Genomic variants from RNA-seq for goats resistant or susceptible to gastrointestinal nematode infection

CURRENT STATUS: POSTED

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DOI: 10.21203/rs.2.16692/v1

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
Genetic resistance, Genomic variants, Goats, Haemonchus contortus
Abstract
Background Gastrointestinal nematodes (GIN) are an important constraint in grazing small ruminant production. Genetic selection for resistant animals is a potential sustainable control strategy. Advances in molecular genetics have led to the identification of several molecular genetic markers associated with genes affecting economic relevant traits.

Results In this study, the variants in the genome of Creole goats resistant or susceptible to GIN were discovered from RNA-sequencing. We identified SNPs, insertions and deletions that distinguish the two genotypes, resistant and susceptible and we characterized these variants through functional analysis. The T cell receptor signalling pathway was one of the top significant pathways that distinguish the resistant from the susceptible genotype with 78% of the genes involved in this pathway showing genomic variants.

Conclusions These genomic variants are expected to provide useful resources especially for molecular breeding for GIN resistance in goats.

Background
Gastrointestinal nematodes (GIN) are one of the most pathogenic parasites in sheep and goats that cause large economic losses. The wide geographic distribution and increasing resistance against anthelmintic molecules require alternative control strategies [1]. Selection for resistant animals using genetic information is a promising strategy. However, selection based on phenotypic traits such as faecal egg count (FEC) has been successfully used [2–4], although this strategy implements a certain degree of uncertainty since FEC is an indirect measure of resistance. The measurements of FEC is also time consuming and costly as it requires animals to be challenged with parasites either naturally or experimentally. On the other hand, selection using information from the genome could provide a faster and more sustainable tool in breeding for GIN resistance. The identification of genomic variation is a main step for understanding the relationship between genotype and phenotype. Single nucleotide polymorphisms (SNPs) showed potential as molecular markers to link genotypes with desired traits in goats, such as milk quality and quantity [5–7], litter size [8,9], growth rate [8,10], fiber quality [11–13] and disease resistance [14].
Recently, Piskol et al. [15] showed that genomic variants detected from RNA sequencing (RNA-seq) data offers a cost-effective and reliable alternative for SNP discovery. They showed that among the SNPs called from the RNA-seq data, more than 98% were also identified by whole-genome sequencing or whole-exome sequencing approaches. RNA-seq technology was developed primarily for mapping and quantifying transcriptomes to analyze global gene expression in different tissues. Besides allowing the detection of differentially expressed genes, since RNA-seq functional genes are sequenced at high coverage, the search for full scale variants (SNPs, insertions and deletions) in coding genes can be performed. Up to date, this technique has been used as a method to detect SNPs in transcribed regions in an efficient and cost-effective way [13,16-19]. In this study, we performed a genomic variant discovery analysis in the abomasal mucosa transcriptomes of resistant and susceptible Creole goats experimentally infected with *Haemonchus contortus*, choosen for its prevalence in sheep and goats throughout the temperate and tropical region of the world, and characterized the variants identified.

Results
Sequencing and SNP discoveries
The RNA-seq produced an average of 15.3 million raw reads per sample. Our read alignment results showed that 99.3% sequencing reads (15.2 million) were successfully aligned to the ARS1 goat’s reference genome with an average 79% paired-end sequencing.

Using RNA-seq reads a total of ~2.33 and ~1.82 million raw genomic variant positions expressed in the abomasal mucosa of resistant and susceptible animals were detected at different time points of infection (Table 1). After variant filtering analysis, we were able to identify 354,598 and 253,218 SNP, 20,463 and 15,645 insertion and 20,397 and 14,841 deletion records for resistance and susceptible kids, respectively. These variants were then used to produce Venn diagrams (Fig. 1) to present common and non-common variants between resistant and susceptible animals. Comparing genomic variants of the two genotypes at different time points of infection, 200,053 SNPs, 10,095 deletions and 8,755 insertions were in common (Fig. 1). To explore the different genomic variants in resistant and susceptible animals, we excluded the common variants and made the subsequent analysis with
non-common variants.

