

Full Length Research Paper

cDNA cloning and polymorphic domains of the major histocompatibility complex (MHC) class I α in two Chinese native chicken breeds

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Major histocompatibility complex (MHC) is a highly polymorphic gene and plays an important role in immune system for vertebrate. To understand the polymorphism character of domestic, we cloned 32 cDNAs of MHC class I α genes of two local chicken breeds in different areas of China. There were 112 variable amino acid residues in all five domains (leader peptide, $\alpha 1$, $\alpha 2$, $\alpha 3$ and TM/CY domains) of the putative α chain, and 76 of them were located in $\alpha 1$ and $\alpha 2$ domains. There were 23 to 25 polymorphic sites with high mutation frequency in $\alpha 1$ and $\alpha 2$ domains. Comparison of chicken with duck, human and mouse revealed that the two domains were highly similar among different species, and some highly polymorphic sites were located at the sites 9, 111(114), 113 (116) and 153 (156). Analysis of the phylogenetic tree indicated no relationship between the breeds and polymorphic alleles. All these results therefore indicate that MHC I class molecule of domestic chickens was more influenced by the pressure of common pathogens rather than geographic differences.

Key words: Chinese native chicken, MHC class I α , $\alpha 1$ and $\alpha 2$ domains, polymorphism.

INTRODUCTION

Major histocompatibility complex (MHC) plays a crucial role in the susceptibility/resistance to pathogens for animals and is also a highly polymorphic gene in vertebrate genomes (Takeshima et al., 2009). This kind of polymorphism is the result of natural selection (Furlong and Yang, 2008). In mammals, MHC gene region spans approximately 4 Mb, while in avian it is greatly reduced in size and gene content (Kaufman et al., 1999). The turkey MHC gene is mapped to two distinct regions (B and Y) of a single chromosome (Chaves et al., 2009) and the exon 2 and exon 3 in chicken MHC class I gene encode the $\alpha 1$ and $\alpha 2$ domains (Hunt and Fulton, 1998). The peptide binding domains (PBD) of $\alpha 1$ and $\alpha 2$ domains in MHC gene appears the most polymorphic domain in vertebrate taxa (Silva and Edwards, 2009). A number of studies have focused on studying and analyzing MHC polymorphism and its evolution process in mammals (Furlong

and Yang, 2008; O'Leary et al., 2009), whales (Xu et al., 2009) wild animals (Koutsogiannouli et al., 2009; Becker et al., 2009) and avian (Ewald and Livant, 2004; Westerdahl et al., 2000).

Restriction fragment length polymorphism (RFLP) has been used to identify MHC allele diversity (Westerdahl et al., 2000), and cDNA-PCR was more often applied to sequencing MHC gene (Silva and Edwards, 2009; O'Leary et al., 2009; Becker et al., 2009). Domestic animals have been artificially selected and bred for a long time. Hence, to know the polymorphic MHC character of domestic chicken in the past evolution, we cloned and analyzed the MHC class I α gene from two local breeds, Wenchang (WC) and Huaibei Partridge (HBP) chicken distributed in the two different areas in China and with diversity of produce and form characters.

MATERIALS AND METHODS

Study materials

Individuals of HB and WC chicken used in the study were derived

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from Breeding Center of Feixi Farming Group in the Province of Anhui (China). In most cases, the family members and breeding records were obtained. Peripheral blood mononuclear cells (PBMCs) were separated from venous blood by density gradient centrifugation. From the GenBank database, we retrieved six amino acid sequences of MHC class I α chains from Leghorn chicken, Silky chicken, *Numida meleagris*, duck, human and mouse with corresponding accession numbers; AY989897, AB178042, AB178051, AB115245, NM_002116 and NM_010380, respectively.

RNA extraction, cDNA synthesis and PCR amplification

Total RNA was isolated using TRIZOL Reagent (Invitrogen). Thereafter, first-strand cDNA was constructed in accordance with the cDNA synthesis kit instructions (TaKaRa Biotechnology, Dalian, China). The primers were designed using Oligo6.0 software, based on known complete cDNA sequence of chicken MHC class I glycoprotein (GenBank accession no. S78682); to amplify overlapping conserved domains from the 5'UTR to the 3'UTR. The sense primer sequence was 5'-GAGAGTGCAGCGGTGCGAGGCGAT-3' and the antisense primer sequence was 5'-AATGCTGGTGTGGACTGTTGGCTC-3'. The PCR amplifications were carried out in a 60 μ l reaction volume containing 50 ng cDNA, 20 pmol of each primer, 0.25 mM of each dNTP, 2.5 U of Taq DNA polymerase and 5 μ l of 10 \times PCR reaction buffer. The PCR was performed by an initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 67°C for 1 min and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min. The products were subjected to electrophoresis in a 0.8% agarose gel and examined after ethidium bromide staining. The resulting PCR fragments were then inserted into a vector (TA-cloning Kit, Takara).

