Polymorphisms of Transporter Associated with Antigen Presentation, Tumor Necrosis Factor-α and Interleukin-10 and their Implications for Protection and Susceptibility to Severe Forms of Dengue Fever in Patients in Sri Lanka

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ABSTRACT

Context: To date, a clear understanding of dengue disease pathogenesis remains elusive. Some infected individuals display no symptoms while others develop severe life-threatening forms of the disease. It is widely believed that host genetic factors influence dengue severity. **Aims:** This study evaluates the relationship between certain polymorphisms and dengue severity in Sri Lankan patients. **Settings and Design:** Polymorphism studies are carried out on genes for; transporter associated with antigen presentation (TAP), promoter of tumor necrosis factor-α (TNF-α), and promoter of interleukin-10 (IL-10). In other populations, TAP1 (333), TAP2 (379), TNF-α (−308), and IL-10 (−1082, −819, −592) have been associated with dengue and a number of different diseases. Data have not been collected previously for these polymorphisms for dengue patients in Sri Lanka. **Materials and Methods:** The polymorphisms were typed by amplification refractory mutation system polymerase chain reaction in 107 dengue hemorrhagic fever (DHF) patients together with 62 healthy controls. **Statistical Analysis Used:** Pearson's Chi-square contingency table analysis with Yates' correction. **Results:** Neither the TAP nor the IL-10 polymorphisms considered individually can define dengue disease outcome with regard to severity. However, the genotype combination, IL-10 (−592/−819/−1082) CCA/ATA was significantly associated with development of severe dengue in these patients, suggesting a risk factor to developing DHF. Also, identified is the genotype combination IL-10 (−592/−819/−1082) ATA/ATG which suggested a possibility for protection from DHF. The TNF-α (−308) GG genotype was also significantly associated with severe dengue, suggesting a significant risk factor. **Conclusions:** The results reported here are specific to the Sri Lankan population. Comparisons with previous reports imply that data may vary from population to population.

Key words: Dengue, dengue hemorrhagic fever, interleukin-10, transporter associated with antigen presentation, tumor necrosis factor-α

INTRODUCTION

The dengue virus (DENV) belongs to the genus *flavivirus*. It has four antigenically related, but distinct, DENV serotypes of infection (DENV-1 to DENV-4) and can be transmitted to humans by *Aedes* mosquitoes that act as a vector and a reservoir of infection. DENV infection can cause a broad spectrum of clinical symptoms ranging from asymptomatic infections or a mild flu-like febrile

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illness (dengue fever [DF]) to the life-threatening severe forms of DF, which include dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue is the most important mosquito-borne viral disease in tropical and subtropical regions, with an estimate of 50 million infections annually by the WHO and an estimate of 390 million infections/year by a study conducted recently using novel risk mapping techniques.[1,2] Less than 3% of these infections will develop into the more severe form of the infection.[3] Approximately, 90% of DHF/DSS cases occur in patients having a secondary infection with a heterologous dengue serotype.[3] This secondary infection carries a higher risk of plasma leakage, which if not appropriately supported clinically with fluid management, can lead to shock. However, a conclusive understanding of the mechanisms underlying why only some patients develop the more severe forms of the infection is lacking.[4]

While epidemiologic factors, ecological factors, and viral variations are factors involved in disease progression, it is believed that genetic heterogeneity of molecules involved in the host immune response to DENV infection contributes to disease progression. Host genetic factors may predispose some individuals to progress to the severe forms of the illness while others may protect individuals.[5-8] Genetic polymorphisms involved in the host immune mechanism can have a significant effect on disease progression due to the importance of the host immune response to the virus. Polymorphisms on transporter associated with antigen presentation (TAP), dendritic cell-specific intercellular adhesion molecule-3-Grabbing nonintegrin, interleukin-10 (IL-10), Vitamin D receptor, cytotoxic T-lymphocyte antigen 4, mannose-binding lectin, JAK1, tumor necrosis factor-α (TNF-α), MICB, and PLCE1 are some of the key molecules in which locus polymorphisms have been associated with DENV disease progression previously.[9-14] TAP is a key component involved in the transport of viral antigenic peptides for loading of major histocompatibility complex Class I molecules within the cell, which play a role in triggering an immunological response from the host. TAP is a key molecule involved in antigen presentation and is thought to help in DENV immune escape and DENV infection.[15-17] Polymorphisms within the gene for TAP have been implicated in cancer, autoimmune disorders and a number of viral infections including DENV itself.[18,19] Polymorphisms in the TAP gene region could influence the selection process that determines which antigen peptides contribute to the pathogenesis of dengue infection, which in turn may determine the severity of the infection. Polymorphisms of TAP1 and TAP2 genes have been typed, and associations have been uncovered for DENV in patients in Puducherry, India.[7,20]

