 birds, and blood samples were obtained via wing vein of each bird. DNA was extracted from blood. The mixed DNA samples of 10 birds within the same breed were sent to BGI Company, Shenzhen, China, for target capture sequencing with selecting Gallus gallus-4.0 as a reference genome.

2.1. Capture and Sequencing of Genomic DNA. Genomic DNA was captured by hybridization in solution to custom-designed cRNA oligonucleotide baits following the manufacturer's protocols (Roche, Switzerland). The raw data of sequencing results are filtered by the following steps. Firstly, we removed the joint pollution in reads. Secondly, we removed the low quality bases (quality value $\leq$ 5 (E)). Through these two steps, we can ensure the high quality of the data.

2.2. Analysis of LEI0258 Marker Sequence in Different Chicken Breeds. According to sequence information of chicken breeds in this study and reference sequence from the NCBI, complete sequences of LEI0258 marker with SNP sites were aligned by using clustalW function of MEGA software.

2.3. Data Analysis. The MHC gene of different chicken breeds can be obtained by the target capture sequencing. The microsatellite marker LEI0258 is located in MHC gene based on what has been previously reported in the literature. Based on the obtained LEI0258 microsatellite sequences in 28 different chicken breeds containing SNP sites, we can

determine different haplotypes according to the number of repeated structure and polymorphic of sequence in LEI0258 microsatellite. The relation between MHC-B haplotypes and disease resistance can be obtained according to previous research (Table 2). We can preliminarily understand the relationship between Chinese local chicken breeds and disease resistance traits. It also can provide the reference data for disease-resistant breeding.

3. Results and Discussions

3.1. The Characteristics of Different Local Chicken Breeds. Chinese local chicken breeds are very much and have their own characteristics. We can put them into the following categories according to their use and their characteristics: the first class for the egg type chickens, including Xianju chicken (XJ), Baier chicken (BE), and Green shelled egg chicken (LD); the second for meat type breeds, including Liyang chicken (LY) and Partridge shank chicken (QM); dual purpose varieties with meat and eggs, including Langshan chicken (LS), Dagu chicken (DG), Beijing fatty chicken (BY), Gushi chicken (GS), Luyuan chicken (Lu), Xiaoshan chicken (XS), Xueshan grass chicken (XC), Rucao chicken (RC), Taihu chicken (TH), and Guangxi yellow chicken (GH). The variety of medicinal chicken is Silky fowl (WG). Other uses of the chicken are Tibetan chicken (ZJ), Chahua chicken (CH), and Gamecock (DJ). Local chicken breeds with high resistance are Gushi chicken (GS), Xueshan grass chicken (XC), and Green shelled egg chicken (LD). These chickens can be used for further research as the high disease-resistance variety resources.

3.2. The Characteristics of LEI0258 Marker. The microsatellite marker LEI0258 (accession no. Z83781) [20] maps to chromosome 16. This marker is located in LOC768783 that belongs to one of the 26 genes on chicken MHC (GenBank AL023516). The LEI0258 alleles show considerable diversity in size not expected for the typical microsatellite marker. Their sequence information including the repeat region and the two flanking regions surrounding the repeat are summarized in Table 1. The entire LEI0258 allele was sequenced, numbered from –78 to –1 for the region immediately upstream of the repeat and 1 to 88 for the region including the last repeat and downstream through the reverse primer. The repeat region consists of two independent repeat elements, a 13 bp repeat of "CTATGTCTTCTTT" and a 12 bp repeat of "CTTTCCTTCTTT". These repeats were replicated from 1 to 28 times for the R13 repeat and from 2 to 20 times for the R12 repeat [19]. In addition to the variation in the number of copies of the R13 and R12 repeats, nine polymorphisms were observed within the flanking sequences. Four polymorphisms were observed in the region upstream of the repeat structure: two insertion or deletion polymorphisms (indels) and two SNPs. Five polymorphisms were observed in the region downstream of the repeat structure: two indels and three SNPs [19].

3.3. Different MHC Haplotypes for Different Chickens. According to the previously obtained haplotypes using LEI0258 allele sequence information [19], we can conclude the repeats numbers and the polymorphisms of different chicken breeds, and define the different MHC-B haplotype for different chickens. The results are shown in Table 1.

The defined MHC-B haplotypes of 26 different chicken breeds in Table 1 were based on those previously known haplotypes using serological and molecular biology methods. As shown in Table 1, different chicken breeds presented structural differences in LEI0258 marker. Firstly, the number of repeat structures was different and this was also what we focused on. All chicken breeds were divided into six categories due to the different structural numbers. All kinds of chicken had a R13 repeat structure with the same number. R12 repeat structure was not the same and 17 numbers of repetitive structure existed in most of the chickens. In addition, some indels and SNPs existed in the upstream and downstream of repeat structure. The existence of these polymorphism loci was helpful to determine haplotypes.

