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Polymorphism analysis and supertype definition of swine leukocyte antigen class I molecules in three-way crossbred (Landrace, Duroc, and Yorkshire) pigs: implications for the vaccine development of African swine fever virus

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Dear Editor,

Swine major histocompatibility complex (MHC) is a highly polymorphic gene in pigs and is also called swine leukocyte antigen (SLA) (Fan et al., 2018). SLA is divided into three major categories, SLA I (SLA-1, -2, -3), SLA II, and SLA III (Smith et al., 2005). SLA I plays an important role in cellular immunity which can eliminate viruses and other foreign antigens in pigs (Macdonald et al., 2010). SLA-1, SLA-2 and SLA-3 are important SLA I alleles and are highly polymorphic. Polymorphism causes extreme difficulties in screening broadly protective cytotoxic lymphocyte (CTL) epitope antigens. The supertype which is built based on peptide-binding specificity of each SLA I can overcome this difficulty to some extent (Thomsen et al., 2013). Three-way crossbred (Landrace, Duroc, and Yorkshire) pigs are widely raised in almost every pig farm in China (approximately 600 million pigs per year). To develop vaccines for this strain of pigs, SLA I alleles and the supertype of SLA I in these three-way crossbred pigs must be studied.

In this study, a total of 164 novel SLA I alleles were identified from three-way crossbred pigs. Detailed procedures of identifying the SLA I alleles are available in supplementary file. We submitted the nucleotide sequences of these new SLA I alleles to the GenBank database and obtained accession numbers: MH294957-MH295122 (Table S1 in Supporting Information). The SLA I allele database is enriched. We have preserved these plasmid clones containing full-length genes of these new SLA class I, and these new alleles may be useful for the scientific community. By MEGA6, the phylogenetic tree was constructed using amino acid sequences of 164 new SLA I alleles and 230 SLA I alleles from Immune Polymorphism Database (IPD) (see supplementary file). These new SLA I alleles did not segregate with the reported ones from IPD but some of the new alleles were concentrated on certain branches, as indicated by the circle mark in Figure S1 in Supporting Information.

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These SLA I alleles were analyzed with the Wu-Kabat method of the PVS website, too. The result showed that the amino acid variation regions were mainly concentrated in the α1 and α2 domains, especially α helix (Figure 1A and B).

In this article, we focused on introducing the supertype into the classification of SLA I. Studies have shown that MHC I molecules of different serotypes can present CTL epitopes with the same or similar function (Fan et al., 2016). These MHC I alleles with these characteristics are classified into the same MHC supertype. For predicting binding peptides, usage of MHC supertypes may minimize the number of predicted epitopes without affecting the group coverage required for the design of peptide-vaccines. This implies the supertype classification method is more practical than the genetic classification method.

The MHCcluster method was used to analyze and build the supertype of SLA I alleles in an unrooted tree visualization (Figure 1C). The MHCcluster method is available in supplementary file. This method can only process up to 200 alleles at the same time, so 145 novel SLA I molecules in three-way crossbred pigs and 55 SLA I molecules from IPD which could reflect the characteristics of their respective branches were selected for supertype studies. Four distinct specificity groups were produced, which were named supertype A, B, C and D. There were alleles dispersed in each supertype, resulting in multiple subtypes for each supertype. The supertype A was divided into three subtypes A1, A2 and A3; the supertype B was divided into three subtypes B1, B2 and B3; the supertype C was divided into three subtypes C1, C2 and C3 (Figure 1C).

