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Alpha tryptase allele of *Tryptase 1* (*TPSAB1*) gene associated with Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in Vietnam and Philippines

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ABSTRACT

We previously reported, significantly higher levels of Chymase and Tryptase in early stage plasma of DSS patients prior to the occurrence of shock suggesting a possible role of mast cells in dengue pathogenesis. To further investigate, we analyzed *CMA1* promoter SNP (rs1800875) and *TPSAB1* gene alleles, which encode the Human Chymase and α- and β- tryptase 1 enzymes respectively, for susceptibility to Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in patients from hospitals in Vietnam (Ho Chi Minh City and Vinh Long) and the Philippines. While the *CMA1* promoter SNP (rs1800875) was not associated with DHF/DSS, the homozygous form of α-tryptase allele was associated with DSS patients in Vinh Long and the Philippines (OR = 3.52, $p < 0.0001$; OR = 3.37, $p < 0.0001$, respectively) and with DHF in Ho Chi Minh City (OR = 2.54, $p = 0.0084$). Also, a statistically significant association was observed when DHF and DSS were combined in Vinh Long (OR = 1.5, $p = 0.034$) and the Philippines (OR = 2.36, $p = 0.0004$); in Ho Chi Minh City when DHF and DSS were combine an association was observed, but it was not statistically significant (OR = 1.5, $p = 0.0505$). Therefore, the α-tryptase might have a possible effect on the susceptibility to severe form of Dengue infection.

Abbreviations: Bp, base pairs; CMA1, chymase human gene 1; CMA1PF, chymase gene forward primer; CMA1PR, chymase gene reverse primer; DEN, Dengue virus serotype; DFD, Dengue fever; DHF, Dengue Hemorrhagic Fever; DNA, deoxy-ribonucleic acid; DSS, Dengue Shock Syndrome; DV, Dengue virus; ELISA, enzyme-linked immunosorbent assay; HCMC, Ho Chi Minh City; Ig, immunoglobulin; IMC, Integrated Management of Childhood Illness; LD, linkage disequilibrium; NK, natural killer cell; OR, odds ratio; *P*, *p*-value probability value; PCMC, Philippine Children's Medical Center; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RITM, Research Institute for Tropical Medicine; RT-PCR, reverse transcription-PCR; SNP, single nucleotide polymorphism; TPSAB1, tryptase human gene alpha-beta 1; TPSAB1PF, tryptase gene forward primer; TPSAB1PR, tryptase gene reverse primer; TPSB2, tryptase human gene alpha-beta 2; VL, Vinh Long Providence; WHO, World Health Organization.

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1. Introduction

Dengue infection causes a wide spectrum of clinical presentation, from asymptomatic and mild Dengue fever (DF), to the most serious forms: Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). It is a global emerging arboviral illness with the number of severe cases rapidly increasing about fivefold within the past 20 years. In Southeast Asian and Latin American countries, majority of the severe cases occur among the pediatric population [1].

DHF is characterized by plasma leakage, thrombocytopenia, coagulation abnormalities, and/or haemorrhage while DSS is accompanied by hypovolemic shock. The pathogenesis of DSS/DHF is not clear. Increased vascular permeability is generally recognized as the hallmark of these severe dengue forms. Currently, no targeted treatments exist to counterbalance the vascular leakage that occur during these severe complicated episodes, partly due to the lack of understanding of the mechanisms of Dengue virus (DV)-induced vascular leakage [2,3].

It has been suggested that increased vascular permeability, which has been implicated in the pathogenesis of asthma and other allergic diseases, is also responsible for the complications in severe Dengue infections. Mast cell activation is closely linked with local or systemic increases in vascular permeability in allergic diseases. Interestingly, recent studies in mouse models suggest that degranulation of mast cells are also involved in Dengue virus infection making them possible targets for novel therapeutic and preventive strategies [4–6]. Chymase and Tryptase are the major proteins specifically stored inside mast cells. They are secreted in the process of degranulation, which is a reflection of the magnitude of mast cell activation. These enzymes have indeed been reported to participate in the allergic process due to their vasoactive, proinflammatory and chemotactic actions [7–9]. We previously reported that on admission, plasma levels of Tryptase and Chymase are significantly elevated among DHF and/or DSS patients compared with either mild DF or febrile illness other than Dengue [10]. Moreover, in studies using mouse model and clinical human specimens with DHF and DSS, direct correlation between mast cell chymase production, and subsequent vascular leakage, were observed [4].

