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*Original article*

# Polymorphic analysis of peptide binding domain of major histocompatibility complex class I in domestic ducks

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## Abstract

Function of duck (*Anas platyrhynchos*) major histocompatibility complex class I (*Anpl*-MHC I) molecules in binding peptides is through the peptide binding groove (PBG), which is thought to be influenced by the high polymorphism of α1 and α2 domains. However, little is known about the polymorphism of *Anpl*-MHC I peptide binding domain (PBD), especially in the domestic duck. Here, we analyzed the polymorphism of forty-eight *Anpl*-MHC I α1 and α2 domains from domestic duck breeds previously reported. All sequences were analyzed through multiple sequence alignment and a phylogenetic tree was constructed. The coefficient of variance of the peptide binding domains (PBDs) from WS, CV, JD, and SX duck breeds was estimated based on the Wu-Kabat variability index, followed by the location of the highly variable sites (HVSs) on reported crystal structure models. Analysis of α1 and α2 domains showed common features of classical MHC class I and high polymorphism, especially in α1 domain. The constructed phylogenetic tree showed that PBDs of domestic ducks did not segregate based on breeds and had a close phylogenetic relationship, even with wild ducks. In each breed, HVSs were mostly located in the PBG, suggesting that they might determine peptide-binding characteristics and subsequently influence peptide presentation and recognition. The combined results of sequence data and crystal structure provide novel valuable insights into the polymorphism and diversity of *Anpl*-MHC I PBDs that will facilitate further studies on disease resistance differences between duck breeds and the development of cytotoxic T-lymphocyte (CTL) epitope vaccines suited for preventing diseases in domestic ducks.

**Key words:** domestic duck, highly variably sites, MHC class I, peptide binding domain, phylogenetic tree, polymorphism

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## Introduction

The major histocompatibility complex (MHC), composed of the MHC I, MHC II, and MHC III genes, is the most polymorphic genetic group that plays an important role in the adaptive immune response. MHC I molecules are encoded by the MHC I locus and play a critical role in presenting pathogen-derived epitope peptides to specific T-cell receptors (TCRs), subsequently resulting in the cytotoxic T-lymphocyte (CTL) response and elimination of pathogens from the host (McMichael et al. 1983, Kane et al. 1984, Jondal et al. 1996). Highly variable polymorphism of MHC I molecules, mostly clustered at α1 and α2 domains, is a common feature closely associated with disease resistance and susceptibility in different species (Lacey et al. 1989, Garcia et al. 1998, Nejentsev et al. 2007), which results in different peptide-binding rules for each MHC molecule (Falk et al. 1991).

Similar to human and mouse MHC I, duck (*Anas platyrhynchos*) MHC I (*Anpl*-MHC I) molecules bind peptides through six pockets in the peptide binding groove (PBG) formed by the two α helixes and the bottom β sheet in α1 and α2 domains (Saper et al. 1991, Jameson et al. 1992, Wu et al. 2017). The highly variable sites (HVSs) of *Anpl*-MHC I molecules are also mainly located in α 1 and α2 domains, especially in the pockets interacting with the peptides directly (Wu et al. 2017, Zhang et al. 2019). Thus, polymorphic analysis of *Anpl*-MHC I α1 and α2 domains in different duck breeds should contribute to elucidate any difference in function associated with susceptibility to various infectious diseases (Kaufman et al. 1995, Wallny et al. 2006, Zhang et al. 2012). However, until now only a few *Anpl*-MHC I alleles have been characterized in some duck lines (Xia et al. 2004, Moon et al. 2005, Fleming-Canepa et al. 2016, Zhang et al. 2017, Zhang et al. 2019). Furthermore, polymorphic analysis of these alleles has not been completed. The scarcity of studies on *Anpl*-MHC I polymorphism and its functional classification are the primary bottlenecks hampering duck CTL immunity research and the development of virus resistant duck lines.

