ISSN: 2997-2728

Research Article

Journal of Biotechnology and Bioinformatics

Bioinformatics Approach is Unraveling Potential Crosstalk between Cytomegalovirus and Epilepsy

Salim Al Rashdi[1] and Nabras Al Mahrami[2*]

[1]*Student, Midical Laboratory Sciences department, Oman College of Health Sciences. Muscat, Oman*

[2]*Bioinformatician, National Genetics Center, Royal Hospital, Ministry of Health, Muscat Oman*

[*]Corresponding Author
Nabras Al Mahrami, Bioinformatician, National Genetics Center, Royal Hospital, Ministry of Health, Muscat Oman.

Submitted: 2024, Jun 01; **Accepted**: 2024, Jun 20; **Published**: 2024, Jun 28

Citation: Al Rashdi, S., Al-Mahrami, N. (2024). Bioinformatics Approach is Unraveling Potential Crosstalk between Cytomegalovirus and Epilepsy. *J biotech bioinform,* 1(2), 01-08.

Abstract

Background: *Cytomegalovirus (CMV) is a double-stranded DNA virus that is known to be associated with congenital disorders. Epilepsy is a neurological disorder that occurs due to the inception of neurotransmitters. It is suggested that cytomegalovirus can affect epilepsy since it can reach the brain. This study aimed to investigate the molecular crosstalk between epilepsy and Cytomegalovirus infection using a bioinformatics approach.*

Methods: *We used gene expression datasets related to each condition retrieved from a public database. Differentially expressed gene analysis was performed for each dataset group separately. The common genes that were significantly expressed under both conditions were subjected to protein-to-protein network analysis and gene enrichment analysis.*

Results: *A total of 192 common genes were identified across the two conditions. The three genes CCL2, CD44, and CCL3 were defined as hub genes in protein-to-protein interaction networks with the highest centrality.*

Discussion: *This finding suggested the essential roles of these molecules in biological systems. Additionally, these genes are involved in inflammatory processing and the immune response.*

Conclusion: *We suggest that inflammatory chemokine molecules may participate in molecular crosstalk between CMV and epilepsy. Therefore, additional investigations are required to demonstrate the role of each suggested molecule in this association.*

Keywords: Cytomegalovirus, Epilepsy, Gene expression, Transcriptomics, Inflammatory Reaction, Biological Network

List of Abbreviations

- Cytomegalovirus (CMV)
- Differential gene expression (DGE)
- Electroencephalography (EEG)
- epidermal growth factor receptor (EGFR),
- Epilepsy (EP)
- Gene Expression Omnibus (GEO)
- Heparan sulfate proteoglycans (HSPGs)
- Human herpesvirus-5 (HHV-5)
- Inducing platelet-derived growth factor-α (pdgfrα),
- Log Fold Change (logFC)
- The National Centre for Biotechnology Information (NCBI)

1. Background

Cytomegalovirus (CMV) is also known as human herpesvirus-5 (HHV-5) and is a double-stranded deoxyribonucleic acid DNA virus that consists of a 235 kb long genome that belongs to *the Herpesviridae* family and is an icosahedral capsid enveloped in shape [1]. Infected people are divided into symptomatic and asymptomatic groups for therapeutic purposes; symptomatic individuals are usually immunocompromised, and individuals with asymptomatic infection are usually in good health, even though asymptomatic individuals do not show the clinical phenotype of the infection as symptomatic individuals. However, these two species have similar genetic expression profiles [2,3]. Patients infected with symptomatic CMV usually suffer from fever, sore throat, fatigue, and swollen glands, and infection may cause infectious mononucleosis and hepatitis, which are similar clinical features to those of the Epstein–Barr virus, which is a member of the same virus family [4]. In severely immunocompromised individuals, CMV can cause damage to the central nervous system, causing encephalitis, myelitis, and myeloradiculitis [5].