Human Chymase is encoded in the *CMA1* gene located on the long arm of chromosome 14 (14q11.2). The polymorphism at the promoter region at −1903 G/A of the gene (rs1800875) has been analysed by several groups for its association with allergy and asthma [11–14]. Human α- and β- tryptase are coded by two tandemly located genes *TPSAB1* and *TPSB2* on the chromosome 16 (16p.13.3). The *TPSAB1* locus codes for the α- and β- tryptase 1, whereas the *TPSB2* gene encodes functional isoforms β-tryptase 2 and β-tryptase 3 [15–17]. α-Tryptase is believed to be constitutive, largely inactive, not stored within the mast cell granules and therefore does not participate in the process of degranulation [19,25]. It is the β-tryptase 1, on the other hand, is an active peptidase involved in the recruitment of neutrophils, initiation of mast cell degranulation and induction of microvascular leakage; hence, it is implicated as a key mediator of allergic inflammation and severity [18–20]. We compared the frequencies of the Chymase and Tryptase gene alleles (*CMA1 and TPSAB1*) in the patients with DHF or DSS and background healthy children using the DNA samples collected by two independent hospital based case control studies conducted in Vietnam in 2002-2005 and in Manila, Philippines in 2008–2009.

2. Subjects and methods

2.1. Study subjects

Vietnamese subjects: The Patients were enrolled in the study previously reported [4]. Briefly, the case control study including healthy control children was performed at the Children's Hospital No. 2 in Ho Chi Minh City (HCMC) ($n = 448$) and the Center for Preventive Medicine in the Vinh Long Province (VL) ($n = 513$) during 2002 to 2005 in Vietnam. The inclusion criteria at the entry point in the hospital were age 6 months to 15 years old, Kinh ethnicity, and must not be related with each other. The patients were diagnosed with dengue using standard procedures for titration of anti-DV IgM and IgG antibodies, virus isolation, and RT-PCR for determination of viral. DV infection was defined by previously established serologic criteria for IgM/IgG Elisa's to DV (DEN 1–4) and Japanese encephalitis virus (kit from Pasteur Institute HCMC) in paired sera, collected in at least three days interval [27]. IgM/IgG ELISAs were considered positive if the ratio of optical density (OD) of test sera to OD of negative control sera was $\geqslant 2.3$[28]. The cases were diagnosed as secondary infection when DV IgM-to-IgG ratio was <1.8 [27]. Buffy coat samples were used to extract genomic DNA by using the QIAamp DNA blood kit (Qiagen, Germany). The 1998 WHO classification criteria [28] were applied to classify patients into DF, DHF and DSS. Our classification met the requirement of the simplified classification system of Integrated Management of Childhood Illness (IMCI), which is based on plasma leakage as a hallmark of severe dengue (DHF/DSS) [29]. 146 patients from Ho Chi Minh City and 218 from Vinh Long Providence were considered DSS patients, 107 patients and 97 were DHF patients, respectively. Vinh Long Providence have a higher percentage of secondary infection (65%) of 315 patients cases confirmed compare to 47% (120 patients) in 253 confirmed cases both DHF and DSS from Ho Chi Minh City.

Healthy unrelated school children living in HCMC ($n = 195$) and VL ($n = 198$), who had no symptoms of Dengue virus infection were recruited as a background population control group for the genetic study and titration of IgM were done according to the standard protocol for MAC-ELISA. In these control groups, 2 cases (1.02%) in HCMC and 13 cases (6.6%) in VL were seropositive by MAC-ELISA.

Philippine subjects: This study included children aged 5–15 years old of Filipino Malay ethnicity ($n = 155$) from two hospitals in Metro Manila, the Philippines—the Research Institute for Tropical Medicine (RITM), located in Alabang, Muntinlupa City, and the Philippine Children's Medical Center (PCMC) in Quezon City, from June 2008 to December 2009. Diagnostic tests used to determine dengue infection included detection of viral RNA by RT-PCR and titration of IgM and IgG antibodies by ELISA (PanBio Dengue IgM and IgG Capture Antibody test, Inverness Medical Innovations, Queensland, Australia). The definition of secondary infection was the same used in the Vietnam population. The 1998 WHO classification criteria [28] was likewise used to classify the Dengue severity. Patients who went into shock (DSS cases) accounted for majority of the patients (121% or 77.6%), while DHF cases were 34 patients. Most of the confirmed cases both DHF and DSS had secondary infection (123% or 80%).

