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Identification of the MICA*070 allele by sequencing and phasing

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Abstract

A novel MICA allele, MICA*070, was defined by sequencing. The new allele differs from the MICA*008:04 sequence in exon 2, encoding a C instead of G corresponding to cDNA nucleotide position 183. This nucleotide substitution is predicted to encode serine instead of arginine at residue 38 of the α1 domain of the MICA molecule.

Keywords

Major histocompatibility complex class I chain-related A genotype; Sequence-based typing; Phasing

The major histocompatibility complex (MHC) class I chain-related A (MICA) gene resides 46 kb centromeric to HLA-B, and plays a major role in the innate immune response [1, 2]. MICA is a highly polymorphic gene, encoding 80 unique alleles that give rise to 63 proteins and 2 null proteins (IMGT/HLA Database v.3.7.0, http://www.ebi.ac.uk/imgt/hla/) [3]. Previous studies have identified strong positive linkage disequilibrium of MICA and HLA-B alleles in ethnically diverse populations [4].

MICA is a ligand for the activating NKG2D receptor. Its role in hematopoietic cell transplantation has been explored in two models: recipient homozygosity and donor-recipient mismatching [5–7]. Patients homozygous for the residue 129 valine were found to be at higher risk for chronic GVHD after HLA-matched sibling HCT compared to patients homozygous for the methionine at this position, independent of acute GVHD [5]. The role of donor-recipient MICA mismatching has been evaluated by two recent studies [6, 7]. MICA mismatching was associated with an increased risk of grades II–IV acute GVHD but not grades III–IV acute GVHD in both HLA 10/10 matched and HLA-mismatched

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Conflict of interest

None of the authors have competing interests.

Supporting Information

The following supporting information is available for this manuscript:

Table S1 All possible 52 nucleotide string combinations descriptive of known pairwise MICA allele combinations according to IMGT/HLA release v3.7.0.

Table S2 Further characterization of Exon 5 for groups of alleles with identical sequences in exon 2, 3 and 4.

Table S3 All possible pairwise sequence combinations descriptive of the heterozygous short tandem repeat (STR).

transplants [6]. The organ system most significantly involved in acute GVHD was the gastrointestinal tract and the association was independent of HLA-B and C mismatches. These data suggested that expression of MICA on the surface of intestinal cells may interact with NK cells through the NKG2D receptor as well as with γδ and αβ T cells. In a second study of patients receiving HLA 12/12 matched unrelated donor transplants, there was no evidence for an association between MICA mismatching and risk of GVHD [7]. Further examination of an independent cohort of 1676 HLA 8/8 matched unrelated donor transplants showed no differences in GVHD risk based on linkage of HLA-B alleles to MICA.

To provide a comprehensive genetic strategy for examining the MICA locus, we developed a sequence-based method that permits the physical linkage of polymorphisms across exons 1–5 of MICA. Our DNA-based approach is an alternative method to traditional cloning. We describe a novel MICA*070 allele in an individual of Caucasian background with the genotype HLA-A*02:01/09/43N, 02:01/09/43N; B*08:01/04, 40:01/07; Cw*07:01/06, 03:04; DRB1*03:01, 01:01; DQB1:02:01/02, 05:01. DNA samples from family members were not available. This study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center (FHCRC).

The sequencing protocol was developed to co-amplify and sequence both alleles of the MICA gene. Genomic DNA was prepared by the salting out method [8]. Oligonucleotide sequences and locations of primers used in this study are presented in Table 1 and Figure 1. A 1131 bp fragment containing exons 2 and 3 was PCR-amplified using primers a and b. A 783 bp fragment containing exons 4 and 5 was amplified using primers c and d. PCR reactions were conducted in a 12 μl volume containing 35–100 ng of genomic DNA, 1X Apex Hot Start Master Mix (Genesee Scientific, San Diego, CA) and 0.42 μM of reverse and forward primers (Table 1, Figure 1). PCR amplification was performed using the ABI GeneAmp®9700 PCR system (Applied Biosystems, Foster City, CA) with the following cycling parameters: activation of the Apex Taq polymerase at 95°C for 15 min; denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 2 min for 30 cycles; a final extension at 72°C for 7 min, and a 4°C hold. Five microliters of the amplified product was electrophoresed on a 2% agarose gel at 125 V for 45 minutes to verify the expected size of the product. Prior to sequencing, 5 μl of the amplified product was purified from the excess dNTPs and PCR primers by adding 2 μl of ExpSAP-IT (USB, Cleveland, OH). The mixture was incubated in an ABI GeneAmp®9700 PCR system for 20 minutes at 37 °C followed by 15 minutes at 80 °C and a 4 °C hold.

