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Can HLA type I and II alleles presence be associated with the clinical spectrum of CHIKV infection?

Authors: Juan C. Rueda[1,2], Ana M. Santos[2], Jose-Ignacio Angarita[2], Eugenia-Lucia Saldarriaga[2], Ingris Peláez-Ballestas[3], Alejandro Silva Espinosa[4], Ignacio Briceño-Balcázar[4], Sofia Arias-Correal[2], Jose Arias-Correal[2], Catalina Villota-Erazo[2,5], Viviana Reyes[2,5], Santiago Bernal-Macías[2,5], Mario H. Cardiel[6], John Londono[2,5]*

[1]Student from the Biosciences Programme, Faculty of Medicine and Engineering, Universidad de La Sabana, Chía, Colombia

[2]Grupo de Espondiloartropatías, Rheumatology Department, Universidad de La Sabana, Chía, Colombia

[3]Rheumatology Unit, Hospital General de México "Doctor Eduardo Liceaga", Mexico City, Mexico

[4]Grupo de Genética Humana, Universidad de La Sabana, Chía, Colombia

[5]Rheumatology Department, Hospital Militar Central, Bogotá, Colombia

[6]Centro de Investigación Clínica de Morelia SC, Morelia, Mexico

* Correspondence:

John Londono

E-mail: john.londono@unisabana.edu.co

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34   **Summary**

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36   Host immune response as well as virulence factors are key in disease susceptibility. There are

37   no known association studies of HLA class I and II alleles with chikungunya (CHIKV) infection in

38   Latin American population. We aim to identify Human Leukocyte Antigen (HLA) alleles present in

39   patients with CHIKV infection when compared to healthy controls, as well as allele association with

40   the clinical spectrum of the disease. A cross-sectional analysis nested in a community cohort was

41   carried out. We included patients 18 years and older with serological confirmation of CHIKV

42   infection. HLA typing of HLA-A, HLA-B and HLA-DRB1 alleles was performed. Two-by-two

43   tables were used to establish associations between allele presence and clinical characteristics. Data

44   from 65 patients with confirmed CHIKV infection were analyzed for HLA typing. CHIKV infection

45   was associated with the presence of HLA-A*68, HLA-B*35, HLA-DRB*01, HLA-DRB1*04 and

46   HLA-DRB1*13 alleles with statistical significance when compared to healthy subjects. A statistically

47   significant relationship was found between the presence of rash in the face or the abdomen and the

48   presence of HLA-DRB1*04. Our study demonstrated that in our cohort, HLA type I as well as type

49   II alleles are associated with CHIKV infection, and specifically an HLA type II allele with

50   dermatological symptoms. Further research is needed to set a path for future investigation on genes

51   outside the HLA system to improve knowledge in the pathophysiology of CHIKV infection and its

52   host-pathogen interaction.

53

54   **Introduction**

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56   Chikungunya virus (CHIKV) is an arbovirus transmitted by the mosquitoes *Aedes (Ae.)*

57   *aegypti* and *Ae. albopictus* (Pialoux, Gaüzère, Jauréguiberry, & Strobel, 2007). CHIKV belongs to

58   the family Togaviridae genus Alphavirus (Thiberville et al., 2013). An infected mosquito bite starts

59   the transmission infecting fibroblasts and macrophages in the dermis (Silva & Dermody, 2017).

60   Replication of the virus causes viraemia, fever, rash, myalgia, arthralgia, and arthritis (Vu, Jungkind,

61   & LaBeaud, 2017). In some cases, CHIKV infection can become chronic where rheumatic symptoms

62   can last for several months to years (Amaral et al., 2019; Vu et al., 2017).

63   The first autochthonous cases of the CHIKV Asian lineage were reported on the Island of

64   Saint Martin in 2013, leading the arrival of the virus to the Western Hemisphere (Cassadou et al.,

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65   2014). By 2015, the virus had spread rapidly to 42 countries of the Caribbean, Central and South

66   America (The Pan American Health Organization., 2015).

67   CHIKV Asian lineage arrived at northern Colombia in August of 2014, spanning through the

68   whole territory with over 100.000 reported cases within the first year using *Ae. aegypti* as the only

69   vector (Campo Carey et al., 2014; Laiton-Donato et al., 2015; Mattar et al., 2015; Pacheco et al.,

70   2017; Padilla et al., 2017; Rodas et al., 2016; Salas Botero, 2014, 2015). Even though the Colombian

71   Health Ministry declared the end of the epidemic by the end of 2015, cases continued to be reported.

72   During 2014 and 2015, the Colombian Rheumatology Association started a study to establish the

73   prevalence of rheumatic diseases in the country using the Community Oriented Program for Control

74   of Rheumatic Diseases (COPCORD) (Chopra, 2013; Chopra & Abdel-Nasser, 2008; Peláez-Ballestas

75   et al., 2011; Quintana et al., 2016). COPCORD is a low-budget, community-oriented program to

76   measure and evaluate pain and disability from rheumatic disorders in developing countries (Chopra,

77   2012, 2013). During the initial phase of the COPCORD study, a CHIKV epidemic struck Colombia

78   from August 2014 to September 2015 (Campo Carey et al., 2014; Rodriguez Reyes, 2017). Because

79   the main complaint in CHIKV is musculoskeletal (MSK) symptoms, the number of cases identified

80   by the COPCORD study increased. Therefore, CHIKV-infected patients had to be distinguished

81   within the studied population.

