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Role of killer cell immunoglobulin-like receptor gene content and human leukocyte antigen–C group in susceptibility to human T-lymphotropic virus 1–associated myelopathy/tropical spastic paraparesis in Peru

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ABSTRACT

Human T-lymphotropic virus 1 (HTLV-1)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) affects approximately 5% of HTLV-1–infected individuals. It is poorly understood why only some infected subjects develop this disease, but host genetic factors may determine susceptibility. The innate immune system may influence disease outcome in HTLV-1–infected individuals because of its role in early immune responses to viral infections. Variation in genes encoding killer cell immunoglobulin-like receptors (KIR) and their human leukocyte antigen (HLA) molecule ligands may affect the risk of HAM/TSP. We performed a two-stage case-control study to examine the distribution of KIR genes and HLA-Cw groups in Peruvian HTLV-1–infected HAM/TSP individuals and asymptomatic carriers. We also tested for epistatic effects between specific KIR genes and HLA-Cw groups. In the first stage, we found several trends toward association with HAM/TSP or proviral load (PVL). However, these results were not replicated in the second stage. In conclusion, this is the first report on KIR gene frequencies in the Peruvian population and may be of significance in hematopoietic stem-cell transplants. Our study did not reveal significant associations between KIR genes and HLA-Cw groups and HAM/TSP or PVL. However, because our study was powered to detect only larger effects, additional studies using larger cohorts are needed.

1. Introduction

Human T-lymphotropic virus 1 (HTLV-1) affects an estimated 15–20 million persons around the world, with areas of high prevalence in Africa, the Caribbean, Japan, Melanesia and South America. In South America, the north of Brazil, Colombia, Guyana, and Peru are endemic zones [1,2] Most HTLV-1–infected individuals remain asymptomatic throughout life. However, between 5% and 10% of infected subjects develop associated diseases, such as adult T-cell lymphoma/leukemia (ATLL) or HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HAM/TSP is a progressive inflammatory disease that affects the ability to walk and is considered one of the most severe diseases associated with HTLV-1 [2].

It is poorly understood why some HTLV-1–infected individuals develop disease, whereas others remain asymptomatic throughout life. The pathogenesis of HTLV-1–associated diseases likely depends on interactions between viral and host factors. Several factors have been linked to HAM/TSP, but only the association with high proviral load (PVL) was consistent across all populations analyzed [3–5]. Nevertheless, the PVL alone cannot explain the risk of HAM/TSP in all HTLV-1–infected subjects because there is a large overlap in PVL between HAM/TSP patients and asymptomatic carriers (ACs).

An increased cytotoxic T-lymphocyte (CTL) response has been observed in HAM/TSP patients compared with ACs, which was associated with a high proviral load of HTLV-1 [6]. This increased CTL response suggests that HTLV-1–specific CTLs fail to eradicate

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the virus and might be considered pathogenic rather than protective [7]. In addition, an autoimmune origin of HAM/TSP caused by molecular mimicry has been hypothesized, as it has been shown that antibodies that recognize the HTLV-1 tax protein can cross-react with the host heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) [8].

The ability to kill virus-infected cells early in the immune response (the innate immune response) suggests that there may be a role for natural killer (NK) cells in determination of the course of HTLV-1 infections. In fact, it has been reported that HTLV-1–infected HAM/TSP patients have lower numbers of NK cells [9] and a decreased activity of NK cells in comparison with ACs [10,11].

The activity of NK cells is partially regulated by the balance between inhibitory and activating signals transmitted by a diverse family of receptors. These receptors are located at the surface of the NK cells and are called killer cell immunoglobulin-like receptors (KIR). KIR are encoded by 14 genes and may transmit either activating or inhibitory signals. Carrying more activating KIR genes has been associated with a stronger immune response and an increased risk for autoimmune diseases, such as psoriasis or rheumatoid arthritis [12–15]. KIR gene receptors have the capacity to recognize the human leukocyte antigen (HLA) class I molecules on target cells. When the expression of certain HLA molecules is downregulated by viral infections or cellular transformation, the KIR and HLA engagement will be altered. Cells that lack expression of self HLA molecules may be destroyed by the cytolytic activity of the NK cells [16].

The HLA-ligands for KIR genes have been divided into two major groups based on amino acid differences of HLA-Cw antigens. KIR2DL2, KIR2DL3 and KIR2DS2 bind HLA-Cw group 1 (Cw*01*03, *07, *08, *12, *13, *14, *1507, *15, and HLA-B*4601 and HLA-B*7301). All have an asparagine at position 80. KIR2DL1 and KIR2 DS1 bind HLA-Cw group 2 (HLA-Cw* 02, *307, *04, *05 *06, *0707, *0709, *1204, *1205, *15, *1602, *17, *18). Group 2 ligands have a lysine at position 80. The ligands for KIR3DL1 are HLA-Bw4-80I molecules [17,18]. Particular combinations of KIR and HLA antigens have been implicated in affecting the course of infectious diseases. KIR3DS1-HLA-B delays the progression to AIDS [19]. KIR2DL3-HLA-Cw1 favors the resolution of hepatitis C virus infection [20]; and the presence of KIR2DL2 and KIR2DS2 predispose to symptomatic herpes simplex virus type 1 infection [21].

