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Genetic Polymorphism of Avian Leukosis Virus Host Receptors in Korean Native Chickens and Establishment of Resistant Line

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ABSTRACT Avian leukosis virus (ALV) is a highly contagious retrovirus that causes tumors and has resulted in great economic loss worldwide owing to its high transmission rate. Various ALV viral subgroups exist, with infections occurring via specific host receptors. The susceptibility or resistance of avian species to the ALV-A and K subgroups is determined by the host receptor, the tumor virus locus A (*tva*) gene, while that to ALV-B depends on another host receptor, the tumor virus locus B (*tvb*) gene. The resistance alleles of *tva* and *tvb* have primarily been identified in China, but none have been detected in Korea. We analyzed the frequencies of *tva* and *tvb* genotypes in White Leghorn (WL), Korean Ogye (KO), and Korean native chicken (KNC) breeds, and assessed the resistance to ALV subgroups. In WL, both *tva* and *tvb* had various genotypes, including susceptibility and resistance alleles, whereas in KO, *tva* and *tvb* resistance alleles were dominant. In KNC, *tva* susceptibility and resistance alleles were mixed, whereas *tvb* resistance alleles were dominant. In addition, we showed that there were differences in the splicing pattern of *tva* transcripts and the expression level of *tvb* transcripts within breeds. Finally, we confirmed that ALV resistance depended on KO and KNC genotypes by *in vitro* infection of chicken embryonic fibroblasts with ALV. These results highlight that some KO and KNC individuals are naturally resistant to ALV subgroups A, B, and K, and will facilitate the preservation of economically superior traits through selective breeding.

(Key words: Avian leukosis virus, Korean native chicken, *tva*, *tvb*)

INTRODUCTION

Avian leukosis virus (ALV) belongs to Group VI of the *Retroviridae* family and causes tumors and immunosuppression in avian species, resulting in significant economic losses. The ALV subgroups known to infect chickens are A-E, J, and K, and are classified depending on viral envelope proteins, which play important roles in interactions between host cells and the virus (Li et al., 2021). Host susceptibility and resistance to ALV are related to the interaction of viral glycoproteins with naturally occurring mutations of host receptors; hence, there are chicken lines that are naturally susceptible and resistant to ALV subgroups A-E, determined by three genetic loci: *tva*, *tvb*, and *tvc* (Barnard et al., 2006).

To infect host cells, ALV-A interacts with TVA, a receptor protein belonging to the low-density lipoprotein receptor (LDLR) family (Bates et al., 1993), and genetic variation of the TVA receptor in chickens determines susceptibility and resistance to ALV-A. A single nucleotide substitution in the LDLR-like domain of the *tva* gene (tva^{r1}) changes the amino acid sequence from cysteine to tryptophan (C40W), thereby lowering the binding affinity of TVA for ALV-A. In another variant, insertion of four nucleotides into exon 1 of the *tva* gene (tva^{r2}) results in a frameshift mutation that blocks TVA receptor expression, leading to infection resistance (Elleder et al., 2004). In addition, deletion of nucleotides in the intronic region of the chicken *tva* gene (tva^{r3}, tva^{r4}, tva^{r5}, and tva^{r6}) affects the branch point signal involved in splicing, resulting in incomplete mRNA splicing and decreased susceptibility to ALV-A (Reinisova et al., 2012; Chen et al., 2015). Further, recent findings demonstrate that ALV-K shares the TVA receptor with ALV-A (Prikryl et al., 2019), and knockout of *Tva* in chicken using the CRISPR/Cas9 system confers resistance to both ALV-A and -K (Koslova et al., 2021).

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The TVB receptor protein belongs to the tumor necrosis factor receptor (TNFR) family and encodes a host receptor for ALV-B, -D, and -E (Adkins et al., 2000). Single nucleotide substitutions in the *tvb* gene regions encoding cysteine-rich domain (CDR) 1 (tvb^{r1}) or CDR3 (tvb^{r3}) induce premature stop codons that generate a truncated TVB receptor and decrease susceptibility of chickens to ALV-B, -D, and -E (Chen et al., 2017; Klucking et al., 2002). In addition, a G to C single nucleotide substitution (tvb^{r2}) induces a cysteine to serine (C125S) amino acid alteration that lowers virus binding affinity (Reinisova et al., 2008), and insertions of two (tvb^{r4}) or one (tvb^{r5}) nucleotides in *tvb* exon 4 induces frameshift mutations, leading to expression of a truncated TVB receptor and reduced susceptibility to ALV-B, -D, and -E infection (Li et al., 2018). TVC is a member of the immunoglobulin superfamily, most similar to mammalian butyrophilins, and introduction of a premature stop codon in the gene encoding TVC confers resistance to ALV-C in chickens (Elleder et al., 2005).

ALV infection represents a substantial economic threat due to its promotion of tumor formation, growth retardation, immunosuppression, and decreased egg production. As ALV has a significant impact on the poultry industry, there have been many studies of Chinse chicken breeds to characterize ALV subgroup strains and the frequency of individuals with resistant alleles (Feng and Zhang, 2016; Su et al., 2018; Cui et al., 2020). Although molecular analysis of the genome of endogenous ALV has been performed in Korea (Kim et al., 2008), there have been no reports of virus resistance alleles in Korean Native chicken breeds. In this study, we analyzed the genome sequences of *tva* and *tvb* in the White Leghorn (WL), Korean Ogye (KO), and Korean Native chicken (KNC) breeds, to determine genotype frequencies and their resistance to ALV subgroups A, B, and K, with the aim of preserving superior traits by selective breeding in the future.

MATERIALS AND METHODS

1. Experimental Animals and Animal Care

The management and experimental use of chickens were approved by the Institute of Laboratory Animal Resources, Seoul National University, South Korea (SNU-190401-1-3). All experimental animals including White Leghorn (WL), Korean Ogye (KO), and Korean Native Chicken (KNC) were cared for according to a standard management program at the University Animal Farm, Seoul National University. The procedure for animal management and blood isolation adhered to the standard operating protocols of our laboratory.

