Niu, Q., Ding, Y., Yang, L., Dunn, J., Zhang, H., Song, J. (2026) Unveiling the functional landscape of A-to-I RNA editing associated with chicken Marek's disease, *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 24047: https://doi.org/10.31274/wcgalp.24047 ©2026 Niu, et al. CC BY-NC-ND

Unveiling the functional landscape of A-to-I RNA editing associated with chicken Marek's disease

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Introduction

Marek's disease (MD) is a highly contagious neuropathic and lymphoproliferative disease of chickens caused by Marek's disease virus (MDV), an oncogenic alphaherpesvirus (Zhu *et al*. 2024). Despite extensive vaccination, occasional outbreaks of MD still occur worldwide, resulting in substantial economic losses and posing a persistent threat to the global poultry industry. A-to-I RNA editing (RESs), represent the most abundant form of RNA editing in vertebrates. RES events have been identified across multiple tissues, providing valuable insights into evolutionary conservation, tissue-specific regulatory patterns, and phenotypic variation in farm animals (Li *et al*. 2025). $CD4^+$ T cells are the primary target of MDV, which infects and transforms them into cancerous lymphomas, resulting in multiple visceral tumors and immunosuppression in chickens (Boodhoo *et al*. 2016). Previous studies have suggested that A-to-I RNA editing was also considered an essential regulator in innate immunity and cell death (Samuel 2012). However, the role of RNA editing in regulating immune responses and disease-resistance for MDV infection in chickens remains largely unexplored. In this study, we investigated the functional characterization of RESs in $CD4^+$ cell for two chicken lines with different infection status of MDV and identified candidate RNA editing sites exhibiting line-specific patterns and differential editing levels associated with MD-resistance. Our study is the first to explore the contribution of RESs in $CD4^+$ T cells to MD-resistance in chickens, providing novel insights into immune-related RNA editing mechanisms.

Materials & Methods

Experimental animals and chicken $CD4^+$ T cell isolation. A total of 12 individuals from two inbred White Leghorn chicken lines (Line 6_3 and Line 7_2) were housed at the Avian Disease and Oncology Laboratory (USDA). Chickens were assigned to MDV-infected and control groups, with 3 biological replicates in each group (Fig.1A). At 5 days post-hatch, infected chickens were intra-abdominally inoculated with MDV strain 648A (500 PFU). At 10 days post-infection, spleens were collected and processed into single-cell suspensions. $CD4^+$ T cells were isolated using PE-conjugated anti-chicken CD4 antibody and EasySep magnetic positive selection. Cell purity was assessed by flow cytometry, yielding 90–95% $CD4^+$ T cells. Total RNA was extracted, cDNA was synthesized using the Smart-seq2 protocol, sonicated, end-repaired, and constructed into Illumina libraries that were sequenced at 50-bp single-end reads on a HiSeq 3000 platform.

Data preprocessing and identification of A-to-I editing events. REDItools3 were utilized to detect the A-to-I editing events based on deduplicated BAMs from RNA-seq (Picardi and Pesole 2013). To obtain the high confidence A-to-I editing sites, we conducted the strict filtrations, including (1) discarding sites with edited reads less than 2 and RNA coverage less than 3; (2) filtering sites overlapping with chicken SNPs; (3) retaining sites identified in at least two individuals; (4) excluding sites with 100% editing level across all samples.

Quantification and comparative analysis of gene expression. DEseq2 was utilized to detect differentially expressed genes (DEG) for pair-wise comparisons. The threshold for identifying DEGs was set to FDR < 0.05 and an absolute log2 fold change more than 1.5. Samples were then classified into edited and unedited groups for each editing site. Wilcoxon rank-sum test was used to assess expression differences of target genes between the two groups.

