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**Original**

# Genetic variation in two candidate genes against gastrointestinal parasites in Colombian Hair Sheep

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## ABSTRACT

**Objective.** To characterize two SNP-type genetic polymorphisms in the GLI1 (rs411868094) and IL20RA (rs419463995) genes, candidates for resistance against gastrointestinal parasites in two biotypes of Colombian hair sheep. **Materials and methods.** From the DNA bank of the Animal Genetics Laboratory of the University of Sucre, 167 samples, belonging to the Ethiopian (n=94) and Sudan (n=73) biotypes, were analyzed by PCR and subsequent bidirectional sequencing of two SNPs in the GLI1 (T>G) and IL20RA (G>A) genes. Allelic and genotypic frequencies, observed (Ho) and expected (He) heterozygosity, F index, and Hardy-Weinberg equilibrium deviations (EHW) were calculated using the GENALEX version 6.5 program. **Results.** For the GLI1 *locus*, the mean genotypic frequencies were 0.155±0.07, 0.370±0.07, and 0.475±0.07 for GG, GT, and TT, respectively. In the Ethiopian biotype, the highest frequencies of the genotype of interest (GG) were found. For the IL20RA *locus*, the AA and AG genotypes had similar and the highest frequency (0.465±0.03) compared to the GG genotype (0.110±0.01). The genotype of interest at this *locus* (AA) was the most frequent in both OPC biotypes. **Conclusions.** The alleles of interest associated with low FEC had a low frequency for the GLI1 gene, but a high frequency for IL20RA. The Ethiopian OPC biotype showed the highest frequencies of the genotypes of interest.

**Keywords:** Genetic diversity; genetic resistance; animal genetic resources (*Source: CAB*).

## RESUMEN

**Objetivo.** Caracterizar dos polimorfismos genéticos tipo SNP en los genes GLI1 (rs411868094) y IL20RA (rs419463995) candidatos a la resistencia contra parásitos gastrointestinales en dos biotipos de ovinos de pelo colombiano. **Materiales y métodos.** Del banco de ADN del laboratorio de Genética Animal de la Universidad de Sucre, se analizaron 167 muestras de ovino de pelo colombiano (OPC), pertenecientes a los biotipos Etíope (n=94) y Sudán (n=73), mediante PCR y secuenciamiento bidireccional dos SNPs en los genes GLI1 (T>G) y IL20RA (G>A). Se calcularon las frecuencias alélicas y genotípicas, la heterocigocidad observada (Ho) y esperada (He), el índice

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F y las desviaciones del equilibrio de Hardy-Weinberg (EHW) con el programa GENALEX versión 6.5. **Resultados.** Para el gen GLI1 para todo el OPC las frecuencias genotípicas promedio fueron 0.155±0.07, 0.370±0.07 y 0.475±0.07 para GG, GT y TT, respectivamente. En el biotipo Etíope, se encontraron las frecuencias más altas del genotipo GG. Para el gen IL20RA en todo el OPC, los genotipos AA y AG tuvieron similar frecuencia (0.465±0.03) y el genotipo GG mostró la frecuencia más baja (0.110±0.01). **Conclusiones.** Las variantes genéticas analizadas fueron polimórficas. Según los reportes de literatura, los alelos de interés por su mejor desempeño contra los parásitos gastrointestinales, tuvieron baja frecuencia en el gen GLI1, pero alta frecuencia en el IL20RA.

**Palabras clave:** Diversidad genética; resistencia genética; recursos genéticos animales (*Fuente: CAB*).

## INTRODUCTIÓN

Sheep (Ovis aries) are characterised as one of the domestic animals with the widest geographical distribution, due to their extraordinary capacity to adapt to different vegetation, climate and management conditions (1,2). In our country, most sheep production is carried out by small-scale producers, who play an important role in the economy, food security and food sovereignty in rural areas. These production systems are mainly based on the Colombian hair sheep breed (OPC) and have low incorporation of technology, with subsequent problems in facilities and precarious reproductive, nutritional, genetic and sanitary management, which are reflected in low production rates and poor business vision (3,4).

