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RESEARCH ARTICLE

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Gene expression analysis identifies hub genes and pathways distinguishing fatal from survivor outcomes of Ebola virus disease

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Abstract

The Ebola virus poses a severe public health threat, yet understanding factors influencing disease outcomes remains incomplete. Our study aimed to identify critical pathways and hub genes associated with fatal and survivor Ebola disease outcomes. We analyzed differentially expressed hub genes (DEGs) between groups with fatal and survival outcomes, as well as a healthy control group. We conducted additional analysis to determine the functions and pathways associated with these DEGs. We found 13,198 DEGs in the fatal and 12,039 DEGs in the survival group compared to healthy controls, and 1873 DEGs in the acute fatal and survivor groups comparison. Upregulated DEGs in the comparison between the acute fatal and survivor groups were linked to ECM receptor interaction, complement and coagulation cascades, and PI3K-Akt signaling. Upregulated hub genes identified from the acute fatal and survivor comparison (FGB, C1QA, SERPINF2, PLAT, C9, SERPINE1, F3, VWF) were enriched in complement and coagulation cascades; the downregulated hub genes (IL1B, 1L17RE, XCL1, CXCL6, CCL4, CD8A, CD8B, CD3D) were associated with immune cell processes. Hub genes CCL2 and F2 were unique to fatal outcomes, while CXCL1, HIST1H4F, and IL1A were upregulated hub genes unique to survival outcomes compared to healthy controls. Our results demonstrate for the first time the association of EVD outcomes to specific hub genes and their associated pathways and biological processes. The identified hub genes and pathways could help better elucidate Ebola disease pathogenesis and contribute to the development of targeted interventions and personalized treatment for distinct EVD outcomes.

KEYWORDS

bioinformatics analysis, Ebola, EVD outcomes, hub genes, PPI

1 | INTRODUCTION

Ebola virus (EBOV) is an extremely pathogenic virus that causes the highly infectious hemorrhagic fever disease, Ebola virus disease (EVD). EVD has a case fatality rate (CFR) of up to 90%, posing a significant global public health threat.[1–3] The largest EVD outbreak in West Africa occurred between 2013 and 2016, with over 28,000 cases and 11,300 fatalities. Guinea, the epicenter of the outbreak, recorded a fatality rate of approximately 60%.[1,4,5] The factors determining survival or fatality in EVD are still not well understood. Advancing our comprehension of these mechanisms is crucial for implementing effective management strategies, developing therapeutic treatments, and formulating vaccines.

Current research on EVD underscores that the severity of the disease and its high CFR are primarily attributed to the host's response to the virus. EBOV disrupts multiple facets of the immune response, triggering cytokine storms, cell death, and multi-organ failure.[6–9] Additional indirect effects, such as the inhibition of Type I Interferons, impairment of dendritic cells, and perturbation of cytokine/chemokine networks, further compromise essential bodily functions.[10,11] Disparities in immune responses have been observed between EVD patients, who survived and those who succumbed. EVD fatalities are linked to robust immune suppression, high viral titers, dysregulated inflammatory responses, and diminished immune cell responses. EVD survivors on the other hand, have shown strong innate immune response, increased immune cell responses and a higher and persistent antibody response.[12–14] Further EVD research, however, is still needed to elucidate the underlying mechanisms of these observations.

Genomic research on pathogenic microorganisms and associated diseases has become imperative for a deeper understanding of their underlying pathogenic mechanisms. Next-generation sequencing and bioinformatics analyses offer an invaluable approach for comprehensive studies of diseases.[15,16] Ebola virus research has employed various approaches, including transcriptomic and proteomic analyses, to identify factors that differentiate disease outcomes.[12,17,18] However, these studies have only made partial progress in identifying transcriptomic and proteomic profiles associated with certain outcomes. Therefore, further investigations are necessary to identify factors associated with EVD outcomes and fully elucidate the mechanisms governing disease outcomes.

Hub genes, as highly connected and influential players in biological networks, hold significant promise in elucidating Ebola disease outcomes.[19–21] Analyzing the network properties of genes associated with different disease courses can unravel critical information about underlying mechanisms. Identifying hub genes provides a holistic view of the molecular interactions that dictate survival or fatality. These hub genes may act as central coordinators in biological processes crucial to the host's response to Ebola virus infection.[22,23] Their identification can serve as key indicators of disease severity, aiding in the classification of patients into distinct prognostic groups. Additionally, hub genes may offer insights into potential therapeutic targets, as their perturbation could have widespread effects on disease-related pathways. The use of hub gene analysis has proven valuable in understanding and identifying therapeutic targets for various diseases, including infectious diseases like COVID-19, neurodegenerative conditions like Alzheimer's, and various cancer types.[24–26] In the specific context of Ebola, unraveling the roles of hub genes may pave the way for a better understanding of the molecular profiles associated with different disease outcomes.

This study seeks to unravel the pathogenic mechanisms of Ebola and delineate disease outcomes. We analyzed publicly available deep sequencing data obtained from the peripheral blood of acutely ill (resulting in either fatal or survival outcomes) and healthy patients. Our analysis aimed to identify highly connected hub genes and associated pathways that distinguish fatal from survival outcomes in Ebola.

