Assignment¹ of the surfactant protein A gene (SFTPA) to bovine chromosome 28q1.8→q1.9 by radiation hybrid mapping

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¹ To our knowledge this is the first time this gene has been mapped.

Rationale and significance

The collectin surfactant protein A (SP-A) is involved in the innate host defense of the lung. It binds to non-self glyco-conjugates on the surface of invading microorganisms. Binding leads to lysis, aggregation and initiation of effector mechanisms that include increased phagocytosis by phagocytic cells and modulation of the inflammatory response (Haagsman, 2002). Across species, the gene encoding SP-A has been found in loci containing genes encoding other collectins. In humans, the collectin gene locus encodes SP-A, surfactant protein D (SP-D) and mannan-binding lectin (MBL) and it maps to chromosome 10q22.3. The genes encoding the bovine collectins conglutinin, collectin 43 (CL-43), collectin 46 (CL-46) and SP-D, have previously been linked to the microsatellites ILSTS099 and IDVGA-8 at chromosome 28 (BTA28) at position q1.8→q1.9 (Gallagher et al., 1993; Hansen et al., 2002, 2003; Gjerstorff et al. 2004). In the present mapping, we find that the gene encoding SP-A maps to the same locus on BTA28.

Materials and methods

For radiation hybrid (RH) mapping, PCR was applied to the 94 clones of the Roslin/Cambridge bovine RH panel (Research Genetics, Huntsville, AL, USA). The primer sequences for amplification of SP-A: 5'-CCA TTA GTT CTC CTT ATC CTC-3' and 5'-TGC AGG CAG TCT TGT CAT TC-3' (amplicon: 850 bp), were derived from a genomic clone, previously identified in our laboratory (GenBank/EMBL accession number: AJ619679). To ensure the specificity of the PCR, the conditions were optimized and the identity of the product was verified by sequencing. Amplification comprised: 5 min of initial denaturation at 94 °C, followed by 35 cycles of 30 s of denaturation at 94 °C, 20 s of annealing at 58 °C and 1 min of elongation at 72 °C. The reactions were finalized with an additional 7 min elongation step at 72 °C. The products were analyzed by 1% agarose gel electrophoresis and the clones were scored into a 94 number vector where 1 is positive and 0 is negative for the SP-A gene.

Results

The obtained SP-A data vector was: ¹1110100000¹⁰, ¹¹0100001101²⁰, ²¹1100001000³⁰, ³¹0010000010⁴⁰, ⁴¹0101101010⁵⁰, ⁵¹0001011100⁶⁰, ⁶¹0000010000⁷⁰, ⁷¹0000000101⁸⁰, ⁸¹0010100000⁹⁰ and ⁹¹1100⁹⁴.

This vector was compared to marker vectors, deposited at The Roslin Radiation Hybrid Database (<http://databases.roslin.ac.uk/radhyb/intro.py>), using the program RHMAP 3.0 (Boehnke et al., 1991). Statistical analysis showed that the SP-A gene is very closely linked to the microsatellite IDVGA-8, located at BTA28q1.8→q1.9 (LOD score 24.55) (Table 1). SP-A was in fact found in all IDVGA-8 positives clones and vice versa. High linkage was found to the SP-D gene and the ILSTS099 marker (LOD scores 14.89 and 7.88, respectively) (Table 1). These results show that the bovine SP-A gene is located in close proximity to the genes encoding conglutinin, CL-43, CL-46 and SP-D.

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Table 1. Results of RH mapping and linkage analysis of bovine SP-A to markers on BTA28

Marker (BTA28)	--	−+	+−	++	<i>P</i> (Break)	Dist (Ray)	LOD
IDVGA-8	64	0	0	28	0.00	0.00	24.55
SP-D	64	0	5	22	0.14	0.15	14.89
ILSTS099	63	1	11	17	0.35	0.43	7.88

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