Unrelated children from a community in Quezon City and a meningococcemia vaccine efficacy study in Muntinlupa City formed the background population group ($n = 268$), which sera were tested for Dengue IgG antibodies using the PanBio Dengue IgG Capture Antibody test by ELISA (Inverness Medical Innovations, Queensland, Australia) and 10 cases (4%) were seropositive for IgG antibodies.

2.2. Ethics statement

The approval of the respective ethical review committees of the Institute of Tropical Medicine, Nagasaki University (No. 140711125) and the Research Institute for Tropical Medicine, Muntinlupa City (No. 2014-005) was obtained before the initiation of the study. Informed consent from the parents or legal guardians

of all participants and assent from participants more than 9 years old were obtained upon enrollment to the study.

2.3. CMA1: the promoter region of CMA1 at position −1903G/A substitution

Genomic DNA was extracted from EDTA-Blood and stored at −20 °C as described elsewhere [30]. The promoter region of CMA1 at position −1903 G/A substitution (rs1800875) was investigated using primer pairs: CMA1 Forward Primer: 5′-GGA-AATGTGAGCAGATAGCGCAGTC-3′ and CMA1 Reverse Primer: 5′-AATCCGGAGCTGGAGAACTCTTGTC-3′. Polymerase chain reaction (PCR) amplifications were performed in a final volume of 30 μl using 10 pmol/μl of forward and reverse primer (CMA1PF, CMA1PR), 10X buffer with 25 mM containing $MgCl_2$, 2.5 mM of dNTPs, 10 ng DNA template and 2 unit of Takara Ex Taq enzyme (Takara Bio. Inc., Shiga, Japan). PCR amplification was then performed for these specimens using Thermal cycler (Biometra, Fr) programmed for 94 °C for 5 min as initial denaturation, 34 cycles of 15 s at 94 °C for denaturation, 60 °C annealing for 15 s, 15 s extension at 72 °C, and a final extension for 10 min at 72 °C. The product from PCR was confirmed by 2% agarose gel electrophoresis.

The restriction cutting of CMA1 gene promoter region at position −1903 for alternative allele G/A substitution was used for restriction fragments length polymorphisms (RFLP) method. The Amplified DNA fragments were genotyped by restriction digestion with enzyme BstXI (New England Biolabs, MA, USA). Digested fragments were determined by electrophoresis using 2% agarose gels, stained with ethidium bromide, and visualizing with UV trans-illuminator. RFLP genotype of individuals were typed in the GG genotype (283 bp, −1903G homozygote), AA genotype (189/94 bp, −1903A homozygote) and the AG heterozygous (283/189/94 bp, −1903A/G).

The accuracy of the RFLP genotype was confirmed by direct sequencing of control samples from Ho Chi Minh City and Vinh Long Providence. To eliminate excess primers and dNTPs, cleaning up of the PCR products was performed using 2 μl of EXOSAP-IT (Ubp, USA) incubated at 37 °C for 15 minutes and then inactivated by heating at 80 °C for 15 min. The sequencing reaction mixture containing 3 ng of Exosap treated PCR amplicon, 2.0 μl of Big Dye V1.1, 5× sequencing buffer, 2 μl of primer (0.8 pmol/μl) and 0.25 μl of Big Dye Terminator V1.1 (Life Technologies, Co., USA) was prepared to a final volume of 10 μl. The sequencing reaction was performed using a Thermal cycler (Biometra, Fr) under the following conditions: 25 cycles of [96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min].The extension products were then purified by gel filtration using Sephadex G-50 (Qiagen, Ger) and sequenced in an ABI 3730 Genetic Analyzer (Life Technologies Co., USA). Finally, sequencing data were analyzed with Sequence Analysis software DNAdynamo (BlueTractorSoftware Ltd).

