Molecular identification of avian leukosis virus subgroup E loci and tumor virus B locus in Chinese indigenous chickens

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ABSTRACT Avian leukosis virus (ALV) subgroup E (*ALVE*) is an endogenous retrovirus in the chicken genome. The chickens carrying *ALVE* locus 3 (*ALVE3*), 6 (*ALVE6*), 9 (*ALVE9*), and 21 (*ALVE21*) have been proved to be susceptible to ALV. Tumor virus locus B (*TVB*) encodes the cellular receptor for ALV subgroups B, D, and E. The insertions of the 4 *ALVE* loci and the genotypes of *TVB* have not been demonstrated in Chinese indigenous chicken breeds. In the present study, the existence of *ALVE3*, *ALVE6*, *ALVE9*, and *ALVE21* were detected in 10 native breeds of Chinese chickens and an introduced breed, the White Leghorn (2 populations in this study, WL1 and WL2), by locus-specific PCR. The PCR products of *ALVE* were further confirmed by sequencing assay. We also surveyed the status of genotypes of *TVB* in Silkie, Beijing You, and White Leghorn (WL1 and WL2) chickens with pyrosequencing assays. The results showed that the carrier frequency of *ALVE3* was 1.3% in the Chinese chicken population, and was 10.3 in WL1 and 49.2% in WL2. The carrier frequency of *ALVE6* was 5.4% in native breeds of Chinese birds, in contrast with 0% in WL1 and 6.8% in WL2. The carrier frequency of *ALVE9* was 0.1% in the Chinese indigenous population, and was 16.0% in WL1 and 11.9% in WL2. The carrier frequency of *ALVE21* was 10.4% in Chinese chickens, whereas *ALVE21* was detected with a frequency of 0% in WL1 and 50% in WL2. The frequency of the *TVB* resistance allele (*TVB**R and *TVB**R′) was 0.4% in Beijing You chickens, whereas it was 70.5% in WL1 and 54.5% in WL2. No carriers of *ALVE3*, *ALVE9*, and *ALVE21* were detected in Silkie fowl, a famous Chinese native breed that has been used as a source for alternative medicine. These results present molecular evidence of *ALVE3*, *ALVE6, ALVE9*, and *ALVE21* insertions and *TVB* genotypes in Chinese indigenous chickens and could provide potential molecular insights into anti-ALV breeding in chickens.

Key words: avian leukosis virus subgroup E, tumor virus locus B, Chinese indigenous chicken, White Leghorn, anti-avian leukosis virus breeding

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INTRODUCTION

The avian leukosis viruses (**ALV**) are retroviruses that consist of 6 subgroups, according to interactions between virus-specific cell receptors and viral envelope glycoproteins (Payne, 1998; Silva et al., 2007; Cheng et al., 2010). Among these retroviruses, ALV subgroup E (*ALVE*) is unique in being an endogenous virus in chickens (Bacon et al., 2000). The others, including *ALVA*, *ALVB*, *ALVC*, *ALVD*, and *ALVJ*, are exogenous retroviruses (Crittenden, 1991; Benkel, 1998; Yu et al., 2008; Cheng et al., 2010). The E-type ALV has been integrated into the chicken genome and can be inherited in a Mendelian manner along with the host genome (Bacon et al., 2000).

Some endogenous proviral loci retain the capacity to code complete infectious retroviruses (Crittenden et al., 1984; Benkel, 1998; Bacon et al., 2000). However, proviruses are often inactive as a result of either hypermethylation or deletion of essential fragments that encode peptides for the infectious viral particles (Baker et al., 1981; Crittenden et al., 1982; Crittenden, 1991; Benkel, 1998; Yu et al., 2008).

