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Short communication

Polymorphisms in the oligoadenylate synthetase gene cluster and its association with clinical outcomes of dengue virus infection

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ABSTRACT

Oligoadenylate synthetases (OAS) play an important role in the immune response against dengue virus. Single nucleotide polymorphisms (SNPs) in the *OAS* genes are known to affect OAS activity and are associated with outcome of viral infections. Polymorphisms in the *OAS1* (rs1131454 and rs10774671), *OAS3* (rs2285932 and rs2072136) and *OAS2* (rs15895 and rs1732778) genes were studied using PCR followed by restriction fragment length polymorphism methods in 109 patients hospitalized for dengue (DEN) and 105 healthy controls (HCs) who have no documented evidence of symptomatic dengue. The two locus haplotype of *OAS2* G-G was significantly higher in all patient groups [DEN vs. HCs, $P = 0.0041$, P corrected (Pc) = 0.012, Odds ratio (OR) 1.73 95% CI 1.16–2.59] while the four locus haplotype of *OAS3-OAS2* C-G-A-G was significantly lower in all dengue patient groups [DEN vs. HCs, $P = 0.0054$, $Pc = 0.0486$, OR 0.09, 95% CI 0.00–0.64] compared to controls. When the six locus haplotypes involving *OAS1*, *OAS3* and *OAS2* polymorphisms were analyzed and compared, the frequency of the haplotype A-A-C-A-G-G was significantly higher [$P = 0.0267$, $Pc = 0.486$, OR 2.34, 95% CI 1.08–4.91] and the frequency of the haplotype A-A-C-G-G-A was significantly lower in DHF cases [$P = 0.014$, $Pc = 0.252$, OR 0.12, 95% CI 0.01–0.85] compared to healthy controls. The results suggest that *OAS1-OAS3-OAS2* haplotypes are associated with differential susceptibility to clinical outcomes of dengue infection.

1. Introduction

Dengue, a re-emerging arboviral disease of humans, is caused by four serotypes of dengue virus (DENV). DENV infections result in a spectrum of manifestations ranging from undifferentiated fever or classical dengue fever (DF) which are milder forms of the disease and dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) which are severe forms of the disease. Heterogeneity in the clinical manifestations of DENV infection is determined by various factors such as age, presence of other chronic diseases, nutritional status, income, viral factors and host immune response associated genetics (Guzman and Kouri, 2003). Among the host immune responses, innate immune responses are important in controlling the DENV infection. Genetic factors affecting innate immune responses might lead to incomplete control of the virus leading to differences in the clinical manifestations of DENV infection.

Components of the innate immune system are the first to sense viral pathogens, induce a response to inhibit the spread of the virus and initiate and modulate adaptive immune responses. RNA sensing pattern recognition receptors when senses a viral RNA, induces interferon-α which in turn activate many genes leading to the production of antiviral proteins (Levy and Garcia-Sastre, 2001). Oligoadenylate synthetases (OAS) are one such family of antiviral proteins and are activated in the presence of double stranded or single stranded RNA with secondary structures and catalyzes the synthesis of 2′-5′ linked oligoadenylate from ATP. These 2′-5′ oligoadenylate then activates ribonuclease L (RNAse L) which degrades the viral RNA (Malathi et al., 2005).

OAS group of enzymes are encoded by three genes present in the 12th chromosome. *OAS1* codes for the p42, p44, p46, p48 and p52 isoforms while *OAS3* encodes p100 and *OAS2* encodes p69 and p71 isoforms of enzymes by alternative splicing (Justesen et al., 2000). Recent studies in mice demonstrated that strains with wild type *Oas1b* allele coding Oas1b protein were resistant to flavivirus infections while strains with mutant alleles were susceptible to flavivirus infections (Mashimo et al., 2002; Perelygin et al., 2002). These observations spurred an interest in the role of *OAS* genes in flavivirus resistance. It has been shown that OAS1 p42 and p46 and OAS3 p100 isoforms inhibit DENV-2 replication (Lin et al., 2009). A single nucleotide polymorphism (SNP) in the *OAS1* gene, rs10774671, has been reported to alter splicing of OAS1 resulting in reduced OAS1 activity and has been shown to be associated with susceptibility to West Nile Virus infections (WNV) (Bonnevie-Nielsen et al., 2005; Lim et al., 2009). Polymorphisms

