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Research Article

Determination of the Tumor Virus B Locus in Turkish Native Chicken Breeds [1][2]

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Abstract

Avian leukosis viruses (ALV) are retroviruses that can induce tumors in chicken. Typically, ALV is controlled through husbandry. However, genetic improvement and/or, selective breeding techniques, offer the unique possibility of true resistance. The tumor virus B (TVB) locus transcribes the cellular receptor that mediates an infection through B, D, and E subgroups of avian leukosis virus. Two single nucleotide polymorphisms (SNPs) at nucleotide positions 172 and 184 of the TVB locus account for the three major 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 Turkish native chicken breeds were investigated using a PCR-RFLP technique to detect. In both Gerze and Denizli breeds, the TVB*S1 allele was common and TVB*S3 was rare, respectively. The TVB*R allele was rare in the Gerze population and absent in the Denizli population. Allele frequencies of TVB*S1, TVB*S3, and TVB*R were evaluated as 0.96, 0.02, and 0.02 in Gerze chickens and 0.98, 0.02, and 0.00, in Denizli chickens, respectively.

Keywords: *Denizli cocks, Gerze fowls, PCR-RFLP, Tumor virus B locus*

Türkiye Yerli Tavuk Irklarında Tümör Viral B Lokusunun Belirlenmesi

Öz

Avian leukosis virusleri (ALV), tavuklarda tümör oluşturan retrovirüslerdir. Tipik olarak, ALV hayvan yetiştirme yöntemleri ile kontrol edilir. Bununla birlikte, genetik gelişme ve/veya selektif ıslah teknikleri, hastalığa direnç için benzersiz olanaklar sunmaktadır. Tümör viral B (TVB) lokusu ALV üç alt grubunun (B, D ve E) viral girişine ortam sağlayan/engelleyen veya aracı olan gruplara özgü yüzey reseptörlerini kodlar. Bu lokusun 172. ve 184. bazlarındaki iki adet tek nükleotit polimorfizmleri TVB*S1, TVB*S3 ve TVB*R allellerini ayırt edilmesine imkan sağlar. TVB*R'nin kodladığı reseptör ALVB, ALVD veya ALVE alt gruplarının hücreye viral girişini engeller. PCR-RFLP yöntemi kullanılarak Türkiye yerli tavuk ırkları olan Denizli ve Gerze tavuklarındaki TVB genotipleri belirlenmiştir. Hem Gerze hem de Denizli ırkında, TVB*S1 alleli yaygın ve TVB*S3 alleli nadir olarak görülmüştür. TVB*R alleli, Gerze popülasyonunda seyrek olarak görülürken Denizli horozu popülasyonlarında tespit edilememiştir. TVB*S1, TVB*S3 ve TVB*R'nin allel frekansları sırasıyla Gerze tavuklarında 0.96, 0.02 ve 0.02, Denizli horozlarında sırasıyla 0.98, 0.02 ve 0.00 olarak hesaplanmıştır.

Anahtar sözcükler: *Denizli horozu, Gerze tavuğu, PCR-RFLP, Tümör viral B lokus*

INTRODUCTION

Avian Leukosis Viruses (ALVs) affect poultry production and cause economic losses through increased tumor mortality and reduced productivity. ALV is a retrovirus and is classified into six major viral subgroups based on virus and cell receptor interaction patterns. One ALV subgroup (subgroup E, or ALVE) is endogenous and encoded by genes within the chicken genome, whereas all other variations, ALVA, B, C, D, and J are exogenous [1]. Three autosomal tumor viral (TV) loci determine subgroup-specific surface receptors on host cells that either mediate

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or block the entry of all ALV viral subgroups. The TV-A, TV-C, and TV-J loci encode receptors for the ALVA, ALVC, and ALVJ subgroups, respectively [2]. The most complex locus is TV-B, or TVB, which encodes receptors for the ALVB, ALVD, and ALVE subgroups. TVB encodes a tumor necrosis factor receptor (TNFR)-related death receptor, and its three alleles determine which ALV subgroups can infect expressing cells [3]. 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 TVB*S1, TVB*S3, and TVB*R. A host cell receptor encoded by the TVB*S1 allele 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 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*R allele is recessive to TVB*S1 and TVB*S3, and the TVB*S3 allele is recessive to TVB*S1 [4].

Using TVB sequence polymorphisms, PCR-RFLP [4] and pyrosequencing methods [5] were developed and validated to distinguish TVB genotypes. Additionally, a new single nucleotide mutation at TVB cDNA position 184 (G→T) of the TVB gene was identified using direct DNA sequencing methods in Chinese native chicken breeds [6].