These identified haplotypes are mainly based on the number of repeated structure, and these haplotypes have appeared in the prior literature [19]. As shown in Table 1, a kind of chicken has a variety of haplotypes; for example, Rucao chicken (RC) has haplotypes of BC/B21.1/BQ/BW1.These haplotypes are similar in the sequence characteristic so that it is difficult to determine the haplotype accurately. In addition, some haplotypes are not previously reported, such as the haplotypes of Chahua chicken (CH) and Tibetan chicken (ZJ). These haplotypes are different from other chickens in the repeat structure. There may be some new haplotypes, and they may have some relations with their characters. Chahua chicken (CH) is unique rare species in China with strong adaptiveness, strong resistance, and high survival rate. Tibetan chicken (ZJ) is an important breed resource which has good ability of adaptivity to high altitude and cold climate. Further studies are needed for unique characteristics of these chickens. Although these results are not particularly accurate reflecting the haplotypes of different chicken, they provide useful reference data for Chinese local chicken that has not been studied before.

3.4. The Relation between MHC-B Haplotypes and Disease Resistance. The MHC-B haplotypes have important significance in the study of disease resistance in chicken. There have been reports that these haplotypes have relation with the resistance to Marek's disease (MD), avian Influenza (AI), Rous sarcoma disease (RS), avian leukosis (AL), infectious bursal disease (IBD), avian infectious bronchitis (IB), *salmonella enteritidis* (*S. enteritidis*), *Escherichia coli* (*E. coli*) and other bacterial diseases. In addition, a number of studies have demonstrated that avian MHC is highly

Table 1: Polymorphisms identified within the LEI0258 alleles of defined MHC haplotypes in different chickens.

Breeds[a]	Upstream polymorphism				R13	R12	Downstream polymorphism					B haplotype
	−61Δ[b]	−30–29TT	−28G	−11G	1		5C	23-29ATTTGAG	33Δ	39A	46T	
WG	G	∘[c]	Δ	∘	1	7	∘	∘	A	T	A	B18
ZJ	G	∘	∘	∘	1	9	∘	Δ	A	T	A	no defined
CH	G	∘	∘	∘	1	10	∘	∘	A	T	A	no defined
LH	G	∘	∘	∘	1	11	∘	∘	A	T	A	B5
LY	G	∘	∘	∘	1	12	∘	∘	A	T	A	B10/B24/B26/B76
GH	G	∘	∘	∘	1	12	∘	Δ	A	T	A	B72/B78
BE	G	∘	Δ	∘	1	12	∘	Δ	A	T	A	B72/B78
Ross	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
GS	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
XC	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
XS	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
YB	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
Lu	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
TH	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
LD	G	∘	∘	∘	1	17	∘	∘	A	T	A	B21.1/BQ/BW1
AK	G	ΔΔ	∘	∘	1	17	∘	∘	A	T	A	BC
DG	G	ΔΔ	∘	∘	1	17	∘	∘	A	T	A	BC
WC	G	ΔΔ	∘	∘	1	17	∘	∘	A	T	A	BC
QM	G	ΔΔ	∘	∘	1	17	∘	Δ	A	T	A	BC
DJ	G	∘	Δ	∘	1	17	∘	∘	A	T	A	BC/B21.1/BQ/BW1
HY	G	∘	Δ	∘	1	17	∘	Δ	A	T	A	BC/B21.1/BQ/BW1
XJ	G	∘	Δ	∘	1	17	∘	∘	A	T	A	BC/B21.1/BQ/BW1
LS	G	∘	∘	∘	1	17	∘	Δ	A	T	A	BC/B21.1/BQ/BW1
RC	G	∘	∘	∘	1	17	∘	Δ	A	T	A	BC/B21.1/BQ/BW1
BY	G	∘	∘	∘	1	17	∘	Δ	A	T	A	BC/B21.1/BQ/BW1
AA	G	∘	∘	∘	1	17	∘	Δ	A	T	A	BC/B21.1/BQ/BW1

[a]The abbreviation of chicken breeds. [b]Δ Defined deletion compared with the reference sequence. [c]∘ is consistent with the reference sequence.

related to the genetic resistance of multiple diseases. Garcia-Camacho (2003) found the activity increase of chicken MHC restricted natural killer cells (NK) that can enhance resistance to Marek's disease virus (MDV) [21]. Vukmanovi (2003) found that the antagonistic effect of autologous peptide on T cell activation can explain some connection between MHC allele and specific chronic diseases [22].