Depending on the existence of endogenous proviral loci, the *ALVE* carriers may be resistant or susceptible to ALV (Bacon et al., 2000; Yu et al., 2008). It was reported that *ALVE* locus 3 (***ALVE3***) was inserted into intron F of the chicken proto-onco gene *hck* (hemopoietic cell kinase; Quintrell et al., 1987; Benkel, 1998) and that the carriers of *ALVE3* were susceptible to ALV (Benkel, 1998; Yu et al., 2008; Benkel et al., 1995). The *ALVE* locus 6 (***ALVE6***) and locus 9 (***ALVE9***) are incomplete proviruses that have unpopular effects on birds because they may induce tolerance to patho-

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genic ALV. Moreover, there is evidence that the prevalence and mortality rates are higher in *ALVE* locus 21 (***ALVE21***) carriers than in the noncarrier population (Tixier-Boichard et al., 1994).

Four cellular receptors of ALV have been identified (Zhang et al., 2007). The tumor virus loci A, C, and J (*TVA*, *TVC*, and *TVJ*) encode cellular receptors for ALVA, ALVC, and ALV-J, respectively. Tumor virus locus B (***TVB***) is the most complex locus among the 4 cellular receptor genes. It encodes cellular receptors for 3 subgroups (ALVB, ALVD, and ALVE*)* and is classified into 4 alleles on the basis of 2 SNP. The *TVB*S1* allele (SNP1 is C, SNP2 is T), which is the most susceptible *TVB* allele, encodes receptors that permit ALVB, ALVD, and ALVE infection. The *TVB*S3* allele (SNP1 is C, SNP2 is A) encodes receptors supporting viral entry of both ALVB and ALVD, but not ALVE. The *TVB*R* allele (SNP1 is T, SNP2 is T) is a resistant allele that encodes an incomplete receptor incapable of inducing any ALVB, ALVD, or ALVE infection. The *TVB*R′* (SNP1 is T, SNP2 is A) and *TVB*R* alleles are distinct alleles, but they have the same function. For the reason that both of them induce the resistance effect, *TVB*R′* and *TVB*R* are referred to as types of R (Zhang et al., 2005). The *TVB*R* allele is completely recessive to *TVB*S3*, and the *TVB*S3* and *TVB*R* alleles are completely recessive to *TVB*S1*. In other words, *TVB*S1/S1* (CT/CT) and *TVB*S1/R* (CT/TT) are susceptible to ALVE, whereas *TVB*R/R* (TT/TT) and *TVB*S3/R′* (CA/TA) are resistant to ALVE (Hunt et al., 2008).

To eliminate the susceptibility of chickens to ALV, the status of the *ALVE* insertion and *TVB* alleles must be established. So far, the prevalence rate of leukosis diseases has been reported as approximately 3 to 30% in Chinese native chickens (Wang et al., 2007); however, no information is available on the epidemiology of *ALVE* and *TVB* in Chinese indigenous chickens. In the present study, we conducted a large-scale investigation on the existence of *ALVE3*, *ALVE6, ALVE9*, and *ALVE21* in 10 Chinese native chicken breeds and an introduced breed, White Leghorn chickens. The *TVB* genotypes were tested in Beijing You (**BJY**), Silkie (**SL**), and White Leghorn chickens (**WL1** and **WL2**).

MATERIALS AND METHODS

Samples

Ten Chinese native chicken breeds and one introduced chicken breed were surveyed: SL (n = 126), BJY (n = 133), Huainan partridge chicken (**HNPC**; n = 120), Wenchang chicken (**WCC**; n = 120), Taihu chicken (**THC**; n = 120), Liyang chicken (**LYC**; n = 120), Rugao chicken (**RGC**; n = 120), Tibetan chicken (**TC**; n = 120), Gushi chicken (**GSC**; n = 118), Hebei Chai chicken (**HBCC**; n = 120), and 2 populations of White Leghorns (n = 29 for WL1, and n = 66 for WL2). The samples of 10 native chicken breeds were randomly collected from 8 provinces in China, as shown in Figure 1. The WL1 originated from a commercial population of White Leghorns in which *ALVE21* had been eliminated via slow-feathering selection (Lowe and Garwood, 1981). However, there was no selection on the other *ALVE* in the WL1. The WL2 birds were randomly collected from a closed breeding population of 2 commercial White Leghorn lines introduced separately into China from the Netherlands and Canada many years ago. During closed breeding, no selection has been conducted to eliminate *ALVE* in this population. In total, 1,260, 1,260, 1,202, and 1,205 samples were detected for the *ALVE3*, *ALVE6*, *ALVE9*, and *ALVE21* loci, respectively. We further detected the genotypes of *TVB* in the SL (n = 83), BJY (n = 114), and the 2 populations of White Leghorn (n = 14 for WL1 and n = 44 for WL2).

