Search of genomic regions influencing faecal egg count, as an indicator of resistance to gastrointestinal nematode infections, based on the analysis of the OvineSNP50 BeadChip

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ABSTRACT: The objective of this study was to perform a preliminary search of genomic regions including Quantitative Trait Loci (QTL) underlying the resistance to gastrointestinal nematode infections (GIN) in a commercial population of Churra sheep by performing linkage (LA) and genome-wide association (GWA) analyses based on SNP-chip data. The studied population included 533 Churra ewes belonging to 15 half-sib families. The ewes and their sires were genotyped with the Illumina 50K BeadChip, whereas measurements of faecal egg count (FEC) were obtained for the ewes using the McMaster method. The LA analysis identified one QTL reaching the 5% chromosome-wise significance level on OAR8, whereas the GWA study found one marker exceeding that significance level on OAR6. A search of candidate genes was performed in the confidence intervals estimated for the QTL detected on these two chromosomes.

Keywords: Gastrointestinal nematode infection; QTL; GWA analysis; Churra sheep

Introduction

In the last few decades, much effort has been developed to understand the host-parasite relationship. The interest of the sheep industry worldwide on this topic (Taylor (2012)) is driven by the persistent problem with GIN in grazing sheep. In these populations the efficient control of the parasites, which was principally based on anthelmintic treatments, is now limited by the increasing development of nematode resistances to several chemical groups of drugs. Previous studies aiming the detection of QTL associated with nematode resistance were based on low density maps of microsatellite markers (http://www.animalgenome.org/QTLdb/sheep). However, the variety of parasites and sheep breed considered in these studies has resulted in lack of agreement among the results. As a consequence, there is merit in carrying out additional studies based on higher marker density to identify genetic variants with a clear effect on the complex trait of parasite resistance. For now, few GWA studies have been reported in sheep in relation to GIN resistance traits (e.g. Kemper et al. (2011); Riggio et al. (2013)). These GWA studies have been conducted in lambs of breeds specialized for meat and/or wool production, whereas similar analyses in adult dairy sheep populations have not yet been reported. In a previous microsatellite-based genome scan performed in Spanish Churra sheep some regions were found to influence faecal egg count (FEC). In the present study, the genotypes generated with the Illumina OvineSNP50 BeadChip were used to identify genomic regions related to this same indicator trait in a different commercial population of Churra sheep by exploiting both LA and GWA analyses.

Materials and Methods

Resource population. Phenotypic and genotypic information for 533 Churra sheep from the Selection Nucleus of the National Association of Churra breeders (ANCHE) was analyzed in the present study. The animals are distributed in 15 half-sib families, with an average family size of 39 daughters per sire (range: 7 to 60). Samples were collected from 17 naturally infected flocks located in the Autonomous Region of Castilla y León. The phenotype trait, FEC, was determined by floating the faeces in zinc sulfate (d=1.33) solution in a McMaster slide and counting the eggs (MAFF (1986)). The samples showed a low level of FEC related to the exceptional scarce precipitation before and during the sampling period. The estimated prevalence of GIN by FEC in flock was 88.2% (mean=42.8 epg) and in sheep was 45.4% (mean=39.4 epg). Presence of Trichostrongylus spp. (49.3%) and Teladorsagia spp. (48.6%) was confirmed in all the flocks.

Data analysis. Prior to further analysis, FEC measurements were log-transformed (LFEC) to get an approximation to the normal distribution. For further analyses, the yield deviations (YD) of raw data were used as dependent variables. The YD estimate was calculated following a multivariate animal model in which LFEC was corrected for the fixed flock effect. DNA samples from a larger population of 1,696 individuals (García-Gámez et al. (2012)) that included the animals with FEC measurements analyzed here had been genotyped with the OvineSNP50 BeadChip. In this study, we performed a quality control (QC) of genotypes for the larger population, and following the steps detailed by (García-Gámez et al. (2012)), but after updating the marker order and genome positions according to the most recent version of the Ovine Genome Assembly, v3.1 (www.livestockgenomics.csiro.au/sheep/oar3.1.php). A total of 43,613 SNP located in the ovine autosomes passed the QC in the larger population. From that subset of markers, the genotypes for the smallest population with LFEC available records were subjected to the analyses presented here.

A 1 cM~1 Mb conversion rate was used to obtain the linkage maps used in the classical LA genome scan, which was performed with the QTLMap software (Filangi et
al. (2010)). The QTL search was performed every 0.1 cM using the software analysis options corresponding to LA. Significance thresholds at the chromosome-wise level were determined by 1,000 permutations, and used to obtain the genome-wise significance thresholds by correcting for the total number of chromosomes under analyses. After conversion of LRT values to the LOD values (Beraldi et al. (2007)), confidence intervals (CI) for the significant QTL were estimated using the 1-LOD drop-off method.

The GWA analysis was performed using the DMU software (available at http://dmu.agrsci.dk) based on a linear mixed model (LMM) as previously explained by García-Gamez et al. (2012). The significance levels corresponding to each analyzed marker were corrected with a Bonferroni correction for the total number of markers analyzed across the individual chromosomes and the genome to obtain the corresponding significance thresholds.

Considering the estimated CI from LA, positional candidate genes were extracted from the Ovine Genome Assembly v3.1, available at the Ensembl database (www.ensembl.org/Ovis_aries/Info/Index) and using Bi-oMart (www.ensembl.org/biomart/martview/). From the initial list of positional candidates, the functional candidates were identified based on the physiological known function and literature reports related to the immune response in nematode resistance.

