Variability at the 3’ untranslated region of the HLA-G gene: a study on patients with AIDS and cytomegalovirus retinochoroiditis

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Cytomegalovirus retinochoroiditis (CMV-R) is the primary cause of blindness among AIDS patients. Since HLA-G is associated with the modulation of the immune response, we hypothesized that variability at the 3’ untranslated region (3’ UTR) of the gene could be implicated in the predisposition to CMV-R. We evaluated whether HLA-G 3’ UTR influences CMV-R development in Brazilian AIDS patients. Peripheral blood DNA was obtained from two groups of patients: (1) AIDS exhibiting CMV-R (n = 40) and (2) AIDS without CMV-R (n = 147). HLA-G 3’ UTR typing was performed using sequencing analysis. Allele, genotype and haplotype frequencies were evaluated using Fisher’s exact test accompanied by the calculation of the odds ratio and its 95% confidence interval (95% CI). The etiologic (EF) and preventive fractions were also estimated. Compared to AIDS patients without CMV-R, AIDS patients with CMV-R showed increased frequencies of the: (1) +3001T allele, (2) the +3001C/T genotype and (3) the UTR-17 (InsTTCGTCGACG) haplotype (EFs = 0.02–0.04). The UTR-3 (DelTCCGACG) haplotype was associated with protection against CMV-R development. Although the risk for developing CMR-V at the population level was relatively low (EF), the identification of HLA-G 3’ UTR variation sites may help to further evaluate the role of post-transcriptional factors that may contribute to the existent immunosuppression caused by HIV per se.

Cytomegalovirus retinochoroiditis (CMV-R) primarily affects patients with AIDS exhibiting CD4 cell counts <50 cells/mm3. Although the frequency of CMV-R has been decreased due to the universal access of patients with AIDS to the combination antiretroviral therapy (cART), retinochoroiditis continues to be an important cause of severe visual loss, particularly in countries where cART is not freely available1. Thus, the identification of AIDS patients prone to develop CMV-R, the early diagnosis and early treatment may precipitate a reduction on the number of years of blindness in patients whose survival is favored by cART2.

Considering that HLA-G has a well recognized immunodulatory role, inhibiting the cytotoxic effects of CD8 and natural killer (NK) lymphocytes3, there is a clear possibility of exploring this gene in the search for innovative immunoregulatory strategies for diagnostic and therapeutic strategies in various conditions, among them AIDS and CMV-R.

Advances in gene sequencing have shown several variation sites along the HLA-G coding and regulatory regions, which may affect protein diversity and gene expression. Variation sites at the HLA-G promoter or 3’ untranslated region (3’ UTR) may affect transcriptional and post-transcriptional gene control by modifying target sites for transcription factors and microRNAs, respectively4. In the Brazilian population, at least 37 single

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nucleotide polymorphisms (SNP) at the promoter and at least 16 variation sites at 3′ UTR have been described for the HLA-G gene.

Several polymorphisms at the HLA-G 3′ UTR segment can influence gene expression. The insertion/deletion (Ins/Del) of 14 base pairs is the best-characterized polymorphism described at 3′ UTR, which has been associated with mRNA stability. The +3142 G/C SNP has also been reported to post-transcriptionally control HLA-G expression, since the presence of a Guanine may increase the affinity of the primary mRNA for the microRNAs (miR)-148a, miR-148b and miR-152. The +3187 A/G polymorphic site is located at the proximity of an AU-rich motif, which also affects mRNA stability. Little information is available regarding other variation sites at HLA-G 3′ UTR, including +3001 T/C, +3003 T/C, +3010 C/G, +3027 C/A, +3035 C/T and +3196 C/G, which are potential binding sites for miRNAs, and thus may post-transcriptionally regulate HLA-G expression.

Since the variation sites at the HLA-G 3′ UTR are in linkage disequilibrium, several 3′ UTR haplotypes have been described, of which at least 15 haplotypes are observed in Brazilians.

