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Detection of quantitative trait loci and putative causal variants affecting somatic cell score in dairy sheep by using a 50K SNP-Chip and whole genome sequencing

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ABSTRACT

This study presents a scan of the ovine genome to identify quantitative trait loci (QTL) influencing the somatic cell score (SCS), a classical indicator of subclinical mastitis in sheep, and a subsequent high-resolution analysis of one of the identified QTL regions based on the analysis of whole-genome sequence data sets. A half-sib commercial population of Churra sheep genotyped with a 50K SNP-Chip was analyzed using linkage analysis (LA) and combined linkage and linkage disequilibrium analysis (LDLA). By LA, 2 5% chromosome-wide significant QTL on OAR5 and OAR25 and one 5% genome-wide significant QTL on ovine chromosome 20 (OAR20) were detected, whereas 22 significant associations were identified by LDLA. Two of the associations detected by LDLA replicated LA-detected effects (OAR20, OAR25). We compared the detected associations with previously reported QTL in sheep and cattle, and functional candidate genes were identified within the estimated confidence intervals. We then performed a high-resolution analysis of the OAR20 QTL region, the most significant QTL region identified by LA that replicated a QTL previously described in Churra sheep for SCS using microsatellite markers. For that, 2 segregating trios of 2 segregating families for the OAR20 QTL (each including the *Qq* sire and 2 daughters, *QQ* and *qq*) were selected for whole-genome sequencing. The bioinformatic analysis of the 6 sequenced samples performed across the genomic interval considered (14.2–41.7 Mb) identified a total of 227,030 variants commonly identified by 2 independent software packages. For the 3 different concordance tests considered, due to discrepancies regarding the QTL peak in the segregating families, the list of mutations concordant with the QTL segregating pattern was processed to identify the variants identified in immune-related genes that show a moderate/high impact on the encoded protein function. Among a list of 85 missense variants concordant with the QTL segregation pattern that were within candidate immune-related genes, 13 variants distributed across 7 genes [*PKHD1*, *NOTCH4*, *AGER*, *ENSOARG00000009395* (*HLA-C*, *Homo sapiens*), *ENSOARG00000015002* (*HLA-B*, *H. sapiens*), *MOG*, and *ENSOARG00000018075* (*BoLA*, *Bos taurus*, orthologous to human *HLA-A*] were predicted to cause deleterious effects on protein function. Future studies should assess the possible associations of the candidate variants identified herein in commercial populations with indicator traits of udder inflammation (SCS, clinical mastitis).

Key words: mastitis, quantitative trait loci, single nucleotide polymorphism-chip, genomic sequencing, genetic marker

INTRODUCTION

In dairy species, the SCC of milk represents a predictive marker of the udder health and is widely used for evaluating milk quality. It also influences milk prices. An increased SCC is either the consequence of an inflammatory process due to the presence of an IMI or, under nonpathological conditions, due to physiological processes such as estrus or an advanced stage of lactation (Raynal-Ljutovac et al., 2007).

Subclinical mastitis constitutes one of the major problems influencing total productivity in dairy sheep. Therefore, resistance/susceptibility to this disease can be considered an important functional trait for the milk production sector. Because SCC provides a measurement of the level of defensive cells that migrate from blood to mammary gland as a response to infection (Gonzalo and Gaudioso, 1985), log-transformed SCC, known as the SCS, can be used as an indicator trait to achieve genetic improvement for mastitis resistance (Shook and Schutz, 1994). Although direct selection

