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# Killer cell immunoglobulin like receptors gene polymorphism in patients with dengue infection, Andaman Islands, India

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## PEER REVIEW

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**Comments**

This is a well designed research study to obtain the possible association of KIRs gene polymorphisms in patients with dengue virus infection. In view of the significant regulatory influences of KIRs on immune function and human health, it is essential to encourage research on KIRs.

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## ABSTRACT

**Objective:** To identify the possible association of killer cell immunoglobulin like receptors (KIRs) gene polymorphisms in patients with dengue virus infection.

**Methods:** Seventeen known KIRs were determined in 30 dengue patients and 40 healthy individuals by the sequence specific primer polymerase chain reaction method. Associations with specific KIR genes were tested using the *Chi*-squared test and Fisher test using EpiInfo 7 software.

**Results:** All frame work genes and pseudo genes were detected among 97% and 98% of all dengue cases and healthy individuals respectively. The total carriage frequency of KIR3DL1 and KIR2DL2 were decrease in dengue patients compared with healthy individuals ( $P=0.0000$  and  $P=0.0005$  respectively) from A haplotype and inhibitory receptors.

**Conclusions:** The KIR polymorphisms may be associated with susceptibility to dengue virus infection. It could be suggested that KIR3DL1 and KIR2DL2 were susceptibility genes for dengue virus. However, these findings provide certain support for hypothesis, that KIR genes influence susceptibility and may play a role in the clearance of dengue virus infection.

## KEYWORDS

Dengue, Killer cells immunoglobulin like receptors, Polymorphisms, Genotypes

## 1. Introduction

Dengue has emerged as a global health problem, as evidenced by a series of epidemics throughout the tropical, subtropical and temperate regions of the world. The WHO has reported that there are 50–100 million infections worldwide every year, now endemic in more than 100 countries and mostly affect Asia, Africa, and the Americas with Southeastern Asia[1]. The pathophysiology of dengue virus (DENV) infection is multifactorial involving complex interactions among viral and host factors. The viral factors include serotype/genotype of the infecting DENV, virulence of the virus and the extent of viremia[2]. Natural killer (NK)

cells are a key component of the innate immune system and are crucial in defense against viruses. NK cells are fast-acting lymphocytes that provide the first line of defense against viral infections, tumor transformation and autoimmune diseases[3].

Killer cell immunoglobulin like receptors (KIRs) are expressed by NK cells and certain T lymphocytes where they regulate specificity and function by interaction with human leucocyte antigen (HLA) class I molecules and located on chromosome 19q13.4, highly polymorphic[4]. KIRs contain both inhibitory and activating receptors. KIR receptors have been characterized in humans that comprise either two (2D) or three (3D) extracellular immunoglobulin

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like domains and either a long (L) or short (S) cytoplasmic tail. Long-tailed receptors carry one or two immunoreceptor tyrosine-based inhibitory motifs that contribute to inhibitory signaling. Short-tailed receptors have a lysine residue in their transmembrane domain that is required for pairing with the immunoreceptor tyrosine-based activation motifs-containing adaptor DAP12<sup>[4]</sup>. Human NK cells largely use KIRs to differentiate between the unhealthy targets from the healthy self<sup>[5]</sup>.

Earlier studies have demonstrated that KIR genes are involved in the pathogenesis of a various of diseases, including rheumatoid arthritis, vasculitis, psoriatic arthritis, type 1 diabetes mellitus, leprosy, hepatitis B virus, psoriasis vulgaris infection and malarial<sup>[6–12]</sup>. However till now, the role of KIR polymorphisms in patients with dengue infection has not been investigated. Therefore, the present study was designed to investigate the KIR gene polymorphisms in dengue cases and healthy individuals by sequence specific primer polymerase chain reaction (SSP-PCR), with special attention given to the association between KIRs and the DENV infection.

## 2. Materials and methods

### 2.1. DNA extraction

During the period from 2011 to 2012, we studied thirty patients (mean age 35 years) with clinical features of DENV infection and positive for either IgM ELISA or reverse transcription-polymerase chain reaction for dengue virus infection. Another forty samples (mean 36.5 years) were obtained from apparently healthy individuals who were negative for IgM and IgG antibodies against dengue infection and who did not have any clinical features of dengue infection. The chromosomal DNA was extracted by using the QIAmp DNA Blood Maxi Kit (Qiagen) and stored at  $-20^{\circ}\text{C}$  before use.

### 2.2. KIR genotyping

Genotyping of KIR alleles were performed to detect the presence or absence of 17 KIR genes such as KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DP1, KIR3DP1 and KIR3DX1 by the SSP-PCR method as described earlier<sup>[13]</sup>.