Purified products (1 μl) were sequenced using the ABI BigDye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Primer e was used to sequence exon 2 in the forward direction; primer b was used to sequence exon 3 in the reverse direction. Primer c was used to sequence exon 4 in the forward direction; primer d was used to sequence exon 5 in the reverse direction (Table 1, Figure 1). Sequenced products were purified using the BigDye XTerminator™ Purification Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Samples were electrophoresed for 2 hours using an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

Exon 2, 3 and 4 sequences were analyzed using Sequencher 5.0 software (Gene Codes Corporation, Ann Arbor, MI). Data from each exon were concatenated from variance tables into a nucleotide string containing all 52 known polymorphic positions of exons 2, 3 and 4 (IMGT/HLA Database v.3.7.0, http://www.ebi.ac.uk/imgt/hla/). The 52-nucleotide string from the sample was imported into a FileMaker Pro v10.0 program (FileMaker Inc., Santa Clara, CA) and cross-referenced against the pairwise combinations of known alleles (Table S1, *Supporting Information*). Allele combinations that show unique polymorphisms in exons 2, 3 and 4 can be definitively genotyped with this method with the exception of 16 alleles

that have identical sequences in exons 2, 3 and 4 (Table S2). Four polymorphic positions and the GCT short tandem repeat (STR) in exon 5 permit delineation of 595 out of 1217 ambiguous allele combinations (Table S1, Table S2; IMGT/HLA Database v.3.7.0, http://www.ebi.ac.uk/imgt/hla/). The exon 5 sequence of the sample was manually compared to all possible pairwise combinations of the eight types of GCT repeats (modified from the approach described by Zou *et al* [11]; Table S3).

The exon 2, 3, 4 and 5 sequences of the MICA*070 allele were consistent with MICA*008:01 and/or MICA*008:04 with a new substitution of G/C corresponding to cDNA nucleotide position 183 in exon 2, which is predicted to encode a non-synonymous amino acid change from arginine (AGG) to serine (AGC) at residue 38 of the α1 domain (Figure 2). Three approaches were taken to verify the substitution and to determine the most likely allele. First, to confirm the presence of the nucleotide substitution, exon 2 was resequenced in forward and reverse directions (amplified with primers a and b, and sequenced with primers e and h; Table 1, Figure 1) from two independent DNA preparations. Second, exon 1 was sequenced to determine whether the sample was homozygous MICA*008:01, homozygous MICA*008:04, or heterozygous. Exon 1 was amplified and sequenced using primers f and g. The sample displayed heterozygous incorporation of both C and T at nucleotide position 21, consistent with MICA*008:01 and 008:04. Finally, to determine whether the novel allele is more closely related to MICA*008:01 or 008:04, DNA was extracted from a lymphoblastoid cell line using the EZ1® DNA tissue kit with the EZ1® Advanced Robot (Qiagen, Valencia, CA). Two oligonucleotide probes were designed to be specific for the novel exon 2 substitution: 5'-TGCCCTGCATTTCTG**G**C-3' and 5'-TGCCCTGCATTTCTG**C**C-3' (Figure 3). The two alleles were separated using the EZ1® HaploPrep kit following the manufacturer's protocol. Exons 1 and 2 of each allele were amplified and sequenced as described above. One allele carried C corresponding to cDNA nucleotide position 21 in exon 1 and G corresponding to cDNA nucleotide position 183 in exon 2, and hence is the MICA*008:01 allele. The second allele carried T corresponding to cDNA nucleotide position 21 in exon 1 which is observed in MICA*008:04 and the novel C substitution corresponding to cDNA nucleotide position 183 in exon 2 (Figure 2). Hence, MICA*070 is a variant of MICA*008:04. The MICA genotype for this individual is MICA*008:01, 070. MICA*070 has been assigned the GenBank JQ522969 accession number. Sequence data for exon 1 in addition to data for exons 2, 3, 4 and 5 permit delineation of 808 of 1217 allele combinations.

