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Integrative analysis of splice-site variants and transcript isoform expression underlying resistance to gastrointestinal nematode infections in sheep

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Introduction

Gastrointestinal nematode (GIN) infections remain a major constraint on sheep production systems worldwide, causing reduced productivity, impaired animal health and welfare, and substantial economic losses (Hayward, 2022). While anthelmintic treatments have been widely used to control GIN infections (Charlier *et al*., 2022), the rapid emergence of drug-resistant parasites has highlighted the need for sustainable, genetics-based approaches to improve host resistance (Sweeney *et al*., 2016). However, genetic resistance to GIN infections is a complex, moderately heritable (h^2 = 0.2-0.4) polygenic trait involving multiple immune and biological pathways (Sweeney *et al*., 2016; Chitneedi *et al*., 2020).

Transcriptomic studies have provided important insights into host–parasite interactions underlying GIN resistance, revealing differential regulation of immune pathways between resistant and susceptible animals (McRae *et al*, 2016; Cunha *et al*., 2025). However, most studies have focused primarily on gene-level expression changes, overlooking the contribution of post-transcriptional regulatory mechanisms. Alternative splicing (AS), for example, is a fundamental process that expands transcriptomic and proteomic diversity by generating multiple mRNA isoforms from a single gene (de Klerk and ‘t Hoen, 2015; Cardoso *et al*., 2018) and plays a critical role in shaping immune responses (Badia-Bringué *et al*., 2024). Genetic variants that disrupt canonical splice donor or acceptor sites can alter splicing patterns, leading to isoform switching, or transcript dysregulation with downstream functional consequences (Anna and Monika, 2018).

Despite growing evidence that splice-site (SS) variants contribute to phenotypic variation in complex traits, their role in GIN resistance in sheep remains poorly characterized. In particular, the integration of SS-associated single nucleotide polymorphisms (SNPs) with mRNA isoform expression has not been systematically explored in the context of host resistance to parasitic infection. Therefore, the objective of this study was to perform an integrative analysis of splice-site SNPs and differentially expressed mRNA isoforms (DEIs) using RNA-Sequencing (RNA-Seq) data from resistant and susceptible Scottish Blackface lambs experimentally challenged with *Teladorsagia circumcincta (T. circumcincta*). By linking genetic variation affecting splicing with transcript isoform dynamics, this study aims to provide novel insights into the molecular mechanisms underlying GIN resistance and to identify functionally relevant candidate markers for genetic improvement in sheep.

Materials & Methods

Animals, Data Source, and Experimental Design. RNA-Sequencing data were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) public repository under accession number GSE63547 (McRae *et al*., 2016). Detailed information on animal management, parasite challenge, phenotyping, and tissue collection is fully described in McRae *et al*. (2016). Briefly, purebred male Scottish Blackface lambs (n = 92) were naturally exposed to GIN under pasture conditions, with resistance assessed using repeated fecal egg count (FEC) measurements. Based on mixed model analyses of two infection rounds, the 10 most resistant (LowFEC; mean FEC=216 eggs per gram (epg)) and 10

most susceptible (HighFEC; mean FEC=1373 epg) lambs were selected. Following parasite clearance and a minimum five-week rest period, lambs were experimentally challenged with 30,000 *T. circumcincta* L3 larvae. Animals were euthanized at 7 and 14 days post-infection (dpi), with 5 animals per phenotype sampled at each time point, and abomasal lymph node tissues were collected for RNA extraction.

RNA Sequencing and Data Processing. Strand-specific RNA-Seq libraries were prepared and sequenced using an Illumina platform to generate 51 bp paired-end reads as described by McRae *et al*. (2016). Raw sequencing data were retrieved from GEO and processed using QIAGEN CLC Genomics Workbench v24.0. Sequence trimming and quality control were performed according to established protocols (Cánovas *et al*., 2014a), and all samples met quality thresholds, including the balanced nucleotide contribution (~25% A, T, G, and C), approximately 50% GC content, and less than 0.1% over-represented sequences, among others.