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). Genotyping of *ALVE* was examined by optimized locus-specific touchdown PCR (Don et al., 1991; Benkel, 1998) with an ABI 9700 PCR instrument (Applied Biosystems, Foster City, CA). The touchdown PCR was used to minimize nonspecific amplification and was carried out in a 20-μL solution: 50 ng of genome DNA, 1× PCR buffer, 0.2 m*M* deoxynucleotide 5′-triphosphates, 0.5 μ*M* for each primer, and 0.75 U of HotStart Taq DNA polymerase (Qiagen, Valencia,

Figure 1. Locations of 10 native chicken breeds in China. 1: Hebei Chai chicken from Hebei province; 2: Beijing You from Beijing; 3: Gushi chicken from Henan province. 4: Taihu, Liyang, and Rugao chickens from Jiangsu province; 5: Huainan partridge chicken from Anhui province; 6: Silkie from Jiangxi province; 7: Wenchang chicken from Hainan province; 8: Tibetan chicken from Tibet.

CA). To identify the insertion status of the 4 *ALVE*, the PCR products were verified by using 2% agarose gels and confirmed by an ABI 3700 sequencer (Applied Biosystems).

The *TVB* was genotyped with a pyrosequencing assay (PyroMark ID, Qiagen). This method is usually used for short-read DNA sequencing and mutation analysis. The 3 primers for *TVB* SNP genotyping referred to the previous research (Zhang et al., 2007).

RESULTS

Genotyping and Sequencing of ALVE3, ALVE6, ALVE9, and ALVE21

The genotypes of the 4 *ALVE* loci for each individual can be directly identified by PCR fragment sizes, as shown in Figure 2. As for *ALVE3*, the smaller fragment (190 bp) represents its insertion (*ALVE3+*), the larger one (270 bp) indicates its absence (*ALVE3–*), and the paired bands represent a heterozygous chicken *ALVE3+/–* (Figure 2A). The fragment size for inserted *ALVE6* is 300 bp (*ALVE6+*), whereas there is no band for the noncarrier *ALVE6* (*ALVE6–*; Figure 2B). It is 450 bp (*ALVE9–*) or 115 bp (*ALVE9+*) for *ALVE9*-homozygous birds, and the paired bands represent *ALVE9+/–* (Figure 2C). The amplification band for the inserted *ALVE21* is 390 bp (*ALVE21+/+*), that for the noninserted *ALVE21* is 510 bp (*ALVE21–/–*), and the paired bands represent the simultaneous presence of a duplicate insertion site that was occupied by *ALVE21*, together with the unoccupied site in *ALVE21+/–* birds (Figure 2D; Tixier-Boichard et al., 1994).

To confirm the genotyping results and map the chromosome positions of *ALVE3*, *ALVE6*, *ALVE9*, and *ALVE21*, the PCR products were sequenced, and sequence alignment was done on National Center for Biotechnology Information (using BLAST) and Ensembl (http://www.ensembl.org/index.html). We found that *ALVE3* was inserted in intron F of the *hck* gene and was mapped onto autosome 20 (Figure 3A), *ALVE6* was located within the long arm of chromosome 1 (Figure 3B), the insertion site of *ALVE9* was within chromosome 6 (Figure 3C), and *ALVE21* was located within sex-chromosome Z (Figure 3D), which was consistent with previous reports (Crittenden, 1991; Benkel, 1998).

