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RNAseq-differentiated gene expression profile of people living with HTLV-1 in the Brazilian Amazon region: a pilot study

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In this study, we aimed investigated the differential gene expression profiles of samples from uninfected individuals (control group) and study groups of asymptomatic human T-lymphotropic virus 1 (HTLV-1) carriers and patients with HTLV-1-associated myelopathy (HAM) by exploratory RNA sequencing (RNA-Seq) analysis. The gene expression profiles of individuals in the asymptomatic group were represented by 3 genes, most associated with cell cycle regulation. The gene expression profiles of individuals in the HAM group were represented by 12 genes, the majority of which are associated with the immune response. The *HLA-A* gene and the non-coding RNA LINC02470 were upregulated in the asymptomatic and HAM groups. The *HLA-DQB1* and *HLA-C* genes were downregulated in the asymptomatic and HAM groups. In this pilot study, although limited in terms of methodological rigor, we showed differential gene expression profiles in different clinical groups of HTLV-1 infection. However, further studies are needed to confirm these findings.

KEYWORDS

HTLV-1, asymptomatic, HAM, transcriptome, RNA-seq, gene expression

1 Introduction

Human T-lymphotropic virus (HTLV) is a deltaretrovirus ([International Committee on Taxonomy of Viruses- ICTV, 2022](https://doi.org/10.1093/ictv/ictv2022)), with approximately 10 million individuals infected worldwide. HTLV-1 infection is most prevalent and considered endemic in parts of southwestern Japan, sub-Saharan Africa, South America, the Caribbean, the Australo-Melanesia region and some regions in the Middle East ([Eusebio-Ponce et al., 2019](https://doi.org/10.1093/ictv/ictv2019)). The second most prevalent type of HTLV infection, HTLV-2 infection, has a limited distribution among intravenous drug users in the United States, Europe, and

Southeast Asia and among Native Americans from North America to South America (Ishak et al., 2020).

Brazil is a country with a high prevalence of HTLV infections (700 thousand to 2 million infected individuals) (Miranda et al., 2022). The State of Pará (northern Brazil) is the site of most epidemiological studies of HTLV infection in the Brazilian Amazon region to date and has a variety of different populations, with prevalence rates ranging from 0.3% to more than 30% and infections occurring mainly in indigenous populations (Abreu et al., 2022; Brito et al., 2022; de Lima et al., 2022; Lopes et al., 2022).

HTLV-1 is associated with inflammatory or lymphoproliferative diseases; two such diseases, i.e., adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy (HAM) are clearly related to the virus, as are other diseases, such as uveitis, dermatitis, strongyloidiasis and tuberculosis (Eusebio-Ponce et al., 2019). Activation of viral genes, such as Tax and HBZ, may be partially related to the pathogenicity of HTLV-1 (Martinez et al., 2019; Brites et al., 2021). However, from the host perspective, the need to understand the factors that trigger different clinical outcomes remains (Vallinoto et al., 2019; Forlani et al., 2021).

In the genomic era, RNA sequencing (RNA-Seq) approaches for obtaining transcriptomes are tools with great potential for answering challenging questions about gene expression profiles of organisms under different conditions and at specific developmental stages and are applied in the analysis of various pathological conditions (Girgenti and Duman, 2018; Schwartz and Stern-Ginossar, 2019). For example, a differential transcriptome of nuclear and cytoplasmic factors that correlates with Tax protein activity has been observed in cell lines transformed by HTLV-1 (Singh et al., 2011). In ATLL models, integrated molecular analyses have revealed transcriptomic abnormalities involving key genes related to cell proliferation, with proliferative potential being associated with specific mutation patterns (Kataoka et al., 2015; Yamagishi et al., 2021).

Microarray analysis has shown that the gene expression of intermediary regulators of the immune response, proliferation and cell migration is increased in individuals with HAM, and the activation of apoptosis factors is likely to regulate signaling involving the proteins encoded by these genes (Menezes et al., 2017; Mozhgani et al., 2019).

In a relatively recent study, a greater number of differentially expressed protein-coding genes were identified and a broader quantitative range of changes in expression levels was detected by RNA-Seq than by microarray analysis in a toxicogenomic evaluation of rat liver tissue (Rao et al., 2018). Specifically, regarding HTLV-1 infection, a meta-analysis revealed that microarray technology can indeed be used to simultaneously measure the expression of tens of thousands of genes in different tissue samples in a cost-effective and high-throughput manner (Jeffery et al., 1999). However, as the results of microarray analysis may not be reproducible or may be influenced by disordered data, the RNA-Seq technique appears to be satisfactory for our research purposes.

In this study, we sought to obtain differential gene expression profiles via exploratory RNA-Seq analysis of samples from HTLV-1-infected individuals (with asymptomatic infection and with HAM) and uninfected individuals inhabiting the Brazilian Amazon region, aiming to identify markers related to the pathogenesis of and/or immune response to HTLV-1 infection.

2 Materials and methods

2.1 Sampling and ethical aspects

Blood samples collected in tubes containing ethylenediamine tetraacetic acid (EDTA) from 18 individuals treated at the Ambulatory of the Tropical Medicine Center of the Federal University of Pará (NMT/UFPA) and at the Fundação Centro de Hematologia e Hemoterapia do Estado do Pará (HEMOPA) were used for this study.

