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Killer-cell immunoglobulin-like receptors and falciparum malaria in southwest Nigeria

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ABSTRACT

Killer-cell immunoglobulin-like receptors (KIRs) are a group of natural killer cell receptors (NKRs) that regulate NK-cell-mediated production of interferon gamma (IFN-γ) in response to infection. These receptors have recently been suggested to influence the severity of clinical *Plasmodium falciparum* malaria infection. We examined the KIR locus in relation to malaria in children from southwest Nigeria. Sequence specific priming (SSP)-PCR was used to detect the *KIR* genes. The presence or absence of fifteen different *KIR* genes was determined in each individual and the proportions compared across 3 clinical groups; asymptomatic malaria, uncomplicated clinical malaria and severe clinical malaria. The genes *KIR2DL5*, *KIR2DS3* and *KIR2DS5* were present in a significantly higher proportion of individuals in the asymptomatic control group than in the malaria cases. Furthermore, *KIR2DS3* and *KIR2DS5* were present in a higher proportion of uncomplicated malaria cases than severe malaria cases. Carriage c-AB2 genotype (which comprises all centromeric *KIR* genes including *KIR2DL5*, *KIR2DS3* and *KIR2DS5*) decreases with severity of the disease suggesting that the KIR AB profile might be associated with protection from severe malaria infection in this population in Nigeria.
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1. Introduction

Infection with *Plasmodium falciparum* has a wide spectrum of manifestations, ranging from mild clinical illness to coma, severe anemia, respiratory distress and shock [1]. The clinical outcome of malaria is determined by factors from the parasite, the human host and the environment [2,3]. Both the innate and adaptive immune responses are triggered by the malaria parasite during infection. Recent findings have shown the importance of innate immune responses in controlling malaria infection [4–6].

Natural killer (NK) cells are a key component of innate immunity. They protect the body from invasion by pathogens including viruses, bacteria and parasites [7,8]. NK cells kill their targets (infected or abnormal cells) without prior sensitization. The effector function of NK cells is regulated by a fine balance between the inhibitory and activating signals transduced by the cascade of natural killer cell receptors (NKR) found on their cell surface. NKRs are structurally categorized into two superfamilies: the immunoglobulin super family which includes the killer cell immunoglobulin-like receptors (KIR) and natural cytotoxicity receptors (NCR) and the C-type lectin family including NKG2 receptors [8,9].

The killer cell immunoglobulin-like receptors (KIRs) are type 1 membrane glycoproteins encoded by a set of highly polymorphic genes located within the leucocyte receptor cluster (LRC) on

Abbreviations: HLA, human leukocyte antigen; NK cell, natural killer cell; SSP, sequence specific priming; NKRs, natural killer cell receptors; LRC, leukocyte receptor cluster.

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human chromosome 19q13.4. KIRs are structurally similar but can be differentiated based on the number of extracellular domains (either 2 or 3), the length of their cytoplasmic tails (long or short) and the type of signal transduced (inhibitory or activatory). With the exception of KIR2DL4, all KIR molecules with a long cytoplasmic tail (KIR2DL or KIR3DL) are inhibitory while those with a short cytoplasmic tail (KIR2DS or KIR3DS) are activating. Individuals differ substantially in their *KIR* gene content, with many haplotypes varying in their profile of genes encoding activating and inhibitory KIR glycoproteins [4,5,10].

Studies of *KIR* gene profiles have been undertaken in relation to infectious diseases including tuberculosis [10] and HIV/AIDS [11–13], and a few studies have explored their associations with malaria [4–6,14,15]. These studies have suggested the importance of KIR genes in the outcome of malaria. However, these studies from different populations have yielded conflicting results which may be due to the population diversity from the different geographical regions [16,17]. Nigeria has one of the highest malaria-associated mortality rates in the world with approximately 24% of infant and 30% of childhood mortality thought to be associated with malaria [18]. In the present study, we determined and compared the distribution of KIR genes in children with asymptomatic, uncomplicated and severe malaria in southwest Nigeria.

2. Materials and methods

2.1. Study area

The study was carried out in Ibadan, southwest Nigeria, a holoendemic area for malaria. Children between the ages of 6–60 months were recruited from Ibadan at the Children's Emergency ward of the University College Hospital, St. Mary's Catholic Hospital, Eleta and the Oni Memorial Hospital. Almost all children (92%) were from the Yoruba ethnic group – the major ethnic population of this sub-region.