2. Genomic DNA Sequencing

Genomic DNA was extracted from blood sample from each chicken drawn from the wing vein. To identify genomic sequence of *tva* and *tvb*, genomic DNA was analyzed by PCR analysis using region-specific primers (TVA seq F: ACA CTG ACA GCG AGG CGT GC, TVA seq R: ACC TCT CCG CAC GAC GTT CT, TVB exon 3 seq F: TCT CCA CGT CTC GGC AGC AC, TVB exon 3 seq R: GGA GAG CCC GAG CAG AGC TG, TVB exon 4 seq F: GCT TTG GCA TGT GGG CAA GG, TVB exon 4 seq R: GCA GCA CGG TGA AGG TGA TG). For sequencing analysis, the PCR amplicons were cloned into the pGEM-T easy vector (Promega) and sequenced using an ABI Prism 3030XL DNA Analyzer (Thermo Fisher Scientific). The sequence was compared against assembled genomes using the Basic Local Alignment Search Tool (BLAST).

3. RNA Isolation, RT-PCR and RT-Quantitative PCR Analysis

Total RNAs from WL, KO and KNC were isolated using Trizol Reagent (Thermo Fisher Scientific) and reverse-transcribed using the Superscript III First-Strand Synthesis System (Thermo Fisher Scientific). The cDNAs of WL, KO and KNCs were amplified with *tva* specific primer (TVA transcript F: CCG GCA TGG TGC GGT TGT TG, TVA transcript R: AGC CAG GTT CCA CGG TCA GC) by RT-PCR and *tvb* 5' and 3' specific primers (qRT Tvb 5' F: TCT TCG CGG AGG TTC AGT, qRT Tvb 5' R: TGG ATA CTC GGT GTA CTC GTC T, qRT Tvb 3' F: TCC CAA AGT GGA AAC CC, qRT Tvb 3' R: CTG CTC TGC CAG ATA AAG G) by RT-quantitative PCR (RT-qPCR). All reaction were performed under the same conditions, containing 100 ng cDNA, 20 X EVA green (Biotium), 10 X PCR buffer, 10 mM each of dNTP, 10 pM of each primer, and 0.5 U *Taq* polymerase

(Bionics). RT-qPCR conditions were as follows: 95°C for 3 minutes followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Melting-cure profiles were analyzed for all amplicons. Each test samples were run in triplicate. Relative quantification of the target gene expression we performed using the $2^{-\Delta\Delta Ct}$ method using housekeeping gene *GAPDH* for control.

4. Culture of Chicken Embryo Fibroblasts

For chicken embryo fibroblast (CEF), fertilized eggs incubated for 6 days were isolated and dissected the limb, and organs from the embryo. The CEF were collected in a 1.5 mL microcentrifuge tube with 1 mL of 0.05% trypsin-EDTA and incubated at 37°C for 10 minutes. After adding the same volume of culture media, CEFs were centrifuged at 1,250 rpm for 5 minutes and the cells were maintained and sub-passaged in DMEM supplemented with 1 X ABAM and 10% FBS. CEFs were incubated at 37°C with an atmosphere of 5% CO_2 and 60%-70% relative humidity.

5. Virus Production and Infection

RCASBP-(A)-CN-EGFP, RCASBP-(B)-CN-EGFP and RCASBP-(K)-CN-EGFP was kindly provided by Dr. Yao and Dr. Nair (Pirbright Institute). Plasmid (5 μg) were mixed with Lipofectamine 2000 reagent (Thermo Fisher Scientific) in Opti-MEM (Thermo Fisher Scientific), and the mixture was applied to 1×10^6 DF-1 cells. The mixture was replaced culture medium 6 h after transfection. One day after transfection we could detect green fluorescence in DF-1 cells, which indicated virus production. Cells were subpassaged, and the medium was changed one day after subpassaging. One day later, the medium containing virus was harvested and frozen at −70°C until use. For viral infection, the medium containing virus was thawed at 37°C and added to individual CEF clones. Four days post-infection, WL, KO and KNC CEFs were observed using fluorescence microscopy (Tu-80; Nikon).

6. Statistical Analysis

Statistical analysis was performed using the GraphPad Prism (GraphPad Software, CA, USA). Significant differences among the groups were evaluated by one-way ANOVA. A value of $P<0.05$ indicated statistical significance.

RESULTS

1. Genotyping Analysis of *tva* and *tvb* Receptor Genes in White Leghorn (WL), Korean Ogye (KO) and Korean Native Chicken (KNC)

To analyze the genotype frequencies of the ALV receptors, *tva* and *tvb*, in WL, KO, and KNC, we sequenced four genetic loci associated with ALV susceptibility and resistance: *tva* exon 1, *tva* intron 1, *tvb* exon 3, and *tvb* exon 4 (Adkins et al., 2000; Elleder et al., 2004; Chen et al., 2015; Chen et al., 2017).

Analysis of *tva* loci revealed three genotypes, $tva^{S/S}$, $tva^{S/R2}$, and $tva^{S/R4}$, encoding TVA receptors for ALV-A and -K in WL. In exon 1, a susceptible allele (tva^{S}) and an allele with a 4 bp insertion (tva^{R2}) were observed, while in intron 1, we detected the susceptibility allele (tva^{S}) and a 5 bp deletion (tva^{R4}) in the variant region. Three resistance genotypes, $tva^{R2/R4}$, $tva^{R4/R5}$, and $tva^{R5/R5}$ (Elleder et al., 2004; Reinisova et al., 2012; Chen et al., 2015) were observed in KO, including the alleles tva^{R2} and tva^{R4}, and a 10 bp deletion in the intronic region (tva^{R5}). For KNC, five *tva* genotypes ($tva^{S/S}$, $tva^{S/R4}$, $tva^{S/R5}$, $tva^{R4/R5}$, and $tva^{R5/R5}$) were observed (Figs. 1A and 1B). These results show that there are differences in *tva* genotypes and their frequencies among the three different Korean chicken breeds analyzed (Table 1).

