



Genetic variation at the TVB locus in Turkish and Iranian native chicken breeds

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ABSTRACT

Avian leukosis viruses (ALVs) are retroviruses that can induce tumors, as well as reduce growth rate and productivity in chicken. The tumor virus B (TVB) locus encodes the cellular receptor that mediates an infection through ALVB, ALVD, and ALVE subgroups of ALV. Two single nucleotide polymorphisms (SNPs) at nucleotide positions 172 and 184 of the autosomal TVB locus account for the three alleles, TVB*S1, TVB*S3, and TVB*R. The receptor encoded by TVB*R allele prevents viral entry into the cell of ALVB, ALVD, or ALVE subgroups. In this study, both SNPs at the TVB locus of 2 Turkish and 7 Iranian native chicken breeds were investigated by using a PCR-RFLP technique to determine genotypes and gene frequencies at TVB locus. The frequency of TVB*S1, and TVB*S3 alleles changed from 0.857 to 1.000, and from 0.022 to 0.143, respectively, in all studied breeds. The TVB*R allele for resistance to ALV-related viruses was not determined. As a result, it can be concluded that Turkish and Iranian native chickens are strongly susceptible to the infection of ALV-related viruses.

Key words: Iran native chicken breeds, PCR-RFLP, Tumor virus B locus, Turkish native chicken breeds

Avian leukosis viruses (ALVs) can cause a variety of transmissible benign and malignant neoplasms gathered under the denomination leucosis/sarcoma disease (Nair and Fadly 2013). ALVs affect poultry production and cause economic losses through increased mortality and reduced productivity. ALVs that occur in chickens have been divided into 6 subgroups (A, B, C, D, E, and J) based on viral envelope glycoproteins and cell receptor interaction patterns. (Coffin *et al.* 1997).

Four autosomal tumor viral (TV) loci transcribe subgroup-specific surface receptors with high affinity to viral envelope glycoproteins on host cells that either mediate or block the entry of all ALV viral sub-groups (Payne and Venugopal 2000). The TV-A, TV-C, and TV-J loci encode receptors for the ALVA, ALVC, and ALVJ subgroups, respectively (Zhang *et al.* 2007). The most complex locus is TVB (tumor virus B subgroup), which encodes a tumor necrosis factor receptor (TNFR)-related death receptor, and its 3 alleles determine which ALV subgroups can infect expressing cells (Adkins *et al.* 2001). Two SNPs at nucleotide positions 172 (C/T) and 184 (T/A) of the TVB

gene cDNA sequence (GenBank accession number AF507016.1) diversify the allelic transcripts and produce the alleles of TVB*S1, TVB*S3, and TVB*R. The host cell receptor encoded by the TVB*S1 (susceptible allele to ALV) mediates viral entry of ALVB, ALVD, and ALVE subgroups. The TVB*S3 allele encodes receptors promoting viral infection with both ALVB and ALVD, but not ALVE. The TVB*R allele, termed as a resistant allele, transcribes an abnormal and truncated receptor that does not allow ALVB, ALVD, or ALVE subgroups to enter the cell and cause infection. The TVB*S1 is completely dominant to the alleles of TVB*S3 and TVB*R. The TVB*S3 is completely dominant to TVB*R (Zhang *et al.* 2005). The TVB*R/*R is resistant to infection of ALV-related viruses while TVB*S1/*- genotypes are susceptible. Using TVB sequence polymorphisms, PCR-RFLP (Zhang *et al.* 2005) and pyrosequencing methods (Zhang *et al.* 2007) were developed and validated to distinguish TVB genotypes.

The Denizli and Gerze are the only two Turkish native chicken breeds. They are named according to provinces in which they originate and are conserved as genetic resources. White marandi, Black marandi, Naked neck, Common breed, Lari, and West azarbaijan are the Iranian native chickens.

ALVs are still considered ubiquitous in commercial chickens notwithstanding the eradication programs instituted by many primary breeding companies (Nair and Fadly 2013). The aim of this study is primarily to determine the genotypes and genes of the TVB locus in Iran native chicken breeds and also to check the status of TVB locus in other Turkish native chicken populations by using PCR-RFLP. This study tries to determine whether Turkish and

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Iranian native chicken breeds are susceptible or resistant to the infection of subgroup ALV- B, D, and E.

MATERIALS AND METHODS

All experimental techniques, including chicken handling and sample collection, were approved by the Animal Experimentations Local Ethics Board of Ankara University. The study was carried out in the Molecular Laboratory of Agricultural Biotechnology Department at Osmangazi University, Eskisehir, Turkey and Genetics Laboratory of Animal Science Department at Faculty of Agriculture in Ankara University, Ankara, Turkey.

Animals: A total of 237 chickens were sampled from 2 Turkish (Denizli; TRD and Gerze; TRG) and 7 Iranian (White Marandi; IRWM, Black Marandi; IRBM, Naked Neck; IRNN, Common Breed; IRCB, Lari; IRLR, West Azarbaijan; IRWA, and an introduced breed, New Hampshire; IRNH) chicken breeds (Table 1). The chickens for TRD and TRG breeds were randomly collected from Lalahan Livestock Central Animal Research Institute in Ankara, Turkey; for IRWM, IRBM, IRNN, IRCB, IRNH breeds from Kerec Research Institute, for IRLR from East Azerbaijan Rearing Central and for IRWA from Urmia Research Institute in Iran.

