Genes and Immunity (2004) 5, 371–374

www.nature.com/gene

FULL PAPER

MICA intron 1 sequences of conserved extended HLA haplotypes: implications for sequencing-based typing

W Shao, ES Lobashevsky, RA Kaslow and MT Dorak
Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA

The human major histocompatibility complex (MHC) class I chain-related gene A (MICA) has a high degree of genetic diversity. Several methods have been used in MICA typing. Recent studies reported different results for the same reference cell lines typed by different methods. By searching the GenBank, we found an indel polymorphism in MICA intron 1 corresponding to the area where one of the sequencing-based typing primers used by others is located. We investigated this polymorphism in 43 reference samples by primer cycle sequencing. This approach revealed three haplotype-specific patterns of polymorphisms in intron 1. This study provided evidence that one of the primers commonly used in MICA typing may fail to amplify both alleles in certain heterozygous combinations. Our data showed a correlation between the three patterns in MICA intron 1 and exon 5 short tandem repeat (STR) alleles. Being neutral ones, the intron 1 and STR polymorphisms appeared to mark the ancestral lineages better than the coding region polymorphisms.
Genes and Immunity (2004) **5,** 371–374. doi:10.1038/sj.gene.6364103
Published online 27 May 2004

Keywords: *MICA; genetic polymorphism; HLA complex; sequencing-based typing*

Introduction

MHC class I polypeptide-related sequence (MIC) genes represent a second lineage of mammalian MHC class I genes.[1] The MICA (MHC class I chain-related gene A) (MICA; Locus ID: 4276) gene is highly polymorphic and has a unique pattern of tissue expression.[2,3] Although MICA protein is MHC class I related, it does not associate with beta-2-microglobulin; it may function as a stress-induced antigen that is broadly recognized by intestinal epithelial γδ T cells and as a ligand for cells expressing the activating natural killer-cell receptor NKG2D.[3] The involvement of MICA molecules in tumor immunology and in the immune response to viral infections and to allografts has recently been suggested.[4–8]

The most recent HLA Nomenclature Committee Report includes 56 MICA alleles (Marsh *et al*[9] and Anthony Nolan Trust, HLA Informatics Group website: http://www.anthonynolan.com/HIG/lists/otherlist.html). This high degree of polymorphism creates a challenge for genotyping similar to that faced with HLA genes. Methods for genotyping include PCR-SSP, SSOP, RSCA,[10–15] along with sequencing-based typing (SBT) usually considered definitive.[2,16–19] Results have differed for the same reference cell lines typed by alternative methods. As examples, the cell line WJR076 was typed as *017 by SBT[2] and PCR-SSP,[12] but also reported as *031.[20] The International Histocompatibility Working Group (IHWG) Cell and Gene Bank Reference Panel (http://www.ihwg.org/shared/cbankover.htm) reports the MICA type of this cell line as heterozygous for *00201/*017. The cell line LBF has been reported as displaying MICA type *011 when typed by SBT,[2] but as *00801 by other methods including RSCA. Whether these discrepancies reflect actual allele differences produced by different methods or merely reporting errors is not clear. In our own MICA typing by IHWG-recognized SBT,[16] we examined all conserved extended haplotypes (CEH). The panel included the cell line WHONP192 (IHW 9140), which had previously been designated heterozygous for CEH 46.1 and 54.1, containing MICA alleles *010 and *01201, respectively. However, our exon 2 sequencing showed a homozygous pattern for allele *010. Upon further scrutiny of those GenBank intron 1 genomic sequences that correspond to the forward primer sequence for exon 2 SBT typing, we found a number of patterns whose alignment suggested an indel polymorphism (Figure 1).

MICA introns 2 and 3 had previously been sequenced mainly to refine MICA allelic assignments, but there is no information on intron 1 sequences.[21] There is already evidence for polymorphism among GenBank entries for this gene; one indel polymorphism is potentially important in the function of forward primers commonly used for exon 2 sequencing. We, therefore, undertook systematic sequencing of intron 1 in reference cell lines representing most of the CEHs.