2 | MATERIALS AND METHODS

2.1 | Datasets

The datasets for this study were obtained from NCBI BioProjects ID PRJNA352396 and PRJNA600939. BioProject PRJNA352396 contains 174 deep sequencing datasets made up of 113 acute fatal, 45 acute survival, and 16 convalescent datasets. BioProject PRJNA600939 contains 472 runs made up of 208 samples from Ebola disease survivors and 264 samples from healthy individuals. A total of 60 samples were obtained for this study (20 acute survival and 20 acute fatal samples from NCBI BioProject PRJNA352396, 20 healthy individual samples from BioProject PRJNA600939).

2.2 | Differential gene expression analysis

The raw fastq files were trimmed to remove adapters and low-quality sequences using the Trim Galore trimming tool. Trimmed data was aligned to the T2T consortium human genome (T2T-CHM13v2.0) using the HISAT2 alignment tool.[27,28] Total aligned read counts were obtained using featureCounts (version 2.0.1),[29] and subsequent differential gene expression (DGE) analysis was performed by utilizing DESeq2 (p-value <0.05, $\log_2$ fold change >1).[30] The fatal and survival outcomes data were

compared to the healthy controls as well as to each other. The Galaxy bioinformatics server (locally installed, version 22.05) was utilized for all the DGE analysis, as described.[31–33] The DEGs were visualized using heatmaps created using Broad Institute's Morpheus heatmap tool (https://software.broadinstitute.org/morpheus). DEGs were subjected to functional analysis by utilizing the Database for Annotation, Visualization, and Integrated Discovery (DAVID).[34,35] Associated KEGG pathways and Gene Ontology (GO) biological processes were recorded.

2.3 | Protein–protein-interactions and hub genes identification

The Cytoscape software platform[36] was used to construct protein–protein-interaction networks (PPIs) among the DEGs using the STRING (search tool for recurring instances of neighboring genes) add-on with a maximum confidence of 0.90. The top 10 hub genes from the PPIs were identified with the MCC (Maximal Clique Centrality) algorithm of CytoHubba add-on of Cytoscape.[37]

2.4 | Gene Set Enrichment Analysis

Hub genes were subject to gene set enrichment analysis (GSEA) using Enrichr[38] to identify linked pathways and biological processes. Enrichr is an intuitive enrichment analysis web-based tool for analysis of gene sets. The tool returns common annotated biological features that represent the gene set provided.

2.5 | Statistical analysis

All statistical analysis was performed using the inbuilt analysis of the bioinformatics tools described above running in a locally installed version Galaxy bioinformatics server environment (version 22.05). Graphs and Venn diagrams associated with our data were created using RStudio statistical and graphics software environment version 2023.06.1 + 524.

3 | RESULTS

3.1 | Identification of differentially expressed genes in acute-fatal and acute-survivor Ebola disease outcomes

To identify genes differentially expressed during Ebola virus infection, the acute survivor and acute fatal groups were compared to the healthy controls and to each other (Figure 1). We identified 9236 upregulated genes and 3962 downregulated genes in the acute fatal group as compared to the control group (panel A); 7771 upregulated genes and 4268 downregulated genes were also identified in the acute survivor group as compared to the control group (panel B). The acute fatal and acute survivor group comparison produced 1478 upregulated genes and 395 downregulated genes (panel C).

3.2 | Identification of pathways and biological processes associated with differentially expressed genes in Ebola disease

To identify important pathways and biological processes linked to the differentially expressed genes (DEGs), functional enrichment analysis was performed. DEGs from the acute fatal and acute survivor comparison were linked to pathways including ECM receptor interaction, complement and coagulation cascades and PI3K-Akt signaling (Figure S1, Data S1). KEGG pathway and GO analysis showed that acute fatal and acute survival group (compared to healthy controls) DEGs were linked to similar pathways and biological processes (Figures S2, S3, Data S2). These pathways and processes showed different levels of enrichment in both groups as shown by their *p*-values. We found that upregulated genes from the acute fatal group (vs. the healthy control group) were more significantly enriched in pathways such as complement and coagulation cascades and calcium signaling. The acute survivor group DEGs on the other hand were more enriched in neutrophil extracellular trap formation, adaptive immune response and cytokine and cytokine receptor interactions (Figures S2, S3, Data S2). These results highlighted crucial pathways, and biological processes significantly associated with EVD outcomes. Pathways central to Ebola pathogenesis fall under the immune, signaling, and regulatory pathway categories[17,39–41] and were the focus of further analysis in this study.

3.3 | Construction of protein–protein interaction networks and identification of hub genes associated with fatal and survival Ebola disease outcomes

Previous reports identified factors contributing to Ebola disease outcomes; these include interferon signaling, acute phase response, and coagulation cascades.[17,40] These factors, however, do not fully explain the mechanisms underlying fatal and survivor EVD outcomes.