Although studies regarding the classical HLA class I and class II profiles of patients with AIDS exhibiting ocular manifestations have been reported so far, there is no information regarding the study of the non-classical HLA-G gene in patients exhibiting both AIDS and CMV-R. The present study was undertaken based on the hypothesis that variation sites at the HLA-G 3′ UTR in patients with AIDS exhibiting or not CMV-R may discriminate variation sites associated with the regulation of the immune checkpoint gene.

### Results

HLA-G 3′ UTR variability was assigned on the basis of the following polymorphic sites, including: 14bp INS/Del (rs371194629) and eight variable sites: +3001 C/T (rs567747015), +3003 C/T (rs1707), +3010 C/G (rs1710), +3027 A/C (rs1719101), +3035 C/T (17179108), +3142 C/G (rs1063320), +3187 A/G (9380142), +3196 C/G (rs1610696).

The frequency of the HLA-G 3′ UTR alleles is shown in Table 1. Only the +3001 T allele was overrepresented in CMV-R AIDS patients compared to AIDS without CMV-R (P = 0.046, OR 18.631 (95% CI 0.885–392.043) and an Etiologic Fraction = 0.024. The +3001C/T genotype frequencies are shown in Table 2. The +3001CC was underrepresented in CMV-R AIDS patients compared to AIDS without CMV-R (P = 0.045, OR 0.053, 95% CI 0.002–0.117) conferring a Preventive Fraction = 0.945, whereas the +3001CT was overrepresented in comparison (P = 0.045, OR 19.026, 95% CI 0.895–404.579, conferring an Etiologic Fraction = 0.047).

Regarding the 3′ UTR haplotypes, the frequency of the UTR-17 (InsTTC CGT GACG) haplotype is higher in patients with AIDS who developed CMV-R compared with patients with AIDS without CMV-R (P = 0.046), conferring an OR 18.557 (95% CI 0.881–390.713) and an Etiologic Fraction = 0.025. In contrast, the UTR-3 (DelTCC GCG ACG) haplotype was underrepresented in CMV-R patients when compared to controls (P = 0.014), conferring an OR 0.285 (95% CI 0.099–0.820) and a Preventive Fraction = 0.116 (Table 3).

### Table 1. Frequency and absolute number of the HLA-G 3′ UTR alleles observed in patients with AIDS exhibiting CMV retinitis (CMV-R: Group I) and in patients with AIDS without CMV-R (Group II).