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for mastitis resistance has been implemented in dairy cattle for over 35 yr in Nordic countries (Østerås et al., 2007) and more recently in France (Govignon-Gion et al., 2016) and Canada (Jamrozik et al., 2013), most countries breed for mastitis resistance indirectly through SCS (Miglior et al., 2005). In dairy sheep, the SCS is considered a functional indicator trait of subclinical mastitis and is one of the factors influencing the price that farmers receive for the milk. In dairy sheep, reported heritability estimates of SCS range between 0.06 and 0.18 (Othmane et al., 2002; Rupp et al., 2003; Legarra and Ugarte, 2005). The SCS is included as a selection target in the breeding scheme of the French Lacaune breed (Barillet et al., 2006). In Churra sheep, although SCS is routinely recorded through the official recording control, the low number of rams under genetic evaluation makes consideration of this trait unfeasible when calculating the selection index. Indirect selection for subclinical mastitis resistance is performed in Churra sheep through the inclusion of udder morphology traits as selection objectives (de la Fuente et al., 1996), with the advantage that these traits are more heritable than SCS. The efficiency of this indirect selection to favor stabilization of SCS would be related to the expected genetic correlations between udder traits and mastitis resistance. Hence, in a Lacaune × Sarda backcross population, Casu et al. (2010) reported high genetic correlations between SCS and both udder attachment (measured as degree of suspension of the udder) and udder depth (−0.42 and −0.50, considering a scale of opposite sign for udder depth than in Churra sheep). These estimates suggest that selection for shallow udders, close to the abdominal wall, and udders with higher degree of suspension would be associated with a genetic response toward lower SCS. Although in Churra sheep genetic correlations have not been estimated between udder morphology traits and mastitis resistance, phenotypic correlations reported in this breed between SCS and udder depth and between SCS and teat size are low but positive (0.13 and 0.18 respectively), which can be explained by the higher frequency of trauma observed in very deep udders and larger teats than standard teat cups (Fernández et al., 1997).

Nevertheless, taking into account the direct influence of SCS on the price of milk, direct selection on this trait would be of great interest for breeders of Churra dairy sheep. As for other traits showing low heritability, marker- or gene-assisted selection would be a feasible strategy to improve resistance to subclinical mastitis not only in Churra sheep but also in other ovine populations devoted to milk production. Detecting genetic variants directly associated with the SCS trait could be exploited to increase the average resistance level of flocks to subclinical mastitis. Historically, the first attempts to identify genes related to this functional trait in dairy sheep populations were genome scans based on microsatellite markers aiming to identify QTL (reviewed by Arranz and Gutiérrez-Gil, 2012). In Churra sheep, an analysis of a half-sib population with 181 markers identified only one chromosome-wide significant QTL for SCS on sheep chromosome 20 (*Ovis aries* 20; **OAR20**). However, the low mapping resolution of this scan together with the differences in marker informativeness among the analyzed families limited the ability to identify reliable candidate genes for this QTL effect (Gutiérrez-Gil et al., 2007).

Presently, SNP chips of medium and high density in sheep provide a substantially improved mapping tool for the identification of QTL that directly control traits of economic interest. In addition, animal scientists currently have access to whole-genome sequence-based technologies, which increases their ability to detect and propose mutations as plausible causal mutations or quantitative trait nucleotides (Sellner et al., 2007).

In Churra sheep, a medium-density 50K SNP-Chip has been used by our research group to map QTL related to milk production traits (García-Gámez et al., 2012) and parasite resistance traits (Atlija et al., 2016). For milk traits, this chip greatly facilitated the identification of the putative causal mutation of a previously described QTL influencing milk protein percentage in Spanish Churra sheep (García-Gámez et al., 2012). In a more recent study, the combination of 50K SNP-Chip genotyping in a commercial population of French dairy sheep and the analysis of whole-genome sequencing information allowed the identification of the causal mutation of an OAR3 QTL influencing mastitis susceptibility in the *SOCS2* gene (Rupp et al., 2015). A custom-made 960-SNP DNA array has been recently used in the Greek Chios breed to confirm previously detected QTL in other sheep breeds and has suggested, for some of the regions, a conserved genetic architecture of mastitis resistance between distinct dairy sheep breeds (Banos et al., 2017).

The objectives of the present study were to (1) perform QTL mapping analyses for the SCS trait using a 50K SNP-Chip based on linkage (**LA**) and combined linkage and linkage disequilibrium (**LDLA**) analyses in the same commercial half-sib population of Churra dairy sheep analyzed for milk production traits by García-Gámez et al. (2012), and (2) exploit the whole-genome sequences of segregating trios to perform a high-resolution study of the genetic variation within the region harboring the most significant QTL detected and assess the polymorphisms showing concordance with the expected QTL genotypes as potential causal variants based on their biological relevance and the physiological effects of the affected gene.