PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Killer Cell Immunoglobulin-like Receptors and Their HLA Ligands are Related with the Immunopathology of Chagas Disease

Christiane Maria Ayo[1], Pâmela Guimarães Reis[1], Márcia Machado de Oliveira Dalalio[2], Jeane Eliete Laguila Visentainer[2], Camila de Freitas Oliveira[1], Silvana Marques de Araújo[2], Divina Seila de Oliveira Marques[3], Ana Maria Sell[2]*

1 Post Graduation Program of Biosciences Applied to Pharmacy, Department of Analysis Clinical and Biomedicine, Maringa State University, Maringa, Parana, Brazil, **2** Basic Health Sciences, Maringa State University, Maringa, Parana, Brazil, **3** Medical Clinic Department, Londrina State University, Londrina, Parana, Brazil

* anamsell@gmail.com, amsell@uem.br

Abstract

The aim of this study was to investigate the influence of killer cell immunoglobulin-like receptor (KIR) genes and their human leucocyte antigen (HLA) ligands in the susceptibility of chronic Chagas disease. This case-control study enrolled 131 serologically-diagnosed Chagas disease patients (59 men and 72 women, mean age of 60.4 ± 9.8 years) treated at the University Hospital of Londrina and the Chagas Disease Laboratory of the State University of Maringa. A control group was formed of 165 healthy individuals - spouses of patients or blood donors from the Regional Blood Bank in Maringa (84 men and 81 women, with a mean age of 59.0 ± 11.4 years). Genotyping of HLA and KIR was performed by PCR-SSOP. KIR2DS2-C1 in the absence of KIR2DL2 ($KIR2DS2^+/2DL2^-/C1^+$) was more frequent in Chagas patients ($P = 0.020$; $Pc = 0.040$; OR = 2.14) and, in particular, those who manifested chronic chagasic cardiopathy—CCC ($P = 0.0002$; $Pc = 0.0004$; OR = 6.64; 95% CI = 2.30–18.60) when compared to the control group, and when CCC group was compared to the patients without heart involvement ($P = 0.010$; $Pc = 0.020$; OR = 3.97). The combination pair $KIR2DS2^+/2DL2^-/KIR2DL3^+/C1^+$ was also positively associated with chronic chagasic cardiopathy. KIR2DL2 and KIR2DS2 were related to immunopathogenesis in Chagas disease. The combination of KIR2DS2 activating receptor with C1 ligand, in the absence of KIR2DL2, may be related to a risk factor in the chronic Chagas disease and chronic chagasic cardiopathy.

OPEN ACCESS

Citation: Ayo CM, Reis PG, Dalalio MMdO, Visentainer JEL, Oliveira CdF, de Araújo SM, et al. (2015) Killer Cell Immunoglobulin-like Receptors and Their HLA Ligands are Related with the Immunopathology of Chagas Disease. PLoS Negl Trop Dis 9(5): e0003753. doi:10.1371/journal.pntd.0003753

Editor: Mauricio Martins Rodrigues, Federal University of São Paulo, BRAZIL

Received: November 20, 2014

Accepted: April 11, 2015

Published: May 15, 2015

Copyright: © 2015 Ayo et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: MMdOD was supported by Fundação Araucária - http://www.fappr.pr.gov.br/. CMA was supported by CAPES—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Summary

Chagas disease is an infection caused by the haemoflagellate protozoan *Trypanosoma cruzi*. It is one of the most important public health problems in Latin America, and was first described by Carlos Justiniano Ribeiro das Chagas, a Brazilian physician and scientist, in 1909. It is mostly vector-borne transmitted to humans by contact with faeces of

Competing Interests: The authors have declared that no competing interests exist.

triatomine bugs. The World Health Organization estimates that about 6 to 7 million people are currently infected with *T*. *cruzi* worldwide. The disease is characterised by acute and chronic phases. The immune response during disease development is crucial for protection because immunological imbalances can lead to heart and digestive tract lesions in chagasic patients. In this work we analysed the role of receptors of immune cells known as Natural Killer cells (killer cell immunoglobulin-like receptor—KIR) and their ligands (Human leukocyte antigens—HLA) in chagasic patients compared to healthy individuals. The uncontrolled activation of NK cells can lead to tissue damage, which, in turn, leads to the development of serious chronic illness. We found that KIR-HLA complex may be related to a risk factor in the chronic Chagas disease and chronic chagasic cardiopathy.

Introduction

Chagas disease, caused by the flagellate parasite *Trypanosoma cruzi*, currently affects around 6 million to 7 million people worldwide and about 25 million have the potential risk of becoming infected [1]. The disease is endemic and its development occurs in acute and chronic phases. The latter may present in different clinical forms, indeterminate, cardiac and/or digestive, with the chronic cardiac form being the most serious [2]. This variation in pathological manifestation has been reported to be related to the complexity of parasite and to differences in host immune response, such as the ability to control parasitaemia, the strength of inflammatory reactions, and the induction of autoimmune-like responses [3–7]. Tissue damage resulting from inflammatory infiltrates and persistence of *T*. *cruzi* in myocardial tissue and changes in microcirculation and commitment of the autonomic nervous system are involved in the pathogenesis of cardiomyopathy. However, the precise pathogenic mechanism of Chagas' heart disease is not completely elucidated [7, 8].

The inflammatory process in the chronic phase of Chagas disease shows signs of cellular activity with $CD4^+$ T and $CD8^+$ T lymphocytes in heart tissue; though fewer numbers of natural killer (NK) cells, macrophages and B cells are also present [9, 10]. In asymptomatic or indeterminate chronic Chagas disease, the presence of circulating NK cells ($CD3^-CD16^+CD56^+$ and $CD3^-CD16^+CD56^{dim}$) coupled with the presence of immunoregulatory ($Treg^-CD4^+CD25^{high}$ and $NKT^-CD3^+CD16^-CD56^+$) or macrophage-like cells ($CD14^+CD16^+$) are responsible for the control the inflammatory mechanisms. However, failure in immunoregulatory mechanisms, with basal levels of NK, NKT and $CD4^+CD25^{high}$ cells, associated with an increased expression of activated $CD8^+$ T cells, are associated with heart disease [11, 12, 13]. The effective function of NK cells is regulated by a balance of activating and inhibitory signals mediated by a diverse set of receptors expressed on their surface, including killer immunoglobulin-like receptors (KIR), which recognize and bind in HLA class I molecules present on the surface of the target cells [14].

