

A Candidate SNP in the Bovine *SP110* Gene is not Associated with Susceptibility to Tuberculosis in Cattle

¹O. Ruiz-Larrañaga, ¹C. Manzano, ¹M. Iriondo, ²J.M. Garrido, ¹M. Aguirre, ²P. Vazquez,
²R.A. Juste and ¹A. Estonba

¹Genetics, Physical Anthropology and Animal Physiology Department, University of the Basque Country, UPV/EHU, Sarriena s/n, 48940 Leioa, Bizkaia, Spain

²Animal Health Department, NEIKER-Teknalia, Berreaga 1, 48160 Derio, Bizkaia, Spain

Abstract: The human *SP110 nuclear body protein (SP110)* gene and the orthologous murine *Intracellular pathogen resistance 1 (Ipr1)* have been reported to be related with susceptibility to tuberculosis. In addition, a recent study reported that SNP c.587A>G (N196S) in the bovine *SP110* gene is associated with susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle. The objective of the present study was to analyze the putative association between SNP c.587A>G in *SP110* gene and susceptibility to *Mycobacterium bovis* infection in cattle. A total of 158 cows (50 infected and 108 non-infected) from a multibreed sample were included in the study. Neither SNP c.587A>G alleles nor genotypes showed significant association with susceptibility to *Mycobacterium bovis* infection in the analyzed cattle population. Nevertheless, other SNPs in this gene must be analyzed in order to rule out the implication of *SP110* gene in susceptibility to bovine tuberculosis.

Key words: Bovine tuberculosis, genetic association, single nucleotide polymorphism, *SP110 nuclear body protein*

INTRODUCTION

Polymorphisms of the human *SP110 nuclear body protein (SP110)* gene have been reported to be associated with tuberculosis (Tosh *et al.*, 2006). Furthermore, the orthologous murine gene *Intracellular pathogen resistance 1 (Ipr1)* has been found to be related to the innate immune response to *Mycobacterium tuberculosis* infection in a mouse model (Pan *et al.*, 2005); on the one hand, *Ipr1* controls mycobacterial replication, and on the other, it appears to induce the death of infected cells. In addition, a recent study reported that SNP c.587A>G (N196S) in exon 5 of the bovine *SP110* gene is associated with susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle (Ruiz-Larrañaga *et al.*, 2010), which causes Johne's disease in ruminants. This non-synonymous polymorphism could modify a possible glycosylation site involving asparagine in the SP110 protein and thus may be one of the causal variants of susceptibility to this infection, unless functional analysis are needed to confirm this fact. For these well established reasons, the *SP110* gene is an attractive candidate bovine tuberculosis susceptibility gene. The objective of this study was to analyze the putative association between SNP c.587A>G in the bovine *SP110*

gene and susceptibility to *Mycobacterium bovis* infection in cattle.

MATERIALS AND METHODS

Analyzed population and tuberculosis status: A total of 158 cows (50 infected and 108 non-infected) from a multibreed sample were included in this study. The tuberculosis status of animals was determined in Neiker-Tecnalia research institute during 2010. Infected animals were those which upon sacrifice in the slaughterhouse were found to present macroscopic lesions. Analysis by means of culture and typing of lung and lymph node samples confirmed that these lesions were caused by *Mycobacterium bovis*. Healthy animals, belonging to two herds in which tuberculosis was confirmed to be present by tissue culture, were tested for cell-mediated immunity using the Bovigam® IFN- γ kit (Prionics, Schlieren, Switzerland) according to the manufacturer's instructions. Animals whose whole blood cultures did not respond to tuberculin Purified Protein Derivate (PPD) were classified as being non-infected or healthy. The isolation of DNA from tissue and blood samples was performed using the Adiapure® DNA extraction and purification kit (Adiagene, Saint Brieuc, France) and the QIAamp® DNA Blood Mini Kit (Qiagen), respectively.

Corresponding Author: O. Ruiz-Larrañaga, Genetics, Physical Anthropology and Animal Physiology Department, University of the Basque Country, UPV/EHU, Sarriena s/n, 48940 Leioa, Bizkaia, Spain, Tel.: +34-94-601-5516; Fax.: +34-94-601-3145

Genotyping and association analysis: The genotyping process and subsequent association analysis were carried out in the University of the Basque Country during 2010. A Taqman assay (Applied Biosystems) was used to genotype SNP c.587A>G in exon 5 of the bovine *SP110* gene. Details of the primers and probes employed are reported elsewhere (Ruiz-Larranaga *et al.*, 2010). Haploview v4.2 (Barrett *et al.*, 2005) and PLINK v2.050 (Purcell *et al.*, 2007) statistical softwares were used for allelic and genotypic association analysis, respectively. A Chi-square test was performed, and *p*-values were calculated for the allelic and genotypic frequencies in infected versus health individuals under different genetic models.

RESULTS AND DISCUSSION

SNP c.587A>G was found to be in Hardy-Weinberg equilibrium in both healthy and infected animal groups ($p>0.05$). The A allele, which is associated with susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle (Ruiz-Larranaga *et al.*, 2010), was found to present a slightly higher frequency in healthy animals (0.886) than in infected animals (0.854). In addition, there was no significant association between c.587A>G alleles and susceptibility to *Mycobacterium bovis* infection in the analyzed cattle population. Results of genotype analysis did not reveal significant association either, under any of the tested models:genotypic, additive, dominant and recessive (Table 1).

Table1: Genotype frequency of SNP c.587A>G in infectedand healthy animals, and nominal p-values for genetic association analysis

Genotype	Infected freq	Non infected freq
AA	0.750	0.810
AG	0.208	0.152
GG	0.042	0.038
Association type	χ^2	Nominal p-value
Allelic	0.603	0.4375
Genotypic	0.765	0.6821
Additive	0.495	0.4819
Dominant	0.706	0.4090
Recessive	0.011	0.9159

These results do not support the implication of SNP c.587A>G in the bovine *SP110* gene in the variability of the individual innate immune response to infection by *Mycobacterium bovis*. Nevertheless, other SNPs in this gene must be analyzed in order to rule out the implication of *SP110* gene in susceptibility to bovine tuberculosis.

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