The individuals were over 18 years of age, of both sexes, had laboratory confirmation of HTLV-1 infection with or without clinical indications of HAM and were not being treated with antiretroviral drugs and/or glucocorticoids. The control group was composed of voluntary blood donors who were seronegative for HTLV-1/2. All individuals belonged to the population of the capital Belém (Pará State) and were ethnically characterized as being of mixed race.

The project was approved by the Ethics Committee for Research with Human Beings of the Institute of Health Sciences of the Federal University of Pará (CAAE: 73782017.8.0000.0018).

2.2 DNA and RNA extraction

Total DNA was extracted from peripheral blood using a PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturer's protocol.

RNA was extracted from blood samples using a TRIzol™ Plus RNA purification kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

2.3 Quantification of leukocytes and the HTLV-1 proviral load

Leukocytes were quantified using a BC-2800Vet hematology analyzer (Mindray, Shenzhen, China) following the manufacturer's recommendations.

The viral load was quantified by qPCR using three target sequences synthesized with the TaqMan® system (Life Technologies, Carlsbad, California, United States) according to a previously described protocol (Tamegão-Lopes et al., 2006). The values obtained were adjusted with respect to the leukocyte count per mm³.

2.4 RNA purification and sequencing

Extracted RNA was purified using a PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) following the manufacturer's recommendations.

After purification, the RNA was quantified using a Qubit RNA HS Assay Kit and a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). For qualitative analysis of RNA, the integrity of the extracted RNA considering the RNA integrity number (RIN) was determined with Bioanalyzer

equipment using an Agilent RNA 6000 Pico Kit (Agilent, Saint Clara, California, United States); RNA transcripts with RIN values of less than 7 were disregarded for downstream analyses.

An RNA library was prepared following the guidelines of the SureSelect Strand-Specific RNA Library Prep System Kit (Agilent, Saint Clara, California, United States) and subjected to paired-end sequencing (2×100) on the NextSeq 500 platform (Illumina, San Diego, California, United States) with the NextSeq 500/550 High Output Kit v2.5 (300 cycles) according to the manufacturer's recommendations.

2.5 Data analysis

The raw sequencing data were analyzed with the miRNA-Seq and RNA-Seq Multiprocess Analysis (miARma-Seq) pipeline using edgeR software for differential expression analysis and the criterion of a false discovery rate (FDR) ≤ 0.05 (Andrés-León and Rojas, 2019). We based our analysis on the reference human genome GRCh38.p13 in the National Center for Biotechnology Information (NCBI) database. The data generated in this study were uploaded to the NCBI public database under accession code GSE224047. Quality control data is available as [Supplementary Data S1](#). Significant gene expressions were represented in reads per million mapped reads (RPKM) in boxplot graphs, showing the medians, their respective interquartile ranges and outliers.

Interprotein interaction networks (interactomes) were inferred using Cytoscape 3.9.1 software (Seattle, Washington, United States) with the GeneMANIA database as a search source; we opted for resulting networks with a maximum of fifty functionally enriched genes and the option of automatic weighting for the selection of networks. Network modularization was carried out using clusterMaker and the MCODE clustering algorithm, followed by selection of the largest network as the main highlight.

Venn diagrams were generated via the Lucidchart website (Utha, United States) according to the number of biological functions of the inferred genes in each network. GraphPad Prism 8.0 software (San Diego, California, United States) was used to graphically visualize gene expression. Multivariate clustering was applied to evaluate similarities between the differential gene expression profiles of the groups, with Ward's method used for agglomeration, selection of the type of Euclidean distance and standardization of the variables (Ayres et al., 2008). Comparison of the proviral load among the groups was performed using the Mann-Whitney test (Ayres et al., 2008).

3 Results

3.1 Sample integrity

Among the samples from the 18 subjects, a satisfactory RIN for downstream analyses was obtained for 10 samples: 4 from participants with HAM, 3 from asymptomatic individuals and 3 from individuals in the control group. All individuals with HTLV-1 infection were female; in the control group, two individuals were male ([Supplementary Table S1](#)). The

log₁₀ values of the proviral load in patients with HAM (4.71 (4.60–4.75)) were greater than those of asymptomatic individuals (0.90 (0.49–1.61); $p = 0.0209$).

