NOTE *Immunology*

Sequence Analysis of the *NRAMP1* Genes from Different Bovine and Buffalo Breeds

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ABSTRACT. The natural resistance associated macrophage protein 1 (*Nramp1*) has been reported to confer resistance or susceptibility to *Mycobacterium bovis*, *Salmonella typhimurium*, and *Leishmania donovani* in the mouse, *Mus musculus*. A Gly and Asp substitution at position 169 of the mouse Nramp protein is invariably associated with the resistant and susceptible phenotypes, respectively. The present study aimed to detect polymorphisms in the *NRAMP1* gene from different cattle and buffalo breeds. Genomic DNAs from five breeds of cattle and four breeds of buffalo were used in the study. Sequencing showed two nucleotide substitutions found in intron 4, three in exon V, and ten in intron 5. An amino acid substitution was observed at nucleotide position 1202 in exon V of the Japanese black, Angus, Philippine and Bangladesh swamp-type buffaloes which coded for Thr, while the Korean cattle, Holstein, African N'dama, In donesian swamp-type buffalo and the Bangladesh river-type buffalo had Ile. All the breeds of cattle and buffaloes tested in this study coded for Gly at the position in exon VI which corresponds to the same amino acid of the murine *Nramp1*-resistant phenotype at position 169. The phylogenetic relationship among the different breeds showed a cluster comprised mainly of cattle and another one mainly of buffaloes.

KEY WORDS: bovine, *NRAMP1*, nucleotide sequence.

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The natural resistance associated macrophage protein 1 (*Nramp1*) has been reported to confer resistance or susceptibility to Mycobacterium bovis, *Salmonella typhimurium*, and *Leishmania donovani* in the mouse, *Mus musculus* [13]. A Gly and Asp substitution at position 169 of the mouse Nramp protein is invariably associated with the resistant and susceptible phenotypes, respectively. Furthermore, these pathogens cause losses in the livestock industry worldwide and are transmissible to the human population despite the widespread application of antimicrobials and vaccination. Bovine (*Bos taurus*) *NRAMP1* cDNA has been cloned and was found to have an 86.9% sequence identity with mouse *Nramp1* [4]. The *NRAMP1* genes of other ruminants have high sequence identity with the bovine *NRAMP1* gene. For example, the *NRAMP1* genes of the bison (*Bison bison*), water buffalo (*Bubalus arnee bubalis*), sheep (*Ovis aries*) and red deer (*Cervus elaphus*) [3, 5–7, 9] were reported to have 99.1%, 98.4%, 97.0% and 96.7% sequence identity with the bovine *NRAMP1* gene, respectively. It was reported in cattle that a microsatellite polymorphism in the 3' untranslated region affected the expression of the *NRAMP1* gene and also affected on *Brucella abortus* replication [1], but it was not associated with *M. bovis* infection [2]. The present study aimed to detect polymorphisms in the *NRAMP1* gene from different cattle and buffalo breeds.

Genomic DNAs from five breeds of cattle and four breeds of buffalo were used in the study. The cattle DNAs used were from Japanese black, African N'dama, Korean, Holstein, and Angus breeds. The buffalo DNAs used were from the Bangladesh swamp-type, Bangladesh river-type, Philippine swamp-type, and Indonesian swamp-type breeds. Based on the sequence of the sheep *NRAMP1* gene [10], the primers for the polymerase chain reaction (PCR) were designed using the Oligo 4.05 program (Oligo Primer Analysis Software, Plymouth, MN). A 433-bp fragment from nucleotide positions 1071 to 1504 was amplified by PCR using the forward primer 5'-CATGAAGCCAACTGGCAAGG-3' and the reverse primer 5'-GAAGCCTGCAAGATGACCAACA-3'. A 419-bp fragment between positions 1433 and 1852 was also amplified using the forward primer 5'-GCGGAAACGAGACCCAGATA-3' and the reverse primer 5'-ACTTTCATTGCCAGCCCCCTC-3'. The PCR was carried out using *Taq* polymerase on a Perkin Elmer DNA Thermal Cycler with a cycling profile comprised of an initial denaturing step of 5 min at 94°C, followed by 33 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on 1% agarose gels and detected by ethidium bromide. Four independent PCR products from each breed were purified using the Geneclean purification kit (Bio 101 Inc., La Jolla, CA) according to the manufacturer's protocol, cloned into pGEM-T Easy vector (Promega, Madison, WI), and at least four clones from each PCR product were subsequently sequenced. The nucleotide sequence was determined using an ABI Prism dRhodamine Terminator cycle sequencing kit (Perkin Elmer, Norwalk, CT) with an ABI 310 Prism genetic analyzer (Perkin Elmer). All of the sequences of *NRAMP1* cDNA were compared with one another among the breeds examined and analyzed using the DNASIS program (Hitachi Co., Tokyo,