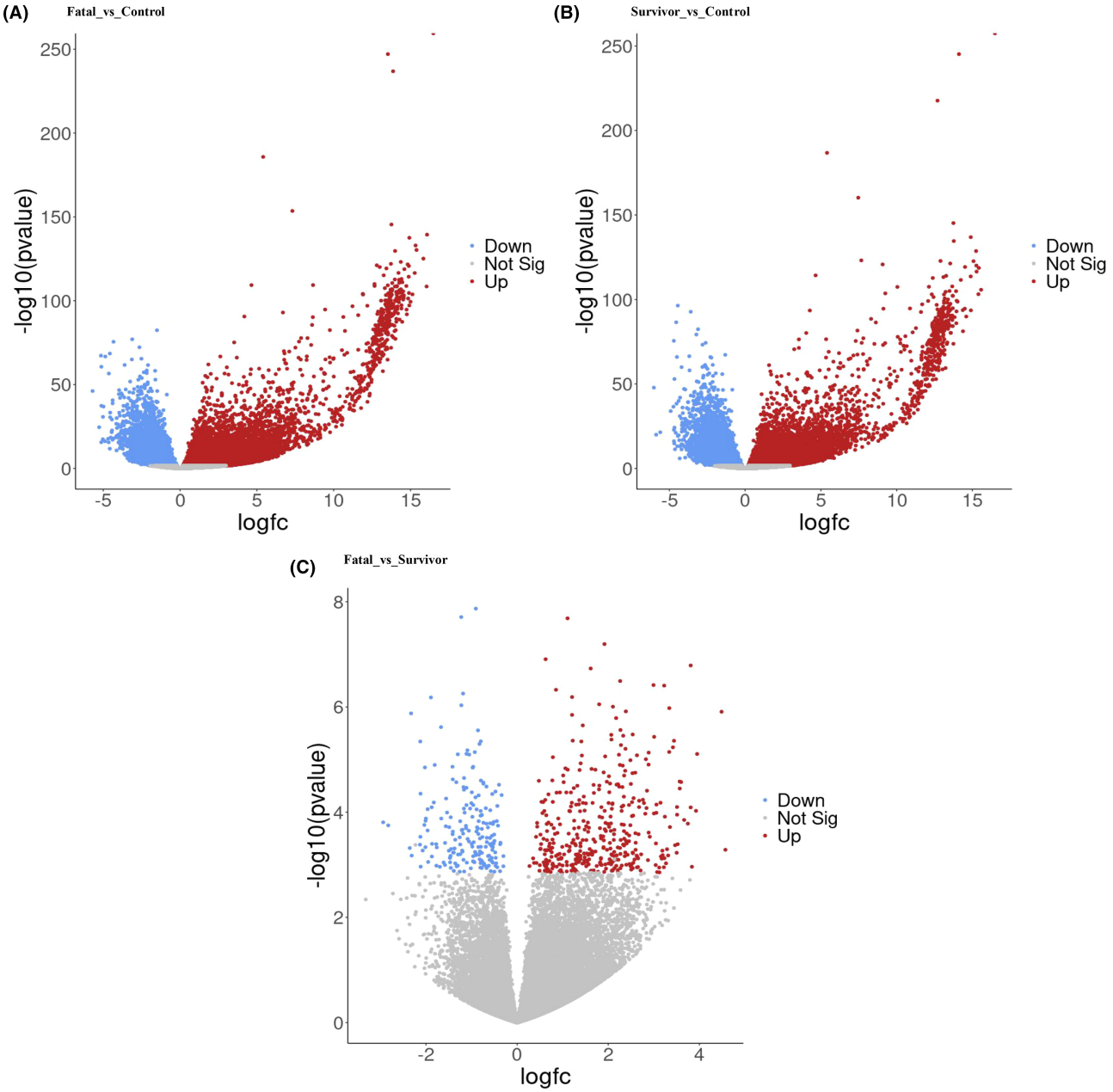

FIGURE 1 Volcano Plots of differentially expressed genes from EVD patients showing (A) fatal compared to healthy controls, (B) survival compared to healthy controls, and (C) fatal compared to survival outcomes. Red represents significantly upregulated genes; blue represents significantly downregulated genes, and gray represents genes not significantly expressed.

To identify important hub genes expressed in different EVD outcomes, we constructed protein–protein interaction (PPI) networks from genes associated with immune system, immune signaling, and regulatory pathways. PPI networks have been shown to be vital in identifying principal elements of biological networks.[42,43] PPIs can be employed to identify essential hub genes in pathways and processes where similarities in expression levels, as seen in our data (Figure S4, Data S1, S2), can be expressed as networks.[37,44,45] The top 10 hub genes identified for each category from the direct fatal/survival comparisons are shown in Figure 2; the expression levels and functions of all hub genes in Figure 2 are shown in Data S3.

The hub genes linked to acute fatal and acute survivor groups as compared to healthy controls were also identified (Figure S5). Common and unique hub genes specifically linked to fatal, or survival outcomes were identified by comparing all the hub genes linked to the outcomes (Figure 3). The upregulated hub genes unique to the fatal group were CCL22, and F2 genes, while CXCL1, HIST1H4F, and IL1A were associated only with the survival group (panel A). The downregulated hub genes common and unique to the fatal group as compared

