

VARIANT DISCOVERY IN GENES IDENTIFIED AS DIFFERENTIALLY EXPRESSED GENES BETWEEN THE ABOMASAL LYMPH NODE TRANSCRIPTOME OF RESISTANT AND SUSCEPTIBLE ADULT SHEEP TO *Teladorsagia circumcincta* INFECTION

Chitneedi, P.K¹., Suárez-Vega, A¹., Martínez Valladares, M^{2,3}., Arranz J.J¹. y Gutiérrez-Gil, B¹.

1 Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, 24071 León.

2 Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain 3 Instituto de Ganadería de Montaña, CSIC-Universidad de León, 24346, Grulleros, León, Spain. pchi@unileon.es



XIX REUNIÓN NACIONAL DE MEJORA GENÉTICA ANIMAL León, 14 y 15 de junio de 2018 Paraninfo de la Facultad de Veterinaria, Universidad de León



Introduction



Teladorsagia circumcincta (Robin et al. 2007)

- ☐ Gastrointestinal nematode (GIN) infections are one of the major health issues facing grazing sheep populations. The resistance/susceptibility trait appears to be a highly complex trait.
- ☐ Several QTL mapping studies have tried to identify genomic regions and mutations^{1,2} but the detection of causal mutations for this trait is still a challenge for the research community.
- ☐ The RNA-Seq technology provides the opportunity to perform gene quantification, differential gene expression and detection of variants with high-throughput transcriptome data from a specific tissue.
- ☐ In a previous study, we identified a list of 106 differential expression genes (DEGs) based on RNA-Seq dataset obtained from the abomasal lymph nodes of 12 adult sheep³.

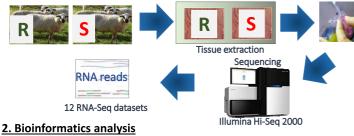
Objective: In the present study, we have performed a variant calling analysis on the same RNA-Seq dataset with a focus on the list of the reported 106 DEGs.

We present a list of functionally relevant variants that could underlie the genetic control of resistance/susceptibility to *T. circumcincta* in adult sheep.

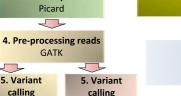
Materials and methods

1. Experimental infection, RNA extraction and RNA-Seq

- 12 adult Churra ewes previously classified as 6 resistant (R) and 6 susceptible (S) to *T. circumcincta* were subjected to an experimental infection (EI). After seven days after, animals were humanitarian sacrificed.
- RNA samples were extracted from abomasal lymph node tissue, sequenced through an Illumina RNA-Seq protocol.



11. Prediction of consequences, genes and deleterious variants VEP Web tool 2. Alignment STAR 10. Common "HIGH" and "Moderate" impact variants SnpSift



5. Variant calling GATK calling SAMTOOLS
6. Variant filtering filtering

GATK

VEP and SnpEff

VEP and SnpEff

National Support of the support of

9. Variant annotation

7. High quality variants of abomasal lymph node transcriptome

Results and Discussion 1. Pre-alignment QC All 12 samples were of high quality 2. Alignment 80.27% reads were uniquely mapped 8.5% reads were mapped to multiple loci 3. Mark duplicates 1,326,960 High quality variants 7. High quality common variants 6.168 Variants 8. Variants in 106 DEG regions (6,104 SNPs, 30 insertions, 34 deletions) 328 Moderate & 4 High 10. Common "HIGH" and "Moderate" Impact Variants (Table 1) impact variants (329 SNPs, 3 insertions, 2 deletions) 11. Prediction of consequences, genes 109 Novel Variants -> and deleterious variants 471 functional consequences in 60 genes

- We found 50 deleterious missense consequences, 25 of them located in 15 genes with known symbol ID.
- Some of these genes harboring missense were related to the immune response according the literature review.

Immune response

BPIFB1⁴, KRT20⁵, SLC38A2⁶, FNDC1⁷.

Cell proliferation in response to skin injury

Table 1: "High" Impact variants identified by both VEP and SnpEff.

<u>S. No</u>	<u>Gene</u>	<u>Location</u>	<u>Consequence</u>
1	LGALS4	OAR14:47718975-47718975	splice acceptor variant
2	SLC38A2	OAR3:139947003-139947003	stop lost
3	ASIC3	OAR4:113023157-113023157	stop gained and splice acceptor variant
4	SULF1	OAR9:46254552-46254553	frameshift variant

Conclusion

- This study has identified genetic variants in genes previously identified as differentially expressed in relation to GIN resistance/susceptibility in adult sheep.
- The variants predicted to have a potential functional impact should be assessed through future studies as potential relevant variation underlying the genetic architecture of sheep GIN resistance in adult sheep.

References

- 1. Coltman et al. 2001. Parasitology, 122: 571–82.
- 2. Atlija et al. 2016. Genet Sel Evol, 48: 4.
- 3. Chitneedi et al. 2018. Vet Res 49.1: 39
- 4. Zhou et al. 2017. Open Med (Wars) 12: 299–307.
- 5. Sen et al. 2012. Proc Natl Acad Sci U S A, 109: 20667–72.
- 6. Carter 2012. Physiol Rev, 92: 1543–76.
- 7. Sigdel et al. 2015. J Immunol Res, 2015: 848790.
- 8. Saarialho-Kere et al. 2002 J Invest Dermatol, 119:14-21.



Acknowledgements



Financial support for this project was received from the LE248U14 project of Junta de Castilla and León Government. P. K. Chitneedi is funded by a predoctoral fellowship from the Junta de Castilla and León Government and the European Social Fund. B Gutiérrez-Gil is funded by the "Ramón y Cajal" Programme (RYC-2012-10230) from the Spanish Ministry of Economy, Industry and Competitiveness (MINECO). M. Martínez-Valladares is also funded by the "Ramón y Cajal" Programme (RYC-2015-18368) from MINECO.