

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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(Human Immunodeficiency
 Virus)가
 (Acquired Immune Defi-

ciency Syndrome) HIV MICA hsp70
 , , , HIV-1 heat-shock promoter
 , MICA 가
 , MICA
 Vδ T 가 MICA Vδ Iγδ T
 (21, 22). , MICA ,
 T
 (intestinal epithelium)
 (1,2). Vδ T αβ T MHC MHC class I, II HLA
 (3,4). , MICA
 Vδ T
 , (innate immunity) 85 homozygous typing cell line
 (5), (HTCLs) 30 MICA
 (6, 7). HIV 가 (23).
 Vδ T 가 MICA
 , Vδ1 T 가 (PCR-SSP, PCR-SSOP, SBT)
 가 HIV 가 MICA
 (8, 9, 10), Vγ9, Vγ2, Vγ3, Vγ4 Vδ1
 가 (11, 12).
 peripheral blood Vγ9 Vδ2 가 HIV MICA
 Vδ+ T HIV 가 HIV
 , anergy (13), , MICA
 Vδ+ T HIV 가 HLA-B
 (14, 15). Vδ 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
 41.2kb , MICA008, MICA010, MICA002, MICA004,
 (16, 17). MIC 6 가 MICA012, MICA009, MICA007 가
 (18), MICA MICB , HIV MICA
 MICC, MICD, MICE, MICF 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°C
1 95°C 10
PCR DNA

2. HLA - B

Ficoll-Hypaque heparin
HLA-B tray
(2 x 10⁶ /ml) well 1 ul
25°C 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
MgCl₂, 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°C 2 가
6% denaturing acrylamide gel 50 watt 4
gel 3 MM
paper 80°C 10-15
Kodak X-Omat AR X-ray film -80°C 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 (NH₄)₂SO₄, 0.1% Tween 20, 15 mM MgCl₂,
450 mM), 25 mM MgCl₂, 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°C 20 , 65°C 50 , 72°C 30 30
1 μg/L ethidium

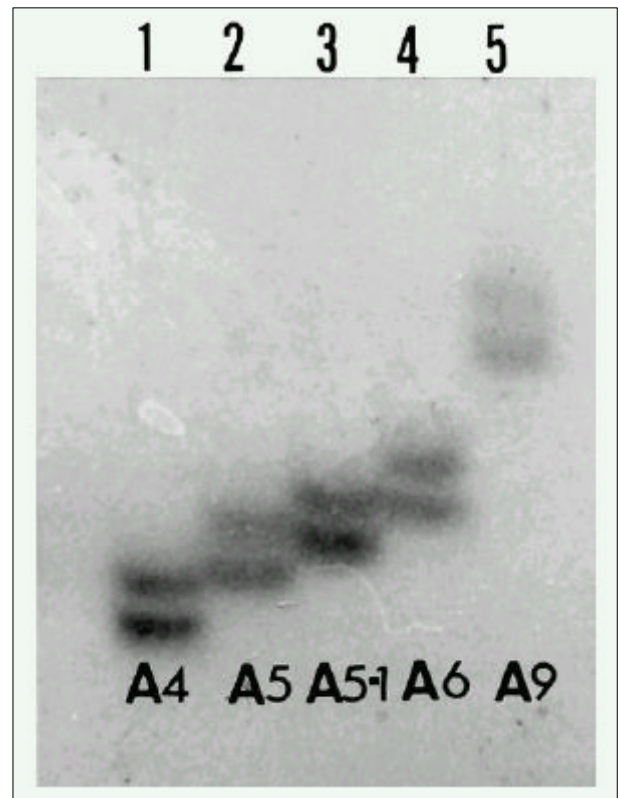


Fig. 1. MICA-TM triplet (GCT) repeat polymorphisms by microsatellite analysis

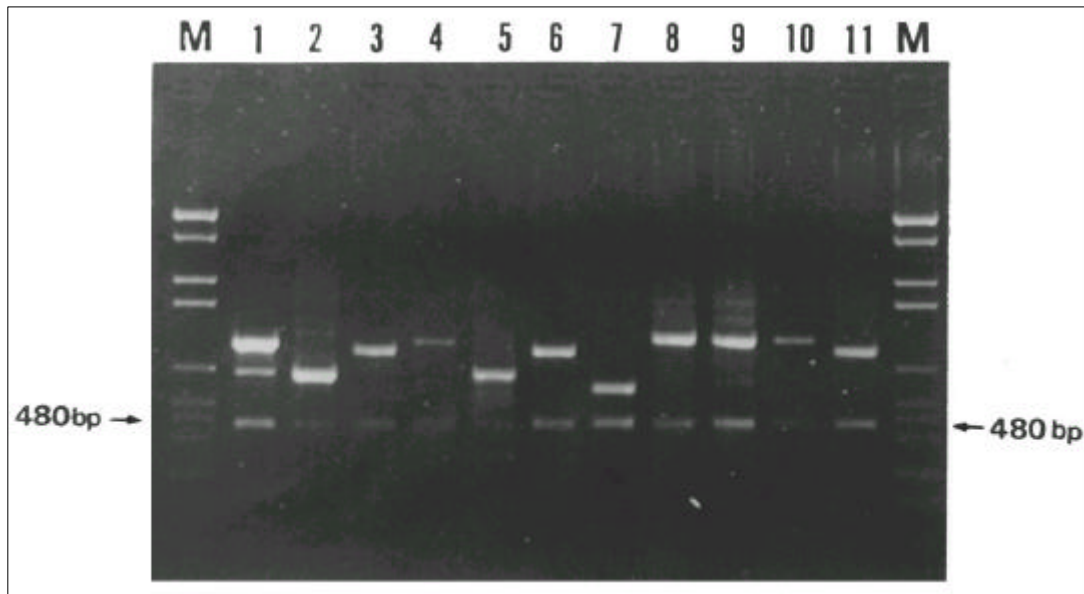


Fig. 2. MICA alleles and group-specific amplicons found in Korean population MICA 002 showed specific amplicons with 777bp and 628bp (lane 1, 2), MICA004 with 744bp (lane 3), MICA007 with 780bp and 628bp (lane 4, 5), MICA008 with 739bp (lane 6), MICA009 with 568bp, 813bp and 799bp (lane 7, 8, 9), MICA010 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, $\chi^2 < 3.147$), 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).

A9 가 HIV (Table 2). HIV (Table 3).

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

Table 2. Gene frequencies of trinucleotide repeat (GCT)_n 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)

Table 3. A two locus haplotype analysis between MICA and trinucleotide repeat (GCT)_n within MICA (MICA_{TM}) in patients with HIV and controls.

MICA	MICA _{TM}	Patients with HIV n=65			Controls n=199		
		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) ≥ 0.03 by direct counting on both groups and chi-square value ≥ 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

MICA

가

, MICA HLA, GvHD (Graft versus Host Disease)

MICA

vs T
가

MICA

가

HIV

MICA MICA-TM (trans-membrane)

MICA

가

MICA019

(25).

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

MICA

null

(blank)가 HLA-B48

, HLA-B48

MICA null

exon3

MICB0107N

가

(27).

MICA null

, HLA-B

MICA

, 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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