| HLA-G position | Allele | Group I (N = 40) | Group II (N = 147) | Odds ratio | Etiologic fraction | P value |
|----------------|--------|-----------------|-------------------|------------|------------------|---------|
| +2960 (14 bp)  | Ins    | 0.3750 (30)     | 0.3741 (110)      |            |                  |         |
|                | Del    | 0.6250 (50)     | 0.6259 (184)      |            |                  |         |
| +3001C/T       | C      | 0.9750 (78)     | 1.0000 (292)      |            |                  |         |
|                | T      | 0.0250 (2)      | 0.0000 (0)        | 18.631 (95% CI 0.885–392.043) | 0.024 | 0.046 |
| +3003C/T       | C      | 0.1625 (13)     | 0.1054 (31)       |            |                  |         |
|                | T      | 0.8375 (67)     | 0.8946 (263)      |            |                  |         |
| +3010C/G       | C      | 0.4625 (37)     | 0.5510 (162)      |            |                  |         |
|                | G      | 0.5375 (43)     | 0.4490 (132)      |            |                  |         |
| +3027C/A       | C      | 0.9750 (78)     | 0.9524 (280)      |            |                  |         |
|                | A      | 0.0250 (2)      | 0.0476 (14)       |            |                  |         |
| +3032C/G       | C      | 0.0125 (1)      | 0.0034 (1)        |            |                  |         |
|                | G      | 0.9875 (79)     | 0.9966 (293)      |            |                  |         |
| +3035C/T       | C      | 0.8875 (71)     | 0.8810 (259)      |            |                  |         |
|                | T      | 0.1125 (9)      | 0.1190 (35)       |            |                  |         |
| +3142C/G       | C      | 0.5385 (42)     | 0.4490 (132)      |            |                  |         |
|                | G      | 0.4615 (36)     | 0.5510 (162)      |            |                  |         |
| +3187A/G       | A      | 0.7250 (58)     | 0.6837 (201)      |            |                  |         |
|                | G      | 0.2750 (22)     | 0.3163 (93)       |            |                  |         |
| +3196C/G       | C      | 0.7179 (56)     | 0.7109 (209)      |            |                  |         |
|                | G      | 0.2821 (22)     | 0.2891 (85)       |            |                  |         |
| +3227A/G       | A      | 0.0405 (3)      | 0.0429 (6)        |            |                  |         |
|                | G      | 0.9595 (71)     | 0.9571 (134)      |            |                  |         |
Due to the systemic immunodepression, patients with AIDS are susceptible to opportunistic pathogens, and one of the most frequent is CMV that, in approximately 85% of cases, induces CMV-R, particularly in patients presenting other risk factors (e.g. CD4+ cells count < 50). Despite the broad availability of cART in many countries, 4.9–7.6% of the patients are believed to develop CMV-R during some evolution stages of AIDS20–24. The individual variability regarding the time of onset and the rate of progression of CMV-R suggest that genetic factors such as histocompatibility alleles and HLA specific haplotypes may be involved in disease susceptibility. The study of genetic susceptibility in infectious diseases usually poses a challenge because most studies compare infected patients with healthy controls, for whom one cannot predict whether or not the individual would be susceptible to or resistant to the infectious agent. In this study, both groups were infected by HIV and developed AIDS, and just one developed CMV-R. Noteworthy, the CMV infection is present in 90% of the healthy Brazilian individuals24 and in about 98% of the patients with AIDS25. Because the previous contact with CMV in Brazilian patients with AIDS is extremely frequent, the development of CMV-R was the dependable variable. Considering that low CD4 cell count itself propitiate an immunosuppressive condition to develop CMV-R17, and also considering that HLA-G has immunosuppressive properties, the study of the regulatory region of the HLA-G gene is of relevance to predict the differential chance of ocular involvement in patients with AIDS. No literature data have been reported regarding the variability at the 3′UTR of the HLA-G gene in patients with AIDS, exhibiting or not CMV-R. Noteworthy, in the present series, just one variable site was associated with CMV-R when this group of AIDS patients was compared with AIDS patients without CMV-R. Accordingly, the +3001T allele, the +3001C/T genotype, and the UTR-17 (InsTTC CGT GACG) haplotype contains the +3001T

