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Short communication

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

K. Alagarasu *, T. Honap, I.M. Damle, A.P. Mulay, P.S. Shah, D. Cecilia

Dengue Group, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001, Maharashtra, India

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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

* Corresponding author. Tel.: +91 020 26006256.
E-mail address: alagarasu@gmail.com (K. Alagarasu).

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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).