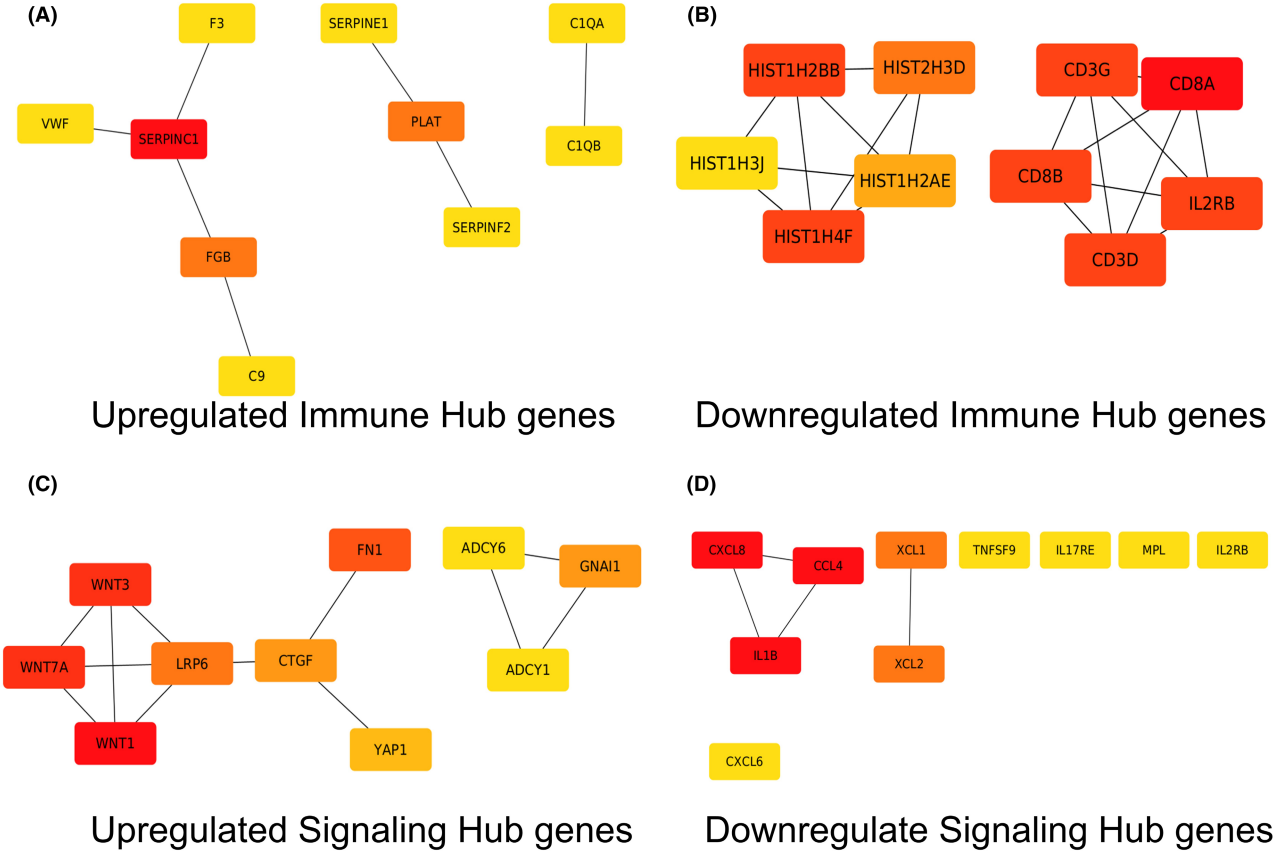

FIGURE 2 Hub genes identified using Cytoscape/cytoHubba analysis of DEGs from acute EVD patients with fatal outcomes compared to those with survival outcomes. (A) Upregulated genes from immune system pathways. (B) Downregulated genes from immune system pathways. (C) Upregulated genes from signaling molecules and signal transduction pathways. (D) Downregulated genes from signaling molecules and signal transduction pathways.

to the survivor group are shown in Figure 3B. Our findings demonstrated that EVD outcomes are linked to specific hub genes that can serve as markers to differentiate these outcomes and help further explain pathogenic mechanisms.

3.4 | Gene Set Enrichment Analysis and identification of unique hub genes linked to fatal and survival Ebola outcomes

To identify important pathways and processes hub genes were significantly enriched in, we performed a Gene Set Enrichment Analysis (GSEA) utilizing Enrichr. The results of GSEA, including pathways and GO biological processes, are shown (Figure 4, Figures S6, S7). Upregulated hub genes from the direct comparison between the acute fatal and acute survivor groups were mostly associated with complement and coagulation cascades (Figures 4A, 5 and Data S4). Downregulated genes, on the other hand, were associated with cytokine and chemokine signaling and immune cell pathways and processes (Figures 4B, 6 and Data S4). In comparison to the healthy group, the upregulated hub genes were mostly associated with cytokine and chemokine interactions, while downregulated genes linked to immune cell signaling and differentiation in both the acute fatal and acute survivor groups (Figures S6, S7, Data S5). These results highlighted that hub genes were linked to important pathways, and biological processes associated with fatal and survivor Ebola disease outcomes.