**Results and Discussion**

The LA analysis for FEC identified one significant QTL at the 5% chromosome-wise level on OAR8, with the peak located at 2 Mb. Six families were significant for this QTL according to the Student-Test provided by the analysis software. The average of the QTL effect in the segregating families was 0.715 in trait units (0.460 SD). For this QTL, the estimated confidence interval (CI) spanned 2.4 Mb (range: 1 to 3.4 Mb). The LA also showed a region on OAR6 was close to the 5% chromosome-wise significance level (maximum located at 88.1 Mb) although did not exceed the threshold. The GWA analysis performed with DMU identified a 5% chromosome-wise significant association for SNP OAR6_83627682.1, located at position 76.601 Mb. The allele substitution effect estimated in trait units for this marker was -0.533 ± 0.113 (0.343 SD). None of the two analyses identified genome-wise significant associations.

Because of the coincident location between the significant result identified by the DMU analysis on OAR6 and the suggestive signal identified on that chromosome by the LA analysis of our resource population, a CI interval was also estimated for the OAR6 QTL based on the LA results. In this case, the CI involved a 12.1 Mb long interval (range 80.5 to 92.6 Mb). Eight families showed a significant Student-Test for this suggestive QTL, whereas the average size of the QTL effect in the segregating families was 0.541 in trait units (0.347 SD). The similar estimated effect for this QTL identified by both, LA and GWA analyses, supports the hypothesis that these two signals are due to the same genetic variation. The search of positional candidate genes yielded a total of 6 and 91 genes for the estimated CI of the OAR8 and OAR6 QTL regions, respectively.

Previous studies have identified significant associations for FEC on OAR8 (Crawford et al. (2006); Marshall et al. (2009); Riggio et al. (2013)), however the corresponding QTL peaks were located at a more distal region of OAR8 than the significant region reported here. Although there is not a clear relationship with parasite resistance for any of the six positional candidates extracted from the OAR6 QTL CI, it is worth mentioning that the product of one of these genes, COL12A1 (type XII collagen), is found in association with type I collagen (COL 1), whose synthesis during parasite infections is suggested to be highly dependent of TH2 cytokines response (Wynn (2004)). Other gene mapping into this CI is SENP6, (SUMO1/Sentrin Specific Peptidase 6), an intrinsic attenuator of the inflammation triggered by Toll Like Receptors (Liu et al. (2013)), which are known to be important in maintaining epithelial barrier function in response to enteric pathogens and parasites (Venugopal et al. (2009)).

The suggestive signal identified on OAR6 by QTLMap and the chromosome-wise significant SNP identified by our GWA study overlap with the CI of the genome-wise significant QTL previously reported for the same trait in a different population of Churra sheep, based on a microsatellite–based genome scan (Gutierrez Gill et al. (2009)). Hence, the results presented in the present work for OAR6 may be considered as the replication of the OAR6 QTL previously reported by Gutierrez Gill et al. (2009). However, the limited power of our statistical analysis, due to the large number of zero records for FEC in the analyzed sample may have influenced on the low significant level of the associations identified. Other associations reported on OAR6 for FEC, based on microsatellite markers (http://www.animalgenome.org/QTLdb/sheep or SNP-chip data (Riggio et al. (2013)) are far away of the QTL reported here in this chromosome.

Among the 91 genes extracted from the estimated CI of the OAR6 QTL, and in addition to the casein coding genes, we have found some interesting functional candidate genes. Four of these six genes, PF4 (Platelet factor 4), CXCL1, CXCL10 (chemokine ligand 1 and 10) and IL8 (Interleukin 8), are CXC chemokines and have a known function in relation to immune regulatory mechanisms as they have a role in cellular proliferation and differentiation of epithelial cells and in recruiting of neutrophils, monocyte and fibroblasts to the site of infections (injury) (Gillitzer and Goebeler (2001)). Among them, the coding product of PF4 stands out due to its role to promote the development of TH2 cytokines and to inhibit the production of TH1 cytokines, the two major mechanisms of the host immune response during parasite infection. On the contrary, the CXCL10 gene has an opposite effect than PF4 (Romagani et al. (2005)). IL-8 is involved in cell migration and has a sig-
significant role in wound healing (Rennekampff et al. 2000)). It is a potent chemoattractant secreted by the basolateral surface of intestinal epithelial cells (IEC) and mediates neutrophiles recruitment from the lamina propria to the epithelium (Kucharzik et al. 2005)). The two other candidates, AREG (Amphiregulin) and EREG (Epiregulin), are members of the epidermal growth factor family, and their main functions are related to cellular proliferation, differentiation and survival of epithelial cells (Inatomi et al. (2006)). AREG is produced by T cells and eosinophils and its absence has an influence on delayed expulsion of T. muris in mouse model (Zaiss et al. 2006)).

No significant associations were found on OAR1, 10 and 14, where the previous genome-scan reported by Gutierrez-Gil et al. (2009) had identified chromosome-wise significant QTL in Churra sheep.

Conclusion

This preliminary study was based on the analysis of OvineSNP50 Beadchip genotypes in a commercial population of Spanish Churra sheep with available data for FEC, which was used as an indicator of the levels of natural infection by gastrointestinal nematodes. The LA analysis identified one novel significant QTL on 5% chromosome-wise level on OAR8, while the GWA study found one SNP exceeding that significance level on OAR6. As the same region detected by the GWA also showed a suggestive significant QTL in LA, we considered these two signals as a possible replication of a previously reported QTL for FEC in Churra sheep. Functional candidate genes have been identified for the OAR6 and OAR8 QTL reported here. Future work will be focused on the analysis of other indicators of nematode resistance such as the serum levels of immunoglobulin A.

Figure 1: Results obtained for faecal egg count based on the linkage analysis (LA; QTLMap software) presented in this study for the 26 ovine autosomes.

Figure 2: Result from the Genome-wide Association study (GWAS; DMU software) for faecal egg count across the whole genome (a) and in a detailed view on chromosome 6 (b).

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