Table 1. Number of SNPs and indels called from transcriptome data.

| Genotype | Days after infection | Raw SNPs | Raw Insertion | Raw Deletion | Filtered SNPs | Filtered Insertion | Filtered Deletion |
|----------|----------------------|----------|---------------|--------------|---------------|-------------------|------------------|
| Resistant | 0                    | 712902   | 37798         | 43176        | 300268        | 14956             | 1684             |
|          | 8                    | 747086   | 38536         | 43672        | 365088        | 17179             | 2017             |
|          | 15                   | 818301   | 42407         | 48589        | 367130        | 17746             | 2026             |
|          | 35                   | 973405   | 49260         | 56391        | 416626        | 19667             | 2249             |
|          | All times            | 2107999  | 104250        | 124743       | 354598        | 20463             | 2039             |
| Susceptible | 0                    | 479135   | 26102         | 29129        | 186577        | 9572              | 1068             |
|          | 8                    | 680515   | 35922         | 40202        | 324887        | 15288             | 1795             |
|          | 15                   | 753841   | 40504         | 44964        | 327288        | 16336             | 1854             |
|          | 35                   | 575608   | 30821         | 33198        | 245840        | 12025             | 1383             |
|          | All times            | 1635811  | 84872         | 99606        | 253218        | 15645             | 1484             |

*Fig. 1. Venn diagram for SNP (a) deletion (b) and insertion (c) variances identified in samples from the resistant and susceptible group.*

Non-common SNPs were annotated with the respective genes. A total of 12,142 and 8,635 genes containing SNPs were identified in the resistant and susceptible animals, respectively. In the samples from the susceptible animals an average of 5.19 SNPs were identified per gene. Meanwhile, the double number of SNPs (10.53 SNPs per gene) were identified in the data from the resistant animals. Among genes containing SNPs, genes with only 1 SNP were more common (1759 for resistant and 2629 for susceptible). Genes with 10 SNPs or less accounted for 69.1% and 88.9% of all SNPs identified in the samples from the resistant and susceptible animals, respectively. Results for SNPs distributions among genes showed that more genes containing one or two SNPs were identified in the susceptible animals, while more genes containing 3 or more SNPs were identified in the resistant animals (Fig. 2).

*Fig. 2. Single nucleotide polymorphism (SNP) distribution among genes.*

Following the same procedure, non-common insertions and deletions were annotated to genes. A total of 4848 and 3292 genes containing insertions and 4660 and 2610 genes containing deletions were identified in data from the resistant and susceptible animals, respectively. Among these genes, genes
with one insertion or deletion were more common and accounted for more than 50% of all insertions or deletions identified. Insertion and deletion distributions among genes are shown in Fig. 3. Data from the resistant animals always contained more insertions and deletions among genes.

*Fig. 3. Insertions and deletions (indels) identified among genes using the data from resistant (R) and susceptible (S) kids.*

All variants (SNPs, insertions and deletions) were combined in two files, one for variants identified in data from resistant animals and one for variants identified in data from susceptible animals. A variant effect prediction analysis was made for both files and the results are shown in Fig. 4. The highest variants ratio was around 50% for intron variants from variants in the resistant (56%) and susceptible (47%) animals, followed by downstream and upstream gene variants (20% and 8% in resistant and 25% and 8% in susceptible animals). A total of 3% of missense variants were predicted in the resistant and 4% in the susceptible animals (Fig. 4).