82   Disease susceptibility can be driven by virulence factors specific to the infective agent as well

83   as host factors like innate and adaptive immune response(Assunção-Miranda, Cruz-Oliveira, & Da

84   Poian, 2013; Kam, Ong, Rénia, Tong, & Ng, 2009; Singh, Tiwari, Mishra, Tiwari, & Dhole, 2012).

85   Regarding host factors, the HLA plays a major role in initiating immune responses HLA molecules

86   are coded on the short arm of chromosome 6, occupying a large portion of the DNA (approximately

87   3500 kilobases) mainly due to its high polymorphism (Abbas, Lichtman, Pillai, & Preceded by:

88   Abbas, n.d.). These polymorphisms allow the immune system to increase the repertoire of peptides

89   presented by HLA molecules, which in turn will affect the susceptibility to infectious diseases. There

90   is evidence that demonstrated HLA class II alleles association to susceptibility or resistance to

91   CHIKV, however none have reported associations with clinical symptoms of CHIKV infection

92   (Bouquillard & Combe, 2009; Chaaithanya et al., 2013; Thanapati, Hande, Das, Gurav, & Tripathy,

93   2014). There is a lack of evidence of association studies of HLA class I and II in the Latin-American

94   CHIKV epidemic. For this reason, we want to determine which HLA alleles are present in the group

95   of patients with positive confirmation for CHIKV infection via ELISA serology and compare to

96   healthy controls. Also, we wanted to evaluate if there is an association between HLA type I and II

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97   alleles and the presence of clinical characteristics of CHIKV in patients with confirmed CHIKV

98   infection.

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100   **Materials and Methods**

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102   *Study population*

103   This was a cross-sectional case-control analysis nested in a community cohort, including

104   patients aged >18 years, from six Colombian cities (Bogotá, Barranquilla, Cúcuta, Cali,

105   Bucaramanga and Medellín). The COPCORD uses a stratified sampling method in three stages.

106   Details on COPCORD sampling are described elsewhere (Rueda et al., 2019).

107   If a patient was positive for COPCORD and CHIKV infection was suspected, a follow-up

108   examination was conducted in the next 7 days by a trained rheumatologist or a rheumatology fellow,

109   during which chikungunya fever was confirmed according to World Health Organization (WHO)

110   criteria. Then, a specific questionnaire was administered including time of disease onset and further

111   symptoms, such as joint, dermatological and gastrointestinal manifestations (World Health

112   Organization (WHO), 2015).

113   Blood samples were also taken. Patients were evaluated only once and were excluded if the

114   examiner suspected or confirmed a rheumatic disease to avoid confusion of symptoms aetiology

115   (arthritis or arthralgia due to the novo autoimmune disease for example). The definitions of arthralgia

116   and arthritis used were taken from Woolf (Woolf, 2003).

117

118   *Case definitions for CHIKV infection according to WHO criteria* (World Health Organization

119   (WHO), 2015)

120   A case was considered suspect based on clinical criteria (acute onset of fever >38.5 ºC and

121   incapacitating joint pain) and epidemiological criteria (residing in or having visited areas that had

122   reported transmission within 15 days prior to the onset of symptoms).

123   A case was confirmed when the patient met laboratory criteria irrespective of clinical

124   presentation (presence of virus-specific IgM or IgG antibodies in a single serum sample collected in

125   the acute or convalescent stage, respectively). Because our population was immunologically naïve

126   (there were no reports of CHIKV infection prior to this epidemic) we considered as positive the

127   presence of virus-specific IgG antibodies in single serum sample during any stage of the disease.

128   CHIKV serology

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129   Enzyme-linked immunosorbent assay (ELISA) was performed according to the

130   manufacturer's instructions (Abcam® ab177848 anti-CHIKV IgM human ELISA kit and ab177835

131   anti-CHIKV IgG human ELISA kit, Abcam, Cambridge, UK). Abcam's anti-CHIKV IgM Human

132   ELISA kit is reported to produce comparable results to the Centers for Disease Control and

133   Prevention (CDC) IgM ELISA (CDC, 2014; Johnson et al., 2016).   Analytical specifications

134   according to manufacturer states a specificity >90% and sensitivity >90% for both IgM and IgG anti-

135   CHIKV.

136   No cross-reactivity against Bordetella pertussis, Chlamydia trachomatis, Chlamydia

137   pneumoniae, dengue virus, tick-borne encephalitis (TBE), Helicobacter pylori, herpes simplex virus

138   2, Leishmania, Mycoplasma, or Schistosoma has been reported for IgM. No cross-reactivity against

139   dengue virus, TBE, cytomegalovirus, Epstein-Barr virus, or Helicobacter pylori has been reported for

140   IgG. The manufacturer reports a 10% rate of misclassification with IgG serology. In our cohort of

141   positive IgG patients with possible CHIKV infection, 10% represents about 5 patients, which is not

142   statistically relevant and therefore does not affect our results.