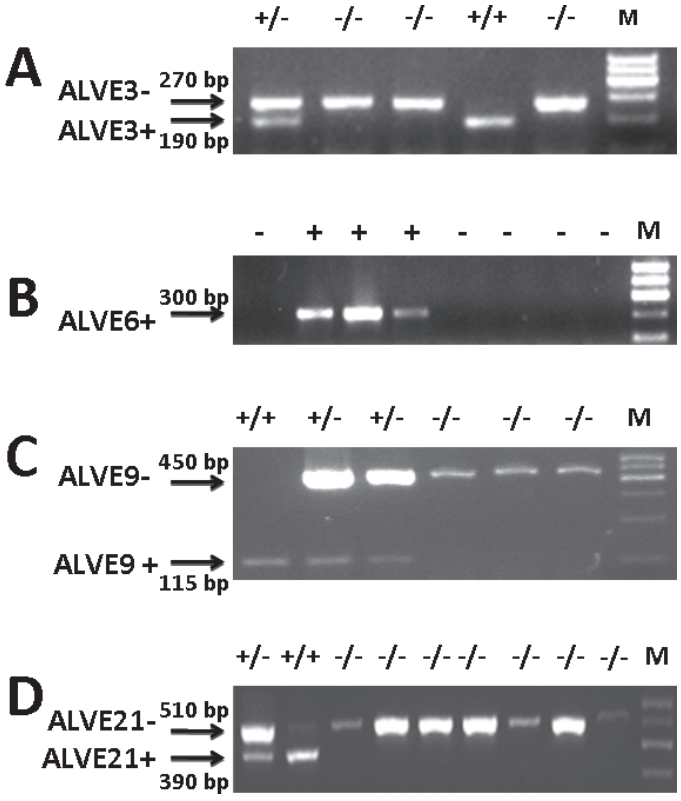

Figure 2. Molecular diagnostic for avian leukosis virus subgroup E (*ALVE*) locus 3 (*ALVE3*; A), locus 6 (*ALVE6*; B), locus 9 (*ALVE9*; C), and locus 21 (*ALVE21*; D) in the chickens using locus-specific PCR. A) Lane 1: *ALVE3* +/– (270 bp plus 190 bp); lanes 2, 3, and 5: *ALVE3* –/– (270 bp); lane 4: *ALVE3* +/+ (190 bp). B) Lanes 1, 5, 6, 7, and 8: *ALVE6* –; lanes 2, 3, and 4: *ALVE6* + (300 bp). C) Lane 1: *ALVE9* +/+ (115 bp); lanes 2 and 3: *ALVE9* +/– (450 bp plus 115 bp); lanes 4, 5, and 6: *ALVE9* –/– (450 bp). D) Lane 1: *ALVE21* +/– (510 bp plus 390 bp); lane 2: *ALVE21* +/+ (390 bp); lanes 3, 4, 5, 6, 7, 8, and 9: *ALVE21* –/– (510 bp). M represents a 100-bp DNA marker.

Carrier Frequency of ALVE3 and ALVE6

The insertion status of *ALVE3* and *ALVE6* was detected in 11 chicken breeds (n = 1,260 for each). The carrier frequency of *ALVE3* and *ALVE6* in each breed is shown in Table 1. No homozygous *ALVE3+/+* individual was identified in the 10 Chinese native breeds and WL1 population, whereas approximately 26% of the WL2 birds were *ALVE3+/+*. A few heterozygous *ALVE3+/–* individuals were detected in HNPC (1.8%), BJY (3.0%), HBCC (16.0%), and TC (4.6%) among the Chinese chicken breeds. In contrast, the ratio of heterozygous *ALVE3+/–* was higher in WL1 (20.7%) and WL2 (46.8%) chickens. Only the *ALVE6* element did not insert in RGC, TC, and WL1, whereas 0.8, 1.7, 0.8, 5.0, 10.0, 10.0, 5.2, and 20.8% positive birds (*ALVE6+*) were detected in the Chinese chicken breeds HNEC, SL, BJY, WCC, THC, HBCC, GSC, and LYC and 6.8% were detected in WL2 birds.