Next, we analyzed the TVB receptor, which is associated with ALV-B, -D, and -E, and found various susceptibility and resistance genotypes ($tvb^{S1/S1}$, $tvb^{S1/S3}$, $tvb^{S3/S3}$, $tvb^{S1/R1}$, $tvb^{S3/R1}$, and $tvb^{R1/R1}$) (Adkins et al., 2000) in WL. By contrast, in KO and KNC, we detected both heterozygosity ($tvb^{S1/R3}$) and homozygosity ($tvb^{R3/R3}$) for a resistance allele (tvb^{R3}) encoding a premature stop codon in *tvb* exon 4 (Chen et al., 2017) (Figs. 1C and 1D). These results reveal that WL carries a variety of *tvb* genotypes, while a resistance allele dominates in KO and KNC through natural selection (Table 2).

2. Validation of *tva* Receptor Splicing Variants in WL, KO and KNC

To determine the *tva* transcript splicing patterns in WL,

Table 1. *TVA* genotypic frequency analysis among different breeds

Line	No.	Genotypic frequency						
		tva $^{S/S}$	*tva* $^{S/R2}$	*tva* $^{S/R4}$	*tva* $^{S/R5}$	*tva* $^{R2/R4}$	*tva* $^{R4/R5}$	*tva* $^{R5/R5}$
White Leghorn	46	0.37 (17/46)	0.11 (5/46)	0.52 (24/46)	0	0	0	0
Korean Ogye	46	0	0	0	0	0.52 (24/46)	0.04 (2/46)	0.44 (20/46)
Korean Native Chicken	47	0.26 (12/47)	0	0.21 (10/47)	0.26 (12/47)	0	0.11 (5/47)	0.17 (8/47)

tva $^{S/S}$: susceptible homozygote for *tva*; *tva* $^{S/R2}$: heterozygote for CGCT insertion; *tva* $^{S/R4}$: heterozygote for ACCCC deletion; *tva* $^{S/R5}$: heterozygote for CGCTCACCCC deletion; *tva* $^{R2/R4}$: heterozygote for CGCT insertion and ACCCC deletion; *tva* $^{R4/R5}$: heterozygote for ACCCC deletion and CGCTCACCCC deletion; *tva* $^{R5/R5}$: homozygote for CGCTCACCCC deletion.

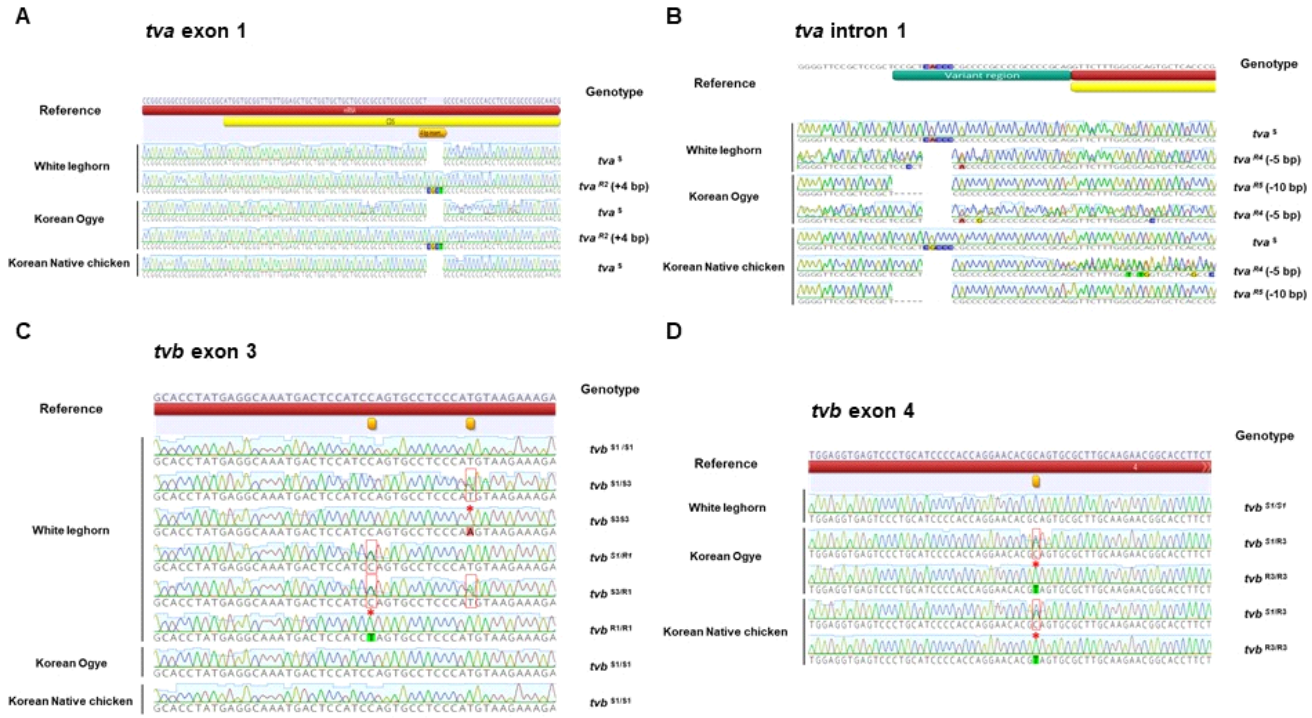

Fig. 1. Sequencing analysis of *tva* and *tvb* genotypes in WL, KO and KNC. (A, B) Representative Sanger sequencing chromatograms and analysis of sequencing patterns of *tva* exon 1 and intron 1 regions amplified from genomic DNA from each breed. (C, D) Representative Sanger sequencing chromatograms and analysis of sequencing patterns of *tvb* exon 3 and exon 4. Yellow boxes in reference sequences are regions containing nucleotide polymorphisms. Asterisks mark substituted nucleotides, and the red box indicates mixed nucleotides.