KIR is a family of 15 closely linked genes and highly polymorphic, on chromosome 19q13.4, that encodes both inhibitory and activating receptors. The receptors molecules may have two or three immunoglobulin-like domains, whereas those with long cytoplasmic tail (2DL and 3DL) are inhibitory due to the presence of ITIMs (tyrosine-based inhibitory motifs), responsible for signal transduction in order to inhibit NK functions. Molecules with short cytoplasmic tail (2DS and 3DS) have an amino acid transmembrane region which allows the association with a particular protein (DAP12), which releases activating signals through ITAMs (tyrosine-based activation motifs) [15].

KIR receptors of NK cells may contribute to the occurrence of different immunological and clinical responses to the same disease in a specific population [16]. Several studies have described the participation of KIR (and their ligands) in infectious diseases, such as AIDS [17, 18], hepatitis C [19, 20], tuberculosis [21, 22], leprosy [23, 24] and malaria [25, 26, 27]. KIR are also involved in autoimmune and inflammatory diseases such as pemphigus foliaceus, psoriasis, scleroderma, rheumatoid vasculitis and Crohn's disease [28, 29, 30, 31, 32], as well as in many types of cancer [33–36] and the survival of transplant patients [37]. The relationship between KIR and their HLA ligands in the immunopathogenesis of chronic chagasic disease remains unknown. Therefore, the aim of this study was to investigate the influence of the *KIR* genes and their HLA ligands in resistance or susceptibility to Chagas disease.

Materials and Methods

This study was approved by the Human Research Ethics Committee of the Maringa State University (COPEP-UEM # 012/2010, CAAE 0296.0.093.000–09). All adults individuals who agreed to participate in this research were informed about the nature of the study and signed an informed consent form.

Subjects

This case-control study enrolled 131 unrelated patients (CCD group) (59 men and 72 women, with a mean age of 60.4 ± 9.8 years) with serologically-diagnosed chronic Chagas disease, living in different municipalities in the north/northwest region of the State of Parana (located in the southern region of Brazil, between 22°29'30"-26°42'59"S and 48°02'24"-54°37'38"W) and treated at the University Hospital of Londrina and the Chagas Disease Laboratory of the State University of Maringa. A control group was formed of 165 healthy, unrelated individuals, who were spouses of the patients or blood donors of the Regional Blood Bank of Maringa (84 men and 81 women, with a mean age of 59.0 ± 11.4 years), living in the same geographical area as the patients and whose serological examination for antibodies against Chagas disease was negative. All individuals (patients and controls) that had participated in this study were monitored and evaluated for clinical symptoms and epidemiological data.

The inclusion criteria of the patient group were: positive laboratory diagnosis of Chagas disease and being in the chronic phase of the disease at the time of the study. The inclusion criteria for the control group were: negative laboratory diagnosis for Chagas disease and living in the same geographical region as the patients. The characteristics of the patients and controls are shown in Table 1.

Due to the great miscegenation in the Brazil population, and after considering the population composition of the state of Parana according to Probst *et al.* [38], patients and controls were classified as a mixed ethnicity population. The risk of population stratification bias, due to differences in ethnic background between patients and controls and variations of allele frequencies according to ethnic background, was minimized by matching patients with controls individuals of the same ethnic background. Mean age, gender rates and residence in the same geographical areas were carefully matching to select the groups.

Serology for *T. cruzi*

Laboratory diagnosis of Chagas disease in patients and controls individuals was carried out by the Regional Blood Bank of Maringa with an ELISA (Enzyme-Linked ImmunoSorbent Assay) test of serum or plasma, using "Chagas III" reagents (GrupoBios, Santiago, Chile) following the manufacturer's instructions. The microplates were read using semi-automatic equipment (ASYS Expert Plus, Cambridge, UK). ELISA cut-off was defined by the formula: cut-off value =

Table 1. Characteristics of the chronic Chagas disease patients and control individuals.

Characteristic	Control N = 165	CCD N = 131	NC N = 87	CCC N = 44
Age (years)				
Mean	59.0 ± 11.4	60.4 ± 9.8	59.0 ± 9.0	63.3 ± 10.5
Minimum	35	35	35	35
Maximum	98	87	87	85
Gender (n as %)				
Female	81 (49.1)	72 (55.0)	53 (60.9)	19 (43.2)
Male	84 (50.9)	59 (45.0)	34 (39.1)	25 (56.8)
Ethnic group* (n as %)				
White	114 (69.1)	96 (73.3)	62 (71.3)	34 (77.3)
Mulatto	36 (21.8)	23 (17.5)	17 (19.5)	6 (13.6)
Black	15 (9.1)	12 (9.2)	8 (9.2)	4 (9.1)
Cardiopathy (n as %)				
Yes		44 (36.6)		
No		87 (66.4)		

CCD: chronic Chagas disease patients; NC: without heart involvement patients, CCC: chronic chagasic cardiopathy patients.
*mixed population (white + mulatto + black) = 165 controls and 131 patients

doi:10.1371/journal.pntd.0003753.t001

(average absorbance of the positive controls + average absorbance of the negative controls) x 0.35; and absorbance equal to or greater than the cut-off value was considered reactive. The indeterminate zone was defined by the absorbance values observed between the cut-off ± 10%. The samples were tested in duplicate and positive and negative controls were included. When the absorbance value was in the indeterminate zone, the ELISA test was repeated in duplicate and the indirect immunofluorescence (IFI) test was performed, according WHO recommendation. The Clinical Immunology Laboratory of the State University of Maringa performed an IFI test with the IMUNOCRUZI® antigen (Biolab, Rio de Janeiro, Brazil) and human anti-immunoglobulin G conjugated to fluorescein (Laborclin, Pinhais, Brazil). Samples were considered positive with titers $\geq$ 40.

Heart examination

The patients with chronic Chagas disease (CCD group) were divided into two distinct groups according to the changes observed in the standard electrocardiography examination at rest. Of the all Chagas disease patients, 44 of them (36.6%), 25 men and 19 women, mean age of 63.3 ± 10.5 years, were considered to have chronic chagasic cardiopathy (CCC group) as they had cardiac signs characteristic of Chagas disease such as right bundle branch block, left anterior hemi-block, unspecific ventricular repolarisation disorders and ventricular and supraventricular premature beats. The remaining 87 (66.4%) patients with chronic Chagas disease, 34 men and 53 women, with a mean age of 59.0 ± 9.0 years, were considered as not having heart disease (NC group) (Table 1).

