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SHORT COMMUNICATION

An Endogenous Avian Leukosis Virus Element in the Genome of Commercial Chickens Showing Emaciation of Unknown Etiology

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ABSTRACT

Endogenous viral loci, designated as avian leukosis virus subgroup E (ALVE), have been found in the genome of commercial chickens. They sometimes modulate the host physiologic processes by changing the expression level of host promoters near an integration site. Therefore, the identification of ALVE locus is necessary for chickens representing abnormal production traits such as body weight and egg production. In this report, an ALVE element is commonly identified in the genome of a laying chickens aged 13 weeks and a Korean native chickens aged 10 weeks showing emaciation using an nested inverse PCR. This element locates near four putative host promoters and body weight related quantitative trait loci (QTL), which can induce body weight change in chickens. In conclusion, we newly report the role of an ALVE element for emaciation of laying and Korean native chickens without the presence of pathogens.

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INTRODUCTION

Endogenous viral loci are identified in the genome of chickens which are known for avian leukosis virus subgroup E (ALVE). There are several ALVEs commonly identified in the genome of commercial chickens (Chen *et al.*, 1998). These elements could not induce neoplasm due to weak or no enhancer activity for cellular oncogenes unlike exogenous avian leukosis virus (ALV) (Feschotte and Gilbert, 2012; Jing *et al*., 2014).

ALVE proviruses are randomly integrated into the host genome although they showed the preferred regions of integration under some conditions. These proviruses are usually found in the region of no essential genetic components in chickens. Thus, most chickens harboring these elements have been showing normal production traits. However, the host physiologic processes are sometimes modulated by *cis*- or *trans*-acting ALVE proviruses depending on insertion site, transcriptional activity and provirus integrity (Benkel, 1998). There were several reports for ALVE elements related to the change of production traits including body weight, egg production and egg weight (Ka *et al*., 2009; Feschotte and Gilbert, 2012). Therefore, the integration sites of ALVE elements need to be evaluated for chickens representing abnormal traits.

MATERIALS AND METHODS

Between 2010 and 2011, four laying chicken flocks and one Korean native chicken flock from various provinces in Korea were submitted to Avian Disease Laboratory (College of Veterinary Medicine, Chungbuk National University) for clinical diagnosis. The submitted chickens commonly represented emaciation without other gross lesions. During routine necropsy procedures, the blood samples were collected and pooled from five flocks, respectively. Viral and host DNA were extracted from each sample using Viral Gene-spin™ DNA/RNA extraction kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. For identifying the integrations sites of proviruses, the nested inverse PCR (IPCR) was performed according to a previous report (Smith and Benkel, 2009). Briefly, the extracted DNA sample was digested overnight using six cutter ECoR I (Enzynomics, Korea) and incubated in a water bath at 65ºC for 20 min to inactivate a restriction enzyme. For circle formation of cutting fragments, the samples were incubated at 12ºC overnight. The nested IPCR was carried out in a PCR Thermal Cycler (Astec, Japan) using two primer pairs, Br-1 (5'-CTTCGGTTGTACGCGGTTAGG AGTC-3') and Br-2 (5'-GTGATAGCTGATTGAATTAT TAATCA-3'), NBr-1 (5'-AGGAGTCCCCTCAGGATAC

AG-3') and NBr-2 (TCATCTTTCGGATGCTACTGG-3') (Smith and Benkel, 2009). PCR products were separated by electrophoresis on 2% agarose gels with 0.5µg/ml ethidium bromide, and identified using ultraviolet trans-illumination. Finally, amplicons were purified using a commercial gel extraction kit (GeneAll, Korea), and nucleotide sequences were obtained by direct sequencing method using an ABI3730XL DNA analyzer (Applied Biosystems, USA). Sequences were manipulated using Bioedit software, version 7.0.9.0 (http://www.mbio.ncsu.edu/bioedit.html). Multiple alignments and restriction site analysis were performed based on nucleotide sequences in CLC sequence viewer 6.7 (CLC bio A/S, Denmark). The quantitative trait loci (QTL) near the integrated site were identified in the chicken QTL database (http://www.animalgenome.org /cgi-bin/QTLdb/GG/index).