2.5. TPSAB1 genotyping: detection by fragment analysis of a deletion in TPSAB1 Intron 4

Comparison of alpha (α) and beta (β) tryptase gene allele sequences of the *TPSAB1* reveals extensive homology in introns as well as exons, with the former having a deletion of 10 base pairs at Intron 4. Fragment analysis was used to detect this 10 bp deletion of the α-tryptase and the complete β-tryptase intron 4 with a size of 131 bp. Following the method described by Pallaoro, Fejzo et al. (1999) PCR primers used were as follows: TPSAB1 Forward Primer: 5′-GCGATGTGGACAATGATGGTGGGTCTG-G-3′ labeled with fluorescent tag (TPSAB1PF-FAM) (BEX Custom synthesis Oligonucleotide, Japan) and TPSAB1 Reverse Primer: 5′-GAAGAGGGTGCAGCCTGAGGAGG-3′ [31]). PCR mixtures containing 10 pmol/μl of forward and reverse primer (TPSAB1PF-FAM, TPSAB1PR), 10× buffer with 25 mM containing $MgCl_2$, 2.5 mM of dNTPs, 10 ng DNA template and 2 unit of Takara Ex Taq enzyme (Takara Bio. Inc., Shiga, Japan) were prepared to a final volume of 30 μl. PCR was then performed using Thermal cycler (Biometra, Fr) programmed for 95 °C for 1 min for initial denaturation; 30 s at 95 °C for denaturation, 66 °C annealing for 45 s, and 45 s extension at 72 °C for 30 cycles; and a final extension for 10 min at 72 °C. Visualization for confirmation of PCR amplification products was done 2% agarose gel electrophoresis with an expected fragment size of 138 bp. Amplified DNA fragments were then diluted 1:10 with double distilled water, and mixed with 100× diluted 500 ROX size standard marker (Life Technologies, Co., USA) and Hi-Di Formamide buffer (Life Technologies, Co., USA). These were then heated to 95 °C for 3 min for denaturation and immediately cooled down in an ice bath for 5 min. The samples were processed using the sequencer ABI 3730 Genetic Analyzer (Life Technologies Co., USA) and analyzed with the Gene Mapper Analysis software (Applied Biosystems). The measures of fragment length were used for allele calling.

2.7. Statistical analysis

Hardy-Weinberg equilibrium, linkage disequilibrium (LD), and haplotype analysis were calculated using PyPopWin32.0.7.0 software (University of Berkely, Berkely, CA) [32]. The strength of association of the given genetic factors with disease severity was assessed using odds ratio (OR) and the significance of association was evaluated by two-sided Fisher's exact test using StatsDirect statistical software (ver.2.8.0, Stats direct Ltd. UK).

3. Results

3.1. Gene frequency of the SNP at position −1903 G/A of the promoter region of Chymase gene (CMA1)

For *CMA1* gene −1903 G/A SNP (rs1800875), the distribution of A and G alleles was not found to be significantly different between cases and controls in both populations from Vietnamese and the Philippines (Table 1). The genotype frequencies fit to the Hardy-Weinberg equilibrium in the total subjects. Direct sequencing of the PCR products from 180 control samples from Vietnam confirmed the amplification of the target promoter region containing the expected *BstXI* restriction site at position −1903.

α- and β- tryptase allele frequency by the detection of 10 bp deletion in intron 4 at position 1239–1249 bp of Tryptase gene (*TPSAB1*).

The gene frequency of the α-tryptase in Vietnam and the Philippines, was fitted to the Hardy–Weinberg equilibrium (Table 2).

As shown in Table 3, the α allele in homozygous form was significantly associated with the development of DHF compared with healthy controls (OR = 2.54, $p = 0.0084$) at HCMC. Development of DSS was significantly associated with the homozygous α-allele compared with healthy controls in patients from VL and the Philippines (OR = 3.52, $p < 0.0001$ and OR = 3.37, $p < 0.0001$, respectively), furthermore when immune response was consider, those with secondary infection and confirmed DSS have a significant increase of susceptibility compare with healthy controls (VL; OR = 4.44, $p < 0.0001$ and the Philippines; OR: 3.77. $p < 0.0001$). When the group of DSS and DHF patients were combined and compared with healthy controls, association of these severe forms with the α allele was noted to be significant in patients from VL and the Philippines (OR = 2.63, $p = 0.0001$ and OR = 3.06, $p = <0.0001$ respectively); however, this association did not reach statistical significance among HCMC subjects (OR = 1.64; $p = 0.11$). A similar

Table 1
Frequency of the promoter region SNP at −1903 A > G of *CMA1* gene in the groups with severe form of Dengue fever (DSS,DHF) and in the controls in Ho Chi Minh City, Vinh Long, Vietnam and Metro Manila, the Philippines.