Identification of differential and group-specific RESs. Using Fisher's exact test, differential RESs were identified for pair-wise comparisons. Only RESs with a mean editing level greater than 0.01 in both groups were retained for differential analysis. Differential RESs were identified based on pooled RNA-read counts for each site. Group-specific RESs were detected using UpSetR across the four groups.

Results

Genome-wide identification of A-I RNA editing sites in chicken CD4$^+$ T-cells

We identified a total of 642 RES events across 12 CD4$^+$ T-cells located in 187 non-redundant positions. We found that majority of RESs (57.8%) were detected in only two samples (Fig.1B). The highest quantity of RESs was detected in the MDV-infected group of Line 7_2. Moreover, we observed significant differences between infected and non-infected groups across the two chicken lines. Line 7_2 exhibited a higher number of RESs in the MDV-infected group than the control group, whereas Line 6_3 displayed an opposing trend (Fig.1C). Significant difference in editing levels was identified between MD-susceptible and MD-resistant lines, where MD-resistant lines showed a higher editing level, suggesting that this pattern may underlie the differential MD resistance of these lines (Fig.1D). Among 187 unique RESs, 145 RESs were mapped to genes, and nearly all of these resided in protein-coding genes. Across all four groups, we found that RESs were predominantly annotated to the 3'UTR region, followed by missense variants and upstream/downstream variants (Fig.1 E), and most of them were predicted to have the indirect regulatory effects or moderate impacts on protein structure (Fig.1F). Moreover, over half of RESs were localized to repeat elements within the chicken genome (Fig.1G).

Differential and group-specific A-to-I editing events for chicken Marek's disease

We employed Fisher's exact test yielding 4, 5, and 2 differential RESs across the same three groups (Fig.2A). For $L6_3$-infection vs. $L6_3$-control, we found the editing site (chr4:17,601,347), located in the 3'UTR of *HMGB3*, presented an upregulated editing level in infected individuals, have a strong negative correlation (r = 0.77) with its gene expression. Conversely, a synonymous site (chr19:7,071,499) with a decreasing editing level in infected individuals show a positive correlation with gene expression (r = 0.74) of LOC101747587 (Fig.2B-D). Integrating DEG results, we identified four differential RESs within two DEGs including ENSGALG00010024056, ENSGALG00010024069 across three groups (Fig.2A). We identified 38, 22, 21, and 50 group-specific RESs for $L6_3$-control, $L6_3$-infection, $L7_2$-control, $L7_2$-infection, respectively. Meanwhile, there were 21 group-shared RESs detected in at least 3 groups (Fig.2E). Functional enrichment analysis was performed for corresponding genes revealed by group-specific RESs (Fig.2F). More importantly, we identified two key Line 63-specific RNA editing sites. These two sites may exert their functions by influencing miRNA binding and by altering amino acid sequences of their target genes, respectively. RNA editing at chr6:26,600,122 within the *MXI1* 3' UTR disrupts the predicted interaction with gga-miR-1759-3p, leading to loss of miRNA binding (Fig.2G). A-to-I RNA editing site at chr16:1,716,632 may result in a threonine-to-alanine substitution at residue 318 of *MHCY57*. Multiple sequence alignment analysis suggested that this amino acid is more conserved after RNA editing across species (Fig.2H). Protein structural analysis revealed that

this threonine-to-alanine substitution was located in a loop region without altering the protein fold of *MHCY57* (Fig.2I).

Discussion

Here, we characterized the first systematic A-to-I RNA editing events in CD4⁺ T cells from different chicken lines with distinct MDV infection statuses. We observed most of A-I editing events in CD4⁺ T cells are enriched in 3'UTR region, which was consistent with previous reports (Li *et al*. 2025). Different from the enrichment of A-to-I RESs in Alu elements in mammals, we found that A-to-I RESs in chickens primarily reside in repetitive elements, particularly in the CR1 family. Previous studies have suggested that RNA editing is associated with viral infection and may contribute to limiting viral infectivity in the host (Huang *et al*. 2024). In this study, we observed the significant difference in the number of RESs between infection and non-infection group in two chicken lines. More importantly, we also found the divergence in RNA editing levels between the two chicken lines with elevated RNA editing level in the MD-resistant Line 6_3 compared with the MD-susceptible Line 7_2, potentially contributing to a more effective immune response against MDV in chicken.