| HLA-G position | Genotype | Group I (N = 40) | Group II (N = 147) | Odds ratio | Etiologic fraction | Preventive fraction | P value |
|---------------|----------|-----------------|-------------------|------------|--------------------|--------------------|---------|
| +2960 (14bp)  | DD       | 0.3750 (15)     | 0.3946 (58)       |            |                    |                    |         |
|               | DI       | 0.5000 (20)     | 0.4626 (68)       |            |                    |                    |         |
|               | II       | 0.1250 (5)      | 0.1429 (21)       |            |                    |                    |         |
| +3001C/T      | CC       | 0.9500 (38)     | 1.0000 (146)      | 0.053 (95% CI 0.002–1.117) | –                   | 0.945               | 0.045   |
|               | CT       | 0.0500 (2)      | 0.0000 (0)        | 19.026 (95% CI 0.895–404.579) | 0.047               | –                   | 0.045   |
|               | TT       | 0.0000 (0)      | 0.0000 (0)        |            |                    |                    |         |
| +3003C/T      | CC       | 0.0250 (1)      | 0.0068 (1)        |            |                    |                    |         |
|               | CT       | 0.2750 (11)     | 0.1973 (29)       |            |                    |                    |         |
|               | TT       | 0.7000 (28)     | 0.7959 (117)      |            |                    |                    |         |
| +3010C/G      | CC       | 0.2000 (8)      | 0.2857 (42)       |            |                    |                    |         |
|               | CG       | 0.5250 (21)     | 0.5306 (78)       |            |                    |                    |         |
|               | GG       | 0.2750 (11)     | 0.1837 (27)       |            |                    |                    |         |
| +3027C/A      | AA       | 0.0000 (0)      | 0.0000 (0)        |            |                    |                    |         |
|               | AC       | 0.0500 (2)      | 0.0952 (14)       |            |                    |                    |         |
|               | CC       | 0.9500 (38)     | 0.9048 (133)      |            |                    |                    |         |
| +3032C/G      | CC       | 0.0000 (0)      | 0.0000 (0)        |            |                    |                    |         |
|               | CG       | 0.0250 (11)     | 0.0068 (1)        |            |                    |                    |         |
|               | GG       | 0.9750 (39)     | 0.9932 (146)      |            |                    |                    |         |
| +3035C/T      | CC       | 0.7750 (31)     | 0.7687 (113)      |            |                    |                    |         |
|               | CT       | 0.2250 (9)      | 0.2245 (33)       |            |                    |                    |         |
|               | TT       | 0.0000 (0)      | 0.0068 (1)        |            |                    |                    |         |
| +3142C/G      | CC       | 0.2821 (11)     | 0.1837 (27)       |            |                    |                    |         |
|               | CG       | 0.5128 (20)     | 0.5306 (78)       |            |                    |                    |         |
|               | GG       | 0.2051 (8)      | 0.2857 (42)       |            |                    |                    |         |
| +3187A/G      | AA       | 0.4750 (19)     | 0.4354 (64)       |            |                    |                    |         |
|               | AG       | 0.5000 (20)     | 0.4966 (73)       |            |                    |                    |         |
|               | GG       | 0.0250 (1)      | 0.0680 (10)       |            |                    |                    |         |
| +3196C/G      | CC       | 0.4359 (17)     | 0.4762 (70)       |            |                    |                    |         |
|               | CG       | 0.5641 (22)     | 0.4694 (69)       |            |                    |                    |         |
|               | GG       | 0.0000 (0)      | 0.0544 (8)        |            |                    |                    |         |
| +3227A/G      | AA       | 0.0000 (0)      | 0.0000 (0)        |            |                    |                    |         |
|               | GA       | 0.0811 (3)      | 0.0857 (6)        |            |                    |                    |         |
|               | GG       | 0.9189 (43)     | 0.9143 (64)       |            |                    |                    |         |

Table 2. Frequency and absolute number of the HLA-G 3′UTR genotypes observed in patients with AIDS exhibiting CMV retinitis (CMV-R: Group I) and in patients with AIDS without CMV-R (Group II).
Table 3. Frequency and absolute number of the HLA-G 3′UTR haplotypes observed in patients with AIDS exhibiting CMV retinitis (CMV-R: Group I) and in patients with AIDS without CMV-R (Group II).