DNA Extraction and Genotyping: Blood samples were collected from the wing vein with sterile syringes into a tube containing EDTA, transported to the laboratory and stored at -20°C until genomic DNA (gDNA) extraction. The gDNA was purified from whole blood samples using a phenol-chloroform method and stored at -20°C until analysis. TVB locus genotypes were identified using the PCR-RFLP method described by Zhang *et al.* (2005). PCR reactions were prepared in a final volume of 25 μL containing as follows; 50–100 ng DNA template, $10\times$ Taq polymerase buffer, 1.5 mM MgCl_2 , 2.5 mM dNTPs, 0.5 U Taq DNA polymerase, and 5 pmol of each primer. Reaction mixtures (15 μL) containing 10 μL of the PCR product and 5 U of restriction enzyme (RE) were incubated for 6 h at 65°C . The restriction fragments were separated on 2.5% agarose gel stained with RedbSafe (iNtRON Biotechnology, Korea) to determine the corresponding genotypes. All primer sequences, PCR product lengths, and REs used for

digestion are shown in Table 2. The genotypic and allele frequencies for TVB locus were estimated by using the PopGene version 1.31 computer software package (Yeh *et al.* 1997) according to formulas by Nei (1987).

RESULTS AND DISCUSSION

In this study, a PCR-RFLP method was used to determine the TVB genotypes of two Turkish and seven Iranian native chicken breeds, and an introduced breed. The TVB PCR-RFLP assay comprises two different PCR reactions (TVB 202 and TVB 303) followed by two independent endonuclease reactions and electrophoresis. We successfully amplified DNA fragments of 202 bp (at position 174) and 303 bp (at position 182) from partial TVB genomic sequences (Table 2). After PCR amplicons were digested with *XbaI* and *NlaIII*, and then allelic variations were detected based on electrophoretic patterns reflecting the presence or absence of the SNP at positions 172 and 184 in TVB202 and TVB303, respectively. Nucleotide substitutions in the SNPs at TVB locus positions 172 and 184 were examined in all chickens based on the criteria established by Zhang *et al.* (2005).

The estimated genotype frequencies for the homozygous genotypes TVB*S1/*S1 and TVB*S3/*S3 ranged from 0.885 to 1.000, and from 0.000 to 0.071, respectively, in all breeds. The range of genotypic frequencies were from 0.043 to 0.143 for the heterozygous genotype TVB*S1/*S3. Among the 237 birds of all population, only two chickens from the IRBM breed were typed as homozygous, the TVB*S3/*S3 genotype. Only 11 chickens with the heterozygous genotype TVB*S1/*S3 were found in all populations. Chickens with the TVB*R/*- genotypes were not found in both Turkish and Iranian native chicken breeds (Table 1).

As shown in Table 1, the common allele at the TVB locus in 9 chicken populations was the TVB*S1 allele (Table 1). The TVB*S3 allele was rare and the resistant allele, TVB*R, was not found in Turkish and Iranian native chicken breeds. TRG, IRWM, IRNH, IRLR and IRWA chicken populations were fixed for the TVB*S1 allele. The frequency of TVB*S1 and TVB*S3 alleles ranged from 0.857 to 1.000, and from 0.022 to 0.143 in studied breeds, respectively.

Table 1. Genotype and gene frequencies at the TVB locus in Turkish and Iranian native chicken breeds

Country	Breed	Abbr.	n	Genotype frequencies			Gene frequencies		
				S1/S1	S1/S3	S3/S3	S1	S3	R
Turkey (TR)	Denizli	TRD	49	0.939	0.061	–	0.969	0.031	–
	Gerze	TRG	16	1.000	–	–	1.000	–	–
Iran (IR)	White Marandi	IRWM	25	1.000	–	–	1.000	–	–
	Black Marandi	IRBM	28	0.786	0.143	0.071	0.857	0.143	–
	Naked Neck	IRNN	26	0.885	0.115	–	0.942	0.058	–
	Common Breed	IRCB	23	0.957	0.043	–	0.978	0.022	–
	New Hampshire	IRNH	25	1.000	–	–	1.000	–	–
	Lari Breed	IRLR	20	1.000	–	–	1.000	–	–
	West Azerbaijan	IRWA	25	1.000	–	–	1.000	–	–

Table 2. Mutation points at TVB locus, primer sequences, PCR product size and restriction endonuclease (RE) (Zhang *et al.* 2005)

Mutation (bp)	Primers	PCR product size (bp)	RE
TVB202 172	F: 5' GGT AAG GCA GTC ACAAGC ATC ACT C 3' R: 5' TAC TCG TCT TTC TTA CAT GGG AGG CTC T 3'	202	<i>Xba</i> I
TVB303 184	F: 5' ACC CCT TCT TGC AGG CAC CTA TGA 3' R: 5' -GGA TGC TGT GCT GCG TGG AGA 3'	303	<i>Nla</i> III