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in the *OAS2* and *OAS3* genes have been shown to be associated with tick borne encephalitis (TBE) virus induced disease (Barkhash et al., 2010). In the present study, we have investigated the polymorphisms in the *OAS1*, *OAS2* and *OAS3* genes which were previously shown to be associated with WNV and TBE disease in a group of patients hospitalized for dengue and healthy controls who had no documented evidence of symptomatic dengue.

2. Methods

2.1. Study subjects

Dengue cases (DEN) include 109 subjects (mean age ± standard deviation (SD) 31.8 ± 12.7) who had a history of hospitalization for dengue during 2007–2010, which had been confirmed by dengue specific IgM ELISA and/or reverse transcriptase polymerase chain reaction. Healthy controls (HCs) consisted of 105 subjects (mean age ± SD 32.6 ± 13.4). HCs had no documented evidence of having symptomatic dengue. Among the dengue cases, 80 cases had DF (mean age ± SD 32.0 ± 12.3). Based on at least two of the DHF defining criteria of the World Health Organization, 29 cases had DHF (mean age ± SD 31.3 ± 14.0) (WHO, 1999). The study was approved by the institutional ethics committee, and a written informed consent was obtained from the study participants before blood collection. All the participants were living in and around Pune, Maharashtra, India and were not related to each other.

2.2. DNA isolation and genotyping

DNA was isolated from the white blood cells using salting out procedure. Polymorphisms in the *OAS1* (rs1131454 and

Table 1
Allele and genotype frequencies of *OAS1* gene polymorphisms in dengue patients and healthy controls.

Alleles/genotypes	Total dengue patients (DEN) (n = 109)**	DF patients (n = 80)**	DHF patients (n = 29)	Healthy controls (HCs) (n = 105)
OAS1 rs1131454 (Exon 3, Gly162Ser)*				
Alleles				
A	53.7 (116)	54.4 (86)	51.7 (30)	56.2 (118)
G	46.3 (100)	45.6 (72)	48.3 (28)	43.8 (92)
Genotypes				
A/A	26.8 (29)	27.8 (22)	24.1 (7)	29.5 (31)
G/A	53.7 (58)	53.2 (42)	55.2 (16)	53.3 (56)
G/G	19.4 (21)	19.0 (15)	20.7 (6)	17.2 (18)
OAS1 rs10774671 (Intron 5, 3′ss@)*				
Alleles				
A	59.6 (130)	59.4 (95)	58.6 (34)	65.2 (137)
G	40.4 (88)	40.6 (65)	41.4 (24)	34.8 (73)
Genotypes				
A/A	33.9 (37)	33.8 (27)	34.5 (10)	42.8 (45)
G/A	51.4 (56)	51.2 (41)	51.7 (15)	44.8 (47)
G/G	14.7 (16)	15.0 (12)	13.8 (4)	12.4 (13)

The allele and genotype frequencies are given in the form of percentages.
Numbers in the parentheses represent allelic or genotypic counts.
@ 3′ss accepting splicing site.
* Barkhash et al. (2010).
** n = 108 in total dengue patients and 79 in DF group for rs1131454.

Table 2
Allele and genotype frequencies of *OAS3* gene polymorphisms in dengue patients and healthy controls.