In a previous study, we characterized the polymorphism of 14 UAA alleles from two duck lines, i.e., WS and CV ducks (Zhang et al. 2017). Later, other 27 *Anpl-MHC I* genes located in locus UAA of SX and JD ducks from China were retrieved from NCBI database (Zhang et al. 2019). Together with five and two UAA alleles reported by Xia et al. (2004) and Moon et al. (2005), respectively, there were 48 UAA alleles isolated from domestic ducks containing complete coding domains by now. In order to illustrate the polymorphism of *Anpl*-MHC I and provide data for their functional study, peptide-binding domains (PBDs) of these 48 UAA alleles from domestic duck breeds were firstly analyzed by sequence alignment and phylogenetic tree was constructed. Then, the coefficient of variance of the PBD region from WS, CV, JD, and SX duck breeds was estimated by the Wu-Kabat method (Kabat et al. 1997), followed by the location of the HVSs in reported crystal structure models. The combined results of sequence data and crystal structure modeling provided valuable insights into the polymorphism and variability of *Anpl*-MHC I PBDs, thereby facilitating the demonstration of disease resistance differences between duck breeds and the development of CTL epitope vaccines better suited for preventing diseases in domestic ducks.

## Materials and Methods

### Sequence collection and analysis

Besides the 41 *Anpl-MHC I* alleles recovered from WS, CV, JD and SX ducks, alleles used to analyze polymorphism of PBD in this study contained five alleles isolated from Pekin ducks and two alleles obtained in White Pekin ducks (also called Cherry valley ducks). Accession numbers deposited in GenBank of the forty-eight UAA alleles were listed in supplementary Table 1.

Alignment of all *Anpl*-MHC I α1 and α2 domains amino-acid sequences were carried out by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and JALVIEW (Waterhouse et al. 2009). Subsequently, phylogenetic tree was constructed in MEGA6 (Zhou et al. 2004) using the neighbor-joining method and p-distance (Saitou et al. 1987). Bootstrap support values were obtained from 1000 replicates.

Other deduced amino acid sequences of *Anpl*-MHC I used to construct the phylogenetic tree were retrieved from NCBI database and the accession numbers were also listed in supplementary Table 1.

### HVS location in three-dimensional UAA structures

The variation coefficients of the α1 and α2 domains were calculated on the PVS website (http://imed.med.ucm.es/PVS/) and HVSs were analyzed through the Wu-Kabat method. Based on *Anpl*-MHC I three-dimensional structures resolved recently (Wu et al. 2017), HVSs (Wu-Kabat index > 6.0) were located using PyMOL Molecular Graphics System. The modeled MHC I molecules from different duck breeds were built by SWISS-MODEL (http://swissmodel. expasy.org).

Supplementary Table 1. GenBank accession numbers of *Anpl-MHC I* alleles in this study.