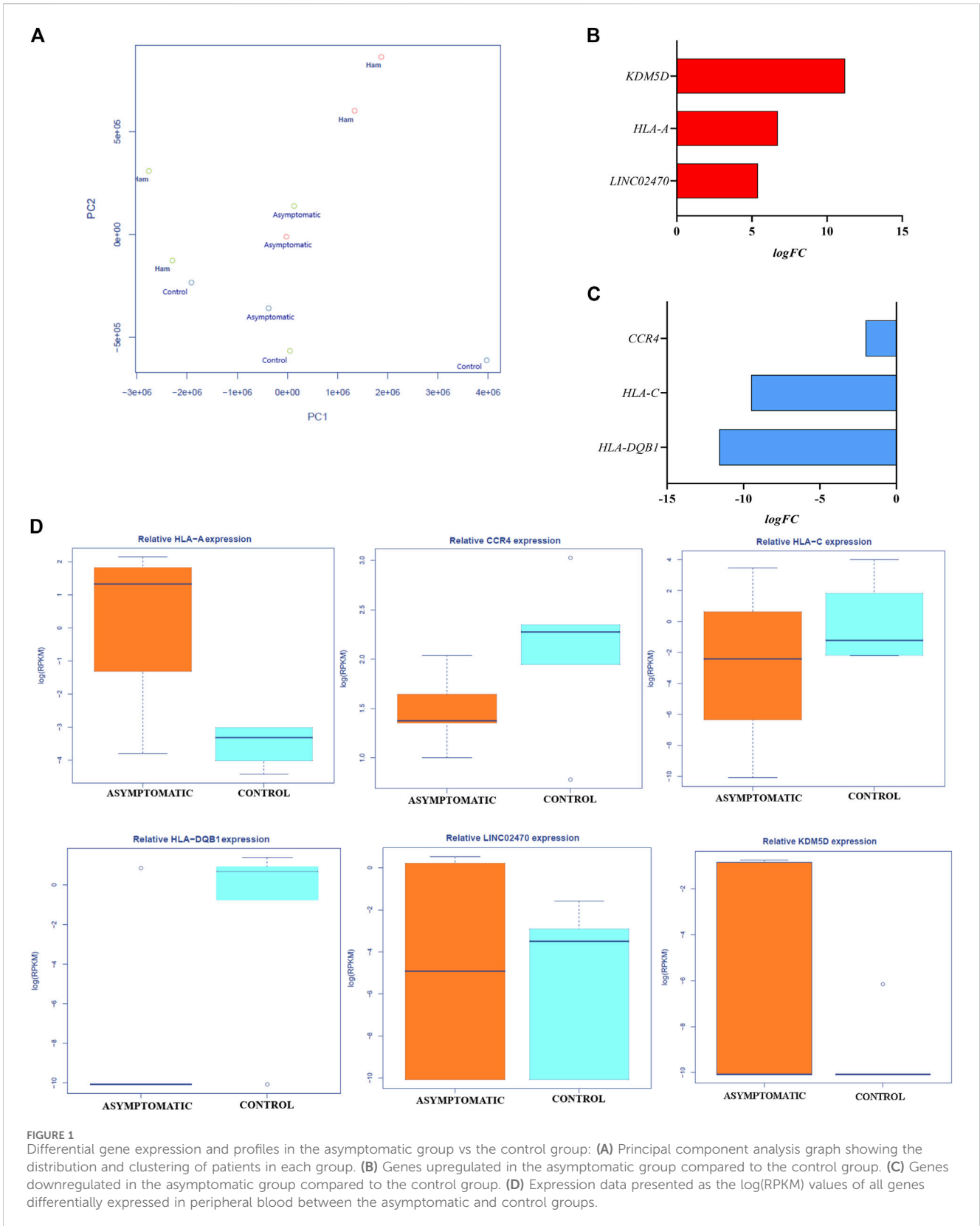
When comparing the groups, we disregarded sex-linked genes ([Supplementary Table S2](#)), resulting in a set of 11,946 genes with differential expression (as determined by the fold change (FC) values). A total of 457 of these genes were significantly differentially expressed among the comparison groups, but only 21 genes had an FDR ≤ 0.05 , as explained below. Principal component cluster analysis (PCA) showed that not all samples were grouped into the same class, however, we observed a tendency for patient samples to cluster in different quadrants from the control group ([Figure 1A](#)).

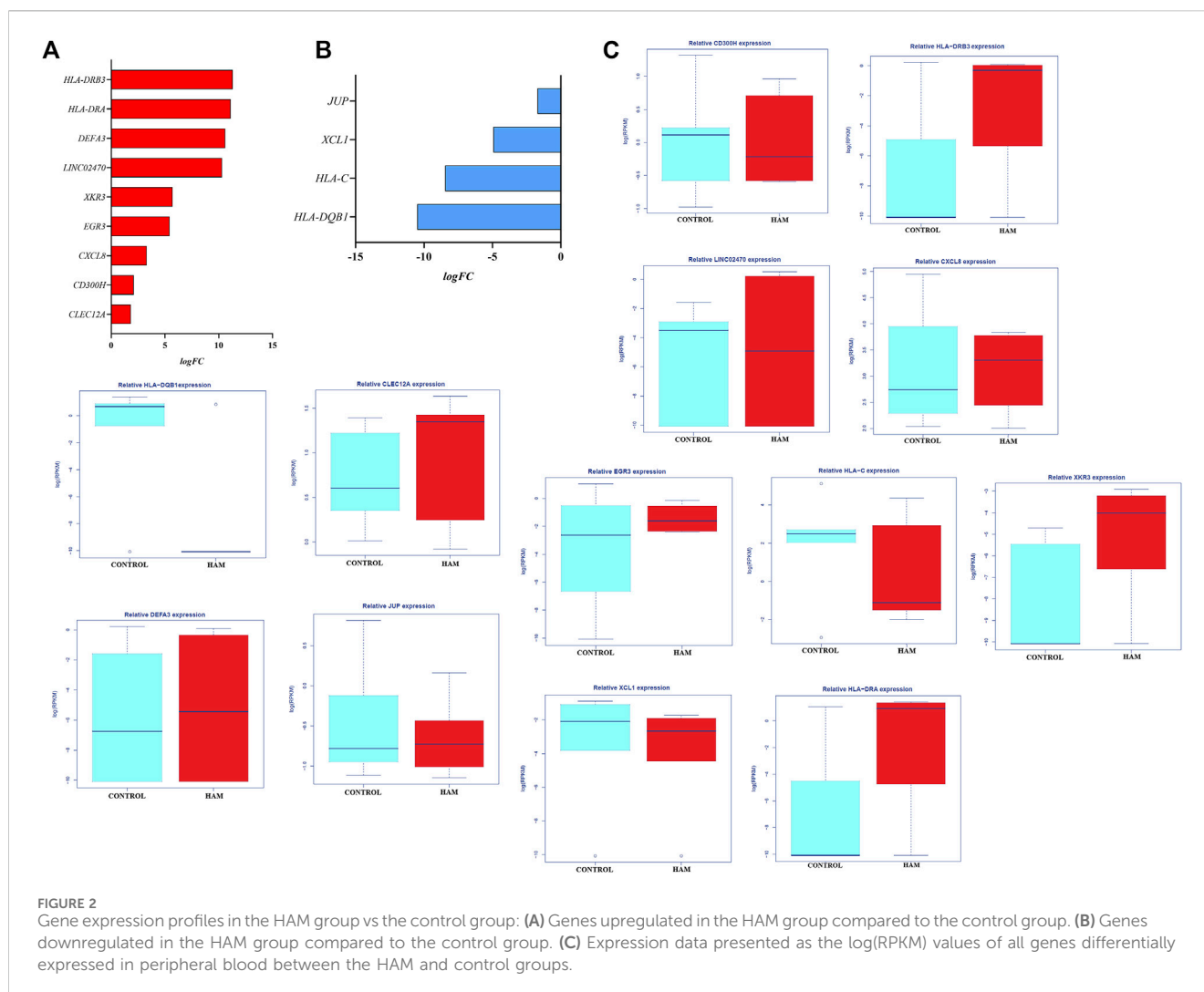
3.2 Differential expression between the control group and the asymptomatic group