KO, and KNC, we amplified the entire coding sequence of the *tva* gene by RT-PCR. For chickens with a *tva*$^{S/S}$ genotype, a long *tva* transcript and a short transcript without a complete transmembrane domain were detected. Nucleotide deletions in the intronic variant region of the resistant alleles, *tva*^{R4} and *tva*^{R5}, affect pre-mRNA splicing, resulting in retention of intron 1 in the mRNA transcript (Chen et al., 2015).

RT-PCR analysis of chicken embryo fibroblast (CEF) cDNA from WL, KO, and KNC showed that, unlike WL, multiple transcripts bands were observed in KO and KNC, due to

Table 2. *TVB* genotypic frequency analysis among different breeds

Line	No.	Genotypic frequency							
		tvb $^{S1/S1}$	*tvb* $^{S1/S3}$	*tvb* $^{S3/S3}$	*tvb* $^{S1/R1}$	*tvb* $^{S3/R1}$	*tvb* $^{S1/R3}$	*tvb* $^{R1/R1}$	*tvb* $^{R3/R3}$
White Leghorn	46	0.04 (2/46)	0.11 (5/46)	0.07 (3/46)	0.33 (15/46)	0.22 (10/46)	0	0.24 (11/46)	0
Korean Ogye	46	0	0	0	0	0	0.15 (7/46)	0	0.85 (39/46)
Korean Native Chicken	47	0	0	0	0	0	0.02 (1/46)	0	0.98 (46/47)

tvb $^{S1/S1}$: susceptible homozygote for *tvb*; *tvb* $^{S1/S3}$: heterozygote for susceptible allele for *tvb*; *tvb* $^{S3/S3}$: susceptible homozygote for *tvb,* contains a SNP (184 T to A); *tvb* $^{S1/R1}$, *tvb* $^{S3/R1}$, *tvb* $^{S1/R3}$: heterozygote for susceptible and resistant allele; *tvb* $^{R1/R1}$: resistant homozygote for *tvb,* contains a SNP (172 C to T); *tvb* $^{R3/R3}$: resistant homozygote for *tvb,* contains premature stop codon in CRD2 domain (Q100*).

incomplete splicing (Fig. 2A). Further sequencing analysis of the PCR products confirmed the presence of four types of transcript lacking intron 1 in WL. In KO, a 4 bp insertion in exon 1 containing intron 1 sequence, generated as a byproduct of incomplete splicing was observed. In KNC, intron 1 retention was observed in both the long and short transcripts, and a mutant form missing some exons was also detected (Fig. 2B).

3. Reduced *tvb* Transcript Expression Levels in KO and KNC

The *tvb*$^{R3/R3}$ genotype, which is not present in WL, is

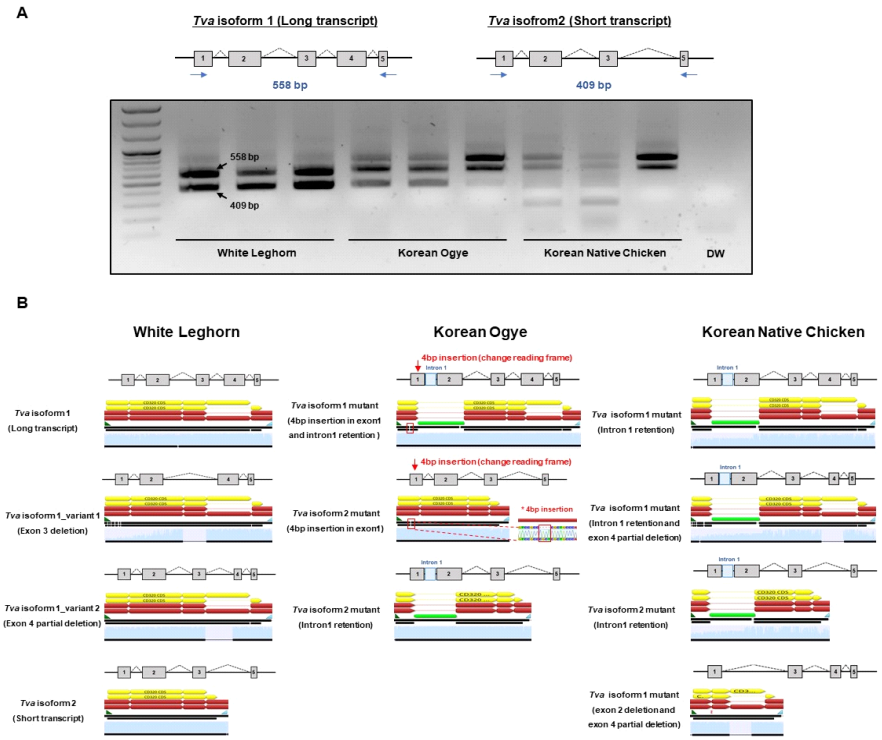

Fig. 2. Splicing patterns and sequencing analysis of *tva* transcripts. (A) Schematic diagram of various *tva* transcripts, including long and short transcripts generated by splicing, and validation of *tva* transcript expression patterns by RT-PCR analysis of samples from WL, KO, and KNC chicken embryo fibroblasts. (B) Sequencing alignment of *tva* transcript sequences generated from each breed (WL, KO, and KNC).

predominant in KO and KNC, and generates a premature stop codon in exon 4, which induces nonsense-mediated RNA decay (Chen et al., 2017). To compare *tvb* transcript stability in a WL susceptible line, KO and KNC resistant lines, we designed primers to amplify the 5' and 3' termini of *tvb* (Fig. 3A). RT-qPCR analysis of both the *tvb* 5' and 3' ends of *tvb* in WL, KO, and KNC CEFs showed that transcript levels were significantly reduced in KO and KNC samples, relative to those from WL (Fig. 3B). These results show that the *tvb* transcript is degraded in KO and KNC, and that there are differences in the level of transcription of *tvb* in KO and KNC relative to WL.

