Original Article

MICA Polymorphism, Linkage Disequilibrium with HLA-B and MICA Antibodies in Thai Kidney Transplant Recipients

Panpimon Luangtrakool MSc[1], Sasijit Vejbaesya MD[1], Komon Luangtrakool PhD[1], Chutima Sermduangprateep MSc[1]

[1] Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: The major histocompatibility complex class I chain-related gene A (MICA) is located in MHC class I region near HLA-B. MICA is one of the polymorphic non-HLA antigens important in kidney transplantation. The information on the distribution of MICA alleles and antibodies in Thai kidney transplant recipients is still lacking.

Objective: To determine MICA allele frequencies, their linkage with HLA-B and MICA antibody frequencies in Thai kidney transplant recipients.

Materials and Methods: One hundred thirty Thai kidney transplant recipients were typed for MICA by PCR-SSP and HLA-B by microlymphocytoxicity. There were 484 recipients tested for MICA antibody screening by LABScreen Mixed and for MICA antibody specificities by MICA single antigen kit. Allele frequencies of MICA, linkage disequilibrium with HLA-B and antibody frequencies of MICA were analyzed.

Results: The four most frequent MICA alleles were MICA*008, *019, *010, *002. The most frequent MICA-HLA-B haplotypes were MICA*010-B46, MICA*008-B60, MICA*019-B75, MICA*018-B18. The most frequent specific antibodies were MICA*007, *002, *001.

Conclusion: The polymorphism of MICA gene and strong linkage disequilibrium with HLA-B were found in Thai kidney transplant recipients similar to the healthy Thais. The specificities of MICA antibodies did not correlate with MICA alleles.

Keywords: MICA, Transplant, Thai

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Major histocompatibility complex class I chain-related genes (MIC) are part of the non-classical MHC genes located on the short arm of chromosome 6. MICA is located approximately 46 kb centrometric to human leukocyte antigen B (HLA-B) and encodes a cell-surface glycoprotein of 383 amino acids. It was found that MICA and HLA-B are in linkage disequilibrium due to the short distance between MICA and HLA-B. The polymorphic molecules of MICA antigens are expressed on endothelial cells, keratinocytes, fibroblast, gastrointestinal epithelium and in several other cell types[1]. The number of alleles is presently more than 70 for MICA with new alleles being continuously discovered[2,3]. MICA polymorphism can induce humoral immune response that may be important in transplantation. There is strong evidence that MICA antibodies are associated with graft failure in kidney transplant recipients[3,4]. The frequency of MICA antibodies in rejected grafts have also been reported[5,6]. The data of MICA polymorphism and linkage disequilibrium with HLA-B were reported in many populations and showed ethnical difference[7], however, the specificities of MICA antibody were reported in only a few studies[4]. In Thais, the data of MICA polymorphisms were reported only in healthy Northeastern Thais[8]. In addition, MICA antibody has never been reported. The aims of the present study were to determine MICA allele frequencies, their association with different HLA-B alleles and MICA antibody frequencies in Thai kidney transplant recipients.

Correspondence to:

Vejbaesya S. Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wang Lang Road, Bangkok 10700, Thailand.
Phone: +66-2-4198081, **Fax**: +66-2-4128419
Email: sasijit.vej@mahidol.ac.th

Materials and Methods

Subjects

One hundred thirty unrelated Thai kidney transplant recipients were typed for MICA and HLA-B polymorphisms, and 484 Thai kidney transplant recipients were tested for MICA antibodies. The sample size was calculated from power and sample size program by proportion of allele frequencies in Northeastern Thais and other populations[8]. The patients were recruited from Siriraj Hospital between

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2008 and 2016. The present study was approved by the Ethics Committee of Siriraj Hospital, Mahidol University, Thailand.

