Short Communication: Identification of Allelic Variation at the Bovine *DRA* Locus by Polymerase Chain Reaction-Single Strand Conformational Polymorphism

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ABSTRACT

The major histocompatibility complex *DRA* locus is noteworthy among the major histocompatibility complex class II loci for the little or no variation reported in many species. In cattle, *DRA* has not been investigated in depth, and the extent of variation at the locus is not yet known. In this study, we used PCR-single strand conformational polymorphism (SSCP) analysis to screen for potential sequence variation in the second exon of bovine *DRA*, which encodes the antigen-presentation groove. Four unique SSCP patterns were detected among 384 cattle from New Zealand. Sequence analysis revealed that these SSCP patterns represent 4 *DRA* alleles; and 3 single nucleotide polymorphisms were detected in exon 2. However, all of the single nucleotide polymorphisms were synonymous, and no amino acid change was therefore expected in this region. The polymorphism detected may be linked to variation elsewhere in the gene that affects its structure or function. **Key words:** cattle, *DRA* locus, polymorphism, polymerase chain reaction-single strand conformational polymorphism

The major histocompatibility complex (MHC) class II molecules are cell-surface glycol proteins that are comprised of noncovalently linked α and β chains. These molecules bind antigen peptides and present them to T lymphocytes for immune recognition and hence play a pivotal role in the initiation of specific immune responses to exogenous antigens. In dairy cattle, there is evidence that certain MHC class II haplotypes are associated with genetic resistance against mastitis (Kelm et al., 1997; Sharif et al., 1998) and bovine leukemia virus infection (Xu et al., 1993; Zanotti et al., 1996).

To investigate the role of MHC genes in immune responsiveness, it is important to have a better understanding of the natural occurrence of MHC polymorphism.

In cattle, there are 2 expressed groups of MHC class II receptors, DQ and DR. For DQ, the α and β chain-encoding genes are highly polymorphic and duplicated in some individual animals, creating extensive diversity for the DQ molecule (Sigurdardottir et al., 1992; Gelhaus et al., 1995; Ballingall et al., 1997). For DR, only one α -chain gene (*DRA*) was identified, and only one β -chain gene (*DRB3*) is known to be functional in cattle (Groenen et al., 1990; Ellis and Ballingall, 1999). Although *DRB3* is highly polymorphic, only one sequence has been reported for bovine *DRA*, despite earlier work that identified RFLP variants (Andersson et al., 1986). As sequence variation has not been reported, the bovine *DRA* gene is thought to be monomorphic (Ellis and Ballingall, 1999). However, this may not be true, especially as *DRA* has not been well studied in cattle, and in sheep the homologue has 3 alleles identified by Southern hybridization (Escayg et al., 1993). Although *DRA* appears to be highly conserved in most mammalian species (Chu et al., 1994; Yuhki and O'Brien, 1997; Smith et al., 2005), polymorphism has been observed in equids (Brown et al., 2004), rhesus macaques (de Groot et al., 2004), and water buffalo (Sena et al., 2003). In this study, we used PCR-single strand conformational polymorphism (SSCP) analysis to examine bovine *DRA* to ascertain whether polymorphism existed at this locus, which may be important in the context of the associations described above between MHC variation and susceptibility to disease in dairy cattle.

Three hundred and eighty-four cattle from 10 New Zealand farms were investigated, and blood samples from these animals were collected on FTA cards (Whatman, Middlesex, UK). Genomic DNA was purified from the blood using a 2-step washing procedure (Zhou et al., 2006).

Two PCR primers were designed based on the published bovine *DRA* sequence (GenBank accession num-

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ber M30120; van der Poel et al., 1990) to amplify the entire exon 2 sequence. These primers were *DRA*-up (5'-TCTTCCTCTCCTGGTTCCCAC-3'), which was located in the 3' end of intron 1, and *DRA*-dn (5'-GCTACAAT TCCCAAGTCTAGG-3'), located in the 5' end of intron 2. Primers were synthesized by Proligo (Boulder, CO). Amplification was performed in a 20- μ L reaction containing genomic DNA on one 1.2-mm disc of washed FTA paper, 0.25 μ M of each primer, 150 μ M of each dNTP (Eppendorf, Hamburg, Germany), 2.5 mM of Mg^{2+} , 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany), and 1 $\times$ the reaction buffer supplied. The thermal profile consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C, with a final extension of 5 min at 72°C. Amplification was carried out in an iCycler (Bio-Rad Laboratories, Hercules, CA). Amplicons with the expected size of 333 bp were visualized by transillumination at 254 nm following electrophoresis at 5 V/cm for 30 min in 1% agarose (Quantum Scientific, Queensland, Australia) gels, using 1 $\times$ Tris-borate-EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA) containing 200 ng/mL of ethidium bromide.