Genotypes/alleles	Vietnam–HCMC						Vietnam–Vinh Long						Philippines–Metro Manila					
	DSS		DHF		Controls		DSS		DHF		Controls		DSS		DHF		Controls	
	n = 87		*n* = 80		*n* = 102		*n* = 88		*n* = 88		*n* = 100		*n* = 121		*n* = 34		*n* = 268	
CMA1	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*
AA	60	0.69	53	0.66	75	0.74	63	0.72	63	0.72	60	0.6	85	0.7	23	0.68	175	0.65
AG	26	0.3	24	0.3	24	0.24	20	0.23	24	0.27	36	0.36	30	0.25	9	0.26	81	0.3
GG	1	0.01	3	0.04	3	0.03	5	0.06	1	0.01	4	0.04	6	0.05	2	0.06	12	0.04
A	146	0.84	130	0.81	174	0.85	146	0.83	150	0.85	156	0.78	200	0.83	55	0.81	431	0.8
G	28	0.16	30	0.19	30	0.15	30	0.17	26	0.15	44	0.22	42	0.17	13	0.19	105	0.2

CMA1, Chymase gene; DHF, Dengue Hemorrhagic Fever; DSS, Dengue Shock Syndrome; VL, Vinh Long Province; HCMC, Ho Chi Minh City; *f*, frequency.

Table 2
Gene frequency of the alleles of *TPSAB1* gene that codes for α or β tryptase in the groups with severe forms of Dengue fever (DHF, DSS) and the controls in Vietnam and the Philippines.

Genotypes/alleles	HCMC Vietnam *n* = 448						Vinh Long Vietnam *n* = 513						Metro Manila Philippines *n* = 423					
	DSS		DHF		Controls		DSS		DHF		Controls		DSS		DHF		Controls	
	n = 146		*n* = 107		*n* = 195		*n* = 218		*n* = 97		*n* = 198		*n* = 121		*n* = 34		*n* = 268	
TPSAB1	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*	*n*	*f*
ββ	55	0.38	32	0.3	85	0.44	53	0.24	39	0.4	76	0.39	23	0.19	4	0.12	89	0.33
αβ	76	0.52	52	0.49	91	0.47	96	0.44	46	0.47	99	0.51	46	0.38	19	0.56	130	0.49
αα	15	0.1	23	0.21	19	0.1	69	0.32	12	0.12	23	0.12	52	0.43	11	0.32	49	0.18
β	186	0.64	116	0.54	261	0.67	234	0.54	124	0.64	251	0.64	92	0.38	27	0.4	308	0.57
α	106	0.36	98	0.46	129	0.33	202	0.46	70	0.36	145	0.37	150	0.62	41	0.6	228	0.43

TPSAB1, *Tryptase AB1 gene*; DHF, Dengue Hemorrhagic Fever; DSS, Dengue Shock Syndrome; HCMC, Ho Chi Minh City; VL, Vinh Long; *n*, number of subjects; *f*, frequency.

tendency for association was observed when the dominant α allele contribution model was used to compare those with severe forms of Dengue with healthy controls in HCMC, VL and the Philippines (Table 3).

4. Discussion

Our study showed no significant association between *CMA1* −1903G allele and Dengue severity. Several studies have looked into the association of this allele with allergic diseases. A positive association between *CMA1* −1903G allele and Japanese patients with atopic eczema, but not in those with asthma, atopic dermatitis or rhinitis, has been reported [21,22]. The same allele was also associated with total serum levels of IgE (<500 IU/mL) among atopic eczema patients [23]. In an Egyptian study, this allele was associated with asthmatic children [14]. On the other hand, no significant association of this allele was observed in several studies among Caucasian patients with asthma and atopic dermatitis [11–13]. Such inconsistent results suggest that *CMA1* −1903 G/A SNP (rs1800875) may not be a strong marker for susceptibility to allergic diseases. Similarly, the results of our study seem to show that this allele is less likely to play a major role in the progression to and pathogenesis of severe dengue infections. Although chymase levels have been found to be elevated in the serum of severe dengue patients [10], this increase may not be attributable to the genotypes of the SNP studied or other mechanisms in transcription, translation and gene expression regulation might be responsible.