| Alleles | GenBank accession no. | Duck lines |
|---|---|---|
| UAA01*WS | KX118673 | |
| UAA03*WS | KX118675 | |
| UAA04*WS | KX118676 | |
| UAA05*WS | KX118677 | WS duck |
| UAA07*WS | KX118679 | |
| UAA08*WS | KX118680 | |
| UAA09*WS | KX118681 | |
| UAA01*CV | KX118683 | |
| UAA02*CV | KX118684 | |
| UAA03*CV | KX118685 | |
| UAA04*CV | KX118686 | CV duck |
| UAA05*CV | KX118687 | |
| UAA06*CV | KX118688 | |
| UAA07*CV | KX118689 | |
| UAA01*JD | MH218820 | |
| UAA02*JD | MH218822 | |
| UAA04*JD | MH218825 | |
| UAA05*JD | MH218827 | |
| UAA06*JD | MH218829 | |
| UAA07*JD | MH218830 | JD duck |
| UAA08*JD | MH218832 | |
| UAA10*JD | MH218835 | |
| UAA11*JD | MH218837 | |
| UAA12*JD | MH218839 | |
| UAA13*JD | MH218841 | |
| UAA14*JD | MH218843 | |
| UAA01*SX | MH218821 | |
| UAA02*SX | MH218823 | |
| UAA03* SX | MH218824 | |
| UAA04*SX | MH218826 | |
| UAA05*SX | MH218828 | |
| UAA07*SX | MH218831 | |
| UAA08*SX | MH218833 | |
| UAA09*SX | MH218834 | SX duck |
| UAA10*SX | MH218836 | |
| UAA11*SX | MH218838 | |
| UAA12*SX | MH218840 | |
| UAA13*SX | MH218842 | |
| UAA14*SX | MH218844 | |
| UAA15*SX | MH218845 | |
| UAA16*SX | MH218846 | |
| *Anpl*-UAA01 | AB115242 | |
| *Anpl*-UBA02 | AB115243 | |
| *Anpl*-UBA01 | AB115244 | Pekin duck |
| *Anpl*-UCA01 | AB115245 | |
| *Anpl*-UDA01 | AB115246 | |
| *Anpl*-U*02 | AY294416 | White Pekin duck |
| *Anpl*-U*03 | AY294417 | |
| UAA*M16.1 | GU245785 | |
| UAA*M16.2 | GU245786 | Mallard duck |
| UAA*M16.3 | GU245787 | |
| UAA*M24.1 | GU245811 | |
| UAA*M24.3 | GU245813 | |
| UAA*M24.5 | GU245815 | |
| UAA*M26.1 | GU245820 | |
| UAA*M26.2 | GU245821 | |
| UAA*M28.2 | GU245828 | |
| UAA*M29.1 | GU245829 | |
| UAA*M30.1 | GU245830 | |
| UAA*M30.2 | GU245831 | |
| UAA*M31.1 | GU245832 | |
| UAA*M31.4 | GU245835 | |
| UAA*M31.5 | GU245836 | |
| UAA*M32.1 | GU245837 | |
| UAA*M35.1 | GU245838 | |
| UAA*M35.2 | GU245839 | |
| UAA*M35.4 | GU245841 | |
| UAA*M35.5 | GU245842 | |
| UAA*M36.1 | GU245844 | |
| UAA*M41.1 | GU245852 | |
| UAA*M48.2 | GU245871 | |
| UAA*M49.1 | GU245872 | |
| UAA*M49.2 | GU245873 | |
| UAA*M49.3 | GU245874 | |
| UAA*M49.4 | GU245875 | |
| UAA*M50.1 | GU245876 | |
| UAA*M50.2 | GU245877 | |
| UAA*M50.3 | GU245878 | |

Table 1. Wu-Kabat plot of amino acid variability and highly variable sites located in the three-dimensional structures of the peptide binding groove (PBG) of the UAA from four duck breeds.

| Residues/Positions | Variability scores | Description of the locations |
|---|---|---|
| Glu/9 | 28.7 | B pocket |
| Ala/24 | 11.182 | B pocket |
| Ala/32 | 6.150 | β-strand |
| Tyr/35 | 6.474 | β-strand |
| Thr/43 | 6.474 | B pocket |
| His/53 | 7.235 | Loop |
| Thr/54 | 6.833 | Loop |
| Asp/60 | 6.833 | α-helix |
| Met/61 | 12.812 | α-helix |
| Val/62 | 18.923 | B pocket |
| Glu/64 | 6.308 | α-helix |
| Phe/66 | 13.667 | B pocket |
| Asp/68 | 12.812 | α-helix |
| Thr/69 | 12.812 | B pocket |
| Val/93 | 10.933 | F pocket |
| Val/95 | 14.643 | C pocket |
| Asp/111 | 12.3 | C/E pocket |
| Leu/128 | 6.56 | Loop |
| Val/150 | 6.833 | E pocket |
| Arg/153 | 7.688 | α-helix |
| Arg/154 | 9.111 | E pocket |

