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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 $\alpha 1$ and $\alpha 2$ 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 a1 and a2 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 $\alpha 1$ and $\alpha 2$ domains showed common features of classical MHC class I and high polymorphism, especially in $\alpha 1$ 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 $\alpha 1$ and $\alpha 2$ 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 $\alpha 1$ and $\alpha 2$ 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 α 2 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 a1 and a2 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 func-

tional 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 α 1 and α 2 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 $\alpha 1$ and $\alpha 2$ 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).



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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
		e v 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	
		William Delain de als
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	
UTATA WIJU.I	00210070	
UAA*M50.2	GU245877	

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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 $\alpha 1$ - and $\alpha 2$ -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 α 1 domain and at positions 99 and 162, a pair of cysteine residues that formed a couple of disulfide bonds in $\alpha 2$ 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 $\alpha 1$ and $\alpha 2$ 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 B2m, 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 $\alpha 2$ domain, there was more polymorphism in the $\alpha 1$ 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 $\alpha 1$ 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).



Polymorphic analysis of peptide binding domain ...

		10	20	30	40	50	60	70	80 ***
Angol-UAA01 Angol-UBA01	EPHSLR	FETAVSDPSP Y	VPQYVA FMT	VGYVDGEAFT S.V.V	Y YDSETRRTEP Y K	RVDWIAAHTDQ RA.N.		FQDTEQNYRMN SRGN.VF.VD	LDTLRE <mark>R</mark> YNQSR M. R
Appl-UBA03		. A	F	V . V	R M	R DNM	NGE N	LRGA. I. VD	
Ampl-UCA01 Ampl-UDA01		. Y	F . T	S V . V V . V		RV.NV		SRGN. IF.V. L.SS. I.L.	F WGP F
UAA01*WS	v .	.HE	F.S	L V	Y K	RA TS . ID	. Y. ERN.R.	S.NN.I.V.	.E
UAA03*WS UAA04*WS	V	.HE .HE	F.S.			RA TS . ID RA TS . ID	ERN.R.		.E
UAA05*WS		.F.GE	FMI	DV.V	R	R TS . ID	. C. ERN . Q.	A.N IF.LD	. E
UAA07*WS UAA08*WS		.F.GE	F F	v.v		R DNM.	NGE N	LRGA. I. VD	. E
UAA09*WS		.F.G	FMI	DV.V	R M . S	R TS . ID		A.NIF.LD	. E R
கூழ1- U№03 கூழ1- U№03			L.F.G	. V		MTS.ID RNM	EWN QN	ND.KIF.V.	<mark>R</mark> a <mark>R</mark> a
UAA01*CV UAA03*CV		.A .YE	GF.G	V . V	R.M.M. R.H.MDS	RDNM			. E <mark>P</mark>
UAA03*CV		YE	L.F.G	V	R	M	. EWN . QN	ND . KIF . V .	<mark>R</mark> a . a
UAA04*CV UAA05*CV	*****		L.F.G				EWN QN		
UAA06*CV		.A	F	v . v	R	R DNM	NGE N	LRGA I VD	.E
UAA07*CV UAA01*JD	· · · · · · · · · · · · · · · · · · ·	.Y.G.E H. E.	L.F	V		MTS.ID			I A
