

## HIV MICA (MHC class I chain-related A)

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### Association of HIV infection with MICA(MHC class I chain-related A) gene alleles

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**Background:** A large number of diseases occur in association with specific HLA-B or-C alleles. Recently a new gene, termed major histocompatibility complex class I chain-related gene A (MICA), has been identified in close proximity to HLA-B. The function of this gene is still unknown. However, it is structurally similar to HLA class I genes. MICA gene is polymorphic and is potentially associated with several diseases. **Methods:** To evaluate the association of MICA gene in Korean patients with human immunodeficiency virus 1 (HIV-1) infections, Polymerase chain reaction-Sequence specific primer (PCR-SSP) was done for MICA alleles in the extracellular exons, and a microsatellite analysis for GCT repeat polymorphisms in the TM exon was also completed. **Results:** In 199 Korean healthy controls, 7 alleles were observed and the frequencies for each allele were MICA008 (44.7%), MICA010 (34.2%), MICA002 (31.7%), MICA004 (23.6%), MICA012 (21.6%), MICA009 (19.6%), and MICA007 (6.5%). When 65 HIV seropositive patients were analyzed, MICA007 allele frequency was significantly higher than in controls (15.4% vs 6.5%, RR=2.6, p<0.04). In contrast, the frequencies of other MICA alleles and microsatellite alleles in the transmembrane region of MICA gene were not significantly different between HIV seropositive patients and controls. The tight linkage between MICA alleles in the extracellular exons and GCT repeat polymorphisms in the TM exon was observed as follows; MICA002/A9, MICA004/A6, MICA007/A4, MICA008/A5.1, MICA010/A5, and MICA012/A4 in both groups. No significant difference between patients and controls was observed in the haplotype frequencies of MICA alleles in the extracellular exons and GCT repeat polymorphisms in the TM exon. **Conclusion:** The data suggest that immune functions related with MICA gene may affect a HIV infections.

**Key Words:** HIV, MICA, PCR-SSP, HLA-B

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Virus)가

(Human Immunodeficiency  
 (Acquired Immune Defi-

ciency Syndrome) HIV MICA hsp70  
 , , , HIV-1 heat-shock promoter  
 (19), MICA 가  
 , MICA  
 . MICA Vδ Iγδ T  
 γδ T 가 (21, 22). , MICA ,  
 T ,  
 (intestinal epithelium)  
 (1,2). γδ T αβ T MHC . MHC class I, II HLA  
 , (3,4). , MICA  
 γδ T  
 , (innate immunity) . 85 homozygous typing cell line  
 (5), (HTCLs) 30 MICA  
 (6, 7). HIV 가 (23).  
 γδ T 가 MICA  
 , Vδ 1 T 가 (PCR-SSP, PCR-SSOP, SBT) ,  
 가 HIV 가 가 MICA  
 (8, 9, 10), Vγ9, Vγ2, Vγ3, Vγ4 Vδ 1  
 가 (11, 12).  
 peripheral blood Vγ9 Vδ2 가 HIV MICA  
 γδ+ T HIV 가 HIV  
 , anergy (13), , MICA  
 γδ+ T HIV 가 HLA-B  
 (14, 15). γδ T . 199 HIV  
 T (ligand) MICA 65 MICA MICA TM  
 MICB가 , 11 kb MIC (transmembrane) GCT  
 (MHC class I chain-related gene) HLA-B 7 MICA  
 4.12kb , MICA008, MICA010, MICA002, MICA004,  
 (16, 17). MIC 6 가 MICA012, MICA009, MICA007 가  
 (18), MICA MICB , HIV MICA  
 MICC, MICD, MICE, MICEF MICA007 가 HIV  
 pseudogene . MICA 43kd 가 383 가  
 (18). MICA MICA MICA transmembrane  
 classical class I , 3 (GCT)  
 (α1, α2, α3 domain), ,  
 carboxyl-terminal . MICA . MICA  
 (epithelial cell line) HIV  
 (gastrointestinal epithelium) (18, 19), 가  
 (keratinocytes),  
 (endothelial cells) (monocytes)  
 , B (20). .