4. Resistance to ALV Subgroup A, B, and K Viruses according to *tva* and *tvb* Genotypes

To verify the role of *tva* and *tvb* genotypes in resistance to ALV subgroups, we infected DF-1 chicken fibroblasts and each CEF clone with subgroup A, B, K, and J viruses. Expression of green fluorescent protein (GFP) was used as a marker to confirm viral replication, in DF-1 positive control cells and the WL #1 line (genotype: $tva^{S/S}$, $tvb^{S1/S1}$), all ALV subgroups entered the cells and replicated. Our results revealed differences in resistance to ALV subgroups, even within the same breeds, according to their genotypes. ALV-B infection in WL line #2 ($tva^{S/R4}$, $tvb^{R1/R1}$) did not lead to GFP expression, indicating that ALV-B could not replicate well in this CEF clone. In KO lines #1 and #2 ($tva^{R2/R4}$, $tvb^{R3/R3}$ and $tva^{R5/R5}$, $tvb^{R3/R3}$), in which both alleles of the *tva* and *tvb* genes confer ALV resistance, GFP expression was almost absent in cells infected with ALV-A, -B, and -K. Finally, ALV-B replication was blocked in KNC line #1 ($tva^{S/S}$, $tvb^{R3/R3}$), whereas replication of ALV-A, -B, and -K was blocked in KNC line #2 ($tva^{R5/R5}$, $tvb^{R3/R3}$). These results show that depending on the genotype, some KO and KNC breeds were naturally resistant to ALV-A, -B, and -K, whereas all breeds were susceptible to ALV-J (Fig. 4).

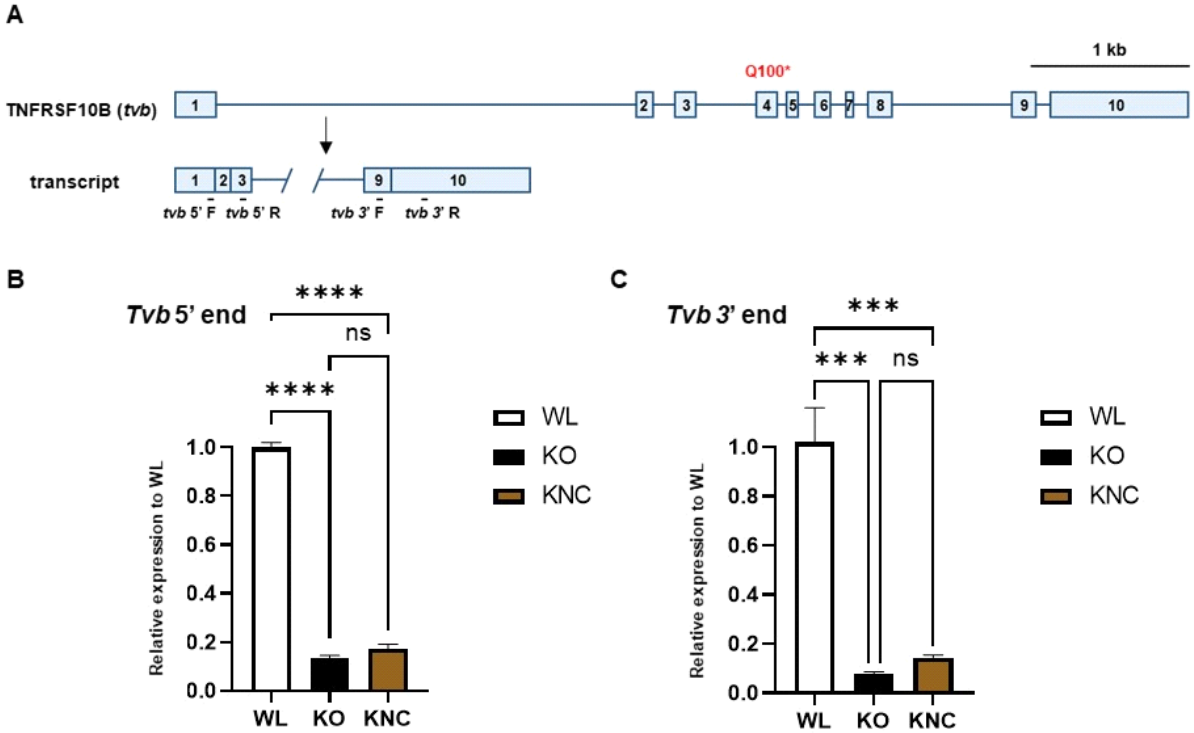

Fig. 3. Nonsense-mediated decay in KO and KNC *tvb* transcripts. (A) Schematic diagram of the location of the premature stop codon in the *tvb* gene and primer sites targeting the 5' and 3' ends of the *tvb* transcript. (B, C) Relative expression levels of mRNA from the 5' and 3' termin of *tvb* in WL, KO, and KNC chicken embryo fibroblasts calculated after normalization using *GAPDH* and WL control samples. Significant differences among groups were determined by one-way ANOVA; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