UAA03*JD		.FAGE	FMI	DV.V	R M . S	R TS . ID	K.ERN.Q.	A.NIF.LD	. E P
UAA04*JD UAA05*JD		. A	F	V.V V.V		RDNM	NGE .N		. E
UAA06*JD UAA07*JD		LI. Y.G.E	M.L.V	. S V . V S V . V		RA.NI Q.N.N.K		SRMN IF VG Q.GH F	E
UAA08*JD			5		Yaaaaaaaaaaa	R	• ¥ • • • • • • •		
UAA10*JD UAA11*JD	· · · · · · · · · · · · · · · · · · ·	. A	FF.S	V.V V	R	RDNM		LRGA.I.VD S.NN.I.V.	.E
UAA13*JD		YI	F . T		Y KK	R N	RE	SRRA. IF.VG	A <mark>P</mark>
UAA 13*JD UAA 14*JD						R	· · · · · · · · ·		<mark>E</mark> R
UAA01*SX		. A	5F			R D NM R D NM		LRGA.I.VD LRGA.I.VD	
UAA03*SX UAA03*SX		• • • • • • • • • • • • • • • • • • •	3 # 3	V . V	Y	R G	1 Y 1 1 1 1 1 1 1 1		. E <mark>E</mark> <mark>R</mark>
UAA04*SX UAA05*SX		. D . G G .	RF . S		H Q	RA.F.N	RQ.	SRGA IF . LD	. E <mark>R</mark>
UAA07*SX					Y	R	X	R	<mark>P</mark>
UAA08*SX UAA09*SX		Y			х хк.	RAN	RE	SRGN. VF.VD	
UAA10*SX		.D.G	RF.S		HQL	R <mark>A.F.N</mark>	RQ	SRGA. IF.LD	.ER
UAA11*SX UAA12*SX		.D.G	RF.S.	HL I D		RA.F.N RA.F.N		SRGA. IF.LD SRGA. IF.LD	. E
UAA 13*SX UAA 14*SX		A	F	AV.V	RM.	RDNM		LRGA.I.VD LRGA.I.VD	. E
UAA 15*SX					Y	R		LKOA	<mark>P</mark>
UAA16*SX	1 9		3		¥	R	. A		
	90	100	110	120	130	140	150	160	170 180
Appl-UAA01		100 VMYGCDLLEDGS			130 DKD TL TYTAADA		150 GTVAERRKY	160 LENTCIEWLRK	
Appl-UBA01	GSYTV <mark>Q</mark> HILQ	VMYGCDLLEDGS R	IRGFD H	SYNG <mark>KDF</mark> IALI G.E. <mark>RE</mark> F	DKDTLTYTAADA R.F	GAQI <mark>I</mark> KR <mark>KW</mark> EED A <mark>TKW</mark> E	GTVAERRKY W.F		VVSYGKDVLERR VM
Appl-UBA01 Appl-UBA02 Appl-UCA01	GSYTVQ . HILQ C.H.LQ	VMYGCDLLEDGS R	IRGFD H SY SFY	S YN G <mark>KD 7</mark> I AL I	DKDTLTYTAADA R.F. W.F.	GAQI <mark>I</mark> KR <mark>KW</mark> EED ATKWE ATKW ATKW.QE	GTVAERRKY W.F R. QW.N		VS YGKD VL E R R
สามาใ- UBA01 สามาใ- UBA03 สามาใ- UCA01 สามาใ- UDA01	.GSYTVQ HILQ C.H.LQ H.WQ H.WQ	VMYGCDLLEDGS RR. H.FR. C.H	IRGFD H S.FY S.FY T.H	SYNGKDFIALI G.E.REF G.E.REF G.D.KDF G.E.RDF	DKDTLTYTAADA R.F. W.F.	GAQIIKR <mark>KV</mark> EED ATKVE ATKV ATKV.QE ATKV.QE	GTVAERRKY W.F R. QW.N E.FL.G.F		VVSYGKDVLERR VM
Appl-UBA01 Appl-UBA02 Appl-UCA01 Appl-UCA01 UAA01*WS UAA01*WS UAA03*WS	GSYTVQ . HILQ C.H.LQ . H.WQ . H.WQ . H.WQ	VMYGCDLLEDGS R H.FR. C.H R. R.	IRGFD H S Y .SF Y Q C TH Y	S YN G K D F I AL 1 G . E . RE F G . E . RE F G . D . K D F G . E . R D F G . E . R D F G . E . R D F	DKDTLTYTAADA R.F. M. R.F. R.F. R.F	GAQITKRKWEED A.T.KW.E A.T.KW.Q A.T.KW.QE A.T.KW.Q A.T.KW.E T.KW.E	GTVAERRKY 		VSYGKDVLERR MRD
4ppl-UBA01 4ppl-UBA02 4ppl-UCA01 4ppl-UDA01 UA401*WS UA403*WS UA403*WS	GSYTV0 HIL0 C . H . L0 H . W0 H . W0 H . W0 H . W0	VMYGCDLLEDGS R H.FR. C.H R. R.	IRGFD H S.F.Y T.H Y Y	S YNGKDF I AL 1 G.E.REF G.D.KDF G.E.RDF G.E.RDF G.E.RDF G.E.RDF	DKDTLTYTAADA R.F. W.F M. R.F	GAQIIKRRWEED A.T.KW.E A.T.KW.E A.T.KW.QE A.T.KW.QE A.T.KW.E T.KW.E T.KW.E	GTVAERRKY W.F QW.N E.FL.G.F F.TM		YVSYGKDVLERR YM RD YM