1. DNA

HIV 65 199

10 ml

Ficoll / Hypaque

106 PCR-K buffer (10X PCR buffer 1 ml, NP-40 40 ul, Tween-20 45 ul, proteinase K (20 mg/ml) 30 ul, D.W 8.8 ml) 1 ml 가 58℃

1 95℃ 10

PCR DNA

2. HLA - B

Ficoll-Hypaque heparin

HLA-B tray

(2 x 10<sup>6</sup> /ml) well 1 ul

25℃ 30 ,

well 5 ul 가 well

eosin Y 5 ul

2 formalin (pH 7.2) 8 ul 가

HLA

3. MICA TM (transmembrane) micro-satellite

MICA TM microsatellite

primer MICA5F 5'-CCTTACCATCTCCA GAAACTGC-3', MICA5R 5'-CCTTTTTTTCAGGGAA AGTGC-3'

MICA 5F [γ-32p] ATP

end labelling . MICA TM microsatellite

PCR DNA (100 ug/ml), Taq polymerase (Boehringer Mannheim), 10X buffer (500 mM KCl, 100 mM Tris-HCl pH 9.0, 15 mM MgCl<sub>2</sub>, 1% Triton X-100), primer (100 ng/ul), 2 mM dNTPs (10X 2 mM dATP, dCTP dGTP, dTTP), end label primer (35 cycles).

50% Urea가 6%

acrylamide gel . PCR DNA 3

ul stop (10 mM NaOH, 95% formamide, 0.05% bromophenol, 0.05% xylene cyanol) 10 ul

97℃ 2 가

6% denaturing acrylamide gel 50 watt 4

gel 3 MM

paper 80℃ 10-15

Kodak X-Omat AR X-ray film -80℃ 12-24

(Fig. 1).

