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Major Histocompatibility Complex Class I Chain–Related A and B (MICA and MICB) Gene, Allele, and Haplotype Associations With Dengue Infections in Ethnic Thais

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Background. Major histocompatibility complex class I chain–related (MIC) A and B (MICA and MICB) are polymorphic stress molecules recognized by natural killer cells. This study was performed to analyze *MIC* gene profiles in hospitalized Thai children with acute dengue illness.

Methods. *MIC* allele profiles were determined in a discovery cohort of patients with dengue fever or dengue hemorrhagic fever (DHF) (n = 166) and controls (n = 149). A replication cohort of patients with dengue (n = 222) was used to confirm specific *MICB* associations with disease.

Results. *MICA*045* and *MICB*004* associated with susceptibility to DHF in secondary dengue virus (DENV) infections (odds ratio [OR], 3.22; [95% confidence interval (CI), 1.18–8.84] and 1.99 [1.07–2.13], respectively), and *MICB*002* with protection from DHF in secondary DENV infections (OR, 0.41; 95% CI, .21–.68). The protective effect of *MICB*002* against secondary DHF was confirmed in the replication cohort (OR, 0.43; 95% CI, .22–.82) and was stronger when *MICB*002* is present in individuals also carrying *HLA-B*18*, *B*40*, and *B*44* alleles which form the B44 supertype of functionally related alleles (0.29, 95% CI, .14–.60).

Conclusions. Given that *MICB*002* is a low expresser of soluble proteins, these data indicate that surface expression of *MICB*002* with B44 supertype alleles on DENV-infected cells confer a protective advantage in controlling DENV infection using natural killer cells.

Keywords. MICA; MICB; gene; allele; haplotype; associations; secondary; dengue; infections; Thais.

Many human immune response genes are located in the major histocompatibility complex (MHC) on chromosome 6, and their products influence T, B, and natural killer (NK) cell responses to virus infections [1]. The timing and intensity of the responses of each of these components can have a profound influence on the outcome of infection. Evidence suggests that these factors are particularly relevant to infection with dengue virus (DENV), for which clinical expression ranges from a mild, self-limited febrile illness (dengue fever [DF]) to a life-threatening plasma leakage syndrome (dengue hemorrhagic fever [DHF]) associated with a cytokine storm [2]. Candidate gene association studies of DENV infections have revealed a variety of genes within the MHC associated with distinct disease phenotypes [1]. In ethnic Thais, allelic variants of classical HLA [3], combinations of HLA alleles or haplotypes encoded by different loci [4], and HLA supertypes or groups of alleles with a shared common function in antigen presentation [5] have all been associated with DENV clinical expression.

Genome-wide association studies (GWASs) of DENV infections in mainland SE Asian populations have detected and replicated an association with the non-classical MHC class I chain–related B (MICB) gene [6–8]. The MICB gene and its functional homologue MICA are located close to the classical class I gene locus HLA-B in the MHC [9]. Both MICA and MICB are polymorphic and encode cell surface and soluble proteins [9] that are recognized by cells with NK function using the most ubiquitous lectinlike activating receptor NKG2D [10]. MIC proteins are considered to be stress-related molecules up-regulated by viruses and inflammation in epithelial cells [11]. Polymorphism of MIC genes can be correlated with increased binding capacity to NKG2D [12] and

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affect surface expression of MIC products [9, 13]. Previous analysis of linkage disequilibrium between MIC genes and the adjacent HLA-B locus has also shown that certain rare extended HLA haplotypes with a complete deletion of the entire MIC region are associated with severe secondary DENV infections in ethnic Thais [4].

Bangkok is considered a major epicenter of DENV amplification and transmission in mainland SE Asia. In this population-dense urban environment, diverse immunological responses to DENV affect the selection of the fittest strains for successful transmission [14, 15]. Given the potential role of MIC gene products in influencing the outcome of DENV infections [4, 6–8], we determined the *MICA* and *MICB* allele profiles in a discovery cohort of Bangkok patients with primary and secondary DF and DHF and compared these groups with ethnically and geographically matched controls. We then tested and confirmed a specific *MICB* allele association in a second replication cohort of DENV-infected patients from Bangkok, and we investigated the effect of linkage disequilibrium between MICB and HLA-B on this association.

