RESEARCH REPORT

FREQUENCIES OF MICA GENE POLYMORPHISM: A COMPARISON BETWEEN INDONESIANS ON BACAN ISLAND AND SUBURBAN JAPANESE

Midori Nishiyama[1], Masanori Takahashi[2], Ken-chi Manaka[3], Betty Roosihermiatie[4], Takao Kuriyama[1] and Kimihiro Nakae[1]

[1]Department of Public Health Sciences, [2]Department of Legal Medicine; [3]Institute for Medical Sciences, Dokkyo University School of Medicine, Mibu, Japan; [4]National Institute of Health System Research and Development, Surabaya, Indonesia

Abstract. MHC class I chain related gene A (MICA) is located near the HLA-B gene on the short arm of human chromosome 6. In the transmembrane (TM) of region of MICA, there is a trinucleotide repeat (GCT/AGC) microsatellite polymorphism in exon 5. Five alleles with 4, 5, 6 and 9 repetitions or 5 repetitions with 1 additional nucleotide insertion (GGCT) are identified and they were named A4, A5, A5.1, A6, and A9 respectively. We report the allele frequencies of 127 Indonesians on Bacan Island and 250 Japanese in the Kanto area. From the genotyping result, the frequency among Indonesians was as follows: A4 15.4%, A5 26.0%, A5.1 16.5%, A6 5.5%, and A9 36.6%. The frequency among Japanese was as follows: A4 20.6%, A5 28.1%, A5.1 10.8%, A6 27.2%, and A9 13.2%. Allele 9 is significantly increased and allele 6 significantly decreased in Indonesians compared with Japanese subjects. The results suggested that MICA microsatellite polymorphism are quite different in each race. Among Indonesians, the frequency of MICA-A9 allele, which was reported to be negatively associated with Behçet's disease, was significantly higher, whereas the MICA-A6 allele frequency, which was reported to be positively associated with Behçet's disease, was significantly lower among Japanese.

INTRODUCTION

The human major histocompatibility complex class I chain-related genes (MICA), spanning over 11 kb of DNA, is located about 40 kb centoromeric to the HLA-B gene on the short arm of human chromosome 6. In the transmembrane (TM) of region of MICA, there is a trinucleotide repeat (GCT/AGC: Alanin) microsatellite polymorphism in exon 5. Five alleles with 4, 5, 6 and 9 repetitions or 5 repetitions with 1 additional nucleotide insertion (GGCT) are identified and they were named A4, A5, A5.1, A6, and A9 respectively (Fodil *et al,* 1996). Interestingly, a number of disease are reported to associate with the MICA gene. In particular, a strong association between Behçet's disease and the MICA gene have been reported and it is worthy of attention (Mizuki *et al,* 1997). Behçet's disease is a multisystemic inflammatory disorder affecting various organs. The highest prevalence of Behçet's disease occurs in a geographical region spanning the Mediterranean, Middle East and East Asia including Japan, and a significant association between HLA-B51 and the disease was found in these high prevalence countries (Verity *et al,* 1999). While patients in South and Southeast Asia are rarely reported, except for North India (Tan *et al,* 1999) and Chinese patients in Singapore (Pande *et al,* 1995). Mizuki *et al* (1997) reported that six GCT repetitions in the transmembrane region of the MICA gene (MICA-A6) had a strong association with Japanese patients with Behçet's disease. According to Salvarani *et al* (2001) the MICA-A9 allele was found to be negatively associated with Behçet's disease.

Thus, we investigate the allele frequencies of Indonesians people who have a very low preva-

Correspondence: Midori Nishiyama, Department of Public Health Sciences, Dokkyo University, School of Medicine, Mibu, Tochigi, 321-0293, Japan.
Tel: +81 (282) 87-2133; Fax: +81 (282) 86-2935
E-mail: m-nishi@dokkyomed.ac.jp

lence of Behçet's disease and compare these with Japanese controls who have a high prevalence as well as the differences in gene distribution.