RESULTS AND DISCUSSION

A number of amplicons including an approximately 900-bp, high-density amplicon were observed by nested IPCR. Among these amplicons, the nucleotide sequences were successfully determined from only an approximately 900 bp-amplicon. This PCR product was identified in only two flocks including a laying chicken flock aged 13 weeks and Korean native chicken flock aged 10 weeks. These flocks represented negative results for avian diseases related with emaciation in chickens including chronic respiratory disease (CRD), coccidiosis, Marek's disease (MD), Reticuloendotheliosis (RE) and exogenous ALV infection by histopathological and molecular examinations (data not shown). An ALVE element of two flocks locates between nucleotide 1762777 and 1762778 within MANBAL-like protein in chromosome 20 of chicken genome in multiple alignments (Figure 1). ALVE could modulate the expression of host promoters within range of approximately 80 kbp from the element (Feschotte and Gilbert, 2012). In this case, there were four putative promoters within 80 kbp distance from an ALVE insertion site including MANBAL (mannosidase, beta A, lysosomal)-like protein, Itchy E3 ubiquitin protein ligase, Formin-like protein and adenosylhomocysteinase (Table 1). The deficiency of mannosidase could decrease the nutrient efficiency in chickens considering mannans as strong anti-nutritive factors (Cho and Kim, 2013). And muscle cachexia can be induced by activated ubiquitin proteasome pathway via increase of HECT E3 ubiquitin ligases, WWP1 in chickens (Matsumoto *et al*., 2008). Formin proteins involve in various cellular processes including cytokinesis, cell polarity, actin cable formation, actin nucleation and limb morphogenesis (Evangelista *et al*., 2003). The inhibition of adenosylhomocysteinase induced the increased level of methionine which increased body weight in broilers (Esteve-Garcia and Mack, 2000), indicating the body weight could be decreased by overexpression of adenosylhomocysteinase gene. Also, this ALVE element locates in the genome of chickens harboring the QTLs affecting body weight in chicken QTL database (Tran *et al*., 2014). In a previous report, this ALVE element was not identified in the genome of laying chickens (Chen *et al*., 2003). Also, we first observed this ALVE element in the genome of Korean native chickens. Therefore, the presence of this ALVE element is first documented in commercial laying and Korean native chickens in this report. The altered expression of putative promoters and QTLs could cause emaciation in two chicken flocks although we could not confirm the exact role of this ALVE element in these flocks due to limited information.

In this report, an ALVE element was newly determined in laying chickens and Korean native chickens, which located within or near the four putative promoters and QTLs having a significant effect on body weights. Considering those flocks negative for any pathogens causing emaciation, it is considered that this ALVE element could affect the emaciation of commercial laying and Korean native chickens partially or fully.

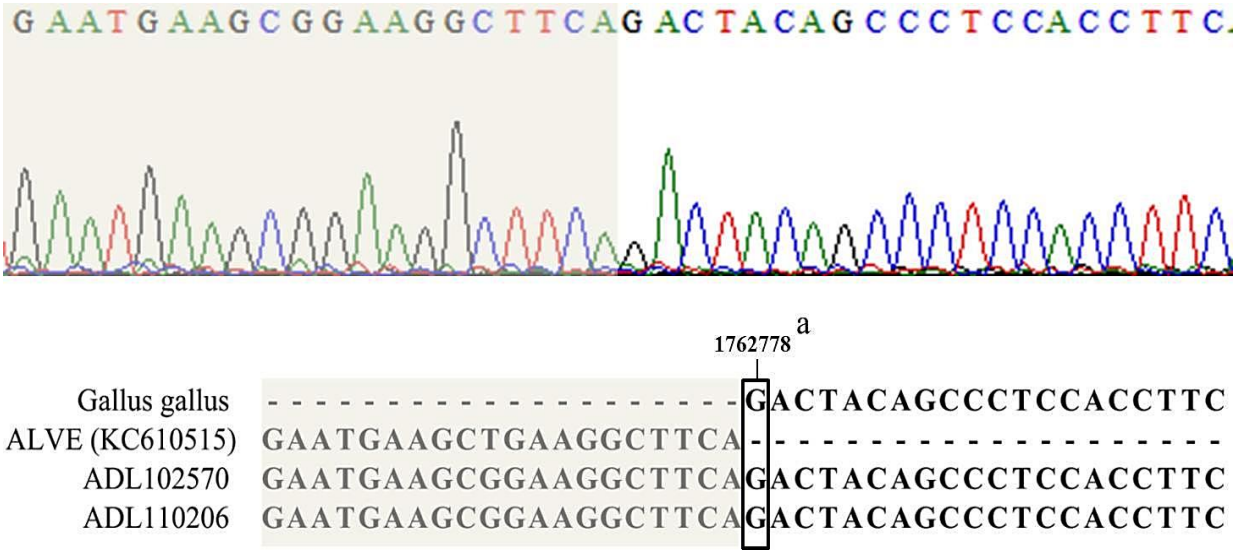

Fig. 1: The integration site of an endogenous avian leukosis virus. The shaded regions represent the ALVE nucleotide sequences and the other regions indicate the chicken host genome. The multiple sequence alignments are performed including the genome of laying chickens (ADL110206), Korean native chickens (ADL102570), reference strain of endogenous avian leukosis virus and the chromosome 20 of chicken genome (1762760 – 1762800 nt position). The slash symbol means the deletion sites.

Table 1: The putative host promoters near the endogenous avian leukosis virus element in the chicken genome

Symbol	Description	Location [B]
LOC431205	MANBAL-like protein [A]	1759710-1765546
ITCH	Itchy E3 ubiquitin protein ligase	1766293-1829963
LOC101748343	Formin-like protein	1829466-1832342
AHCY	Adenosylhomocysteinase	1839315-1861689

[A]MANBAL=mannosidase, beta A, lysosomal-like; [B]Nucleotide numbering in chromosome 20 of chicken genome

Conclusions: We newly proposed the role of a new endogenous viral locus for decreased body weight in commercial laying and Korean native chickens. Therefore, the presence of this element should be evaluated for commercial chickens showing emaciation without infection of specific pathogens.

Author's contribution: BS developed the overall research idea and performed molecular experiments. BS, EO, YJ, JS and JN did necropsy for chickens representing emaciations and collected tissue samples. IP supervised overall procedures including experiments and articles. All authors approved the manuscript.

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