143

144   *HLA typing*

145   Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes for

146   extraction of deoxyribonucleic acid (DNA) using the commercial Wizard Genomic DNA Purification

147   kit (reference PROMEGA® trademark A1120) according to merchant specifications. After extraction

148   of DNA, concentration was adjusted to 80 ± 20 μg/μl, electrophoresis was performed to verify the

149   DNA, and then stored at -80 °C until processing of HLA.

150   Typing was performed using single specific primer-polymerase chain reaction (SSP-PCR)

151   commercial kit from Biotest® HLA-mark SSPtray ABDR Bio- Rad®. It contains a plate with 96

152   wells which determines alleles A, B, and β1 chain within the DR. The PCR product was verified by

153   gel electrophoresis in 2% agarose, bands were visualized by ultraviolet light, and photo

154   documentation was analyzed later by the Bio-Rad® program SSP HLA Typing V 1.2.0.0 Software

155   Medical Bio-Rad® Diagnostics GmbH.

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157   *Control population*

158   As controls, we use 100 unrelated healthy individuals from the same geographic regions, and

159   ethnicity of the patients. Controls were matched by gender and age. Control population had no

160   history or symptoms of rheumatic disease or infectious disease and were randomly ascertained from a

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161   list of transplant donors. The control population was obtained 2 years prior to the CHIKV epidemic

162   in Colombia.

163

164   *Statistical analysis*

165   Descriptive analysis was made using means and standard deviation (SD) for continuous

166   variables and count and percentages for categorical variables. Two by two tables were used to

167   establish associations between categorical variables. Odds ratios (OR) were calculated for

168   associations with 95% confidence intervals (CI), and Student's t-test used to compare means,

169   regarding $p < 5\%$ as statistically significant. Allele frequency (AF) among the patient population was

170   calculated using Genepop program on-line version (Rousset, 2008). The comparison between the

171   different allele frequencies in the patient group and the control population was performed using *Chi^2*,

172   with Bonferroni correction. Hardy-Weinberg equilibrium was tested using Arlequin® statistical

173   software version 3.5. SPSS version 22.0 (IBM, Armonk, NY, USA) was used for data analysis. To

174   validate the statistically significant allele frequencies a power calculation was made.

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176   *Ethical considerations*

177   This study was carried out according to the Declaration of Helsinki 2013. Informed consent

178   was obtained, prior to the patients' admission. The study was approved by the ethics committee from

179   La Universidad de La Sabana (study approval MED-197-2015) and the Hospital Militar Central

180   (study approval 106-2016).

181

182   **Results**

183

184   548 (8.4%) were included in our study as suspected for CHIKV infection from the the 6528

185   people surveyed in the COPCORD study. Of those, 295 (53.8%) were positive for CHIKV IgG or

186   IgM, fulfilling the WHO criteria for confirmed CHIKV infections. 65 were analyzed for HLA typing.

187

188   *Demographics, clinical characteristics, and disability*

189   Most patients were female, mestizo, with a median age of 45.2 years, living in 1 and 2 strata.

190   The socio-economic variable is in relation with a social stratification system implemented by law in

191   Colombia in 1990, where urban populations are classified into different strata with similar economic

192   characteristics (scale from 1 to 6, being 1 the lowest income and 6 the highest) ("Colombia - Social

193   Stratification by Law | Ifhp.Org," n.d.). Details are depicted in Table 1.

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194   Patients were characterized by presence of symmetric arthralgia (n: 60; 92.3%) of knees (n:

195   45; 69.2%) and hands (n: 39; 60.0%), symmetric arthritis (n: 31; 47.7%) of hands (n: 17; 26.2%) and

196   feet (n: 17; 26.2%), fatigue (n: 56; 86.2%), fever (n: 50; 76.9%), myalgia (n: 43; 66.2%) of the

197   extremities (n: 32; 49.2%), pruritic (n: 28; 43.1%) rash (n: 41; 63.1%) on the face (n: 28; 43.1%), and

198   diarrhea (n: 21; 32.3%) (Fig 1).

199   According to the health assessment questionnaire disease index (HAQ-DI), most patients

200   described mild to moderate disability (n: 37; 56.9%), while visual analogue scale (VAS) on pain and

201   disability were referred as severe (n: 25; 38.5% and n: 36; 55.4% respectively). See Table 2 for

202   details.

203   Most patients had no problems in all dimensions (mobility, self-care, usual activities, anxiety/

204   depression) from the EuroQol 5 dimensions 3 levels (EQ-5D-3L) disability scores, except in

205   pain/discomfort where most patients had some problems (Fig 2).