4 | DISCUSSION

The pathogenic mechanisms of Ebola and factors delineating EVD outcomes are still not well understood. In this study, we identified essential hub genes and pathways associated with fatal and survival outcomes in EVD. We identified 13,198 DEGs in the fatal and 12,039 DEGs in the survival group compared to healthy controls, and 1873 DEGs in the acute fatal and survivor groups comparison. Upregulated DEGs in the acute fatal versus survivor group comparison were linked to ECM receptor interaction, complement and coagulation cascades, and PI3K-Akt signaling. Upregulated hub genes identified

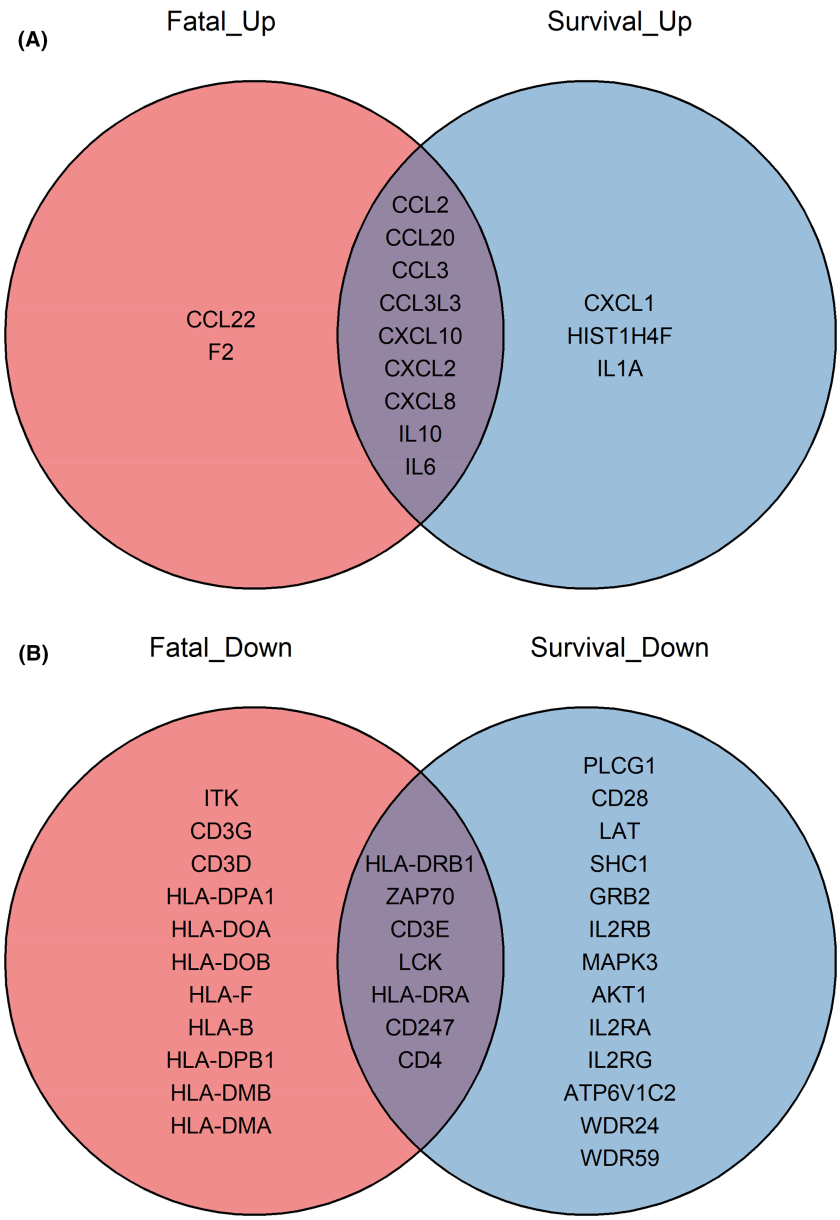

FIGURE 3 Venn diagram showing hub genes common and unique to fatal and survival outcomes. (A) Upregulated hub genes from fatal and survival groups versus healthy controls (B) downregulated hub genes from fatal and survival groups versus healthy controls.

from the acute fatal and survivor direct comparison (FGB, C1QA, SERPINF2, PLAT, C9, SERPINE1, F3, VWF) were enriched in complement and coagulation cascades; the downregulated hub genes (IL1B, 1L17RE, XCL1, CXCL6, CCL4, CD8A, CD8B, CD3D) were associated with immune cell processes. Additional hub genes were identified by comparing fatal and survivor cases to healthy controls; of these genes, CCL22 and F2 were unique to fatal outcomes, while CXCL1, HIST1H4F, and IL1A were upregulated hub genes unique to survival outcomes. Collectively, our findings demonstrate for the first time the expression of specific hub genes and pathways closely linked to fatal and survival EVD outcomes.

Our study identified 9236 upregulated and 3962 downregulated genes in the acute fatal group; 7771 upregulated and 4268 downregulated genes were also identified in the acute survivor group compared to healthy controls. Liu et al. identified 2200 upregulated genes in the acute-fatal group, and 1300 upregulated genes in the acute-survivor group compared to a convalescent control group.[17] In our study, we employed a healthy EBOV-uninfected control group compared to the convalescent group used in the Liu et al. study. The use of the healthy uninfected control group presents a more accurate baseline for cellular gene expression.[46,47] Furthermore, our use of the latest T2T-CHM13v2.0 T2T human genome reference, provides us the opportunity for newer discovery which was not previously available.[48,49]

We found that DEGs from the acute fatal and acute survival groups were linked to pathway and processes such as complement and coagulation cascades, neutrophil extracellular trap formation, cytokine–cytokine receptor interactions and calcium signaling. The complement and coagulation cascades and calcium

FIGURE 4 GSEA of hub genes with their enriched pathways and biological processes in fatal compared to survival groups. (A) Upregulated hub genes network. (B) Downregulated hub genes network.

signaling were more enriched in the fatal group. These findings are in line with previous findings that linked coagulation to Ebola pathogenesis and associated it with vascular leakage and the multiple organ failure seen in fatal EVD outcomes.[50,51] The survivor group showed enrichment in extracellular trap (NET) formation, Th1 and Th2 cell differentiation, and adaptive immune response. Staples (2020),[52] has reported NETs formation during Ebola infection but their role in pathogenic mechanisms, or disease outcomes have not been elucidated. Survivors of EVD, however, have been shown to mount a well-balanced immune response that includes appropriate T cell activation supportive of survival.[13,50] Thus, our findings are aligned with these previous observations, as well as to an additional study that revealed similar results[17] These results re-emphasize the association of fatal EVD to significant activation of complement and coagulation factors while showing that the survivor group benefits from proper activation of immune cell processes.