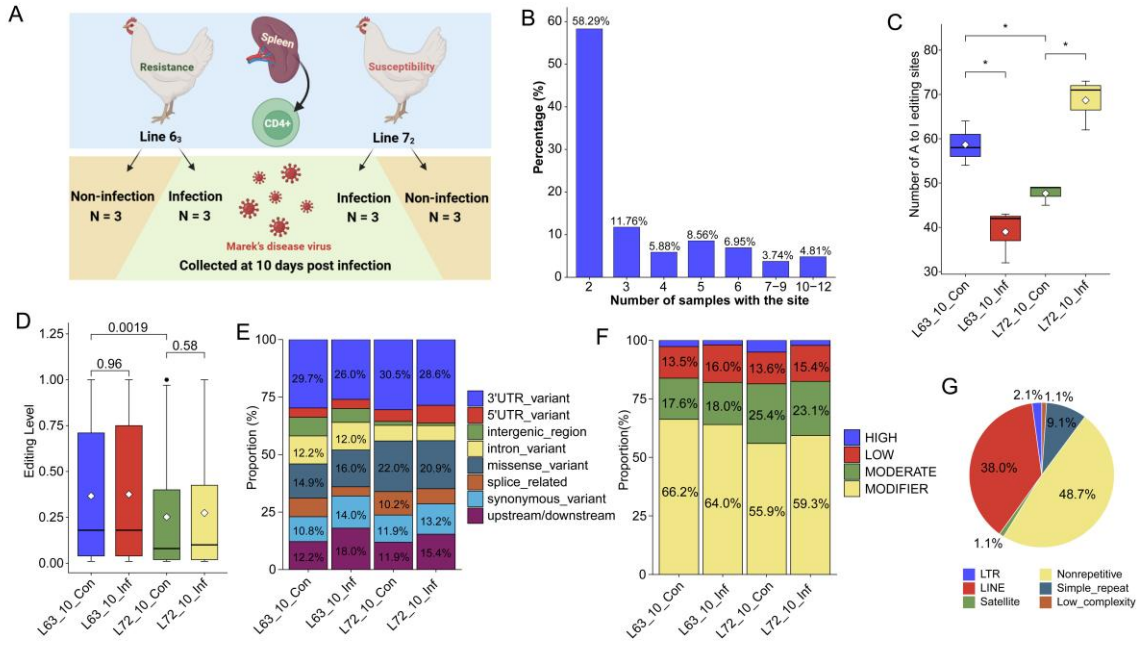

Figure 1 A-to-I RNA editing in CD4⁺ T cells from two chicken lines with different infection status. A. The experimental design for the identification of RNA editing in chicken CD4⁺ cells. B. The distributed percentage of A-to-I editing sites (RESs) across individuals. C. Boxplot for the comparison of the number of RESs across four group. *, $P \leq 0.05$ (Wilcoxon rank-sum tests). D. Boxplot for the comparison of the number of RESs across four group. *P*-values are marked in the plot (Wilcoxon rank-sum tests). E. Comparison of genomic annotation for RESs across groups. F. Functional effect of RESs on protein structure. G. Distribution of RESs across different chicken repeat elements.

Conclusions

Our findings advance our understanding of post-transcriptional RNA editing mechanisms underlying MD resistance, provide mechanistic insights into ADAR-mediated RNA editing across chicken lines with distinct resistance phenotypes, and offer valuable implications for vaccine improvement and disease-resistant breeding in poultry.