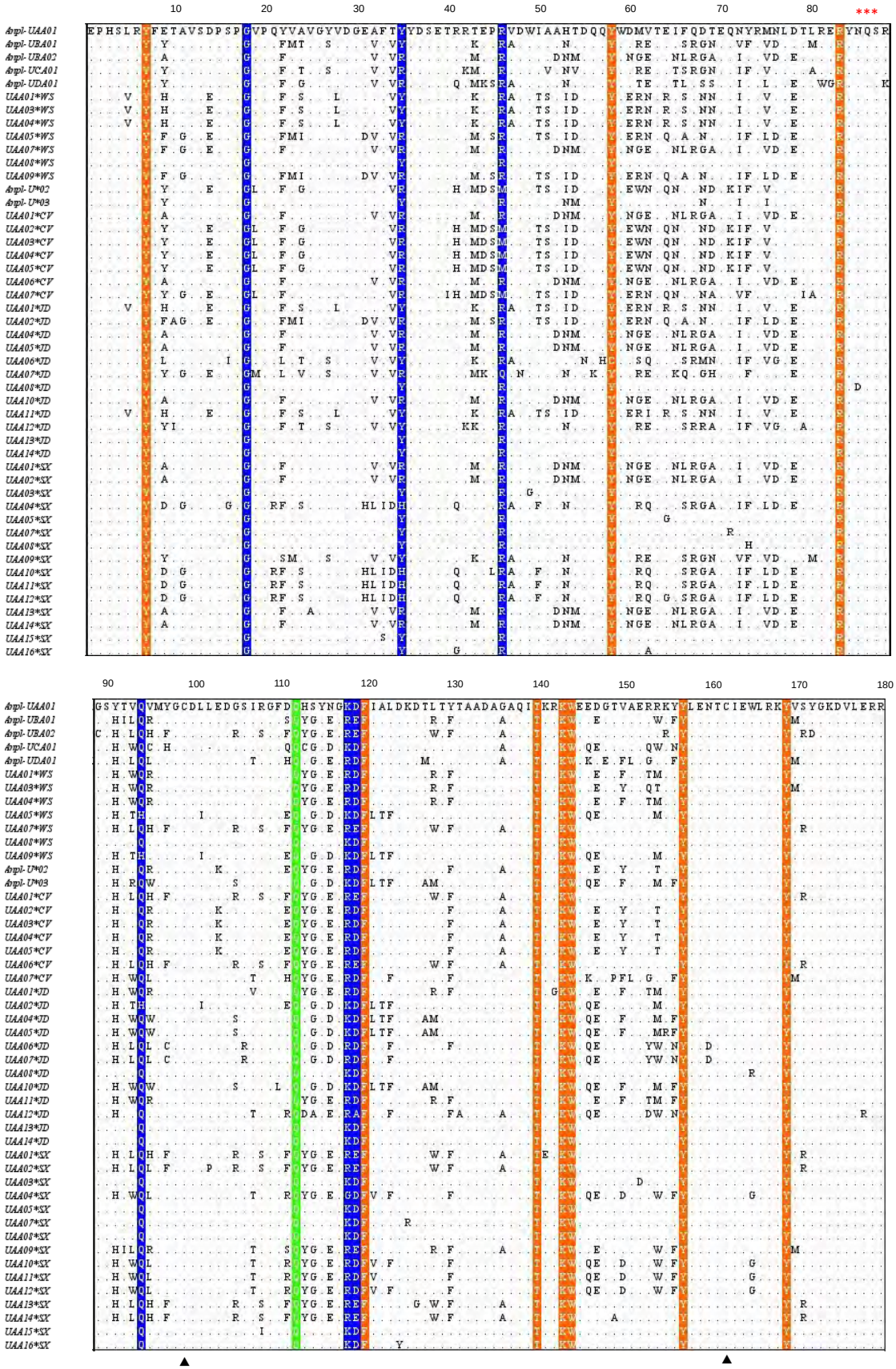
## Results

### Common features and polymorphism analysis of PBDs

To assess α1- and α2-domain polymorphism, amino acid sequences of 48 UAA alleles were aligned. As shown in supplementary Fig. 1, all sequences possessed identical length, including 180 amino acids. At positions 85-87, an N-linked glycosylation site (NQS) in α1 domain and at positions 99 and 162, a pair of cysteine residues that formed a couple of disulfide bonds in α2 domain were conserved in every sequence. All amino acids involved in peptide anchoring including 7Y, 58Y, 83R, 120F, 140T, 143K, 144W, 157Y, and 169Y were conserved in PBDs. Moreover, in α1 and α2 domains, one (112Q) out of two and nearly half (18G, 35R, 46R, 94Q, 117K, and 118D) relatively conserved residues that interacted with CD8 molecules and β2m, were found. These results indicated that PBDs of alleles derived from the five domestic duck breeds had the common features and functions of classical MHC class I molecules; this finding was consistent with previous studies on *Anpl*-MHC I characterization (Wu et al. 2017, Zhang et al. 2017, Zhang et al. 2019). However, high polymorphism was also demonstrated in the PBD domain. Compared to the α2 domain, there was more polymorphism in the α1 domain (supplementary Fig. 1), especially in the residues known to comprise the B and F pockets that act as primary anchor sites for peptides located in the α1 domain (Wu et al. 2017, Zhang et al. 2019).