Briefly, PCR was performed in 12.5  $\mu\text{L}$  of reaction mixtures containing 100 ng of genomic DNA, 10 pmol of each primer, 2 mmol/L deoxyribonucleotide triphosphate (Bangalore Genei, India), 2.5 mmol/L  $\text{MgCl}_2$  and 1 IU of DNA *Taq* polymerase (Bangalore Genei, India) and carried out in a GeneAmp PCR system 2720 (Applied Biosystems, USA). The PCR conditions were as follow as:  $95^{\circ}\text{C}$  for 2 min for initial denaturation was followed by 10 cycles of  $94^{\circ}\text{C}$  for 10 seconds and  $65^{\circ}\text{C}$  for 40 seconds, then 20 cycles of  $94^{\circ}\text{C}$  for 20 seconds,  $61^{\circ}\text{C}$  for 20 seconds and  $72^{\circ}\text{C}$  for 30 seconds. The amplified PCR products were electrophoresed in 2.5% agarose gels, containing pre-stained with ethidium bromide and viewed under UV trans-

illuminator. Furthermore, genotypes observed in this study were defined as haplotype combinations, AA, AB, and BB<sup>[14]</sup>.

### 2.3. Statistical analysis

Associations with specific KIR genes were tested using the *Chi*-squared test and Fisher test using EpiInfo 7 software ([www.cdc.gov/epiinfo](http://www.cdc.gov/epiinfo)).  $P < 0.05$  (two-tailed) was considered statistically significant.

## 3. Results

### 3.1. Carrier frequency of each KIR genes

A total of 30 dengue patients and randomly selected 40 healthy individual DNA samples were analyzed to look for an association with KIR genes in DENV infection. The number of individuals carrying each KIR gene, individual KIR gene frequencies, inhibitory/activating KIR gene and interactions were counted. The carrier frequency of each KIR genes among patients with dengue virus infection and healthy individuals is shown in Table 1.

**Table 1**

Comparison of carrier frequency of KIR genes A haplotype B haplotype and framework/pseudo genes in dengue cases and healthy individuals.

KIR alleles		Dengue (n=30)% F (N)	Healthy individuals (n=40)% F (N)	P value
A haplotype	2DL1	100.0 (30)	100.0 (40)	–
associated KIR genes	2DL3	83.3 (25)	85.0 (34)	0.886 0
	3DL1	20.0 (6)	100.0 (40)	0.000 0
	2DS4	56.6 (17)	77.5 (31)	0.110 0
	2DL2	63.0 (19)	97.5 (39)	0.000 5
B haplotype associated KIR genes	2DL5	86.6 (26)	82.5 (33)	0.886 9
	3DS1	53.3 (16)	62.5 (25)	0.599 3
	2DS1	50.0 (15)	40.0 (16)	0.554 9
	2DS2	76.6 (23)	75.0 (30)	0.903 9
	2DS3	46.6 (14)	65.0 (26)	0.197 1
	2DS5	46.6 (14)	60.0 (24)	0.386 6
	Framework genes/ pseudo genes	2DL4	97.5 (29)	100.0 (40)
	3DL2	97.5 (29)	97.5 (39)	–
	3DL3	97.5 (29)	97.5 (39)	–
	2DP1	97.5 (29)	97.5 (39)	–
	3DP1	100.0 (30)	100.0 (40)	–

All frame work genes and pseudo genes were detected among 97% and 98% of all dengue cases and healthy individuals respectively. Both groups contain, KIR2DL4 (96.6% and 100%), KIR3DL2 (96.6% and 97.5%), KIR3DL3 (96.6% and 97.5%), KIR2DP1 (96.6% and 97.5%), KIR3DP1 (100.0% and 100.0%) and KIR3DX1 (96.6% and 97.5%) genes were found respectively.

In A haplotype, the maximum frequencies of two alleles KIR2DL1 and KIR3DL1 were from healthy individuals, KIR2DL1 were observed 100.0% from dengue cases. Although, B haplotype the minimum frequencies of one allele KIR2DS1 of 40% were from healthy individuals, alleles KIR3DL1, KIR2DS1 and KIR2DS5 were observed 20.0%, 50.0% and 46.6% from dengue cases respectively. However, the total carriage frequency of KIR3DL1 and KIR2DL2 were lower in dengue patients than in healthy individuals ( $P=0.0000$  and  $P=0.0005$ , respectively).

### 3.2. Inhibitory KIRs in dengue cases and healthy individuals

Further, these 17 KIR genes contain activating receptors and inhibitory receptors. The activating receptors were observed in dengue cases and healthy individuals. The difference in the dengue cases and healthy individuals was not statistically significant. The frequency of KIR2DL2 (corrected  $P=0.0005$ , OR=0.044, CI: 0.001–0.358) and KIR3DL1 (corrected  $P=0.0000$ , OR=0.000, CI: 0.000–0.036) was statistically significant. The remaining alleles were not different in statistical significance in inhibitory receptors.

The frequency of KIR genes carrying the different heterozygous and homozygous genotypes.

Furthermore, representative of AA, BB and AB genotypes and their assumed haplotype combinations were observed from dengue patients and healthy individuals. The highest frequencies of genotypes in patients and controls were homozygote BB genotype and heterozygote AB, respectively.

