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Genetic Variation at the TVB Locus in Chinese Native Chicken Breeds

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Abstract: Tumor Virus locus B (TVB) encodes the cellular receptor for ALV subgroups B, D and E. Two Single Nucleotide Polymorphisms (SNPs) at nucleotide positions 172 (C/T) and 184 (T/A) together differentiate the allelic transcripts for TVB*S1, TVB*S3 and TVB*R. Genetic diversity at the TVB locus of chickens was investigated in Chinese indigenous chickens and the White Leghorns. An earlier undescribed nucleotide substitution at 184 (G to T) has been identified in Chinese native chicken breeds using the direct sequencing. Researchers found that the novel allele TVB*S′ was in all of Chinese native breeds but TC and homozygous TVB*S'/*S' genotype found in ZZG, XSBNG and CHC, theirs frequencies were 0.28, 0.26 and 0.57, respectively. Except CHC, Chinese native chicken breeds have higher homozygous TVB*S1/*S1 frequency than WL populations. The resistant allele TVB*R was found in WL populations and TC flocks with a frequency of 0.44 and 0.11, respectively.

Key words: Genetic variation, tumor virus locus B, Chinese native chicken, anti-avian leukosis virus breeding, homozygous

INTRODUCTION

Avian Leukosis Viruses (ALVs) are divided into six major viral subgroups (A to E and J) based on infection interference patterns and virus receptor usage (Payne, 1998). Five of the subgroups (ALVA to ALVD and ALVJ) are exogenous avian viruses and the other subgroup (ALVE) is an endogenous virus. The exogenous viruses of subgroups ALVA to ALVD and the endogenous virus ALVE initiate the cell entry phase of infection through an interaction between surface units on viral subgroup-specific glycoprotein envelope and subgroup-specific surface receptors on host cells. After decades of studies, four autosomal Tumour Virus (TV) loci encoding cellular receptors for six ALV subgroups have been identified and characterized (Zhang *et al*., 2007). TVA and TVC encode the receptors for subgroup ALVA and subgroup ALVC, respectively. They carried either dominant susceptibility alleles (e.g., TVA*S, TVC*S) or recessive resistance alleles (e.g., TVA*R, TVC*R). The susceptibility alleles code for functional virus receptors whereas the resistance alleles are expressed as non-functional receptors or as the absence of receptor (Elleder *et al*., 2004). The susceptibility allele code for functional ALVJ receptors has been found but the resistance alleles not yet (Chai and Bates, 2006).

TVB is the most complex locus among the 4 cellular receptor genes. It encodes the cellular receptor for ALV subgroups B, D and E. TVB*S1 encodes a cellular receptor mediating infection of subgroups B, D and E. TVB*S3 encodes the receptor for two subgroups, B and D (Adkins *et al*., 2001). TVB*R encodes a dysfunctional receptor that is incapable of mediating any ALV-B, ALV-D or ALV-E infection (Klucking and Young, 2004). TVB*S1 is completely dominant to TVB*S3 and TVB*R and TVB*S3 is completely dominant to TVB*R. Two Single Nucleotide Polymorphisms (SNPs) at nucleotide positions 172 (C/T) and 184 (T/A) of *TVB* gene cDNA sequences (GenBank accession numbers AF161713, AF161712 and AF507016.1) together differentiate the allelic transcripts for TVB*S1, TVB*S3 and TVB*R. Additionally, some points of the *TVB* gene is important for subgroup E interaction determinants (Klucking and Young, 2004).

Because ALV can affect production traits included age at sexual maturity, egg production, egg weight, fertility, hatchability, nonspecific mortality and body weight (Payne, 1998), a long-term and more effective control of the economic loss and contaminations induced by ALV may lie in reducing genetic susceptibility of commercial egg-layer and broiler populations through selection for genetic resistance to ALV in the breeder

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flocks. Three methods for check polymorphism of the *TVB* gene were reported such as Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay (Zhang *et al*., 2005) and a medium-throughput or a high-throughput assay (Zhang *et al*., 2007; Yang *et al*., 2011) but those methods have the limitation. The goal of this study was to survey the status of genotypes of TVB in Chinese native chicken breeds and an introduced breed, White Leghorn chickens with method of direct DNA sequencing.