KIR and HLA genotyping

DNA from all samples was extracted by the salting-out method adapted by Cardozo *et al.* [39]. The concentration and purity of DNA were verified using NanoDrop 2000 equipment (Thermo Scientific, Wilmington, USA). KIR and HLA-A, B and C were genotyped according

manufacturer's instructions by Polymerase Chain Reaction-Sequence Specific Oligonucleotide Probes protocols with Luminex technology (One Lambda Inc., Canoga Park, CA, USA). First, target DNA was PCR-amplified using group specific primers sets. Each PCR product was biotinylated, which allowed later detection using R-Phycoerythrin-conjugated Strepavidin (SAPE). Each PCR product was denatured and allowed to hybridise to complementary DNA probes conjugated to fluorescently coded microspheres. After washing the beads, bound amplified DNA from the test samples was tagged with SAPE. A flow analyser, the LABScan 100, identified the fluorescent intensity of PE (phycoerithrin) on each microsphere. The fluorescent intensity varied based on reaction outcome, and was expected to be 1000 or above for control positive probes. The data were interpreted using a computer program (HLA Fusion 2.0 Research, One Lambda).

Some HLA-KIR ligands specificities belonging to the group C1, C2 and Bw4 were considered according to Kulkarni *et al.* [40] and Thananchai *et al.* [41]. HLA molecules from the C1 group include the specificities from *HLA-C*01*, **03*, **07*, **08*, **12*, **14 *16* and are ligands of KIR2DL2, KIR2DL3 and KIR2DS2. HLA molecules from C2 group that include *HLA-C*02*, **04*, **05*, **06*, **07*,**15*, **17*, and **18* specificities interact with KIR2DL1 and KIR2DS1. HLA-Bw4 epitopes (*HLA-A*23*, **24*, **32; HLA-B*13*, **27*, **44*, **51*, **52*, **53*, **57*, **58*) are recognized by KIR3DL1 and KIR3DS1. Specificities from *HLA-A*03* and/or—*A*11* are KIR3DL2 ligands.

Based on the content of the genes, two types of KIR genotypes have been described and are designated AA and BX (BB and AB). The main distinction between them is the number of genes encoding activating receptors. Individual genotypes were determined to be AA when the genes *KIR2DL1*, *KIR2DL3*, *KIR2DL4*, *KIR2DS4*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3*, *KIR2DP1* and *KIR3DP1* were present. The presence of one or more of the following genes: *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* and *KIR3DS1* characterised the genotype BX (defined according http://www.allelefrequencies.net).

Statistical analysis

KIR, *HLA* and KIR-HLA frequencies were obtained by direct counting. Comparisons of the frequencies of KIR ligands, *KIR* genes, KIR AA and BX genotypes and KIR with or without ligands between patients and controls were performed using the Chi-square test with Yates' correction or Fisher's Exact Test. The associations of genetic trait between chronic Chagas disease and controls were measured by OR (Odds Ratio) and the 95% confidence interval (95% CI). Statistical analyses were performed using the Open Epi program: Open Source Epidemiologic Statistics for Public Health, version 2.3.1 (http://www.openepi.com/Menu/OE_Menu.htm). *P*-values ≤ 0.05 were considered statistically significant. A correction for multiple testing was done by multiplying the *P*-values by the number of the tests (Bonferroni correction). A Hardy-Weinberg equilibrium fit was performed by calculating expected genotype frequencies and comparing with the observed values using Arlequin software (version 3.1).

Results

HLA and *KIR* genotypes frequency distribution in the studied populations was in Hardy-Weinberg equilibrium. The frequencies of *KIR* genes in the control group were similar to those found by Rudnick *et al.* [42] in individuals of the north/northwest region of the state of Paraná.

Distribution of *KIR* gene frequencies among controls, chronic Chagas disease patients (CCD), patients without heart involvement (NC), and chronic chagasic cardiopathy patients (CCC) is shown in Table 2. CCC presented a lower frequency of *KIR2DL2* when compared to the control group (31.8% vs. 53.3%; $P = 0.017$; OR = 0.41; 95% CI = 0.20–0.83); however, significance was lost after Bonferroni correction ($Pc = 0.27$). No significant differences were found in

Table 2. Distribution of *KIR* genes in healthy controls, chronic Chagas disease patients and in groups of patients with and without heart involvement.

KIR genes	Control N = 165 n (%)	CCD N = 131 n (%)	NC N = 87 n (%)	CCC N = 44 n (%)
KIR2DL1	162 (98.2)	131 (100)	87 (100)	44 (100)
KIR2DL2	88 (53.3) [a]	49 (37.4)	35 (40.2)	14 (31.8) [a]
KIR2DL3	146 (88.5)	119 (90.8)	79 (90.8)	40 (90.9)
KIR2DL4	165 (100)	131 (100)	87 (100)	44 (100)
KIR2DL5	102 (61.8)	73 (55.7)	53 (60.9)	20 (45.5)
KIR2DP1	162 (98.2)	130 (99.2)	87 (100)	43 (97.7)
KIR2DS1	67 (40.6)	57 (42.7)	43 (49.4)	14 (31.8)
KIR2DS2	92 (55.8)	62 (48.1)	40 (46.0)	22 (50.0)
KIR2DS3	59 (35.8)	29 (22.1)	17 (19.5)	12 (27.3)
KIR2DS4	155 (93.9)	124 (94.7)	81 (93.1)	43 (97.7)
KIR2DS5	69 (41.8)	59 (41.8)	44 (50.6)	15 (34.1)
KIR3DL1	158 (95.8)	127 (96.9)	84 (96.6)	43 (97.7)
KIR3DL2	165 (100)	131 (100)	87 (100)	44 (100)
KIR3DL3	165 (100)	131 (100)	87 (100)	44 (100)
KIR3DP1	165 (100)	131 (100)	87 (100)	44 (100)
KIR3DS1	63 (38.2)	54 (41.2)	41 (47.1)	13 (29.5)

CCD: chronic Chagas disease patients, NC: without heart involvement patients, CCC: chronic chagasic cardiopathy patients.

[a] $P = 0.017$; $Pc = 0.27$; OR = 0.41; 95% CI = 0.20–0.83 (CCC vs Controls)

doi:10.1371/journal.pntd.0003753.t002

the distribution of other *KIR* genes between all of the analysed groups. According to expected the *KIR* framework genes, *KIR2DL4*, *KIR3DL2*, *KIR3DL3* and *KIR3DP1* were present in all samples, which were important internal controls.

The frequencies of the HLA class I ligands of the KIR (A3 and/or A11, Bw4, C1 and C2, in homozygosity and heterozygosity) were analysed and were similar between groups. An exception was found for the specificities of the *HLA-A*03* and/or—*A*11*, ligands of KIR3DL2, which were lower in chronic Chagas disease patients (CCD) (19.1% vs. 30.3%; $P = 0.036$; $Pc = 0.144$; OR = 0.54; 95% CI = 0.31–0.94), but the significance was lost after Bonferroni correction.