### Phylogenetic analysis of PBDs

To determine the phylogenetic relationship of PBDs of UAA alleles from domestic duck breeds and other species, amino acid phylogenetic trees were constructed. As shown in Fig. 1, all sequences clustered together showed no separation of lineages among different duck species, not even between domestic and wild ducks. In addition, PBDs of the five domestic duck lines under present study did not segregate with each other and at least three breeds were located in every branch, suggesting their close evolutionary relationship. Furthermore, UAA sequences isolated from the same individual were located at different clusters, such as UAA06*-JD and UAA08* JD, recovered from the JD-4 duck (Zhang et al. 2019), which were found distributed on different branches (Fig. 1).



Supplementary Fig. 1. Alignment of α1 and α2 domains of the 48 UAA sequences from domestic ducks. The conserved amino acids that form disulfide bonds and the N-linked glycosylation site are denoted by triangles and by red asterisks, respectively. The conserved amino acids involved in peptide binding are highlighted in orange. The residues interacting with β2m and CD8 molecules are highlighted in blue and green, respectively.


Fig. 1. Phylogenetic tree of α1 and α2 domains of *Anpl*-MHC I molecules constructed in MEGA6 using the neighbor-joining method and p-distance. Bootstrap values were obtained from 1000 replicates.

Table 2. Wu-Kabat plot of amino acid variability and highly variable sites located in the three-dimensional structures of the peptide binding groove (PBG) of the UAA from WS duck breed.

| Residues/Positions | Variability scores | Description of the locations |
|---|---|---|
| His/9 | 9.0 | B pocket |
| Ser/24 | 7.5 | B pocket |
| Arg/64 | 6.5 | α-helix |
| Ser/66 | 9.0 | B pocket |
| Trp/93 | 9.0 | F pocket |

Table 3. Wu-Kabat plot of amino acid variability and highly variable sites located in the three-dimensional structures of the peptide binding groove (PBG) of the UAA from CV and White Peking duck breeds.

| Residues/Positions | Variability scores | Description of the locations |
|---|---|---|
| Gly/61 | 8.0 | α-helix |
| Leu/93 | 8.0 | F pocket |
| His/95 | 8.0 | C pocket |
| Phe/111 | 8.0 | C/E Pocket |

## HVS distribution and location in the three-dimensional structure of PBDs

To investigate HVSs and their distribution, α1 and α2 domains of the 43 sequences [excluding the five alleles isolated from Pekin ducks, as their name and sequence did not correspond in different reports (Xia et al. 2004, Wu et al. 2017)], were analyzed using the Wu-Kabat method. As shown in Table 1, there were 21 HVSs, most of which clustered at amino acids 60 to 69. At amino acid positions P9, P24, P61, P62, P66, P68, P69, P93, P95, and P111, higher variability scores (>10) were recorded; among them, variability of P9 obtained the highest score (28.7), but in individual analysis of the four duck breeds, it showed lower variation [WS: 9.0; CV<6.0; JD:24.0; SX: 10.0 (Zhang et al.

2019)] than that in the analysis of all breeds combined. In the analysis of the CV duck line, there were no HVSs in the seven sequences. When *Anpl*-U*02 and *Anpl*-U*03 obtained from White Pekin duck were introduced, four HVSs with high variability scores (8.0) were located at P61, P63, P95 and P111.

The HVSs composing the PBD were located in the three-dimensional structure of UAA and over half of them clustered in pockets in the whole (Table 1); among them, amino acids at P9, P24, P43, P62, P66, and P69 were located in the B pocket, P95 and P111 (which was also in the E pocket) in the C pocket, P150 and P154 in the E pocket, and P93 in the F pocket. The HVSs of UAA alleles from WS (Table 2), SX and JD duck lines (Zhang et al. 2019) were also clustered mostly in the pockets, and most of them in the B pocket, which is one of the two primary anchor sites for peptide-binding (Wu et al. 2017). However, the four HVSs of the CV duck line were different, three of which were located in the C, E and F pockets of the α2 domain (Table 3).