The Denizli and Gerze are the only two Turkish native chicken breeds. They are named after the cities from which they originate and are conserved as genetic resources. Denizli cocks are well known for their long crowing and Denizli and Gerze breeds are reared for hobby purposes [7]. To the best of our knowledge, there are some published study about genetic diversity of Turkish native chicken populations, Denizli and Gerze, at molecular level but there are no reports about ALV infection events or the TV locus status of Turkish native chicken populations [7-9]. The aim of this study was to determine the genotype of the TVB locus in Turkish native chicken breeds using the PCR RFLP method.

MATERIAL and METHODS

All experimental techniques, including animal handling and sample collection, were approved by the Medical and Surgical Experimental Research Center Committee (TICAM) of Eskisehir Osmangazi University with the Decision No: 2014/419-1. The study was carried out in the Molecular Genetics Laboratory of the Agricultural Biotechnology Department at Osmangazi University, Eskisehir, Turkey.

Animals

In this study, a total of 175 chickens were genotyped from original populations of two Turkish native chicken breeds (Denizli, n = 148; and Gerze, n = 27).

DNA Extraction and Genotyping

Genomic DNA 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.[4]. PCR was performed to amplify the polymorphic TVB locus regions. Each 25 µL PCR reaction mixture contained: 50-100 ng DNA template, 10 X *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 the appropriate temperature *(Table 1)*. The resulting restriction fragments were separated by agarose gel electrophoresis and stained using RedSafe to determine the corresponding genotypes. All oligonucleotides, PCR profiles, PCR amplicon lengths, and REs used for digestion of PCR amplicons are shown in *Table 1*. TVB locus allele frequencies were calculated using the PopGene version 1.31 computer software package [10].

Table 1. *The oligonucleotides, PCR profile and product lengths, and restriction endonuclease (RE) for digestion of PCR products [4]*

Mutation	Primers	PCR				RE and Incubation Temperature
		Profile			Product Size bp	
TVB202 172. bp	F: 5′ GGT AAG GCA GTC ACAAGC ATC ACT C 3′ R: 5′TAC TCG TCT TTC TTA CAT GGG AGG CTC T 3′	94°C	05 min	30 cycle	202	*XbaI* 65°C
		94°C	60 sec			
		56°C	60 sec			
		72°C	45 sec			
		72°C	05 min			
TVB303 184. bp	F: 5′ ACC CCT TCT TGC AGG CAC CTA TGA 3′ R: 5′ -GGA TGC TGT GCT GCG TGG AGA 3′	94°C	03 min	30 cycle	303	*NlaIII* 65°C
		94°C	60 sec			
		60°C	60 sec			
		72°C	60 sec			
		72°C	05 min			

RESULTS

In this study, we used PCR-RFLP to examine the TVB genotypes of the only two Turkish native chicken breeds, Denizli and Gerze. 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 and 303 bp from partial TVB genomic sequences *(Table 1)*. After PCR amplicons were digested with *XbaI* and *NlaIII*, allelic haplotypes were detected based on electrophoretic patterns reflecting the presence or absence of the SNP at positions 172 or 184 in TVB202 or TVB303, respectively. Nucleotide substitutions in the SNPs at TVB locus positions 172 *(Fig. 1a)* and 184 *(Fig. 1b)* were examined in all chickens based on the criteria established by Zhang et al.[4].

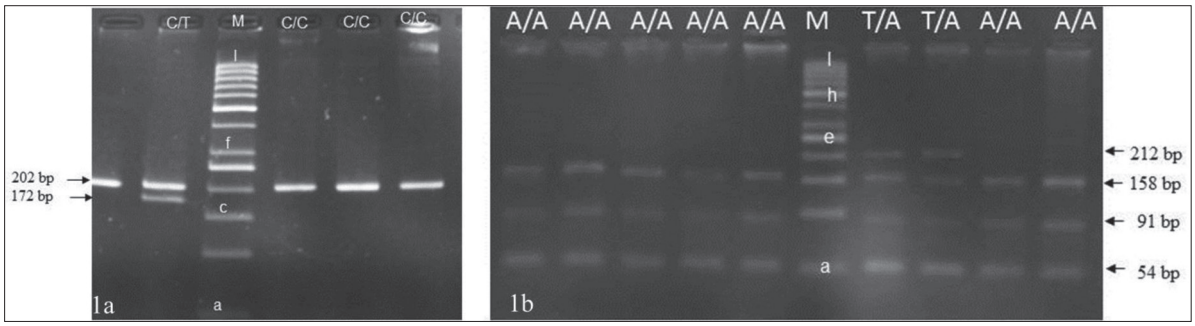

Fig 1. a- The gel-image of 202 bp PCR products after treatment with *XbaI* restriction enzyme and **b-** 303 bp PCR products after treatment with *NlaIII* restriction enzyme (M, 50 bp Fermentas® GeneRuler DNA ladder, a:50 bp, c: 150 bp, e:250 bp, f:300 bp h:500 bp and l:1000 bp)