#### 4. MICA

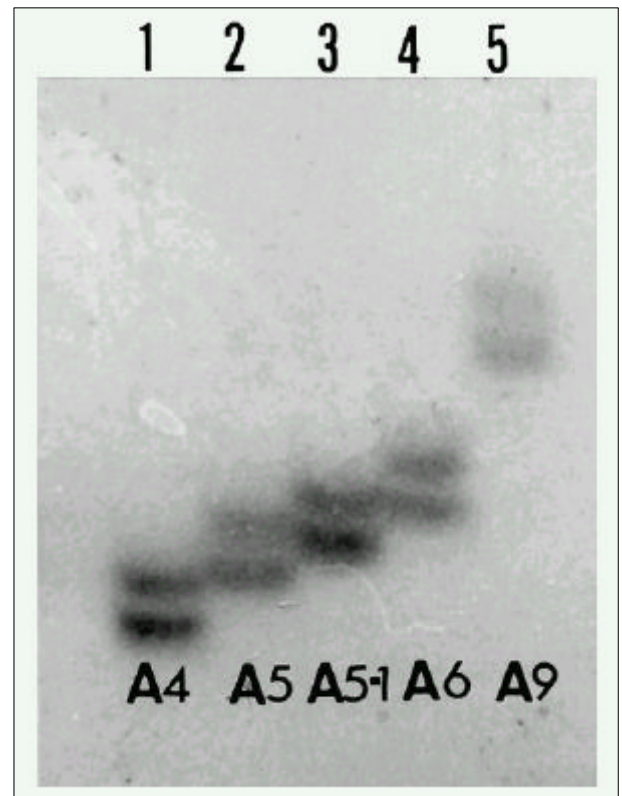
MICA exon 2, 3, 4 PCR-SSP

(23). PCR

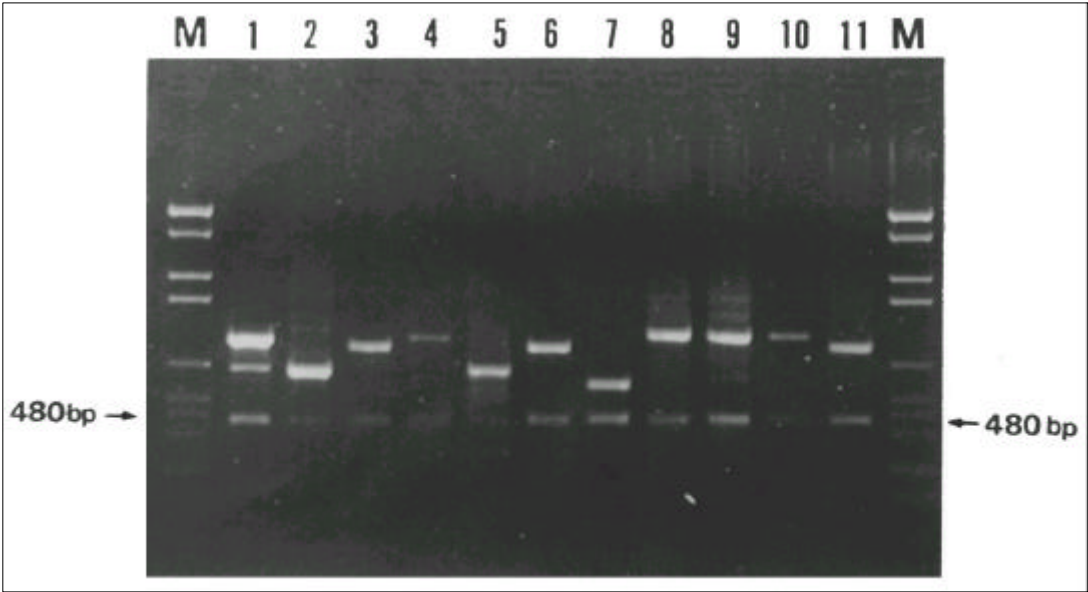
DNA 25 ng, 10X buffer (750 mM Tris-HCl pH 8.8, 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Tween 20, 15 mM MgCl<sub>2</sub>, 450 mM), 25 mM MgCl<sub>2</sub>, 2 mM dNTPs, internal control primers (conserved homologous sequences of human growth hormone ; 480bp) 2 uM, antisense, sense sequence-specific primer 1uM, Taq polymerase 0.4 units (5 U/μl; Boehringer Mannheim)

10 ul 94℃ 20 , 65℃ 50 , 72℃ 30 30

1 μg/L ethidium



**Fig. 1.** MICA-TM triplet (GCT) repeat polymorphisms by microsatellite analysis



**Fig. 2.** MICA alleles and group-specific amplicons found in Korean population MICA 002 showed specific amplicons with 777bp and 628bp (lane 1, 2), MICA004 with 744bp (lane 3), MICA007 with 780bp and 628bp (lane 4, 5), MICA008 with 739bp (lane 6), MICA009 with 568bp, 813bp and 799bp (lane 7, 8, 9), MICA010 with 799bp (lane 10), and MICA012 with 741bp (lane 11)

**Table 1.** The frequencies of the MICA gene alleles in patients with HIV and controls

MICA	Patients with HIV n=65 (%)	Controls n=199 (%)
002	17(26.2)	63(31.7)
004	16(24.6)	47(23.6)
007	10(15.4)a	13(6.5)
008	33(50.8)	89(44.7)
009	10(15.4)	39(19.6)
010	18(27.7)	68(34.2)
012	9(13.8)	43(21.6)
Blank	2(3.1)	7(3.5)

a ; p<0.04, RR=2.6

1.0% agarose gel 100 V 1  
(Fig. 2).

5)

Chi-square

5

two-tailed Fisher's

5

(Relative risk (RR)) Woolf Haldane's  
. Two-loci (HF)  
(LD) Mattiuz  
. LD LD= $\sqrt{(d/N)} - [\sqrt{(b+d)(c+d)}/N]$   
a, b, c, d ++, +-, -+, -- N  
. HF PAB  
= PA X PB + LD PA PB  
genotype .

1. MICA

MICA MICA008  
(44.7%), MICA010 (34.2%), MICA002 (31.7%), MICA  
004 (23.6%), MICA012 (21.6%), MICA009 (19.6%),  
MICA007 (6.5%) (Table 1).  
HLA-B48 가 MICA  
(blank) 3.5% . HIV  
MICA MICA007  
가 가  
(relative risk=2.6, p<0.04)(Table 1). MICA

가 . / A4 (HF=0.03, LD=0.03,  $\chi^2 < 31.47$ ), MICA008 / A5.1 (HF=0.10, LD=0.08,  $\chi^2 < 58.32$ ), MICA009 / A6 (HF=0.10, LD=0.08,  $\chi^2 < 72.15$ ), MICA010 / A5 (HF=0.18, LD=0.13,  $\chi^2 < 106.46$ ), MICA012 / A4 (HF=0.11, LD=0.10,  $\chi^2 < 124.09$ )가 (Table 3), (25).