MATERIALS AND METHODS

Patients and Controls

Unrelated ethnic Thai children who had serologically and virologically confirmed DENV infections, were between the ages of 3 and 14 years, and weighed >20 kg were enrolled in Bangkok between 1994 and 2007, with fully informed consent, as described elsewhere [3]. Clinical diagnoses of DF and DHF were assigned by an expert physician reviewer guided by the 1997 World Health Organization (WHO) criteria [16, 17]. A total of 250 patients with DF and 150 with DHF were available for MIC allele analysis. DHF was further classified into increasing grades of clinical severity, DHF grades 1, 2, 3, and 4, according to WHO criteria [3]. Serological responses to DENV were measured with both enzyme immunoassay and hemagglutination inhibition test. DENV serotypes (types 1, 2, 3, and 4) were identified by isolation in *Toxorhynchites splendens* mosquitoes or reverse-transcription polymerase chain reaction (PCR), as described elsewhere [17, 18]. The infecting DENV serotype was determined in all but 8 patients (data not shown).

Primary or secondary infections were defined on the basis of DENV-specific immunoglobulin M–immunoglobulin G serum antibody ratios and the titer of hemagglutination inhibition antibodies, according to WHO guidelines [18, 19]. On this basis, 26% of patients with DF (65 of 250) had primary infections, and 74% (184 of 250) had secondary infections. By contrast, 8% of patients with DHF (12 of 150) had primary infections, and 92% (138 of 150) had secondary infections. One hundred forty-nine unrelated, ethnically and geographically matched, normal healthy Thai blood donors with no clinical history of autoimmune or malignant disease, whose families were known to have resided in the Bangkok metropolitan area for many generations, were used as population controls. Previous exposure to DENV in this control group was unknown. Human use approval was granted by the institutional review boards of the Thai Ministry of Public Health, the Office of the US Army Surgeon General, and the University of Rhode Island.

Study Design

Molecular genotyping was performed on full-length genomic DNA prepared from whole blood, as described elsewhere [3, 20]. PCR with sequence-specific primers and direct sequencing were used to MICA and MICB type 96 patients DF, 81 with DHF, and 149 controls in a discovery cohort, as described elsewhere [19, 21]. Deduced MICA and MICB antigen or phenotype frequencies (PFs) were determined using the following formula; PF (%) = n/N × 100, where n represents the number of individuals with a given MIC allele, and N the total number of individuals MIC typed in each of the patient and control groups.

A second cohort of 154 patients with DF and 69 with DHF were screened for the *MICB*002* allele in a replication study, using a combination of forward and reverse PCR sequence-specific primers (Supplementary Tables 1 and 2) selected to specifically identify the presence or absence of *MICB*002* (as given in Supplementary Table 3), using the same methods and conditions as above [20]. The presence or absence of *MICB*002* identified with this screening method in the replication cohort was confirmed by means of direct sequencing, as described elsewhere [21]. HLA-B locus class I allele and supertype profiles were available for all but 10 patients and all the controls, as described elsewhere [3–5]. Linkage disequilibrium indices D and D' for *MICB* and *HLA-B* were determined in the Thai control panel (Supplementary Table 4), using Arlequin software (version 3.1) [22] and haplotype frequency estimation software [23].

Statistical Analysis

MICA and *MICB* PFs were compared between each patient group (stratified for primary or secondary infections and disease severity) and the controls and were tested for associations using the χ^2 test for heterogeneity (2 × 2 contingency tables). Differences were considered significant at $P < .05$. Bonferroni-corrected P (P_c) values for multiple comparisons and random MIC allele association (in the discovery cohort) depended on the number and type of comparisons made, namely, the number of *MICA* (n = 17) or *MICB* (n = 9) alleles identified, combined with the number of patient and control group comparisons (n = 8; primary DF vs controls, secondary DF vs controls, secondary DHF vs controls, all secondary DENV infections vs controls, all DENV infections vs controls, primary DF vs secondary DF, primary DF vs secondary DHF, and secondary DF vs secondary DHF). This equates to 17 + 8 or 25 comparisons for *MICA,* and 9 + 8 or 17 comparisons for *MICB.* Differences were considered highly significant at $P_c < .05$.