Our phased sequencing method provides an approach for investigating the role of MICA in disease and complements the techniques are currently available [10, 11]. MICA*070 provides evidence for the diversity of the MICA genetic locus. Although a family study was not available to assign the HLA-B haplotype to MICA*070, our data suggest that complete characterization of exons 1, 2, 3, 4 and 5 is required to definitively type the majority of the alleles of this locus. MICA*070 furthermore sheds light on potential functional consequences of sequence variation in the α1 domain. The 63 known MICA proteins to date all encode arginine at residue 38 of the α1 domain. The MICA*070 allele is the first reported sequence to have a substitution at this residue. Interestingly, crystallographic studies indicate a role for residue 38 in interactions with the NKG2D receptor [12]. Future studies are indicated to define the significance of the residue 38 serine substitution in ligand-receptor interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

EWP designed the study and procured funding. DM designed the reagents and performed genotyping. All authors contributed to data analysis and preparation of the manuscript. This work was supported by grants CA100019 and CA18029 (EWP, MM, DM) from the US National Institutes of Health, and Research on Allergic Disease and Immunology (Health, and Labor Science Research Grant), the Ministry of Health, Labour and Welfare of Japan (SM).

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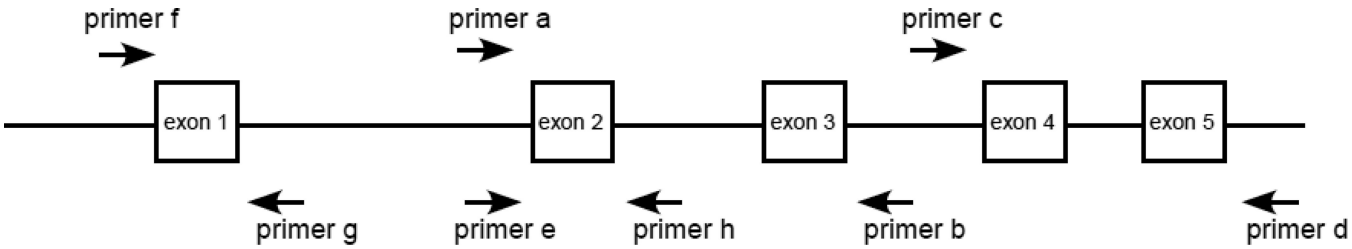

Fig. 1. MICA amplification and sequencing primers (not to scale)

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AA Residue[1]                -20                -15                -10                 -5                  1
MICA*001          ATG GGG CTG GGC CCG GTC TTC CTG CTT CTG GCT GGC ATC TTC CCT TTT GCA CCT CCG GGA GCT GCT GCT G|AG CCC
MICA*008:01:01    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -|-- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -|-- ---
MICA*008:04       --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -|-- ---
MICA*070          --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -|-- ---