Among the 175 chickens investigated, only one Gerze breed chicken, and no Denizli breed chickens, had the TVB*R allele. Gerze chickens are conserved in the city of Gerze, where they originated, and the entire population of this flock was genotyped (n = 27). The common allele at the TVB locus in both chicken populations was the TVB*S1 allele. The TVB*S3 allele was rare in both Turkish native chicken breeds and the TVB*R allele was not detected in Denizli chicken populations. Moreover, among the 141 birds of the Denizli population, five cocks of the Pekmez Kefi variety and only two hens were found to have the TVB*S1/S3 genotype, whereas all others were homozygous for the TVB*S1 allele. In Gerze breed hens, only one of 27 chickens had the TVB*S3/R genotype. The frequency of TVB*S1, TVB*S3, and TVB*R alleles were evaluated as 0.96, 0.02, and 0.02 in Gerze and 0.98, 0.02, and 0.00 in Denizli chickens, respectively.

DISCUSSION

For the first time, TVB locus genetic polymorphisms were determined and allele frequencies calculated for Turkish native chicken breeds. Molecular information revealing whether the Turkish native chicken breeds examined are or are not genetically resistant to any of ALVB, ALVD, and ALVE infections will aid conservation efforts.

In addition to PCR-RFLP techniques [11], other studies have used sequencing [6] and pyrosequencing methods [5] to examine the TVB locus polymorphism. Our findings are consistent with those of studies performed in other countries.

A study conducted in India, using the PCR-RFLP technique, detected TVB*S1 alleles in the White Leghorn (WL) line and in native Kadaknath hens, whereas the TVB*R allele was not detected in either breed. Furthermore, Kadaknath chickens were clearly homozygous for the TVB*S1 allele [11].

Zhang et al.[5] researched the genetic diversity at the TVB locus in 36 broilers and 16 laying chicken lines in the USA using the pyrosequencing method. They found that, in broiler lines, the frequency of TVB*S1/S1, TVB*S1/R, and TVB*R/R genotypes were 83%, 14% and 3%, respectively. On the other hand, the frequency of TVB*S1/S1 and TVB*R/R genotypes in laying lines was 44% and 15%, respectively.

Using the pyrosequencing method, a Chinese study of 258 chickens, consisting of two domestic chicken breeds and two WL populations, found the frequency of the TVB*R/R genotype in one of the WL populations to be 0.53, and one of the native breeds was found to be homozygous for TVB*S1/S1. Among domestic breeds, only one chicken was found to have the TVB*S1/R genotype [12]. Another study, conducted with a total of 1428 chickens from ten domestic Chinese breeds and 15 commercial broiler flocks, the frequencies of TVB*S1, TVB*S3, and TVB*R alleles varied between 0.71 and 0.91, 0.00 and 0.09, and 0.04 and 0.29, respectively. Eleven of the 25 breeds were homozygous for the TVB*S1 allele and five breeds of those had the TVB*R/R resistant genotype at varying frequencies (ranging from 0.03 to 0.15) [13].

Yu et al.[6] used the sequencing method to examine the genetic variation at the TVB locus in a total of 459 chickens from nine domestic and WL breeds in China. They detected the TVB*R allele in only two of the nine breeds. The TVB*R

allele frequency was determined to be 0.44 in WL and 0.11 in Tibetan chicken breeds and the common allele was TVB*S1. Additionally, a new G→T mutation was detected at the TVB locus and called TVB*S'. Furthermore, the TVB*S1/S' genotype was observed in other native Chinese breeds with the exception of Tibetan chicken breeds [6].

In general, our 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 that the TVB*R resistance allele is seldom observed in native chickens. Consistent with the results of analyses in Chinese [6,12,13] and Indian [11] domestic chicken breeds, the TVB*R allele was rarely observed in Turkish native breeds. These results are parallel to the observation that the TVB*R allele frequency in native chicken breeds is lower than that observed in WL populations [6].

In this study, all animals of Gerze breed were genotyped in Gerze city. The existing number of animals in the Gerze population is very low, resulting in a restricted study population size. Yang et al.[12] reported that the frequency of the TVB*R allele might be higher given a larger sample size. Here, the TVB*R allele was rarely detected in the Gerze breed, yet it is possible that chickens genetically resistant to ALV would have been identified with a larger sample size. While there are no vaccines for ALV diseases, genetic resistance is very important to protect chickens [14]. Additionally, Liao et al.[13] noted that the TVB*R allele could be used to improve laying performance and reduce lymphoid leukosis in chickens. Therefore, selection of chickens with TVB*R alleles could improve lifespan as well as egg and meat production in native breeds.

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