Regarding sanitary management, the assessment of livestock health conditions in developing countries for the identification of priority diseases to be controlled revealed that gastrointestinal parasitic infections (GPI) were one of the most important problems in sheep and goats (5,6). Gastrointestinal parasitic infestations with Haemonchus contortus, Teledorsagia circumcincta, Trichostrongyles sp, Nematodirus sp. impose severe restrictions on the production of small ruminants, especially those reared by marginal producers in a low external input system (7) as are most of the sheep production systems in our country.

PGI cause large economic losses to producers, in terms of body weight loss, low wool and milk production, direct cost of anthelmintic drugs, male and female infertility, anaemia, submandibular oedema, respiratory problems and losses due to mortality, among others (8). For example, the annual cost of treatment for Haemonchus contortus was estimated at USD 26 million in Kenya, USD 46 million in South Africa, USD 103 million in India and USD 436 million in Australia (9,10). In Italy, losses from Cysticercus tenuicollis were estimated at 330000 euros (11). These losses in Colombia have not been clearly determined.

The correct strategy for PGI control must be based on knowledge of the parasite species found in the sheep flock and their prevalence, the hosts and breeds, the local climate, the flock size and the management practices used (8,12). However, it is not always easy to take the above into account, so the use of anthelmintic drugs for the control of PGI is common. The indiscriminate use of these has further complicated the treatment of PGI. With negative consequences related to the emergence of resistant parasite strains, the presence of drug residues in animal products and increases in treatment costs (8,12).

As an alternative to the use of drugs, the selection of genetically resistant animals has been suggested for PGI control (6,13,14). This selective breeding process for PGI resistance uses faecal egg count (FEC), antibody assays, packed cell volume and anaemia score by the FAMACHA® method as indicators of resistance (7,15). However, these classical selection methods for this phenotype are slow and costly, and the accuracy of selection depends on many factors that can be difficult to control, such as variations in natural helminth infection and seasonal environmental load, among others (7,12).

These difficulties suggest that the process of selecting PGI-resistant animals would be more efficient if based on indirect estimates, such as those generated from molecular marker information. In addition to this, the estimated heritability for the trait FEC ranged from 0.18 to 0.46 in lambs (16) and from 0.17 to 0.31 in postpartum ewes(17). These mean heritability values suggest that PGI resistance can be improved through genetic selection.

The first approach in terms of genetic selection to understand the genotype-phenotype relationship is quantitative trait loci (QTL). Among the QTL related to PGI resistance, 45 have been reported on resistance to Haemonchus spp., 22 on Trichostrongyles spp., 11 on Nematodirus spp. and 6 on Strongyles spp. However, identification of candidate genes has been elusive. The lack of consensus overlap between reported QTL has made it difficult to identify candidate genes and genetic markers for selection in sheep (18).

Because of this, genome-wide association studies in several sheep breeds have associated certain polymorphisms in the GLI1 and IL20RA genes as candidates for resistance against PGI (13-15,19). These polymorphisms have not been characterised in the OPC breed. Therefore, the aim of this research was to characterise two SNP-like genetic polymorphisms in the GLI1 (rs411868094) and IL20RA (rs419463995) genes as candidates for resistance against gastrointestinal parasites in two biotypes of Colombian hair sheep.

## MATERIALS AND METHODS

**DNA samples.** Samples from the DNA bank of the Animal Genetics Laboratory of the University of Sucre, which is composed of 167 samples, belonging to the biotypes Ethiopian (n=94) and Sudan (n=73), were used in this research.

**PCR amplification of the fragments of interest.** In the GLI1 gene (GLI family zinc finger 1), the rs411868094 (T>G) polymorphism located at position 3:173101518, amplified with the primers 5-AGAACCCTGGGCAGATTACC-3 and 5-AGTCAGCGCCCAGCAGATTGAA-3, was studied in a fragment of 230 base pairs. In the IL20RA gene (Interleukin 20 receptor alpha), the rs419463995 (G>A) polymorphism located at position 8:66786499 was studied, using the primers 5-TACAGCCCCCCCAAGAAGCAAGCAGT-3 and 5-AGGGTTTTTACAAAGACGGGGGGG-3, in a fragment of 250 base pairs (13).

PCR reactions were carried out in a final volume of 50l containing 10 ng DNA, 250 nM of each primer and 1X MangoMixTM super mix (Bioline©-USA). The amplification profile of the two fragments included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, hybridisation at 60°C for 30 seconds, extension at 72°C for 30 seconds, a final extension at 72°C for 5 minutes. The reactions were performed on an Eppendorf® Mastercycler® Nexus Thermal Cyclers.