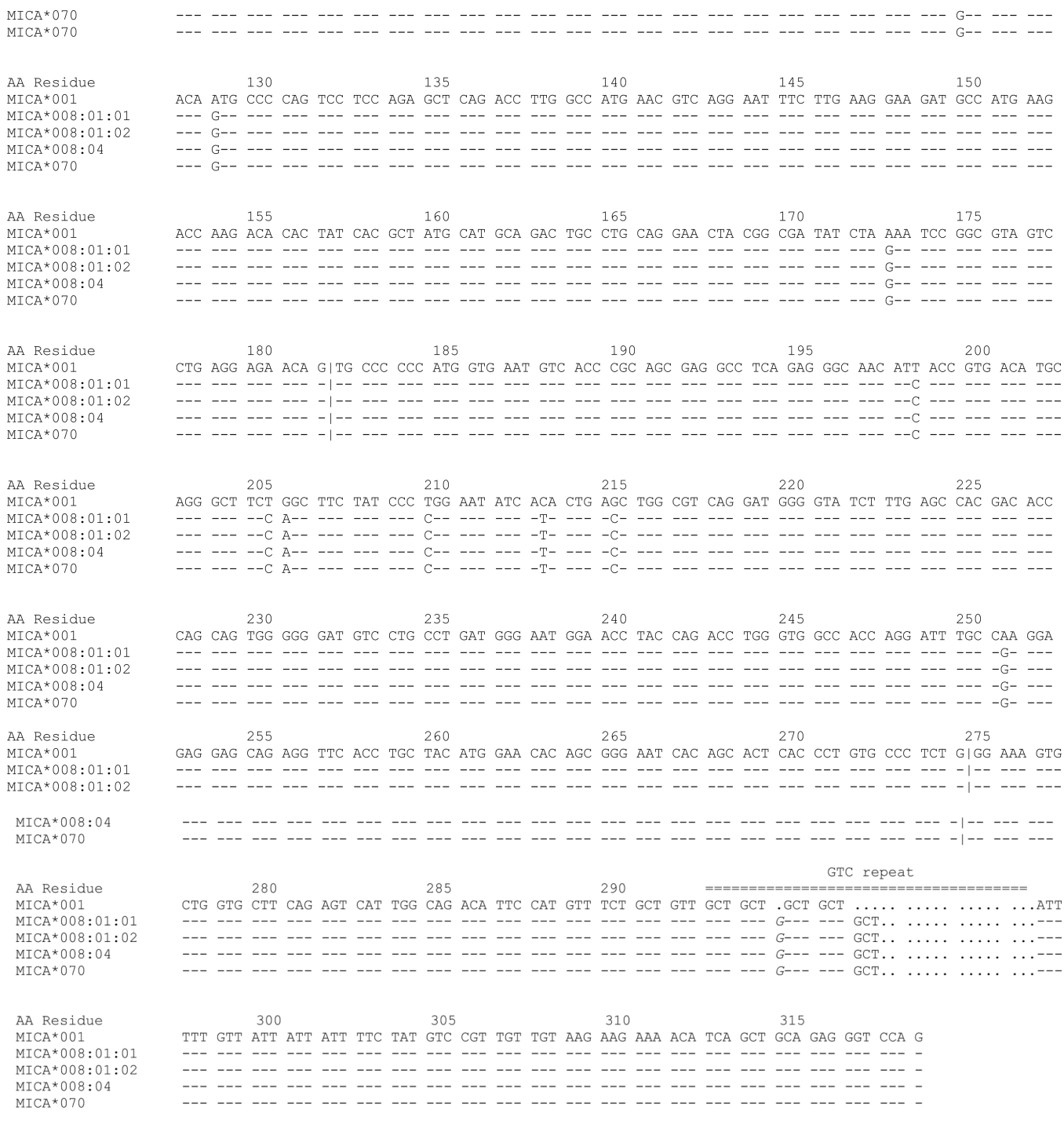
AA Residue                5                  10                 15                  20                  25
MICA*001          CAC AGT CTT CGT TAT AAC CTC ACG GTG CTG TCC TGG GAT GGA TCT GTG CAG TCA GGG TTT CTC ACT GAG GTA CAT
MICA*008:01:01    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T G-- --- --- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T G-- --- --- ---
MICA*008:04       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T G-- --- --- ---
M MICA*070        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T G-- --- --- ---