Carrier Frequency of ALVE9 and ALVE21

The insertion status of *ALVE9* and *ALVE21* was detected in 11 chicken breeds (n = 1,202 for *ALVE9* and n = 1,205 for *ALVE21*), and their carrier frequency in each breed is shown in Table 2. No homozygous *ALVE9+/+* individual was identified among the 10 Chinese native breeds, whereas 8.0 and 3.4% of *ALVE9+/+* were detected in WL1 and WL2 birds, respectively. In the TC, 2.7% were heterozygous individuals (*ALVE9+/–*). In contrast, the frequencies of *ALVE9+/–* was 16.0 and 16.9% in WL1 and WL2, which were much higher than in Chinese birds. No *ALVE21* element was inserted in SL fowl or WL1, whereas 15.3 and 0.9% positive

Table 1. Genotype and carrier frequencies of avian leukosis virus subgroup E (*ALVE*) locus 3 (*ALVE3*) and locus 6 (*ALVE6*) in the chickens

		ALVE3 genotype frequency			*ALVE3* carrier frequency			*ALVE6* carrier frequency	
Breed[1]	No.	+/+	+/−	−/−	+	−	No.	+	−
HNPC	111	0	0.018	0.982	0.009	0.991	120	0.008	0.992
SL	118	0	0	1	0	1	120	0.017	0.983
BJY	133	0	0.030	0.970	0.015	0.985	120	0.008	0.992
WCC	113	0	0	1	0	1	120	0.050	0.950
THC	119	0	0	1	0	1	120	0.100	0.900
HBCC	119	0	0.160	0.840	0.080	0.920	120	0.100	0.900
RGC	117	0	0	1	0	1	120	0	1
GSC	118	0	0	1	0	1	96	0.052	0.948
LYC	112	0	0	1	0	1	120	0.208	0.792
TC	109	0	0.046	0.954	0.023	0.977	120	0	1
WL1	29	0	0.207	0.793	0.103	0.897	25	0	1
WL2	62	0.258	0.468	0.274	0.492	0.508	59	0.068	0.932
Total	1,260						1,260		

[1]HNPC = Huainan partridge chicken from Anhui province; SL = Silkie from Jiangxi province; BJY = Beijing You from Beijing; WCC = Wenchang chicken from Hainan province; THC = Taihu chicken from Jiangsu province; HBCC = Hebei Chai chicken from Hebei province; RGC = Rugao chicken from Jiangsu province; GSC = Gushi chicken from Henan province; LYC = Liyang chicken from Jiangsu province; TC = Tibetan chicken from Tibet; WL1 = first population of White Leghorns; WL2 = second population of White Leghorns.

birds (*ALVE21*+/+) were detected in the BJY and GSC breeds, respectively. The *ALVE21*+/− was found in HNPC (11.6%), BJY (55.0%), WCC (3.5%), THC (3.1%), HBCC (9.9%), RGC (5.3%), GSC (7.1%), LYC (51.5%), and TC (32.1%) among the Chinese chicken breeds and was found in 100% of WL2 birds. The BJY had the highest carrier frequency of *ALVE21*+ (42.8%) among the Chinese chicken breeds.

Genotypes and Genotypic Frequency of the TVB Gene

To ascertain the potential for tolerance induction of *ALVE21*+/+ or *ALVE21*+/− of the BJY, we detected the genotypes of the *TVB* gene in BJY chickens and also compared them in SL, WL1, and WL2 chickens (n = 258). We found that the *TVB* gene was located within chromosome 22, which was consistent with a previous report (Zhang et al., 2005). Four *TVB* genotypes were tested in the chickens, and the pyrograms are shown in Figure 4. The frequency of *TVB* genotypes detected is listed in Table 3. The susceptibility frequencies of the *TVB*S1/S1* (Figure 4A) and *S1/R* (Figure 4C) genotypes in the WL1 population were 11.8 and 35.3%, respectively, whereas 52.9% of the WL1 birds were resistant (*TVB*R/R*; Figure 4D). For the WL2 birds, the genotypic frequencies of susceptibility (*TVB*S1/S1* and *S1/R*) were 18.2 and 52.3%, respectively, and those of resistant birds (*TVB*R/R* and *S3/R′*, Figure 4B) were 27.3 and 2.2%, respectively. In contrast, all the BJY and SL birds were susceptible genotypes (*TVB*S1/S1* or *S1/R*).

