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BRIEF REPORT — POLYMORPHISM REPORT

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Dinucleotide repeat polymorphism in the third intron of the *NRAMP2/DMT1* gene

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Abstract Nramp1 (natural resistance-associated macrophage protein 1), encoding a polytropic integral membrane protein, was isolated as a candidate of the mouse *Lsh/Ity/Bcg* locus, which regulates macrophage activation for antimicrobial activity against intracellular pathogens. The *NRAMP2* gene was cloned from human genome as a homologue of *NRAMP1*. We found a polymorphic dinucleotide repeat in the third intron of the *NRAMP2* gene. This polymorphism will be a useful genetic marker to study disease associated with susceptibility to infection with intracellular pathogens.

Key words *NRAMP2* · *DMT1* · Dinucleotide repeat · Iron transport

Introduction

In the mouse, natural (innate) resistance of the host to infection with intracellular pathogens such as Leishmania, Salmonella, and Mycobacterium is controlled by the *Lsh/Ity/Bcg* locus. The *Nramp1* (natural resistance-associated macrophage protein 1) gene was positionally cloned from the *Lsh/Ity/Bcg* locus in the mouse genome (Vidal et al. 1993), revealing that the gene product has features characteristics of an integral membrane protein, including 12 putative transmembrane segments. Expression of the *Nramp1* gene is controlled in a tissue-specific manner and is limited exclusively to phagocytic cells. The cDNA and genomic DNA for the corresponding human counterpart of *Nramp1* were isolated and characterized (Kishi 1994; Kishi et al. 1996). Afterward a second gene, *NRAMP2*, was identified in the mouse and human genomes (Gruenheid et al. 1995; Kishi and Tabuchi 1997), and we isolated the human *NRAMP2* gene and determined the complete DNA sequence, which is approximately 42 kb in length, containing 17 exons (GenBank accession number AB015355) (Kishi and Tabuchi 1998). Gunshin et al. (1997) electrophysiologically identified a rat divalent metal transporter, *DMT1*, and found it to be an isoform of *Nramp2*. *Nramp2/DMT1* exihibits an unusually broad substrate range, including Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, and Pb^{2+}, and mediates active proton-coupled transport. The functional characteristics of *NRAMP1* and *NRAMP2* were determined by expression in a divalent metal transporter-disrupted strain of yeast cells (Tabuchi et al. 1999). Therefore a better understanding of genes responding to infection with intracellular pathogens as well as of divalent metal transport in the cells is important from the biological and the clinical points of view. To investigate the relationship between genetic variations at the *NRAMP2/DMT1* locus and the host susceptibility to infections with various ferrophilic intracellular pathogens, including several Mycobacteria species, we characterized a polymorphic microsatellite in the *NRAMP2/DMT1* gene.

Source and description

A sequence of a microsatellite was identified in the third intron of the *NRAMP2* gene through genome sequence analysis. Both the TA and CA repeats appear to be polymorphic. Polymerase chain reaction (PCR) primers were designed to amplify a fragment containing the dinucleotide repeat region.

PCR primer sequence

Forward: 5′-GTTCAGCAGCCTCAGGTTTAAT-3′
Reverse: 5′-CTATGGTTGTGCCACTGCACT-3′

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A

GTTCAGCAGC CTCAGGTTTA ATTTTGGCTG GCAATCTCTG AAGACTATAT
CTATATATCT ATATATATAT ATACACACAC ACACACACAC ACACACCCCA
TCTATATCTA TCTATCTCTG TCTGTCTGTC TGTCTGTCCG TCTATATATC
TATCTATCTA TCTATCTATC TGTCTATCTA ATCTTTTTTA CAGACAGGGT
CTTGCTCTGT CATCTAGGCT CCAGTGCAGT GGCACAACCA TAG

B

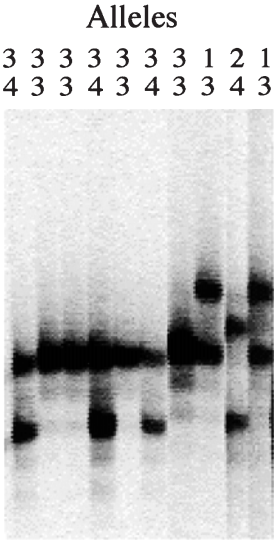

Fig. 1. A Nucleotide sequence of the polymorphic repeat and the flanking region of allele 3 at the natural resistance-associated macrophage protein 2 (*NRAMP2*)/divalent metal transporter 1 (*DMT1*) locus. Sequences used for forward and reverse primers are *underlined*. **B** Infrared fluorescence image showing a polymorphic repeat at the *NRAMP2* locus in ten unrelated individuals

PCR conditions

PCR was performed in 7.5-μl volumes of a mixture containing 50 ng of genomic DNA, 1 unit of KOD Dash DNA polymerase (Toyobo, Osaka, Japan), 1 × PCR buffer (120 mM Tris HCl [pH 8.8], 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 10 μg/ml bovine serum albumin, 1.25 mM each of deoxyribonucleotide triphosphate [dNTP]s, 2.5 mM $MgCl_2$), 0.5 pmol of an infrared fluorescence-labeled forward primer, and 0.5 pmol of nonfluorescence reverse primer. The cycle conditions were 95°C for 2 min, and then 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension step of 7 min at 72°C, in a GeneAmp 9 600 thermocycler (Perkin Elmer Cetus, Foster City, CA, USA). The PCR products were electrophoresed in 0.25-mm-thick denaturing 7% polyacrylamide gels at 1 500 V for 1–2 h, using a LI-COR 4 000L automated DNA sequencing apparatus (LI-COR, Lincoln, NE, USA). The sizes of the alleles were determined by comparison with a sequencing ladder of a control plasmid.

Polymorphism and allele frequency

Frequency. Four alleles were detected in 140 chromosomes of DNA from 70 unrelated Japanese individuals (Table 1).

Table 1. Size and frequency of the alleles of the polymorphic microsatellite from genomic DNA of 70 unrelated Japanese individuals

Allele	Size (bp)	Frequency
1	247	0.014
2	245	0.021
3	243	0.743
4	239	0.221

A representative infrared fluorescence image of the repeat polymorphism is shown in Fig. 1A. The observed frequency of heterozygotes was 0.45.

Mendelian inheritance. Codominant inheritance was observed in three two-generation families.

Chromosomal localization. The *NRAMP2* gene was localized to chromosome 12q13 (Vidal et al. 1995).

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