Sequencing and handling of MHC class I gene sequences

All sequences were obtained from randomly chosen clones. Nucleotide sequencing was performed by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The nucleotide data were obtained by sequencing the forward and reverse strand. This might remove some results of sequencing artefact. Sequence analysis was aligned using ClustalX 1.83 package (Thompson et al., 1997). Wu-kabat index system (Wu and Kabat, 1970) was applied to identify polymorphism in α 1 and α 2 domains. We modeled three-dimensional model of MHC class I structure using the SWISSMODEL web server (<http://www.expasy.ch/swissmod/SWISS-MODEL.html>), which could search for similar templates of available structures from the protein data bank (PDB), while SWISS-Pdb viewer 4.01 was used to generate a three-dimensional image (Arnold et al., 2006; Schwede et al., 2003; Guex and Peitsch, 1997). In addition, phylogenetic tree of amino acid sequences were created using MEGA3.1 program (Kumar et al., 2004) by neighbor-joining method (Saitou and Nei, 1987) and the bootstrap test was carried out with 1000 iterations.

RESULTS

Alignment of MHC class I α sequences of two Chinese chicken breeds

We cloned 17 and 15 of MHC class I α sequences of HBP and WC breeds, respectively. All the full-length cDNAs contained a complete coding sequence (CDS) including

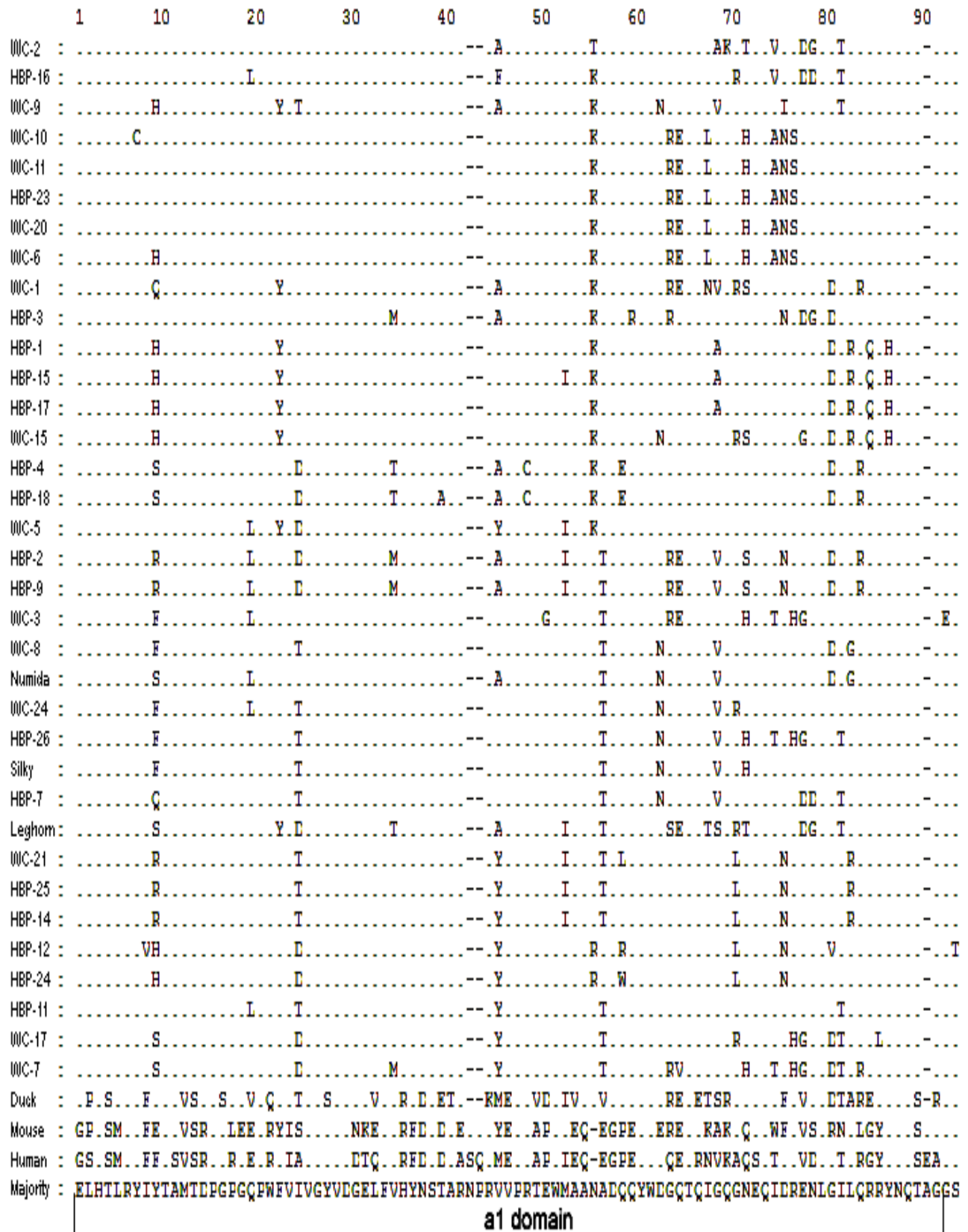
1068 or 1035 bp, and were composed of 7 or 8 exons. Some sequences were shorter by 33 bp by sequencing, which revealed a lack of exon 7. The full-length sequences deduced encoded a 355 or 344 amino acid polypeptide including 21 amino acid residues of a leader peptide, followed by 88 residues of the α 1 domain, 91 residues of the α 2 domain, 91 residues of the α 3 domain and 53 or 64 residues of the TM/CY domain. Alignment of the amino acid sequences revealed 112 variable amino acid sites in α chain, of which 5, 36, 40 and 31 sites were located in the signal peptide, α 1, α 2, α 3 and TM/CY domains respectively. There were 76 variable sites in the α 1 and α 2 domains (Figure 1). The result show that mutations were located mainly in the two domains, and this was similar to other chicken breeds such as Leghorn, Silky chicken and *N. meleagris*.