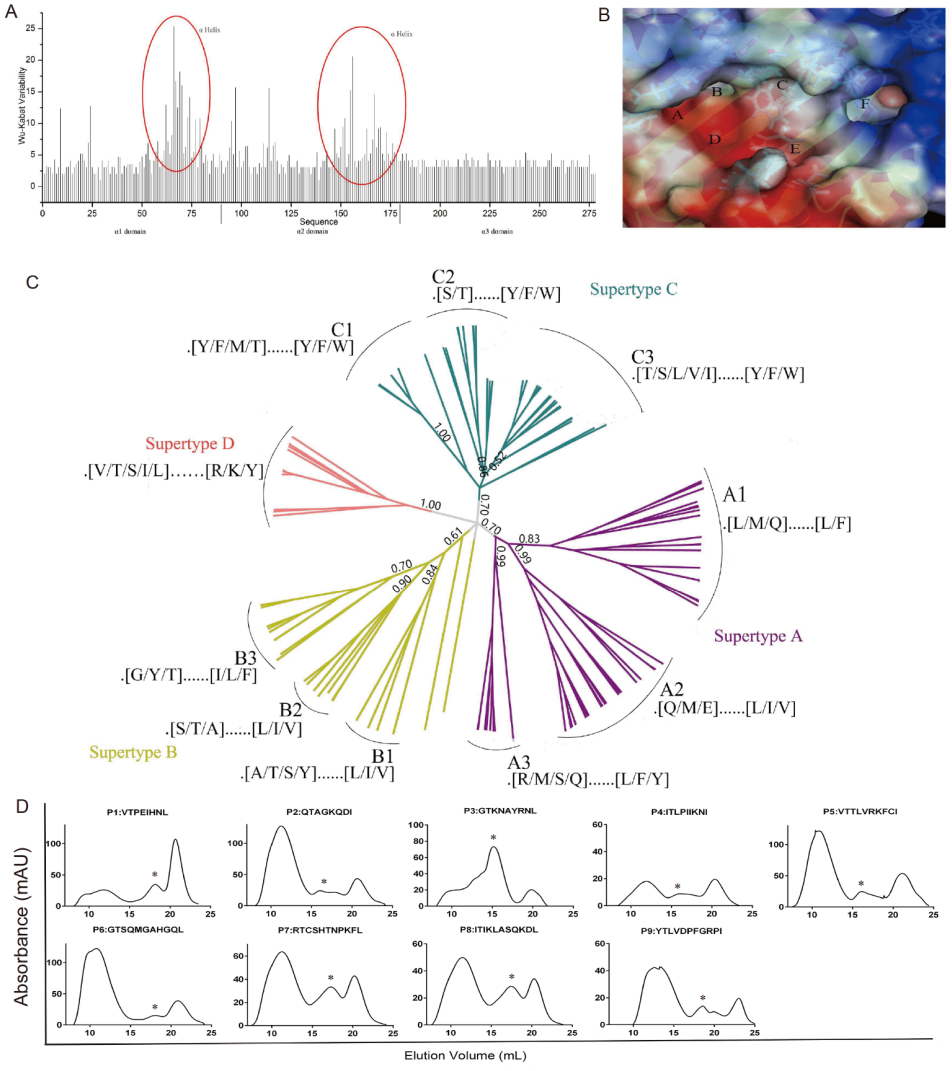

Figure 1 Supertypes and the amino acid variation regions of SLA I molecules. A, Wu-Kabat variability at amino acids of SLA I is shown. The amino acid variation regions were mainly concentrated in the α1 and α2 domains, especially α helix. B, The modelling crystal structure of MH295054. A–F indicate pocket A–F. The red area is negatively charged and the blue area is positively charged. The amino acids with Wu-Kabat variation values greater than 10 were located in the structure (structure is shown as a stick model). C, Supertype of SLA I molecules. The amino acid motifs for each subtype are shown. D, The binding of peptides from P72 in ASFV identified by gel filtration with MH295054. P3, P7 and P8 could form stable complexes with MH295054. The specific peak is marked with "*".

The supertype was built based on the specificities of binding motifs. Table S2 (in Supporting Information) lists the amino acid motifs for each subtype. The alleles in subtype A1 had a preference for hydrophobic amino acids (L or F) in C-terminal position and amino acids (L, M or Q) in position 2. The alleles in subtype A2 had a preference for hydrophobic amino acids (L, I or V) in C-terminal position and amino acids (Q, M or E) in position 2. The subtype A3 was defined because the second amino acid in motifs was (R, M, S or Q), and the last amino acid was (L, F or Y). Motifs of these three subtypes were very similar, so they were classified as supertype A. The alleles in subtype B1 and B2 had a preference for hydrophobic amino acids (L, I or V) in C-terminal position. Amino acids in position 2 were different; they were (A, T, S or Y) and (S, T or A), respectively. The subtype B3 was defined because the second amino acid in motif was (G, Y or T), and the last amino acid was (I, L or F). The alleles in subtype C1, C2 and C3 shared a same peptide binding specificity for hydrophobic amino acids (Y, F or W) in C-terminal position, but had different amino acids in position 2, which were (Y, F, M or T), small aliphatic amino acids (S or T) and (T, S, L, V or I), respectively. The subtype C1, C2 and C3 were classified as a supertype C because amino acids (Y, F or W) in C-terminal position were similar. The amino acid motifs of supertype D were different from other supertypes, especially the last amino acids which preferred aromatic and basic amino acids (R, K or Y). The small, aliphatic and aromatic amino acids (V, T, S, I or L) frequently appeared on the second amino acid residue of this supertype motifs. In the motifs of these four supertypes, the amino acid residues at position 2 were diverse, while the ones at C-terminal position were less variable, mainly hydrophobic amino acids such as L, I, V, F, and Y (except for supertype D). The SLA I alleles at the different locus could be located at the same supertype, such as MH294966 (SLA-1), MH295050 (SLA-2) and MH295113 (SLA-3), belonging to supertype A (Table S3 in Supporting Information). The result also suggests the SLA I alleles at the different loci could have similar peptide binding.