Because we found a significant association between severe forms of Dengue fever and the homozygous α-tryptase allele of the *TPSAB1* gene as shown in Table 3, the expression of α-tryptase in mast cells or in basophils [24] is suspected to affect susceptibility to DHF and DSS. α- and β- tryptase are soluble proteases, and proteolytic activation leads them to assemble into a mature tetrameric form and storage in mast cell granules, however it has been considered this process occurs with β-tryptase and confine to α-tryptase, which is continuously secreted, as a result of a deletion in the *TPSAB1* gene [9,19]. In our previous study, using the same plasma samples from Vietnamese patients, plasma levels of tryptase increased significantly in DHF and DSS thus suggesting that degranulation of mast cells is a key process in the pathogenesis of severe Dengue [10]. Note, however, that the tryptase in serum, whose levels had been found to be elevated in that study, included both α-tryptase and ß-tryptase. Our present observation of increased susceptibility to severe dengue in those with the homozygous form of α-tryptase allele might suggest that expression of this allele might not be constitutive as previously believed. The elevation in total serum plasma, believed to be largely β-tryptase mediated, may in fact be attributable to the effects of α-tryptase. In a study involving Caucasian families with at least two asthmatic siblings (per family) reported a significant association of α-tryptase allele and the disease severity in asthma [25]. This study also reported that more than one copy of α alleles may be present in one gene and that the presence of more copies are associated with increased atopy severity. Similarly, a study involving healthy subjects reported significantly higher levels of plasma tryptase in those with genotypes having at least one α-tryptase allele than in those without [26]. Taken together, α-tryptase allele may contribute to the degree of vascular leakage, and consequently to the severity of a disease via mechanisms that are still not well elucidated. This may possibly be related to the plasma levels of α-tryptase or that α-tryptase might be a competitive inhibitor to β-tryptase tetramerization inside the granules in the setting of severe dengue.

At this point, we are not able to demonstrate the plasma levels of α-tryptase. However, the finding of the strong effect of homozygous α-tryptase on the pathogenesis of DHF and DSS encourages further investigation of the mast cell activation mechanism during the acute phase of dengue infection. With previous studies reporting a significant association of the α-allele with atopy, it would be

Table 3
Association of *TPSAB1 alleles* α and β with the severe forms of Dengue fever (DHF, DSS) patients and healthy controls in Vietnam and the Philippines.

TPSAB1		αα vs ββ + αβ			αα + αβ vs ββ		
		HCMC	Vinh Long	Metro Manila	HCMC	Vinh Long	Metro Manila
DSS vs controls		15/146 vs 19/195	69/218 vs 23/198	52/121 vs 49/268	91/146 vs 110/195	165/218 vs 122/198	98/121 vs 179/268
	OR	1.06	**3.52**	**3.37**	1.3	**1.94**	**2.12**
	95%CI	0.48–2.3	**2.05–6.21**	**2.03–5.56**	0.81–2.03	**1.24–3.03**	**1.23–3.74**
	p value	>0.999	**<0.0001**	**<0.0001**	0.32	**0.0021**	**0.0052**
DSS with primary infection vs controls		7/70 vs 19/195	17/77 vs 23/198	7/17 vs 49/268	26/70 vs 85/195	30/77 vs 76/198	2/17 vs 89/268
	OR	1.03	**2.15**	3.13	0.76	1.02	0.27
	95%CI	0.35–2.71	**1.004–4.53**	0.95–9.6	0.42–1.39	0.6–1.81	0.03–1.2
	p value	>0.9999	**0.03**	0.05	0.4	>0.9999	0.1
DSS with secondary infection vs controls		8/76 vs 19/195	52/141 vs 23/198	43/94 vs 49/268	29/76 vs 85/195	23/141 vs 76/198	18/94 vs 89/268
	OR	1.089	**4.44**	**3.77**	0.8	**0.31**	**0.48**
	95%CI	0.4–2.76	**2.48–8.1**	**2.18–6.5**	0.44–1.42	**0.17–0.54**	**0.25–0.86**
	p value	0.82	**<0.0001**	**<0.0001**	0.5	**<0.0001**	**0.01**
DHF vs controls		23/107 vs 19/195	12/97 vs 23/198	11/34 vs 49/268	75/107 vs 110/195	58/97 vs 122/198	30/34 vs 179/268
	OR	**2.54**	1.07	**2.14**	**1.81**	0.93	**3.73**
	95%CI	**1.24–5.20**	0.46–2.38	**0.88–4.92**	**1.07–301**	0.55–1.57	**1.25–14.97**
	p value	**0.0084**	0.85	**0.067**	**0.026**	0.8	**0.0099**
DHF with primary infection vs controls		18/45 vs 19/195	3/33 vs 23/198	3/5 vs 49/268	17/63 vs 85/195	14/33 vs 76/198	0 vs 89/268
	OR	**3.7**	0.76	**0.05**	**0.48**	1.18	–
	95%CI	**1.67–8.11**	0.14–2.77	**0.74–81.4**	**0.24–0.92**	0.51–2.65	–
	p value	0.0006	>0.9999	0.005	0.03	0.7	–
DHF with secondary infection vs controls		5/44 vs 19/195	9/63 vs 23/198	8/29 vs 49/268	15/44 vs 85/195	25/63 vs 76/198	4/29 vs 89/268
	OR	1.2	1.3	1.7	0.67	1.06	0.32
	95%CI	0.33–3.56	0.48–3.05	0.61–4.3	0.31–1.39	0.56–1.96	0.08–0.97
	p value	0.78	0.66	0.22	0.31	0.88	0.035
DSS vs DHF		15/146 vs 23/107	69/218 vs 12/97	52/121 vs 11/34	91/146 vs 75/107	165/218 vs 58/97	98/121 vs 30/34
	OR	**0.42**	**3.28**	1.57	0.7	**2.09**	0.57
	95%CI	**0.19–0.89**	**1.64–7.02**	0.66–3.91	0.4–1.24	**1.21–3.6**	0.13–1.86
	p value	**0.02**	**0.0002**	0.32	0.23	**0.005**	0.44
DSS + DHF vs controls		38/253 vs 19/195	81/315 vs 23/198	63/155 vs 49/268	166/253 vs 110/195	223/315 vs 122/198	128/155 vs 179/268
	OR	1.64	2.63	3.06	1.47	1.5	2.36
	95%CI	0.88–3.12	**1.56–4.56**	**1.91–4.9**	**0.98–2.20**	**1.02–2.23**	**1.42–3.99**
	p value	0.11	**0.0001**	**<0.0001**	**0.0505**	**0.034**	**0.0004**