206

207   *Allele frequencies and clinical associations*

208   The HLA-A alleles A*02 (n: 25; 38.5%), A*24 (n: 21; 32.3%), and A*68 (n: 10; 15.4%) were

209   the most frequent in the patients' group, being the latter, the only allele associated with CHIKV

210   infection (p=0.005; OR: 8.90, CI: 1.88- 42.13) when compared to healthy subjects (Table 3). HLA

211   A*29 was a protective factor for CHIKV infection (p=0.002; OR: 0.10, CI: 0.02-0.44).

212   B*35 was the most frequent HLA-B allele in CHIKV infected patients (47.7%; n: 31) with

213   statistically significant association (p=0.03; OR: 2.01, CI: 1.06-3.86) (Table 4).

214   The most common HLA-DRB1 allele in CHIKV infected patients was DRB1*04, followed

215   by DRB1*13 and DRB1*01, all of which showed association with CHIKV infection (see Table 5 for

216   details). The power of the study according to the allele frequency were 86%, 55%, 92%, 100% and

217   81% for A*68, B*35, DRB1*01, DRB1*04 and DRB1*13, respectively.

218   When evaluating the association between symptoms of CHIKV infection and the frequency of

219   HLA alleles, only the presence of rash on the face and abdomen was associated with HLA-DRB1*04.

220   In figure 3 are depicted the 2 most frequent alleles in CHIKV infected population.

221   Haplotype frequencies were calculated; however, no differences were found when comparing

222   CHIKV infected patients with controls.

223   There was no association between the presence of frequent alleles in the HLA-studied locus

224   and the disability scales.

225

226   **Discussion**

227

228   Our study demonstrated the presence of five HLA class I and II alleles in CHIKV infected

229   patients when compared to healthy subjects. Specifically, HLA-A*68, HLA-B*35, HLA-DRB1*01,

230   HLA-DRB1*04 and HLA-DRB1*13. Of interest, HLA-A*68 and HLA-DRB1*04 had the strongest

231   association with CHIKV infection. In fact, HLA-DRB1*04 was the only allele associated with the

232   presence of rash in the abdomen and the face. No other alleles were statistically associated with other

233   clinical symptoms or disability scores. Only class I alleles (HLA-A*29 and HLA-B*46) were

234   associated with reduced risk of CHIKV infection as well as rash in the abdomen and the face (HLA-

235   B*35). None of the HLA-DRB1 alleles showed protection for CHIKV infection.

236   To our knowledge, in Latin American population there are no studies associating the presence of

237   HLA class I and II alleles with CHIKV infection. In India, two studies have reported the association

238   of HLA alleles with CHIKV infection (Chaaithanya et al., 2013; Thanapati et al., 2014). One study

239   demonstrated the presence of HLA-DRB1*04-HLA-DQB1*03 haplotype with susceptibility to

240   CHIKV infection, and the presence of HLA-DRB1*11 and HLA-DRB1*11-HLA-DQB1*03

241   haplotype with resistance to the infection (Thanapati et al., 2014). The other study reported a lower

242   frequency of HLA-DQB1*03:03 allele in CHIKV infected patients with statistical significance when

243   compared to normal subjects (Chaaithanya et al., 2013). In this study, although HLA-DRB1*01 and

244   HLA-DRB1*04 were more frequent in CHIKV infected patients, the difference was not statistically

245   significant. Interestingly enough, in 21 patients from Reunion Island with chronic CHIKV infection

246   who developed rheumatoid arthritis (RA) after the infection, HLA-DRB1*01 and DRB1*04 alleles

247   were frequently found (Bouquillard & Combe, 2009). Taking this into account, special interest

248   should be given to research of HLA-DRB1 alleles in CHIKV infected patients and its role in the

249   pathophysiology of the disease.

250   Other studies have shown association of alleles like those found in our study and other

251   infectious diseases. For example, in Poland, researchers found that the presence of HLA-DRB*13

252   was protective against pulmonary tuberculosis (Dubaniewicz, 2000). Severe and non-severe cerebral

253   malaria was associated with an increased frequency of HLA-B*46 in Thai patients (Hananantachai et

254   al., 2005). In Mexico, HLA-DRB1*04:07 was associated with susceptibility to localized cutaneous

255   leishmaniasis (Olivo-Díaz et al., 2004). Also, Mexican patients exhibited an increased frequency of

256   HLA-B*35 when compared to healthy controls suggesting susceptibility to Chagas cardiomyopathy,

257   and lower frequencies of HLA-A*68 implying protection (Cruz-Robles, Reyes, Monteón-Padilla,

258   Ortiz-Muñiz, & Vargas-Alarcón, 2004). A study found significant increase of HLA-DRB1*01-

259   DQB1*05:01 haplotype in Chagas cardiomyopathy from Venezuelan patients (Colorado, Acquatella,

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260   Catalioti, Fernandez, & Layrisse, 2000). Regarding viral infections, researchers found a trend toward

