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Short communication. IL-1 family members as possible candidate genes affecting economically important traits in cattle

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Abstract

Health problems in cattle cause considerable economic losses for the producers. Proinflammatory cytokines have been shown to play important roles in different aspects of animal health and production. In the present work, interleukin-1β and interleukin-1 receptor antagonist genes (*IL1B* and *IL1RN,* respectively) have been localized on bovine chromosome 11 (BTA11q22). The precise location for *IL1RN* is reported and discrepancies concerning the exact position of *IL1B* (BTA 11q22 or BTA 11q23/11q24) are solved. In addition, the BTA11q22 region has been shown to carry several quantitative trait loci intervals affecting cattle production. Thus, it may be interesting to consider these two cytokines in order to develop more efficient breeding schemes and improve the accuracy and intensity of selection programs.

Additional key words: breeding program, cytokines, disease, embryonic loss, stillbirth, yield grade.

Resumen

Nota corta. Miembros de la familia IL-1 como posibles genes candidato que afectan a rasgos de importancia económica en el ganado vacuno

Los problemas sanitarios causan grandes pérdidas económicas a los ganaderos. Se ha demostrado que las citoquinas proinflamatorias desempeñan importantes papeles en distintos aspectos relacionados con la salud y la producción animal. En este trabajo se han localizado los genes que codifican para la interleuquina-1β y el antagonista del receptor de la interleuquina-1 (*IL1B* y *IL1RN,* respectivamente) en el cromosoma 11 bovino (BTA11q22). De este modo, se ha establecido la localización precisa de *IL1RN* y se han resuelto las discrepancias alrededor de la posición exacta de *IL1B* (BTA 11q22 ó BTA 11q23/11q24). Además, se ha visto que la región BTA11q22 es portadora de varios intervalos cromosómicos que afectan a caracteres productivos de interés en ganadería. De manera que sería interesante tener en cuenta estas dos citoquinas a la hora de desarrollar eficientes esquemas de producción y de mejorar la precisión y la intensidad de los programas de selección.

Palabras clave adicionales: citoquinas, enfermedad, mortalidad neonatal, pérdida de embriones, programas de mejora, tasa de rendimiento.

The detection of loci affecting economically important traits represents a major objective of livestock genomics. It should ultimately lead to more efficient breeding schemes and improve the accuracy and intensity of selection programs (Georges and Andersson*,* 1996; Gregory *et al*., 2002). Good health is essential for high production, longevity, and welfare of cattle. Diseases like mastitis and bovine spongiform encephalopathy cause great costs to farmers. The resistance to several diseases is a complex phenomenon and is influenced by many genes and, to a great extent, by environmental factors (Holmberg and Andersson-Eklund*,* 2004).

It has been observed that several diseases affecting cattle have an inflammatory component mediated by cytokines (Mrak and Griffin, 2001), which are soluble polypeptide factors that control the growth and differentiation of cells involved in immune and haematopoietic systems.

In this context, interleukin-1 (IL-1) is a pluripotent, proinflammatory cytokine family consisting of three structurally related polypeptides that exert their

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functions by interacting with specific receptors in the cell membrane (IL1R1 and IL1R2). The first two are interleukin-1α (IL-1α) and interleukin-1β (IL-1β), two agonists that have a broad spectrum of both beneficial and harmful biological actions. The third is interleukin-1 receptor antagonist (IL-1Ra), which inhibits the activity of interleukin-1. The IL-1 family orchestrates inflammatory and host defence responses in the periphery (Dinarello, 1996). IL-1 activates T-cells, upregulates expression of adhesion molecules, and induces expression of a number of other proinflammatory cytokines and other inflammation-associated proteins that form an amplifying cascade of inflammatory responses (Mrak and Griffin, 2001). It can also be a participant in degenerative events arising from inappropriate activation of the immune system. The association of polymorphisms in IL-1 genes with risk for several diseases underscores a role for IL-1 in pathology (McDowell *et al.*, 1995; Donaldson *et al.,* 2001).

Previous reports describing *IL1B* and *IL1RN* as good candidate genes modulating scrapie resistance/susceptibility in sheep, revealed a significant overexpression of both cytokines in the cerebellum of infected animals (Marcos-Carcavilla *et al*., 2007).

As there is a great similarity between ovine and bovine genomes, in the present work, the primers designed for ovine *IL1B* and *IL1RN* genes in the report mentioned above (Marcos-Carcavilla *et al*., 2007) were used in order to carry out the physical mapping of both genes in cattle. The objective was to verify the locations of these genes in the bovine genome and to check if these locations correspond to any QTL affecting any health trait in this species.