Bold letter shows the significant number.
TPSAB1, tryptase 1 gene; DHF, Dengue Hemorrhagic Fever; DSS, Dengue Shock Syndrome; VL, Vinh Long Providence; HCMC, Ho Chi Minh City; OR, odds ratio; *p* value, Exact Fisher's two sided; 95%CI, Fisher exact 95% confidence interval, using StatsDirect statistical software (ver2.8.0, Stats direct Ltd. UK).

important to elicit the history of atopy or any allergic conditions and rule them out as a potential, confounding factor.

Author Contributions

Study was conceived and designed by CV, MK and KH. The experiments were performed by CV, MK, ADR. CV, MK, and KH analyzed the data. CV, ADR and KH wrote the manuscript. All authors contributed the data collection and approved the final version for publication.

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References

[1] WHO: Dengue and severe dengue: Fact sheet N°117, 2014.
[2] Gubler DJ. Dengue and Dengue Hemorrhagic Fever. Clin. Microbiol. Rev. 1998;11(3):480.
[3] Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin. Microbiol. Rev. 2009;22(4):564.
[4] St John AL, Rathore APS, Raghavan B, Ng M-L, Abraham SN, Medzhitov R. Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage. eLife 2013;2:e00481.
[5] Avirutnan P, Matangkasombut P. Unmasking the role of mast cells in dengue. eLife 2013;2:00767.
[6] St John AL, Rathore APS, Yap H, Ng M-L, Metcalfe DD, Vasudevan SG, et al. Immune surveillance by mast cells during dengue infection promotes natural killer (NK) and NKT-cell recruitment and viral clearance. Proc. Natl. Acad. Sci. 2011;108(22):9190.
[7] E. Chugunova, Biological function of mast cell chymase. Department of Molecular Biosciences, vol Veterinaria Uppsala, Swedish University of Agricultural Sciences, 2004, p. 51.
[8] Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat. Immunol. 2005;6(2):135.
[9] Hallgren J, Pejler G. Biology of mast cell tryptase. FEBS J. 2006;273(9):1871.
[10] Furuta T, Murao LA, Lan NTP, Huy NT, Huong VTQ, Thuy TT, et al. Association of mast cell-derived VEGF and proteases in Dengue Shock Syndrome. PLoS Negl. Trop. Dis. 2012;6(2):e1505.
[11] Forrest S, Dunn K, Elliott K, Fitzpatrick E, Fullerton J, McCarthy M, et al. Identifying genes predisposing to atopic eczema. J. Allergy Clin. Immunol. 1999;104(5):1066.
[12] Pascale E, Tarani L, Meglio P, Businco L, Battiloro E, Cimino-Reale G, et al. Absence of association between a variant of the mast cell chymase gene and atopic dermatitis in an Italian population. Hum. Hered. 2001;51(3):177.
[13] Iwanaga T, McEuen A, Walls AF, Clough JB, Keith TP, Rorke S, et al. Polymorphism of the mast cell chymase gene (CMA1) promoter region: lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. Clin. Exp. Allergy 2004;34(7):1037.
[14] Hossny EM, Amr NH, Elsayed SB, Nasr RA, Ibraheim EM. Association of polymorphism of the mast cell chymase gene promoter region (−1903 G/A) and (TG)n(GA)m repeat downstream of the gene with bronchial asthma in children. J. Investig. Allergol. Clin. Immunol. 2008;18(5):376–81.
[15] Akin C, Soto D, Brittain E, Chhabra A, Schwartz LB, Caughey GH, et al. Tryptase haplotype in mastocytosis: relationship to disease variant and diagnostic utility of total tryptase levels. Clin. Immunol. 2007;123(3):268.