Two (6.6%) were heterozygous (AB genotypes), which in other words is a combination of A and B haplotypes, 28 (93.3%) showed a combination of B haplotypes from 30 dengue cases. However, none of the genotypes had a combination of A haplotype. In healthy individuals, 26 (65%) heterozygous AB genotypes were observed to be combination of A and B haplotypes, 14 (35%) homozygous BB genotypes were observed to be combination of B haplotypes and none of the homozygous AA genotypes were observed. Interestingly, both the homozygous BB genotypes and heterozygous AB genotypes ( $P=0.000003$ ) differences were statistically significant.

## 4. Discussion

NK cells are an essential component of innate immunity and provide a crucial initial defense against pathological organisms during the time (0–5 d) when the adaptive immune system is processing antigen<sup>[15]</sup>. A striking feature of KIR genes is their lack of conservation among species and their rapid evolution, which cannot be accounted solely by divergence in HLA class I molecules<sup>[16]</sup>. If KIR gene evolution were pathogen driven, some of the diversity would be expected to correlate with resistance or sensitivity to certain infectious diseases. Several genetic studies on viral infection have revealed an influence of HLA–KIR gene interactions on disease outcome<sup>[17]</sup>. This data clearly presented that there was a difference in the frequencies of these KIR genes in dengue patients and healthy individuals. Recently, most of the studies focused on human genetic factor relevant to infectious diseases in HLA genetic alteration<sup>[18]</sup>.

The present study establishes the presence/absence of 17 KIR genes in dengue infected and healthy controls. Earlier studies have suggested the possibility of KIR3DL1 evolution maintains variation in KIR3DL1 cell–surface expression levels, potentially due to the effect of such variation on functional capacity<sup>[19]</sup>. In the two unrelated viral infections, hepatitis C virus and human T lymphotropic virus type 1, possession of the KIR2DL2 gene enhanced both protective and damaging HLA class I–restricted anti–viral immunity<sup>[20]</sup>.

The inhibitory KIR genes 2DL2 is linked together as a cluster in the centromeric half of the KIR gene complex. Investigation of these frequencies relative to the frequencies of the haplotype combination AB/BB revealed that haplotypes with both KIR3DL1 and KIR2DL2 account for a proportion of the A and B haplotypes. A similar distribution among the leprosy cases, with a reduced frequency of AA haplotype in borderline patients (20.0%) compared with controls (33.9%), was observed but it was not statistically significant<sup>[9]</sup>.

A recent study on NK cell dysfunction in overactive immune responses to H1N1 infection showed KIR3DL1/S1 and KIR2DL2/L3 allotypes and cognate HLA ligands in H1N1<sup>[21]</sup>. However, indicating these two alleles KIR3DL1 and KIR2DL2 susceptibility of DENV infection. In this present study the dengue cases were associated with reduced quantity of the inhibitory KIR3DL1 allele and an increased quantity of the activatory KIR3DS1 allele. This data clearly showed there was a difference in the frequencies of KIR alleles. The presence of inhibitory receptor KIR3DL1 and another inhibitory receptor KIR2DL2 was statistically significant in patients with DENV infection compared to healthy individuals. This association might reduce the risk for DENV infection. These results revealed a role of inhibitory KIRs. However, we may conclude that inhibitory KIRs, in synergy with T cells, are a major factor of the outcome of persistent viral infection. Although obtained in a relative small cohort of study subjects, it confirms that NK cells play a role in this phenomenon and suggest a possible explanation for the increased NK activity seen in dengue patients.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

KIRs are a family of highly polymorphic activating and inhibitory receptors that serve as key regulators of human NK cells. The authors have attempted to identify the possible association of KIRs gene polymorphisms in patients with DENV infection.

#### Research frontiers

The authors collected DNA samples from a total 30 dengue patients and randomly selected 40 healthy individual and analyzed the samples to explore an association with KIR genes in DENV infection. The number of individuals carrying

each KIR gene, individual KIR gene frequencies, inhibitory/activating KIR gene and interactions were counted.

### Related reports

Earlier studies have demonstrated that KIR genes are involved in the pathogenesis of a various of diseases, including malaria (Yindom *et al.*, 2012). However till now, the role of KIR polymorphisms in patients with dengue infection has not been investigated. Therefore, the present study was designed to investigate the KIR gene polymorphisms in dengue cases.

### Innovations & breakthroughs

KIR genes are involved in the pathogenesis of a various of diseases, including rheumatoid arthritis, vacuities, psoriatic arthritis, type 1 diabetes mellitus *etc.* The present study was designed to investigate the KIR gene polymorphisms in dengue cases and healthy individuals by SSP-PCR, with special attention given to the association between KIRs and the DENV infection.

### Applications

Dengue is a serious public health problem and vector control is the only option to control the spread of the disease. No vaccine or specific treatment is available for dengue fever. Hence, these findings that KIR genes influence susceptibility and may play a role in the clearance of DENV infection, will be useful for develop a novel tool to fight against dengue infections.

### Peer review

This is a well designed research study to know the possible association of KIRs gene polymorphisms in patients with DENV infection. In view of the significant regulatory influences of KIRs on immune function and human health, it is essential to encourage research on KIRs.

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