261   increased early HIV-1 infection in infants presenting HLA-A*29 via breast milk (Farquhar et al.,

262   2004). In another study, hepatitis C clearance was found to associated with HLA-DRB1*01:01 and

263   HLA-DRB1*04:01 after a multiple logistic regression analysis (McKiernan et al., 2004).

264   The most amount of research in the role of HLA and disease has been made in autoimmune

265   diseases. A meta-analysis in Latin American population found risk associations between HLA-

266   DRB1*04 and type 1 diabetes (T1D), HLA-DRB1*01:01 and *04:04 and RA; and HLA-

267   DRB1*13:01 and autoimmune hepatitis (AIH) (Rojas et al., 2010). Moreover, some alleles confer

268   risk for more than one autoimmune disease. For example, HLA-DRB1*04:05 was associated with

269   increased risk for RA, AIH and T1D. HLA type I alleles has also been found to be associated with

270   autoimmune diseases. A study in 49 patients with birdshot retinochoroidopathy found the presence

271   of HLA-A*29 in 95.5% (Baarsma, Priem, & Kijlstra, 1990). In Japanese patients with psoriatic

272   arthritis, the presence of HLA-B*46 was associated with severe sacroiliitis (Ikumi et al., 2018), while

273   Kawasaki disease was associated with increased frequencies of HLA-B*35 in Korean children (Oh et

274   al., 2008). Also in Korea, a study found association between HLA-B*35 and Moyamoya disease

275   (Han et al., 2003).

276   The relationship between host genetics and disease can be variable. The presence of an allele

277   like HLA-DRB1*13 is associated with CHIKV infection (susceptibility), on the other hand, the same

278   allele confers protection for AIH. On the contrary, while HLA-A*29 and B*46 protect against

279   CHIKV infection, they are associated with birdshot retinochoroidopathy and severe sacroiliitis in

280   psoriatic arthritis, respectively. To explain the relationships between HLA, autoimmune and

281   infectious diseases, two hypotheses have been proposed (Matzaraki, Kumar, Wijmenga, &

282   Zhernakova, 2017). The first hypothesis affirms that pathogen pressure on the human genome has led

283   to selection of host defense genes that protects against infections. However, this advantageous

284   selection may also increase the risk of developing autoimmune diseases. The second hypothesis

285   suggests that pathogens can trigger autoimmunity by molecular mimicry, epitope spreading bystander

286   activation or cryptic antigens (Ercolini & Miller, 2009).

287   Studies on host's genetics and CHIKV infection have included other genes aside the HLA

288   system. Researchers in India reported that single nucleotide polymorphisms (SNPs) in OAS gene

289   cluster that codes for various oligoadenylate synthetases, influenced the risk of developing clinical

290   symptoms in CHIKV infected patients (Chaaithanya et al., 2016). A study on CHIKV-infected

291   patients belonging to multiple ethnicities reported that a noncoding SNP in the toll like receptor 3

292   (TLR3) gene, rs6552950, is associated with disease severity and CHIKV specific neutralizing

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293 antibody response (Her et al., 2015). Also, three polymorphisms of TLR7 (rs179010, rs5741880,

294 rs3853839) and one in TLR8 (rs3764879) were significantly associated with CHIKV infection in a

295 cohort in India. In that study, the polymorphisms of TLR7 had higher risk of developing fever, rash,

296 joint pain and higher levels of interferon α (Dutta & Tripathi, 2017). These compelling evidence

297 proofs the complexity between the host's immune response and genetic factors and infectious

298 diseases like CHIKV infection.

299   Our study has limitations due to the small sample size and inability to perform DQ typing.

300 Therefore, our results should be interpreted with caution. A small sample can decrease the possibility

301 of other associations that could potentially be significant if the sample is larger. On the other hand,

302 some statically significant associations can lose power due to smaller sample. In our cohort, this was

303 the case of HLA-B*35 in patients with CHIKV. Although a statically significant association was

304 found due to a small sample size, the power of the study did not reach the expected 80%. This can be

305 explained by the high frequency of HLA-B*35 in Latin American populations, where in Mexico and

306 Peru frequencies ranges between 33.8%, and 34.4%, while in other populations like Mauritanian

307 (8.6%), and Sri Lankan (10.9%) frequencies are lower (De Pablo et al., 2000; Falcón-Lezama et al.,

308 2009; Hamed et al., 2018; Malavige et al., 2011). Also, patients were not followed in time to

309 establish symptoms persistence, chronic CHIKV and the presence of the HLA alleles. This would

310 help elucidate the role of host genetic factors in the pathophysiology of chronic CHIKV disease.

311 Nevertheless, and despite the aforementioned, we were able to identify susceptibility and protection

312 for CHIKV infection in a Latin American cohort.

313

314 **Conclusion**

315   Our study demonstrated the role of HLA alleles in the risk and protection for CHIKV

316 infection and clinical symptoms in a Latin American cohort. Our results suggest the need of further

317 research and set a path for future investigation on genes outside the HLA system to help elucidate the

318 pathophysiology of the CHIKV infection as well as its interaction with its host.