## Discussion

To date, 113 duck UAA sequences (containing complete CDs) have been reported (Xia et al. 2004, Moon et al. 2005, Fleming-Canepa et al. 2016, Zhang et al. 2017, Zhang et al. 2019); most of them were isolated from wild ducks. Although there are abundant duck resources in China, only five duck breeds have been studied to obtain MHC I sequences (Xia et al. 2004, Moon et al. 2005, Zhang et al. 2017, Zhang et al. 2019). Furthermore, some researches indicate that UAA obtained from domestic duck breeds are characterized with high polymorphism (Xia et al. 2004, Moon et al. 2005, Wu et al. 2017, Zhang et al. 2017, Zhang et al. 2019). However, a complete polymorphic analysis of the five domestic duck breeds is needed for determining their degree of polymorphism precisely and defining a novel, functional classification of the main duck breeds of China. In this study, 48 UAA alleles from the five domestic duck breeds were collected to analyze their polymorphism, especially in the PBD domains that constitute the binding epitope-peptides of the functional domain. Overall, the analysis found characteristics identified in UAA molecules from other duck breeds, such as the length of PBD, the cysteine residues involved in the formation of disulfide bonds and the residues interacting with β2 m and CD8 molecules (Moon et al. 2005, Fleming-Canepa et al. 2016, Zhang et al. 2019). Moreover, in agreement with results from previous studies of duck UAA polymorphism (Zhang et al. 2019), HVSs of UAA molecules were found located in α1 and α2 domains, especially in α1 domains. As one MHC class I gene bias expressed in ducks may make pathogen escape within an individual easy (Fleming-Canepa et al. 2016), high polymorphism in functional domains in the population may convey an advantage, as it allows for more antigenic epitope-peptides that may potentially result in the eventual elimination of pathogens from the host.

In the phylogenetic analysis of all UAA PBDs, some alleles from different duck breeds belonging to the same clusters or alleles from the same breeds were distributed in different branches of the phylogenetic tree. According to the results, there was no significant divergence of UAA among different duck breeds; furthermore, the data indicated that α1 and α2 domains of duck UAA alleles hold a close phylogenetic relationship that suggests a concerted functional evolution regardless of whether they are domestic or wild ducks.

In comparison with WS, JD, and SX duck lines, the CV duck line showed the lowest allelic diversity, which was closely related to the extensive artificial selection for a pure-line variety. The result of HVS analysis also suggested that White Pekin ducks from which *Anpl*-U*02 and *Anpl*-U*03 were recovered, and CV ducks, from which UAA01*CV- UAA07*CV were isolated, belonged to different pure-line varieties (Wang 1986).

According to the crystal structure of *Anpl*-UAA*01 (Wu et al. 2017), UAA molecules bind peptides through six pockets in the PBG formed by the two α helixes and the β sheet in α1 and α2 domains, with B and F pockets being the primary, vital anchor-sites for peptide presentation. The combined analysis of HVSs and their location in the crystal structure of UAA indicated that most HVS variability is localized within the PBG, and over half of the HVSs are localized at B and F pockets, either in the overall analysis of all breeds combined or in the analysis of each separate breed. However, the CV duck line showed fewer HVSs than local elite duck breeds (WS, SX and JD ducks), regardless of numbers or variation coefficients. Moreover, there were no HVSs located in the B or F pockets, which is not advantageous to disease resistance. Thus, the UAA alleles of local elite duck breeds could present more pathogen-derived epitope peptides (Bingham et al. 2009, Liang et al. 2011, Li et al. 2015, Liu et al. 2017) and they may be far more resistant to infectious disease than CV ducks. In the analyzed duck breeds, no HVSs were located in the A or D pockets. The exceptional clustering of HVSs might be specific to duck breeds examined here; alternatively, it may be a consequence of the limited sample size.

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