The plasma leakage characteristic of severe dengue infections is suspected to be caused by malfunction of vascular endothelial cells induced by cytokines.[21] DF and DHF patients have been reported to have significant differences in cytokine levels, and the involvement of cytokines in DENV pathogenesis has been identified previously.[22,23] Plasma levels of cytokines such as TNF-α and IL-10 have been found to be significantly higher in DHF patients in comparison to DF patients.[24,25] Considering that the production of cytokines can be regulated by genetic polymorphisms and different genetic variants have been associated with DENV, we decided to study these particular polymorphic sites on TNF-α and IL-10 in relation to the Sri Lankan population.

The genetic variation on the promoter region of TNF-α at positions −308 results in two allelic forms. The presence of guanine (G) defines the common variant and the presence of adenine (A) defines the less common variant. TNF-α (−308) A allele is associated with increased gene transcription of TNF-α, in comparison to the common allele G.[26] Several studies have shown higher levels of TNF-α in patients with DHF and DF in comparison to controls. Some of these studies show plasma levels of TNF-α are higher in patients with DHF than patients with DF.[24] TNF-α (−308) A allele is a possible risk factor of DHF in a cuban and venezuelan population and the TNF-α (−308) GG genotype has been associated with protection from DHF.[8,27] However, studies conducted by Vejbaesya et al. (2009) in a Thai population and García-Trejo *et al.* in Mexico did not find an association between TNF-α (−308) polymorphisms and dengue disease severity.

Genetic variation on the IL-10 promoter regions −1082, −819 and −592 influence IL-10 production.[28] However, in previous studies, IL-10 promoter (−1082) A/G, (−819) C/T, (−592) C/A polymorphisms investigated individually did not show any significant association with dengue disease severity in Cuban and Venezuelan populations.[8,27] However, the IL-10 genotype combination IL-10 (−1082/−819/−592) ACC/ATA was reported to be a DHF risk factor in Cuban patients.[8]

Hence, the purpose of the present study was to evaluate the relationship between certain polymorphisms and the genetic susceptibility to the severe forms of DF in patients in Sri Lanka. The polymorphisms reported on here; TAP1 (333), TAP2 (379), TNF-α (−308), and IL-10 (−1082, −819, −592) have been previously associated with DENV disease pathogenesis as mentioned above and therefore chosen to be tested within the Sri Lankan population group investigated here. This information is important to

determine if the host genetic background could play an important role in defining the clinical outcome of dengue infections in Sri Lankan patients.

MATERIALS AND METHODS

Sample collection

A total of 107 patients with suspected severe dengue infection, who were admitted to a Tertiary Care Hospital in Colombo, were recruited to the study following informed written consent. The study was approved by the Ethics Review Committee of the University of Sri Jayewardenepura, Sri Lanka. The severity of their dengue infection was classified based on the WHO 2011 guidelines.[25,29] Accordingly, all patients who were classified as having DHF either had clinical or laboratory evidence of fluid leakage. Based on the 2011 WHO diagnostic criteria, shock was defined as lowering of pulse pressure to 20 mmHg or less or the presence of signs of poor capillary perfusion (cold extremities, poor capillary refill or a rapid pulse rate). All clinical features such as the presence of fever, vomiting, diarrhea, myalgia, blood pressure, pulse rate and volume, general status of the patient, presence of any bleeding manifestations and presence of any possible fluid accumulation in the pleural cavity and abdomen were monitored several times a day from the time of admission to hospital, until they were discharged. Patients presenting with clinical symptoms of DHF and DSS were grouped together under the category of DHF based on the WHO 2011 criteria. Among 107 patients, 26 patients were classified as having DSS and 81 patients were classified as DHF. Laboratory investigations such as the full blood count were done several times a day and aspartate aminotransferase, alanine aminotransferase once during the critical phase, in all patients until they were discharged from hospital. Serum electrolytes, coagulation profiles, chest radiography and ultrasound scans of the abdomen were only done in selected patients who developed severe dengue due to resource limitations. Patients with DF were not recruited, as our aim was to determine the genetic polymorphisms that are associated with severe dengue or DHF, and therefore, we compared the genetic polymorphisms in patients with more severe forms of dengue, with those who had a past mild or asymptomatic dengue infection.