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320

321 **Author Contributions**

322   JCR, AMS, JIA, ELS, IPB, ASE, IBB, SAC, JAC, VR, CVE, SBM, MHC, JL:

323 conceptualization, supervision, funding acquisition. methodology, data curation, writing-original

324 draft preparation, and writing-review and editing.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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545 **Tables**

Table 1. Demographic features of CHIKV infected patients

Features	Patients (n=65)	Controls (n=100)
Mean age in years (SD)	45.22 ± 16.6	46.40 ± 18.4
Females	46 (70.8%)	64 (64.0%)
Education level		
Basic	23 (35.4%)	30 (30.0%)
Medium	22 (33.8%)	43 (43.0%)
Technical	10 (15.4%)	15 (15.0%)
Professional	7 (10.8%)	9 (9.0%)
None	3 (4.6%)	3 (3.0%)
Ethnicity		
Mestizo	34 (52.3%)	51 (51.0%)
Caucasian	26 (40.0%)	38 (38.0%)
Afro-American	4 (6.2%)	8 (8.0%)
Amerindian	1 (1.5%)	3 (3.0%)
Socioeconomic strata		
Stratum 1	24 (36.9%)	32 (32.0%)
Stratum 2	22 (33.8%)	28 (28.0%)
Stratum 3	13 (20.0%)	20 (20.0%)
Stratum 4	4 (6.2%)	18 (18.0%)
Stratum 5	2 (3.1%)	2 (2.0%)

CHIKV: chikungunya virus

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Table 2. Disability of CHIKV infected patients

	Patients (n=65)
HAQ-DI (mean, SD)	0.21 ± 0.47
Without disability	22 (33.8%)
Mild to moderate disability	37 (56.9%)
Moderate to severe disability	4 (6.2%)
Severe to very severe disability	2 (3.1%)
Pain VAS (mean, SD)	52.92 ± 33.48
Without pain	16 (24.6%)
Mild pain	10 (15.4%)
Moderate pain	14 (21.5%)
Severe pain	25 (38.5%)

CHIKV: chikungunya virus; HAQ-DI: health assessment questionnaire disease index; VAS: visual analogue scale

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Table 3. Allele frequencies of HLA A in CHIKV and healthy controls

Allele	CHIKV (n: 65)	Controls (n: 100)	Allele frequency		OR (CI)	*p*	BC *p*
			CHIKV	Controls			
A							
*01	12 (18.5)	12 (12.0%)	0.092	0.071			
*02	25 (38.5%)	41 (41.0%)	0.200	0192			
*03	10 (15.4%)	7 (7.0%)	0.083	0.045			
*10	1 (1.5%)	0 (0.0%)	0.008	0.000			
*11	5 (7.7%)	4 (4.0%)	0.042	0.026			
*23	5 (7.7%)	12 (12.0%)	0.042	0.051			
*24	21 (32.3%)	28 (28.0%)	0.167	0.167			
*25	5 (7.7%)	8 (8.0%)	0.033	0.019			
*26	1 (1.5%)	9 (9.0%)	0.008	0.038			
*28	0 (0.0%)	11 (11.0%)	0.000	0.045	0.05 (0.003-1.02)	0.002	0. 052
*29	2 (3.1%)	24 (24.0%)	0.017	0.141	0.10 (0.02-0.44)	<0.001	0.002
*30	6 (9.2%)	11 (11.0%)	0.050	0.071			
*31	9 (13.9%)	8 (8.0%)	0.075	0.051			
*32	2 (3.1%)	5 (5.0%)	0.017	0.032			
*33	2 (3.1%)	3 (3.0%)	0.008	0.019			
*34	2 (3.1%)	0 (0.0%)	0.017	0.000			
*36	1 (1.5%)	1 (1.0%)	0.008	0.006			
*43	2 (3.1%)	0 (0.0%)	0.017	0.000			
*66	0 (0.0%)	1 (1.0%)	0.000	0.006			
*68	10 (15.4%)	2 (2.0%)	0.083	0.006	8.90 (1.88-42.13)	<0.001	0.005
*74	4 (6.1%)	0 (0.0%)	0.033	0.000			
*80	0 (0.0%)	1 (1.0%)	0.000	0.006			

HLA: human leukocyte antigen; CHIKV: chikungunya virus; OR: odds ratio; CI: 95% confidence interval; BC: Bonferroni correction

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Table 4. Allele frequencies of HLA B in CHIKV and healthy controls