| HLA-G haplotype | 2960 (14 bp) | 3001 | 3003 | 3010 | 3027 | 3032 | 3035 | 3142 | 3187 | 3196 | 3227 | Group I | Group II | Odds ratio, etiologic fraction (EF) or preventive fraction (PF) | P value |
|-----------------|-------------|------|------|------|------|------|------|------|------|------|------|----------|----------|------------------------------------------------|--------|
| UTR-1           | DEL         | C    | T    | G    | C    | G    | C    | G    | C    | G    | G    | 0.2632 (20) | 0.3007 (83) |                                            |        |
| UTR-2           | INS         | C    | T    | C    | C    | G    | G    | A    | G    | G    | G    | 0.2368 (18) | 0.2500 (69) |                                            |        |
| UTR-3           | DEL         | C    | T    | C    | C    | G    | G    | A    | G    | C    | C    | 0.0526 (4)  | 0.1630 (45) | OR = 0.285 (95% CI 0.099–0.820) PF = 0.116 | 0.014  |
| UTR-4           | DEL         | C    | C    | G    | C    | G    | C    | A    | C    | G    | G    | 0.1579 (12) | 0.0978 (27) |                                            |        |
| UTR-5           | INS         | C    | T    | C    | C    | G    | T    | G    | A    | C    | G    | 0.0658 (5)  | 0.0616 (17) |                                            |        |
| UTR-6           | DEL         | C    | T    | C    | G    | C    | G    | C    | A    | C    | G    | 0.0789 (6)  | 0.0290 (8)  |                                            |        |
| UTR-7           | INS         | C    | T    | C    | A    | G    | T    | G    | A    | C    | G    | 0.0132 (1)  | 0.0507 (14) |                                            |        |
| UTR-10          | DEL         | C    | T    | C    | C    | G    | G    | A    | G    | A    | G    | 0.0395 (3)  | 0.0217 (6)  |                                            |        |
| UTR-13          | DEL         | C    | T    | C    | C    | G    | T    | G    | A    | C    | G    | 0.0000 (0)  | 0.0036 (1)  |                                            |        |
| UTR-15          | INS         | C    | T    | C    | C    | G    | G    | A    | C    | G    | G    | 0.0263 (2)  | 0.0036 (1)  |                                            |        |
| UTR-17          | INS         | T    | T    | C    | T    | G    | C    | G    | A    | C    | G    | 0.0263 (2)  | 0.0000 (0)  |                                            | 0.046  |
| UTR-18          | DEL         | C    | T    | G    | C    | G    | C    | A    | C    | A    | G    | 0.0263 (2)  | 0.0072 (2)  |                                            |        |
| UTR-49          | DEL         | C    | T    | G    | C    | G    | C    | G    | G    | G    | G    | 0.0000 (0)  | 0.0072 (2)  |                                            |        |
| UTR-50          | INS         | C    | T    | C    | C    | G    | G    | G    | G    | G    | G    | 0.0132 (1)  | 0.0036 (1)  |                                            |        |

allele, and they were all associated with susceptibility to CMV-R. The +3001C/T variable site is uncommon in healthy Brazilian individuals, but has been encountered in African populations. This allele was also associated with infectious diseases, including leprosy.

Although some polymorphic sites at the HLA-G 3′UTR (14 bp Ins/Del, +3142C/G and +3187A/G) have been functionally studied, the +3001C/T has not, and in analogy to other variation sites it may be a different target for microRNA binding. In addition to the low frequency of +3001C/T, the etiologic fractions conferred by the +3001T allele, +3001C/T genotype and UTR-17 haplotype were also low, varying from 0.02 to 0.04, thus contributing to 2–4% of the CMV-R susceptibility at the population level. This result indicates that the +3001C/T variation may act in concert with other variable sites at HLA-G 3′UTR or surrounding variants. Indeed, the +3001T allele is in linkage disequilibrium with the 14 bp Insertion, +3142G and the +3187A alleles, which are included at the +3001C/T variable site.

The classical HLA class I molecules (HLA-A, -B and -C) play a major role on the defense against viruses by presenting virus-derived peptides to cytotoxic TCD8 cells, and the lack of classical class I molecules on the surface of virus infected cells provides a signal for the cytotoxic action of NK cells. In this aspect, HLA-G may inhibit the cytotoxic activity of both CD8 and NK cells, facilitating virus escape from the host immune response and permitting virus spread. Although HLA-A, HLA-B and HLA-C form common extended haplotypes due to the linkage disequilibrium among these genes, the most striking linkage disequilibrium of the HLA-G gene is with
the HLA-A gene\textsuperscript{28,30}, and the typing of both classical and non-classical class I genes is important to understand the association of HLA genes with virus disorders.