Two primer pairs designed from ovine *IL1B* intron 2 (5'-GCAGGAGGCGCAGCAGACAT-3') and intron 3 (5'-GAACAAGCCGGCCCAGAACACT-3') and *IL1RN* intron 3 (5'-ATGATTCTGKGGGTTGACYAGGAT-3' and 5'-GGCTTGCCTTTTTGGAGTTATC-3'), that displayed 400 and 200 bp amplicons respectively, were used to screen a bovine bacterial artificial chromosome (BAC) library (Eggen *et al.,* 2001). PCR was performed on PTC-100 thermocyclers in a final volume of 15 µl containing 1x standard buffer supplemented with 125 µM dNTP, 1.5 mM $MgCl_2$, 0.5 µM of each primer, and 0.03 U μl^{-1} Taq polymerase (Promega). The following PCR conditions were used: denaturation at 94°C for 5 min; 30 amplification cycles of denaturation at 94°C for 45 s, annealing at 55ºC for 30 s, and extension at 72°C for 45 s; a final 5 min extension at 72°C.

Two BAC clones containing bovine *IL1B* (311C4) and *IL1RN* (699F7) genes were identified. These BAC clones belong to a contig previously mapped to bovine chromosome 11 (BTA11) (Schibler *et al.,* 2006). Seven BAC end sequences of this contig are homologous with sequences of human chromosome 2, between 106 and 113 Mb. Comparative mapping among man, cattle and mouse suggested that these clones should be located on BTA11q12 or BTA11q22. This location was confirmed by cytogenetic mapping. Following the protocol described by Hayes *et al*. (1991), bovine *IL1B* and *IL1RN* containing BACs were labelled by nick translation with biotin-14-dATP (BioNick 18247-015 labelling system, Invitrogen life technologies). Then, they were used as probes for *in situ* hybridization on R-banded bovine chromosome preparations obtained by late 5-bromo-2'-deoxyuridine incorporation and stained with propidium iodide (RBP-bands). More than 10 metaphase spreads per gene were analysed. The result of FISH (fluorescence *in situ* hybridization) confirms the assignment of bovine *IL1B* and *IL1RN* to BTA 11q22 (Fig. 1). This is consistent with previous reports mapping both genes on BTA11 (Band *et al.,* 2000). In the present work, the bovine *IL1RN* gene location has been reported more accurately than in previous reports (Band *et al.,* 2000) and discrepancies concerning the exact position of the bovine *IL1B* gene (BTA 11q22; López-Corrales *et al.*, 1998) or BTA 11q23/11q24 (http://dga.jouy.inra.fr/cgi-bin/lgbc/nResu_loc.operl?BASE=cattle&LOCI=IL1B) have been solved. The confirmation that *IL1B* and *IL1RN* genes are positioned on BTA11q22 is interesting because various QTL regions affecting several health traits in cattle have been described on bovine chromosome 11 (Holmberg and Andersson-Eklund, 2004; Schulman *et al.,* 2004; Polineni *et al.,* 2006, http://bovineqtl.tamu.edu). The physical and genetic locations of several markers and the above mentioned QTL intervals are represented in Figure 1. This information has been compiled by consulting different web sites (http://dga.jouy.inra.fr/cgi-bin/lgbc/; http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9913; http://dga.jouy.inra.fr/cgi-bin/lgbc/intro2.pl?BASE=cattle; http://rubens.its.unimelb.edu.au/%7Ejillm/jill.htm; http://www.cgd.csiro.au/; http://www.thearkdb.org/browser?species=cow; http://bovineqtl.tamu.edu/). This could be helpful because the QTL intervals are described as regions within markers, which, in general, are located only by linkage mapping. Thus, taking into account markers that have been positioned by both