MICA and HLA-B typing

The MICA genotyping was performed for exons 2, 3, and 4 by using polymerase chain reaction sequence-specific primers (PCR-SSP) for the 48 MICA alleles[9]. The cycling conditions for MICA amplification were 96°C for one minute, followed by five cycles of 96°C for 20 seconds, 70°C for 45 seconds, and 72°C for 25 seconds, 21 cycles of 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 30 seconds, four cycles of 96°C for 30 seconds 55°C for one minute and 72°C for 90 seconds. The PCR products were electrophoresed through 2.0% agarose gel containing 1.0 ug/ml ethidium bromide, which were run for 20 minutes at 100 V in 0.5X TBE buffer (pH 8.0) and visualized under a UV transilluminator. The relative size of PCR products was determined by comparison against the migration of a ΦX174 DNA-HaeIII Digest (BioLab). Successful PCR amplifications were verified with the HGH internal control primer, which generate a 485 bp amplicon. The method for MICA typing was validated by external quality control program (UCLA). HLA-B typing was done by microlymphocytotoxicity assay[10].

Detection of MICA antibodies

The kidney transplant recipients were tested for HLA and MICA antibodies using commercial kits (LABScreen Mixed, One Lambda, Inc) by the Luminex technique. The patient's serum was incubated with the beads. After washing, the beads were incubated with R-phycoerythrin (PE) conjugated goat anti-human IgG. The Luminex fluorocytometer detected the fluorescent emission of PE and a dye signature from each bead. The patients positive for MICA screening were confirmed by MICA single antigen kit (One Lambda, Inc). The antibody specificity was based on normalized mean fluorescence intensity (MFI) greater than 2,000.

Data analysis

Allele frequencies (AF) of MICA were calculated using the following formula: AF = the sum of the allele/2n×100; where n is the sum of the total number of individuals analyzed. HLA-B serotype gene frequencies (GF) were calculated using the following formula: $GF = (1-\sqrt{1-f})\times 100$, where f is the frequency of the corresponding antigen[11]. Arlequin statistical analysis software v3.5.1.2 (University of Bern, Switzerland) was used for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between two alleles at two loci of MICA and HLA-B. Statistical significance was defined at the 5% level after the p-value was adjusted using Bonferroni's correction, by multiplying the p-value by the number of independent comparisons performed[11]. The frequencies of MICA antibodies were calculated directly.

Results

The number of MICA alleles were 15 and HLA-B antigens were 26 in 130 Thai kidney transplant recipients. Deviation from HWE proportions was observed at MICA locus (p=0.007), however HLA-B distribution was consistent with HWE proportions (p>0.05). The four most frequent MICA alleles were MICA*008 (20.0%), *019 (18.08%), *010 (16.92%), and *002 (12.69%). These alleles combined accounted for 67.67% of the MICA gene pool. The four most frequent gene frequencies for HLA-B were B46 (11.86%), B60 (9.70%), B75 (9.28%), and B13

Table 1. Allele frequencies (AF) of MICA and gene frequencies (GF) of HLA-B in kidney transplant recipients

MICA	n	AF (%)	HLA-B	n	GF (%)
*002	33	12.69	7	4	1.55
*004	14	5.39	8	3	1.16
*007	1	0.39	13	21	8.43
*008	52	20.00	14	1	0.39
*009	8	3.08	18	19	7.60
*010	44	16.92	27	10	3.92
*011	1	0.39	35	7	2.73
*012	12	4.62	38	7	2.73
*015	1	0.39	39	7	2.73
*017	5	1.92	44	12	4.73
*018	15	5.77	46	29	11.86
*019	47	18.08	48	1	0.39
*027	9	3.46	51	11	4.32
*045	16	6.15	52	4	1.55
*049	2	0.77	54	1	0.39
			55	7	2.73
			56	7	2.73
			57	4	1.55
			58	12	4.73
			60	24	9.70
			61	8	3.13
			62	20	8.01
			63	1	0.39
			70	1	0.39
			75	23	9.28
			77	4	1.55

MICA = major histocompatibility complex class I chain-related gene A; HLA-B = human leukocyte antigen B

(8.43%) as shown in Table 1.

Forty-nine MICA-HLA-B haplotypes occurred in all recipients and 32 MICA-HLA-B haplotypes occurred at least two times. Eighteen of the 32 MICA-HLA-B haplotypes with a frequency greater than 1% showed significant LD (Table 2). Five most frequent haplotypes were MICA*010-B46 (11.54%), followed by MICA*008-B60 (10.38%), MICA*019-B75 (7.69%), MICA*018-B18 (5.77%), and MICA*045-B13 (5.77%).