Polymorphism of the α 1 and α 2 domains

Mutation frequency was divided into three levels by Wu-kabat index analysis: score = 1 indicated no mutation, 1 < score < 4 indicated a low degree of variation, while score \geq 4 indicated a high degree of variation. As shown in Figure 2, a total of 68 amino acid positions were all replaced in α 1 and α 2 domains of MHC class I α in both chicken breeds, accounting for 38% of the total residues in two domains. Moreover, 23 sites had high mutation frequency (score \geq 4) in two domains of HBP, and 10 and 13 of polymorphic sites were in α 1 and α 2 domains, respectively. There were 25 sites with high mutation frequency (score \geq 4) in the two domains of WC, 13 of polymorphic sites in the α 1 domain, and others in the α 2 domain. Further analysis of sequences revealed four and six main peaks (score \geq 8) in the two domains of HBP and WC, respectively; with four main sites (score \geq 8) and all were located at the residues 9, 111, 113 and 153 in both chicken breeds.

High polymorphic sites in α 1 and α 2 domains among different species

Comparison of high polymorphic sites of the α chain revealed a structural similarity between chicken breeds and other species (Table 1). There were six highly variable sites (9, 69, 111, 113, 149 and 153) with scores (\geq 8) in α 1 and α 2 domains of chicken by Wu-kabat index analysis. Similarly, the sites (9, 66, 97, 111 and 113) appeared in duck with high mutation frequency (score \geq 8), which had 28 polymorphic residues (score \geq 4) in α 1 and α 2 domains, 11 of them had scores \geq 8 (Xia et al. 2004). Moreover, the α 1 and α 2 domains of HLA-A and H-D in human and mouse (Bjorkman and Parham 1990; Pullen et al. 1992) revealed nine and 19 sites with highly variable sites (score \geq 8), respectively, including the sites (9, 114, 116 and 156) in human corresponding to the sites (9, 111, 113 and 153) in chicken.