Correspondence: Dr MT Dorak, School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 4LP, UK. E-mail: dorak@openlink.org
Received 08 March 2004; revised 08 April 2004; accepted 08 April 2004; published online 27 May 2004

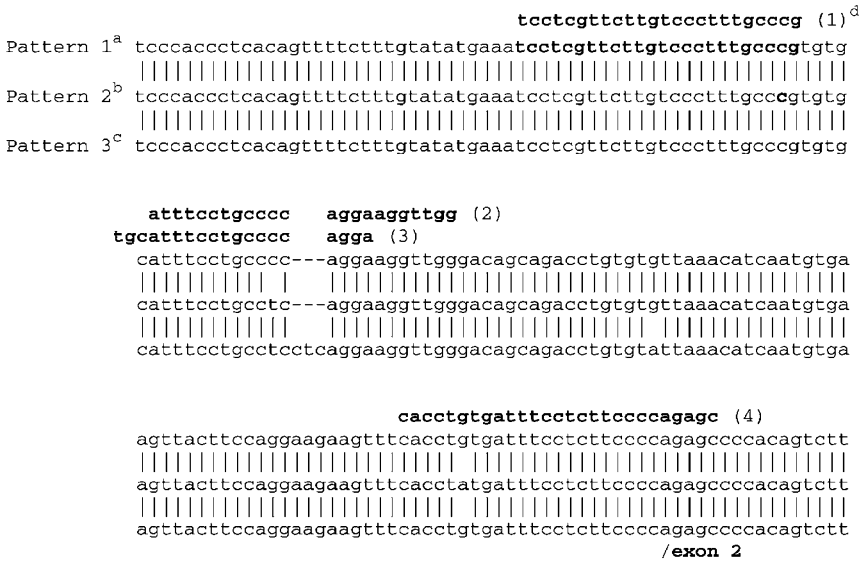

Figure 1 MICA intron 1 sequences in GenBank and location of the primers. [a]Sequence Gl: 1405892. The forward primer of the IHWG MICA typing protocol (exon 2 SBT) was based on this sequence.[16] [b]Sequences Gl: 1764043, Gl: 1764051 and Gl: 3451361. [c]Sequences Gl: 557554/CEH 57.1; Gl: 557557/CEH 18.2; Gl: 2317763/Pan trogodytes; Gl: 33300640/MICE; Gl: 27544409/MICB. There are more than 10 other sequences in GenBank with this pattern in intro 1 (Gl: 27544411, Gl: 1764045, Gl: 3273727, Gl:5002624, Gl: 18857822, GlL3451357, Gl: 25137029, Gl: 18873627, Gl: 5926710, GI: 5672627, Gl: 19572503). [d]Primers shown are: (1) generic MICA amplification forward primer; (2) exon 2 forward primer used by IHWG and Katsuyama *et al*,[16] (3) foward primer to amplify MICA gene by Obuchi *et al*,[19] (4) newly designed exon 2 sequencing primer, which spans the intron–exon junction. One nucleotide mismatch between this primer and pattern 2 sequence towards the 5′-end did not interfere with sequencing reaction.

Results and discussion

We observed the three intron 1 sequence patterns expected from the sequences available in GenBank (Web Figure A, see Supplementary Information). The findings for all CEHs (Table 1) suggested that the primers used by the IHWG, Katsuyama *et al* and Obuchi *et al* may fail to amplify both alleles when present in certain heterozygous combinations, because of competition for the sequences that match fully and partially to the forward primer used. The primers used by those other investigators will fully match the MICA intron 1 sequences only if they include pattern 3, but only partially match the others. This may result in failure to amplify alleles with patterns 1 and 2. This discrepancy was observed during our own efforts to type reference cell lines using the primers described previously.[16] The cell line WHONP192, which is heterozygous for CEH 46.1 and 54.1, showed an unexpected homozygous pattern in exon 2, but was heterozygous in exons 3 and 4 (not shown). Sequencing of MICA intron 1 in the cell lines T7526/WHONP439 and KT3 predicted this result because CEH 46.1 has pattern 1 sequence in intron 1, while CEH 54.1 has pattern 3. We redesigned the forward exon 2 SBT primer and were able to amplify both MICA exon 2 fragments in WHONP192. This new primer (5′-CAC CTG TGA TTT CCT CTT CCC CAG AGC) corresponds to sequence lying between nucleotide positions 6926 and 6952 in the reference MICA sequence (GI:1405892). The exon 2 forward primers used by others[2,17,18] are also outside the polymorphic region in intron 1.