Out of the 484 kidney transplantation recipients, 38 were positive by MICA antibody screening and 35 (7.25%) were confirmed positive by MICA single antigen assay. The specificities of MICA antibodies in 35 recipients are shown in Table 3. The most frequent antibodies were MICA*007 (71.43%), followed by MICA*002 (62.86%) and MICA*001 (57.14%). The least frequent antibody detected was MICA*005 (5.71%). Only seven (20%) had antibody specific to single MICA allele, whereas 28 (80%) had antibody specific to multiple MICA alleles. The MFI value of MICA antibodies were between 2,000 to 30,000. The lowest and the highest of MFI value were MICA*002 antibodies (2,005 and 30,088). The MFI value of MICA*005 was strong (16,030 to 18,449). Of the 35 graft recipients with positive MICA antibodies by single antigen, 22 (62.8%) had HLA antibodies, and 13 (37.1%) had only MICA antibodies.

Table 2. Haplotype frequencies (HF) of MICA-HLA-B in kidney transplant recipients

MICA	HLA-B	n	HF (%)	D'	*p*-value
*002	35	7	2.69	1.00	<0.0001
*002	38	7	2.69	1.00	<0.0001
*002	58	12	4.62	1.00	<0.0001
*004	44	12	4.62	1.00	<0.0001
*008	7	4	1.54	1.00	<0.005
*008	39	5	1.92	0.64	<0.05
*008	60	27	10.38	1.00	<0.0001
*009	51	6	2.31	0.74	<0.0001
*010	46	30	11.54	0.96	<0.0001
*010	62	10	3.85	0.40	<0.0001
*012	55	4	1.54	0.55	<0.0001
*012	56	3	1.15	0.40	<0.0001
*017	57	5	1.92	1.00	<0.0001
*018	18	15	5.77	1.00	<0.0001
*019	75	20	7.69	0.76	<0.0001
*019	77	4	1.54	1.00	<0.0001
*027	61	7	2.69	0.77	<0.0001
*045	13	15	5.77	0.93	<0.0001

MICA = major histocompatibility complex class I chain-related gene A; HLA-B = human leukocyte antigen B; D' = relative linkage disequlibrium value

Table 3. MICA antibody frequencies in 35 kidney transplant recipients using MICA single antigen beads

MICA antibodies	Frequencies, n (%)	Range of MFI
001	20 (57.14)	2,320.23 to 23,186.44
002	22 (62.86)	2,005.60 to 30,088.47
004	6 (17.14)	2,022.48 to 23,206.21
005	2 (5.71)	16,030.49 to 18,449.00
006	4 (11.43)	3,248.00 to 24,701.84
007	25 (71.43)	2,908.52 to 25,340.67
008/027*	10 (28.57)	2,221.83 to 22,389.83
009	7 (20.00)	2,247.16 to 24,122.24
012	16 (45.71)	2,755.20 to 17,322.64
015	15 (42.86)	2,000.87 to 26,433.11
017	14 (40.00)	2,418.76 to 22,264.69
018	15 (42.86)	2,384.45 to 17,581.22
019	8 (22.86)	2,661.29 to 22,647.01
028	5 (14.29)	2,044.40 to 23,772.75
046	5 (14.29)	3,601.70 to 26,358.65

MICA = major histocompatibility complex class I chain-related gene A; MFI = mean fluorescence intensity

* Antibody against MICA*008 and *027 cannot be differentiated by One Lambda MICA single antigen beads

Discussion

Fifteen MICA alleles were detected in the present study, despite the high number of MICA alleles described. MICA*008 was the most frequent allele (20.00%), followed by MICA*019 (18.08%), MICA*010 (16.92%), and MICA*002 (12.69%). MICA*008 and *002 were also common in many populations including Chinese, Japanese, Korean, and Caucasian populations[12-15]. MICA*010 was also found frequently (more than 10%) in Asians including Chinese, Japanese, and Korean populations[12-14]. However, MICA*019 was found less frequently (less than 5%) in other populations than in Thais[12-15]. Most of the studies showed MICA polymorphism in healthy individuals[7,8,12-15]. Previous study in Brazilian population reported MICA alleles in kidney transplant candidates and showed that MICA polymorphism was different from that in healthy population[16]. However, the MICA allele diversity in Thai kidney transplant recipients in the present study was similar to healthy Northeastern Thais[8] and normal controls in Bangkok (data not shown). The deviation from Hardy-Weinberg proportions may be from non-healthy individuals. In addition, the gene frequencies of HLA-B in kidney transplant recipients were not different from normal