Sixty-two dengue seropositive individuals who had never been hospitalized due to a febrile illness were recruited as healthy controls following informed written consent. As they never had a febrile infection that warranted hospital admission and were found to have dengue specific IgG antibodies confirming seropositivity, they were considered to have had a mild/asymptomatic past dengue infection.

Serology

Acute dengue infection was confirmed by testing the serum samples that were collected after day 6 of illness with a commercial capture-IgM and IgG enzyme-linked immunosorbent assay (ELISA) (Panbio, Brisbane, Australia). The ELISA was performed and the results were interpreted according to the manufacturer's instructions. This ELISA assay has been validated as both sensitive and specific for primary and secondary DENV infections.[30,31] Patients who only had DENV specific IgM were classified as having a primary dengue infection while those who had a positive result for both IgM and IgG were classified as having a secondary dengue infection.

Dengue virus-specific real time-polymerase chain reaction

DENV RNA was extracted from serum using QIAamp viral RNA mini kit (Qiagen CA, USA). RNA was reverse transcribed and the polymerase chain reaction (PCR) was performed using primers and conditions as previously described.[32] When determining the serotype of the infecting DENV, positive controls for DEN1, DEN2, DEN3 and DEN4 were used in all experiments.

DNA extraction and genotyping

Human genomic DNA was extracted from whole blood using the QIAamp DNA Mini Kit (Qiagen CA, USA). Samples were stored at −20°C until further use. Polymorphisms on TAP, TNF-α and IL-10 were identified by amplification refractory mutation system (ARMS-PCR) methodology using primers designed previously.[33-35] The primers and ARMS-PCR conditions used are given as Tables S1 and S2. All PCR reagents were from Promega Corporation, USA.

Polymorphic residues investigated were: Codon 333 on TAP1 (ATC to GTC, Ile to Val) and codon 379 on TAP2 (GTA to ATA, Val to Ile) gene regions; codon (−308) on the promoter region of TNF-α (A to G); and codons (−1082) (A to G), (−819) (C to T), and (−592) (A-C) on the promoter region of IL-10.

DNA regions selected were amplified and PCR products were separated by 2% agarose gel electrophoresis and stained with ethidium bromide. PCR fragments from randomly selected samples were sequenced to confirm the presence of the suspected polymorphisms.

Table S1: PCR primers and primer protocol

Gene	Primers used	Primer sequences (5'-3')
TAP1 (333)	TAP1/ARMS1 TAP1/ARMS2 TAP1/ARMS3 TAP1/ARMS4 (Powis *et al.*, 1993)	CCCTGCACTGAGATTTGCAGACCTCTGGAG GATCAGTGTCCCTCACCATGGTCACCCGGA GGGCAGAAGGAAAAGCAGAGGCAGGGTCAC ACCTGGGAACATGGACCACAGGGACAGGGT Amplified product size: 533 bp, 351 bp, 241 bp
TAP2 (379)	TAP2/ARMS1 TAP2/ARMS2 TAP2/ARMS3 TAP2/ARMS4 (Powis *et al.*, 1993)	TTGGAGGGCTGCAGACCGTTCGCAGTTTTG GAGACCTGGAACGCGCCTTGTACCTGCGCG ACAACCACTCTGGTATCTTACCCTCCTGAT ACATAGCTCCCCACGCTCTCCTGGTAGATC Amplified product size: 427 bp, 328 bp, 158 bp
TNF-α (−308)	R primer F1G primer F2A primer (Perrey *et al.*, 1999)	TCTCGGTTTCTTCTCCATCG ATAGGTTTTGAGGGGCATGG AATAGGTTTTGAGGGGCATGA Amplified product of 184 bp
IL-10 (−592)	R primer F1C primer F2A primer (Kingkeow *et al.*, 2011)	CAAGCCCCTGATGTGTAGA CTGTGACCCCGCCTGTC CTGTGACCCCGCCTGTA Amplified product of 600 bp
IL-10 (−819)	R primer F1C primer F2T primer (Perrey *et al.*, 1999)	AGGATGTGTTCCAGGCTCCT CCCTTGTACAGGTGATGTAAC ACCCTTGTACAGGTGATGTAAT Amplified product of 233 bp
IL-10 (−1082)	R primer F1G primer F2A primer (Perrey *et al.*, 1999)	CAGTGCCAACTGAGAATTTGG CTACTAAGGCTTCTTTGGGAG ACTACTAAGGCTTCTTTGGGAA Amplified product of 258 bp