In the comparison between the control group and the asymptomatic group, 6 genes had an FDR ≤ 0.05 , of which 3 were upregulated ([Figure 1B](#)) and 3 were downregulated ([Figure 1C](#)) in the asymptomatic group. Among the upregulated genes, lysine demethylase 5 days (*KDM5D*) was upregulated by approximately 11-fold (logFC: 11.20; $p = 1.33 \times 10^{-7}$; FDR: 0.0002), human leukocyte antigen of the A locus (*HLA-A*) was upregulated by approximately 7-fold (logFC: 6.72; $p = 5.54 \times 10^{-6}$; FDR: 0.032), and the noncoding gene *LINC02470* was upregulated by approximately 5-fold (logFC: 5.40; $p = 4.76 \times 10^{-6}$; FDR: 1.86×10^{-5}). Among the downregulated genes, a chemokine receptor gene (*CCR4*) was downregulated by approximately 2-fold (logFC: -2.01 ; $p = 3.86 \times 10^{-5}$; FDR: 0.03), human leukocyte antigen of the C locus (*HLA-C*) was downregulated by approximately 10-fold (logFC: -9.5 ; $p = 2.02 \times 10^{-6}$; FDR: 0.02), and human leukocyte antigen of the DQB1 locus (*HLA-DQB1*) was downregulated by approximately 12-fold (logFC: -11.6 ; $p = 7.49 \times 10^{-6}$; FDR: 0.007). [Figure 1D](#) shows the gene expression data as the RPKM values for all genes differentially expressed in peripheral blood between the asymptomatic and control groups.

3.3 Differential expression between the control group and the group with HAM

In the comparison between the control group and the HAM group, 13 genes exhibited an FDR ≤ 0.05 , 9 of which were upregulated ([Figure 2A](#)) and 4 of which were downregulated ([Figure 2B](#)) in the HAM group. Among the upregulated genes, human leukocyte antigen of the DRB3 locus (*HLA-DRB3*) was upregulated by approximately 11-fold (logFC: 11.28; $p = 2.95 \times 10^{-24}$; FDR: 1.7×10^{-20}), and C-type lectin domain family gene 12 (*CLEC12A*) was upregulated by approximately 2-fold (logFC: 1.83; $p = 0.005$; FDR: 0.01). Among the downregulated genes, plakoglobin (*JUP*) was downregulated by approximately 2-fold (logFC: -1.71 ; $p = 8.1 \times 10^{-5}$; FDR: 0.037), and *HLA-DQB1* was downregulated by approximately 10-fold (logFC: -10.49 ; $p = 2.35 \times 10^{-5}$; FDR: 0.012). [Figure 2C](#) shows the gene expression data as the RPKM values of all genes differentially expressed in peripheral blood between the control and HAM groups.