Alleles/genotypes	Total dengue patients (DEN) (n = 109)	DF patients (n = 80)	DHF patients (n = 29)	Healthy controls (HCs) (n = 105)
OAS3 rs2285932 (Exon 6, Ile438Ile)*				
Alleles	67.0 (146)	67.5(108)	65.5 (38)	71.4 (150)
C	33.0 (72)	32.5 (52)	34.5 (20)	28.6 (60)
T				
Genotypes	37.6 (41)	38.7 (31)	34.5 (10)	47.6 (50)
C/C	58.7 (64)	57.5 (46)	62.1 (18)	47.6 (50)
C/T	3.7 (4)	2.8 (3)	3.4 (1)	4.8 (5)
T/T				
OAS3 rs2072136 (Exon 8, Ser567Ser)*				
Alleles				
G	63.3 (138)	65.0 (104)	58.6 (34)	69.5 (146)
A	36.7 (80)	35.0 (56)	41.4 (24)	30.5 (64)
Genotypes				
G/G	42.2 (46)	43.7 (35)	37.9 (11)	46.7 (49)
A/G	42.2 (46)	42.5 (34)	41.4 (12)	45.7 (48)
A/A	15.6 (17)	13.8 (11)	20.7 (6)	7.6 (8)

The allele and genotype frequencies are given in the form of percentages.
Numbers in the parentheses represent allelic or genotypic counts.
* Barkhash et al. (2010).

rs10774671), *OAS3* (rs2285932 and rs2072136) and *OAS2* (rs15895 and rs1732778) genes were studied using polymerase chain reaction followed by restriction fragment length polymorphism method as described earlier (Barkhash et al., 2010; Fedetz et al., 2006).

2.3. Statistical analysis

Allele and genotype frequencies were calculated by direct counting. Genotype frequency distributions were tested for their confirmation to Hardy–Weinberg equilibrium using the Chi square test. Pairwise linkage disequilibrium (LD) between the *OAS1*, *OAS3* and *OAS2* gene polymorphisms was computed using Haploview software version 4.2 and haplotype frequencies were inferred (Barrett et al., 2005). Allele, genotype and haplotype frequencies were compared between different study groups using the Chi square test or Fisher's exact test as appropriate. For allele and haplotype frequencies, *P* values with Yate's correction and odds ratio (OR) with 95% confidence limits (CI) were calculated using Statcalc program, Epi info version 6.0.4, CDC, Atlanta, GA, July 1996). For haplotype associations, the *P* values were further subjected to Bonferroni's correction, in which the *P* values were multiplied with the number of haplotypes. For genotypic associations, *P* values with OR adjusted for gender and age were calculated by logistic regression using the SNPstats program (Sole et al., 2006). A *P* value less than 0.05 were considered significant. Function prediction analysis of *OAS* gene polymorphisms was carried out using the FastSNP web

Table 3
Allele and genotype frequencies of *OAS2* gene polymorphisms in dengue patients and healthy controls.

Alleles/genotypes	Total dengue patients (DEN) (*n* = 109)	DF patients (*n* = 80)	DHF patients (*n* = 29)	Healthy controls (HCs) (*n* = 105)
OAS2 rs15895 (3′UTR/Exon11, Ter720Trp)*				
Alleles				
G	83.0 (181)	83.0 (133)	83.0 (48)	77.1 (162)
A	17.0 (37)	17.0 (27)	17.0 (10)	22.9 (48)
Genotypes				
G/G	67.9 (74)	68.0 (54)	69.0 (20)	61.0 (64)
A/G	30.3 (33	31.0 (25)	28.0 (8)	32.3 (34)
A/A	1.8 (2)	1.0 (1)	3.0 (1)	6.7 (7)
OAS2 rs1732778 (3′ Flanking region)*				
Alleles				
G	76.6 (167)	75.0 (120)	81.0 (47)	71.0 (149)
A	23.4 (51)	25.0 (40)	19.0 (11)	29.0 (61)
Genotypes				
G/G	55.1 (60)	52.5 (42)	62.1 (18)	47.6 (50)
A/G	43.1 (47)	45.0 (36)	37.9 (11)	46.7 (49)
A/A	1.8 (2)	2.5 (2)	0 (0)	5.7 (6)