*Fig. 4. Variant effect prediction in resistant and susceptible animals.*

After the GO enriched analysis, 10,736 and 8,538 genes containing genomic variants in the resistant and susceptible animals were assigned with one or more GO terms. The top 10 significant GO terms under the categories of biological processes, molecular functions, and cellular components of these annotated genes are shown in Fig. 5. Six to seven of the top 10 terms under each GO category were similar whatever the genotype. For the cellular component, the major category that was identified in the data from the resistant but not the susceptible animals was the mitochondrial matrix. For the molecular function, phosphoric ester hydrolase activity was the most represented term for the variants identified in data from the resistant animals which were not present in data from the susceptible ones. The most significant GO terms in the category of biological process, were phospholipid metabolic process and macroautophagy which were identified only in the data from the resistant animals.

*Fig 5. Gene ontology for genes containing variance for data from the resistant and susceptible genotypes.*

The top 10 significant KEGG pathways for genes containing genomic variants identified in data from
both genotypes are presented in Fig. 6. Mitogen-activated protein kinase (MAPK) signaling pathway, T cell receptor signaling pathway, hepatitis B and longevity regulating pathway were the top significant pathways identified only in data from the resistant animals. Activation of T lymphocytes is a key event of the host adaptative immune response. Therefore, we focused on T cell receptor signaling pathway that was identified in the top KEGG pathways for the resistant animals. The T cell receptor signaling pathway and the number of genomic variants for each gene in this pathway in the data from the resistant animals are presented in Fig. 7. A total of 100 genes are known to control this pathway in *Capra hircus* breeds. At least one genomic variants was identified in 78 genes out of these 100 genes (Fig. 7).

*Fig 6. KEGG pathways for genes containing genomic variants in the data from the resistant and susceptible animals.*

*Fig 7. Number of genomic variants for each gene in the T cell receptor signalling pathway in the resistant animals.*

**Discussion**

In this study, we used data from RNA-seq data of Creole kid goats abomasal mucosa samples at four time point after infection with *H. contortus* of two genotype, resistant and susceptible, to identify putative gene-related genomic variants. To our knowledge, this is the first study to identify genomic variants from RNA-seq data in goats infected with a GIN. Other studies identified SNPs in goats using RNA-seq analysis for climate adaptation traits [17] and fiber quality [13].

One benefit to variant calling from RNA-seq is the focus on genes/transcripts that are expressed in the target tissue. However, there are some concerns about the information provided by such data. It was previously shown that some false positive SNPs identified in cDNA arise from alignment of a read to the wrong gene which represents a problem in gene families with highly conserved domains when using short sequence reads [16,22]. This situation has also been observed in regions associated with sequence repeats [23]. Therefore, one great challenge of using Illumina sequencing for transcriptome analysis is the short read length. We have used the Illumina TruSeq RNA sample prep kit that generated read lengths of 2 X 75 bases pair with paired-end reads to increase the base coverage.
within expressed genes in a sample and as a result improved variant detection sensitivity.

Studies for GIN infection in cattle indicated that GO terms associated with genes that were differently expressed between resistant and susceptible cattle were predominantly related to lipid metabolism and the top function of regulatory networks identified was associated with lipid metabolism [24]. Our results showed that the most significant GO term associated with genes containing variants in resistant animals was phospholipid metabolic process. T cells are a subset of lymphocytes that have a central role in adaptive immune response. T cell receptor is a complex of integral membrane proteins on the surface of T cells, which takes part in the activation of T cells in response to antigen recognition and eventually results in cellular proliferation, differentiation, cytokine production, and/or activation-induced cell death [25]. In the present study, we found that genes containing genomic variants that distinguish resistant goats were associated with T cell receptor signalling pathway in KEGG pathway enriched analysis. Mitogen-activated protein kinase (MAPK) is a highly conserved module that is involved in various cellular functions, including cell proliferation, differentiation and migration. Besides, the MAPK has been shown to play a key role in transduction extracellular signals to cellular responses [26]. The MAPK signalling pathway was in the top significant pathways identified from genes containing genomic variants of the resistant genotype. This pathway was previously reported to be regulated by differently expressed genes of resistant and susceptible Yichang White goats and indicated to play significant role in the resistance of this goat breed to GIN infection [27].