PCR: Polymerase chain reaction, IL-10: Interleukin 10, TAP: Transporter associated with antigen presentation, TNF-α: Tumor necrosis factor-α, ARMS: Amplification refractory mutation system

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc. version 4.03) using the Pearson's Chi-square contingency table analysis with Yates' correction. A $P < 0.05$ was considered statistically significant. An odds ratio (OR) >1 was interpreted as posing a risk for developing severe dengue or DHF and an OR <1 was interpreted as a possible implication of protection from developing severe dengue. This analysis was performed for the individual polymorphisms as well as allelic genotype combinations to investigate the hypothesis that certain alleles or genotype combinations may predispose individuals to progress to the severe forms of dengue or DHF.

RESULTS

According to the IgM, IgG, and DENV PCR results, the 107 patients studied were confirmed to have DHF. Of 107 patients, 26 patients had shock and 27 had clinical evidence of either a pleural effusion or ascites. Bleeding manifestations were seen in 43 (40.2%) patients. Of these 43 patients, 30 had mucosal or skin bleeding only (petechie and bleeding from the gums), while only 15 had significant bleeding from the gastrointestinal tract. 11 out of 15 patients who had significant gastrointestinal bleeding also had shock. Fifteen of 107 patients had primary DENV infections while 92 patients had secondary DENV

Table S2: PCR amplification protocols

Gene	PCR reaction mix (50 ml)	Amplification conditions
TAP1 (333)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer mix of 4: 8 μl DNA template: 1 μl Distilled water: 26.1 μl Taq DNA polymerase: 0.4 μl	95°C: 5 min 94°C: 1 min 58°C: 2 min ⎫ 35 cycles 72°C: 2 min ⎭ 72°C: 10 min
TAP2 (379)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer mix of 4: 8 μl DNA template: 1 μl Distilled water: 26.1 μl Taq DNA polymerase: 0.4 μl	95°C: 5 min 94°C: 1 min 58°C: 2 min ⎫ 35 cycles 72°C: 2 min ⎭ 72°C: 10 min
TNF-α (−308)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer forward: 3 μl 10 μM primer reverse: 3 μl DNA template: 1 μl Distilled water: 28.2 μl Taq DNA polymerase: 0.3 μl	95°C: 1 min 95°C: 15 s 63°C: 1 min ⎫ 10 cycles 72°C: 40 s ⎭ 95°C: 20 s 59°C: 1 min ⎫ 25 cycles 72°C: 50 s ⎭ 72°C: 10 min
IL-10 (−592)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer forward: 3 μl 10 μM primer reverse: 3 μl DNA template: 1 μl Distilled water: 28.2 μl Taq DNA polymerase: 0.3 μl	94°C: 2 min 95°C: 30 s 61°C: 1 min ⎫ 30 cycles 72°C: 1 min ⎭ 72°C: 10 min
IL-10 (−819)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer forward: 3 μl 10 μM primer reverse: 3 μl DNA template: 1 μl Distilled water: 28.2 μl Taq DNA polymerase: 0.3 μl	94°C - 2 min 95°C: 30 s 61°C: 1 min ⎫ 30 cycles 72°C: 1 min ⎭ 72°C: 10 min
IL-10 (−1082)	5 × reaction buffer: 10 μl 25 mM MgCl₂: 3 μl 10 mM dNTP: 1.5 μl 10 μM primer forward: 3 μl 10 μM primer reverse: 3 μl DNA template: 1 μl Distilled water: 28.2 μl Taq DNA polymerase: 0.3 μl	94°C: 2 min 95°C: 30 s 61°C: 1 min ⎫ 30 cycles 72°C: 1 min ⎭ 72°C: 10 min

PCR: Polymerase chain reaction, IL-10: Interleukin 10, TAP: Transporter associated with antigen presentation, TNF-α: Tumor necrosis factor-α

infections. All patients recruited for the study were of Sri Lankan descent and nationality.