MATERIALS AND METHODS

Subjects

We investigated 127 Indonesians volunteers living on Bacan Island, Maluku Province and 250 Japanese volunteers living in the Kanto area. Bacan Island is one of three main islands on the Bacan subdistrict located 127º-125ºE and 0º15′-0ºS in Indonesia (Fig 1). Subjects were selected from all 11 villages surrounding, the work area of the Health center in Labuha. Inhabitants of the 11 villages were informed of the purpose of the study and voluntarily asked to participate in genetic examination. Consent for participation in the study was provided by the subjects themselves or their legal guardians. Venous blood samples were kept frozen before transporting to Japan for blood examination. After arrival at our laboratory, the samples were stored at -80º. Japanese volunteers were recruited through a series of information meetings held in Dokkyo University School of Medicine. With informed consent for genetic examination, we took venous blood samples and stored them separately at -80º until DNA analysis.

Analysis of triplet repeat polymorphism of the MICA gene

Genomic DNA was extracted from blood samples using a DNA extractor QIAamp DNA mini kit (Qiagen GmbH, Germany) following the protocol of the manufacturer or Phenol-Chloroform method. For analysis of microsatellite repeat polymorphism in the TM region of the MICA gene, PCR primers flanking the TM region (MICA5F, 5′-CCTTTTTTTCAGGGAAAGTGC-3′; MICA5R, 5′-CCTTACCATCTCCAGAAA CTGC-3′) as previously described (Mizuki *et al*, 1997), were ordered to design by Espec Oligo Service Corp in Japan. Each amplification reaction contained 70-120 ng of high molecular weight DNA, 2.0 mM $MgCl_2$, 0.01% gelatin, 2.0 mM of dNTP (dATP, dCTP, dGTP, and dTTP), 10 pmol of each primer and 0.25 unit of Goldtag DNA polymerase (AmpliTag Gold™; Perkin Elmer) with a PCR buffer supplied from Perkin Elmer in a final volume of 25 μl. The PCR cycle conditions were as follows: initial denaturation for 13 minutes at 95ºC, followed by 10 cycles of 94ºC for 1 minute, 64ºC for 1 minute, 70ºC for 1.5 minutes, and 20 cycles of 90ºC for 1 minute, 64ºC for 1 minute, 70ºC for 1.5 minutes, with a final extension for 10 minutes at 72ºC. After con-

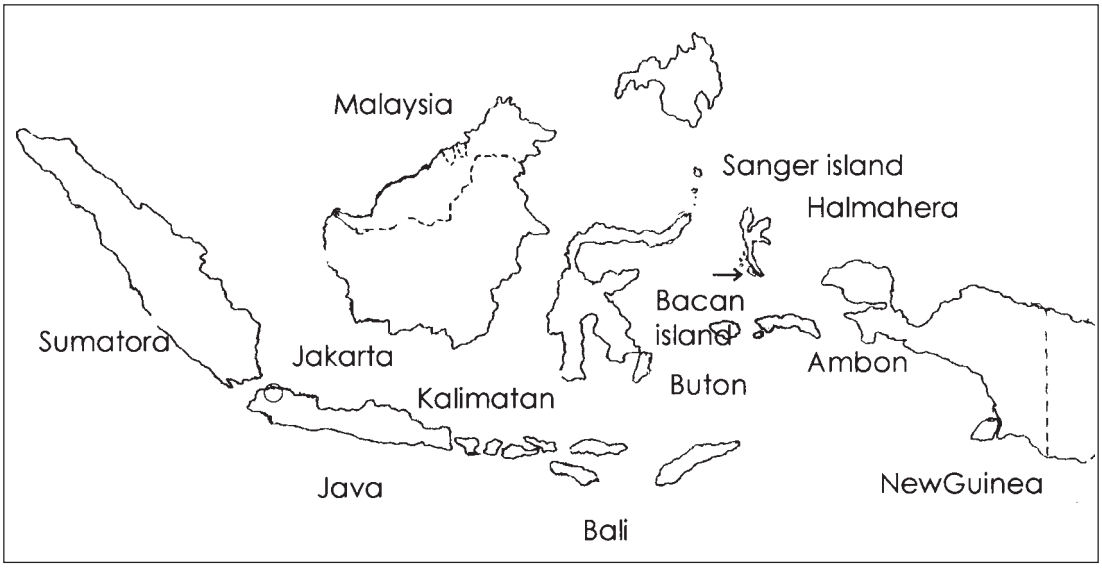

Fig 1–Bacan island is located adjacent to Halmahera island, North Maluku, in between Sulawesi and New Guinea islands.