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the risk of either disease severity (OR with 95% CI >1.0) or protection (OR with 95% CI <1.0). Previous (a priori) power calculations demonstrated that all patient and control groups were of sufficient size to achieve ≥80% power to detect *MIC* allele differences of 11%–21% ($P < .05$) [4], except for the primary DHF group (n = 12), which was underpowered and thus deemed too small for inclusion in the analysis. To analyze the effect of the presence or absence of *HLA-B* alleles in the B44 supertype group on *MICB* allele associations with dengue, an additional 4 comparisons were added to the above MICB correction factor for patient and control group comparisons (n = 8), for a total of 12 comparisons used to correct *P* values derived with χ^2 tests.

RESULTS

MICA Alleles in Dengue Discovery Cohort

MICA antigen or PFs in our discovery cohort of patients with primary DF, secondary DF, or secondary DHF are given in Table 1, together with the equivalent PF in our ethnically and geographically matched Bangkok controls. Only a relatively rare allele *MICA*045* demonstrated a significant association with susceptibility to patients with secondary DHF compared with controls ($P < .05$; P_c, not significant).

Table 1. Major Histocompatibility Complex Class I Chain–Related A Antigen or Phenotype Frequencies in the Thai Dengue Discovery Cohort[a]

Discovery Cohort (MICA)	Patients or Controls, No. (PF, %)			
	Primary	Secondary		Controls
	DF (n = 40)	DF (n = 56)	DHF (n = 70)	(n = 149)
*MICA*002*	12 (30.0)	15 (26.8)	20 (28.6)	35 (23.5)
*MICA*004*	3 (7.5)	4 (7.1)	3 (4.3)	20 (13.4)
*MICA*007*	0	1 (1.8)	1 (1.4)	4 (2.7)
*MICA*008*	15 (37.5)	20 (35.7)	24 (34.3)	51 (34.2)
*MICA*009*	3 (7.5)	7 (12.5)	6 (8.6)	7 (4.7)
*MICA*010*	11 (27.5)	15 (26.8)	22 (31.4)	43 (28.9)
*MICA*012*	1 (2.5)	6 (10.7)	4 (5.7)	15 (10.1)
*MICA*015*	0	0	0	3 (2.0)
*MICA*016*	0	0	0	1 (0.7)
*MICA*017*	3 (7.5)	2 (3.6)	2 (2.9)	10 (6.7)
*MICA*018*	5 (12.5)	6 (10.7)	4 (5.7)	12 (8.1)
*MICA*019*	16 (40.0)	14 (25.0)	25 (35.2)	54 (36.2)
*MICA*027*	3 (7.5)	5 (8.9)	3 (4.3)	17 (11.4)
*MICA*033*	0	0	1 (1.4)	1 (0.7)
*MICA*038*	0	0	0	1 (0.7)
*MICA*044*	0	0	0	1 (0.7)
*MICA*045*	5 (12.5)	3 (5.4)	12 (17.1)[b]	9 (6.0)[b]

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; MICA, major histocompatibility complex class I chain–related A; PF, phenotype frequency.

[a]Bonferroni-corrected *P* values (P_c) were calculated as $P \times 25$ (17 MICA alleles + 8 patient group and control comparisons). Eleven patients identified with primary DHF were excluded from the analysis owing to relatively low numbers and lack of statistical power (see Materials and Methods).

[b]Significant difference for *MICA*045* between patients with secondary DHF infections and controls: $\chi^2 = 6.8$; $P = .009$; $P_c = .23$; odds ratio, 3.22; 95% confidence interval 1.18–8.84.

MICB Alleles in Dengue Discovery Cohort

MICB PF in the discovery cohort of patients and controls are presented in Table 2. Overall the frequency of *MICB*002* was reduced in all patient groups compared with the controls ($P < .05$; P_c, not significant). The protective effect of *MICB*002* was relatively more significant in patients with secondary DHF ($P < .05$). By contrast, a less significant association was also observed between *MICB*004* and susceptibility to all DENV infections combined, and particularly secondary DHF ($P < .05$; P_c, not significant) (Table 2).