Our study found IL10, CCL3, CCL3L3, CXCL2, CXCL10, CCL2, CXCL8, CCL20, IL6, F2, CCL22 CXCL1, IL1A, and HIST1H4F/H4C6 as upregulated hub genes in fatal and survivor groups (vs. control). In addition, the upregulated hub genes F2 and CCL22 were highly linked to the acute fatal group, while CXCL1, IL1A, and HIST1H4F/H4C6 were associated with the acute survivor group. F2 and CCL22 have been shown to play roles in coagulation issues and immune cell recruitment.[53–56] F2 plays a role in the coagulation abnormalities observed during Ebola virus infection.[7,53] The infection triggers widespread activation of the coagulation cascades, resulting in excessive consumption of coagulation factors, including prothrombin (F2).[11] Prothrombin is converted to thrombin during the coagulation process, and

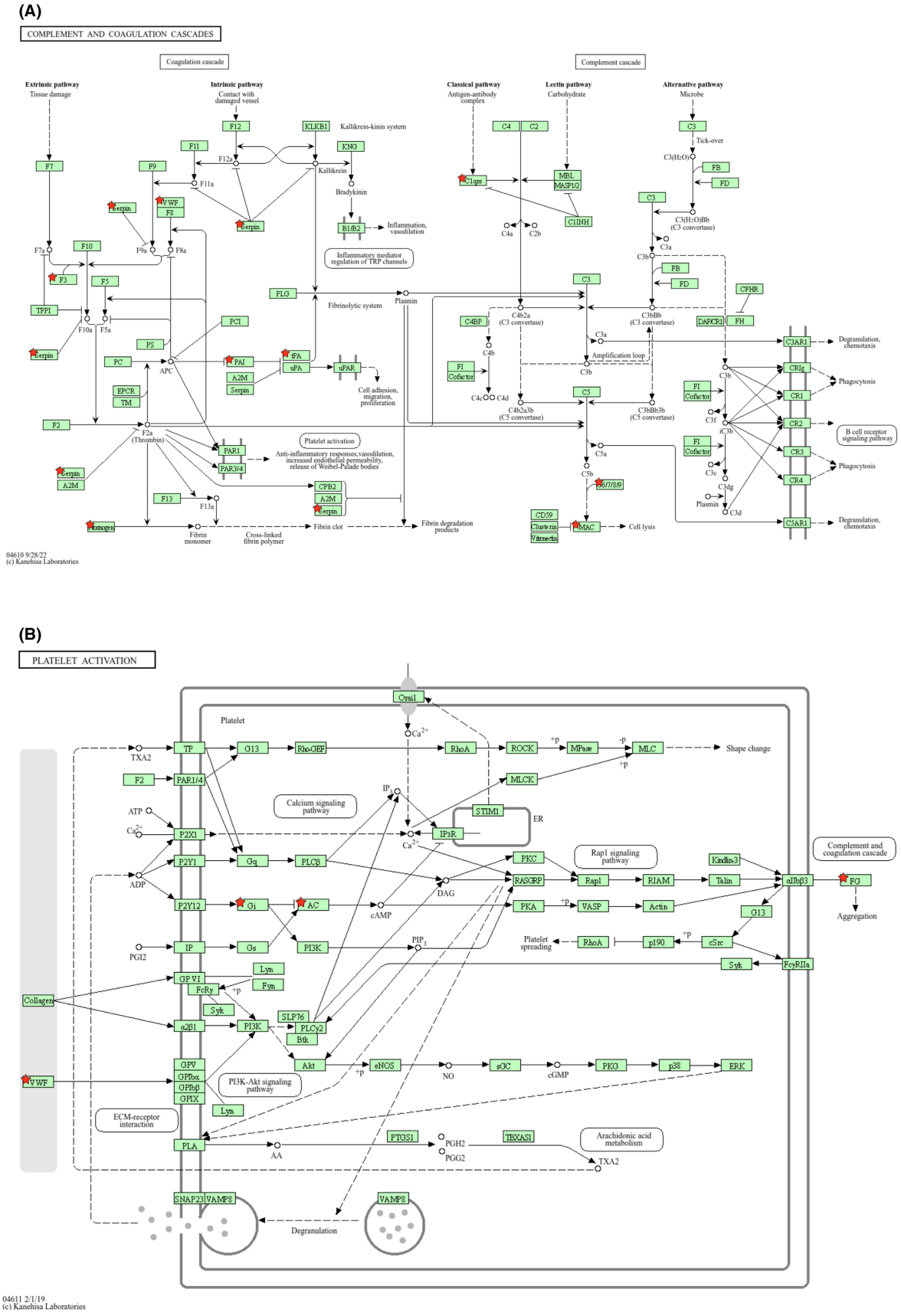

FIGURE 5 KEGG pathways associated with upregulated hub genes from fatal versus survival comparison. (A) Complement and coagulation cascades. (B) Platelet activation. Hub genes are shown in red stars.