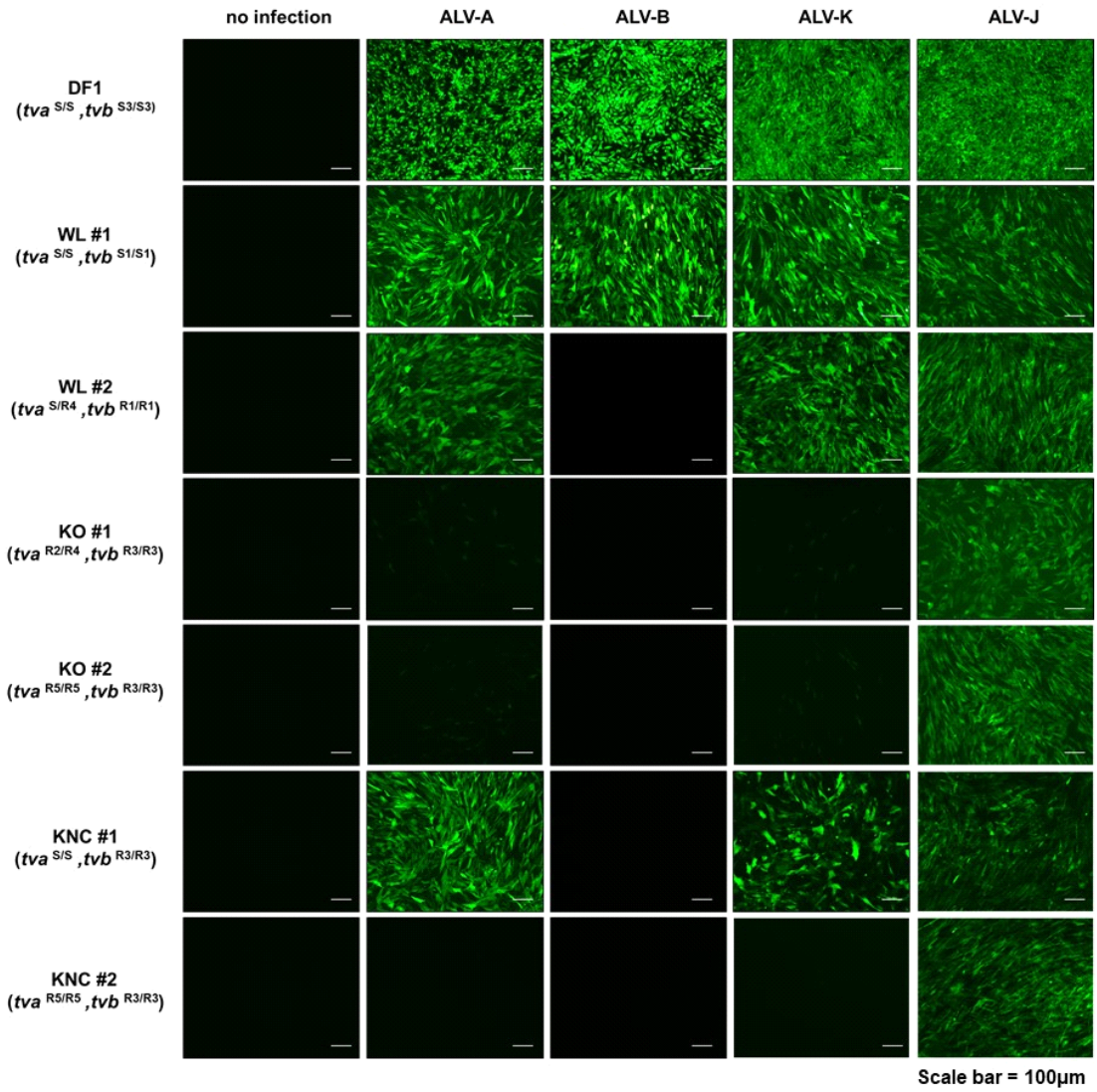

Fig. 4. Infection of WL, KO and KNC CEF clones with ALV subgroup A, B, K and J. Representative images of viral infection of cells from individuals with different genotypes within each breed. GFP expression in ALV-infected CEF clones was examined under a fluorescence microscope. DF-1 cells and ALV-J infection were used as positive controls. Scale bar=100 μm.

DISCUSSION

Multiple ALV subgroups, classified based on their glycoproteins, have significant impacts on the poultry industry. Although considerable efforts to eradicate and prevent ALV infection are ongoing, there is currently no vaccine that can adequately control ALV, and the continuous spread of ALV in Asian countries remains a problem (Feng and Zhang, 2016).

To inhibit viral replication in host cells, it is important to block the interaction between the viral glycoprotein and its host receptor. Indeed, there have been many reports that resistance to ALV subgroups can be naturally acquired by nucleotide mutations in host receptors. Single nucleotide polymorphisms in the genes encoding the TVA and TVB receptors affect viral binding affinity, while insertions or deletions of multiple nucleotides induce frameshift mutations, ultimately affecting viral resistance (Elleder et al., 2004; Reinisova et al., 2008; Reinisova et al., 2012; Chen et al., 2015; Li et al., 2018).

Our analysis revealed significant differences in genotypes and their frequencies among breeds. Genotypes comprising

homozygous susceptibility and heterozygous susceptibility and resistance alleles for the TVA receptor were present in WL ($tva^{S/S}$, $tva^{S/R2}$, and $tva^{S/R4}$), while only resistance alleles were observed in KO ($tva^{R2/R4}$, $tva^{R4/R5}$, and $tva^{R5/R5}$), and susceptibility and resistance alleles were mixed in KNC ($tva^{S/S}$, $tva^{S/R4}$, $tva^{S/R5}$, $tva^{R4/R5}$, and $tva^{R5/R5}$). The difference in genotypes for the TVB receptor was more pronounced, with WL possessing various alleles ($tvb^{S1/S1}$, $tvb^{S1/S3}$, $tvb^{S3/S3}$, $tvb^{S1/R1}$, $tvb^{S3/R1}$, and $tvb^{R1/R1}$), whereas in KO and KNC, the resistant genotype ($tvb^{R3/R3}$) was dominant, although some heterozygous genotype ($tvb^{S1/R3}$) was detected.