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Table 1. Nucleotide substitutions in the region between the positions 1071 and 1852 (781 bp) of the *NRAMP1* gene from the different bovine and buffalo breeds. The amino acid substitution from Thr to Ile at position 1202 is indicated

Position	Intron 4		Exon 5			Intron 5									
Breed	1130	1140	1202	1260	1290	1316	1319	1341	1348	1405	1418	1426	1462	1634	1664
Jap. Blk	g	g	c^{Thr}	t	c	t	t	c	t	g	a	g	g	a	a
Korean	-	-	t^{Ile}	-	-	-	-	-	-	-	-	-	-	-	-
Holstein	-	-	t^{Ile}	-	-	-	-	-	-	-	-	-	-	-	-
Angus	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N'Dama	t	-	t^{Ile}	-	-	-	-	-	-	-	-	-	a	g	-
Phil.Swamp	-	c	-	c	-	-	-	-	c	-	c	a	-	-	-
Indo.Swamp	-	c	t^{Ile}	c	-	-	-	-	c	-	c	a	-	-	g
Bang.Swamp	t	-	-	-	a	c	-	a	c	a	c	-	-	-	g
Bang. River	-	c	t^{Ile}	c	a	-	g	-	c	-	-	a	-	-	-

Abbreviations are as follows: Japanese black (Jap. Blk.), African N'dama (N'Dama), Philippine (Phil.), Indonesia (Indo.), Bangladesh (Bang.), Swamp-type buffalo (Swamp), and River-type buffalo (River).

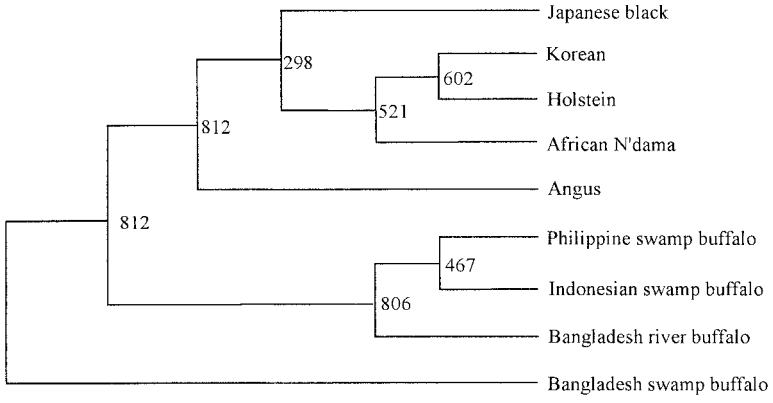

Fig. 1. A phylogenetic tree of bovine *NRAMP1* was constructed using the neighbor-joining method [11].

Japan). The phylogenetic analyses were done using the neighbor-joining (NJ) method [11]. Alignment of multiple sequences was done using the CLUSTAL X program [12].

The 781-bp region between positions 1071 and 1852 of the *NRAMP1* covers the area between intron 4 and intron 6. Since the region of the murine *Nramp1* which is a match for exon VI of the bovine *NRAMP1* is related with the amino acid substitution causing the resistant or susceptible phenotypes, it would be interesting to determine the sequence of exon VI and the adjacent region of the *NRAMP1* gene. As shown in Table 1, two nucleotide substitutions were found in intron 4, three in exon V, and ten in intron 5. There were no nucleotide substitutions found in exon VI or in intron 6. An amino acid substitution was observed at nucleotide position 1202 only in exon V. Japanese black, Angus, Philippine and Bangladesh swamp-type buffaloes coded for Thr (ACT) at this position, while the Korean cattle, Holstein, African N'dama, Indonesian swamp-type buffalo and the Bangladesh river-type buffalo had Ile (ATT). We will study in the future whether this amino acid exchange might influence the resistivity against bacterial infection. It was reported in the mouse that the resistant *Nramp1* genotype carries Gly at position 169 in exon VI but the sensitive type has Asp [8]. All the breeds of cattle and buffaloes tested in this study coded for Gly at the position in exon VI, which corresponds to the same amino acid of the murine *Nramp1*-resistant phenotype at position 169. Based on these substitutions in the sequence, a phylogenetic tree was constructed as shown in Fig. 1. The phylogenetic relationship among the different breeds showed a cluster comprised mainly of cattle and another one mainly of buffaloes.

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