The amplified products were visualised by electrophoresis on 1.5% agarose gels, stained with GelRedTM and a 100 base pair molecular weight marker. Products where amplification was evident were quantified using a NanoDrop 2000TM spectrophotometer (Thermo Fisher Scientific). Products with at least 100ng/l were sent for bidirectional sequencing to Macrogen.

**Data analysis.** Electropherograms were edited and aligned using Geneious Prime© software (Version 2019.1). According to the relative positions of the SNPs (GLI1 rs411868094 T>G and IL20RA rs419463995 G>A) the animals were genotyped. From a genotyping matrix, allele and genotypic frequencies, observed (Ho) and expected (He) heterozygosity, deviations from Hardy-Weinberg equilibrium (EHW) and Wright's F-statistics, FST, FIS and FIT were calculated from a molecular analysis of variance assuming the OPC biotypes as the components of the population structure using Arlequin version 3.5.2.2 (20). Allele and genotypic frequencies were compared between biotypes using Fisher's test at 5% significance using jamovi Ver.2.2.

## RESULTS

Table 1 shows the allele and genotypic frequencies for the genes evaluated. For the GLI1 locus, the frequency of the T allele in both biotypes was higher, although, in the Ethiopian biotype, the frequency of the G allele was significantly higher ($p<0.05$) than for the Sudan biotype. Overall, the OPC population showed a higher frequency of the TT genotype as well as for the Ethiopian (0.42) and Sudan (0.53) biotypes. The heterozygous genotype showed similar frequency in the subpopulations evaluated, while the frequency of the GG genotype was higher in the Ethiopian biotype. The differences in the frequencies of the homozygous genotypes (GG and TT) between the biotypes was significant ($p<0.05$).

In IL20RA gene, the frequency of A and G alleles (Table 1) in both biotypes was similar ($p>0.05$) with higher value in the A allele over G. The frequencies of AA and AG genotypes were different between biotypes ($p<0.05$), with mean values for the whole population of 0.465±0.03 and 0.465±0.03, respectively. In the Ethiopian biotype, genotype AA was the most frequent genotype, on the other hand, the genotype with the highest value observed in the Sudan biotype was AG**.**

**Table 1.** Allele and genotypic frequencies in GLI1 and IL20RA genes in the Ethiopian and Sudanese biotypes of OPC.

| | Allele frequencies | | Genotypic frequencies | | |
|---|---|---|---|---|---|
| **GLI1** | | | | | |
| **Biotype** | **G** | **T** | **GG** | **GT** | **TT** |
| **Ethiopian** | 0.39 | 0.61 | 0.21 | 0.37 | 0.42 |
| **Sudan** | 0.28 | 0.72 | 0.10 | 0.37 | 0.53 |
| **OPC** | 0.335 ±0.07* | 0.665 ±0.07* | 0.155 ±0.07* | 0.370 ±0.07 | 0.475 ±0.07* |
| **IL20RA** | | | | | |
| **Biotype** | **A** | **G** | **AA** | **AG** | **GG** |
| **Ethiopian** | 0.69 | 0.31 | 0.49 | 0.39 | 0.12 |
| **Sudan** | 0.67 | 0.33 | 0.44 | 0.46 | 0.10 |
| **OPC** | 0.680 ±0.01 | 0.320 ±0.01 | 0.465 ±0.03* | 0.465 ±0.03* | 0.110 ±0.01 |

* Indicates statistical differences ($p<0.05$) in frequencies between biotypes.

Genetic diversity indices for the GLI1 gene are presented in table 2. Ho values were lower than expected under EHW, this difference was greater in the Ethiopian biotype, which meant significant deviations from EHW for this biotype and not for Sudan, extending to OPC. Likewise, FST, FIS and FIT values were significantly different from zero.

The Ho value was lower than the He value in the Ethiopian biotype for the IL20RA gene and the opposite was true for the Sudan biotype (Table 2). However, the heterozygote deficit in Ethiopian and excess in Sudan did not signify deviations from Hardy-Weinberg equilibrium (EHW), which was confirmed by the non-significant values of FST, FIS and FIT.