Studies on the role of KIR or particular KIR-HLA combinations on HTLV-1 proviral load and HAM/TSP susceptibility are lacking. In the present case-control study, we evaluated the association of KIR genes and HLA-Cw with the susceptibility to HAM/TSP among Peruvian HTLV-1–infected subjects. In addition, we tested for epistatic effects between specific KIR genes and HLA-Cw groups.

2. Subjects and methods

2.1. Subjects

All HTLV-1–infected subjects were recruited from the HTLV-1 cohort of the Institute of Tropical Medicine, Alexander von Humboldt in Lima, Peru. The clinical diagnosis of HAM/TSP was made by one or two experts according to international guidelines [22,23]. Subjects with a subnormal neurological examination or some other clinical manifestations previously associated with HTLV-1 infection were not included in the current study (see Supplementary Table 1 for an overview). All selected subjects were genetically unrelated. Their origin was defined as Andean if both parents were born in the Andes, or as Mestizo if at least one parent was not born in the Andes. Patients with known Asian or African ancestry were not included in the study.

We performed a case-control study in two stages to avoid false-positive findings. In the first stage, we analyzed samples from 55 HAM/TSP patients and 109 ACs. In the second stage, we included 85 HAM/TSP patients and 146 ACs. This study was approved by the Research Ethics Committee of the Universidad Peruana Cayetano Heredia, and written informed consent was obtained from all participants.

2.2. Proviral load

DNA was extracted from peripheral blood mononuclear cells (PBMC) using the Genomic Prep Blood DNA Isolation Kit (Amersham Biosciences UK Ltd, Buckinghamshire, UK). To determine the PVL, we used SYBR Green-based real-time quantitative polymerase chain reaction (PCR) on an iCycler Thermal Cycler (Bio Rad, Hercules, CA), with human endogenous retrovirus 3 as reference gene [3]. The PVL was expressed as the number of HTLV-1 copies per 10^4 PBMC.

2.3. Genotyping

DNA was extracted from EDTA-treated blood samples using the Genomic Prep Blood DNA Isolation Kit (Amersham Biosciences UK Ltd, UK).

In the first stage, KIR genotyping was performed by multiplex PCR for the KIR genes *2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS4s*, *2DS5,* and *3DS1* [24]. The frequency of each KIR gene was calculated as the percentage of positive individuals among all individuals evaluated.

The genotyping in the second stage was performed by singleplex reactions, using sequence-specific primers [25]. The data obtained in the first stage with the multiplex genotyping reactions were also confirmed with the singleplex reactions to avoid differences due to the use of different primer sets and techniques.

In both stages, we used the primers described by Jones to determine HLA-Cw group [26]. As some of the primers cross-react with specific HLA-Bw alleles, reactions to detect HLA-Bw alleles *0713, *6702, *5401, *5402, and *5507 were included to increase specificity of the HLA-Cw subgroup typing [26].

The genetic substructure of a population and an admixture may lead to spurious associations in genetic association studies. Therefore, we typed 37 unlinked ancestry-informative markers (AIMs), thus enabling us to correct for population stratification. These genetic markers were selected based on their large differences in allele frequency between Native Americans and European Americans [27] and were located in regions not previously associated with HAM/TSP. Based on other studies [28], we anticipated that this number of markers would be sufficient to detect and correct for population stratification. AIMs were genotyped by Kbiosciences (http://www.kbioscience.co.uk).

2.4. Statistical analysis

For univariate analysis, *i.e.*, comparisons of frequencies of KIR genes, HLA-C groups and specific combinations of KIR genes and HLA-C groups between HAM/TSP patients and ACs, we used the Fisher's exact test. The Mann–Whitney *U* test was used to compare continuous variables.

For multivariate analysis, logistic regression was carried out to evaluate the association of KIR genes, HLA-Cw group, and specific combinations of KIR genes and HLA-Cw group with HAM/TSP, including age, gender, and logarithmically transformed PVL as covariates. To investigate whether a putative genetic effect acted through the PVL, the analysis was carried out both with and without the PVL term. If a gene solely acts through PVL, the association between gene and disease status will no longer be significant when the PVL term is included in the model. Conversely, if a gene remains significantly associated with the diseases status, the effect of the gene is, at least in part, independent of PVL.

To deal with potential population stratification problems, we conducted a principal component analysis on the AIMs using the EIGENSOFT software [29] and included the first three principal