One major problem for goats and sheep genomic analysis was that the functional analysis was not available with goats or sheep as reference species due to the lack of data for C. hircus and Ovis aries in functional analysis programs. Therefore, almost all previous publications in goats and sheep genomic filled used human [27-29] or bovine [30] genome as reference for functional analysis. Nowadays, the availability of goat genome reference within Ensemble and KEGG pathways made the variants effect prediction and pathways analysis possible and more specific for goats. Our study considers, as one of the first studies, to use the goat genome as reference in the functional analysis.

Conclusion
The present study showed the possibility to use RNA-seq data as an efficient and cost-effective
method to detect genomic variants in transcribed genomic regions. It implements an additional use of such high throughput data and is a great resource to gain further knowledge of animal resources. Genomic variants in genes involved in T cell receptor signaling pathway plays a role in GIN resistance in goats. This work provides valuable resources for genomic differences between resistant and susceptible goats to GIN infection and serves as a basis towards developing genomic markers for GIN resistance in goats.

Methods

Ethics statement
All animal care handling techniques and procedures as well as the procedures for experimental infection, tissue sampling and slaughtering were approved by the French Ethic Committee n°069 (Comité d’Ethique en Matière d’Expérimentation Animale des Antilles et de la Guyane, CEMEAAG) authorized by the French Ministry of Higher Education, Research and Innovation. The experiment was performed at the INRA Experimental Facilities PTEA (Plateforme Tropicale d’Expérimentation sur l’Animal) according to the certificate number A 971-18-02 of authorization to experiment on living animals issued by the French Ministry of Agriculture.

Animals and experimental design
The breeding value (BV) of each goat of the experimental flock of PTEA was estimated since 1995 by using FEC at 11 months of age (when available) following natural mixed infection on pasture and taking into account the FEC of its ascendants and pedigree. The Eight 9-month old Creole kids were chosen on the basis of their extreme estimated BV in their cohort. Before the experiment the kids were reared at pasture with a limited level of GIN contamination (FEC < 500). The FEC of the 8 kids (n = 4 resistant and n = 4 susceptible), were not statistically different. The averages BV of the 2 groups of animals were distant by 1.04 genetic standard deviation. The animals were drenched with moxidectine (Cydectine®, Fort Dodge Veterinaria S. A., Tours, France, 300 µg/kg) and housed indoors under worm-free conditions in a single pen, one month before the start of the experiment. Kids were orally infected with a single dose of 10,000 H. contortus third-stage larvae (L3) in two consecutive challenges. Each challenge lasted for 5 weeks with 8 weeks interval between the end of challenge 1 and the start of challenge 2. Four weeks before the second experimental infection a fistula was
surgically implanted in the abomasum of each animal to allow abomasal mucosa sampling at 0, 8, 15 and 35 days post infection (dpi).

**Surgical procedure**
The custom designed abomasal cannula consisted of a flexible plastic tube with a length of 7 cm and a diameter of 2 cm with a rounded base of 4 cm in diameter. This flexible plastic was chosen to limit the possibility of mechanical abrasion of the mucosal surface of the abomasum. The animals were fasted 16 h before cannula insertion surgery. The animals were premedicated with ketamine (2mg/kg IV, Le Vet Pharma, Wilgenweg, Netherlands), xylazine (0.2mg mg/kg IM, Le Vet Pharma, Wilgenweg, Netherlands) and oxytetracycline (20 mg/kg IM, Eurovet Animal Health, Handelsweg, Netherlands). The animals were positioned in left lateral recumbency. Skin over the surgical site was shaved and prepped with povidone iodine (Vétédine, Laboratoire Vetoquinol S. A., Lure, France). A ventral midline incision was made to locate and exteriorize the abomasum. A 3 cm purse-string suture (Silk 2–0) was placed midway between the lesser and greater curvature and a stab incision was made in the center to insert the cannula. Then, the purse-string suture was tightened and tied off. To maintain the abomasum in an anatomically correct position, another stab incision was made in the abdominal wall at 10cm from the laparotomy incision on the right paramedian area to enable the cannula to be passed freely through. An external flange was placed over the external part of cannula and fixed with adhesive fabric plaster strip. A sterile compress was inserted into the cannula as stopper. After the surgical procedure, all the animals were housed individually with free access to fresh water and hay.