Interestingly, the MICA intron 1 sequence patterns showed an association with exon 5 short tandem repeat (STR) alleles (Table 1). All CEHs with intron 1 sequence pattern 2 had the STR allele A6, while those with pattern 1 had STR allele A5 or A5.1. There was, however, no relation to exon 2–4 polymorphisms or to *HLA-B* types. Thus, two neutral polymorphisms in the initial intron are associated with two in the final exon, but polymorphisms in the intervening coding region have developed independently. This finding agrees with the demonstration of balancing selection for functional MICA polymorphisms.[22,23] Several lines of evidence suggest that the haplotypes bearing pattern 3 of intron 1 sequence represent the ancestral lineage. This pattern is shared by the common chimpanzee gene (GI:2317763), by MICB (GI:27544409) and the pseudogene MICE (GI:33300640). While the examples of patterns 1 and 2 all have unique exon 5 STR polymorphisms, haplotypes with intron 1 pattern 3 include a heterogeneous group of exon 5 alleles. A practical implication of these findings is that the three patterns described here in intron 1 in conjunction with the STR polymorphisms in exon 5 mark ancestral lineages better than the coding region polymorphisms.

Our findings exemplify the influence of DNA polymorphisms on the performance of a primer in both PCR and/or sequencing reactions for detecting those polymorphisms. This concern should diminish as data in GenBank increase. The intron 1 polymorphisms appeared to cluster the MICA allelic lineages into three groups, but offered no advantage over the exon 5 STR polymorphism in distinguishing MICA alleles that share the same coding region polymorphisms. The design of primers for any method of MICA typing should take into account possible polymorphisms in the introns, exons 2–4 and exon 5 STR.

Table 1 Characteristics of the reference samples used in MICA intron 1 sequencing[a]

IHW no.	*Name*	*CEH*	*HLA-B*	*HLA-DRB1*	*MICA-ex2–4*	*MICA-ex5*	*MICA-int1*
10W9031	BOLETH BO	62.1	1501	0401	010	A5	Pattern 1
10W9060	CB6B-CGB1B	62.3	1501	1301	010	A5	Pattern 1
10W9094	CF996	64.1	1401	0701	019	A5	Pattern 1
10W9146	COL, E	62.2	1501	0401	010	A5	Pattern 1
10W9105	FPAF FPF F	35.5	3502	1104	016	A5	Pattern 1
10W9041	J0528239	35.5	3502	1104	016	A5	Pattern 1
10W9076	T7526	46.1	4601	0901	010	A5	Pattern 1
10W9139	WHONP439	46.1	4601	0901	010	A5	Pattern 1
10W9066	TAB089, TAB	46.2	4601	0803	010	A5	Pattern 1
10W9042	TISI	35.4	3508	1103	016	A5	Pattern 1
10W9037[b]	SWEIG007	Not CEH	4002	1101	027	A5	Pattern 1
10W9090	AWELLS	44.1	4402	0401	00801	A5.1	Pattern 1
KOREA[c]	B37DR10	37.1	3701	1001	00801	A5.1	Pattern 1
10W9093	BER	13.1	1302	0701	00801	A5.1	Pattern 1
10W9022	COX	8.1	0801	0301	00801	A5.1	Pattern 1
10W9097	EMJ	60.3	4001	1302	00801	A5.1	Pattern 1
10W9069	MADURA	60.2	4001	0801	00801	A5.1	Pattern 1
10W9098	MT14B	60.1	4001	0404	00801	A5.1	Pattern 1
10W9318	PGF	7.1	0702	1501	00801	A5.1	Pattern 1
10W9047	PLH	47.1	4701	0701	00801	A5.1	Pattern 1
10W9001	SA	7.2	0702	01	00801	A5.1	Pattern 1
10W9070[b]	LUY	Not CEH	5101	0803	00901	A6	Pattern 2
10W9286	AKIBA	52.1	5201	1502	00901	A6	Pattern 2
10W9106	MANIKA	50.1	5001	0701	00902	A6	Pattern 2
10W9050	MOU-MANN	44.3	4403	0701	004	A6	Pattern 2
10W9051	PITOUT	44.2	4403	0701	004	A6	Pattern 2
10W9021	RSH, RSHD	42.1	4201	0302	004	A6	Pattern 2
10W9108	CAR, ML	35.2+55.1	35, 55	0101,0401	019	A4, A9	Pattern 3
10W9008	DO208915	18.1	1801	1501	018	A4	Pattern 3
10W9019	DUCAF	18.2	1801	0301	001	A4	Pattern 3
10W9039	JVM	18.3	1801	1102	001	A4	Pattern 3
10W9092	BM92	51.1	5101	0404	00701	A4	Pattern 3
10W9053	HOR	44.4	4403	1302	004	A4	Pattern 3
10W9107	KT3, LKT3	54.1	5401	0405	01201	A4	Pattern 3
10W9052	DBB	57.1	5701	0701	017	A9	Pattern 3
10W9157	HAU, ML	58.1	5801	0301	00201	A9	Pattern 3
10W9006	WT100BIS	35.2	3501	0101	00201	A9	Pattern 3
10W9026	YAR	38.1	3801	0402	00201	A9	Pattern 3
10W9012[b]	WJR076	Not CEH	5701	16	017	A9	Pattern 3
10W9079	LWAGS	65.1	1402	0102	011	A6	Pattern 3
10W9002	MZO	65.1	1402	0102	011	A6	Pattern 3
10W9078	PMG	65.1	1402	0102	011	A6	Pattern 3
10W9055	HO301	65.2	1402	1302	011	A6	Pattern 3