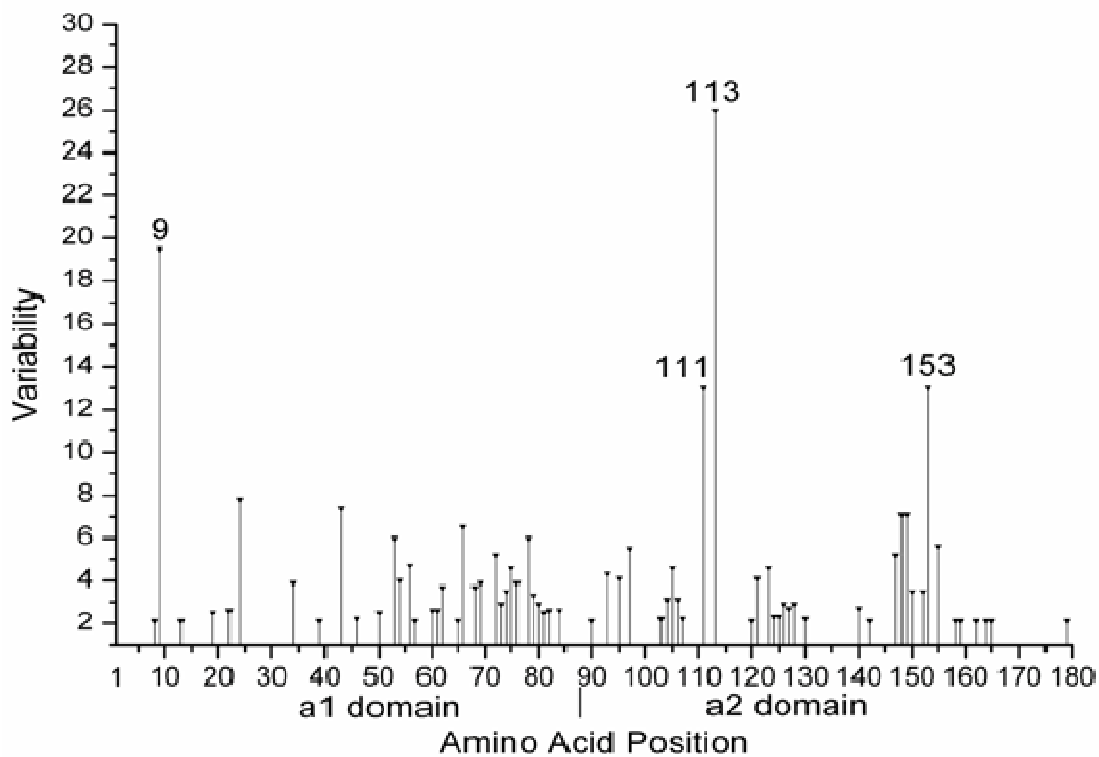
a1 domain

Figure 1. Alignment of amino acid sequences of $\alpha 1$ and $\alpha 2$ domains of MHC class I in chickens, duck, human and mouse. The numbers above the sequences represent amino acid positions. Dots represent identity in amino acid residues. Dashes represent the indel positions in the sequence alignment.