*MICB*002* Allele Frequency in Replication Dengue Cohort

Given that the strongest association with a relatively common MIC-encoded allele in the discovery cohort was with *MICB*002,* we screened a second replication cohort of DENV-infected patients in Bangkok with a panel of MICB-specific PCR primers (Supplementary Tables 1–3) designed to unambiguously detect the presence or absence of *MICB*002* in ethnic Thais (Table 3). The frequency of *MICB*002* was significantly reduced in all patient groups combined versus the Bangkok controls ($P_c < .05$), indicating a strong protective effect of this allele against symptomatic dengue, particularly after secondary exposure to DENV ($P_c < .05$). This effect was more pronounced in patients with secondary DHF ($P_c < .05$), thus replicating and confirming the original observation in the discovery cohort (Table 2).

Table 2. Major Histocompatibility Complex Class I Chain–Related B Antigen or Phenotype Frequencies in the Thai Dengue Discovery Cohort[a]

Discovery Cohort (MICB)	Patients or Controls, No. (PF, %)			
	Primary	Secondary		Controls
	DF (n = 40)	DF (n = 56)	DHF (n = 70)	(n = 149)
*MICB*002*	19 (47.5)[b]	24 (42.8)[b,c]	21 (30.0)[b,c,d]	76 (51.0)[b,c,d]
*MICB*003*	4 (10.0)	2 (3.6)	3 (4.3)	10 (6.7)
*MICB*004*	17 (42.5)[e]	25 (44.6)[e]	34 (48.6)[e,f]	48 (32.2)[e,f]
*MICB*005:02*	19 (47.5)	34 (60.7)	46 (65.7)	91 (61.1)
*MICB*005:03*	3 (7.5)	2 (3.6)	1 (1.4)	9 (6.0)
*MICB*008*	5 (12.5)	7 (12.5)	8 (11.4)	14 (9.4)
*MICB*009N*	2 (5.0)	4 (7.1)	4 (5.7)	14 (9.4)
*MICB*013*	0	1 (1.8)	3 (4.3)	4 (2.7)
*MICB*014*	4 (10.0)	2 (3.6)	2 (2.9)	5 (3.4)

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; MICB, major histocompatibility complex class I chain–related B; PF, phenotype frequency.

[a]Bonferroni-corrected *P* values (P_c) were calculated as $P \times 17$ (9 MICB alleles + 8 patient group and control comparisons). Eleven patients identified with primary DHF were excluded from the analysis owing to relatively low numbers and lack of statistical power (see Materials and Methods).

[b]Significant difference for *MICB*002* between all patients with DENV infections and controls: $\chi^2 = 4.9$; $P = .03$; $P_c = .45$; odds ratio (OR), 0.60; 95% confidence interval (CI), .38–.97.

[c]Significant difference for *MICB*002* between patients all patients with secondary dengue virus (DENV) infections and controls: $\chi^2 = 6.5$; $P = .01$; $P_c = .19$; OR, 0.53; 95% CI, .32–.89.

[d]Significant difference for *MICB*002* between patients with secondary DHF infections and controls: $\chi^2 = 8.5$; $P = .004$; $P_c = .06$; OR, 0.41; 95% CI, .21–.68.

[e]Significant difference for *MICB*004* between all patients with DENV infections and controls: $\chi^2 = 6.1$; $P = .01$; $P_c = .24$; OR, 1.78; 95% CI, 1.09–2.89.

[f]Significant difference for *MICB*004* between patients with secondary DHF infections and controls: $\chi^2 = 5.4$; $P = .02$; $P_c = .34$; OR, 1.99; 95% CI, 1.07–2.13.

Table 3. *MICB*002* Antigen or Phenotype Frequency in the Thai Replication Cohort of Dengue Virus-Infected Patients[a]

Replication Cohort (MICB)	Patients or Controls, No. (PF, %)			
	Primary	Secondary		Controls
	DF (n = 25)	DF (n = 129)	DHF (n = 68)	(n = 149)
*MICB*002*	8 (32.0)[b]	46 (35.7)[b,c,d]	21 (30.9)[b,c,e]	76 (51.0)[b,c,d,e]

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; MICB, major histocompatibility complex class I chain–related B; PF, phenotype frequency.

[a]Bonferroni-corrected *P* values (P_c) were calculated as $P \times 8$ (for the 8 patient group and control comparisons). One patient was identified with primary DHF in the replication cohort and excluded from the analysis owing to singleton value and lack of statistical power (see Materials and Methods).