The allele and genotype frequencies are given in the form of percentages.
Numbers in the parentheses represent allelic or genotypic counts.
* Barkhash et al. (2010)

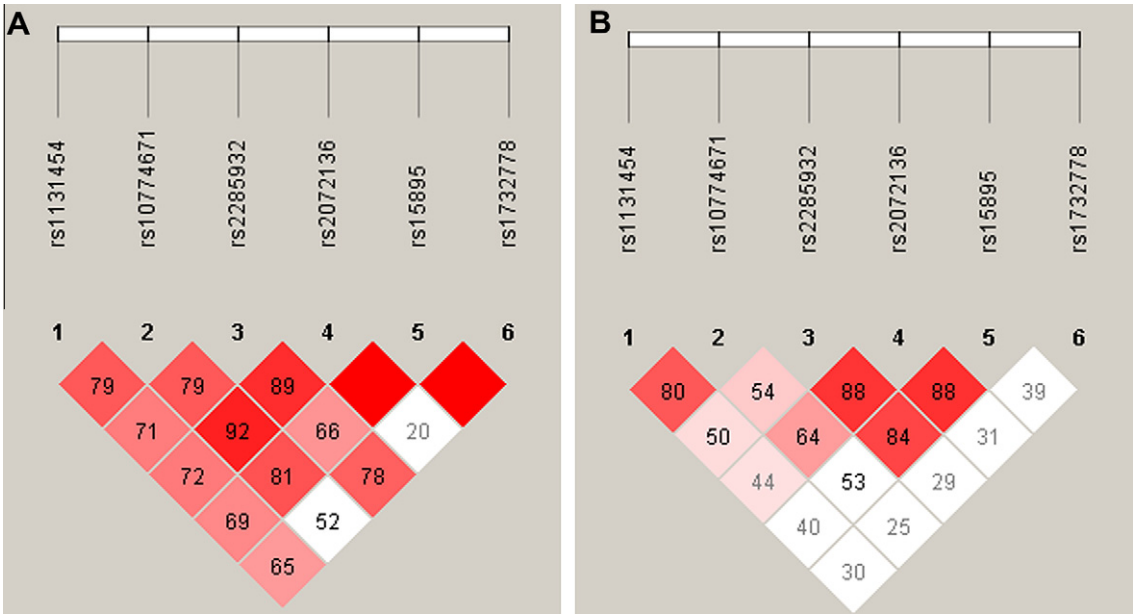

Fig. 1. Pattern of linkage disequilibrium of OAS gene cluster polymorphisms in the healthy controls (A) and dengue patients (B). Graphical representation of OAS gene cluster with the locations of polymorphisms in *OAS1* (rs1131454 and rs10774671), *OAS3* (rs2285932 and rs2072136) and *OAS2* (rs15895 and rs1732778) genes. Numbers in the boxes represent the strength of LD (D′) value multiplied by 100. In red boxes without any numbers, the D′ value is 100. The intensity of the red color of the boxes represents strength of linkage disequilibrium (D′) with dark red boxes having high LD and white boxes having low LD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Frequency of *OAS1*, *OAS3* and *OAS2* haplotypes in dengue patient groups and healthy controls.