[a]HLA-B, HLA-DRB1, MICA typing results are from published reports.
[b]SWEIG007, LUY and WJR076 do not represent any CEH.
[c]This sample is not an IHW cell line.

Material and methods

Cell lines

A panel of International Histocompatibility Workshop (IHW) reference cell lines was analyzed for polymorphisms in MICA intron 1. Full HLA typings of these cell lines ($n = 42$), obtained as DNA from the ECACC (Salisbury, UK) or IHWG Cell and Gene Bank (Seattle, WA), are described below (Table 1); further details are available elsewhere (Cattley[24], Ohashi and Tokunaga[25] and ECACC and IMGT/HLA Cell Bank). CEH assignments were made according to the latest update.[26] One sample derived not from a cell line but from an anonymous subject carrying CEH 37.1 (homozygous for B*37–DRB1*10); it was kindly provided by Dr Myoung Park (Seoul National University College of Medicine, Korea). Of the samples sequenced, 40 represented 36 CEHs (Table 1).

Primer cycle sequencing

We used primer cycle sequencing to screen 43 samples. We first amplified the whole MICA gene fragment (2001 bp) using the primers originally described by Fodil *et al*[2] with slight modifications: MICAF 5′-TCC TCG TTC TTG TCC CTT TGC CCG-3′ (nucleotides 6819–6842) and MICAR 5′-CTG CCC CCT TCC CTT CCC AAA TT-3′ (nucleotides 8996–9019). These new primers have MICA-specific 3′-end nucleotides. PCR products were purified using spin columns (Ultrafree-MC Centrifugal Filter Units, Millipore, Billerica, MA, USA) to remove excess primers and dNTPs, and subjected to cycle sequencing

using the Cy5-labelled forward sequencing primer: MICINT1F 5′-Cy5-GAA GAC TGT GGG GCT CTG G-3′. This primer spans the exon 1/intron 1 junction; last three nucleotides are in intron 1. The sequencing reactions were performed using Thermo Sequenase Primer Cycle Sequencing Kit (Amersham Biosciences Corp, Piscataway, NJ, USA). The amount of primer in the reaction was 2 pmol and the total reaction volume was 6 μl. The termination reactions were run in the DNA thermal cycler (MJ Research Inc., Waltham, MA, USA) for 20 cycles in the following thermal profile: 30 s at 95°C, 30 s at 57.5°C and 1 min at 72°C. The reactions were loaded on ALFexpress II DNA Analysis System (Amersham Biosciences Corp, Piscataway, NJ, USA). Electropherograms were analyzed manually.