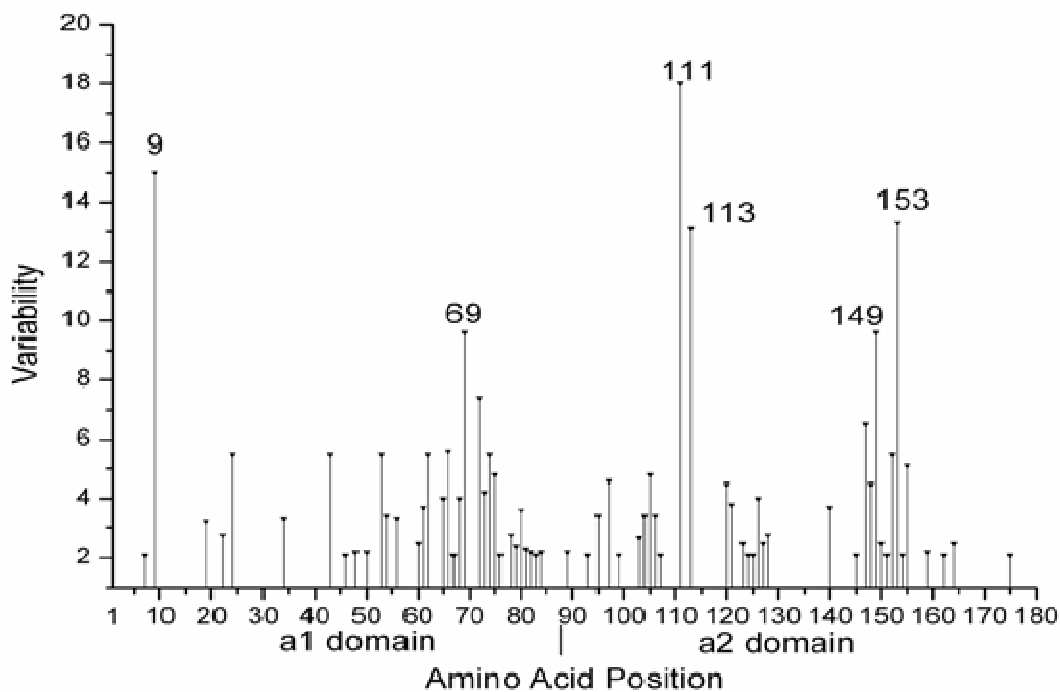
	100	110	120	130	140	150	160	170	180
WOC-2	R	-	S Y	G R	-	E S E P	R N	M	
HBP-16	R	-	S Y	G R	-	E S E P	R N	M	
WOC-9	L	-	S Y	L R	-	V D D	S N	A	
WOC-10		GGSP-	F	L	S	-	V	G	
WOC-11		GGSP-	F	L	S	-	V	G	
HBP-23		GGSP-	F	L	S	-	V R	G	
WOC-20	R	GGSP-	F	L	S	-	VR	G	
WOC-6		GGSP-	F	L	S	-	V	G	
WOC-1		GGSP-	F	L	S	-	V		
HBP-3	L	-	D	G	-	L	M		
HBP-1	A	GSP-	Y M	T G	-	E S E P	R N		
HBP-15	A	GSP-	Y M	T G	-	E S E P	R S		
HBP-17	A	GGSP-	Y M	L M R	G	-	E S E P	R N	
WOC-15		GSP-	Y M	T G	-	V	R S		
HBP-4		GAP-S	Y S	G	S	-	E S E P	R R N	
HBP-18		GAP-S	Y S	G	S	-	E S E P	R R N	
WOC-5		G P-	Y M	T G	G	-	D Y V L		
HBP-2	S	-	A	V G L	-	Y	L		
HBP-9	S	-	A	V G L	-	Y	L		R
WOC-3		-		G	S	-	E Y	L	
WOC-8		-	Y T	G	-		N		
Numida	R N	-	S D	G A R	-	Y	L		
WOC-24	L	-	S	L G	-	L			
HBP-26	L	-	S	L G	-	L			
Silky	L	-	S D	L G M	-	L			
HBP-7	L	-	D	G	-	D Y	L		
Leghorn	L	-	S D		-	D Y	L		
WOC-21	F	-	R S	L M R	-	E S E P	R N		
HBP-25	F	-	R S	L M R	-	E S E P	R N		
HBP-14	F	-	R S	P M R	-	E S E P	R N		
HBP-12	T	-		L A E M R	S	-	E Y	R R	
HBP-24	T	-		L A E M R	S	-	E Y	R R	
HBP-11	T	-		L A E M R	S	-	E Y	R R	
WOC-17	S	-	D R	G	S	-	E Y	R R	R
WOC-7	A	-	S Y	L M R	-	V E Y	R N	A	
Duck	W C H	-	S F Q C G	R L L Y	D A A Q I	T	Q N N I	R S	D V E
Mouse	L I Q S	I G S W R L L	L F E Y	I N E L R W	D M A Q I R	- Q S A	H Y A G E	H L K N	N T L T
Human	I I	V G S R L	R D	K Y I N E L R S W	D M A Q I	- A A H E	Q L R A D G	L N	E T Q T
Majority	HTVQWMYGCDILEDGTIFIRGYHQEAYDGRDFIAFRDRTMTFTAAVPEAVPTKRRKWEQEGGVAEGWRQYLEETCWEVLBRRYVEYGRKAEIGRR								

a2 domain

Figure 1. Contd.



a



b

Figure 2. Wu-Kabat plot of amino acid variability in the $\alpha 1$ and $\alpha 2$ domains of MHC class I of the Haibei Partridge chicken (a) and Wenchuang chicken (b). Polymorphic sites had a Wu-Kabat score ≥ 4 . The Wu-Kabat scores ≥ 8 are marked with their positions.