[b]Significant difference for between all patients with dengue virus (DENV) infections and controls: χ^2 = 11.0; P < .001; P_c = .007; odds ratio (OR), 0.49; 95% confidence interval (CI), .31–.77.

[c]Significant difference between all patients with secondary DENV infections and controls: χ^2 = 10.1; P = .001; P_c = .01; OR, 0.50; 95% CI, .31–.78.

[d]Significant difference between patients with secondary DF infections and controls: χ^2 = 6.6; P = .01; P_c = .08; OR, 0.53; 95% CI, .32–.89.

[e]Significant difference between patients with secondary DHF infections and controls: χ^2 = 7.7; P = .006; P_c = .046; OR, 0.43; 95% CI, .22–.82.

Moreover, the frequency of *MICB*002* in all secondary DHF infections, in both the discovery (Table 2) and replication cohorts (Table 3) combined (42 of 138 [30.4%]), was significantly lower than in the controls (76 of 149 [51.0%]; χ^2 = 12.5; P < .001; P_c = .007; OR, 0.42; 95% CI, .25–.70).

*MICB*002* and the HLA-B44 Supertype in Discovery and Replication Cohorts

Profound linkage disequilibrium is known to exist between alleles of MICB and the adjacent HLA-B gene loci [9]. In our Bangkok population controls, *MICB*002* is in linkage disequilibrium with *HLA-B*18, B*40,* and *B*44* (Supplementary Table 4), which together form the HLA-B44 supertype, a functionally related group of alleles that share a common preference to bind and present antigenic peptides of a similar structure to the immune system [24]. Thus, we tested both our discovery and replication cohorts for the effect of *MICB*002* and the presence or absence of B44 supertype alleles on dengue disease association (Table 4). The presence of both *MICB*002* and a B44 supertype allele (*HLA-B*18, B*40,* and *B*44*) together was strongly protective against the development of secondary DHF, compared with the controls (P_c < .05) and both patients with primary DF (P_c < .05) and those with secondary DF (P < .05). By contrast, the absence of both *MICB*002* and a B44 supertype allele was significantly associated with susceptibility to DHF in secondary DENV infections (P < .05; Table 4).

DISCUSSION

Cells with NK activity perform a variety of cytolytic and immunoregulatory functions using an extensive array of diverse receptors, many of which recognize either increased or decreased expression of classical and non-classical HLA class I gene products on the surface of cells infected with viruses [1], including DENV [1, 25]. NK cells can kill DENV-infected cells in the absence of antibody and independent of any pathway using antibody-dependent cellular cytotoxicity [26], thus indicating that direct recognition of DENV-induced target ligands occurs via activating NK receptors. The most ubiquitous activating lectinlike NK cell receptor (NKG2D) recognizes the stress-induced MHC-encoded MICA and MICB proteins [9]. Engagement of MIC proteins by NKG2D triggers NK cells and costimulates antigen-specific CD8 αβ T cells [11], which are important in controlling DENV infections [2].

MICA and MICB can be expressed at the surface of virus-infected cells or released as soluble proteins (sMICA/B) after proteolytic cleavage from the cell surface [9, 27]. sMICA/B can block NKG2D-bearing NK cells in the periphery before they engage with membrane-bound MIC on virus-infected cells [27]. Virus-induced sMICA/B can also down-regulate NKG2D and induce a state of unresponsiveness or anergy in NK cells

Table 4. *MICB*002* and HLA-B44 Supertype Combinations or Haplotype Frequencies in the Combined Thai Discovery and Replication Cohorts[a]

Combined Discovery and Replication Cohorts (*MICB*002*, HLA-B44 Supertype Combinations)		Patients or Controls, No. (HF, %)			
		Primary	Secondary		Controls
*MICB*002*	HLA-B44 supertype	DF (n = 65)	DF (n = 178)	DHF (n = 135)	(n = 149)
Positive	Positive	17 (26.2)[b]	35 (19.7)[c]	13 (9.6)[b,c,d]	40 (26.8)[d]
Positive	Negative	11 (16.9)	32 (17.9)	29 (21.5)	36 (24.2)
Negative	Positive	10 (15.4)	36 (20.2)	25 (18.2)	18 (12.1)
Negative	Negative	27 (41.5)	75 (42.1)	68 (50.4)[e]	55 (36.9)[e]

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; HF, haplotype frequency; MICB, major histocompatibility complex class I chain–related B.