References

1 Bahram S, Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. *Proc Natl Acad Sci USA* 1994; **91**: 6259–6263.
2 Fodil N, Laloux L, Wanner V *et al.* Allelic repertoire of the human MHC class I MICA gene. *Immunogenetics* 1996; **44**: 351–357.
3 Bahram S. MIC genes: from genetics to biology. *Adv Immunol* 2000; **76**: 1–60.
4 Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stastny P. Identification of MICA as a new polymorphic alloantigen recognized by antibodies in sera of organ transplant recipients. *Hum Immunol* 2000; **61**: 917–924.
5 Hankey KG, Drachenberg CB, Papadimitriou JC *et al.* MIC expression in renal and pancreatic allografts. *Transplantation* 2002; **73**: 304–306.
6 Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 2002; **419**: 734–738.
7 Salih HR, Rammensee HG, Steinle A. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 2002; **169**: 4098–4102.
8 Cerwenka A, Lanier LL. NKG2D ligands: unconventional MHC class I-like molecules exploited by viruses and cancer. *Tissue Antigens* 2003; **61**: 335–343.
9 Marsh SG, Albert ED, Bodmer WF *et al.* Nomenclature for factors of the HLA system, 2002. *Eur J Immunogenet* 2002; **29**: 463–515.
10 Mendoza-Rincon J, Arguello JR, Perez-Rodriguez M *et al.* Characterization of the MICA polymorphism by sequence-specific oligonucleotide probing. *Immunogenetics* 1999; **49**: 471–478.
11 Zhang Y, Lazaro AM, Lavingia B, Stastny P. Typing for all known MICA alleles by group-specific PCR and SSOP. *Hum Immunol* 2001; **62**: 620–631.
12 Ahmad T, Marshall SE, Mulcahy-Hawes K *et al.* High resolution MIC genotyping: design and application to the investigation of inflammatory bowel disease susceptibility. *Tissue Antigens* 2002; **60**: 164–179.
13 Collins RW, Stephens HA, Clare MA, Vaughan RW. High resolution molecular phototyping of MICA and MICB alleles using sequence specific primers. *Hum Immunol* 2002; **63**: 783–794.
14 Perez-Rodriguez M, Arguello JR, Fischer G *et al.* Further polymorphism of the MICA gene. *Eur J Immunogenet* 2002; **29**: 35–46.
15 Zhang Y, Han M, Vorhaben R, Giang C, Lavingia B, Stastny P. Study of MICA alleles in 201 African Americans by multiplexed single nucleotide extension (MSNE) typing. *Hum Immunol* 2003; **64**: 130–136.
16 Katsuyama Y, Ota M, Ando H *et al.* Sequencing based typing for genetic polymorphisms in exons, 2, 3 and 4 of the MICA gene. *Tissue Antigens* 1999; **54**: 178–184.
17 Visser CJ, Tilanus MG, Tatari Z *et al.* Sequencing-based typing of MICA reveals 33 alleles: a study on linkage with classical HLA genes. *Immunogenetics* 1999; **49**: 561–566.
18 Yao Z, Volgger A, Helmberg W *et al.* Definition of new alleles of MIC-A using sequencing-based typing. *Eur J Immunogenet* 1999; **26**: 225–232.
19 Obuchi N, Takahashi M, Nouchi T *et al.* Identification of MICA alleles with a long Leu-repeat in the transmembrane region and no cytoplasmic tail due to a frameshift-deletion in exon 4. *Tissue Antigens* 2001; **57**: 520–535.
20 Fischer G, Arguello JR, Perez-Rodriguez M *et al.* Sequence-specific oligonucleotide probing for MICB alleles reveals associations with MICA and HLA-B. *Immunogenetics* 2000; **51**: 591–599.
21 Yao Z, Volgger A, Keller E, Chandanayingyong D, Albert ED. Allelic variation in the intron 2 and 3 of the MICA gene. *J Biol Regul Homeost Agents* 1999; **13**: 47–50.
22 Marsh SG, Packer R, Heyes JM *et al.* The International Histocompatibility Workshop cell line panel. In: Charron D (ed). *Genetic Diversity of HLA: Functional and Medical Implications*. EDK: Paris, 1997: 633–647.
23 Prasad VK, Yang SY. Allele assignment for HLA-A, -B, and -C genes to the Tenth International Histocompatibility Workshop cell lines. *Tissue Antigens* 1996; **47**: 538–546.
24 Cattley SK, Williamson JF, Tay GK, Martinez OP, Gaudieri S, Dawkins RL. Further characterization of MHC haplotypes demonstrates conservation telomeric of HLA-A: update of the 4AOH and 10IHW cell panels. *Eur J Immunogenet* 2000; **27**: 397–426.
25 Ohashi J, Tokunaga K. Computer simulation analysis suggests weak balancing selection operative at the MICA locus. *Hereditas* 2000; **133**: 25–28.
26 Elsner HA, Schroeder M, Blasczyk R. The nucleotide diversity of MICA and MICB suggests the effect of overdominant selection. *Tissue Antigens* 2001; **58**: 419–421.

Supplementary Information accompanies the paper on Genes and Immunity's website (http://www.nature.com/gene).