3.4 Differential expression between the asymptomatic group and the group with HAM

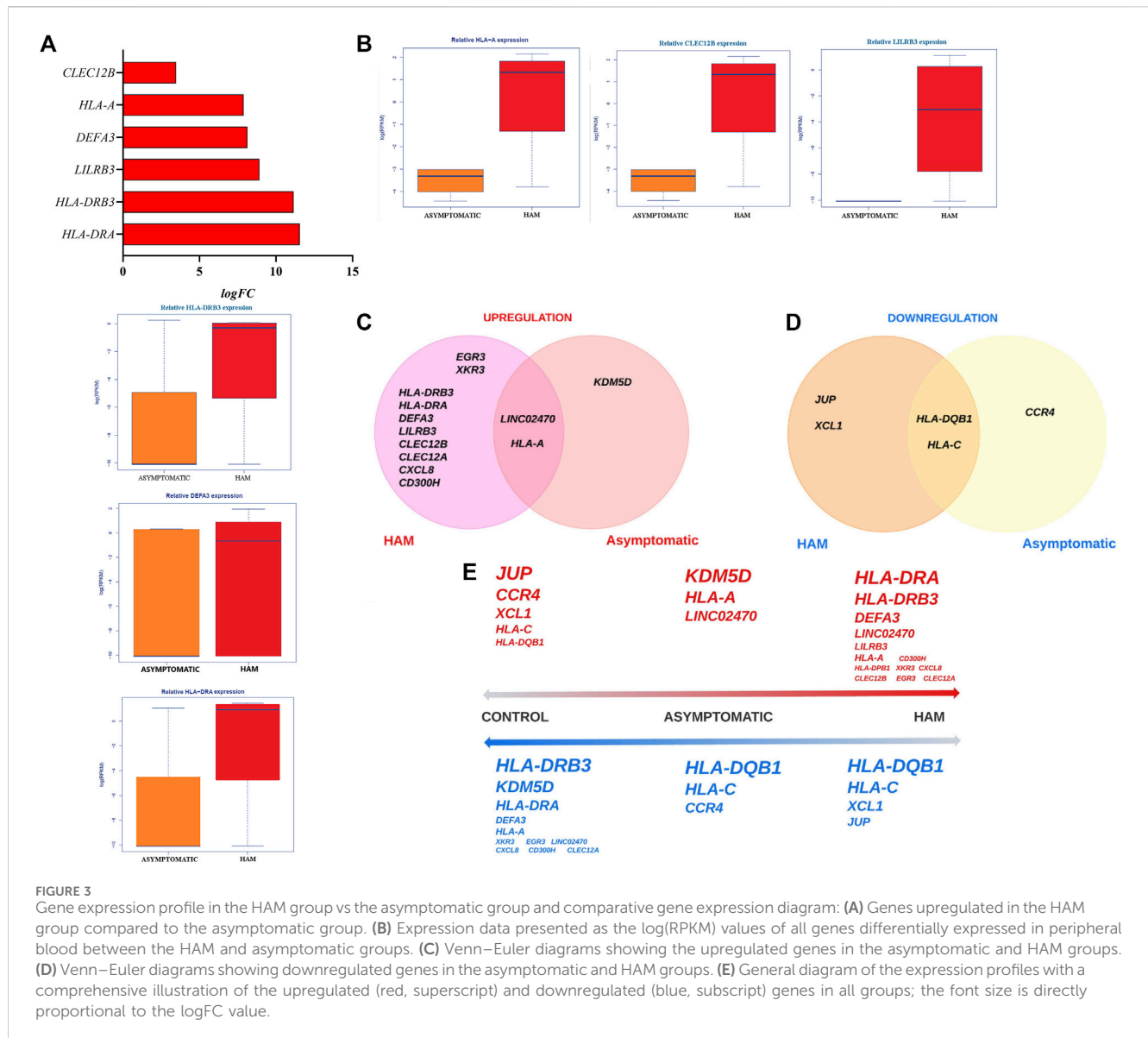
In addition, 6 genes were upregulated, with an $FDR \leq 0.05$, in the HAM group compared to the asymptomatic group (Figure 3A). Among these genes, human leukocyte antigen of the DRA locus (*HLA-DRA*) was approximately 12-fold more highly expressed ($\log FC: 11.55$; $p = 0.01$; $FDR: 6.05-27$), and *CLEC12B* was approximately 3-fold more highly expressed ($\log FC: 3.47$; $p = 3.67-05$; $FDR: 0.007$). Figure 3B shows the gene expression data measured in RPKM of all genes differentially expressed in peripheral blood between the asymptomatic and HAM groups.

More genes were upregulated in the HAM group than in the asymptomatic group. The *KDM5D* gene was the only gene upregulated only in the asymptomatic group, whereas *LINC02470* and *HLA-A* were upregulated in both groups (Figure 3C). Fewer genes were downregulated than upregulated in either group; *CCR4* was the only downregulated gene in the asymptomatic group, *HLA-DQB1* and *HLA-C* were downregulated in both groups, and *JUP* and chemokine ligand type 1 (*XCL1*) were downregulated only in the HAM group (Figure 3D).

We constructed a diagram with all the positively regulated and negatively regulated genes ordered by FC. In the qaul we highlighted the upregulated genes in red and the downregulated genes in blue in each group; The font size of each word is related to the FC, the larger fonts indicate the most regulated genes in each specific degree (Figure 3E). In the control group, the *JUP* gene was the most upregulated, and the *HLA-DQB1* gene was the most downregulated. In the asymptomatic group, *KDM5D* was the most upregulated; in the HAM group, *HLA-DRA* was the most upregulated. In both the asymptomatic and HAM groups, *HLA-DQB1* and *HLA-C* were the most downregulated with respect to the control group.

3.5 Interactome

Interaction networks were inferred for the genes differentially expressed in each group; by enrichment analysis, these genes were categorized into four functional groups based on general biological properties (Figure 4). The interactome of the control group was formed by seven types of network categories, among which the physical interaction type network accounted for approximately 77.64% of the interactions. The largest cluster encompassed the *CCR4*, *XCL1* and *JUP*