In this study, we reported that susceptibility or protection against CMV-R in patients with AIDS is associated with specific variation sites at the HLA-G 3′UTR, and these findings deserve further studies to evaluate specific post-transcriptional factors that may target these gene segments, and consequently the differential production of HLA-G. The genetic susceptibility to produce differential levels of immunomodulatory molecules such as HLA-G, in concert with the immunosuppression caused by the HIV infection, may further influence host immunosurveillance, propitiating the concomitant appearance of other viral infections, including CMV-induced complications.

Methods

All investigation was performed following the guidelines of the National Committee of Ethics in Research (CONEP) Brazil. The study was submitted to the Ethics Committee of the University Hospital of the Ribeirão Preto Medical School, demanding the use of samples stored at a Bank of Biological Samples (protocols # 1525/1998, #8992/2001 and #7581/2007). The Ethics Committee approved the use of the stored samples, waiving the informed consent (protocol #4084/2009). All additional patients who were further included in the study signed informed consent.

The samples were obtained from two groups of individuals: Group I (n = 40) patients with AIDS and CMV-R diagnosed according to current criteria; and Group II (n = 147), patients with AIDS diagnosed according to current criteria and without CMV-R diagnosis, regardless of the evolutionary phase of the latter or of whether or not they had started antiviral treatment.

DNA was obtained using a salting-out procedure. The 3′UTR of the HLA-G gene was amplified by the polymerase chain reaction (PCR) using with two primers: HLAG8R—GTC TTC CAT TTA TTT TGT CTCT and HLAG8F—TGT GAA ACA GCT GCC CTG TGT in a final volume of 25 μL, containing ultrapure deionized water and amplification buffer (0.2 M Tris–HCl, 0.5 M KCl, pH 8.5, 0.2 mM dNTP, 1.50 mM d MgCl\textsubscript{2}, 5 pmol of each primer, 0.5 U platinum polymerase Platinum (Invitrogen, Carlsbad, CA, USA), and 200 ng genomic DNA. PCR-conditions consisted of an initial cycle at 94 °C for 5 min followed by 30 cycles of three stages: 95 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min, followed by a final stage at 72 °C for 7 min. The final product of the amplification was sequenced (Genetic Analyzer ABI PRISM 310 (Applied Biosystems, Foster City, CA, USA) with the HLAG8R—GTC TTC CAT TTA TTT TGT CTCT primer using the BIGDYE Terminator v3.1 device (Applied Biosystems, Foster City, CA, USA).

HLA-G 3′UTR alleles and genotypes were estimated by direct counting using the GENEPOP 3.4 software\textsuperscript{31}. Linkage disequilibrium was evaluated using the ARLEQUIN v.3.1\textsuperscript{32} software and adherence to Hardy–Weinberg equilibrium was determined by the Guo and Thompson test\textsuperscript{33} using the GENEPOP 3.4 software\textsuperscript{31}. Since the base sequencing in the gamete phase was unknown, the haplotypes were inferred using the PHASE\textsuperscript{34} and Expectation–Maximization\textsuperscript{35} methods.

The allele, genotype and haplotype frequencies were compared between groups by Fisher’s exact test with the calculation of the Odds Ratio (OR) and 95% confidence interval (95% CI). The level of significance was set at P ≤ 0.05. We also estimated the etiologic fraction that indicates the contribution of each allele, genotype, or haplotype to the susceptibility to CMV-R, and the preventive fraction, that indicates how much these factors contribute to protection against the development of CMV-R.