Infection with CMV is not restricted by age or sex, even though the main risk factors for CMV infection are fluid contact with infected individuals, where it can be transmitted through milk feeding and delivery in the case of congenital CMV infection and other fluids, such as urine, saliva, blood and blood, or through transplantation [4]. CMV transplantation can occur due to the entry of CMV into a latent phase in which the virus remains dormant in infected cells until it is donated to a patient, which may lead to the activation of latent CMV. Additionally, CMV can reactivate in immunocompromised situations, such as AIDS, chemotherapy or nutrient deficiency [4]. Cytomegalovirus is usually a mild infection, but in the case of a compromised immune individual, the rate of virus replication reaches a high level, causing serious end-organ disease since CMV can infect a wide range of epithelial and fibroblasts [6]. CMV introduces its genome to host cells by inducing PH-independent fusion, during which the virus enters without its envelope inside a fibroblast. Additionally, CMV can induce endocytosis, during which the whole virus enters an epithelial cell with its envelope [6]. The CMV envelope contains glycoprotein M, glycoprotein N, glycoprotein B, glycoprotein H, UL130, and UL131, which work to induce platelet-derived growth factor-α (PDGFRα), epidermal growth factor receptor (EGFR), heparan sulfate proteoglycans (HSPGs) and integrins [6].

Congenital Cytomegalovirus (CMV) is an infectious condition in which infants are infected with CMV through their mothers; they are usually unaware of their infectious condition before delivery, which leads to congenital defects in the developing brain [4]. Not all infected babies show signs and symptoms of CMV; however, some infected conditions are symptomatic and show signs at birth, such as jaundice, rash, low birth weight, hepatosplenomegaly, and retinitis, and some of them experience seizure episodes. These signs can lead to long-term health problems, including vision loss, motor development delay, microcephaly, seizures, and hearing loss [4]. Tegument proteins are proteins that exist in the space between the virus capsid and its envelope in all herpesviruses [7]. Treatment for CMV can be administered in the case of symptomatic congenital cytomegalovirus in the form of drugs such as valganciclovir to improve the development outcome [4].

Epilepsy (EP) is a neurological noncontagious disorder of the central nervous system (CNS) caused by repetitive uncontrolled electric rushes in the whole brain or in part leading to unintended or involuntary movements [8]. There are 50 million people affected by epilepsy, which is considered to be the most common neurological disorder worldwide [8]. Epilepsy is classified into two main categories: focal seizures and generalized seizures, where focal seizures involve specific involuntary movements of the body part. In contrast, generalized seizures involve the whole body [8]. Essentially, epilepsy is not restricted by specific age or sex; however, the effect of seizure episodes may vary between males and females, where males generally experience more severe seizure episodes, while women experience more seizure fluctuations [9]. Epileptic patients develop symptoms such as loss of consciousness, awareness, disturbances in movement, and loss of vision; additionally, they tend to experience physical injuries because of sudden involuntary movements, which may lead to fractures and bruising, which can cause psychological problems [8]. The incidence of EP in Arab countries is 6.9 per 1000 individuals, with the leading risk factors being parental consanguinity and family history [10]. Clinical manifestations and electroencephalography (EEG) images are used to diagnose epilepsy caused by different etiologies, such as trauma or damage in the brain; congenital abnormalities; encephalitis; and brain tumors, such as gliomas [8]. Genetic variance and mutations play a role in activating seizures, for example, *UBE3A, CDKL5,* and genes that encode ion channels in neurons, such as *KCNQ2* [11-13]. Infectious agents may cause epilepsy to develop by inducing inflammatory-mediated agent responses in brain tissue to affect cytokine secretion, leading to hyperexcitability [14]. Generally, epilepsy is caused by an error in neurotransmitter receptors such as NMDA and GABA, which enhance or inhibit the electrical signals that may lead to seizure episodes [15]. People who have epilepsy are treated with surgery in cases of focal seizure or with antiepileptic drugs such as oxcarbazepine and carbamazepine for focal and generalized epilepsy, respectively, which are selected based on patient clinical history [16].

There is notable evidence of the involvement of cytomegalovirus in epilepsy [17,18]. For example, Lin *et al* studied 112 neurological patients from January 2012 to December 2014, and the results showed that 96 patients with epilepsy had the expression of the pp67 mRNA of CMV. Another study conducted magnetic resonance imaging (MRI) on patients who had symptomatic congenital CMV. The results showed that the second most abnormal finding on MRI was polymicrogyria, which is related to epilepsy [19]. Overall, previous studies have shown that there is an association between cytomegalovirus infection and epilepsy, but the exact pathogenesis is still unclear. This study aimed to investigate the molecular crosstalk between Cytomegalovirus and epilepsy via transcriptomic and biological network approaches.