**Biopsy sampling procedure**
Biopsy specimens were taken from the abomasal mucosa using a flexible endoscope (FG–24V, Pentax, France). The biopsies samples of 2×2×2mm taken with the endoscopic forceps with window (model KW1815S) were quickly snap frozen into liquid nitrogen and stored at −80°C until RNA extraction. The animals were restrained in a harness made with a surgical drape allowing animal legs to protrude and which exposed the cannula. No sedation was used since no signs of discomfort or pain were observed during or after the procedure. The sterile compress inserted into the cannula was removed and the abomasal contents collected. The endoscope was introduced into the abomasal lumen and 3 biopsies
per animal and per time points were taken from the abomasal folds of the fundic mucosa. At each time point the whole fundic mucosa was observed and no sign of mucosal injury due to the previous sampling was observed.

RNA extraction and sequencing
Total RNA was extracted using the NucleoSpin® RNA isolation kit (Macherey-Nagel, Hoerdt, France) following the manufacturer’s instructions, except that DNase digestion was performed with twice the indicated amount of enzyme. The total RNA concentration was measured with NanoDrop 2000 (ThermoScientific TM, France). The RNA integrity was verified using an Agilent Bioanalyzer 2100 (Agilent Technologies, France) with a RNA Integrity Number of > 7.5. The extracted total RNA was stored at -80°C until sequencing.

High-quality RNA from all samples was processed for the preparation of cDNA libraries using an Illumina TruSeq RNA sample prep kit for mRNA analysis following the Illumina’s protocols. After quality control and quantification, cDNA libraries were pooled in groups of 6 and sequenced on 5 lanes on the HiSeqTM 2000 (Illumina® NEB, USA) to obtain approximatively 30 million reads (100 bp paired-end) for each sample with insert sizes ranging from 200 to 400 base pairs.

Bioinformatics analysis
The quality control check on raw reads in FASTQ format was processed using FASTQC. The remaining reads were aligned to the Capra hircus genome (assembly ARS1 from NCBI) using the Burrows-Wheeler Aligner (BWA). Genomic variants including SNPs as well as small insertions and deletions (indels) were detected using mpileup in SAMtools. Variant filtering criterion: A detected variant was kept only if met four criteria: the read depth was more than 10, the quality score was over 20, the minor allele frequency was over 0.05 and the variants present at least in 50% of the group individuals and replicates.

Variant statistics and functional annotation
SNPs, insertions and deletions were compared between samples from the resistant and susceptible groups. Common variants were excluded and only different variants between the two groups were kept for the subsequent analysis. Variant information for distribution among genes was calculated within the R program after merging variants with the respective annotated genes. The effect of the
variants (SNPs, insertions and deletions) on genes were determined using variant effect predictor (VEP) web interface tool provided by Ensemble online tools (https://www.ensembl.org/info/docs/tools/vep/index.html) within the goat genome reference (Assembly: ARS1) and results were extracted as.txt file for graphical interface using the R program. Genes containing variants were annotated with Gene Ontology (GO) terms under the categories of biological processes, molecular functions, and cellular components using clusterProfiler R package [20]. The Bonferroni-corrected P-value $\leq 0.05$ was used as the threshold. Additionally enriched KEGG pathways for genes containing variants were identified using the same package. The Pathview package was used for visualization [21]. The R version 3.5.1 was used.