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References

1. Holland, G. N. et al. Relationship between opacity of cytomegalovirus retinitis lesion borders and severity of immunodeficiency among people with AIDS. IOVS. 60, 1853–1862. https://doi.org/10.1167/iovs.18-26517. PubMed (2019).
2. Gupta, P. K., Patel, N. V., Patel, S. D. & Patel, K. J. Cytomegalovirus retinitis in acquired immunodeficiency syndrome patients: A problem worth giving attention to. Indian J. Sex. Transm. Dis. 35, 21–24. https://doi.org/10.4103/0253-7184.132411. PubMed (2014).
3. Carosella, E. D., Howangyin, K. Y., Favier, B. & Lemaoult, J. HLA-G-dependent suppressor cells: Diverse by nature, function, and significance. Hum. Immunol. 69, 700–707. https://doi.org/10.1016/j.humimm.2008.08.280. PubMed (2008).
4. Lucena-Silva, N. et al. The +3187A/G HLA-G polymorphic site is associated with polar forms and reactive reaction in leprosy. Mol. Genet. Genomic Med. 1, 123–130. https://doi.org/10.1002/mgg3.14. PubMed (2013).
5. Castelli, E. C. et al. HLA-G variability and haplotypes detected by massively parallel sequencing procedures in the geographicaly distinct population samples of Brazil and Cyprus. Mol. Immunol. 83, 115–126. https://doi.org/10.1016/j.molimm.2017.01.020. PubMed (2017).
6. Dias, F. C., Castelli, E. C., Collares, C. V. A., Moreau, P. & Donadi, E. A. The role of HLA-G molecule and HLA-G gene polymorphisms in tumors, viral hepatitis, and parasitic diseases. Front. Immunol. 6, 9. https://doi.org/10.3389/fimmu.2015.00099.eCollection. PubMed (2015).
7. Rousseau, P. et al. The 14 bp deletion-insertion polymorphism in the 3′ UT region of the HLA-G gene influences HLA-G mRNA stability. Hum. Immunol. 64, 1005–1010. https://doi.org/10.1016/j.humimm.2003.08.347. PubMed (2003).
8. Rehmann, V. et al. Association of soluble HLA-G plasma levels with HLA-G alleles. Tissue Antigens 57, 15–21. https://doi.org/10.1034/j.1399-0039.2001.057001015.x. PubMed (2001).
9. Hvid, T. V., Hyleniou, S., Rorbye, C. & Nielsen, L. G. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 55, 63–79. https://doi.org/10.1007/s00251-003-0547-z. PubMed (2003).
10. Veit, T. D. & Chies, J. A. Tolerance versus immune response-microRNAs as important elements in the regulation of the HLA-G gene expression. Transpl. Immunol. 20, 229–231. https://doi.org/10.1016/j.trim.2008.11.001. PubMed (2009).
11. Tön, Z. et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am. J. Hum. Genet. 81, 829–834. https://doi.org/10.1086/521200. PubMed (2007).
12. Yie, S., Li, L., Xiao, R. & Librach, C. L. A single base-pair mutation in the 3’ untranslated region of HLA-G mRNA is associated with pre-eclampsia. Mol. Hum. Reprod. 14, 649–653. https://doi.org/10.1093/molehr/gan059.PubMed (2008).
13. Oliveira, M. L. G. et al. Extended HLA-G genetic diversity and ancestry composition in a Brazilian admixed population sample: Implications for HLA-G transcriptional control for case-control association studies. Hum. Immunol. 79, 790–799. https://doi.org/10.1016/j.humimm.2018.08.005.PubMed (2018).
14. Porto, I. O. et al. MicroRNAs targeting the immunomodulatory HLA-G gene: A new survey searching for microRNAs with potential to regulate HLA-G. Mol. Immunol. 65, 230–241. https://doi.org/10.1016/j.molimm.2015.01.030.PubMed (2015).
15. Rodrigues, M. L. V. et al. Frequency of HLA class 1 and 2 alleles in Brazilian patients with AIDS and cytomegalovirus retinitis. Acta Ophthalmol. Scand. 81, 514–516. https://doi.org/10.1034/j.1600-4240.2003.00098.x.PubMed (2003).
16. De Marro, A. L. et al. Susceptibility to toxoplasmatocinchororiditis is associated with HLA alleles reported to be implicated with rapid progression to AIDS. Dis. Markers. 33, 309–312. https://doi.org/10.3233/DMA-2012-00935.PubMed (2012).