Haplotypes	Total dengue patients (DEN) (*n* = 109)	DF cases (*n* = 80)	DHF cases (*n* = 29)	Healthy controls (HCs) (*n* = 105)
OAS1				
rs1131454 and rs10774671				
A-A	49.5	49.4	49.5	52.3
G-G	35.6	34.9	37.4	30.9
G-A	10.8	10.8	10.9	12.9
A-G	4.1	4.8	2.2	3.9
OAS3				
rs2285932 and rs2072136				
C-G	32.3	34.2	26.7[a]	41.8[a]
C-A	35.1	33.9	38.8	29.6
T-G	31.1	30.2	31.9	27.7
T-A	1.5	0	2.6	0.9
OAS2				
rs15895 and rs1732778				
G-G	62.6[b]	61.2[c]	65.8[d]	48.9[b,c,d]
G-A	21.3	23.2	17.0	28.2
A-G	14.6	14.7	15.3	22.0

[a] DHF vs. HCs: *P* = 0.035, *Pc* = 0.140, OR 0.53, 95% *CI* = 0.26–1.03.
[b] DEN vs. HCs: *P* = 0.0041, *Pc* = 0.012, OR 1.73, 95% CI 1.16–2.59.
[c] DF vs. HCs: *P* = 0.015, *Pc* = 0.045, OR 1.69, 95% CI 1.09–2.61.
[d] DHF vs.HCs: *P* = 0.020, *Pc* = 0.060, OR 2.01, 95% CI 1.06–3.90.

Table 5
Frequency of *OAS3-OAS2* haplotypes in dengue patient groups and healthy controls.

Haplotypes *OAS3-OAS2*[@]	Total dengue patients (DEN) (*n* = 109)	DF cases (*n* = 80)	DHF cases (*n* = 29)	Healthy controls (HCs) (*n* = 105)
CAGG	26.9[a]	25.7	29.8[b]	19.0[a,b]
CGGG	20.0	20.7	17.8	20.7
TGAG	14.1	13.8	15.1	17.5
CGGA	11.0	12.6	6.7	15.8
TGGG	13.6	13.1	15.4	8.3
CAGA	7.5	7.2	9.0	10.6
TGGA	3.2	3.7	1.4	2.0
CGAG	0.4[c]	0.6[d]	0.3	4.6[c,d]
TAGG	1.8	1.5	2.5	0.8

[@] The order of nucleotides in the haplotype is based on the order of SNP alleles in the chromosome (rs2285932-rs2072136- rs15895- rs1732778).
[a] DEN vs. HCs: *P* = 0.050, *Pc* = 0.450, OR 1.55, 95% CI 0.96–2.51.
[b] DHF vs. HCs: *P* = 0.057, *Pc* = 0.513, OR 1.82, 95% CI 0.87–3.68.
[c] DEN vs. HCs: *P* = 0.0054, *Pc* = 0.0486, OR 0.09, 95% CI 0.00–0.64.
[d] DF vs. HCs: *P* = 0.0157, *Pc* = 0.153, OR 0.12, 95% CI 0.01–0.93.

Table 6
Frequency of *OAS1-OAS3-OAS2* haplotypes in dengue patient groups and healthy controls.

Haplotypes *OAS1-OAS3-OAS2*[@]	Total dengue patients (DEN) (*n* = 109)	DF cases (*n* = 80)	DHF cases (*n* = 29)	Healthy controls (HCs) (*n* = 105)
A-A-C-A-G-G	21.4	19.0	26.7[a]	14.4[a]
G-G-T-G-A-G	12.5	12.3	13.1	15.5
A-A-C-G-G-G	12.3	13.4	8.4	13.1
A-A-C-A-G-A	7.3	6.8	9.5	10.8
A-A-C-G-G-A	6	7.8	1.6[b]	10.9[b]
G-G-T-G-G-G	10.7	10.4	11.6	5.8
G-A-C-G-G-G	6.1	5.9	7.3	7.6
G-G-T-G-G-A	4.3	4.4	3.6	2.1
G-G-C-G-G-A	2.8	2.9	2.0	2.3
G-A-C-A-G-G	2.1	2.1	2.4	2.3
G-G-C-G-G-G	2.4	1.5	4.7	1.7
G-G-C-G-A-G	0.4	0.5	0.4	2.9
A-G-T-G-A-G	1.0	2.0	0	1.1
G-G-C-A-G-G	3.7	5.2[c]	0	0.7[c]
A-A-T-A-G-G	1.8	0	2.5	0.8
A-A-T-G-A-G	1.5	2.0	0	0.8
G-A-C-A-G-A	0	1.2	0	0
A-G-T-G-G-G	0	0	0.5	0