AA Residue               30                  35                 40                  45                  50
MICA*001          CTG GAT GGT CAG CCC TTC CTG CGC TGT GAC AGG CAG AAA TGC AGG GCA AAG CCC CAG GGA CAG TGG GCA GAA GAT
MICA*008:01:01    --- --- --- --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:04       --- --- --- --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*070          --- --- --- --- --- --- --- --- -A- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Residue               55                  60                 65                  70                  75
MICA*001          GTC CTG GGA AAT AAG ACA TGG GAC AGA GAG ACC AGA GAC TTG ACA GGG AAC GGA AAG GAC CTC AGG ATG ACC CTG
MICA*008:01:01    --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:04       --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*070          --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Residue               80                  85                  90                  95                 100
MICA*001          GCT CAT ATC AAG GAC CAG AAA GAA G|GC TTG CAT TCC CTC CAG GAG ATT AGG GTC TGT GAG ATC CAT GAA GAC AAC
MICA*008:01:01    --- --- --- --- --- --- --- --- -|-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- -|-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*008:04       --- --- --- --- --- --- --- --- -|-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
MICA*070          --- --- --- --- --- --- --- --- -|-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Residue              105                 110                 115                 120                 125
MICA*001          AGC ACC AGG AGC TCC CAG CAT TTC TAC TAC GAT GGG GAG CTC TTC CTC TCC CAA AAC CTG GAG ACT AAG GAA TGG
MICA*008:01:01    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- ---
MICA*008:01:02    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- ---
```



[1]Amino acid (AA) residue is numbered according to the IMGT/HLA database v.3.7.0 (http://www.ebi.ac.uk/imgt/hla/).
Exon boundaries are marked with vertical lines.
MICA*008:01:01 and MICA*008:01:02 differ in intron 1 which was not sequenced in this study.
MICA*070 encodes C at cDNA nucleotide position 183 in exon 2 (in bold). This is predicted to encode a non-synonymous amino acid change from arginine (AGG) to serine (AGC) at residue 38 of the α1 domain.
The short tandem repeat (STR) of $(GCT)_n$ is marked with double underline.
The insertion of G present in the MICA*008 family is marked in italics.

Fig. 2. Alignment of MICA*008:01, MICA*008:04 and MICA*070

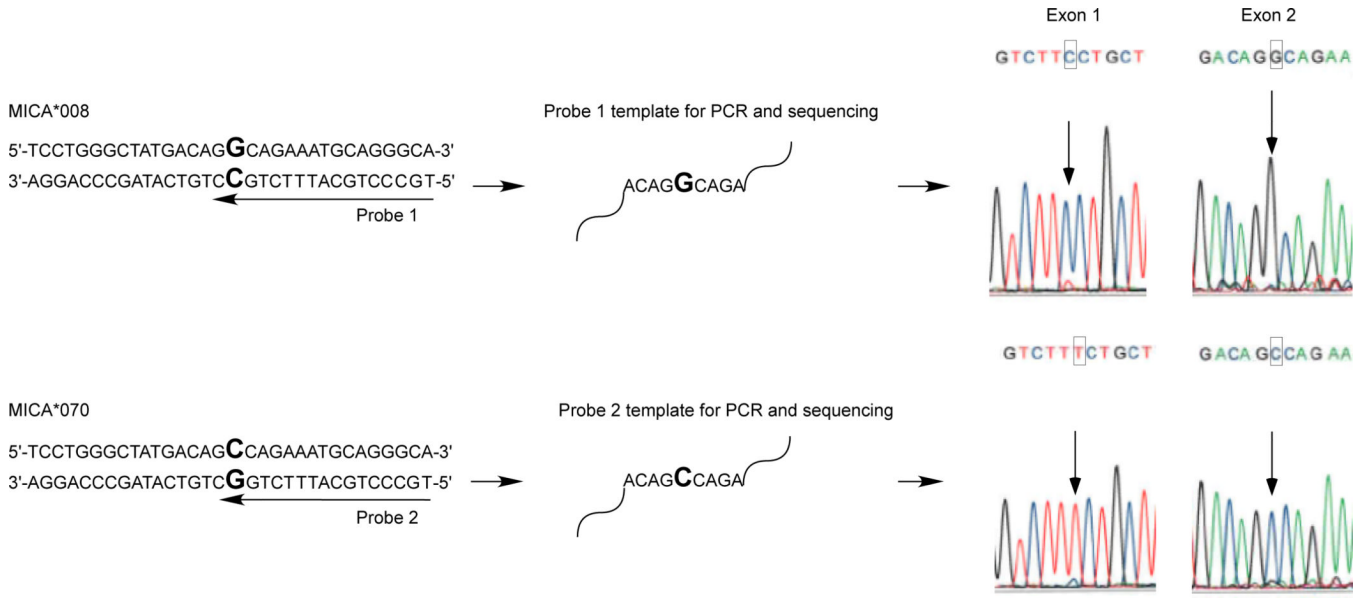


Fig. 3. Phasing of the new allele

Two oligonucleotide probes were designed to separate MICA*008 from MICA*070 using the Qiagen HaploPrep kit. Separated alleles were amplified and sequenced to determine the linkage of exon 1 to exon 2. Original chromatograms depict the novel exon 2 substitution at cDNA nucleotide position 183 in MICA*070 compared to MICA*008.

Table 1

Oligonucleotide primers used for amplification and sequencing

Primer name[1]	Sequence 5'-3'	Orientation	Location[2]
Primer a	CCCCCTTCTTCTGTTCATCA	Forward	10561–10580
Primer b	CCAACAGGAAATGCCTTCAT	Reverse	11673–11692
Primer c	CCAGAGTGAGGACAGACTTGC	Forward	12025–12045
Primer d	CATGCCTATCTTTGCAGGAG	Reverse	12744–12763
Primer e	ATTTCCTGCCCCAGGAAGGTTGG	Forward	10658–10680
Primer f	TCGTGATTGGCCCTAAGTTC	Forward	3714–3733
Primer g	CCGAGGAGGACTGAAAAGTG	Reverse	4092–4111
Primer h	CCTGCTGAGTTCCACTGAC	Reverse	11131–11152

[1] Primers b, c, d, f and g were designed using the Primer3 program v. 0.4.0 (http://frodo.wi.mit.edu/) [9], primers a and h according to reference 10 and primer e according to reference 11.

[2] Numbered according to NCBI reference of MICA sequence (http://www.ncbi.nlm.nih.gov/nuccore/NC_000006.11?report=genbank&from=31367561&to=31383090).