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Identification of single nucleotide polymorphisms in the bovine solute carrier family 11 member 1 (*SLC11A1*) gene and their association with infection by *Mycobacterium avium* subspecies *paratuberculosis*

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ABSTRACT

Johne's disease is a chronic enteritis caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) that causes substantial financial losses for the cattle industry. Susceptibility to MAP infection is reported to be determined in part by genetic factors, so marker-assisted selection could help to obtain bovine populations that are increasingly resistant to MAP infection. Solute carrier family 11 member 1 (*SLC11A1*) was adjudged to be a potential candidate gene because of its role in innate immunity, its involvement in susceptibility to numerous intracellular infections, and its previous association with bovine MAP infection. The objectives of this study were to carry out an exhaustive process of discovery and compilation of polymorphisms in *SLC11A1* gene, and to perform a population-based genetic association study to test its implication in susceptibility to MAP infection in cattle. In all, 57 single nucleotide polymorphisms (SNP) were detected, 25 of which are newly described in *Bos taurus*. Twenty-four SNP and two 3′-untranslated region polymorphisms, previously analyzed, were selected for a subsequent association study in 558 European Holstein-Friesian animals. The SNP c.1067C > G and c.1157–91A > T and a haplotype formed by these 2 SNP yielded significant association with susceptibility to MAP infection. The c.1067C > G is a nonsynonymous SNP that causes an amino acid change in codon 356 from proline to alanine (P356A) that could alter SLC11A1 protein function. This association study supports the involvement of *SLC11A1* gene in susceptibility to MAP infection in cattle. Our results suggest that SNP c.1067C > G may be a potential causal variant, although functional studies are needed to assure this point.

Key words: paratuberculosis, solute carrier family 11 member 1 (*SLC11A1*), single nucleotide polymorphism, haplotype

INTRODUCTION

Johne's disease, caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), is a chronic enteritis characterized by progressive weight loss and profuse diarrhea. Johne's disease occurs worldwide and is highly prevalent in domestic ruminants, approximately 20% in cattle of several European countries (Nielsen and Toft, 2009). It is currently recognized as one of the main diseases of dairy cattle in industrialized countries and results in substantial financial losses every year for the dairy industry (Hasonova and Pavlik, 2006). Beyond this financial damage, bovine MAP infection may pose a public health risk, as MAP has been linked to Crohn's disease in humans (Juste et al., 2009).

Currently, there is no treatment for Johne's disease and the only control system that consistently yields good results is vaccination, which has proved notably successful in sheep and cattle (Köhler et al., 2009). Vaccination for MAP has met resistance in some countries because of its potential interference with the diagnosis of bovine tuberculosis, but it is highly beneficial financially because it reduces the number of clinically and subclinically infected animals (Juste et al., 2002). However, vaccination prevents only disease and not infection, so new strategies are required to help eradicate the infection and reduce its financial impact.

Genetic factors are involved in intracellular infections; MAP infection heritability is estimated to be in a moderate range, with 0.102 being the most reliable value (Koets et al., 2000; Mortensen et al., 2004; Gonda

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et al., 2006). The use of pre-existing genetic factors, selecting against susceptible animals by marker-assisted selection combined with classical breeding programs (Dekkers, 2004), may be a viable alternative in combating Johne's disease. To implement such a technique, genetic markers associated with susceptibility to MAP infection must be identified. Some of the studies conducted to date point to various chromosome regions or QTL associated with paratuberculosis (Gonda et al., 2007; Settles et al., 2009), and different genes have been highlighted as potentially involved in the mechanism of susceptibility to MAP infection (Estonba et al., 2005; Mucha et al., 2009; Pinedo et al., 2009a,b).