DISCUSSION

Avian leukosis has caused huge economic losses in the poultry industry; however, no effective vaccine is available. Anti-disease breeding from a genetic angle is an alternative method to prevent avian leukosis infection. Numerous studies suggest that the carriers of *ALVE3*, *ALVE6*, *ALVE9*, *ALVE21*, and the susceptible allele

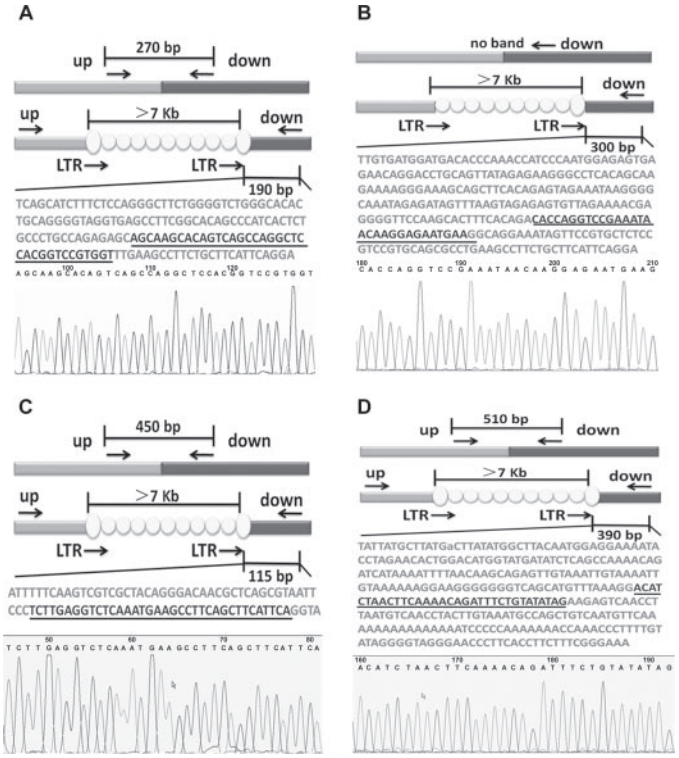

Figure 3. The sequencing results of the locus-specific PCR products for avian leukosis virus subgroup E (*ALVE*) locus 3 (*ALVE3*; A), locus 6 (*ALVE6*; B), locus 9 (*ALVE9*; C), and locus 21 (*ALVE21*; D). The upper panel represents the chromosomes that lack the endogenous viral element or integrated proviral element. Arrows mark the locations of the locus-specific PCR primers. The primers "up" and "down" lead to a negative band, whereas the primers "LTR" and "down" yield a positive band. For *ALVE*-integrated birds, the distance between primers "up" or upstream "LTR" and "down" was too long to amplify effectively under standard PCR conditions. The lower sequencing maps show the underlined sequence of *ALVE3* (A), *ALVE6* (B), *ALVE9* (C), and *ALVE21* (D).

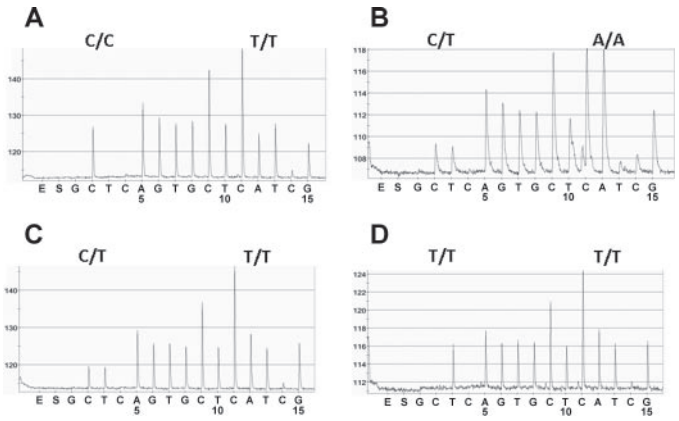

Figure 4. Pyrograms of the observed tumor virus locus B (*TVB*) genotypes by pyrosequencing assay. A) The *TVB*S1/S1* genotype. The first and the second SNP are C/C and T/T, respectively. B) The *TVB*S3/R′* genotype. The first and the second SNP are C/T and A/A, respectively. C) The *TVB*S1/R* genotype. The first and the second SNP are C/T and T/T, respectively. D) The *TVB*R/R* genotype. The first and the second SNP are T/T and T/T, respectively.