[16] Caughey GH, Raymond WW, Blount JL, Hau LWT, Pallaoro M, Wolters PJ, et al. Characterization of human γ-tryptases, novel members of the chromosome 16p mast cell tryptase and prostasin gene families. J. Immunol. 2000;164(12):6566.
[17] Soto D, Malmsten C, Blount JL, Muilenburg DJ, Caughey GH. Genetic deficiency of human mast cell alpha-tryptase. Clin. Exp. Allergy 2002;32(7):1000.
[18] Huang C, De Sanctis GT, O'Brien PJ, Mizgerd JP, Friend DS, Drazen JM, et al. Evaluation of the substrate specificity of human mast cell tryptase βI and demonstration of its importance in bacterial infections of the lung. J. Biol. Chem. 2001;276(28):26276.
[19] Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. Immunol. Rev. 2007;217(1):141.
[20] Trivedi NN, Tamraz B, Chu C, Kwok P-Y, Caughey GH. Human subjects are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations. J. Allergy Clin. Immunol. 2009;124(5):1099.
[21] Mao XQ, Shirakawa T, Enomoto T, Shimazu S, Dake Y, Kitano H, et al. Association between Variants of mast cell chymase gene and serum IgE levels in eczema. Hum. Hered. 1998;48(1):38.
[22] Mao XQ, Shirakawa T, Yoshikawa T, Yoshikawa K, Kawai M, Sasaki S, et al. Association between genetic variants of mast-cell chymase and eczema. Lancet 1996;348(9027):581.
[23] Tanaka, Sugiura, Uehara, Sato, Hashimoto T, Furuyama. Association between mast cell chymase genotype and atopic eczema: comparison between patients with atopic eczema alone and those with atopic eczema and atopic respiratory disease. Clin. Exp. Allergy 1999;29(6):800.
[24] Voehringer D. Protective and pathological roles of mast cells and basophils. Nat. Rev. Immunol. 2013;13(5):362.
[25] Abdelmotelb AM, Rose-Zerilli MJ, Barton SJ, Holgate ST, Walls AF, Holloway JW. Alpha-tryptase gene variation is associated with levels of circulating IgE and lung function in asthma. Clin. Exp. Allergy 2014;44(6):822.
[26] Min H-K, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase levels in healthy adults. J. Allergy Clin. Immunol. 2004;114(1):48.
[27] Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J. Clin. Microbiol. 1992;30(3):545.
[28] WHO: Dengue Hemorrhagic Fever: diagnosis, treatment, prevention, and control. Geneva, World Health Organization, 1997, p. 1.
[29] WHO: Dengue, Dengue Hemorrhagic Fever and Dengue Shock Syndrome in the context of the integrated management of childhood illness. World Health Organization, 2005, p. 34.
[30] Lan NTP, Kikuchi M, Huong VTQ, Ha DQ, Thuy TT, Tham VD, et al. Protective and enhancing HLA Alleles, HLA-DRB1*0901 and HLA-A*24, for severe forms of Dengue virus infection, Dengue Hemorrhagic Fever and Dengue Shock Syndrome. PLoS Negl. Trop. Dis. 2008;2(10):e304.
[31] Pallaoro M, Fejzo MS, Shayesteh L, Blount JL, Caughey GH. Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. J. Biol. Chem. 1999;274(6):3355.
[32] Lancaster AK, Single RM, Solberg OD, Nelson MP, Thomson G. PyPop update – a software pipeline for large-scale multilocus population genomics. Tissue Antigens 2007;69:192.