The solute carrier family 11 member 1 (***SLC11A1***, formerly *NRAMP1*) gene, is expressed mainly in the phagosomes of cells belonging to the immune system, such as macrophages and neutrophils. The role of the SLC11A1 protein is to prevent intracellular bacterial growth. The mechanism seems to be linked to the transporting of divalent metal ions, mainly Mn^{2+} and Fe^{2+}, with protein SLC11A1 playing a leading role in the cellular recycling of the latter (Soe-Lin et al., 2008). The involvement of the *SLC11A1* gene in infections by intracellular pathogens including mycobacteria has been shown in mice, humans, and various domestic animals (Vidal et al., 1993; Gazouli et al., 2008; Sanchez-Robert et al., 2008). Recently, a strong genetic influence of *Slc11a1* on the innate susceptibility of mice to infection with MAP has been established (Roupie et al., 2008). For cattle, a genetic association has been described between a microsatellite-type polymorphism $(GT)_n$ in the 3′-untranslated region (**UTR**) of the gene and *Brucella abortus* infection in Holstein-Friesian cattle (Adams and Templeton, 1998). In the same region of the gene, Estonba et al. (2005) analyzed a larger fragment described previously (Hořín et al., 1999), which includes the $(GT)_n$ microsatellite described by Adams and Templeton (1998) and a further adjacent $(GT)_n$ repeat. This study detected a genetic association with MAP infection in a naturally infected Holstein-Friesian herd.

The present study, however, is not only focused in one region of *SLC11A1* but goes further in an attempt to cover the whole functional variability of the *SLC11A1* gene in cattle, including all exons and their flanking intronic regions, UTRs, and part of the promoter region. With the aim of testing for its potential involvement in susceptibility to MAP infection, we conducted a SNP discovery approach on bovine *SLC11A1* gene and performed a candidate gene type genetic association test between these SNP and MAP infection in the Holstein-Friesian breed. In this study we also sought to establish the relationship between microsatellite $(GT)_n$ of Adams and Templeton (1998), the fragment analyzed by Estonba et al. (2005) in the same 3′-UTR, and the SNP covering the whole length of the *SLC11A1* gene.

MATERIALS AND METHODS

SNP Discovery and Selection

Comparative sequencing was used to identify novel SNP polymorphisms in *SLC11A1*. The gene was divided into 11 fragments (N1_1 to N1_11) covering all the exons, flanking intronic regions, promoter, and UTR (Supplemental Table 1; available online at http://www.journalofdairyscience.org/). Primers were designed with the Primer3 program, using Btau_3.1; ENSBTA00000015520 sequence from the Ensembl database (http://www.ensembl.org/index.html) as reference. Fragments N1_1 to N1_10 were sequenced in 15 individuals from 14 bovine breeds: Blonde, Limousin, Holstein-Friesian (2), Gelbvieh, Red Angus, Jersey, Guernsey, Salers, Pirenaica, Terreña, Betizu, Monchina, Beefmaster, and Brangus. The SNP found were then validated in a sample of 85 Holstein-Friesians. Because the N1_11 fragment includes several microsatellites, it was cloned and then sequenced in 17 Holstein-Friesians using a TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) to avoid sequencing errors.

DNA was purified from blood samples with a QIAamp Mini Kit (Qiagen, Hilden, Germany). Fragments were amplified in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) and sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with both forward and reverse primers. Sequences were detected using 3100 Avant and 3130xl Genetic Analyzer equipment and analyzed with SeqScape v2.5 software (Applied Biosystems) to discover SNP.

As a complementary strategy, nucleotide sequences for the bovine *SLC11A1* gene stored in the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) nucleotide database were compared with the reference sequence, and those SNP not described previously were noted. Finally, *SLC11A1* SNP from IBISS3 (Hawken et al., 2004), NCBI dbSNP, Ensembl, and Animal Genome databases were also compiled (http://www.livestockgenomics.csiro.au/IBISS3/; http://www.ncbi.nlm.nih.gov/snp/; http://www.ensembl.org/index.html; http://www.animalgenome.org/, respectively). For creating the SNPlex marker set for the association study, SNP were selected according to their frequencies in the 85 Holstein-Friesian samples and their methodological compatibilities.