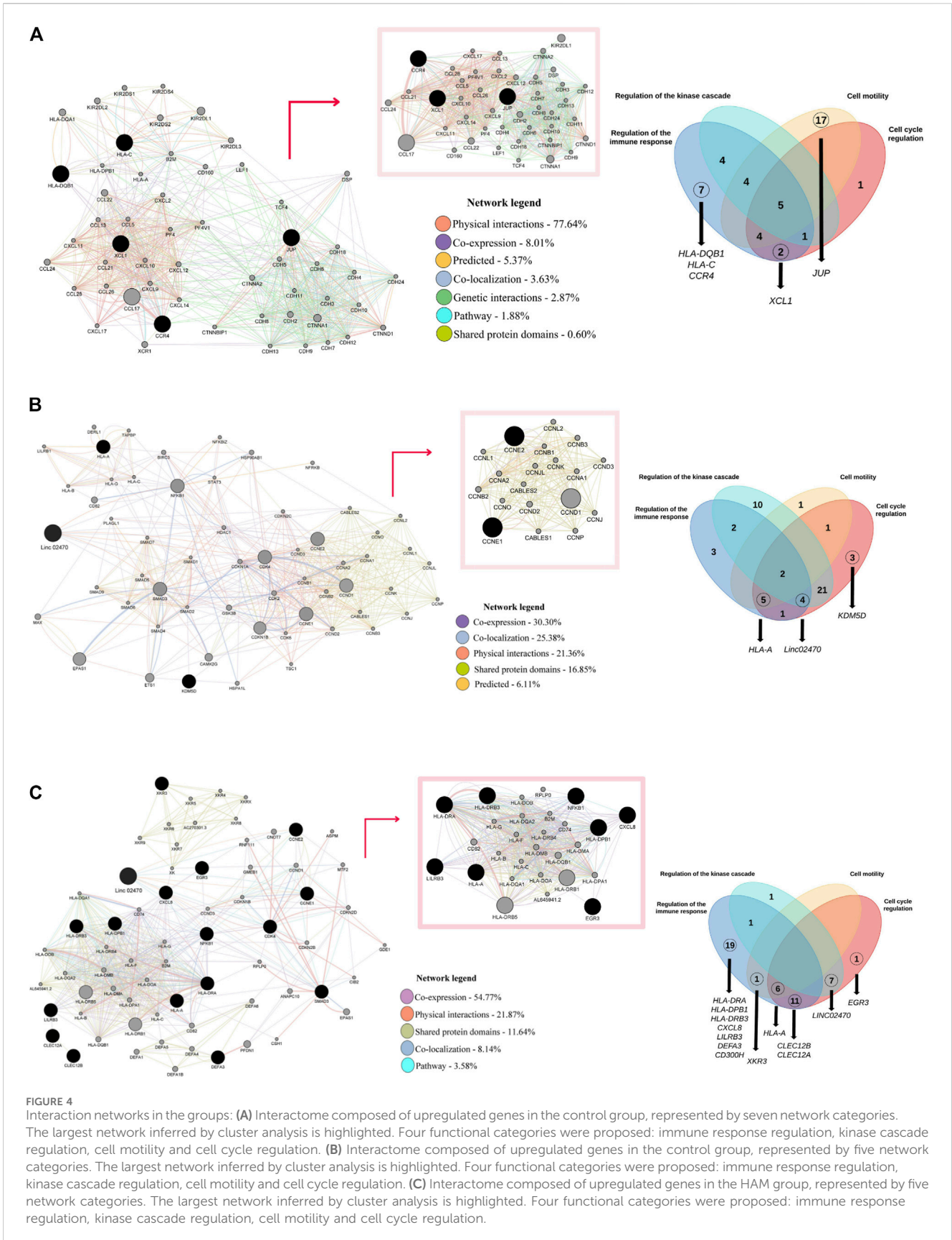
genes, with a clear predominance of categories such as physical interaction and genetic interaction. Most of the genes shown were grouped as immunoregulatory genes, among which *HLA-DQB1*, *HLA-C* and *CCR4* are tightly related to the immune response. *XCL1* clustered into both the immune response and cell cycle regulation categories, and *JUP* clustered narrowly among cell motility genes (Figure 4A).

The interactome of the asymptomatic group was formed by five types of network categories, among which the coexpression type network accounted for 30.3% of the interactions. The largest cluster encompassed genes of the G1-type cyclin family (*CCNE2* and *CCNE1*), which are some of the genes that interact with the noncoding RNA *LINC02470*; in this cluster, we observed a predominance of the prediction type category among the interactions. Most of the genes clustered as cell cycle regulation genes, among which all three genes upregulated in the asymptomatic group were included, although the *HLA-A* gene also clustered in the immune regulation and cell motility categories, and the RNA *LINC02470* clustered in the immune regulation and kinase cascade regulation categories (Figure 4B).

The interactome of the HAM group was formed by five types of network categories, among which the coexpression type network accounted for 50.77% of the interactions. The largest cluster encompassed the genes *HLA-A*, *LILRB3*, *HLA-DRB3*, *HLA-DRA*, *HLA-DPB1*, *CXCL8*, *EGR3* and *NFKB1* (*NFKB1* specifically interacts with the *LINC02470* RNA axis). Notably, all network categories were represented in this cluster, although the strongest interactions were observed in the physical interaction type category. Most of the genes clustered as immunoregulatory genes, and only an early growth response gene (*EGR3*) and the RNA *LINC02470* clustered in the kinase cascade regulation and cell cycle regulation categories (Figure 4C).

4 Discussion

In the present study, we reported different differential gene expression profiles between people living with HTLV-1. We observed that individuals with HAM showed greater diversity in



the number of differentially expressed genes and gene expression profiles.

The *HLA-DQB1* and *HLA-C* genes were downregulated in individuals with both clinical profiles of infection, possibly suggesting the importance of these genes in the anti-HTLV-1 immune response. Both HLA class I and class II proteins are important in the immune response to HTLV-1 infection (Reith and Mach, 2001). Studies have shown that some *HLA-DQB1* alleles associated with HAM are upregulated in different blood cell lines and even in uninfected individuals (Yamano et al., 1997; Zajacova et al., 2018). This observation suggests that the HLA class II-mediated antigen presentation pathway may be effective in maintaining the response to HTLV-1 infection but that enhancement of this proinflammatory profile may be detrimental, especially at the level of the nervous system, as previously suggested (Enose-Akahata et al., 2017).