[@] The order of nucleotides in the haplotype is based on the order of SNP alleles in the chromosome (rs1131454-rs10774671-rs2285932-rs2072136-rs15895-rs1732778).
[a] DHF vs. HCs: *P* = 0.027, *Pc* = 0.486, OR 2.34, 95% CI 1.08–4.91.
[b] DHF vs.HCs: *P* = 0.014, *Pc* = 0.252, OR 0.12, 95% CI 0.01–0.85.
[c] DF vs. HCs: *P* = 0.0125, *Pc* = 0.216, OR 5.55, 95% CI 1.08–54.11.

server (Yuan et al., 2006). Power calculations were performed using G*Power version 3 (Faul et al., 2007).

3. Results

3.1. Allele and genotype frequencies

The allele and genotype frequencies of *OAS1*, *OAS3* and *OAS2* gene polymorphisms were not different between healthy controls and different dengue patient groups. The genotypic distributions confirmed to Hardy–Weinberg equilibrium in healthy controls ($P > 0.05$) (Tables 1–3).

3.2. Linkage disequilibrium pattern among OAS genes and haplotype frequencies

Since all the OAS genes are present in the same chromosome in a cluster, we performed linkage disequilibrium (LD) analysis and predicted haplotype frequencies using Haploview software. LD analysis revealed that LD patterns were different between cases and controls (Fig. 1).

The haplotype frequencies of *OAS1* gene (rs1131454–rs1077467) were not different between the study groups. Among the *OAS3* haplotypes (rs2285932–rs2072136), C-G haplotype was observed at a lower frequency in dengue patient groups compared to HCs with statistical significance reaching only in DHF cases [$P = 0.035$, $Pc = 0.140$, OR = 0.53, 95% CI 0.26–1.03]. The G-G haplotype of *OAS2* gene (rs15895 and rs1732778) was significantly higher in cases irrespective of whether the group is DF or DHF [DEN vs. HCs: $P = 0.0041$, $Pc = 0.012$, OR 1.73, 95% CI 1.16–2.59; DF vs. HCs: $P = 0.015$, $Pc = 0.045$, OR 1.69, 95% CI 1.09–2.61; DHF vs. HCs: $P = 0.020$, $Pc = 0.060$, OR 2.01, 95% CI 1.06–3.90] (Table 4).

When *OAS3-OAS2* haplotype frequencies were compared, the frequency of C-A-G-G haplotype was higher in dengue patient groups while the frequency of C-G-A-G was significantly lower in dengue patient groups [DEN vs. HCs: $P = 0.0054$, $Pc = 0.0486$, OR 0.09, 95% CI 0.00–0.64] compared to HCs (Table 5).

When the six locus haplotypes involving *OAS1*, *OAS3* and *OAS2* polymorphisms were analyzed and compared, the frequency of the haplotype A-A-C-A-G-G was significantly higher in DHF cases compared to HCs [DHF vs. HCs: $P = 0.027$, $Pc = 0.486$, OR 2.34, 95% CI 1.08–4.91]. The frequency of the haplotype A-A-C-G-G-A was significantly lower in DHF cases compared to HCs. [DHF vs. HCs: $P = 0.014$, $Pc = 0.252$, OR 0.12, 95% CI 0.01–0.85] (Table 6).

4. Discussion

The results suggest that *OAS1*, *OAS3* and *OAS2* gene polymorphisms *per se* may not be associated with DF and DHF. Earlier, it has been shown that 'A' allele of rs10774671 was associated with susceptibility to WNV infection while polymorphisms in the *OAS3* (rs2285932 and rs2072136) and *OAS2* (rs15895 and rs1732778) genes have been shown to be associated with clinical outcomes of TBEV infection (Lim et al., 2009; Barkhash et al., 2010). It is possible that the sample size in the present study is limited in power to detect minor associations observed between different study groups.