firming the success of the amplification reaction by 2% agarose gel electrophoresis, PCR products, pre-heated to 95ºC (then immediately chilled on ice) were loaded onto 8% polyacrylamide gels containing 8 M urea, electrophoresed at 40 W, 48ºC for 3 hours and 30 minutes, stained with silver with Promega's DNA silver staining system to identify trinucleotide repeat (GCT/AGC) microsatellite polymorphism at the MICA gene.

DNA sequencing

From the samples genotyped with the above method, several samples of each homozygosity type was selected for DNA sequencing to identify the nucleotide order. DNA sequencing is conducted using BigDye Terminator Cycle Sequencing FS Ready Reaction Kit. Each amplification reaction contained 350-400 ng of the PCR products, 3.2 pmol of the forward or reward primer, 8 μl of pre-mixture (A,C,G,T-Big Dye Terminator, Tris-HCl, Buffer, AmpliTag DNA Polymerase, FS) and distilled water in a final volume of 20 μl. The PCR cycle conditions were as follows: hot start at 96ºC followed by 25 cycles of 94ºC for 10 seconds, 50ºC for 5 seconds, 60ºC for 4 minutes, then stored at 4ºC. The amplified products were purified using SigmaSpin™ Post-Reaction Purification Columns and analyzed by an automated fluorescent DNA sequencing machine (Applied Biosystem model 377 sequencer).

Data analysis

Allele frequencies and phenotype frequencies were estimated by direct counting. Statistical analysis was performed using SPSS 11.0 for windows. Peason's χ^2 test was used to compare significant differences between allele frequency of Indonesians and Japanese groups.

RESULTS

As the result of electrophoresis, fragment size of MICA-A4 allele shows 180 bp, A5 allele shows 183 bp, A5.1 allele shows 184 bp, A6 allele shows 186 bp and A9 allele shows 195 bp (Fig 2). MICA-genotypes are classified into A4/A4, A5/A4, A5/A5, A5.1/A4, A5.1/A5, A5.1/A5.1, A6/A4, A6/A5, A6/A5.1, A6/A6, A9/A4, A9/A5, A9/A5.1, A9/A6, and A9/A9. There are 5 types of homozygosity and 10 types of heterozygosity. Each genotyping frequency of samples is shown in Table 1. There were 49 cases of homozygosity (38.6%) and 78 heterozygosity (61.4%) in Indonesians and 104 homozygosity (44.3%) and 131 heterozygosity (55.7%) in Japanese. Power of discrimination was 0.89 in Indonesians and 0.91 in Japanese. Allele frequency of Indonesians were as follow; A4:15.4%, A5:10.8%, A5.1: 16.5%, A6:5.5%, and A9:36.6%. That of Japanese were as follow; A4: 21.0%, A5:287.6%, A5.1:11.2%, A6:27.0%, and A9:13.2% (Table 2).