Quantification of Differentially Expressed mRNA Isoforms. Trimmed reads were aligned to the annotated ovine reference genome Oar_rambouillet_v3.0 (ARS-UI_Ramb_v3.0) using the Large Gap Read Mapping (LGRM) tool in CLC Genomics Workbench v24.0 (Cardoso *et al*., 2018). The Transcript Discovery tool was subsequently used to identify both known and novel mRNA isoforms. Expression levels were normalized to reads per kilobase per million mapped reads (RPKM), facilitating comparisons between groups (Cánovas *et al*., 2014b). Differentially expressed mRNA isoforms (DEIs) between LowFEC and HighFEC groups at 7- and 14-dpi were identified with significance defined as Fold discovery rate (FDR) < 0.01 and |Fold change (FC)| ≥ 2 (Asselstine *et al*., 2022). Associated gene annotations were retrieved using Ensembl BioMart (Ensembl Genes release 115), and functional enrichment analysis was performed using Reactome.

SNP Discovery and Splice-Site Variants Identification. Trimmed RNA-Seq reads were aligned to the annotated Oar_rambouillet_v3.0 reference genome using the RNA-Seq analysis tools in CLC Genomics Workbench v24.0. To improve variant calling accuracy, reads from individuals within each phenotype group were merged into a single composite dataset prior to variant detection (Lam *et al*., 2020). The functional SNPs were identified separately for the LowFEC and HighFEC groups using the Fixed Ploidy Variant Detection workflow. The Predict Splice Site Effect function was subsequently applied to identify SNPs with predicted splice site effects (SSEs) (Cunha *et al*., 2025). Functional consequences of predicted SS SNPs, including splice-site effects, variant impact classification (high or moderate), and associated amino acid changes, were assessed using Ensembl Variant Effect Predictor (VEP), followed by pathway enrichment analysis using Reactome.

Integration of Splice-Site SNPs and mRNA Isoforms. The co-localization of SS-associated SNPs with DEIs was assessed using shared genomic coordinates. Genomic overlaps were identified using the findOverlaps function from the GenomicRanges package in R Studio. Overlapping co-localizations identified at both the gene and isoform levels were considered putative functional variants associated with splicing differences between LowFEC and HighFEC lambs in response to *T. circumcincta* infection. Functional annotation of overlapping genes was performed using Reactome.

Results & Discussion

Differential mRNA Isoform Expression. Analysis of mRNA isoform expression identified 240 DEIs mapping to 124 genes at 7 dpi, and 216 DEIs mapping to 109 genes at 14 dpi between LowFEC and HighFEC lambs. Among these, six genes (*ACSL4*, *FERMT3*, *MRPL49*, *QSER1*,

SESN3, and *TFG*) were consistently identified at both 7 and 14 dpi in resistant versus susceptible sheep. Functional analysis of mRNA isoform-associated genes at 7 dpi revealed enrichment of metabolic pathways related to fatty acid metabolism, oxidative stress regulation, and transcriptional programs associated with immune cell differentiation, indicating early regulatory differences between resistant and susceptible animals ($p<0.05$). In contrast, at 14 dpi, resistant sheep displayed enrichment of pathways related to leukocyte migration, epithelial junction organization, and MAPK signaling, consistent with the establishment of a coordinated immune response relative to susceptible animals.

Splice-Site SNPs Discovery. In total, 450 and 442 SNPs with predicted SSEs were identified in the LowFEC and HighFEC groups, respectively, and were group-specific, using CLC Genomic Workbench v24.0. The VEP analysis of SS-associated SNPs revealed distinct functional patterns between resistant and susceptible sheep. After filtering for high- and moderate-impact SS SNPs with predicted amino acid altering consequences, 46 SNPs mapping to 20 genes were uniquely identified in the resistant group, whereas 48 SNPs mapping to 37 genes were unique to the susceptible group.
Metabolic pathway enrichment analysis showed that resistance-associated SS SNPs were enriched in pathways related to immune regulation, mitochondrial fatty acid β-oxidation, and transcriptional control. Notably, enrichment of interleukin-37 signaling and interleukin-1 family signaling suggests tightly regulated inflammatory responses ($p<0.05$). In contrast, susceptibility-associated SS SNPs were enriched in pathways related to tryptophan catabolism, extracellular matrix organization, platelet activation, and innate immune responses ($p<0.05$). Multiple pathways associated with collagen biosynthesis, degradation, and fibril assembly indicate ongoing tissue remodeling and damage in susceptible animals. Additional enrichment of platelet activation, hemostasis, and neutrophil degranulation pathways supports a model of chronic inflammation and immune dysregulation.