2. Materials and Methods

2.1 Data Collection

The transcriptomic datasets were collected from the Gene Expression Omnibus (GEO) database at the National Centre for Biotechnology Information (NCBI) [20]. The keywords "Epilepsy" and "Cytomegalovirus" were used for dataset analysis. The results have been limited to samples of only. For more precise data selection, the inclusion criteria were as follows: i) the dataset must contain samples from disease and control groups, ii) the dataset must contain at least eight samples, iii) the dataset can be called by the R packages "GEO2R" or "DESeq2", and iv) the expression profiling has been performed by expression microarray or RNA-sequencing. The exclusion criteria were as follows: i) samples collected from species other than *Homo sapiens*, ii) a dataset without controls, and iii) datasets associated with multiple diseases. The collected transcriptomic data are shown in Table 1.

S. NO	Accession ID	Platform	Sample count (case/control)	Reference
1	GSE108211	GPL10558	CMV (148/10)	(Ouellette et al., 2020)
2	GSE206198	GPL18573	CMV (15/15)	(Fulkerson et al., 2020)
3	GSE17948	GPL8300	CMV (12/4)	(Chan, Nogalski and Yurochko, 2009; Chan et al., 2010)
4	GSE186334	GPL20301	EP (46/22)	(Gomes-Duarte et al., 2022)
5	GSE134697	GPL16791	EP (17/2)	(Kjær et al., 2023)
Total collected samples			CMV (175/29), EP (63/24)	

Table 1. The Collected Datasets Were Retrieved from the GEO Database with the Key Search Terms "Epilepsy" and "Cytomegalo Virus" According to the Defined Criteria

2.2 Differential Gene Expression Analysis

With R Studio, differential gene expression (DGE) was conducted for all the retrieved datasets separately. In detail, R Studio is an integrated development environment for the R programming language [21]. It provides a collection of different functions as packages. By using the limma and DESsq2 packages, DGE was conducted in three main steps. First, the datasets were divided into sample and control groups, followed by normalization. Then, variance and dispersion measures were calculated for groups of genes in each dataset separately by comparing the mean and variance to predict the scatterplot of the samples. Finally, the log fold change (logFC) was calculated with the p value for each gene. For visualization purposes, volcano plots were created for each dataset to identify the significantly scattered up- and downregulated genes (see Figure 1).

2.3 Identification of Common and Unique Genes

All the datasets were filtered to determine significant up- or downregulated genes using a p value = 0.005. This threshold was defined according to the common practice analysis pipeline recommendation. Furthermore, the datasets were modified to create two separate datasets for each condition. Additionally, the duplicated genes have been removed. These datasets were subsequently input into the Multiple List Comparator Tool from MolBioTools to define the common and unique genes between CMV and EP [22]. Common genes were defined as significantly up- or downregulated genes in both the CMV and EP datasets, while unique genes were defined as significantly up- or downregulated genes in both the CMV and EP datasets. This technique enabled the characterization of molecules involved in both conditions for more in-depth analysis. The numbers of common and unique genes are presented in the Venn diagram (see Figure 2).

2.4 Functional Enrichment Analysis

The list of common genes between CMV and EP was defined as the input for functional enrichment analysis, considering that each gene might be representative of known biological characteristics such as biological processes and molecular functions. This analysis considers the overlap between the significantly expressed genes with annotated biological features and ranks them according to their involvement in the entire system. The Cluster Profiler package was the main library used for this analysis, and the results are presented in Figure 4.

2.5 Protein–Protein Network

A network approach was used to define the hub gene products that interact with most of the molecules in the system. The initial step of network construction was to use a list of common genes between CMV and EP as input in the STRING database. This database is an online application that has been commonly used to construct networks by using extracted gene symbols [23]. We applied STRING to retrieve a maximum of 20 "partner" genes for each input gene symbol in the query list to construct networks that contained additional genes that neighbor each gene in the list. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a freely available online tool that is consistent with other biological databases used to find the latest associations between genes and proteins. We constructed a network with different types of connections among the genes through text mining, experimental links, database sources, coexpression, neighborhood, gene fusion, and cooccurrence. The constructed network (see Figure 5) was downloaded to Cytoscape for topological feature analysis.