A recent genome-wide analysis of KNC, Chinese indigenous breeds, and commercial breeds identified local adaptation and selection signatures related to innate immune responses in KO and KNC (Cho et al., 2022). In addition, a selection signature related to the innate immune response to *Salmonella* infection was identified in Korean indigenous goats using genome-wide analysis (Kim et al., 2019). Therefore, the natural occurrence of *tva* and *tvb* resistance alleles in KO and KNC can be considered selection signatures that have developed over long periods of time in the Korean Peninsula.

Recently reported data demonstrate that the avian TVA receptor protein belongs to the LDLR family, is an ortholog of mammalian CD320, and acts as a vitamin B_{12} transporter as well as a viral receptor (Krchlikova et al., 2021). Further, *tva* knockout chickens generated using CRISPR/Cas9 technology are not only resistant to ALV-A and -K, but also exhibit vitamin deficiency (Koslova et al., 2021). Growth retardation due to vitamin deficiency was not observed in KO and KNC individuals resistant to ALV subgroups, and further studies are needed to determine whether there are other genes that can compensate for lack of vitamin uptake by the TVA receptor.

The TVB receptor protein belongs to the TNFR family, which regulates dendritic cell and natural killer cell interactions apoptosis in response to DNA-damage stimulation, chronic inflammation and tumorigenesis induced in TNFR knockout mice (Finnberg et al., 2005; Finnberg et al., 2008; Iyori et al., 2011). TNFR is involved in several signaling pathways, but the function of the TNFR family member, *tvb*, in chicken remains unknown. Therefore, studies to determine whether other TNFR family genes are activated in KO and KNC in place of *tvb* are required. Both KO and KNC are resistant to ALV subgroups due to naturally occurring mutations in TVA and TVB proteins that have arisen during evolution; however, further studies are needed to validate the effects of these changes on the biological functions of TVA and TVB other than as viral receptors.

Lines naturally resistant to ALV-A, -B, and -K have been reported previously; however, no ALV-J resistant lines have been discovered in nature. Instead, an ALV-J resistant chicken, with viral binding disrupted by removal of the tryptophan 38 residue of the ALV-J receptor, was recently generated using the CRISPR/Cas9 system (Koslova et al., 2020). Therefore, construction of a multiple ALV resistant line, by selective breeding of the CRISPR/Cas9-generated ALV-J resistant line with naturally ALV-A, B, and K resistant lines, is expected to contribute greatly to the poultry industry.

적 요

조류 백혈병 바이러스는 암을 유발할 수 있는 전염성이 높은 레트로바이러스의 하나이다. 이는 높은 전파력으로 인해 전 세계적으로 큰 경제적 손실을 초래하고, 다양한 바이러스 하위 그룹이 존재하여 특정 숙주 수용체를 통해 감염이 일어난다. 백혈병 바이러스 A형과 K형 바이러스에 대한 조류 종의 민감성 또는 저항성은 숙주 수용체인 tumor virus locus A(*tva*)의 유전자형에 의해 결정되고, 바이러스 B형은 숙주 수용체인 tumor virus locus B(*tvb*)의 유전자형에 의존한다. 대부분 중국에서 *tva, tvb* 저항성 대립 유전자가 밝혀졌지만, 한국에서는 연구된 바가 없다. 본 연구에서는 자연계에 존재하는 화이트 레그혼, 오골계 그리고 한국 재래 닭의 *tva*, *tvb* 유전자의 유전형 빈도를 분석하고, 백혈병 바이러스 감염에 대한 저항성을 확인하고자 하였다. 화이트 레그혼에서는 *tva*와 *tvb* 모두 민감성 및 저항성 대립유전자를 포함하여 다양한 유전자형을 가지고 있으며, 오골계에서는 *tva*와 *tvb*에 대한 저항성 대립 유전자가 존재하였다. 한국 재래 닭에서는 *tva*의 경우 민감성과 저항성 대립유전자가 섞여 있으며, *tvb*의 경우 저항성 대립 유전자형이 존재하였다. 또한, 계통에 따라 *tva* 전

사체의 스플라이싱 패턴과 *tvb* 전사체의 발현 수준에 차이가 있음을 확인하였다. 마지막으로 닭 배아 섬유아세포에서 조류 백혈병 바이러스 시험관내 감염을 통해 오골계 및 한국 재래 닭에서 유전자형 의존적으로 저항성을 획득함을 확인하였다. 이러한 결과는 일부 오골계와 한국 재래 닭이 조류 백혈병 바이러스 아형인 A, B 그리고 K형에 자연적으로 내성이 있으며 선택적 육종을 통해 경제적으로 우수한 형질을 보존할 수 있을 것으로 사료된다.

(색인어: 조류 백혈병 바이러스, 한국 재래닭, *tva*, *tvb*)

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REFERENCES

Adkins HB, Brojatsch J, Young JA 2000 Identification and characterization of a shared TNFR-related receptor for subgroup B, D, and E avian leukosis viruses reveal cysteine residues required specifically for subgroup E viral entry. J Virol 74(8):3572-3578.

Barnard RJ, Elleder D, Young JA 2006 Avian sarcoma and leukosis virus-receptor interactions: from classical genetics to novel insights into virus-cell membrane fusion. Virology 344(1):25-29.

Bates P, Young JAT, Varmus HE 1993 A receptor for subgroup-a rous-sarcoma virus is related to the low-density-lipoprotein receptor. Cell 74(6):1043-1051.

Chen WG, Liu Y, Li AJ, Li XJ, Li HX, Dai ZK, Yan YM, Zhang XH, Shu DM, Zhang HM, Lin WC, Ma JY, Xie QM 2017 A premature stop codon within the tvb receptor gene results in decreased susceptibility to infection by avian leukosis virus subgroups B, D, and E. Oncotarget 8(62): 105942-105956.

Chen WG, Liu Y, Li HX, Chang S, Shu DM, Zhang HM, Chen F, Xie QM 2015 Intronic deletions of tva receptor gene decrease the susceptibility to infection by avian sarcoma and leukosis virus subgroup A. Scientific Reports 5.