As can be seen from Table 2, some haplotypes have a strong resistance such as B2, B12, and B21, and some have poor resistance such as B5, B13, B15, and B19. For example, B5 and B13 showed significant susceptibility to specific virus. But for the majority of haplotypes, they are related to different resistance for different diseases. For instance, B2 haplotype is resistant to MD, IBD, and RS and is susceptible to ALV; B21 haplotype has strong resistance to MD, AI, and IBD. But for AL, IB, and *Escherichia coli*, B21 haplotype shows weak resistance; B12 is resistant to AL and RS and is susceptible to IBDV; B19 is resistant to AL but is susceptibility to MDV and IBDV.

B21 haplotype exists in most local chicken breeds and these can be seen in Table 1. As can be seen from Table 2, B21 haplotype is resistant to many diseases. These results explain that the local chicken breeds are not susceptible to disease compared with those commercial varieties. Based on the relationship between disease resistance and local chicken haplotypes, the resistant blood type haplotypes (such as B21) can be a candidate marker for high resistance and these will be applied to breeding for disease resistance if the resistant haplotype can be inherited stably. Furthermore, for those not defined haplotypes, we can continue to carry out experiment for verifying their resistance to disease. All these can provide useful basis for breeding resistant chicken.

Table 2: The relation between MHC-B haplotypes and disease resistance.

B haplotype	Disease	The association with disease	References
B23	MD	Susceptibility	Schat [23]
B21	MD	Strong resistance	Briles [24] Blankert [25]
	AI	Resistance	Boonyanuwat [26]
	IBD	Good immune response	Bacon [27]
	AL	Poor resistance	Bacon [4]
	IB	Susceptibility	Bacon [28]
	E. coli	susceptibility	Macklin [29]
B19	MD	Susceptibility	Briles [24] Blankert [25]
	IB	Susceptibility	Juul-Madsen [30]
	AL	Strong resistance	Bacon [4]
B18	SE	Susceptibility	Cotter [31]
B17	MD	Poor resistance	Schat [23]
B15	MD	Susceptibility	Briles [24]
	IBD	Poor immune responses	Bacon [27]
	AL	Poor resistance	Bacon [4]
	RS	Susceptibility	Nordskog [32]
	IB	Resistance	Bacon [28]
	SE	Susceptibility	Cotter [31]
B13	MD	Susceptibility	Briles [24]
	AI	Susceptibility	Boonyanuwat [26]
	IBD	Moderate resistance	Bacon [27]
	AL	Poor resistance	Bacon [4]
	RS	Poor resistance	Schierman [33]
	IB	Poor resistance	Bacon [4]
	E. coli	Resistance	Macklin [29]
B12	IBD	Poor immune responses	Bacon [27]
	AL	Strong resistance	Bacon [4]
	RS	Suppressing tumor growth	Plachy [34]
B6	MD	Resistance	Briles [24]
	RS	Associated with tumor regression	Schierman [33]
B5	MD	Susceptibility	Briles [24]
	IBD	Poor immune responses	Bacon [27]
	AL	Poor resistance	Plachy [34]
	RS	Susceptibility	Collins [35]
B4	IBD	Strong resistance	Juul-Madsen [30]
B3	MD	Susceptibility	Briles [24]
B2	MD	Resistance	Briles [24]
	IBD	Good immune response	Bacon [27]
	AL	Susceptibility	Bacon [4]
	RS	Susceptibility	Collins [35]
B1	RS	Promote tumor growth	Nordskog [32]
	SE	High mortality rate	Pevzner [36]

4. Ethics Statement

Experiments were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals of Yangzhou University (Yangzhou University, China, 2012) and the Standards for the Administration of Experimental Practices (Jiangsu, China, 2008). All operations were performed according to recommendations proposed by the European Commission (1997).

5. Conclusions

This research focused on the sequence of LEI0258 marker in different varieties of chicken, which can be sequenced by the target capture sequencing. We could define different haplotypes of different chickens according to the number of repeated structures and polymorphism of microsatellite sequences and we obtained the relation between MHC-B haplotypes and disease resistance. The observed results showed the most Chinese local chicken breeds had resistant haplotypes (B21) and these blood type haplotypes can be a candidate marker of high resistance for breeding. Some undefined haplotypes were also observed that may have some association with disease resistance and need further experimental verification. All results can provide the reference data for disease-resistant breeding and MHC gene function study in poultry.

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