Allele	CHIKV (n: 65)	Controls (n: 100)	Allele frequency		OR (CI)	*p*	BC *p*
			CHIKV	Controls			
B							
*07	10 (15.4%)	11 (11.0%)	0.080	0.065			
*08	7 (10.8%)	6 (6.0%)	0.045	0.029			
*13	0 (0.0%)	2 (2.0%)	0.000	0.006			
*14	1 (1.5%)	5 (5.0%)	0.009	0.029			
*15	6 (9.2%)	2 (2.0%)	0.054	0.012			
*17	1 (1.5%)	0 (0.0%)	0.009	0.000	4.98 (0.97-25.49)	0.001	0. 053
*18	5 (7.7%)	6 (6.0%)	0.045	0.035			
*27	1 (1.5%)	7 (7.0%)	0.000	0.041			
*35	31 (47.7%)	31 (31.0%)	0.250	0.159	2.02 (1.06-3.86)	0.001	0.03
*38	6 (9.2%)	8 (8.0%)	0.045	0.047			
*39	5 (7.7%)	8 (8.0%)	0.036	0.047			
*40	11 (17.0%)	14 (14.0%)	0.098	0.065			
*41	1 (1.5%)	1 (1.0%)	0.009	0.006			
*42	3 (4.6%)	3 (3.0%)	0.027	0.018			
*44	6 (9.2%)	28 (28.0)	0.054	0.159			
*46	1 (1.5%)	1 (1.0%)	0.009	0.006	0.26 (0.10-0.67)	<0.001	0.005
*47	1 (1.5%)	2 (2.0%)	0.009	0.012			
*48	5 (7.7%)	0 (0.0%)	0.045	0.000			
*49	3 (4.6%)	11 (11.0%)	0.027	0.065	18.27 (0.99-336.31)	0.001	0.050
*50	2 (3.1%)	0 (0.0%)	0.018	0.000			
*51	8 (12.3%)	12 (12.0%)	0.071	0.071			
*52	1 (1.5%)	5 (5.0%)	0.009	0.029			

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*53	1 (1.5%)	2 (2.0%)	0.009	0.012
*54	0 (0.0%)	1 (1.0%)	0.000	0.006
*55	2 (3.1%)	1 (1.0%)	0.018	0.006
*57	1 (1.5%)	4 (4.0%)	0.009	0.012
*58	1 (1.5%)	4 (4.0%)	0.009	0.024
*64	1 (1.5%)	0 (0.0%)	0.009	0.000

HLA: human leukocyte antigen; CHIKV: chikungunya virus; OR: odds ratio; CI: 95% confidence interval; BC: Bonferroni correction

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Table 5. Allele frequencies of HLA DRB1 in CHIKV and healthy controls

Allele	CHIKV (n: 65)	Controls (n: 100)	Allele frequency		OR (CI)	*p*	BC *p*
			CHIKV	Controls			
DR							
*01	15 (23.1%)	5 (5.0%)	0.127	0.050	5.70 (1.95-16.59)	<0.001	0.001
*03	11 (17.0%)	8 (8.0%)	0.085	0.100			
*04	31 (47.7%)	11 (11.0%)	0.237	0.138	7.37 (3.33-16.30)	<0.001	<0.001
*07	13 (20.0%)	11 (11.0%)	0.102	0.113			
*08	8 (12.3%)	14 (14.0%)	0.068	0.175			
*09	1 (1.5%)	1 (1.0%)	0.008	0.013			
*10	2 (3.1%)	2 (2.0%)	0.017	0.013			
*11	3 (4.6%)	4 (4.0%)	0.025	0.037			
*12	3 (4.6%)	3 (3.0%)	0.025	0.037			
*13	16 (24.7%)	8 (8.0%)	0.127	0.087	3.75 (1.50-9.39)	<0.001	0.004
*14	9 (13.9%)	5 (5.0%)	0.076	0.062	3.05 (0.97-9.56)	0.004	0.055
*15	8 (12.3%)	12 (12.0%)	0.068	0.150			
*16	4 (6.1%)	2 (2.0%)	0.034	0.025			

HLA: human leukocyte antigen; CHIKV: chikungunya virus; OR: odds ratio; CI: 95% confidence interval; BC: Bonferroni correction

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564   Figures
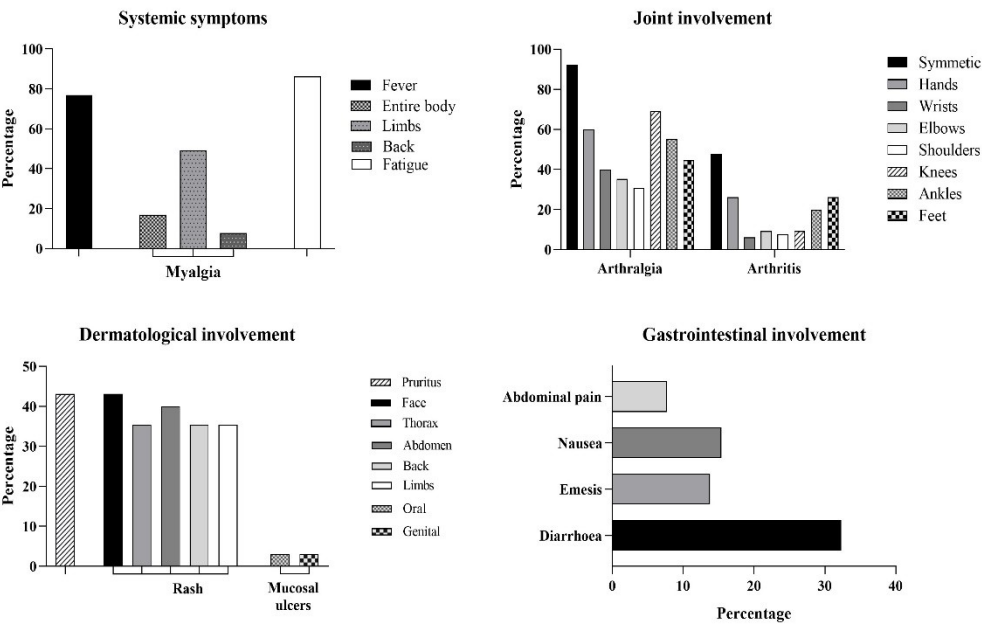