Cho Y, Kim JY, Kim N 2022 Comparative genomics and selection analysis of Yeonsan Ogye black chicken with whole-genome sequencing. Genomics 114(2):110298.

Cui N, Cui X, Huang Q, Yang S, Su S, Xu C, Li J, Li W, Li C 2020 Isolation and identification of subgroup J Avian Leukosis virus inducing multiple systemic tumors in parental meat-type chickens. Front Vet Sci 7:614854.

Elleder D, Melder DC, Trejbalova K, Svoboda J, Federspiel MJ 2004 Two different molecular defects in the Tva receptor gene explain the resistance of two tvar lines of chickens to infection by subgroup A avian sarcoma and leukosis viruses. J Virol 78(24):13489-13500.

Elleder D, Stepanets V, Melder DC, Senigl F, Geryk J, Pajer P, Plachy J, Hejnar J, Svoboda J, Federspiel MJ 2005 The receptor for the subgroup C avian sarcoma and leukosis viruses, Tvc, is related to mammalian butyrophilins, members of the immunoglobulin superfamily. Journal of Virology 79(16):10408-10419.

Feng M, Zhang X 2016 Immunity to avian leukosis virus: where are we now and what should we do? Front Immunol 7:624.

Finnberg N, Gruber JJ, Fei P, Rudolph D, Bric A, Kim SH, Burns TF, Ajuha H, Page R, Wu GS, Chen Y, McKenna WG, Bernhard E, Lowe S, Mak T, El-Deiry WS 2005 DR5 knockout mice are compromised in radiation-induced apoptosis. Mol Cell Biol 25(5):2000-2013.

Finnberg N, Klein-Szanto AJ, El-Deiry WS 2008 TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. J Clin Invest 118(1):111-123.

Iyori M, Zhang T, Pantel H, Gagne BA, Sentman CL 2011 TRAIL/DR5 plays a critical role in NK cell-mediated negative regulation of dendritic cell cross-priming of T cells. J Immunol 187(6):3087-3095.

Kim JY, Jeong S, Kim KH, Lim WJ, Lee HY, Kim N 2019 Discovery of genomic characteristics and selection signatures in Korean Indigenous goats through comparison of 10 goat breeds. Front Genet 10:699.

Kim YJ, Park SI, Park SJ, Kim HH, Jung YW, Kwon JT, Jang BG, Kim HK, Cho KO 2008 Molecular analysis of endogenous avian leukosis/sarcoma virus genomes in Korean chicken embryos. J Vet Med Sci 70(1):17-23.

Klucking S, Adkins HB, Young JAT 2002 Resistance to infection by subgroups B, D, and E avian sarcoma and leukosis viruses is explained by a premature stop codon within a resistance allele of the tvb receptor gene. Journal of Virology 76(15):7918-7921.

Koslova A, Trefil P, Mucksova J, Krchlikova V, Plachy J, Krijt J, Reinisova M, Kucerova D, Geryk J, Kalina J, Senigl F, Elleder D, Kozich V, Hejnar J 2021 Knock-out of retrovirus receptor gene tva in the chicken confers resistance to avian leukosis virus subgroups A and K and affects cobalamin (vitamin B_{12})-dependent level of methylmalonic acid. Viruses 13(12):2504.

Koslova A, Trefil P, Mucksova J, Reinisova M, Plachy J, Kalina J, Kucerova D, Geryk J, Krchlikova V, Lejckova B, Hejnar J 2020 Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus. Proc Natl Acad Sci U S A 117(4):2108-2112.

Krchlikova V, Mikesova J, Geryk J, Barinka C, Nexo E, Fedosov SN, Kosla J, Kucerova D, Reinisova M, Hejnar J, Elleder D 2021 The avian retroviral receptor Tva mediates the uptake of transcobalamin bound vitamin B_{12} (cobalamin). J Virol 95(8):e02136-20.

Li H, Tan M, Zhang F, Ji H, Zeng Y, Yang Q, Tan J, Huang J, Su Q, Huang Y, Kang Z 2021 Diversity of avian leukosis virus subgroup J in local chickens, Jiangxi, China. Sci Rep 11(1):4797.

Li XJ, Chen WG, Zhang HM, Li AJ, Shu DM, Li HX, Dai ZK, Yan YM, Zhang XH, Lin WC, Ma JY, Xie QM 2018 Naturally occurring frameshift mutations in the tvb receptor gene are responsible for decreased susceptibility of chicken to infection with avian leukosis virus subgroups B, D, and E. Journal of Virology 92(8):e01770-17.

Prikryl D, Plachy J, Kucerova D, Koslova A, Reinisova M, Senigl F, Hejnar J 2019 The novel avian leukosis virus subgroup k shares its cellular receptor with subgroup A. Journal of Virology 93(17):e00580-19.

Reinisova M, Plachy J, Trejbalova K, Senigl F, Kucerova D, Geryk J, Svoboda J, Hejnar J 2012 Intronic deletions that disrupt mrna splicing of the tva receptor gene result in decreased susceptibility to infection by avian sarcoma and Leukosis virus subgroup A. Journal of Virology 86(4):2021-2030.

Reinisova M, Senigl F, Yin XQ, Plachy J, Geryk J, Elleder D, Svoboda J, Federspiel MJ, Hejnar J 2008 A single-amino-acid substitution in the Tvb(S1) receptor results in decreased susceptibility to infection by avian sarcoma and leukosis virus subgroups B and D and resistance to infection by subgroup E *in vitro* and *in vivo*. Journal of Virology 82(5):2097-2105.

Su Q, Li Y, Li W, Cui S, Tian S, Cui Z, Zhao P, Chang S 2018 Molecular characteristics of avian leukosis viruses isolated from indigenous chicken breeds in China. Poult Sci 97(8):2917-2925.

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