[a]Bonferroni-corrected *P* values (P_c) were calculated as $P \times 12$ (4 *MICB*002*, B44 haplotypes + 8 patient group and control comparisons (see Materials and Methods).

[b]Significant difference in combination of *MICB*002*-positive and B44 supertype–positive alleles in patients with secondary DHF and primary DF infections: χ^2 = 9.4; P = .002; P_c = .03; OR, 0.30; 95% CI, .13–.71.

[c]Significant difference in combination of *MICB*002*-positive and B44 supertype–positive alleles in patients with secondary DHF infections and those with secondary DF infections: χ^2 = 6.0; P = .01; P_c = .18; OR, 0.44; 95% CI, .21–.90.

[d]Significant difference in combination of *MICB*002*-positive and B44 supertype–positive alleles in patients with secondary DHF infections and in controls: χ^2 = 13.8; P < .001; P_c = .002; odds ratio (OR), 0.29; 95% confidence interval (CI), .14–.60.

[e]Significant difference in absence of *MICB*002* and B44 supertype alleles between patients with secondary DHF infections and controls: χ^2 = 5.2; P = .02; P_c = .24; OR, 1.73; 95% CI, 1.05–2.87.

[27], which is a powerful form of immune evasion. DENV infection has been associated with the release of sMICB in vivo [28] and the up-regulation of a host proteolytic enzyme matrix metalloproteinase 9, which cleaves MICB from the cell surface [29, 30], as well as up-regulation of classic HLA class I surface expression in vitro [31].

Exon-specific *MICA*008* allele associations with DENV infections have been reported in Cuban patients [32]. These associations were not replicated in our Thai discovery cohort (Table 1). By contrast, *MICA*045* was increased in frequency in our secondary DHF discovery cohort, but this allele was not identified in the previous Cuban study [32]. A polymorphic dimorphism in the second external protein domain of MICA at position 129 (valine [Val]/methionine [Met]) is known to affect NKG2D target affinity and NK cell activation [12]. Our PCR primer panel was designed to identify this dimorphism [20]; however, no significant variation in MICA position 129 Val or Met frequencies were observed between our patient and control groups (data not shown), indicating that there is no major association between low-affinity (129Val) or high-affinity (129Met) MICA alleles with dengue infection and NK cell activation via NKG2D.

By contrast, 3 large GWASs performed in mainland SE Asian populations have identified and replicated a specific association with a MICB single-nucleotide polymorphism (SNP rs3132468-C) in some 5614 Vietnamese and Thai patients exposed to DENV [6–8]. More recently, this association with has been replicated in a smaller group of Colombian patients with dengue [33]. The MICB rs3132468 SNP is located in intron 6 [8], some 2000 base pairs downstream from exons 2 and 3, which encode the structural variants of MICB [9] analyzed in our study (Tables 2–4). Thus, the dimorphic polymorphism characterized by SNP rs3132468T/C is noncoding and unlikely to be located in any known promoter or enhancer elements. However, MICB rs3132468-C is in linkage disequilibrium with another MICB SNP (rs3828916-C) [8], which is located in a 5'-upstream regulatory region of exon 2 of the MICB allele encoding *MICB*004* [34].

Given that *MICB*004* was significantly increased in frequency in our discovery cohort, particularly in patients with secondary DHF (Table 2), this would indicate that the original GWAS observations of MICB associations in dengue cohorts may have a structural basis in terms of specific MICB protein variants influencing disease outcome. Furthermore, analysis of MICB messenger RNA expression in Epstein-Barr virus–transformed lymphoblastoid cell lines has shown that copy number of the original MICB rs3132468-C SNP associated with susceptibility to DENV infection is correlated with lower MICB expression [8], albeit in a relatively low number of cell lines (n = 20). However, measuring MICB gene expression in EBV-transformed cell lines is intrinsically difficult to interpret given that EBV, like other large DNA viruses, is known to down-regulate MIC gene expression [9, 35].