of *TVB* (*TVB*S1*) reduce the resistance of the host to ALV (Benkel, 1998; Bacon et al., 2000; Benkel et al., 1995; Tixier-Boichard et al., 1994). Eliminating the carriers of the 4 *ALVE* and *TVB*S1* from the ancestral breeders is a practical breeding strategy to defend the chickens against ALV infection (Bacon et al., 2000). The present study was the first large-scale investigation to detect the status of *ALVE3*, *ALVE6*, *ALVE9*, *ALVE21*, and *TVB*S1* in Chinese chicken breeds.

The average carrier frequency of *ALVE3* was 1.3% in Chinese native chickens, compared with 10.3% in WL1 and 49.2% in WL2. The carrier frequency of *ALVE6* was 5.4% in Chinese native birds, in contrast with 0% in WL1 and 6.8% in WL2. The frequency of the *ALVE9* insertion was much lower in the Chinese native population (0.1%), which was detected only in TC, but was 16% in WL1 and 11.9% in WL2. The carrier frequency of *ALVE21* (10.4%) was obviously higher than the carrier frequency of the other 3 *ALVE* in Chinese native chickens. Notably, no insertion of *ALVE3*, *ALVE9*, and *ALVE21* was found in any SL samples, and the carrier frequency of *ALVE6* was very low in that breed (1.7%). The SL were randomly collected from a commercial population, and there was no selection to eliminate *ALVE*. The SL is a famous native chicken breed in China and Southeast Asia countries. The eggs and meat of SL fowl have been credited with medicinal and health-promoting properties for thousands of years (Toyosaki and Koketsu, 2004). In recent decades, the medicinal chemical and biochemical components of SL meat and eggs have been determined by using modern scientific approaches. The results have shown that the SL whole eggs indicated significant oxidative stability causing restricted generation of hydroperoxides until 8 d of storage (Muroya et al., 2000; Toyosaki and Koketsu, 2004; Chen et al., 2008). However, any association with the absence of the *ALVE3*, *ALVE9*, and *ALVE21* insertions in the SL genome related to the medicinal value of SL fowl needs further study.

The first population of White Leghorns, WL1, was used as a control for our study. The WL1 birds were collected from a commercial population of White Leghorns in which *ALVE21* had been eliminated. All the samples from WL1 birds in the present study were *ALVE21*−, which is consistent with the background of the WL1 population or, alternatively, the accuracy of the *ALVE* locus-specific PCR used in the study.

Given the fact that *ALVE3*, *ALVE6*, and *ALVE9* insertions are of low frequency in Chinese chickens, it may be possible to eliminate these elements by molecular selection and thereby improve the resistance of Chinese chickens to ALV.

Table 2. Genotype and carrier frequencies of avian leukosis virus subgroup E (*ALVE*) locus 9 (*ALVE9*) and locus 21 (*ALVE21*) in the chickens

		ALVE9 genotype frequency			*ALVE9* carrier frequency			*ALVE21* genotype frequency			*ALVE21* carrier frequency	
Breed[1]	No.	+/+	+/−	−/−	+	−	No.	+/+	+/−	−/−	+	−
HNPC	117	0	0	1	0	1	112	0	0.116	0.884	0.058	0.942
SL	114	0	0	1	0	1	126	0	0	1	0	1
BJY	116	0	0	1	0	1	111	0.153	0.550	0.297	0.428	0.572
WCC	119	0	0	1	0	1	113	0	0.035	0.965	0.018	0.982
THC	101	0	0	1	0	1	98	0	0.031	0.969	0.018	0.985
HBCC	110	0	0	1	0	1	111	0	0.099	0.901	0.050	0.950
RGC	119	0	0	1	0	1	114	0	0.053	0.947	0.026	0.974
GSC	92	0	0	1	0	1	113	0.009	0.071	0.920	0.044	0.956
LYC	118	0	0	1	0	1	103	0	0.515	0.485	0.257	0.743
TC	112	0	0.027	0.973	0.013	0.987	109	0	0.321	0.679	0.161	0.839
WL1	25	0.080	0.160	0.760	0.160	0.840	29	0	0	1	0	1
WL2	59	0.034	0.169	0.797	0.119	0.881	66	0	1	0	0.500	0.500
Total	1,202						1,205					