The ARMS-PCR fragments produced for genotyping were sequenced to confirm the accuracy of the system.

Transporter associated with antigen presentation polymorphisms

Table 1 shows the distribution of the TAP1 (333) and TAP2 (379) genotype variants and genotype frequencies. The genotype distribution for both polymorphisms did not defer significantly between severe dengue patients and controls. Genotype combinations of the two TAP

Table 1: Comparison of TAP, IL-10, and TNF-α single nucleotide polymorphisms between DHF patients and controls

Variable	DHF n = 107 (%)	Controls n = 62 (%)	P	OR	95% confidence limits
TAP1 (333) genotype					
Ile/Ile	66 (61.7)	34 (54.8)	0.48	1.33	0.71-2.50
Ile/Val	37 (34.6)	25 (40.3)	0.56	0.78	0.41-1.49
Val/Val	4 (3.7)	3 (4.8)	0.96	0.76	0.16-3.53
TAP2 (379) genotype					
Val/Val	87 (81.3)	52 (83.9)	0.83	0.84	0.36-1.92
Ile/Val	20 (18.7)	10 (16.1)	0.83	1.19	0.52-2.75
Ile/ Ile	0 (0)	0 (0)	Null	Null	Null
IL-10 (−592) genotype					
A/A	28 (26.2)	18 (29.0)	0.82	0.87	0.43-1.74
A/C	56 (52.3)	31 (50.0)	0.89	1.10	0.59-2.05
C/C	23 (21.5)	13 (21.0)	0.91	1.03	0.48-2.22
IL-10 (−819) genotype					
C/C	24 (22.4)	14 (23.0)	0.87	0.99	0.47-2.10
C/T	60 (56.1)	33 (53.2)	0.84	1.12	0.60-2.10
T/T	23 (21.5)	15 (24.2)	0.83	0.86	0.41-1.80
IL-10 (−1082) genotype					
A/A	56 (52.3)	26 (41.9)	0.25	1.52	0.81-2.86
A/G	40 (37.4)	31 (50.0)	0.15	0.60	0.32-1.12
G/G	11 (10.3)	5 (8.1)	0.84	1.31	0.43-3.95
TNF (−308) genotype					
G/G*	95 (88.8)	47 (75.8)	0.04	2.53	1.10-5.83
A/G	10 (9.3)	13 (21.0)	0.06	0.39	0.16-0.95
A/A	2 (1.9)	2 (3.2)	0.97	0.57	0.08-4.16

Values are represented as *n*. A percentage value is indicated in parenthesis. *P*-value was calculated by χ² and *P* < 0.05 was considered statistically significant. Statistically significant results have been highlighted in bold and the use of an asterisk. OR: Odds ratio, IL-10: Interleukin 10, TAP: Transporter associated with antigen presentation, DHF: Dengue hemorrhagic fever, TNF: Tumor necrosis factor

polymorphic sites were also investigated [Table 2] without showing any statistically significant results.

Tumor necrosis factor-α polymorphisms

Table 1 shows the distribution of the TNF-α genotype frequencies for the −308 position for severe dengue patients and controls. Even though a higher frequency of genotype G/G was observed in both the severe dengue patients as well as the control group, there was a statistically significant association of the G/G genotype with severe dengue patients ($P = 0.04$, OR = 2.53, confidence interval [CI] = 95%).

Interleukin-10 polymorphisms

As shown in Table 1, IL-10 genotype data for positions −592, −819 and −1082 on the promoter region, considered individually did not show statistically significant results for an association with either of the conditions.

Genotype combinations of the polymorphic sites on IL-10 were then evaluated [Table 2] and contrary to the

results of the individual positions, a certain IL-10 genotype combination, IL-10 (−592/−819/−1082) CCA/ATA was found to be significantly associated with the development of the severe form of DF ($P = 0.04$, OR = 2.42, CI = 95%). While the frequency of the genotype combination, ATA/ATA also implied an association with risk of severe dengue the *P*-value failed to reach significance.

The genotype combination IL-10 (−592/−819/−1082) ATA/ATG was found to have a statistically significant association with the control group with a $P = 0.02$ and an OR = 0.09 with a 95% CI.

DISCUSSION

Results obtained from the patients with DHF were compared with the healthy control group for all of the polymorphisms investigated. While serotyping was carried out for some of the samples due to sample and resource limitations it could not be completed. Only the DENV1 serotype was found, and this matches with other existing epidemiological data. However, these data were not used as it was beyond the scope of this study.