2.2. Selection of human subjects

A total of 554 children, comprising 200 with asymptomatic malaria, 153 with uncomplicated clinical malaria and 201 with severe malaria as defined by World Health Organization criteria (WHO, 2000), were recruited from the Children's Emergency ward and Children Out-patient Clinic of the three hospitals. The inclusion criteria were a blood film positive for *P. falciparum*-infected erythrocytes and having no other co-existing illness. Asymptomatic malaria was defined as presence of asexual *P. falciparum* in peripheral thick blood smears, an axillary temperature of <37.5 °C and an absence of malaria-related symptoms. Uncomplicated malaria was defined as presence of asexual parasitaemia and a temperature of >37.5 °C without severe malaria symptoms. Severe malaria was sub-categorized into severe malaria anaemia (defined as packed cell volume of <15%) and cerebral malaria (unrousable coma which persisted for more than 30 min after a convulsion, Blantyre score of $\leqslant$2). Ethical approval was obtained from the joint University of Ibadan/University College Hospital ethical review committee. Informed consent was obtained from the parents of all children recruited for the study.

The mean age of the children in the asymptomatic, uncomplicated and severe malaria groups were 58.3, 47.7, and 41.1 months, respectively. The mean body temperature was highest in the severe malaria group with a temperature of 38.0 °C while it was lowest in the asymptomatic control group with a temperature of 36.6 °C. The proportion of females was similar in each group (48.0%, 50.3% and 47.8% in asymptomatic, uncomplicated and severe malaria cases, respectively).

2.3. Sample collection and processing

Five milliliters of venous blood was obtained from each child, collected into a sterile tube with EDTA anticoagulant. Thick and thin blood smears were prepared and stained with Giemsa. The thick film was examined for the presence of malaria parasites while the thin film was used to estimate parasite density. The parasites were counted against 200 white blood cells and densities calculated based on an assumed total white blood cell of 8000/μL. DNA was extracted from whole blood samples using the Nucleon BACC I Kit (Tepnel Life Sciences, UK). The DNA samples were stored at −20 °C prior to genotyping. The quality and concentration of the DNA samples were tested using the Nano Drop Spectrophotometer ND – 1000 (NanoDrop Technologies, Inc., Wilmington, USA).

2.4. Genotyping

Polymerase chain reaction – sequence specific priming (PCR-SSP) technique was used to type 14 functional *KIR* genes (*KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3,* and *KIR3DS1*) and 1 pseudogene (*KIR2DP1*). This technique involved the use of 60 KIR specific primers to amplify conserved regions of the gene of interest as previously described [19]. Briefly, this protocol entailed the use of two pairs of KIR-specific primers (in separate PCR reactions) to amplify two segments of different sizes from the same KIR gene (if present). The PCR products were stained with ethidium bromide during electrophoresis in 2% agarose gel. An electronic gel picture was taken and scored for the presence or absence of specific bands by two independent scientists. Samples with discrepant results were repeated and the gene considered present if one of the reaction pairs was consistently positive. The use of two pairs of primers to detect the same gene was to limit false negative results given that this population has not been typed for KIR genes before. The absence of specific bands on both reactions was confirmed by repeating the typing to make sure that the gene was actually absent. Each reaction also contained a pair of internal control primers amplifying a 796 bp fragment from the third intron of HLA-DRB1 gene to check for PCR efficiency.

2.5. Statistical analysis

Analyses were performed using STATA version 10 (Stata Corporation, TX, USA). The proportions of individuals with each *KIR* gene type were compared between asymptomatic, uncomplicated and severe malaria groups. Multivariate analysis was performed using logistic regression, adjusting for age, gender, ethnicity and parasite density, p values of < 0.05 were considered significant after Bonferroni correction for multiple comparisons. Differences in the frequencies of activating and inhibitory *KIR* genes across the 3 clinical groups were determined using one-way analysis of variance (ANOVA), with a post test for linear trend. The centromeric and telomeric motifs of each KIR genotype were assigned using a technique adapted from Cooley et al. [20] and Pyo et al. [21].

3. Results

3.1. KIR gene frequencies

Fourteen functional *KIR* genes and the pseudogene *2DP1* were detected in the study population, with *KIR2DL1* and *KIR3DL1* being present in 100% of individuals (Fig. 1 and Supplementary Table S1). The proportions of individuals with inhibitory *KIR* genes were generally higher than those for activating *KIR* genes. The most

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