In this study, the genotypes of the TVB locus were determined in Turkish and Iranian native chicken breeds by using PCR-RFLP. For the first time, TVB locus genetic polymorphisms were determined and allele frequencies calculated for Iranian native chicken breeds. Molecular information revealing whether or not the Turkish and Iranian native chicken breeds examined are genetically resistant to any of ALVB, ALVD, and ALVE infections will aid conservation efforts. TVB*S3 allele is resistant to ALVE but susceptible to ALVB and ALVD. The TVB*S3/S3 genotype could be used to protect the native chicken breeds against the ALVE infection since TVB*S3/S3 genotype transcribes receptor that blocks ALVE from entering an infection cycle in chickens (Zhang *et al.* 2007).

In a previous study (Kaya 2018) on the TVB locus status of Turkish native breeds, only one Gerze (n=27) chicken (TVB *S3/*R genotype) had the TVB*R allele and it was not found in Denizli chickens. The common allele at the TVB locus in both chicken populations was the TVB*S1 allele and the TVB*S3 allele was rare in both Turkish native chicken breeds. The frequency of TVB*S1 and TVB*S3 alleles were evaluated as 0.96, and 0.02, in Gerze and 0.98, and 0.02 in Denizli chickens, respectively, in previous research. However in the current study, TRG was typed as homozygous TVB*S1/*S1 and the estimated gene frequencies for TVB*S1 and TVB*S3 were 0.969, and 0.031 in TRD, respectively.

In general, the results presented here are in agreement with those of similar studies around the world. Taken together, these studies show that the TVB*S1 allele is the common allele and the TVB*R resistance allele is rarely observed in native chickens. Consistent with the results of studies in Chinese (Yang *et al.* 2011; Yu *et al.* 2012), Indian (Chatterjee 2013), and Turkish (Kaya 2018) domestic chicken, the TVB*R allele was rarely observed in indigenous breeds. TVB*R allele was not detected in Indian native breed (Chatterjee 2013). Among 1,217 chickens from 10 Chinese indigenous breeds, only one chicken was found to have the TVB*S1/*R genotype (Yang *et al.* 2011). In another study, conducted with a total of 363 chickens from 10 domestic Chinese breeds, only one breed had the TVB*R allele with a frequency of 0.11 (Yu *et al.* 2012). These results are parallel to the observation that the TVB*R allele frequency in native chicken breeds is lower than that observed in White Leghorn populations (Zhang *et al.* 2007). Contrary to this situation, in total 442 birds from 10 native Chinese breeds were genotyped and the resistant allele TVB*R was

detected in 6 of the 10 Chinese local chickens, ranged the frequencies from 0.04 to 0.14, whereas the resistant genotype TVB*R/*R was only detected in one native breed with the frequencies at 0.03 (Liao *et al.* 2014).

The TVB*S3 encodes receptors that permit ALV-B and ALV-D infection but block ALV-E from entering an infection cycle (Zhang *et al.* 2007). In this study, the TVB*S3 allele was found in only four of the 9 breeds studied, and its frequency ranged from 0.031 to 0.143 in those four breeds. Among the 237 birds of all population, only two chickens from the IRBM breed were found as the TVB*S3/S3 homozygous genotype. Only 11 chickens with the heterozygous genotype TVB*S1/*S3 were found in all populations. In spite of this circumstance, the TVB*S3/S3 genotype was not detected in native breeds tested in China (Yu *et al.* 2012), India (Chatterjee 2013), and Turkey (Kaya 2018). Also, the TVB*S3 allele was not found in indigenous breeds investigated in China (Yu *et al.* 2012) and India (Chatterjee 2013).

In some studies conducted with broiler, egg-layer, and laboratory chicken lines from multiple major commercial companies, universities and research institutes, TVB*R/*R genotype frequency ranged from 0.03 to 0.15, and 6% were typed as homozygous TVB*S3/S3. Chickens with the heterozygous genotype TVB*S3/*R were found as 0.03 (Zhang *et al.* 2007). In another study with commercial broiler line in China, the estimated genotypic frequencies for TVB*S1/*R, and TVB*R/*R varied from 0.08 to 0.34, and from 0.03 to 0.15, respectively (Liao *et al.* 2014).

In this study, the number of chickens in some breeds are to be considered very small. Yang *et al.* (2011) reported that the frequency of the TVB*R allele might be higher given a larger sample size. Although the TVB*R allele was not detected in this study, it is possible that chickens genetically resistant to ALV might be identified with a larger sample size populations, especially Gerze breed in which the TVB*R allele was previously detected (Kaya 2017). While there are no vaccines for ALV diseases, genetic resistance is very important to protect chickens (Min and Xiquan 2016). Genetically resistant chickens are resistant to infection and tumor induction by ALVs of the subgroups concerned, and they usually fail to develop antibodies.

As a conclusion, based on the genotypic and gene frequencies for TVB locus, it was propounded that both Turkish and Iranian native chickens were genetically susceptible to the infection of ALV. These findings have to be taken into consideration for the conservation programmes and selection progress in future planning.

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