MATERIALS AND METHODS

Chicken breeds: Nine Chinese native chicken breeds and one introduced chicken breed were surveyed: Jiangshan Black-Bone Chicken (JSBBC; n = 48), Wumeng Black-Bone chicken (WMBBC; n = 48), Ningdu Sanhuang (NDSH; n = 46), Zhangzhou Game (ZZG; n = 47), Xishuangbanna Game (XSBNG; n = 46), Wenchang Chicken (WCC; n = 36), Tibetan Chicken (TC; n = 57), Chahua Chicken (CHC; n = 35) and White Leghorns (WL; n = 96). The samples of nine native chicken breeds were randomly collected from 8 provinces in China. The WL originated from a pure line population of White Leghorns in which ALVE21had been fixed via slow-feathering selection. Researchers detected the genotypes of TVB in the eight Chinese native chicken breeds and White Leghorn populations.

DNA extraction and genotyping: A whole blood sample for each bird was used for genomic DNA isolation by the Phenol-Chloroform Method. The quantity and quality of DNA were measured via an ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE). The TVB was genotyped with direct DNA sequencing. Purified PCR products were quantified and normalized with sterile ultrapure (MilliQ) water. Because the PCR product has nearly 70% G and C nucleotide content, 0.5 μL Dimethyl Sulfoxide (DMSO; 2.5%) was added to break the GC bonds. Low volume DNA sequencing was done by a fluorescence detection system and compared to the consensus sequence (ABI Applied Biosystems 3730 DNA Analyzer (accuracy 99.96%), Sequencer program 4.0.5 (Gene codes). The sequencing electropherogram was analyzed by software and polymorphisms were displayed as chromatogram files and visualized (Finch TV V1.4.0, Geospiza). Heterozygotes (G/T) were further verified by Mutation Surveyor 3.0 (Soft Genetics).

RESULTS

New mutation at nucleotide positions 184 (G/T) of TVB locus in Chinese native chicken breeds: The direct sequencing results showed that there was one single nucleotide mutation at cDNA 184 (G→T) of *TVB* gene in Chinese native chicken breeds and this mutation resulted in an amino acid change (glycine→cysteine) at residue 62 of TVB which is a critical determinant for subgroup E viral infection. Researchers named this allele of TVB is TVB*S'.

The genetic variability of the *TVB* gene and genotype frequencies of Chinese native chicken breeds and White Leghorn populations: Population types, number of lines and sample sizes are presented in Table 1. The novel allele TVB*S′ was in all of Chinese native breeds but TC, the estimated gene frequency varied from 0.03-0.74. TVB*S1 was the most common allele in all chicken breeds, theirs frequencies varied from 0.26-0.97. TVB*S3 was at a very low frequency (0.01) in populations of White Leghorn and was not detected in all Chinese native chicken breeds. The resistant allele, TVB*R was found in WL populations and TC flocks with a frequency of 0.44 and 0.11, respectively.

Considering genotype frequencies, Chinese native chicken breeds have higher homozygous TVB*S1/*S1 frequency than WL populations except CHC. The 19% of White Leghorn were typed as TVB*R/*R whereas none of Chinese native chicken breeds was found. TVB*S'/*S' genotype found in ZZG, XSBNG and CHC, theirs frequencies were 0.28, 0.26 and 0.57, respectively but

Table 1: *TVB* gene and genotypic frequencies of Chinese native chicken breeds and White Leghorn flocks