17. Biber-Saloum, T. G. et al. HLA-C alleles and cytomegalovirus retinitis in Brazilian patients with AIDS. J. Ophthalmol. 2018(830), 3830104. https://doi.org/10.1155/2018/3830104.eCollection.PubMed (2018).
18. Sun, X., Stephens, J. C. & Zhao, H. The impact of sample size and marker selection on the study of haplotype structures. Hum. Genomics. 1, 179–193. https://doi.org/10.1186/1471-7364-1-3-179.PMC (2004).
19. Northfield, J. W., Harcourt, G., Lucas, M. & Klenerman, P. Immunology of viral co-infections with HIV. Arch. Immunol. Ther. Exp. (Warsz). 53, 3–12 (2005) [PMID: 15761372].
20. Ociepka, P. et al. Cytomegalovirus retinitis screening and treatment in human immunodeficiency virus patients in Malawi: A feasibility study. Open Forum Infect. Dis. 6, ofz439. https://doi.org/10.1093/ofid/ofz439.eCollection.PubMed (2019).
21. Shi, Y. et al. Prevalence and clinical management of cytomegalovirus retinitis in AIDS patients in Shanghai, China. BMC Infect. Dis. 11, 326–334. https://doi.org/10.1186/1471-2334-11-326.PubMed (2011).
22. Castelli, E. C. et al. Insights into HLA-G genetics provided by worldwide haplotype diversity. Front. Immunol. 5, 476. https://doi.org/10.3389/fimmu.2014.00476.eCollection.PubMed (2014).
23. Cunha, A. A., Marin, I. J., Aquino, V. H. & Figueiredo, L. T. Diagnosis of cytomegalovirus infections by qualitative and quantitative PCR in HIV infected patients. Rev. Inst. Med. Trop. Sao Paulo. 44, 127–132. https://doi.org/10.1590/0036-4665200200030003.PubMed (2002).
24. Martelli-Palomino, G. et al. Polymorphic sites at the 3′ untranslated region of the HLA-G gene are associated with differential hla-g soluble levels in the Brazilian and French population. PLoS ONE 8, e71742. https://doi.org/10.1371/journal.pone.0071742.eCollection.PubMed (2013).
25. Dehghani, N. H. et al. Tumor necrosis factor region polymorphisms are associated with AIDS and with cytomegalovirus retinitis. AIDS. 23, 1641–1647. https://doi.org/10.1097/QAD.0b013e32832f5591.PubMed (2009).
26. Sabbagh, A. et al. Worldwide genetic variation at the 3′ untranslated region of the HLA-G gene: Balancing selection influencing genetic diversity. Genes Immun. 15, 95–106. https://doi.org/10.1038/jen.2013.67.PubMed (2014).
27. Castelli, E. C., Mendes-Junior, C. T., Viana de Camargo, J. L. & Donadi, E. A. HLA-G polymorphism and transitional cell carcinoma of the bladder in a Brazilian population. Antigens. 72, 149–157. https://doi.org/10.10111/j.1399-0039.2008.01091.x.PubMed (2008).
28. Carlini, F. et al. Association of HLA-A and non-classical HLA class I alleles. PLoS ONE 11, e0163570. https://doi.org/10.1371/journal.pone.0163570.eCollection.PubMed (2016).
29. Rouxset, F. Genepop007: A complete re-implemention of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x (2008) [PMID: 21585727].
30. Excoffier, L. & Slatkin, M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol. Biol. Evol. 12, 921–917. https://doi.org/10.1093/oxfordjournals.molbev.a040269 (1995) [PMID: 7476138].

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Author contributions

L.M.V., M.L.V.R.* and E.A.D. contributed equally for the design of the work; interpretation of data; and have drafted the work and revised it. E.C.C. was responsible for implementing the HLA-G sequencing technique in the Institution and collaborated in the review of the work. N.H.S.D., L.M.V. and M.L.V.R. were responsible for the acquisition of data. C.T.M.-J. was responsible for data analysis and collaborated in the review of the work. J.F. collaborated in the interpretation of the data and in the review of the work.

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Competing interests

The authors declare no competing interests.

Additional information

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