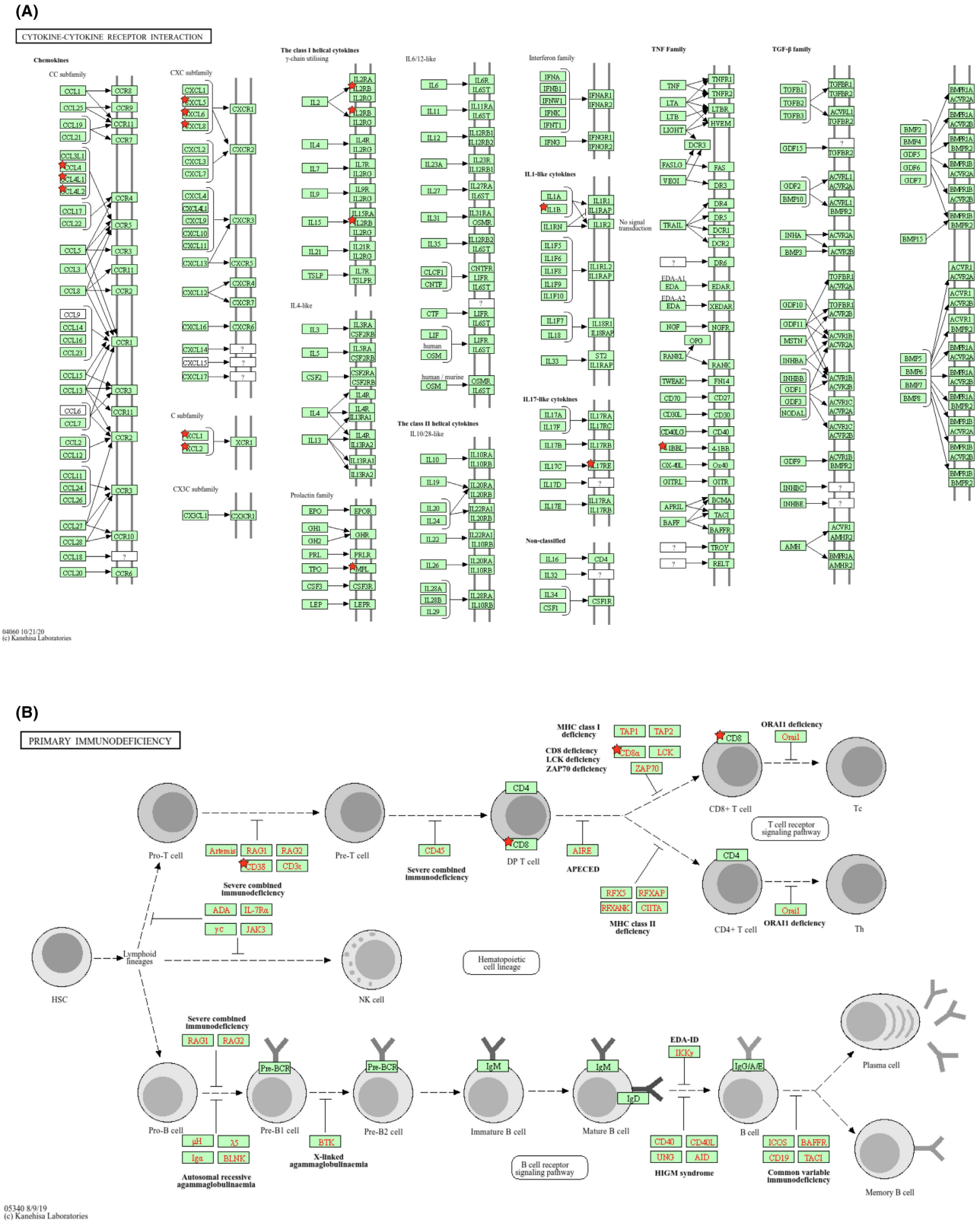

FIGURE 6 KEGG pathways associated with downregulated hub genes from fatal versus survival comparison. (A) Cytokine–cytokine receptor interactions. (B) Primary immunodeficiency. Hub genes are shown in red stars.

elevated levels of thrombin contribute to microvascular thrombosis and subsequent hemorrhage due to the depletion of coagulation factors and platelets. Thrombin activation leads to disseminated intravascular coagulation (DIC), hemorrhagic manifestations, and endothelial dysfunction, all of which are critical components of the severe vascular and systemic effects observed in EVD.[7,11,53] On the other hand, CCL22 plays a significant role in immune evasion and modulation during Ebola virus infection. Its function in attracting regulatory T cells (Tregs) and impacting dendritic cell function is crucial for the virus to evade immune detection and suppression, weakening the host's antiviral response.[57] The recruitment of Tregs by CCL22 promotes the inhibition of effector T-cell responses, which are crucial for viral control and clearance.[55,58] Additionally, CCL22's contribution to the cytokine storm observed in severe EVD cases intensifies the systemic inflammatory response and leads to severe disease manifestations, including tissue damage, vascular leakage, and multi-organ failure.[10,11,59] The coordinated dysregulation of F2 and CCL22 genes, given their functions, would create an atmosphere for coagulation abnormalities and increased infectivity of the Ebola virus; this may decrease patients' chances of survival in the acute fatal group.