The distribution of the frequencies of the KIR and their HLA ligands (KIR2DL2-C1; KIR3DL3-C1; KIR2DS2-C1; KIR2DL1-C2; KIR2DS1-C2; KIR3DL1-Bw4, KIR3DS1-Bw4) are listed in Table 3. The KIR2DL2-C1 (16.8% vs. 32.1%; $P = 0.036$; OR = 0.43; 95% CI = 0.24–0.75), the KIR3DL2-A3/11 pair (19.1% vs. 30.3%; $P = 0.037$; OR = 0.54; 95% CI = 0.31–0.94) and KIR2DL2 in the presence of the ligands in the homozygous state (KIR2DL2-C1/C1) (4.5% vs. 19.5% $P = 0.031$; OR = 0.23; 95% CI = 0.04–0.89) had lower frequency in the patients with chronic Chagas disease, but the significance was lost after Bonferroni correction.

The correlation between the distribution of activating and inhibitory KIR and their respective HLA ligands is shown in Table 4. An increased risk or susceptibility of developing chronic Chagas disease (12.2% vs. 4.2%; $P = 0.020$; $Pc = 0.040$; OR = 2.14; 95% CI = 1.25–7.88) and chronic chagasic cardiopathy (22.7% vs. 4.2%; $P = 0.0002$; $Pc = 0.0004$; OR = 6.64; 95% CI = 2.30–18.60) was observed for the KIR2DS2^{+}/2DL2^{-}/C1^{+} combination (KIR2DS2 and the C1 ligand in the absence of KIR2DL2). This correlation was also observed when CCC was compared to the NC (22.7% vs. 6.9%; $P = 0.010$; $Pc = 0.020$; OR = 3.97; 95% CI = 1.34–11.79). Susceptibility was also observed when KIR2DL3 was present, KIR2DS2^{+}/2DL2^{-}/KIR2DL3^{+}/C1^{+} combination, for CCD (10.7% vs. 4.2%; $P = 0.050$; $Pc = 0.10$; OR = 1.06; 95% CI = 1.1–6.9) and

Table 3. Distribution of KIR and their respective HLA ligands in Chagas disease patients and controls.

KIR—HLA ligands	Control N = 165 n (%)	CCD N = 131 n (%)	NC N = 87 n (%)	CCC N = 44 n (%)
2DL1-C2	80 (48.4)	57 (43.5)	35 (40.2)	22 (50.0)
2DL2-C1	53 (32.1) [a]	22 (16.8) [a]	14 (16.1)	9 (22.5)
2DL3-C1	75 (45.5)	54 (41.2)	33 (37.9)	21 (47.7)
3DL2-A3/A11	50 (30.3) [b]	25 (19.1) [b]	18 (20.7)	7 (15.9)
3DL1-Bw4	113 (68.5)	94 (71.8)	60 (69.0)	34 (77.3)
2DS1-C2	37 (22.4)	22 (16.8)	16 (18.4)	6 (13.6)
2DS2-C1	53 (32.1)	27 (20.6)	15 (17.2)	12 (27.3)
3DS1-Bw4	47 (28.5)	40 (30.5)	29 (33.3)	11 (25.0)
2DL1-C2C2	40 (24.2)	25 (19.1)	19 (21.8)	6 (13.6)
2DL2-C1C1	19 (11.5)	19 (14.5)	17 (19.5) [c]	2 (4.5) [c]
2DL3-C1C1	37 (22.4)	43 (32.8)	30 (34.5)	13 (29.5)
2DS1-C2C2	13 (7.9)	14 (10.7)	13 (14.9)	1 (2.3)
2DS2-C1C1	20 (12.1)	26 (19.8)	20 (23.0)	6 (13.6)
2DL2/2DL2-C1C2	9 (5.5)	2 (1.5)	1 (1.1)	1 (2.3)
2DL2/2DL3-C1C2	44 (26.7)	20 (15.3)	12 (13.8)	8 (18.2)
2DL3/2DL3-C1C2	31 (18.8)	34 (26.0)	21 (24.1)	13 (29.5)
2DL2/2DL2-C1C1	3 (1.8)	6 (4.6)	5 (5.7)	1 (2.3)
2DL2/2DL3-C1C1	16 (9.7)	13 (9.9)	12 (13.8)	1 (2.3)
2DL3/2DL3-C1C1	21 (12.7)	30 (22.6)	18 (20.7)	12 (27.3)

CCD: chronic Chagas disease patients; NC: without heart involvement patients, CCC: chronic chagasic cardiopathy patients.

[a] *P* = 0.036; *Pc* = 0.108; OR = 0.43; 95% CI = 0.24–0.75 (CCD vs Controls)

[b] *P* = 0.037; *Pc* = 0.10; OR = 0.54; 95% CI = 0.31–0.94 (CCD vs Controls)

[c] *P* = 0.031; *Pc* = 0.093; OR = 0.23; 95% CI = 0.04–0.89 (CCC vs NC)

Bw4 = *HLA-A*23,*24,*32; HLA-B,*13,*27,*44,*51,*52,*53,*57,*58*

Group C1 = *HLA-C*01,*03,*07,*08,*12,*14,*16*

Group C2 = *HLA-C*02,*04,*05,*06,*07,*15,*17,*18*

doi:10.1371/journal.pntd.0003753.t003

when CCC was compared to NC (18.2% vs. 5.7%; *P* = 0.041; *Pc* = 0.08; OR = 3.64; 95% CI = 1.12–11.91), although it was lost after Bonferroni correction. However, the susceptibility of developing disease remained for CCC (18.2% vs. 4.2%; *P* = 0.004; *Pc* = 0.004; OR = 5.02; 95% CI = 1.71–14.73) when compared to controls.

No significant difference was observed in the AA and BX genotype frequencies between all groups. Also, there was no significant difference in the frequencies of AA genotypes when the complete forms of the *KIR2DS4* gene or its variants (deleted form) were present.

Discussion

To the best of our knowledge, this is the first study of KIR and the HLA ligands in the immunopathology of chronic Chagas disease. The identification of the role of KIR and KIR-HLA ligand pairs in the Chagas disease development could improve our understanding of the role of NK cells in the immunopathogenesis of this disease.

The current study shows that the distribution of the *KIR* genes highlights differences in the frequency of *KIR2DL2* when chronic chagasic cardiopathy patients (CCC) were compared to controls, although the significance was lost after correction for multiple testing. *KIR2DL2* and *KIR2DL3* segregate as alleles of a single locus, and are both common and recognise C1 [43].

Table 4. Distribution of activating KIR plus inhibitory KIR and their respective ligands in chronic Chagas disease and controls.