**Table 2.** Genetic diversity indices in the GLI1 and IL20RA genes in the Ethiopian and Sudanese biotypes of OPC.

| **GLI1** | | | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Ho** | **He** | **EHW** | **$F_{ST}$** | **$F_{IS}$** | **$F_{IT}$** |
| **Etíope** | 0.37 | 0.48 | 4.70* | | | |
| **Ethiopian** | 0.37 | 0.40 | 0.32$^{ns}$ | 0.020* | 0.166* | 0.010* |
| **OPC** | 0.370 ±0.06 | 0.440 ±0.06 | 4.89* | | | |
| **IL20RA** | | | | | | |
| **Biotype** | **Ho** | **He** | **EHW** | **$F_{ST}$** | **$F_{IS}$** | **$F_{IT}$** |
| **Etíope** | 0.39 | 0.43 | 1.32$^{ns}$ | | | |
| **Ethiopian** | 0.47 | 0.44 | 0.22$^{ns}$ | 0.06ns | 0.048ns | 0.42ns |
| **OPC** | 0.430 ±0.06 | 0.435 ±0.01 | 0.296$^{ns}$ | | | |

Ho: observed heterozygosity, He: expected heterozygosity, FST: population structure index, FIS: intra-biotype inbreeding coefficient, FIT: population inbreeding coefficient, EHW: Hardy-Weinberg equilibrium, * statistical differences ($p<0.05$), ns: not significant.

## DISCUSSION

The present study evaluated two genetic polymorphisms in the GLI1 and IL20RA genes, proposed as candidates for gastrointestinal parasite resistance in sheep.

Breeding programmes aimed at improving host resistance to PGI may help alleviate this problem in the long term. Genetic variation in host resistance exists for the major PGI species affecting sheep. For example, the indigenous sheep breeds Maasai (21), Rhon (22), St Agnes (23) and Black Belly (24) have been reported to have relatively better resistance against PGIs. Similarly, high genetic variation within commercial breeds to PGI has been demonstrated in Merino (25), Romney (26), Scottish Blackface (27) and IIle de France (28) sheep among others. However, this intra-breed variation for the PGI resistance trait in Colombian Criollo hair and wool sheep has not been determined.

Despite the above, studies have been carried out in our country to characterise the parasitic agents that affect production systems. In a study carried out in five municipalities in the department of Antioquia in Colombia, an infection rate of 86.6% was found in sheep, with the

most prevalent nematodes being Haemonchus Contortus (66.3%), Oesophagostomum spp. (38.9%), Trichostrongylus spp. (34.7%) and Ostertagia spp. (24.2%) (29). In another study carried out in the same department in sheep and goat production systems under confinement, semi-confinement and grazing, they found that 76% of the animals were infected, where 69. 5% had low parasite loads (less than 200 eggs/g of faeces), with a high prevalence of infection by Trichostrongylidae, with Haemonchus contortus (61.3%), Teladorsagia (Ostertagia) circumcincta (25.5%) and Trichostrongylus sp (21.5%) being the most frequent parasites(30). In Creole breed animals, raised extensively and semi-extensively, without rotation of paddocks in the department of Boyacá, in 637 samples of faecal matter, they observed a prevalence of 89.4% of parasitized sheep, finding 63% of the Eimeriidae family, followed by Trichostrongylidae (47. 4%), Dyctiocaulidae (38.1 %) and Strongylidae with a prevalence of 21.5 %, as well as Fasciolidae (6.3%), Trichuridae (5.7%), Anoplocefalidae (2.4%), Toxocaridae (1.3%), Taeniidae (0.3%) and Capillaridae (0.2%) (8). On the other hand, high prevalences of trichostrtrongylids (97.70%) and Eimeria spp (81.61%) were observed in OPC sheep in the department of Córdoba (Colombia) (31). The above characterisation effort should be completed by correlating parasite loads with the different breed groups.

Periasamy et al. (13) proposed a lower PGI egg count associated with the GG genotype in the GLI1 gene in sheep. In OPC biotypes this genotype was not the most frequent in the population reaching an average of 0.16±0.07, with higher frequency in the Ethiopian biotype than in the Sudanese. The proposal of this variant, as a major gene for this trait, could be interesting for OPC breeders, as the high frequency of the G allele (0.34±0.07) would allow a selection process in favour of it, in order to increase its frequency. Several authors stress that host genetics significantly affects FEC, being this an important phenotypic marker for the resistance of different gastrointestinal parasites in sheep (6,7,12-15).