While results obtained for TAP1 and TAP2, polymorphisms within an Indian population demonstrated a significant association with disease progression.[7] The results obtained for the Sri Lankan population investigated here did not show any significant association with disease severity. Combinations of TAP genotypes were also investigated and compared with the control group but did not show statistically significant results. It has been seen from previous studies that associations for a number of polymorphisms change from population to population and do not remain a constant across all population groups worldwide.[5]

The analysis of the TNF-α −308 polymorphic site demonstrated an association between the G/G polymorphism and the development of severe dengue ($P = 0.04$, OR = 2.53, CI = 95%) suggesting that this polymorphism may be a risk factor for developing DHF among patients in Sri Lanka. This interpretation has been made cautiously keeping in mind that the *P*-value is just below the cut-off value of 0.05 and a study with a larger sample size will help to confirm these findings. This is the 1st time that the G/G genotype has been demonstrated as a risk factor in any population. The G/G genotype has been associated with protection from DHF in a population in Venezuela by Fernandez-mestre *et al*. Studies conducted by Vejbaesya *et al*. in Vietnam, García-Trejo *et al*. in Mexico and a recent study in India did not find any association between TNF-α −308 polymorphisms and dengue disease severity.[6,13,36] The

Table 2: Comparison of IL-10 promoter and TAP genotype combinations between DHF patients and controls

Gene	Variable	Genotype Combination	DHF $n = 107$ (%)	Controls $n = 62$ (%)	*P*-value	OR	95% confidence limits
IL-10	−592/−819/−1082	A/A, C/C, A/A	1 (1)	2 (3)	0.63	0.28	0.02-3.19
		A/A, C/C, A/G	0	0	Null	Null	Null
		A/A, C/C, G/G	0	0	Null	Null	Null
		A/A, C/T, A/A	2 (2)	2 (3)	0.97	0.57	0.08-4.16
		A/A, C/T, A/G	2 (2)	2 (3)	0.97	0.57	0.08-4.16
		A/A, C/T, G/G	0	1 (2)	0.78	0.19	0.01-4.76
		A/A, T/T, A/A	19 (18)	6 (10)	0.23	2.01	0.76-5.36
		A/A, T/T, A/G*	1 (1)	6 (10)	0.02	0.09	0.01-0.75
		A/A, T/T, G/G	3 (3)	0	0.47	4.19	0.21-82.46
		A/C, C/C, A/A	0	0	Null	Null	Null
		A/C, C/C, A/G	0	1 (2)	0.78	0.19	0.01-4.76
		A/C, C/C, G/G	0	0	Null	Null	Null
		A/C, C/T, A/A*	34 (32)	10 (16)	0.04	2.42	1.10-5.34
		A/C, C/T, A/G	20 (19)	15 (24)	0.51	0.72	0.34-1.54
		A/C, C/T, G/G	2 (2)	2 (3)	0.97	0.57	0.08-4.16
		A/C, T/T, A/A	0	2 (3)	0.25	0.11	0.005-2.38
		A/C, T/T, A/G	0	0	Null	Null	Null
		A/C, T/T, G/G	0	0	Null	Null	Null
		C/C, C/C, A/A	6 (6)	4 (6)	0.91	0.86	0.23-3.18
		C/C, C/C, A/G	13 (12)	6 (10)	0.81	1.29	0.46-3.59
		C/C, C/C, G/G	4 (4)	2 (3)	0.8	1.16	0.21-6.56
		C/C, C/T, A/A	0	0	Null	Null	Null
		C/C, C/T, A/G	0	0	Null	Null	Null
		C/C, C/T, G/G	0	0	Null	Null	Null
		C/C, T/T, A/A	0	0	Null	Null	Null
		C/C, T/T, A/G	0	1 (2)	0.78	0.19	0.01-4.76
		C/C, T/T, G/G	0	0	Null	Null	Null
TAP	333/379	Ile/Ile, Val/Val	56 (52)	30 (48)	0.73	1.17	0.63-2.19
		Ile/Ile, Ile/Val	10 (9)	4 (6)	0.71	1.49	0.45-4.99
		Ile/Ile, Ile/Ile	0	0	Null	Null	Null
		Ile/Val, Val/Val	26 (24)	20 (32)	0.35	0.67	0.34-1.35
		Ile/Val, Ile/Val	11 (10)	5 (8)	0.84	1.30	0.43-3.95
		Ile/Val, Ile/Ile	0	0	Null	Null	Null
		Val/Val, Val/Val	3 (3)	2 (3)	0.75	0.86	0.14-5.33
		Val/Val, Ile/Val	1 (1)	1 (2)	0.73	0.57	0.04-9.37
		Val/Val, Ile/Ile	0	0	Null	Null	Null