controls in Bangkok Thais[10]. We identified 49 MICA-HLA-B haplotypes in kidney transplant recipients. A large number of these haplotype were found only sporadically, whereas 18 haplotypes had frequency of more than 1% and were significant. MICA*002, *008, *010, *012, and *019 showed association with several HLA-B alleles similar to Northeastern Thais, however, specific HLA-B showed linkage with single MICA allele. This one-way linkage disequilibrium was similar to other populations and might suggest that the origin of MICA alleles is older than major branches of HLA-B[15]. Several MICA-HLA-B haplotypes previously observed in other populations were found in Thais. MICA*008-B7, MICA*002-B35, and MICA*009-B51 were frequent in Caucasians[13]. MICA*009-B51, MICA*010-B46, and MICA*045-B13 were also frequent in Chinese population[12]. In Thais, in the present study and Northeastern Thais MICA*008-B60, MICA*010-B46, and MICA*019-B75 were more frequent than in other populations[12-16]. There were a few differences of haplotype frequencies between kidney transplant recipients and healthy Northeastern Thais. MICA*008-B60 was more frequent, whereas MICA*002-B58 was less frequent in the present study than in the Northeastern Thais. However, the differences were not significant.

In contrast to MICA alleles, which were reported in many studies in different populations, the information on the specificities of MICA antibodies was found in only a few studies[4]. The authors analyzed Thai kidney transplant recipients with anti MICA antibodies, which no previous data existed, and showed 7.23% had MICA antibodies. Most of the patients with MICA antibodies had HLA antibodies. The specificities of MICA antibodies were also analyzed and most of the patients had polyspecific antibodies similar to other studies[17,18]. In Caucasians, the most frequent MICA antibodies were antibodies against MICA*018, *019, and *001, but antibodies against MICA*004 and *009 were rare[18,19]. This was different from the present study, as antibodies against MICA*004 and MICA*009 were also frequent in Thais. Interestingly, MICA antibody frequencies did not correlate with expected frequencies based on MICA allele frequencies. MICA*001 and MICA*007 were rare in Thai population, however, the antibodies for these alleles were detected with high frequency in renal graft recipients. This was similar to previous study in other population that found little correlation between MICA alleles and antibodies[18]. The antibodies might be induced by epitopes found in microorganisms or allergen or allergens cross-react with MICA epitopes similar to natural HLA antibodies[20]. In addition, denatured MICA molecules could be the cause of non-specific reaction[18]. In the present study, MICA*002 was the frequent allele and antibody. Therefore, if MICA*002 was found to be donor specific antibody, this might be important in kidney transplantation. To compare MICA antibody frequencies with other studies was difficult due to many variations between studies. Previous study has shown that there are differences between the kits from different companies[19]. In addition, there are no reference standards of sera for antibody detection and variation in cut-off MFI. Therefore, many factors should be considered when analyzing the role of MICA antibody. The strength of MICA antibodies was also important as there was evidence that only patients with high MFI values developed acute rejection[19]. The present data of MICA alleles and antibodies might be useful in understanding MICA sensitizing event in kidney transplant recipients.

Conclusion

The diversity of MICA allele and linkage disequilibrium with HLA-B was found in Thai kidney transplant recipients similar to the healthy Thais. In addition, high frequencies MICA antibodies did not correlate with MICA alleles.

What is already known on this topic?

Previous study in Caucasians reported MICA polymorphism in kidney patients and found difference in MICA polymorphism from healthy individuals. The specificities of MICA antibodies in kidney transplant recipients were reported only in Caucasians.

What this study adds?

The polymorphism of MICA in Thai kidney transplant recipients and linkage disequilibrium with HLA-B were similar to healthy individuals, which was different from previously reported in Caucasians. The specificities of MICA antibodies in Thai kidney transplant recipients were different from those in Caucasians and there was no correlation between MICA antibodies and alleles.

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Potential conflicts of interest

The authors declare no conflict of interest.

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