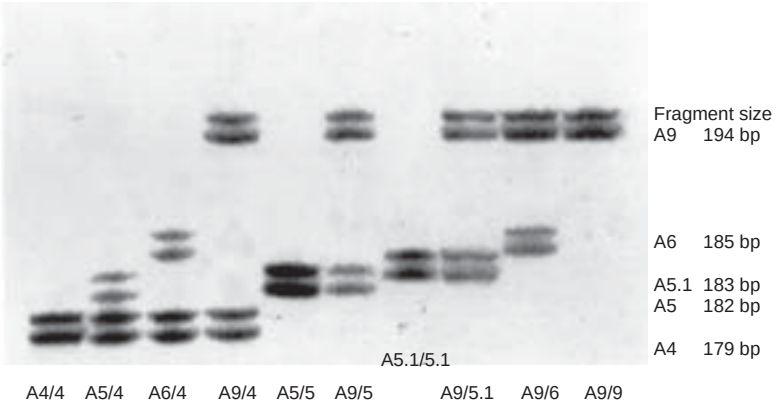

Fig 2–Eight percent polyacrylamide gels containing 8 M urea show the PCR products of MICA gene polymorphism. A4 allele produces a 179 bp fragment, A5: 182 bp, A5.1: 183 bp, A6: 185 bp and A9: 194 bp.

Table 1
MICA genotype frequencies among Indonesians on Bacan island and Japanese in the Kanto area.

Phenotype	Indonesians		Japanese	
	No.	%	No.	%
A9/A9	18	14.2	17	6.8
A9/A6	7	5.5	16	6.4
A9/A5.1	14	11.0	2	1.2
A9/A5	22	17.3	7	2.8
A9/A4	14	11.0	6	2.4
A6/A6	2	1.6	25	10.0
A6/A5.1	2	1.6	15	6.0
A6/A5	-	0	29	11.6
A6/A4	1	0.78	25	10.0
A5.1/A5.1	9	7.1	6	2.4
A5.1/A5	4	3.1	20	8.0
A5.1/A4	6	4.7	6	2.4
A5/A5	15	11.8	33	13.2
A5/A4	8	6.3	16	6.4
A4/A4	5	3.9	26	10.4
Total	127	100	250	100

Table 2
MICA gene frequencies among Indonesians on Bacan Island and Japanese in the Kanto area.

Allele	Gene frequencies	
	Indonesians (%)	Japanese (%)
A9	93 (36.6)	66 (13.2)
A6	14 (5.5)	135 (27.0)
A5.1	42 (16.5)	56 (11.2)
A5	66 (26.0)	138 (27.6)
A4	39 (15.4)	105 (21.0)
Total	254 (100)	500 (100)

Pearson's chi-square: Value=108.2, df=14, p<0.000

The allele frequencies among Indonesians was quite different to that of Japanese people. The MICA-A9 allele frequency among Indonesians was higher, and the MICA-A6 allele frequency was significantly lower than Japanese (p<0.000) (Table 3).

Each MICA allele was analyzed by an automated fluorescent DNA sequencing machine. The result of DNA sequencing of each allele is shown in Fig 3. Four different repeats (A4, A5, A5.1, A6 and A9) were identified and A9 allele have two different nucleotide from the other alleles. In addition, the A5.1 allele showed 1-bp insertion in the microsatellite region similar to previous re-

Table 3
A9 and A6 allele frequencies among Indonesians and Japanese.

	A9 allele[a]			A6 allele[b]		
	Without	Hetero	Homo	Without	Hetero	Homo
Indonesians (n=127)	52 (40.9%)	57 (44.9%)	18 (142%)	115 (90.6%)	10 (7.9%)	2 (1.6%)
Japanese (n=250)	201 (80.4%)	32 (12.8%)	17 (6.8%)	140 (56.0%)	85 (34.0%)	25 (10.0%)

Pearson's chi-square: [a]Value=61.2, df=2, p<0.000, [b]Value=46.0, df=2, p<0.000

Fig 3–Each MICA allele (A4, A5, A5.1, A6, and A9) was analyzed by an automated fluorescent DNA sequencing machine. There was a trinucleotide repeat (GCT) microsatellite polymorphism.

*A9 allele have two different nucleotides to other alleles (T→C, C→T).

+A5.1 allele showed 1-bp insertion in the microsatellite region similar to previous reports (GCT→GGCT).

ports (Fodil *et al,* 1996; Perez-Rodeigues *et al,* 2000).

DISCUSSION

The MICA gene phenotype and allele frequencies among Indonesians and Japanese people were estimated from the results of the present study.