To screen for allelic variants of the *DRA* gene, all amplicons were subjected to SSCP analysis. A 0.7- μ L aliquot of each amplicon was mixed with 7 μ L of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95°C for 5 min, samples were cooled rapidly on wet ice and then loaded on 16 $\times$ 18 cm, 14% acrylamide:bisacrylamide (37.5:1; Bio-Rad Laboratories) gels. Electrophoresis was performed in Protean II xi cells (Bio-Rad Laboratories), at 320 V for 18 h at 12°C in 0.5 $\times$ TBE buffer. Gels were silver-stained according to the method of Sanguinetti et al. (1994). Under the established conditions, 4 unique SSCP binding patterns were resolved, with one or a combination of 2 of these patterns being observed for individual cattle (Figure 1). This was consistent with there being homozygous or heterozygous genotypes at the *DRA* locus.

To determine the nucleotide sequences corresponding to individual SSCP patterns, genomic DNA representatives of these unique SSCP patterns were selected for amplification using the proofreading enzyme Pwo SuperYield DNA polymerase (Roche Applied Science, Mannheim, Germany), and the amplicons were subsequently ligated into the pCR 4 Blunt-TOPO vector (Invitrogen, Carlsbad, CA). A 2- μ L aliquot of the ligation mixture was used to transform competent *Escherichia coli* cells (One Shot INV α F', Invitrogen). Between 10 and 15 insert-positive colonies for each transformation were picked and incubated overnight in Terrific broth (Invitrogen) at 37°C, in a shaking rotary incubator (225

Figure 1. The PCR-single-strand conformational polymorphism of the bovine *DRA* gene. Representative genotypes for the 4 unique SSCP patterns corresponding to 4 allelic sequences *01011, *01012, *01013, and *01014 (GenBank accession nos. DQ821713, DQ821714, DQ821715, and DQ821716, respectively) are shown.

rpm). Plasmid DNA was recovered from bacterial cells by boiling for 10 min in 0.8% Triton X-100 solution, and 1 μ L of the supernatant was used as a template for PCR under the conditions described previously. Amplimers from clones and the corresponding genomic DNA were run adjacent to each other on SSCP gels for comparison of the banding patterns, and only those clones for which the patterns matched those of the corresponding genomic DNA templates were selected for DNA extraction and subsequent DNA sequencing.

Plasmid DNA was extracted from overnight cultures using a QIAprep Spin Miniprep kit (Qiagen). The DNA was sequenced in both directions using the M13-forward and reverse primers at the Waikato DNA Sequencing Facility (University of Waikato, Hamilton, New Zealand). Identical sequences obtained from at least 3 separate clones from different animals, or 2 independent PCR amplifications from the same animal, were subjected to further sequence analysis. Sequence analysis was carried out using DNAMAN (Version 4.0, Lynnon BioSoft, Vaudreuil, Canada) and revealed 4 different nucleotide sequences. One sequence was identical to the published bovine *DRA* sequence, whereas the remaining 3 sequences were novel but shared high

Table 1. Bovine *DRA* allelic variation detected in exon 2

Nucleotide position ¹	Single nucleotide polymorphism	Allele			
		*01011 ²	*01012	*01013	*01014
53	C/T	C	T	C	C
116	G/A	G	G	G	A
197	C/T	C	C	T	C
Frequency (n = 384)		0.64	0.03	0.24	0.09

¹Nucleotide positions are relative to the first nucleotide of exon 2 of the bovine *DRA* gene.

²Identical to the published bovine *DRA* sequence M30120 (van der Poel et al., 1990).

sequence homology to the known bovine *DRA* sequence. This suggests that these sequences represent allelic variants of the bovine *DRA* gene.

Sequence alignment showed there were 3 single nucleotide polymorphisms (SNP) in the bovine *DRA* exon 2 region, which creates 4 alleles in total (Table 1). The bovine *DRA* allele sequences detected here were named as *01011, *01012, *01013, and *01014 according to the BoLA allele nomenclature (Davies et al., 1997; <http://www.projects.roslin.ac.uk/bola/bolahome.html>), and deposited into the NCBI GenBank with the accession numbers DQ821713 to DQ821716.

All of these SNP were synonymous, and no amino acid polymorphism was predicted in the $\alpha 1$ domain of the bovine *DRA* molecule. However, the functional significance of these SNP cannot be ignored because it has been reported that synonymous mutations in coding regions may act alone or in combination with other mutations in the same transcript to influence mRNA stability and translation, thereby causing functional effects (Duan et al., 2003). Additionally the SNP may be linked to variation in other *DRA* gene regions with functional or structural significance. Alternatively, the lack of polymorphism at the amino acid level may be as a result of critical structural or functional constraints in the exon 2 region (Chu et al., 1994).

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