4 mj- UBA01 Amj- UBA02 Amj- UCA01 Amj- UDA01 UAA01+WS UAA03+WS UAA04+WS UAA04+WS UAA04+WS UAA07+WS	G S Y T V Q . H I L Q C H L Q . H W Q . H W Q . H W Q . H W Q . H T H . H Z Q	VMYGCDLLEDGS R	IRGFD H 	SYNGKD FIAL1 G.E.REF. G.D.KDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.D.KDFLTF. G.D.KDFLTF.	DKDTLTYTAADA R.F. M. R.F. R.F. R.F	GAQITKRKWEED A.T.KW.E A.T.KW.Q A.T.KW.QE A.T.KW.Q A.T.KW.E T.KW.E	GTVAERRKY W.F QW.N E.FL.G.F F.TM		YVSYGKDVLERR YM RD YM
4 ppl-UBA01 Appl-UBA02 Appl-UCA01 Appl-UDA01 UDA01+WS UAA03+WS UAA04+WS UAA05+WS UAA05+WS UAA08+WS	G S Y T V Q . H I L Q C H L Q . H WQ . H WQ . H WQ . H WQ . H WQ . H WQ . H J Q	VMYGCDLLEDGS R.H.F.R.C.H. I R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R	IRGFD H S S Y S F Q C T H 7 S F Y C C T H 7 S F Y	SYNGKDFIALI G.E.REF. G.E.REF. G.D.KDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.D.KDFLTF G.E.REF. KDF.	DKDTLTYTAADA R.F. M. R.F. R.F. R.F	GAQIXKESVEED A.T.S.V.E A.T.S.V.QE A.T.S.V.QE A.T.S.V.QE T.S.V.E T.S.V.E T.S.V.QE A.T.S.V.QE A.T.S.V.QE A.T.S.V.QE	GTVAERRKY W.F R. QW.N E.FL.G.F F.TM. Y.QT. F.TM. M.		YVSYGKDVLERR YM RD YM
4 ppl-UEA01 A ppl-UEA02 A ppl-UEA01 A ppl-UEA01 ULA01+WS ULA03+WS ULA03+WS ULA03+WS ULA07+WS ULA07+WS ULA07+WS ULA09+WS A ppl-UP42	GSYTV HILO C.H.LO H.WO H.WO H.WO H.WO H.WO H.TH H.TH H.TH	VMYGCDLLEDGS R H F R C H R R R 	IRGFD H 	SYNGKD FIAL1 G.E.REF. G.E.REF. G.D.KDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.REF. 	DKDTLTYTAADA R.F W.F M. R.F R.F R.F W.F 	GAQITKREWEED A. T. 200 E A. T. 200 E A. T. 200 A T. 200 A T. 200 E T. 200 E	GTVAERRKY W.F QW.M.F F.FL G.F F.TM. Y.QT. F.TM. M. M. Y.T		YVSYGKDVLERR YM RD YM
4 ppl-UBA01 4 ppl-UBA02 4 ppl-UCA01 4 ppl-UDA01 UDA01*WS UAA03*WS UAA03*WS UAA05*WS UAA05*WS UAA05*WS UAA05*WS UAA05*WS UAA09*WS	GSYTV0 . HIL0 C.H.L0 . H.W0 . H.W0 . H.W0 . H.W0 . H.W0 . H.TH . H.TH . H.TH	VMYGCDLLEDGS R.H.F.R.C.HR. C.HR. R.R.R.R.R.R.R.R.R.R.R.R.R.R.R.R	IRGFD H 	SYNGKD 7 IAL1 G.E.REF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.RDF. G.E.REF. S.C.KDFLTF. G.D.KDFLTF.	DKDTLTYTAADA R.F. M. R.F. R.F. R.F	GAQITKREWEED A. 7. 2007 A. 2007 A. 7. 2007 A	GTVAERRKY W.F QW.M.F F.FL G.F F.TM. Y.QT. F.TM. M. M. Y.T		YVSYGKDVLERR YM RD YM
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фр. URA01 фр. URA02 фр. URA02 фр. UCA01 фр. UCA01 фр. UCA01 ида04W85 ULA054W85 ULA054W85 ULA054W85 ULA054W85 ULA054W85 ULA054W85 ULA054W85 ULA054W70 ULA054C7 ULA054C7 ULA054C7	G S Y T V G . H I L G C . H I G . H WG . H WG . H WG . H WG . H WG . H WG . H T H T H T H T H T H T H T H C H T H C H C H C H C H C H C H C H C H C H C	VMYGCDLLEDGS R	IRGFD H 	SYNGKD ALI G.E.RS G.E.RS G.E.RS G.E.RD G.E.RD G.E.RD G.E.RD G.E.RD G.D.KD LTF G.E.RS G.D.KD LTF G.E.RD G.E.RD G.E.RD G.E.RD G.E.RD G.E.RD	DEDTLTYTAADA R.F W.F M. R.F R.F R.F W.F W.F AM. W.F AM. F F F F	GAQIIIKREWEED A. T. EV. E A. T. EV. E A. T. EV. E A. T. EV. E T. EV. E T. EV. E T. EV. E A. T. EV. E A. T. EV. QE A. T. EV. QE A. T. EV. QE A. T. EV. E A. T. EV. E	GIVAERRKY 		VSYGKDVLERR M
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Supplementary Fig. 1. Alignment of $\alpha 1$ and $\alpha 2$ 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 $\beta 2m$ and CD8 molecules are highlighted in blue and green, respectively.