Integration of Splice-Site SNPs and mRNA Isoforms. Integration of SS SNPs with DEIs identified overlapping genes and isoforms, suggesting potential effects on alternative splicing and transcript diversity. Many overlaps involved novel or previously uncharacterized isoforms, emphasizing the value of isoform-level analyses for uncovering regulatory mechanisms missed by gene-level approaches. These overlapping candidates were considered putative functional variants influencing alternative splicing in response to *T. circumcincta* infection.
At 7 dpi, 5 and 3 DEIs overlapped with SS associated SNPs uniquely identified in the LowFEC and HighFEC groups, respectively, while at 14 dpi, 1 and 3 such overlapping isoforms were detected. Notably, immune response-associated genes were identified through this integrative approach, including the *FERMT3* gene, involved in leukocyte adhesion and immune cell trafficking; the *NCOA7* gene, associated with oxidative stress regulation; the *TEP1* gene, linked to telomerase function and cellular maintenance; and the *SENP6* gene, which plays a role in protein desumoylation and chromatin organization. The functional roles of these genes suggest that SS SNPs may influence immune-cell adhesion, transcriptional and post-transcriptional regulation, and cellular stress responses during GIN infections.

Conclusions

This study integrated splice-site SNPs with mRNA isoform expression to elucidate regulatory mechanisms underlying GIN resistance in sheep. Resistant lambs exhibited splice-associated variation and mRNA isoform expression patterns enriched for immune regulation, metabolic efficiency, and cellular homeostasis, whereas susceptible lambs showed signatures of tissue remodeling, stress response, and immune dysregulation. Overlapping splice-site SNPs and DEIs identified the *FERMT3*, *NCOA7*, *TEP1*, and *SENP6* genes as functional candidate genes

that may mediate GIN resistance, providing evidence for the involvement of splicing-related regulatory mechanisms in GIN-infected sheep.

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References

Hayward A.D. (2022) International Journal for Parasitology 52:843-853. doi.org/10.1016/j.ijpara.2022.09.004

Charlier J., Bartley D.J., Sotiraki S., Martinez-Valladares M., Claerebout E., von Samson-Himmelstjerna G., and Rinaldi L. (2022) Advances in Parasitology 115:171-227. doi.org/10.1016/bs.apar.2021.12.002

Sweeney T., Hanrahan J.P., Ryan M.T., and Good B. (2016) Parasite Immunology 38:569-586. doi.org/10.1111/pim.12347

Chitneedi P.K., Arranz J.J., Suárez-Vega A., Martínez-Valladares M., and Gutiérrez-Gil B. (2020) Animal Genetics 51:266-277. doi.org/10.1111/age.12894

McRae K.M., Good B., Hanrahan J.P., McCabe M.S., Cormican P., Sweeney T., and Keane O.M. (2016) Veterinary Parasitology 224:96-108. doi.org/10.1016/j.vetpar.2016.05.014

Cunha S.M., Schenkel F.S., Karrow N.A., Mallard B., and Cánovas Á. (2025) Scientific Reports 15:37287. doi.org/10.1038/s41598-025-21335-5

de Klerk E., and AC't Hoen P. (2015) Trends in Genetics 31:128-139. doi.org/10.1016/j.tig.2015.01.001

Cardoso T.F., Quintanilla R., Castelló A., González-Prendes R., Amills M., and Cánovas Á. (2018) BMC Genomics 19:145. doi.org/10.1186/s12864-018-4515-2

Badia-Bringué G., Lavín J.L., Casais R., and Alonso-Hearn M. (2024) Frontiers in Immunology 15:1354500. doi.org/10.3389/fimmu.2024.1354500

Anna A., and Monika G. (2018) Journal of Applied Genetics 59:253-268. doi.org/10.1007/s13353-018-0444-7

Cánovas A., Rincón G., Bevilacqua C., Islas-Trejo A., Brenaut P., Hovey R.C., and Medrano J.F. (2014a) Scientific Reports 4:5297. doi.org/10.1038/srep05297

Cánovas A., Reverter A., DeAtley K.L., Ashley R.L., Colgrave M.L., Fortes M.R., and Thomas M.G. (2014b) PLoS One 9:e102551. doi.org/10.1371/journal.pone.0102551

Asselstine V., Medrano J.F., and Cánovas A. (2022) BMC Genomics 23:222 doi.org/10.1186/s12864-022-08430-x

Lam S., Zeidan J., Miglior F., Suárez-Vega A., Gómez-Redondo I., Fonseca P.A., and Cánovas A. (2020) BMC Genomics 21:703. doi.org/10.1186/s12864-020-07107-7