The GLI1 gene belongs to the zinc finger type 1 family and is located on sheep chromosome 3. GLI proteins are multifunctional transcription factors that can act as activators or repressors of transcription, central nervous system development and the gastrointestinal tract (32). Genetic and biochemical studies indicate that GLI1 regulates the expression of different target genes and is a transcriptional activator of the SHH (Sonic hedgehog) signalling pathway, which is involved in regulating the formation and surveillance of progenitor cells in animal embryonic development by moderating histogenesis and organogenesis through cellular processes of proliferation, differentiation, migration and cell survival, which favour the formation of morphogenetic fields (33). Similarly, in cerebellar development, the importance of the SHH protein for granule cell proliferation during the perinatal period, also controlling the growth of the ventral cerebellum/brain cortex, is well established (34). Postnatally, this pathway remains active at sites where tissue renewal continues into adulthood and during the processes of damage and repair of cell populations (33,34).

In the IL20RA gene, the AA genotype was significantly associated with lower PGI egg counts (13). In this study the AA genotype presented the highest frequency for OPCs (0.47±0.03) with higher frequency in the Ethiopian biotype (0.49) than in Sudan (0.44). The IL20RA gene is located on chromosome 8 of sheep NC_040264.1 and is an alpha subunit of the Interleukin 20 receptor. This gene belongs to the type ll family of cytokine receptors, by binding to its ligands, such as interleukin IL-19, IL-20 and IL-24, IL20RA can form a functional heterodimeric receptor with IL20RB. The cytokines of the IL-20 subfamily are mainly produced by immune cells, such as myeloid cells, lymphocytes and leukocytes, and act on epithelial tissues to enhance epithelial innate immunity, however, immune-related functions are shared by many other cytokines, in addition to their unique roles in driving tissue protection and regeneration (35). These functions of cytokinins can be seen in the different investigations where they have detected the presence of signalling of this subfamily as in the case of Pampinta lamb (36) and in a population of sheep where in a panel of 13 SNPs within 7 genes they observed IL2RA as a candidate gene related to exposure to H. contortus (37). Additionally in sheep Djallonke and Sahelian Yaro et al. (38) by means of gene ontology identified IL20RA involved in biological processes, such as immune response and chemotaxis to Haemonchus and Trypanosoma infestation. Meanwhile, this gene has also shown that the signalling pathway promotes the formation of a favourable immune microenvironment in malignant cells(39). This research confirms

the association of the II-type cytokine receptor family with nematode resistance, reinforcing the role of the host response to parasites.

In the GLI1 gene, the variant analysed (rs411868094) showed deviations from Hardy-Weinberg equilibrium, with a low value of genetic differentiation ($p<0.05$) between biotypes. The deficit of intra-biotype heterozygotes (FIS) was highest in Ethiopian. This deficit extended to the whole OPC population, although with lower intensity (FIT). The low number of heterozygotes leads to an increase in the frequency of homozygous genotypes. The report by Periasamy et al (13) proposes that animals with the GG genotype have a lower PGI egg count, but paradoxically, this genotype is not the most frequent genotype in OPC. This suggests that there has been no selection for this genotype in this breed. It is also possible that the variant studied here does not have this positive association or even that other genes are related to PGI resistance.

Our results showed that the variant studied in the IL20RA gene is in Hardy-Weinberg. This occurs when the population is randomly mated and there is an absence of migration, genetic drift or selection, resulting in stable allele frequencies between generations (40). This ensures that in the OPC, no breeding processes aimed at increasing resistance to PGI have been carried out.

In conclusion, the genes evaluated are polymorphic in OPC, the alleles of interest associated with low FEC had low frequency for the GLI1 gene, but high frequency for IL20RA. The Ethiopian biotype of OPC showed the highest frequencies of the genotypes of interest. The loci under study had high genetic diversity, no significant deviations from Hardy-Weinberg equilibrium were found in IL20RA but significant deviations were found in GLI1, evidence of the absence of breeding schemes in favour of the genotypes of interest.

## Conflict of interest

The authors declare no conflict of interest.

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