CXCL1, IL1A, and HIST1H4F/H4C6 on the other hand, are known to play roles in immune cell recruitment and inflammation.[60–63] CXCL1 rapidly recruits neutrophils to infected tissues during viral infections. Neutrophils enhance the inflammatory response and help control viral infections through the release of antimicrobial proteins and reactive oxygen species (ROS).[64] A study found that reduced early neutrophil recruitment correlated with better survival outcomes in EVD.[65] IL1A is a pro-inflammatory cytokine that plays a significant role in the immune response to infections including EVD. It stimulates the production of various cytokines and chemokines, which amplifies the inflammatory response.[66] IL1A also activates endothelial cells, promoting the recruitment of immune cells to the sites of infection. However, excessive production of IL1A can lead to a hyper-inflammatory state known as a cytokine storm, which can result in severe tissue damage, organ failure, and poor survival outcomes.[67] The coordination among CXCL1, IL1A, HIST1H4F/H4C6, and their associated genes may lead to a more robust immune response against infection, giving the acute survivor patient group a better chance at survival.

Downregulated hub genes in the fatal group were primarily from the HLA group, while AKT1, ATP6V1C2, CD28, GRB2, IL2RA, IL2RB, IL2RG, LAT, and MAPK3 were found as hub genes in the survival group only (vs. control). The pathways and processes associated with these hub genes were mainly related to immune cell pathways and antigen processing and presentation, which were significantly more enriched in the fatal group as compared to the survivor group. The HLA group of genes plays a major role in the induction and regulation of immune response.[68–70] HLA molecules present viral antigens to T cells, facilitating the detection and clearance of the virus.[69] Efficient presentation of viral antigens is essential for mounting a robust antiviral response. Ebola virus can evade immune surveillance by downregulating HLA class I molecules on the surface of infected cells. This impairs the recognition of infected cells by cytotoxic T lymphocytes (CTLs), allowing the virus to evade destruction.[71] The down regulation of these genes indicates a significant suppression of the body's immune response in the case of the fatal group, allowing the virus to evade immune clearance.[72,73] On the other hand, the survivor group would benefit from the body's ability to recognize Ebola virus proteins and effectively clear the virus. These findings were further validated in the study of DGE between the acute-fatal group and the acute-survival group.

Our study found that fatal Ebola cases (compared to survivors) had upregulated hub genes such as FGB, C1QA, SERPINF2, F3, SERPINC1, and C1QB, which were enriched in complement and coagulation cascades. Conversely, hub genes related to immune and inflammatory signaling such as CXCL8, CCL4, CD3G, CD8A, CXCL, and IL1B were downregulated in the fatal versus survivor comparison. The upregulated genes have been reported to contribute to severe immune and organ dysfunction observed in fatal Ebola cases.[74,75] The downregulation of immune and inflammatory signaling may lead to a more pronounced immune system suppression in the acute fatal group compared to the acute survival group. Therefore, our findings suggest a significant association of fatal outcomes in EVD with potent immunosuppression and a significant overactivation of the complement and coagulation systems. This can result in severe immune and tissue/organ dysfunction, significantly reducing the chances of recovery for patients in the acute fatal group.[10,59]

In this study we identified hub genes and pathways associated with fatal and survival Ebola disease outcomes. Our results demonstrate for the first time the association of EVD outcomes to specific hub genes and their associated pathways and biological processes. Fatal EVD outcomes are closely linked to activation of complement and coagulation cascades and a suppression of antigen processing and presentation and immune cell activation and response. Survivors on the other showed an upregulation in genes linked to immune activation and inflammatory response suggesting a robust and controlled immune response.

In summary, we have explored the highly connected hub genes associated with EVD to identify factors that differentiate fatal from survival EVD outcomes. We have provided insights into critical molecular interactions and factors contributing to different EVD outcomes. These findings contribute specific hub genes that could serve as factors for differentiating Ebola disease outcomes and improve identification of patients in acute need of extra care. By identifying critical molecular interactions and hub genes, this study contributes to the development of targeted interventions and personalized treatment for distinct EVD outcomes. The hub genes identified could also serve as therapeutic targets for drug discovery to improve outcomes for those diagnosed with EVD.

AUTHOR CONTRIBUTIONS

Clay Gloster: Conceptualization (equal); funding acquisition (lead); project administration (lead); supervision (supporting); writing—review and editing (supporting); **Perpetua Muganda:** Conceptualization (equal); funding acquisition (supporting); project administration (supporting); supervision (lead); methodology (equal); writing—original draft (supporting); writing—review and editing (equal); **Christopher Doss:** Conceptualization (supporting); funding acquisition (supporting); project administration (supporting); supervision (supporting); writing—review and editing (supporting) **Melvin Mensah-Bonsu:** Methodology (equal); investigation (lead); visualization (lead); writing—original draft (lead); formal analysis (lead); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data generated/analyzed during the current study are available in the Supplemental files. Raw data files analyzed were obtained from the NCBI/SRA database (SRA datasets SRP092544 and SRP241739).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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