Table 1. Amino acid positions with high variability (Wu-kabat index ≥ 4.0) in MHC class I $\alpha 1$ and $\alpha 2$ domains of chicken (HBP and WC), duck, mouse (H-D) and human (HLA-A).

Position		Chicken (HBP)	Chicken (WC)	Duck	Human	Mouse
Bird	Mammal					
$\alpha 1$ domain						
9	9	19.5	15	12.1	9.1	6.5
24	24	7.8	5.5	8		6.5
27	45			4.5		7.4
32	62			6	13.9	5.2
43	63	7.4	5.5		4.2	10.4
51	66			4.5		6.8
52	67			4.5		
53	69	6.	5.5	6		
54	70	4		8		4.3
56	73	4.7				6.5
60	77			4.5		
61	80			12.1		4.3
62	81		5.5	6		
65	82		4	6		
66	89	6.5	5.6	9		4.3
68			4	12.1		
69			9.6	12.1		
72		5.2	7.4			
73			4.2			
74			5.5			
75		4.6	4.8	6		
76				4.5		
78		6				
$\alpha 2$ domain						
93	95	4.3		6	5.4	8.7
95	97	4.1		15	5.8	10.8
97	99	5.5	4.6	8		13
105	114	4.6	4.8		8.3	8.1
111	116	13	18	15	6.3	8.7
113	150	26	13.1	10		4.9
116	152			6	5.9	4.3
120	155		4.5			10.4
121	156	4.1			8.3	9.3
123		4.6				
126			4			
127						
128						
146				4.5		
147		5.2	6.5			
148		7.1	4.5			
149		7.1	9.6			
150				4.5		
152			5.5			
153		13	13.3	4.5		
154				4.5		
155		5.6	5.1			
156				4.5		

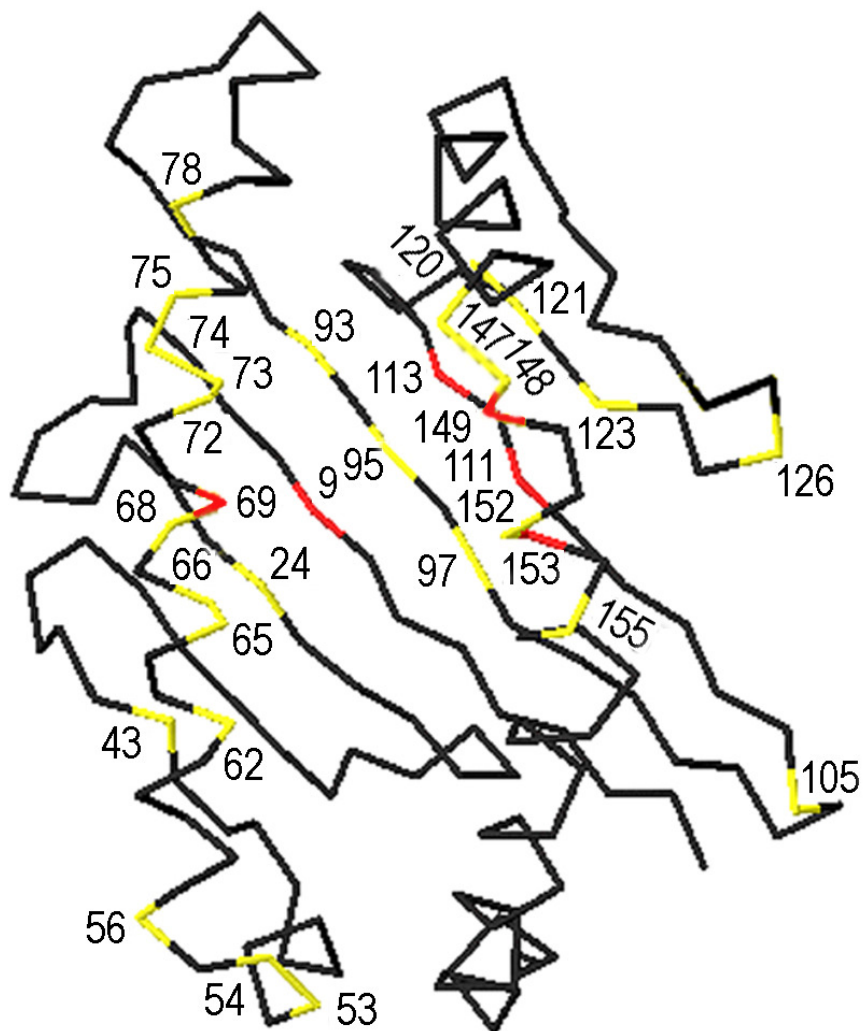


Figure 3. The backbone trace of $\alpha 1$ and $\alpha 2$ regions in MHC class I of chicken based on the crystal structures reported, sequences and sites in Figure 1 and Table 1. Amino acid residues in the model are colored individually (Wu-Kabat score ≥ 4 – yellow, Wu-Kabat score ≥ 8 – red).