The *HLA-C* gene was the second most downregulated gene in patients with HTLV-1 infection. Specifically, one previous study indicated the association of genetic variability in *HLA-C* with clinical aspects of HTLV-1 infection, based on the role of this gene in infections with other retroviruses (Penova et al., 2021). Furthermore, a genome-wide association study (GWAS) in an HTLV-1 endemic population suggested that haplotypes formed by classical HLA class II alleles in linkage disequilibrium with *HLA-C* alleles act as protagonists in immune homeostasis during infection (Lei and Takahama, 2012). This observation supports our findings of joint downregulation of HLA class I and II molecules in patients with HTLV-1 infection.

In addition, we observed an interaction network formed by upregulated *XCL1*, *CCR4* and *JUP* only in the control group. There is no direct interaction pathway between *XCL1* and *CCR4*; however, both participate in directional cell migration during immune surveillance and inflammation and are expressed in the same cell lineages (Aktary et al., 2017; Yoshie, 2021). Beyond its role in cell adhesion, in this context, *JUP* may function in T-cell lineage differentiation and the regulation of proinflammatory responses (Yu et al., 2010; Saeidi et al., 2023). These findings first appear controversial, considering that the same genes are expressed in individuals with different clinical profiles of HTLV-1 infection: cells that express *CCR4* are the preferred target cells for viral transmission and/or persistence, as seen mainly in ATLL (Bai et al., 2017), *XCL1* has been shown to be a serum marker of myelopathy (Haynes et al., 2010), and the *JUP*-regulated signaling pathway is associated with secondary cancer cell lines in patients with ATLL (Tricarico et al., 2020). However, data show these regulatory pathways are activated to promote immune control of infection, but it is chronic activation due to viral persistence that causes systemic damage, as immune homeostasis of the host response is expected for satisfactory control (Watanabe et al., 2020).

Most of the genes represented in the gene expression profiles in the asymptomatic group are related to cell cycle regulation processes. Lysine demethylase 5D (*KDM5D*), the most upregulated gene in asymptomatic individuals, and its paralog *KDM5C* encode lysine demethylases that function as epigenetic regulators of large chromatin-modifying complexes and that strongly influence gene expression and processes related to DNA repair, including the recruitment of other epigenetic modifiers and transcription factors to specific sites on chromatin (Niederer et al., 2014).

Studies have shown that alterations in host DNA occur in the asymptomatic phase of HTLV-1 infection (Yeh et al., 2016). In this phase, the HTLV-1 provirus exhibits a tendency to integrate into transcriptionally active regions that favor the expression of viral genes (Chu et al., 2021). Indeed, differential expression of nuclear and cytoplasmic factors was observed in a transcriptomic analysis of cell lines transformed by HTLV-1, which correlated with the activity of viral proteins (Kataoka et al., 2015). Additionally, alterations in the native process of DNA demethylation were found to presuppose the presence of previously detected malignancies in patients with ATLL in a Brazilian population (Pessôa et al., 2023).

We highlighted the upregulation of the noncoding RNA *LINC02470* in both clinical profiles of HTLV-1 infection. The immediate target genes of this noncoding RNA (*NFKB*, *SMAD3*, *CDK4*, *CCNE1* and *CCNE2*) were visualized in interaction networks, with the interactomes of *CCNE1* and *CCNE2* notable in the asymptomatic group. These findings contribute to the knowledge about the prominent control of cell activity already observed in asymptomatic individuals, as E-type cyclins also represent key components of the central cell cycle machinery, as they primarily phosphorylate several regulatory proteins of the G1 and S phases (Nath et al., 1999; Itoh et al., 2018).

A recent Brazilian study showed the expression of relevant microRNAs (miRNAs) in the control of the G1/S transition of the cell cycle in a patient with ATLL after maintenance therapy based on CHOP chemotherapy followed by IFN- α and zidovudine, with the *CCNE1* gene being one of the targets of the interaction pathway (Pessôa et al., 2023). In another Brazilian study, differential expression of 128 miRNAs, 34 of which were novel, was detected in asymptomatic patients, with the most evident biological functions of the differentially expressed miRNAs also related to the control of cell proliferation, differentiation and apoptosis (Valadão de Souza et al., 2020). These findings support our finding of the prevalence of differentially expressed noncoding RNAs interfering with pathways related to cell cycle control among patients with different clinical profiles of HTLV-1 infection. The differences in the RNA expression profiles observed in different Brazilian cohorts may be due to several factors, including the ethnic origin of the population under investigation, as well as the presence of other associated clinical conditions.

Our data showed that the *HLA-A* gene was expressed in asymptomatic individuals, a contradictory finding if we evaluate whether the cellular control generated by viral infection leads to marked maintenance of the immune response (Andrade et al., 2013). Interestingly, one study showed that HLA class I expression induces cell proliferation through functional inhibition of the retinoblastoma protein (Rb) mediated by an interaction with the E-type cyclin pathway (Lubben et al., 2007).