[1]HNPC = Huainan partridge chicken from Anhui province; SL = Silkie from Jiangxi province; BJY = Beijing You from Beijing; WCC = Wenchang chicken from Hainan province; THC = Taihu chicken from Jiangsu province; HBCC = Hebei Chai chicken from Hebei province; RGC = Rugao chicken from Jiangsu province; GSC = Gushi chicken from Henan province; LYC = Liyang chicken from Jiangsu province; TC = Tibetan chicken from Tibet; WL1 = first population of White Leghorns; WL2 = second population of White Leghorns.

Table 3. Genotypic frequencies and allelic frequency of tumor virus locus B

		Genotypic frequency				Allelic frequency			
Breed[1]	Number	S1/S1	R/R	S1/R	S3/R′	S1	R	S3	R′
BJY	114	0.991	0	0.009	0	0.996	0.004	0	0
SL	83	1	0	0	0	1	0	0	0
WL1	17	0.118	0.529	0.353	0	0.295	0.705	0	0
WL2	44	0.182	0.273	0.523	0.022	0.444	0.534	0.011	0.011
Total	258								

[1]BJY = Beijing You from Beijing; SL = Silkie from Jiangxi province; WL1 = first population of White Leghorns; WL2 = second population of White Leghorns.

Compared with *ALVE3*, *ALVE6*, and *ALVE9*, the tolerance induced by *ALVE21* is much more complicated. The locus is sex linked and may be related to the slow-feathering gene. In poultry breeding and production, the slow-feathering feature is widely used in feather-sexing identification (Lowe and Garwood, 1981). Thus, it may be a long and gradual process to exclude the carrier of *ALVE21* in Chinese indigenous chickens. In addition, numerous studies have disclosed that it is necessary to induce an infection program when ALV bind with a specific cellular receptor (TVB; Bacon et al., 2000; Zhang et al., 2005; Hunt et al., 2008). The *TVB*R/R* encodes a cellular receptor that is dysfunctional and incapable of permitting any ALVB, ALVD, and ALVE infection. Thus, the *TVB*R/R* is a resistant genotype for the 3 subgroups of ALV. The genotype of *TVB* is of importance for complete provirus *ALVE21* infection. Only when the birds have both the *ALVE21* locus and *TVB*S1/S1* can the complete provirus *ALVE21* induce immunity tolerance in the host. Therefore, detecting the genotypes of *TVB* is necessary for selecting ALV-resistant birds among Chinese native chicken breeds. Although we did not find individual *TVB*R/R* in the samples, 1 birds with *TVB*S1/R* was detected in BJY chickens. If more samples could be detected, birds with the genotype *TVB*R/R* could be found in Chinese native chickens.

Besides *ALVE21*, the complete endogenous virus loci included *ALVE1*, *ALVE2*, *ALVE7*, *ALVE10*, *ALVE11*, *ALVE12*, *ALVE14*, and *ALVE18*. Previous work reported that *ALVE10*, *ALVE11*, and *ALVE12* loci could express complete viruses only with the presence of *ALVE1* (Crittenden, 1991). We first screened the insertion status of the *ALVE1* locus in SL and BJY chickens and found that all of them were homozygous negative (*ALVE1−/−*). For the *ALVE14* and *ALVE18* loci, although they were proposed as complete elements, their function was still unclear (Crittenden, 1991). Therefore, the functions of these endogenous viruses should be explored and more molecular information provided for anti-disease breeding in chickens.

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