2. MICA Transmembrane (GCT) HIV MICA transmembrane (GCT) (Table 2). HIV

3. MICA (GCT) (Table 3).

4. HLA - B 199 MICA MICA transmembrane (GCT) HIV 22 HLA-B (Table 4). HIV HIV slow progression HLA-B27

MICA002 / A9 (HF=0.15, LD=0.13,  $\chi^2 < 172.44$ ), MICA004 / A6 (HF=0.11, LD=0.08,  $\chi^2 < 67.33$ ), MICA007

**Table 2.** Gene frequencies of trinucleotide repeat (GCT)<sub>n</sub> within MICA gene in patients with HIV and Controls

Microsatellite Alleles	Patients with HIV n=65(%)	Controls n=199(%)
A4	19(29.2)	61(30.7)
A5	29(44.6)	98(49.2)
A5.1	18(27.7)	41(20.6)
A6	26(40.0)	80(40.2)
A9	15(23.1)	57(28.6)

MICA Vδ1Vδ (intestinal epithelial) Vδ T . , MICA MICA - 가

**Table 3.** A two locus haplotype analysis between MICA and trinucleotide repeat (GCT)<sub>n</sub> within MICA (MICATM) in patients with HIV and controls.

Patients with HIV n=65					Controls n=199		
MICA	MICATM	LD	HF	XX	LD	HF	XX
002	A9	0.10	0.11	45.57	0.13	0.15	172.44
004	A6	0.08	0.11	19.95	0.08	0.11	67.33
007	A4	0.06	0.07	21.10	0.03	0.03	31.47
008	A5.1	0.11	0.14	24.14	0.08	0.10	58.32
009	A6	0.05	0.07	12.31	0.08	0.10	72.15
010	A5	0.10	0.14	25.01	0.13	0.18	106.46
012	A4	0.06	0.07	25.29	0.10	0.11	124.09

HF; haplotype frequency, LD; linkage disequilibrium (delta value)

Two-locus haplotypes frequencies (HF)  $\geq 0.03$  by direct counting on both groups and chi-square value  $\geq 19.95$  were listed. Table 4. Distribution of HLA-B alleles between patients with HIV and controls

**Table 4.** Distribution of HLA-B alleles between patients with HIV and controls

HLA-B Alleles	Patients with HIV n=22 (%)	Controls n=199 (%)
7	2(9.1)	14(7.0)
8	0	2(1.0)
13	0	15(7.5)
14	0	9(4.5)
27	4(18.2)	12(6.0)
35	0	20(10.0)
37	0	2(1.0)
38	0	5(2.5)
39	1(4.5)	1(0.5)
44	7(31.8)	52(26.0)
46	1(4.5)	22(11.0)
48	2(9.1)	8(4.0)
51	2(9.1)	30(15.0)
52	3(13.6)	10(5.0)
54	0	29(15.0)
55	1(4.5)	6(4.5)
56	1(4.5)	0
57	0	1(0.5)
58	2(9.1)	29(14.5)
59	0	6(3.0)
60	0	16(8.0)
61	8(36.4)	46(23.0)
62	7(31.8)	42(21.0)
67	1(4.5)	4(2.0)
75	0	3(1.5)

exon 2, 3, 4 non-synonymous

MICA  
가  
, MICA HLA  
GvHD (Graft versus Host Disease)

MICA  
MICA  
가  
HIV

MICA MICA-TM (trans-membrane)

MICA  
가  
MICA019  
(25). MICA002, 004, 007, 008, 012  
MICA009 (19.6% vs 31.5%; Korean vs Japanese),  
MICA010 (34.2% vs 19.2%; Korean vs Japanese)  
(25).

MICA  
MICA  
MICA  
MICA  
HLA-B  
가  
MICA002/B35,  
MICA004/B44, MICA007/B27, MICA008/B60, B61,  
MICA009/B51, B52, MICA010/B62, MICA012/B54, B55  
(25). MICA  
null (blank) HLA-B48

MICA large-scale  
(100kb) (26).  
HLA-B48 4% (Table 4),  
HLA-B48 homozygotes 가 PCR-SSP  
MICA PCR  
HLA-B48  
heterozygote 가

MICA PCR  
null (blank)가 HLA-B48

, HLA-B48 MICA null  
exon3  
MICB0107N 가 (27).  
MICA null  
MICA  
HLA-B

, MICA  
가

- HLA-B (28).  
가  
가  
HLA-B  
MICA HLA-B  
가  
HIV 22 HLA-B  
HLA-B27  
HIV 가  
HIV MICA  
MICA007 가 HIV  
(relative risk=2.6, p<0.04). MICA  
007 HLA-B27 가  
(25), B27 HIV slow progression  
(29). , MICA007  
HLA-B27 HIV  
slow progression  
MICA  
MICA-TM GCT ,  
HLA-B HIV  
HIV  
HIV HLA class I class II  
MICA HIV  
MICA HLA
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