Spatial orientation of highly polymorphic sites in $\alpha 1$ and $\alpha 2$ domains

To understand the role of high variable amino acid residues in the spatial structure of MHC class I α chain, we simulated the three-dimensional structure of $\alpha 1$ and $\alpha 2$ domains according to the crystal structure reported (Koch et al., 2007). As shown in the Figure 3, the $\alpha 1$ and $\alpha 2$ domains appeared as a configuration based on two α helices and eight β sheets, which formed antigen peptide binding groove in the intramolecular structure. Most of the highly variable residues were located in the α helices or β sheets, especially the sites (62, 65, 66, 68, 69, 72, 73, 74, 75 and 78) in the $\alpha 1$ helix and the sites (152, 153 and 155) in the $\alpha 2$ helix of the molecular middle domain. In addition, three amino acid residues with mutation frequency (score ≥ 8) were located in the β sheet, with

only one (site 153) in the α helix.

Phylogenetic analysis of $\alpha 1$ and $\alpha 2$ domains

Furthermore, we constructed phylogenetic trees based on 38 amino acid sequences of $\alpha 1$ and $\alpha 2$ domains in MHC class I α as aligned by MEGA software (Figure 4). The homology of all alleles among the four breeds ranged from 77.7 to 99.5%, and these sequences were divided into several groups. The evolution of MHC class I α among duck, mouse and human had familiar way, but the sequences of MHC class I α in chicken breeds were distributed in different branches without rules, which revealed no correlation among breeds. This result suggests that MHC class I α chains of different chicken breeds retain similar genetic characters from ancestral