African swine fever (ASF) is an acute, hemorrhagic, and severe infectious disease with a mortality rate of 100%. There is currently no effective commercial vaccine against ASFV. The live attenuated vaccine provides greater level of protection, but there are biosafety issues such as virulence re-emergence and gene recombination. Generally ASFV inactivated vaccine is not very effective, so the role of only single humoral immunity in anti-ASFV is not very clear. Some studies have shown that cellular immunity plays an important role in protection against ASFV (Oura et al., 2005). Therefore, the development of vaccines containing cellular immunity caused by CTL epitopes is a good strategy for the development of ASFV vaccines. Protein P72 of ASFV which is structural protein is identified as highly immunogenic. The protein is commonly used as the goal protein of ASFV subunit vaccines (Kollnberger et al., 2002).

SLA-1*0401 and SLA-2*hs0202 belong to supertype C and A, respectively, whose crystal structures have been reported (Zhang et al., 2011; Fan et al., 2016). There is no relevant report on crystal structures of SLA I molecules in supertype B and D, and there are more alleles in supertype B than in supertype D. Therefore, this paper focuses on the simulation structure and peptide binding of SLA I molecules in supertype B. According to the similarity of genetic sequences, MH295054 which is an allele in supertype B may reflect the characteristics of other alleles in this supertype to some extent. Therefore, the crystal structure of MH295054 was simulated using Swiss-model in order to study binding peptides of SLA I in supertype B (Figure 1B). In the simulated structure, the amino acids with Wu-Kabat variation values greater than 10 were located on the pockets or α helix. The D and E pockets in the modeling crystal structure were negatively charged (Figure 1B), so these two pockets prefer the positively charged amino acids K, R, and H. This caused the third, third to last and fourth to last amino acids favor K, R, and H in SLA-binding peptides. In supertype B, the motifs .[T].....[I/L] covered subtypes B1, B2 and B3. We used these two motifs to analyze for P72 of ASFV and considered the K, R, and H at the corresponding positions. Nine potential CTL epitopes were identified including P1:VTPEIHNL, P2:QTAGKQDI, P3:GTKNAYRNL, P4:ITLPIIKNI, P5:VTTLVRKFCI, P6:GTSQMGAHGQL, P7:RTCSHTNPKFL, P8:ITIKLASQKDL and P9:YTLVDPFGRPI (Table S4 in Supporting Information).

The recombinant proteins of heavy chain and of β_2m of MH295054 were expressed as inclusion bodies, and purification of MH295054 assembled with CTL epitopes peptides were performed by chromatography on a Superdex 200 increase 10/300 GL (GE Healthcare) size-exclusion column. As shown in Figure 1D, the three CTL epitope peptides, namely P3, P7 and P8, could form stable complexes with MH295054, and peptides P1, P2, P4, P5, P6 and P9 could not form stable complexes with MH295054. This result indicates that these peptides P3, P7 and P8 could be as candidate peptide antigens to develop subunit vaccines which could cover alleles in supertype B.

In summary, we defined 164 novel SLA I alleles from three-way crossbred pigs and added them to the NCBI database. We also explored the polymorphisms and functional diversities of SLA I alleles. Supertype of SLA I molecules was built with a large sample for the first time, and the amino acid motifs for alleles in each supertype were analyzed. These help to determine the CTL epitope for swine virus more accurately. The CTL epitopes of ASFV which had high animal coverage were identified by these motifs in supertype B. These epitopes including P3, P7 and P8 would be candidate peptides for the development of the ASFV vaccine

which could cover swines with alleles in supertype B. The supertype of SLA I will be very useful to develop the cellular immune vaccines for swine virus.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

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