It was revealed that the frequencies of MICA gene polymorphism among Japanese and Indonesians populations were quite different. Among Indonesians, the frequency of the MICA-A9 allele, which was reported to be negatively associated with Behçet's disease (Salvarani *et al,* 2001) was significantly higher, and the MICA-A6 allele frequency, which was reported to be positively associated with Behçet's disease (Mizuki *et al,* 1997) was significantly lower than Japanese.

Though research of MICA gene polymorphism has been conducted in many countries, few have been in South Eastern Asia. According to Mizuki *et al* (1997) the frequency of Japanese controls was as follows. A9: 16.5%, A6:25.7%, A5.1:9.2%, A5:31.6%, and A4:17.0%. From research in Korea, A9:15.2%, A6:26.5%, A5.1:19.1%, A5:26.2%, and A4:13.0% (Park *et al,* 2002). These previous results correlated with the present study.

According to Yabuki *et al* (1999) A9:16.3%, A6:28.8%, A5.1:27.5%, A5:8.0%, and A4:17.5% among Greek people. Among Italian controls, A9:19.9%, A6:29.2%, A5.1:26.5%, A5:11.9%, and A4:12.4% (Salvarani *et al,* 2001). These previous studies were conducted in countries known to have a high prevalence of Behçet's disease and the frequency of the MICA-A6 allele was as high as Japanese controls. On the other hand, Liu *et al* (2002) reported that the frequencies of MICA polymorphism in Taiwan were as follows: A9:10%, A6:5%, A5.1:25%, A5:37%, and A4:23%, and the A6 allele frequency was much lower than in Japanese and other countries with a high prevalence of Behçet's disease. The prevalence of the disease was reported considerably lower in Taiwan than in Japan (Chung *et al,* 1986). A study of Latvia reported that the A6 frequency was substantially low (Berzina *et al,* 2002).

As in a study of Japanese patients with Behçet's disease (Mizuki *et al,* 2000), MICA-A6 was found to be strongly associated with Behçet's

disease in the Greek sample (Yabuki *et al*, 1999). A recent study of Behçet's disease in Korea also reported that MICA-A6 had a strong association with Behçet's disease patients (Park *et al*, 2002). In Spanish research, however, the association of Behçet's disease is stronger with HLA-B51 rather than with the MICA gene (Gonzalez-Escribano *et al*, 1999). The frequency of MICA-A6 was not significantly higher in Italian patients than in controls, but the MICA-A9 allele was found to be negatively associated with Behçet's disease (Salvarani *et al*, 2001). HLA-B51 was indicated as the most important susceptibility gene in that disease (Salvarani *et al*, 2001). The study of Behçet's disease patients of Japan, Greece or Italy, showed that only HLA-B51 was found to be significantly associated with Behçet's disease in all three populations (Mizuki *et al*, 2000). The analysis of a study in Israel indicated that association between MICA and Behçet's disease resulted secondarily from a strong linkage disequilibrium with HLA-B51 (Cohen *et al*, 2002). There are several views regarding the association between MICA and Behçet's disease, and it remains undecided.

There were many previous studies regarding the association between MICA and various diseases; for example type 1 diabetes (Gambelunghe *et al*, 2000; Bibao *et al*, 2002; Shtauvere-Bramens *et al*, 2002; Zake *et al*, 2002), ulcerative colitis (Seki *et al*, 2001; Sugimura *et al*, 2001), or psoriatic arthritis (Gonzalez-Escribano *et al*, 2001). There is a strong linkage showing disequilibrium between the HLA-B antigens and the MICA alleles. Strong association of A4 with B18 and B27; A5 with B62; A5.1 with B7, B8 and B60; A6 with B44, B51, and B52; and A9 with B35 were reported in a previous study (Mizuki *et al*, 1997). Thus, association between MICA allele and diseases was reported to be probably the secondary result from HLA antigens (Mizuki *et al*, 2000; Cohen *et al*, 2002).

From our present study, however, it was suggested that MICA allele frequencies were considerably different among different races. The diseases that had race sensitivities such as Behçet's disease probably have genetic factors of onset. Further investigation of MICA gene and that disease are required.

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