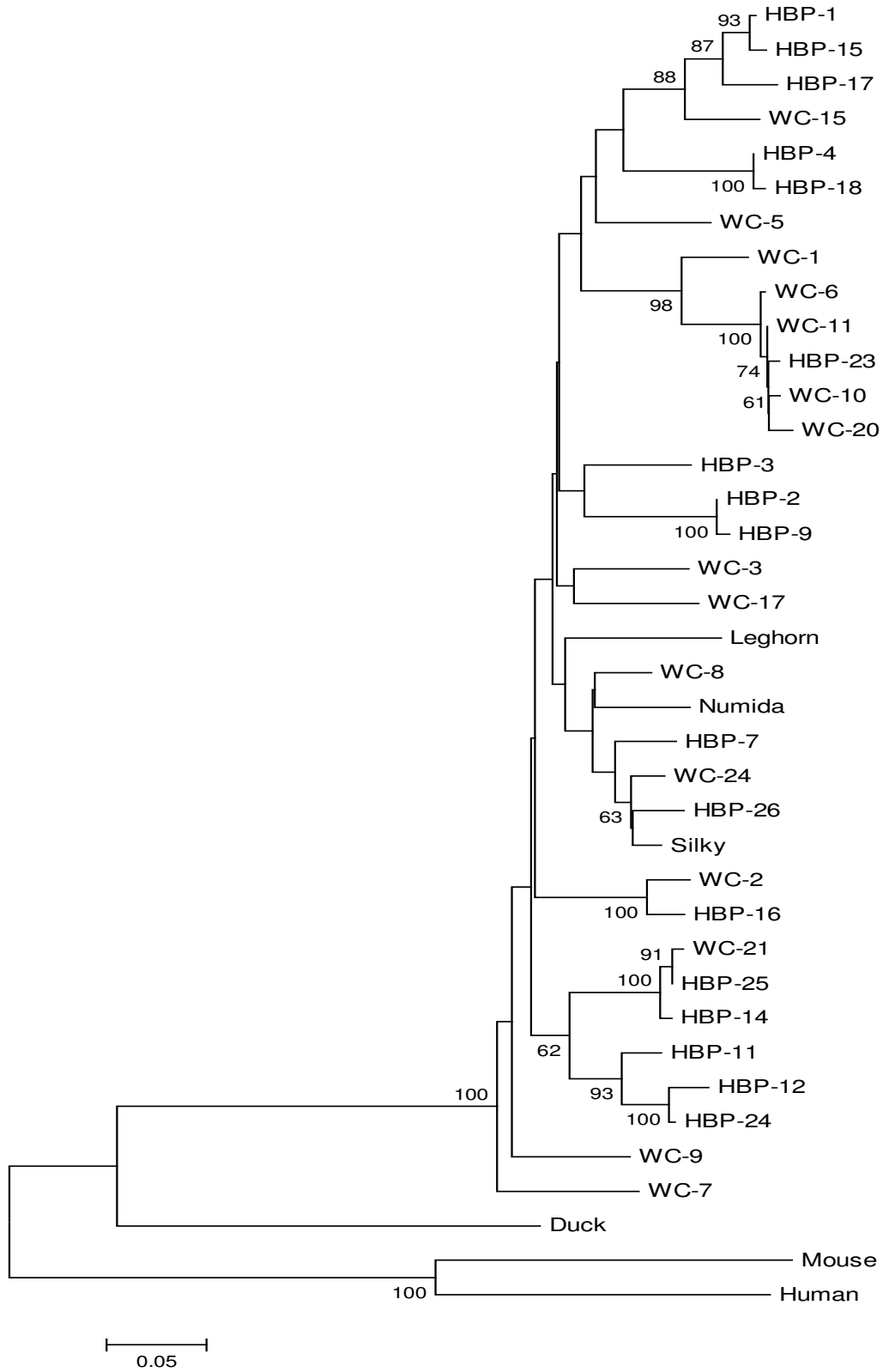


Figure 4. Phylogenetic tree of chicken MHC I created by the neighbor-joining method based on the amino acid sequences aligned in Figure 1 (see Figure 1 for sequence references). Genetic distance is indicated at the bottom. The reliability of the cluster analyses are tested by bootstrap confidence limits and indicated as percent success per 1,000 bootstrap trials with values above 50% presented on nodes.

MHC gene, though they present some distinct diversity in the character and production during long-term breeding.

DISCUSSION

Comparison of predicted structure of these MHC class I α chain indicated obvious features: first the diversity of these polymorphic amino acid residues, whose score of mutation frequency was higher than or equal to 4 on the Wu-kabat index occurred in a1 and a2 domains, especially in the side chain that contacted with the antigen in PBD. This was similar to the mammalian MHC class II DRB, even as Furlong and Yang (2008) found that almost all amino acid residues inferred to be under positive selection were in the PBD and in contact with the antigen side chains, although residues outside of but close to the PBD. In the evolution process, mutation of the amino acid sequence occurs in the non-PBD, destroying PBD structure integrity which makes MHC molecules fail in binding antigen peptides, while some mutations within the PBD increase the potential of MHC binding antigen peptides. The polymorphic genes of MHC are regarded as essential genes for individual fitness under conditions of selection (Eizaguirre et al., 2009), and it is just these negative or positive selections that drive MHC allelic diversity at loci for antigen presentation (Goto et al., 2009).

We all know that MHC gene descended from a common ancestry and came into being polymorphism under the environmental pressure. In birds, a comparison of MHC class I gene sequences between the quail and chicken in phylogenetic analysis showed that the quail MHC genes were duplicated after the separation of these two species from their common ancestor (Shiina et al., 2004). The two breeds analysed in our study were bred in two Chinese areas that were thousands of miles apart. No relationship was found between breeds feature and MHC class I α gene in phylogenetic trees. Similarly, Koutsogiannouli et al. (2009) surveyed the level of MHC genetic diversity of European and Asia brown hares (*Lepus europaeus*) in natural populations, and did not find a strong phylogeographic signal in the full-length exon 2 α -DQA alleles' phylogeny. Xu et al. (2009) found that three MHC alleles (DQB, DRA and MHC-I) of cetaceans showed similarity and identity when compared with the phylogenetic trees. Moreover, Gu and Nei (1999) thought that the MHC evolution in mammals is in agreement with the birth-and-death model of evolution. Under conventional breeding conditions, the animal MHC are subjected to likely pathogen-mediated selection as adaptive processes, rather than breed and geographical differences, although further studies still need to be implemented.

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