

Novel MICA Allele Identified Through Phased Sequencing

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The major histocompatibility complex (MHC) class I chain-related gene A (*MICA*) encodes a protein similar in structure to MHC class I cell surface receptors that plays a role in innate immune responses. *MICA* is involved in tumor surveillance, viral infections, and autoimmune diseases. *MICA* genes are highly polymorphic, with 80 different alleles encoding 63 proteins and 2 null proteins. Like other human leukocyte antigen (HLA) molecules, *MICA* proteins have three immunoglobulin-like domains exposed on the cell surface, ($\alpha 1$, $\alpha 2$, $\alpha 3$), a transmembrane and a cytoplasmic domain. Most of the known polymorphisms are located in the $\alpha 2$ domain of *MICA*, and can lead to altered immune responses. Researchers in Dr. Effie Pettersdorf's group in the Clinical Research Division developed a sequence-based method to link polymorphisms across exons 1-5 of the *MICA* gene and identified a novel polymorphism in the $\alpha 1$ domain.

MICA is expressed on epithelial cells, especially of the gastrointestinal (GI) tract, fibroblasts, endothelial cells, monocytes and dendritic cells. *MICA* binds the activating NKG2D receptor on natural killer (NK) cells and certain T-cells to induce cytolytic activity and/or cytokine production. Immune responses in solid organ transplant recipients are mounted against different protein forms of *MICA* alleles. Antibodies can be generated by mismatched amino acids in transplant recipients and have been found against *MICA* in organ transplants that are rejected. Presence of *MICA* antibodies is associated with decreased kidney transplant survival and also seen for heart transplants. *MICA* mismatching in hematopoietic cell transplantation has been associated with increased risk of graft-versus-host disease (GVHD), with allogeneic transplanted T-cells or NK cells targeting mismatched *MICA* proteins in the GI tract of the transplant recipient.

For transplant recipients, donors are matched for most HLA antigens to reduce GVHD. Most of the genes that code for HLA antigens are located in the same region of the genome, known as the MHC region. *MICA* is located in this region, located 46 kilobases centromeric to *HLA-B*, an HLA antigen used to match donor and recipients. Because of the close proximity, *MICA* alleles are linked to inheritance of *HLA-B* alleles. By mapping the MHC region for all polymorphisms, recipients and donors can be matched better to reduce the risks of GVHD or graft rejection in transplant recipients.

Previously, *MICA* alleles have been genotyped through sequence-specific primers, sequence-specific oligonucleotide probes, or direct sequencing of the gene to identify nucleotide changes. Typing of the transmembrane region (exon 5) of *MICA* has commonly been performed by PCR and sizing of the amplified fragments that consist of variable numbers of GCT nucleotide repeats. Research technician Dawn Morgan and colleagues in the Pettersdorf lab developed a new method to co-amplify and sequence both alleles of *MICA* across exons 1-5 and to physically link polymorphisms across the exons using haplotype-specific extraction (HSE) to separate diploid genomic DNA into its haploid components. Using this method, 52 known polymorphisms in exons 2, 3, and 4 can be genotyped for polymorphic allele combinations with the exception of 16 alleles that have identical sequences in exons 2, 3, and 4. 595 of 1217 ambiguous allele combinations can further be identified by sequencing exon 5 in the reverse direction to examine the four polymorphic positions and the GCT short tandem repeat in this exon.

By using this method, the researchers identified the novel *MICA**070 allele in an individual with Caucasian background. This allele was similar to sequences of alleles *MICA**008:01 and/or *MICA**008:04 in exons 2, 3, 4, and 5. Two oligonucleotide probes were designed to separate the *MICA**008 allele in exon 1 and *MICA**070 allele in exon 2 using HSE and determine the linkage of the two alleles, whether they occur on the same DNA strand of the chromosome. This phasing showed that the *MICA**070 allele is a variant of the *MICA**008:04 allele, with the additional substitution of G to C in nucleotide position 183 in exon 2. This results in a non-synonymous amino acid change of arginine to serine at residue 38 of the α 1 domain. This novel allele increases the diversity of the *MICA* genetic locus, and suggests that complete characterization of exons 1-5 is required to type the majority of the *MICA* polymorphisms.

A homodimer of NKG2D receptors binds the α 1 and α 2 domains of *MICA* (see figure), as determined by structural analysis in the laboratory of Dr. Roland Strong in the Basic Sciences Division (Li *et al.*, 2001). Since the amino acid substitution of serine at residue 38 instead of arginine is within the region of NKG2D binding, future studies are needed to determine the significance of this amino acid change in *MICA* and NKG2D interactions. Disruption or weakening of *MICA* interaction with NKG2D may alter the activation of NK cells or the modulation of T-cells (Steinle *et al.*, 2001).

[Moran D., Morishima S., Malkki M., and Petersdorf E.W.](#) 2012. Identification of the *MICA*(*)070 allele by sequencing and phasing. *Human Immunology*. Epub ahead of print, doi: 10.1016/j.humimm.2012.11.009.

Also see: [Zou Y. and Stastny P.](#) 2010. Role of *MICA* in the immune response to transplants. *Tissue Antigens*. 76:171-6.

[Li P., Morris D.L., Willcox B.E., Steinle A., Spies T., and Strong R.K.](#) 2001. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nature Immunology*. 5:443-51.

[Steinle A., Li P., Morris D.L., Groh V., Lanier L.L., Strong R.K., and Spies T.](#) 2001. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics*. 53:279-8

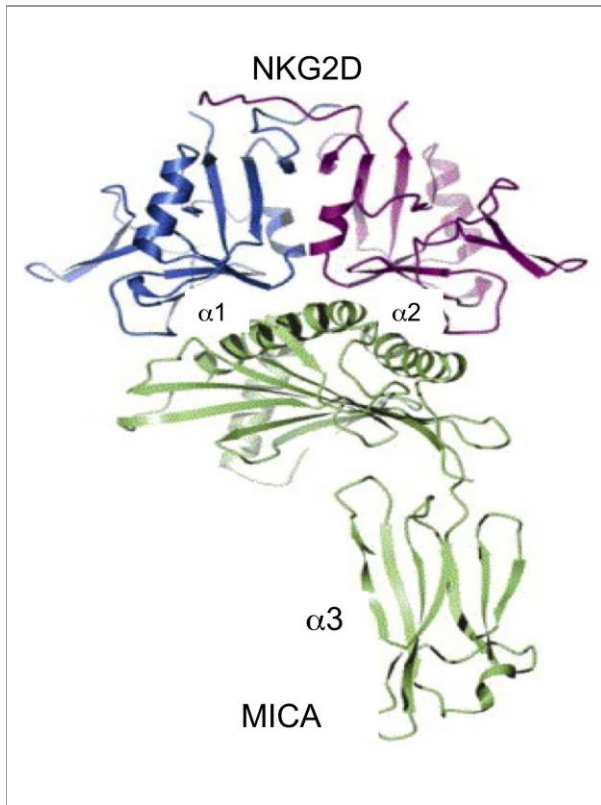


Image adapted from Li et al., 2001

Ribbon diagram showing crystal structures of NKG2D bound to MICA. The NKG2D homodimer is colored in blue and magenta, MICA is green with domains labeled. NKG2D recognizes the alpha1 and alpha2 domains of MICA. The novel MICA*070 allele has a serine substitution at amino acid 38 in the alpha1 domain, which may impact binding to NKG2D.