		Genotypic frequency							Allelic frequency			
Breeds	Number	S1S1	S1R	S1S3	S3R	S'S'	RR	S1S'	S1	S3	S'	R
WL	96	0.29	0.50	0.01	0.01	-	0.19	-	0.55	0.01	-	0.44
TC	57	0.79	0.21	-	-		-	-	0.89	-	-	0.11
NDSH	46	0.89	-	-	-	-	-	0.11	0.95	-	0.05	-
JSBBC	48	0.87	-	-	-	-	-	0.13	0.94	-	0.06	-
WMBBC	48	0.90	-	-	-	-	-	0.10	0.95	-	0.05	-
ZZG	47	0.32	-	-	-	0.28	-	0.40	0.52	-	0.48	-
XSBNG	46	0.41	-	-	-	0.26	-	0.33	0.58	-	0.42	-
WCC	36	0.94	-	-	-	-	-	0.06	0.97	-	0.03	-
CHC	35	0.09	-	-	-	0.57	-	0.34	0.26	-	0.74	-

homozygous TVB*S3/*S3 not found in all flocks. The novel genotype TVB*S1/*S' was found in NDSH, JSBBC, WMBBC, ZZG, XSBNG, WCC and CHC flocks with a frequency of 0.11, 0.13, 0.10, 0.40, 0.33, 0.06 and 0.34, respectively. The TVB*S1/*R genotype frequency of WL and TC populations is 0.50 and 0.21, respectively and 1% were typed as TVB*S3/*R in WL populations.

DISCUSSION

An earlier undescribed nucleotide substitution at base position 184 (G/T) of TVB locus in Chinese native breeds has been identified using the direct DNA sequencing. Compared with the PCR-restriction fragment length polymorphism assay (Zhang *et al*., 2005), medium-throughput assay and high-throughput assay (Zhang *et al*., 2007), direct DNA sequencing can identify precisely any novel base substitutions within the sequence to analys domain that may occur in tested samples. The position of the nucleotide substitution is exactly the same as a previously described mutation TVB*S3 (Adkins *et al*., 2000) indicating for the first time that nucleotide substitutions at the same position in the sequence of TVB can cause resistance for ALV-E. The nucleotide substitution of G to T at base position 184 as described here (designated TVB*S′) is the first point mutation identified among chickens causing TVB nonsense mutation. In addition, this report provides evidence for the first time that two different nucleotide substitutions at the same base position in the chicken *TVB* gene lead to the generation of two different mutant alleles. Thus, in this case at TGT (Cys) codon 62 was found to have changed to GGT (Gly) whereas in TVB*S3, a nucleotide substitution has been found not only in the same codon but also at the same base position (TGT to AGT). In the case of TVB*S3, the mutation caused the loss of binding to endogenous subgroup E viral glycoproteins (Brojatsch *et al*., 1996), maybe in the present case, it caused the same research. Although, the type of nucleotide substitution was different between the two, the fact that a single base position became the target of the mutation twice in TVB locus may indicate that this position is more selective pressure on the chicken population because of ALVE presence.

In this study, most of Chinese native breeds have the allele of TVB*S′ do not in the populations of White Leghorn used as a control. And total of 74 lines representing pure-line chicken populations managed by multi-major commercial broiler and egg-layer companies and academic institutes have also not this new allele of TVB*S′ (Zhang *et al*., 2007). Researchers can infer that Chinese native chicken breeds maybe have a different evolution way for resistance of subgroup ALVE, compare with commonly chicken breeds used for layer, meat and experiment. In all of Chinese native breeds, TVB*R was just detected in TC chickens with gene frequency 0.11 whereas White Leghorn in this study has higher TVB*R frequency (0.44). Because TVB*S3 and TVB*R can resistance to ALV-E infection (Klucking *et al*., 2002), the number and frequency of *ALV-E* genes is one of causes that the frequencies difference of TVB*S′ (or TVB*S3) and TVB*R in different chicken populations. The earlier study shows that Chinese native chicken breeds have the lower frequency of TVB*R than White Leghorn populations (Yang *et al*., 2011) and layers have the higher frequency of TVB*R than broilers (Zhang *et al*., 2007). The consistent presence of *ALV-E* genes in White Leghorn populations would potentially provide a uniform expression of ALV-E proteins. The uniform expression of *ALV-E* genes in a line of WL as opposed to Chinese native chicken breeds may somehow affect the type of TVB receptors (Zhang *et al*., 2007). Therefore, the functions of these endogenous viruses should be explored and more molecular information provided for anti-disease breeding in chickens.

CONCLUSION

These results present molecular evidence of *TVB* genotypes in Chinese native chickens and could provide potential molecular insights into anti-ALV breeding in chickens.

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