KIR-HLA Ligand		Control N = 165 n (%)	CCD N = 131 n (%)	NC N = 87 n (%)	CCC N = 44 n (%)
KIR-C1					
	$2DS2^{+}/2DL2^{-}/C1^{+}$	7 (4.2) [a,b]	16 (12.2) [a]	6 (6.9) [c]	10 (22.7) [b, c]
	$2DS2^{-}/2DL2^{+}/C1^{+}$	6 (3.6)	4 (3.1)	1 (1.1)	3 (6.8)
	$2DS2^{+}/2DL3^{-}/C1^{+}$	12 (7.3)	9 (6.9)	6 (6.9)	3 (6.8)
	$2DS2^{-}/2DL3^{+}/C1^{+}$	51 (30.0)	53 (40.5)	34 (39.1)	19 (43.2)
	$2DS2^{+}/2DL2^{-}/2DL3^{-}/C1^{+}$	0 (0.0)	2 (1.5)	0 (0.0)	2 (4.5)
	$2DS2^{-}/2DL2^{-}/2DL3^{+}/C1^{+}$	45 (27.3)	50 (38.2)	33 (37.9)	17 (48.6)
	$2DS2^{+}/2DL2^{-}/2DL3^{+}/C1^{+}$	7 (4.2) [d, f]	14 (10.7) [d]	5 (5.7) [e]	8 (18.2) [e,f]
	$2DS2^{-}/2DL2^{+}/2DL3^{+}/C1^{+}$	5 (3.)	3 (2.3)	1(1.1)	2 (4.5)
	$2DS2^{+}/2DL2^{+}/2DL3^{-}/C1^{+}$	12 (7.3)	7 (5.3)	6 (6.9)	1 (2.3)
	$2DS2^{+}/2DL2^{+}/2DL3^{+}/C1^{+}$	54 (32.7)	30 (22.9)	23 (26.4)	7 (15.9)
KIR- C2					
	$2DS1^{+}/2DL1^{-}/C2^{+}$	3 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)
	$2DS1^{-}/2DL1^{+}/C2^{+}$	70 (42.4)	43 (32.8)	26 (29.9)	21 (47.7)
	$2DS1^{+}/2DL1^{+}/C2^{+}$	47 (28.5)	36 (27.5)	29 (33.3)	7 (15.9)
KIR- Bw4					
	$3DS1^{+}/3DL1^{+}/Bw4^{+}$	41 (24.8)	35 (26.7)	25 (28.7)	10 (22.7)
	$3DS1^{+}/3DL1^{-}/Bw4^{+}$	7 (4.2)	3 (2.3)	2 (2.3)	1 (2.3)
	$3DS1^{-}/3DL1^{+}/Bw4^{+}$	72 (43.6)	60 (46.3)	36 (41.4)	24 (54.5)
KIR-HLA- A3/11					
	$3DL2^{+}/A3^{+}/A11^{+}$	50 (30.3) [g]	25 (19.1) [g]	18 (20.7)	7 (15.9)
	$3DL2^{+}/A3^{-}/A11^{+}$	17 (10.6)	6 (4.6)	5 (5.8)	1 (2.3)
	$3DL2^{+}/A3^{+}/A11^{-}$	33 (20.0)	19 (14.5)	13 (14.9)	6 (13.6)
	$3DL2^{+}/A3^{-}/A11^{-}$	118 (71.5)	106 (80.9)	69 (79.3)	37 (84.1)

CCD: chronic Chagas disease patients; NC: without heart involvement patients, CCC: chronic chagasic cardiopathy patients.

[a] $P = 0.020$; $Pc = 0.040$; OR = 2.14; 95% CI = 1.25–7.88 (CCD vs Controls)

[b] $P = 0.0002$; $Pc = 0.0004$; OR = 6.64; 95% CI = 2.30–18.60 (CCC vs Controls)

[c] $P = 0.010$; $Pc = 0.020$; OR = 3.97; 95% CI = 1.34–11.79 (CCC vs NC)

[d] $P = 0.050$; $Pc = 0.100$; OR = 1.06; 95% CI = 1.1–6.9 (CCD vs Controls)

[e] $P = 0.040$; $Pc = 0.080$; OR = 3.64; 95% CI = 1.12–11.91 (CCC vs NC)

[f] $P = 0.004$; $Pc = 0.008$; OR = 5.02; 95% CI = 1.71–14.73 (CCC vs Controls)

[g] $P = 0.036$; $Pc = 0.144$; OR = 0.54; 95% CI = 0.31–0.94 (CCD vs Controls)

doi:10.1371/journal.pntd.0003753.t004

Although KIR2DL2 and KIR2DL3 exhibit quantitative differences in specificity and avidity for the HLA-C1 ligand, they qualitatively differ in their genetics, functional effect, and clinical influence [44, 45]. In the current study, *KIR2DL3* was not significantly different in any of the comparisons, revealing a possible distinction between these receptors in NK-cell regulation in the cardiac form of Chagas disease.

KIR were analysed in the presence of their respective HLA ligands. HLA class I genes are located on chromosome 6 and *KIR* genes are on chromosome 19, which allows independent inheritance and expression of both. The independent segregation of these genes, coupled with the high specificity of KIR for certain HLA allotypes, allows the expression of KIR molecules for which the HLA ligand is not present, or *vice versa*; this results in a lack of functionality of NK cells due to a lack of signalling [40]. Moreover, depending on the combination of KIR and

HLA ligands, NK cells may exhibit different degrees of response due to an excess of inhibition or activation, a balance between inhibition and activation, or even an undetermined behaviour [46, 47].

In this study, the pair KIR3DL2-HLA-A3/A11 was less frequent in chronic Chagas disease: *KIR3DL2* is a framework gene that is present in almost all genotypes, meaning that the difference in the frequency of this inhibitory KIR-HLA pair may be related to differences in the frequencies of HLA-A3/A11 ligands and not reflecting participation in the pathogenesis of Chagas disease.

However, the results indicate that the interaction of KIR2DL2 and KIR2DS2 with HLA-C1 may have an important role of NK cells in the development of Chagas disease. KIR2DS2 and KIR2DL2 share the same ligand most likely because of the homology of the same extracellular portion, although there is a difference in the function of activating or inhibiting NK cells, respectively. In this study, KIR2DL2 in the presence of the HLA-C1 ligand was less frequent in chronic Chagas disease patients than in controls and those patients with chronic chagasic cardiopathy also had a lower frequency of KIR2DL2 with the homozygous C1 (KIR2DL2-C1/C1) compared to the group without heart failure, although the significance was lost after correction for multiple testing. No association of KIR2DS2-C1 was found between Chagas disease and controls.