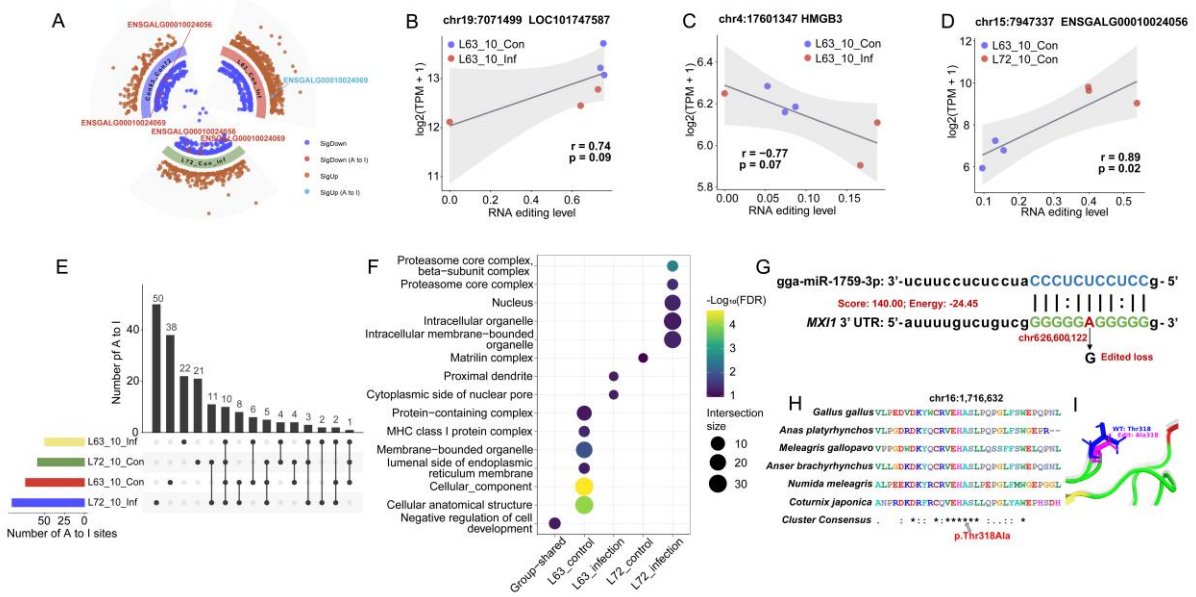

Figure 2 Comparative analysis pinpointed two key RESs for chicken Marek's disease. A. Differential analysis across Line 6_3 vs Line 7_2, and infected and non-infection group in Line 6_3 and line 7_2. B-D. Editing level of differential RES (chr19:7,071,499) between infected and non-infected group shows correlation with target genes (B) LOC101747587 and (C) *HMGB3*, and (D) ENSGALG00010024056. E. Upset plot show the group-specific and group-shared A-to-I editing sites among four groups with different infection status in two chicken lines. F. The GO analyses for target genes of group-specific and group-shared RESs. G. The gga-miR-1759-3p binding site in the *MXI1* 3'UTR. H. Multiple sequence alignment for orthologous sequence of *MHCY57* across species. I. Comparison of protein structure between before editing (wild) and after editing (edit).

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture (NIFA) National Research Initiative Competitive Grants (grant nos. 2008-35204-04660 and 2010-65205-20588).

References

Boodhoo, N., A. Gurung, S. Sharif, *et al*. 2016. Veterinary Research, 47: 119.
Huang, M., A. Mark, J. Pham, *et al*. 2024. PLOS ONE, 19: e0307450.
Li, C., W. Lv, Z. He, *et al*. 2025. Nucleic Acids Research, 53: gkaf534.
Picardi, E., and G. Pesole. 2013. Bioinformatics, 29: 1813-14.
Samuel, C. E. 2012. Curr Top Microbiol Immunol, 353: 163-95.
Zhu, Z.-J., M. Teng, Y. Liu, *et al*. 2024. npj Vaccines, 9: 109.