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

Thamizhmani Ramanathan, Chaaithanya Itta Krishna, Sugunan Attayur Purushottaman, Vijayachari Paluru*

Regional Medical Research Centre (ICMR), Dollygunj, Port Blair-744101, Andaman & Nicobar Islands, India

PEER REVIEW

Peer reviewer

Dr. N. Arunachalam, Scientist "G", Centre for Research in Medical Entomology, Madurai-625 002, India.
E-mail: arunachalam50@gmail.com

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 immunoglubulin 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 Southeasern 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

*Corresponding author: Vijayachari Paluru, Director, Regional Medical Research Centre (Indian Council of Medical Research), Dollygunj, Port Blair, Andaman and Nicobar Islands-744101, India.
Tel: +91 3142 251158, +91 3142 251164
Fax: +91 3142 251163
E-mail: pblicmr@sancharnet.in
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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[4]. Human NK cells largely use KIRs to differentiate between the unhealthy targets from the healthy self[5].

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 malaria[6-12]. 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 fourty 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 °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[13].

Briefly, PCR was performed in 12.5 μL 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 $MgCl_2$ 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 °C for 2 min for initial denaturation was followed by 10 cycles of 94 °C for 10 seconds and 65 °C for 40 seconds, then 20 cycles of 94 °C for 20 seconds, 61 °C for 20 seconds and 72 °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[14].

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). $P<0.05$ (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.

Table1

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)	0.8860
	3DL1	20.0 (6)	100.0 (40)	0.0000
	2DS4	56.6 (17)	77.5 (31)	0.1100
B haplotype	2DL2	63.0 (19)	97.5 (39)	0.0005
associated KIR genes	2DL5	86.6 (26)	82.5 (33)	0.8869
	3DS1	53.3 (16)	62.5 (25)	0.5993
	2DS1	50.0 (15)	40.0 (16)	0.5549
	2DS2	76.6 (23)	75.0 (30)	0.9039
	2DS3	46.6 (14)	65.0 (26)	0.1971
	2DS5	46.6 (14)	60.0 (24)	0.3866
Framework genes/	2DL4	97.5 (29)	100.0 (40)	-
pseudo genes	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).