Declarations

Availability of data and materials

All data generated during this study are available in the NCBI SRA repository. All other relevant data are included in this published article.

Competing interests

The authors declare no competing interests.

Funding

This study was funded by the Project MALIN (La Région Guadeloupe and Fonds Européens FEDER). H. M.A was supported by a doctoral fellowship from the project European Graduate School in Animal Breeding and Genetics.

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Acknowledgements

The authors want to give thanks to the Duclos team for care and handling of the animals: F. Pommier, F. Nimirf, F. Periacarpin, C. Deloumeaux and M. Jean-Bart. A special thanks to Dr. Harry Archimede for his valuable contribution for the surgical procedure.

References

1. Jabbar A, Iqbal Z, Kerboeuf D, Muhammad G, Khan MN, Afaq M. Anthelmintic resistance: The state of play revisited. Life Sci. 2006;79(26):2413–31.

2. Morris C, Wheeler M, Watson T, Hosking B, Leathwick D. Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. New Zeal J Agric Res. 2005;48(1):1–10.

3. Karlsson LJE, Greeff JC. Selection response in fecal worm egg counts in the Rylington Merino parasite resistant flock. Aust J Exp Agric. 2006;46:809–11.

4. Kemper KE, Palmer DG, Liu SM, Greeff JC, Bishop SC, Karlsson LJE. Reduction of faecal worm egg count, worm numbers and worm fecundity in sheep selected for worm resistance following artificial infection with Teladorsagia circumcincta and Trichostrongylus colubriformis. Vet Parasitol. 2010;171(3-4):238–46.

5. Li Z, Chen Z, Lan X, Ma L, Qu Y, Liu Y, et al. Two novel cSNPs of weaver gene in Chinese indigenous goat and their associations with milk yield. Mol Biol Rep. 2010;37:563–9.

6. Hou JX, An XP, Song YX, Wang JG, Ma T, Han P, et al. Combined effects of four SNPs within goat PRLR gene on milk production traits. Gene. 2013;529:276–81.

7. An X, Song Y, Hou J, Wang S, Gao K, Cao B. Identification of a functional SNP in the 3’-UTR of caprine MTHFR gene that is associated with milk protein levels. Anim Genet. 2016;47:499–503.

8. Xiong Q, Chai J, Li X, Suo X, Zhang N, Tao H, et al. Two tagSNPs in the prolactin
receptor gene are associated with growth and litter traits in Boer and Macheng Black crossbred goats. Livest Sci. 2016;193(October):71–7.

9. Zhang R, Lai F, Wang J, Zhai H, Zhao Y, Sun Y. Analysis of the SNP loci around transcription start sites related to goat fecundity trait base on whole genome resequencing. Gene. 2018;643(December 2017):1–6.

10. Ma L, Qin Q, Yang Q, Zhang M, Zhao H, Pan C, et al. Associations of six SNPs of POU1F1-PROP1-PITX1-SIX3 pathway genes with growth traits in two chinese indigenous goat breeds. Ann Anim Sci. 2017;17(2):399–411.

11. Zhou J., Zhu X., Zhang W, Qin F, Zhang S., Jia Z. A novel single-nucleotide polymorphism in the 5’ upstream region of the prolactin receptor gene is associated with fiber traits in Liaoning cashmere goats. Genet Mol Res. 2011;10(4):2511–6.

12. Wang X, Zhao ZD, Xu HR, Qu L, Zhao HB, Li T, et al. Variation and expression of KAP9.2 gene affecting cashmere trait in goats. Mol Biol Rep. 2012;39(12):10525–9.

13. Wang L, Zhang Y, Zhao M, Wang R, Su R, Li J. SNP discovery from transcriptome of Cashmere goat skin. Asian-Australasian J Anim Sci. 2015;28(9):1235–43.