Our study was designed to focus on identifying exon-specific MICB allele profiles in a carefully defined cohort of patients exposed to DENV in the urban setting of Bangkok, a recognized hot zone of DENV transmission in mainland Southeast Asia [14]. In this cohort, *MICB*002,* a relatively common allele in most populations [34], seems to be associated with protection against developing DHF in secondary DENV infections (Tables 2 and 3). A similar trend may also have been evident in a previous Cuban study [32]. There is some variability of *MICB*002* allele frequencies across different populations [34, 36–38]. Nevertheless, our urban Thai control *MICB*002* allele frequency (Table 2) is equivalent to other Thai [38] and non-Thai populations in this region [34].

Structurally, the protein product of *MICB*002* does not have any unique amino acids in the first and second extracellular MICB protein domains, particularly at the known contact sites with NKG2D receptors [39]. *MICB*002* shares a patchwork of polymorphisms with the other MICB alleles but has a unique allele-defined protein profile [40]. Functionally, cell surface expression of *MICB*002* has been reported to be reduced compared with other MICB alleles, which is largely attributed to a 2-base nucleotide deletion in the 5' promoter region of *MICB*002* [13], although this effect varies depending on the reporter cell lines used to measure MICB transcription and on heat shock stimulation [27].

Nevertheless, *MICB*002* is also associated with relatively low production of sMICB proteins [27]. Thus, a low sMICB-producing allele (*MICB*002)* seems to provide protection against secondary DHF (Tables 2–4), suggesting that reduced MICB expression confers some advantage in controlling DENV infection, particularly if not released as a soluble protein. However, if functionally retained and expressed on DENV-infected cells *MICB*002* may act as a beacon to early NKG2D-mediated immune responses, capable of reducing viral load and severe disease in individuals undergoing secondary infection with DENV. Inhibition of sMICB is being explored to enhance cancer immunotherapy [41, 42], and our findings suggest that this approach might have clinical value in DENV infections.

The MICA and MICB gene loci are located adjacent to HLA-B in the class I region of the human MHC [9]. A characteristic feature of the MHC is formation of stable allele combinations encoded by different loci forming genetic haplotypes, which vary in composition and frequency between different ethnic groups [1]. *MICB*002* is in linkage disequilibrium with *HLA-B*18, B*40,* and *B*44* (Supplementary Table 4), which form the B44 supertype [23] that has been shown to be protective against DHF after secondary DENV infections in our cohort [5]. In our Bangkok Thai patients and controls with full HLA-B and MICB allele profiles available, the presence of both *MICB*002* and a B44 supertype allele gave the most significant protective association against secondary DHF (Table 4). This indicates that some MHC gene combinations can act synergistically to influence disease expression in previously DENV-exposed individuals.

Taken together with previous HLA allele, haplotype, and supertype associations identified in the same Bangkok cohort [3–5], the current data suggest there is a hierarchy of multifactorial effects and associations, now including MICB expression, influencing DENV disease outcome. In the last several years, one vaccine against dengue has been licensed and another has shown positive results over the first year after vaccination in a critical phase III trial [43, 44]. However, the efficacy of both vaccines is influenced by host factors, particularly previous DENV exposure. The potential for MICB genes to modulate the association of HLA with clinical dengue disease will need to be considered in studies to further define host genetic factors with vaccine efficacy.

In conclusion, we have investigated previous GWAS-defined intron-located MICB SNP associations with susceptibility to severe DENV infections [6–8, 33] by analyzing exon-specific MICA and MICB allele profiles in an urban Thai cohort of DENV-infected patients in a case-control targeted gene association study. *MICB*002* was associated with protection against developing severe secondary DHF infections in both our discovery and replication cohorts. *MICB*002* is a low expresser of soluble proteins [27] and has also been implicated as a protective genetic factor in other noninfectious inflammatory conditions in Far Eastern populations [45]. Because sMICB is known to block NKG2D-baring cells in the periphery and impede the cytotoxic and immunoregulatory activities of NK and T cells [27], the expression and retention of membrane-bound *MICB*002* together with certain functionally related HLA-B alleles [5] seems to provide a significant protective advantage against the development of DHF or severe disease in secondary DENV infection.

Notes

Disclaimer. The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the official views of the National Institutes of Health, the US Army, or the US Department of Defense.

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