We thus explored whether this interaction pathway is applicable and found that *HLA-A* is involved in the interaction. The physical interaction network model was very conclusive in inferring the interactome of candidate genes. The most parsimonious interactions among the *HLA-A*, Rb and *CCNE1* proteins involved four intermediate pathways, represented by *PACS1-MYC*, *CD8A-CDK1*, *TAP1-PSMD10* and *CALR-CDK2* protein interactions (Supplementary Figure S1). These interaction pathways have not yet been clarified; however, some lines of evidence indicating their plausibility have been published. For example, interactions of *HLA-A* with *PACS1* and *MYC* have

been described, even as a pathway deregulated in individuals with HIV infection (Zhao and Day, 2001; Lubben et al., 2007). In addition, the MYC oncoprotein appears to interact with Rb (Zhao and Day, 2001) and CCNE1 (Nakayama et al., 2016) in cell cycle maintenance.

Collectively, these data suggests a complex system of interactions in which *HLA-A* signaling activates an alternative pathway that regulates the cell cycle. In this context, strategic control of HLA expression can spontaneously inhibit the expression of interferons (IFNs), as suggested by studies that evaluated signaling pathways shared between these factors (Browne et al., 2006; Jørgensen et al., 2020). This possibility could justify the lack of detection of genes related to IFNs in infected individuals, in contrast to expectations (Bidkhorri et al., 2020).

Most genes that were upregulated in asymptomatic individuals were also expressed in individuals with HAM, indicating a transient and relevant gene expression profile during the progression of infection. Over time, the active contribution of the cellular machinery in asymptomatic individuals likely results in a balance in the expression of viral genes and infected cells, with the targeted immune response being a selection (Chu et al., 2021). However, notorious immune dyshomeostasis is a characteristic of HAM pathogenesis and is strongly hypothesized to be a risk determinant (Cook et al., 2013). This observation may clarify the upregulation of 11 genes related to the immune response, especially HLA class II genes, in patients with HAM.

Our results reinforce the immunological plasticity of HLA types in the anti-HTLV response. Although the balance of *HLA-A* and *HLA-DR* expression seems to favor clinical progression, the expression of *HLA-C* and *HLA-DQB* suggests an attempt to control the infection. These data are plausible if we consider that this plasticity is well recognized, even among alleles of a single HLA type (Andrade et al., 2013).

We inferred that the non-HLA genes expressed in the HAM group may interact in the HLA regulation pathway. This inference seems logical given that *NFKB* (Souri et al., 2019) and *EGR3* (Li et al., 2012) regulate *HLA* expression in active T cells; moreover, interestingly, some of the miRNAs downregulated in patients with HAM may favor the expression of *NFKB* and likely promote cellular senescence (de Souza et al., 2023). There is no clear interaction pathway between the protein products of the *CXCL8* and *HLA* genes; however, the coexpression state of the factors observed in our study may be linked to the relationship between *CXCL8* and the activation of CD4⁺ T lymphocytes (Crespo et al., 2018). Finally, members of the *LILRB3* gene family may be associated with maintenance of the immune response through HLA interactions (Bashirova et al., 2014).

An immunological counteroffensive is likely mounted via the expression of the *DEFA3* gene in individuals with HAM, as defensins constitute a family of antimicrobial peptides related to the antimicrobial activity of phagocytes, inflammatory fluids and epithelial secretions (Ganz, 2003). The mechanism by which the defensin pathway may influence the pathogenesis of HTLV-1 infection. However, in one study, it was proposed that one of the functional outcomes of the serum vitamin D imbalance in individuals with HAM is an imbalance in defensin production and a consequent impact on the antiviral response (Derakhshan et al., 2020).

In the present study, we used RNA-Seq analysis to investigate differences and similarities in gene expression profiles among noninfected subjects, asymptomatic carriers of HTLV-1 and patients with HTLV-1-associated HAM. Although the small sample size is a limitation of our study, which may require the reconsideration of further conclusions and inferences, we believe that the results are interesting and demonstrate a gene expression profile suggestive of an association with the clinical progression of HTLV-1 infection. However, further multicenter studies utilizing RNA-Seq analysis in a larger cohort, as well as quantitative analysis of gene expression by quantitative PCR (qPCR), are needed to confirm our results. Finally, we recognize that future studies involving functional assays or *in vitro* experiments will help to elucidate the functional significance of the gene expression alterations identified herein.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found in the Gene Expression Omnibus (GEO) repository, accession number GSE224047 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE224047>).

Ethics statement

The studies involving humans were approved by Comitê de Ética em Pesquisa da Universidade Federal do Pará. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LS: Formal Analysis, Investigation, Methodology, Software, Validation, Writing—original draft, Writing—review and editing. RF: Investigation, Methodology, Resources, Writing—review and editing. VA: Conceptualization, Funding acquisition, Supervision, Writing—review and editing. MF: Formal Analysis, Investigation, Methodology, Writing—review and editing. CD: Investigation, Methodology, Writing—review and editing. RR: Data curation, Methodology, Writing—review and editing. SP: Data curation, Investigation, Methodology, Writing—review and editing. CD: Investigation, Methodology, Resources, Writing—review and editing. MS: Writing—review and editing. IV: Conceptualization, Project administration, Writing—review and editing. AC: Conceptualization, Investigation, Methodology, Supervision, Writing—review and editing. AR: Conceptualization, Funding acquisition, Project administration, Supervision, Writing—original draft, 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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frnar.2024.1419833/full#supplementary-material>

SUPPLEMENTARY DATA S1

Sequencing quality control summary data.

SUPPLEMENTARY DATA S2

Summary of sequencing quality control.

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