The difference in LD pattern between the cases and controls suggested that the haplotype frequencies might differ between cases and controls. The two locus haplotype analyses revealed that the *OAS1* haplotypes were not associated with dengue disease manifestations further corroborating the lack of association of *OAS1* alleles or genotypes with dengue. The *OAS3* (rs2285932–rs2072136) haplotype, C-G haplotype might be associated with reduced risk of DHF while the G-G haplotype of *OAS2* gene (rs15895 and rs1732778) may be associated with increased risk of symptomatic dengue requiring hospitalization.

The results from *OAS3-OAS2* and *OAS1-OAS3-OAS2* haplotype analyses suggest that haplotypes from *OAS* gene cluster involving *OAS1*, *OAS3* and *OAS2* might affect susceptibility to dengue disease severity. The *OAS2-OAS3* haplotype C-G-A-G and *OAS1-OAS3-OAS2* haplotype A-A-C-G-G-A might be associated with increased OAS activity and confer an advantage against development of symptomatic dengue requiring hospitalization while the *OAS2-OAS3* haplotype C-A-G-G and *OAS1-OAS3-OAS2* haplotype A-A-C-A-G-G might have reduced OAS activity leading to increased DENV replication before the development of acquired immune responses and might increase the risk of DHF. The 'A' allele of rs10774671 of *OAS1* has been shown to be associated with increased replication of WNV in primary human lymphoid tissues (Lim et al., 2009). The polymorphisms studied in the *OAS3* gene are not known to cause any amino acid changes. However, function prediction studies using FastSNP revealed that *OAS3* polymorphisms might affect mRNA splicing regulation and hence OAS3 activity. The 'A' allele of rs2072136 of *OAS3* has been shown to be associated with decreased response to IFN-α treatment of hepatitis B virus infected patients (Ren et al., 2011). Hence it is possible that C-G haplotype of *OAS3* gene might be associated with increased OAS activity leading to reduced viral replication and reduced risk of DHF. It is also possible that *OAS3* SNPs might be in LD with some other functional polymorphisms in the *OAS3* gene or *OAS* gene cluster. In the present study, *OAS3* SNPs were observed to be in LD with a SNP (rs15895) in the *OAS2* gene. The G allele of rs15895 of *OAS2* is known to code for normal OAS2 isoform while the 'A' allele of rs15895 codes for an isoform which has eight amino acids less than the usual isoform (Barkhash et al., 2010). It is possible that shorter and longer forms of OAS2 might differ in their activity and contribute to the associations observed with dengue. The cumulative effect and epistatic interactions of all the polymorphisms might also contribute to expression and activity of *OAS* genes.

The present study is limited by the sample size. Based on the frequency of *OAS2* haplotype G-G which is found to be higher in all patient groups, the study is underpowered (50%) to detect the observed differences (odds ratio of 1.73) between healthy controls and total dengue patients. The study has power of 80% to detect an odds ratio of 2.25 and above. Since the samples were collected retrospectively, the present study also suffers from lack of data on infecting serotype and the immune status of the cases (primary or secondary). Hence larger studies with data on infecting serotypes and immune status are needed. Further functional studies are needed to evaluate the effect of SNPs in the *OAS* gene cluster on OAS expression and activity. Understanding the mechanisms behind the association of *OAS* haplotypes with dengue might be useful in designing OAS based therapeutics for dengue. To the best of our knowledge, this is the first study to show an association between polymorphisms in the OAS gene cluster and dengue disease pathogenesis. To conclude, the present study suggests that *OAS1-OAS3-OAS2* haplotypes are differentially associated with symptomatic dengue requiring hospitalization.

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