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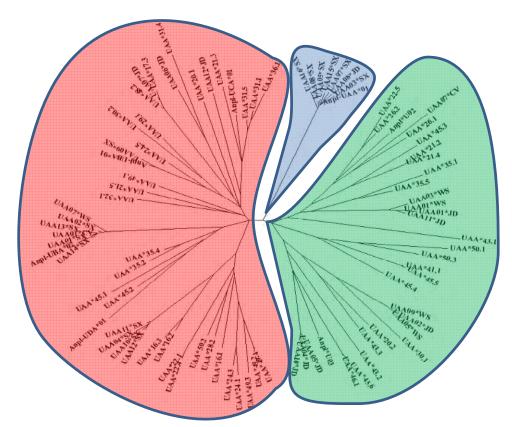


Fig. 1. Phylogenetic tree of α1 and α2 domains of *Anpl*-MHC I molecules constructed in MEGA6 using the neighbor-joining method and p-distance. Bootstrap values were obtained from 1000 replicates.

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.

9.0	D maaltat
	B pocket
7.5	B pocket
6.5	α-helix
9.0	B pocket
9.0	F pocket
	6.5 9.0

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, $\alpha 1$ and $\alpha 2$ 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.

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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 E pocket, and P93 in the F pocket. the 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 $\alpha 2$ 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 $\beta 2$ 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 $\alpha 1$ and $\alpha 2$ domains, especially in $\alpha 1$

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 $\alpha 1$ and $\alpha 2$ 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 α 1 and α 2 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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