Values are represented as *n*. A percentage value is indicated in parenthesis. *P*-value was calculated by χ^2 and $P < 0.05$ was considered significant. Statistically significant results have been highlighted in bold and the use of an asterisk. IL-10: Interleukin 10, TAP: Transporter associated with antigen presentation, DHF: Dengue hemorrhagic fever, OR: Odds ratio

A (−308) allele has been identified as the high expression allele of TNF-α, in comparison to the common allele G.[26] Studies have shown plasma levels of TNF-α are higher in patients with severe dengue.[24] The G allele is reported as the low expression allele and in the Sri Lankan patients studied here, appears to be predominant among patients with severe dengue, contrary to the theory that the presence of the high expression allele explains high TNF-α levels and increased vascular permeability in severe dengue patients.[8] However, what is not yet known is whether variation in the level of this cytokine is a primary cause for severe dengue or just reflects a secondary downstream effect of inflammation.

IL-10 is a major pleiotropic cytokine and high levels of IL-10 have been associated with DHF.[25] As seen in Table 1, IL-10 genotype data for positions (−592), (−819), and (−1082) on the promoter region imply that neither of these single polymorphisms considered individually can define the dengue disease outcome with regard to severity in Sri Lanka. Studying genotype combinations can provide a more accurate understanding of gene effects; therefore, we looked at genotype combinations of IL-10 as shown in Table 2. Our results show that the IL-10 genotype combination, IL-10 (−592/−819/−1082) CCA/ATA was found to be significantly associated with the development of DHF ($P = 0.04$, OR = 2.42, CI = 95%) suggesting a risk factor to developing severe dengue. Once again the interpretation has been made keeping in mind that the *P*-value is just below the cut-off value of 0.05. Interestingly,

this result was seen among Cuban patients previously in a study conducted by Perez *et al.* in 2010. The IL-10 (−592/−819/−1082) CCA/ATA genotype combination has been previously related to low expression of IL-10.[37] IL-10 is an important anti-inflammatory cytokine and immune regulator. This result may imply that insufficient expression of IL-10 would result in inefficient immune regulation and resultant severity of disease. However, this does not explain the high levels of IL-10, also repeatedly reported in severe dengue patients previously.[25] The IL-10 genotype combination IL-10 (−592/−819/−1082) ATA/ATG was found to be associated with protection from severe dengue in Sri Lankan patients studied here. This association has not been reported previously in other populations. Other genotype combination associations seen within the Cuban population were not evident among the Sri Lankan patients investigated here. While certain other genetic variants appeared to be associated with risk of severe dengue, the *P*-values failed to reach significance. It is however, important to note that the IL-10 (−819) and (−592) polymorphisms are in linkage disequilibrium with each other.[37,38]

CONCLUSIONS

The IL-10 genotype combination, IL-10 (−592/−819/−1082) CCA/ATA was significantly associated with the development of DHF suggesting a risk factor to developing severe dengue, the IL-10 genotype combination IL-10 (−592/−819/−1082) ATA/ATG suggests a possibility of protection from DHF and the TNF-α (−308) GG genotype was found to be significantly associated with severe dengue suggesting a significant risk factor in Sri Lankan patients. Analysis of the results obtained here together with previous studies from different populations reveals that associations between host genetics and the clinical outcomes are complex. The results reported here are specific to the Sri Lankan population. Further studies with increased sample size and categorization of patients by infecting serotype may lead to a better understanding of the Sri Lankan situation. It is still unclear how many genes contribute to dengue susceptibility and the extent to which these genes interact with each other to cause severe disease. More data obtained from different population groups are crucial to a complete understanding of genetic associations to dengue infections. These results would help to understand the extent to which dengue pathogenesis could be genetically predicted and possibly help identify risk groups in populations where dengue is endemic.

Conflicts of interest

There are no conflicts of interest.

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