To evaluate the C1-reactive KIR2D NK cells, the combination of one pair (inhibitory) in the absence of the other pair (activating) was analysed (the KIR2DL3 was included as well). The results are shown in Table 4. It was possible to observe that KIR2DS2-C1 in the absence of KIR2DL2 (KIR2DS2^{+}/2DL2^{-}/C1^{+} combination) was more frequent in Chagas patients and, in particular, in those who manifested chronic chagasic cardiopathy when compared to the control group, and when chronic chagasic cardiopathy patients were compared to the without heart involvement patients. In addition, KIR2DS2^{+} in the absence of KIR2DL2 and the presence of KIR2DL3 (KIR2DS2^{+}/2DL2^{-}/KIR2DL3^{+}/C1^{+}) was also significantly more common in the CCC group compared to the NC group.

The data obtained in this study indicated a possible susceptibility related to the activating KIR2DS2 and its C1 ligand in the absence of KIR2DL2, for the development of Chagas disease and chronic chagasic cardiopathy. The KIR2DL2-C1 pair has a strong inhibitory effect on NK cells and the KIR2DL3-HLA-C1 pair caused weaker inhibitory signals [48]. Although the same ligand can have affinities for both activating (-DS) and inhibitory (-DL) receptors of NK cells for some KIR, the affinity for inhibitory KIR is higher than for its homologous activating KIR [46]. David *et al.* [49] showed that when KIR2DL2 and KIR2DS2 were co-expressed, NK cell inhibition overrode NK cell activation. Thus, if inhibitory KIR are absent from the surfaces of NK cells, group C HLA ligands will bind to activating receptors, inducing the effector function of the NK cells and consequently greater inflammation and tissue injury. This predisposes to chronic Chagas disease and the manifestations of chronic chagasic cardiopathy. Although the KIR2DS2^{+}/2DL2^{-}/KIR2DL3^{+}/C1^{+} combination presents one activating and one inhibitory KIR, the activating signals generated by KIR2DS2^{+}/C1^{+} appeared to be stronger than the inhibitory signals generated by KIR2DL3^{+}/C1^{+}. In other studies, the KIR2DL2-C1 pair was associated with protection against chronic myeloid leukaemia [50] systemic sclerosis [51] and kidney cancer patients [52] and KIR2DS2 (KIR2DS2^{+}/2DL2^{-}/C1^{+}) was more common for other autoimmune diseases [30, 53].

The *KIR2DL2* and *KIR2DS2* genes are in strong linkage disequilibrium, as observed among patients (Δ' = 0.99; *P*-value = 0.004) and controls (Δ' = 1.0; *P*-value = 0.0001). However, in this study, there were 8 controls (4.8%) and 4 patients (3.1%) where only *KIR2DL2* was present, and 10 controls (6.1%) and 18 patients (13.7%) with *KIR2DS2* (activating) but without *KIR2DL2* (inhibitory). Within the sub-groups, three patients with cardiopathy (6.8%) and one

without heart disease (1.2%) had only *KIR2DL2*, whilst *KIR2DS2* alone was present in 11 patients with cardiopathy (25.0%) and 7 patients without heart disease (8.1%).

The immune response during infection by *T. cruzi* determines the development of the different clinical manifestations of Chagas disease: it is associated with both the pathogenesis and protective effects that control tissue damage [12]. Several studies have shown that there is an increase in the frequency of circulating NK cells in chronic chagasic cardiopathy patients, and uncontrolled activation of NK cells and pro-inflammatory monocytes can also lead to tissue damage, which, in turn, leads to the development of serious chronic illness [11, 12, 13]. For other hand, Chagas cardiopathy could be linked to autoimmunity [9, 54, 55]. In this study, we investigated KIR-HLA ligand as a risk factor in Chagas disease and these results could corroborate to the NK immunopathogenic mechanism in the Chagas disease understanding.

In this KIR-HLA ligands study, a possible risk factor for the development of Chagas disease and chronic chagasic cardiopathy related to the activating KIR2DS2 and its C1 ligand in the absence of KIR2DL2 was found. To better understanding the role of NK cells and the expression or KIR in the immunopathogenesis of Chagas disease, others studies would be done like histological analysis in the CCC and NK cytotoxicity assays.

Conclusion

The combination of KIR2DS2 activating receptor with C1 ligand, in the absence of KIR2DL2, may be related to a risk factor in the chronic Chagas disease and chronic chagasic cardiopathy.

Acknowledgments

The authors are grateful to all of the volunteers who participated in this study, the Regional Blood Bank of Maringa, the University Hospital of Londrina and the Chagas Disease Laboratory of the State University of Maringa.

Author Contributions

Conceived and designed the experiments: AMS MMdOD JELV SMdA. Performed the experiments: CMA PGR CdFO DSdOM. Analyzed the data: AMS CMA. Contributed reagents/materials/analysis tools: MMdOD AMS JELV SMdA DSdOM. Wrote the paper: CMA AMS JELV.

References

1. WHO. Chagas disease (American trypanosomiasis)—Fact sheet 340.
2. Andrade LO, Machado CRS, Chiari E, Pena SDJ, Macedo AM. Differential tissue distribution of diverse clones of *Trypanosoma cruzi* in infected mice. Mol Biochem Parasitol. 1999; 100: 163–172. PMID: 10391378
3. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simoes MV. Pathogenesis of chronic Chagas heart disease. Circulation. 2007; 115: 1109–1123. PMID: 17339569
4. Arce-Fonseca M, Ballinas-Verdugo MA, Reyes PA, Aranda-Fraustro A, Monteon VM. Autoantibodies to human heart conduction system in Chagas' disease. Vector Borne Zoonotic Dis. 2005; 5: 233–236. PMID: 16187891
5. Manoel-Caetano FS, Silva AE. Implications of genetic variability of Trypanosoma cruzi for the pathogenesis of Chagas disease. Cad Saude Publica. 2007; 23: 2263–2274. PMID: 17891288
6. Souza PE, Rocha MO, Rocha-Vieira E, Menezes CA, Chaves AC, Gollob KJ, et al. Monocytes from patients with indeterminate and cardiac forms of Chagas disease display distinct phenotypic and functional characteristics associated with morbidity. Infect Immun. 2004; 72: 5283–5291. PMID: 15322024
7. Cunha-Neto E, Nogueira LG, Teixeira PC, Ramasawmy R, Drigo SA, Goldberg AC, et al. Immunological and non-immunological effects of cytokines and chemokines in the pathogenesis of chronic Chagas disease cardiomyopathy. Mem Inst Oswaldo Cruz. 2009; 104: 252–258. PMID: 19753481
8. Biolo A, Ribeiro AL, Clausell N. Chagas cardiomyopathy—where do we stand after a hundred years? Prog Cardiovasc Dis. 2010; 52: 300–316. doi: 10.1016/j.pcad.2009.11.008 PMID: 20109600