Figure 1. Clinical features of CHIKV infected patients
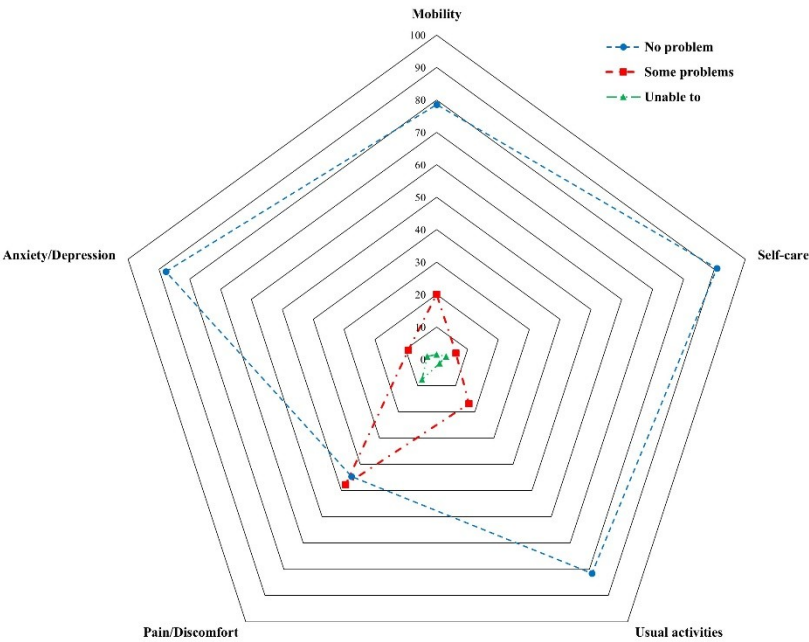CHIKV: chikungunya virus

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Figure 2. EQ-5D-3L in CHIKV infected patients
EQ-5D-3L: EuroQol 5 dimensions 3 levels; CHIKV: chikungunya virus

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Figure 3. HLA alleles and CHIKV symptoms

HLA B*35

HLA DRB1*04

CHIKV: chikungunya virus; HLA: Human leukocyte antigen; NS: not significant

	n (%)	Odds ratio (95% Confidence interval)	p value
Joint involvement			
Arthralgia	30 (50.0)	5.35 (0.24-116.31)	NS
	27 (43.5)	0.81 (0.04-13.70)	NS
Arthritis	13 (21.7)	0.51 (0.18-1.42)	NS
	14 (22.6)	1.00 (0.36-2.71)	NS
Systemic symptoms			
Fever	23 (38.3)	1.00 (0.30-3.30)	NS
	21 (33.9)	0.92 (0.28-2.96)	NS
Myalgia	18 (30.0)	0.54 (0.18-1.62)	NS
	18 (29.0)	0.75 (0.25-2.18)	NS
Fatigue	26 (43.3)	1.00 (0.22-4.43)	NS
	23 (37.1)	0.61 (0.14-2.54)	NS
Dermatological involvement			
Rash			
Face	**9 (15.0)**	**0.28 (0.09-0.83)**	**0.02**
	16 (25.8)	**3.20 (1.11-9.15)**	**0.02**
Thorax	8 (13.3)	0.41 (0.14-1.22)	NS
	12 (19.4)	2.08 (0.71-6.06)	NS
Abdomen	**8 (13.3)**	**0.27 (0.09-0.82)**	**0.01**
	16 (25.8)	**4.33 (1.45-12.88)**	**0.007**
Back	8 (13.3)	0.41 (0.14-1.22)	NS
	12 (19.4)	2.08 (0.71-6.06)	NS
Limbs	7 (11.7)	0.45 (0.14-1.39)	NS
	11 (17.7)	1.55 (0.53-4.47)	NS
Mucosal ulcers	2 (3.3)	2.07 (0.17-24.14)	NS
	2 (3.2)	2.53 (0.21-29.56)	NS
Gastrointestinal involvement	11 (18.3)	0.75 (0.26-2.13)	NS
	12 (19.4)	1.21 (0.43-3.35)	NS

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