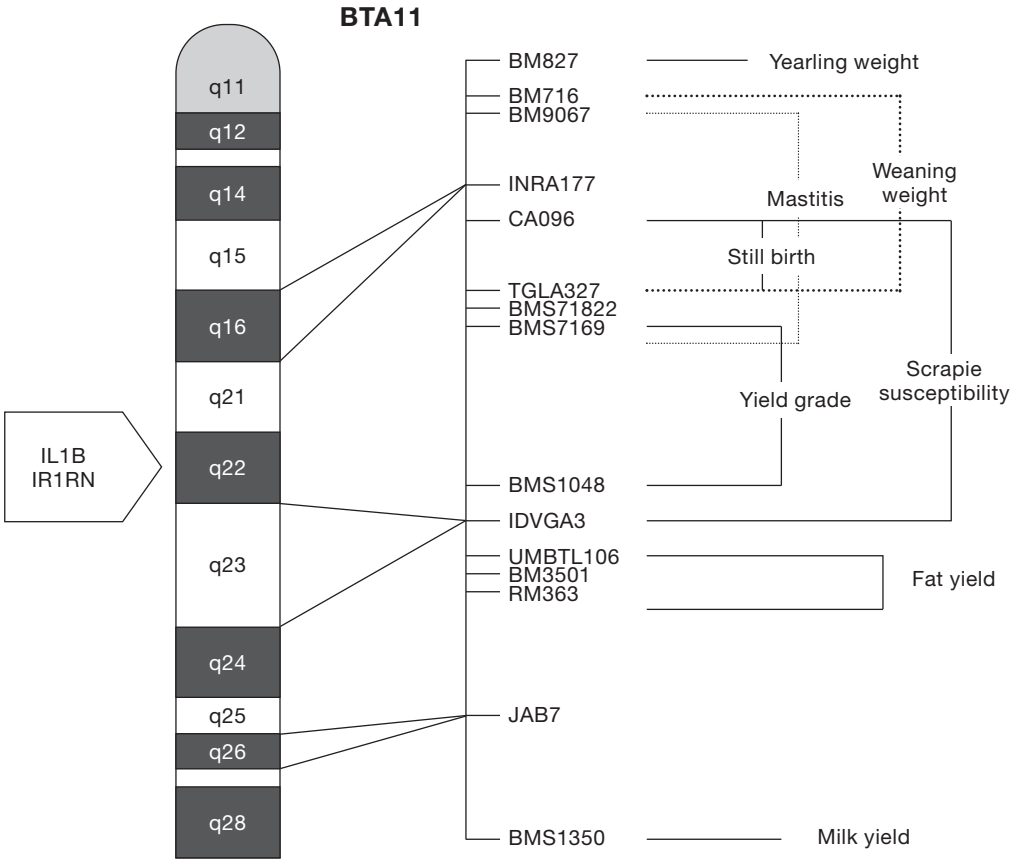

Figure 1. Schematic representation of bovine *IL1B* and *IL1RN* genes with regard to previous QTL regions described for BTA11. Left, cytogenetic location for both genes as it has been established in the present work (q22). Right, representation of the genetic locations for several markers and different QTL intervals affecting various economically important traits in cattle. Correspondence between the two maps for INRA177, IDVGA3, and JAB7 markers is also shown.

cytogenetic and linkage approaches (INRA177, IDVGA3, and JAB7), it is possible to infer the location of these QTL regions on the cytogenetic map. Therefore, the position 11q22 is between INRA177 (11q16) and IDVGA3 (11q23), whereas the other possible location for the *IL1B* gene (11q23-24) is between IDVGA3 (11q23) and JAB7 (11q26) markers. The interval within INRA177 and IDVAG3 contains QTL regions for mastitis, weaning weight, still birth and yield grade, whereas the other possibility contains only one region affecting fat yield (Fig. 1). With regard to these traits and the role that IL1-β and IL1-Ra might play, Lund *et al*. (1994) described a moderately high positive genetic correlation between clinical mastitis and other diseases, indicating that cows that are susceptible to mastitis also tend to be more predisposed to other health problems. Furthermore, studies using mastitis as a model have confirmed that one cause of early embryonic loss in ruminants and other species is infectious diseases or activation of immune response at sites outside the reproductive tract where IL-1 and other cytokines appear to be involved. Thus, IL-1β could cause embryonic loss by stimulating endometrial prostaglandin synthesis and decreasing endometrial cell proliferation (Martal *et al*., 1997; Hansen *et al*., 2004). On the other hand, IL-1β overproduction seems to be related to a reduction in food intake and body weight gain in mice (Lawrence and Rothwell, 2001). In the same way, previous studies (Matsuki *et al*., 2003) showed that IL-1 receptor antagonist deficient mice present a lean phenotype due to an abnormal lipid metabolism.

Additionally, previous reports describing these cytokines as positional and functional candidate genes modulating scrapie susceptibility in sheep (Marcos-Carcavilla *et al.*, 2007), place ovine *IL1B* and *IL1RN* genes on OAR 3p22, between CA096 and IDVAG3 markers. In the present work, the position assigned to the bovine counterparts is also between these two markers

(Fig. 1). Thus, as scrapie and bovine spongiform encephalopathy share a lot of features affecting the neurodegeneration process, it is conceivable that IL1-β and IL1-Ra also have a role in bovine spongiform encephalopathy. However, a whole-genome scan mapping QTL for bovine spongiform encephalopathy resistance/susceptibility (Zhang *et al*., 2004) identified two genome-wide significant QTL on BTA17 and X/Y and four genome-wide suggestive QTL on BTA 1, 6, 13 and 19; but none on BTA11 where *IL1B* and *IL1RN* are located in cattle. Several arguments could explain this discrepancy: i) the small number of families (four) and markers (two for BTA 11) analysed in the bovine study, ii) the possibility of having no variance at the QTL locus in the bovine families studied, iii) in spite of being two transmissible spongiform encephalopathies, bovine spongiform encephalopathy and scrapie are two different diseases with specific characteristics, thus, it is possible that IL-1β and IL-1Ra have a different behaviour in both cases.

Anyway, IL-1 family, a major mediator of inflammation, performs numerous functions related to host defence mechanisms by regulating not only the immune system, but also the neural and endocrine systems. IL-1 is produced by a wide variety of cells and its receptors are expressed on a wide range of immune, neural, and endocrine cells (Tocci, 1997). The overexpression of IL-1β that occurs in systemic and neuronal diseases, amplifies established IL-1 driven cascades, which, because of the excessive expression of IL-1, become degenerative and self-propagating (Griffin and Mrak, 2002). All this information points to *IL1B* and *IL1RN* as good positional and functional candidate genes affecting several health traits with economic relevance to cattle production.

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