9. Reis DD, Jones EM, Tostes S, Lopes ER, Gazzinelli G, Colley DG, et al. Characterization of inflammatory infiltrates in chronic chagasic myocardial lesions: presence of tumour necrosis factor-alpha+ cells and dominance of granzyme A+, CD8+ lymphocytes. Am J Trop Med Hyg. 1993; 48: 637–644. PMID: 8517482
10. Higuchi MD, Ries MM, Aiello VD, Benvenuti LA, Gutierrez PS, Bellotti G, et al. Association of an increase in CD8+ T cells with the presence of *Trypanosoma cruzi* antigens in chronic, human, chagasic myocarditis. Am J Trop Med Hyg. 1997; 56: 485–489. PMID: 9180594
11. Vitelli-Avelar DM, Sathler-Avelar R, Dias JCP, Pascoal VP, Teixeira-Carvalho A, Lage OS, et al. Chagasic patients with indeterminate clinical form of the disease have high frequencies of circulating CD3+CD16–CD56+ natural killer T cells and CD4+CD25 high regulatory T lymphocytes. Scand J Immunol. 2005; 62: 297–308. PMID: 16179017
12. Vitelli-Avelar DM, Sathler-Avelar R, Massara RL, Borges JD, Lage OS, Lana M, et al. Are increased frequency of macrophage-like and natural killer (NK) cells, together with high levels of NKT and CD4+CD25high T cells balancing activated CD8+ T cells, the key to control Chagas' disease morbidity? Clin Exp Immunol. 2006; 145: 81–92. PMID: 16792677
13. Sathler-Avelar R, Vitelli-Avelar DM, Teixeira-Carvalho A, Martins-Filho AO Innate immunity and regulatory T-cells in human Chagas disease: what must be understood? Mem Inst Oswaldo Cruz. 2009; 104: 246–251. PMID: 19753480
14. Ljunggren HG, Kärre K. In search of the "missing self": MHC molecules and NK cell recognition. Immunol Today. 1990; 11: 237–244. PMID: 2201309
15. Middleton D, Gonzelez F. The extensive polymorphism of KIR genes. Immunology. 2010; 129: 8–19. doi: 10.1111/j.1365-2567.2009.03208.x PMID: 20028428
16. Green S, Pichyangkul S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Nisalak A, et al. Early CD69 expression on peripheral blood lymphocytes from children with dengue haemorrhagic fever. J Infect Dis. 1999; 180: 1429–1435. PMID: 10515800
17. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert J, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Gen. 2002; 31: 429–434.
18. Alter G, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med. 2007; 204: 3027–3036. PMID: 18025129
19. Montes-Cano MA, Caro-Oleas JL, Romero-Gómez M, Diago M, Andrade R, Carmona I, et al. HLA-C and KIR genes in hepatitis C virus infection. Hum Immunol. 2005; 66:1106–1109. PMID: 16571411
20. Marangon AV, Silva GF, de Moraes CF, Grotto RM, Pardini MI, de Pauli DS, et al. KIR genes and their human leukocyte antigen ligands in the progression to cirrhosis in patients with chronic hepatitis C. Hum Immunol. 2011; 72: 1074–1078. doi: 10.1016/j.humimm.2011.08.017 PMID: 21920398
21. Méndez A, Granda H, Meenagh A, Contreras S, Zavaleta R, Mendoza MF, et al. Study of KIR genes in tuberculosis patients. Tissue Antigens. 2006; 68: 386–389. PMID: 17092251
22. Lu C, Shen YJ, Deng YF, Wang CY, Fan G, Liu YQ, et al. Association of killer cell immunoglobulin-like receptors with pulmonary tuberculosis in Chinese Han. Genet Mol Res. 2012; 11: 1370–1378. doi: 10.4238/2012.May.15.7 PMID: 22653583
23. Franceschi DS, Mazini OS, Rudnick CC, Sell AM, Tsuneto LT, de Melo FC, et al. Association between killer-cell immunoglobulin-like receptor (KIR) genotypes and leprosy in Brazil. Tissue Antigens. 2008; 72: 478–482. doi: 10.1111/j.1399-0039.2008.01127.x PMID: 18778326
24. Jarduli LR, Alves HV, de Souza-Santana FC, Marcos EV, Pereira AC, Dias-Baptista IM, et al. Influence of KIR genes and their HLA ligands in the pathogenesis of leprosy in a hyperendemic population of Rondonópolis, Southern Brazil. BMC Infect Dis. 2014; 14: 438. doi: 10.1186/1471-2334-14-438 PMID: 25117794
25. Taniguchi M, Kawabata M. KIR3DL1/S1 genotypes and KIR2DS4 allelic variants in the AB KIR genotypes are associated with Plasmodium-positive individuals in malaria infection. Immunogenetics. 2009; 61: 717–730. doi: 10.1007/s00251-009-0401-z PMID: 19859704
26. Yindom LM, Forbes R, Aka P, Jonha O, Jeffries D, Jallow M, et al. Killer-cell immunoglobulin-like receptors and malaria caused by *Plasmodium falciparum* in The Gambia. Tissue Antigens. 2012; 79: 104–113. doi: 10.1111/j.1399-0039.2011.01818.x PMID: 22220719
27. Hirayasu K, Ohashi J, Kashiwase K, Hananantachai H, Naka I, Ogawa A, et al. Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. PLoS Pathog. 2012; 8: e1002565. doi: 10.1371/journal.ppat.1002565 PMID: 22412373
28. Augusto DG, Lobo-Alves SC, Melo MF, Pereira NF, Petzl-Erler ML. Activating KIR and HLA Bw4 ligands are associated to decreased susceptibility to Pemphigus Foliaceus, an autoimmune blistering skin disease. PLoS One. 2012; 7: e39991. doi: 10.1371/journal.pone.0039991 PMID: 22768326

29. Luszczek W, Mánczak M, Cislo M, Nockowski P, Wi niewski A, Jasek M, et al. Gene for the activating natural killer cell receptor, KIR2DS1, is associated with susceptibility to psoriasis vulgaris. Hum Immunol. 2004; 65: 758–766. PMID: 15310528

30. Momot T, Koch S, Hunzelmann N, Krieng T, Ulbricht K, Schmidt RE, et al. Association of killer cell immunoglobulin-like receptors with scleroderma. Arthritis Rheum. 2004; 50: 1561–1565. PMID: 15146426