14. Minozzi G, Mattiello S, Grosso L, Crepaldi P, Chessa S, Pagnacco G. First insights in the genetics of caseous lymphadenitis in goats. Ital J Anim Sci. 2017;16:31–8.

15. Piskol R, Ramaswami G, Li JB. Reliable Identification of Genomic Variants from RNA-Seq Data. Am J Hum Genet. 2013;93:641–51.

16. Cánovas A, Rincon G, Islas-Trejo A, Wickramasinghe S, Medrano JF. SNP discovery in the bovine milk transcriptome using RNA-Seq technology. Mamm Genome. 2010;21:592–8.

17. Sharma U, Banerjee P, Joshi J, Kumar vijh R. Identification of SNPs in Goats ( Capra hircus) using RNA-Seq Analysis. Int J Anim Vet Adv. 2012;4(4):272–83.

18. Martínez-Montes AM, Fernández A, Pérez-Montarelo D, Alves E, Benítez RM, Nuñez Y,
et al. Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing. Anim Genet. 2017;48(2):151-65.

19. Pareek CS, Błaszczyk P, Dziuba P, Czarnik U, Fraser L, Sobiech P, et al. Single nucleotide polymorphism discovery in bovine liver using RNA-seq technology. PLoS One. 2017;12(2):1-26.

20. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. Omi A J Integr Biol. 2012;16(5):284-7.

21. Luo W, Brouwer C. Pathview: An R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics. 2013;29(14):1830-1.

22. Cirulli ET, Singh A, Shianna K V., Ge D, Smith JP, Maia JM, et al. Screening the human exome: A comparison of whole genome and whole transcriptome sequencing. Genome Biol. 2010;11(5):1-8.

23. Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. Genomics. 2008;92(5):255-64.

24. Li RW, Rinaldi M, Capuco A V. Characterization of the abomasal transcriptome for mechanisms of resistance to gastrointestinal nematodes in cattle. Vet Res. 2011;42(1):114.

25. Huse M. The T-cell-receptor signalling network. J Cell Sci. 2009;122:1269-73.

26. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res. 2002;12(1):9-18.

27. Bhuiyan AA, Li J, Wu Z, Ni P, Adetula AA, Wang H, et al. Exploring the Genetic Resistance to Gastrointestinal Nematodes Infection in Goat Using RNA-Sequence. Int J Mol Sci. 2017;18:751-67.

28. McRae KM, Good B, Hanrahan JP, McCabe MS, Cormican P, Sweeney T, et al. Transcriptional profiling of the ovine abomasal lymph node reveals a role for timing
of the immune response in gastrointestinal nematode resistance. Vet Parasitol. 2016;224:96–108.

29. Chitneedi PK, Suárez-Vega A, Martínez-Valladares M, Arranz JJ, Gutiérrez-Gil B. Exploring the mechanisms of resistance to Teladorsagia circumcincta infection in sheep through transcriptome analysis of abomasal mucosa and abomasal lymph nodes. Vet Res. 2018;49(1):1–11.

30. Ahmed AM, Good B, Hanrahan JP, McGettigan P, Browne J, Keane OM, et al. Variation in the Ovine abomasal lymph node transcriptome between breeds known to differ in resistance to the gastrointestinal nematode. PLoS One. 2015;10(5):1–17.

Figures

Figure 1
Venn diagram for SNP (a) deletion (b) and insertion (c) variances identified in samples from the resistant and susceptible group
Figure 2

Single nucleotide polymorphism (SNP) distribution among genes
Insertions and deletions (indels) identified among genes using the data from resistant (R) and susceptible (S) kids.

Variant effect prediction in resistant and susceptible animals.
Figure 5

Gene ontology for genes containing variance for data from the resistant and susceptible genotypes
KEGG pathways for genes containing genomic variants in the data from the resistant and susceptible animals

Figure 6
Figure 7

Number of genomic variants for each gene in the T cell receptor signalling pathway in the resistant animals