31. Yen JH, Moore BE, Nakajima T, Scholl D, Schaid DJ, Weyand CM, et al. Major histocompatiblity complex class I recognizing receptors are disease risk genes in rheumatoid arthritis. J Exp Med. 2001; 193: 1159–1167. PMID: 11369787

32. Hollenbach JA, Ladner MB, Saeteurn K, Taylor KD, Mei L, Haritunians T, et al. Susceptibility to Crohn's disease is mediated by KIR2DL2/KIR2DL3 heterozygosity and the HLA-C ligand. Immunogenetics. 2009; 61: 663–671. doi: 10.1007/s00251-009-0396-5 PMID: 19789864

33. Carrington M, Wang S, Martin MP, Gao X, Schiffman M, Cheng J, et al. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. J Exp Med. 2005; 201: 1069–1075. PMID: 15809352

34. Ashouri E, Dabbaghmanesh MH, Rowhanirad S, Bakhshayeshkaram M, Ranjbar Omrani G. Activating KIR2DS5 receptor is a risk for thyroid cancer. Hum Immunol. 2012; 73: 1017–1022. doi: 10.1016/j.humimm.2012.07.325 PMID: 22836040

35. Wi niewski A, Jankowska R, Passowicz-Muszyńska E, Majorczyk E, Nowak I, Frydecka I, et al. KIR2DL2/S2 and HLA-C C1C1 genotype is associated with better response to treatment and prolonged survival of patients with non-small cell lung cancer in a Polish Caucasian population. Hum Immunol. 2012; 73: 927–931. doi: 10.1016/j.humimm.2012.07.323 PMID: 22836042

36. De Re V, Caggiari L, De Zorzi M, Talamini R, Racanelli V, D' Andrea M, et al. Genetic Diversity of the KIR/HLA System and Outcome of Patients with Metastatic Colorectal Cancer Treated with Chemotherapy. PLoS One. 2014; 9: e84940. doi: 10.1371/journal.pone.0084940 PMID: 24497922

37. Franceschi DS, de Souza CA, Aranha FJ, Cardozo DM, Sell AM, Visentainer JEL. Importance of immunoglobulin-like receptors in allogeneic hematopoietic stem cell transplantation. Rev Bras Hematol Homoter. 2011; 33: 126–130. doi: 10.5581/1516-8484.20110033 PMID: 23284260

38. Probst CM, Bompeixe EP, Pereira NF, de O Dalalio MM, Visentainer JE, Tsuneto LT, et al. HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Paraná, Brazil. Hum Biol. 2000; 72: 597–617. PMID: 11048789

39. Cardozo DM, Guelsin GA, Clementino SL, Melo FC, Souza CD, Braga MA, et al. DNA extraction from coagulated human blood for application in genotyping techniques for human leukocyte antigen and immunoglobulin-like receptors. Rev Soc Bras Med Trop. 2009; 42: 651–656. PMID: 20209349

40. Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Semin Immunol. 2008; 20: 343–352. doi: 10.1016/j.smim.2008.06.003 PMID: 18635379

41. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, et al. Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol. 2007; 178: 33–37. PMID: 17182537

42. Rudnick CC, Franceschi DS, Marangon AV, Guelsin GA, Sell AM, Visentainer JEL. Killer cell immunoglobulin-like receptor gene diversity in a Southern Brazilian population from the state of Paraná. Hum Immunol. 2008; 69: 872–876. doi: 10.1016/j.humimm.2008.09.002 PMID: 18848853

43. Khakoo SI, Carrington M. KIR and disease: a model system or system of models? Immunol Rev. 2006; 214: 186–201. PMID: 17100885

44. Moesta AK, Parham P. Diverse functionality among human NK cell receptors for the C1 epitope of HLA-C: KIR2DS2, KIR2DL2, and KIR2DL3. Front Immunol. 2012; 22: 336.

45. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science. 2004; 305: 872–874. PMID: 15297676

46. Williams AP, Bateman AR, Khakoo SI Hanging in balance. KIR and their role in disease. Mol Interv. 2005; 5: 226–240. PMID: 16123537

47. Nelson GW, Martin MP, Gladman D, Wade J, Trosdale J, Carrington M. Cutting edge heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. J Immunol. 2004; 173: 4273–4276. PMID: 15383555

48. Bashirova AA, Martin MP, McVicar DW, Carrington M. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defence. Annu Rev Genomics Hum Genet. 2006; 7: 277–300. PMID: 16824023

49. David G, Djaoud Z, Willem C, Legrand N, Rettman P, Gagne K, et al. Large spectrum of HLA-C recognition by killer Ig-like receptor (KIR) 2DL2 and KIR2DL3 and restricted C1 specificity of KIR2DS2:

dominant impact of KIR2DL2/KIR2DS2 on KIR2D NK cell repertoire formation. J Immunol. 2013; 191: 4778–4788. doi: 10.4049/jimmunol.1301580 PMID: 24078689

50. Middleton D, Diler AS, Meenagh A, Sleator C, Gourrad PA. Killer immunoglobulin-like receptors (KIR2DL2 and/or KIR2DS2) in presence of their ligand (HLA-C1 group) protect against chronic myeloid leukaemia. Tissue Antigens. 2009; 73: 553–560. doi: 10.1111/j.1399-0039.2009.01235.x PMID: 19493232

51. Salim PH, Jobim M, Bredemeier M, Chies JA, Schlottfeld J. Killer cell immunoglobulin-like receptor (KIR) genes in systemic sclerosis. Clin Exp Immunol. 2010; 160: 325–330. doi: 10.1111/j.1365-2249.2010.04095.x PMID: 20082621

52. Al Omar S, Middleton D, Marshall E, Porter D, Xinarianos G, Raji O, et al. Associations between genes for killer immunoglobulin-like receptors and their ligands in patients with solid tumors. Hum Immunol. 2010; 71: 976–981. doi: 10.1016/j.humimm.2010.06.019 PMID: 20600442

53. Lowe DP, Cook MA, Bowman SJ, Briggs DC, UK Sjogren's Interest Group. Association of killer cell immunoglobulin-like receptors with primary Sjogren's syndrome. Rheumatology. 2009; 48: 359–362. doi: 10.1093/rheumatology/ken503 PMID: 19181658

54. Cunha-Neto E, Teixeira PC, Nogueira LG, Mady C, Lanni B, Stolf N, et al. New concepts on the pathogenesis of chronic Chagas cardiomyopathy: myocardial gene and protein expression profiles. Rev Soc Bras Med Trop. 2006; 39: 59–62. PMID: 17605209

55. Cooke A, Zaccone P, Raine T, Phillips JM, Dunne DW. Infection and autoimmunity: are we winning the war, only to lose the peace? Trends Parasitol. 2004; 20: 316–321. PMID: 15193562