2.6 Topology Analysis

To investigate the topological aspects of the protein interaction network, Cyto Hubba plugged in Cyto scape was used. We calculated topological features, including degree, closeness, and betweenness [24]. We selected these topological parameters because they are the main properties that define the role of the node in the network system. Additionally, they are the most commonly

implemented topological parameters for node ranking in network systems. The definition of each parameter is as follows: 1. Degree centrality is an essential property that influences a node connection and is characterized by the number of a node's connections to other nodes in a network. 2. The betweenness centrality measures the number of times a node appears to bridge along the shortest path connecting two nodes. 3. The closeness centrality is defined based on the reciprocal of the sum of the shortest distances between two nodes in a network. The results of this analysis are presented in Table 2 and Figure 6.

3. Results

3.1 Differential Gene Expression Analysis

Six datasets were retrieved, four from CMV and two from EP. GES108211, GES241027, and GES17948 were analyzed with GEO2R, while GSE206198, GES186334, and GSE134697 were analyzed with the help of DESeq2, where each dataset was analyzed individually for DEGs (see Figure 1). Merging the significantly expressed genes for each condition and

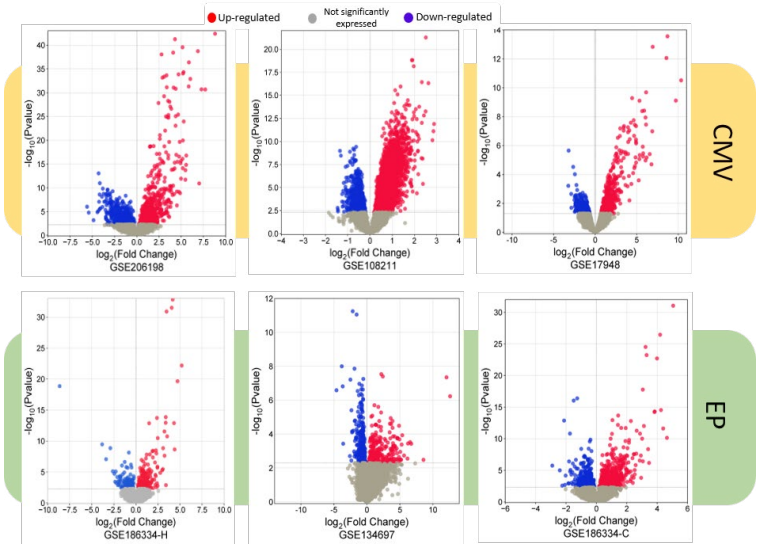

Figure 1: Volcano Plots for Differentially Expressed Genes for Each Dataset. The X-Axis Depicts the Degree of Expression by LOGFC, While the Y-Axis Depicts the Confidentiality of Expression (P value)

removing duplications revealed 4296 genes for CMV and 1188 genes for EP. There were 192 genes common to both CMV and EP (Figure 2).

3.2 Protein–Protein Network Analysis and Hub Gene Prediction

The common genes (n = 192) were used as inputs in the STRING database to construct the protein-to-protein interaction network (Figure 3). The network contained nodes (yellow and cyan boxes) that represented the gene products. Each node is labeled with a gene symbol. The interactions of the proteins with each other are represented by a connected line. The cyan boxes are the highly connected nodes representing the hubs. Hubs play an essential role in maintaining the integrity of networks and mostly have crucial functions in the system. In topology analysis, three different algorithms, namely, degree, betweenness and closeness

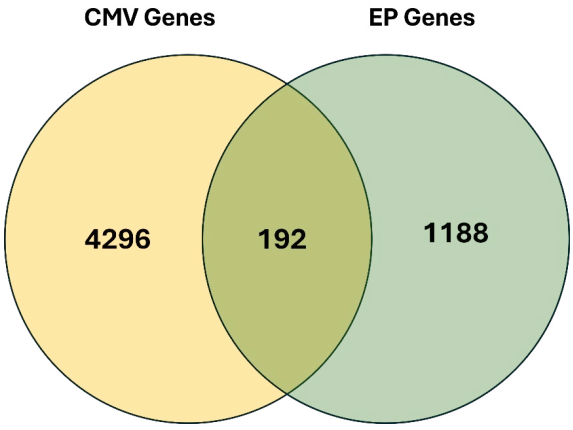

Figure 2: Venn Diagram Represents the Common and Unique Genes for CMV and EP

Centrality, were used to rank the hub genes. We ranked the genes of the top 10 hubs from the highest to lowest centrality (see Table 2). Among the ranked genes, CCL2, CD44 and CCL3 are the three genes with the highest centrality.

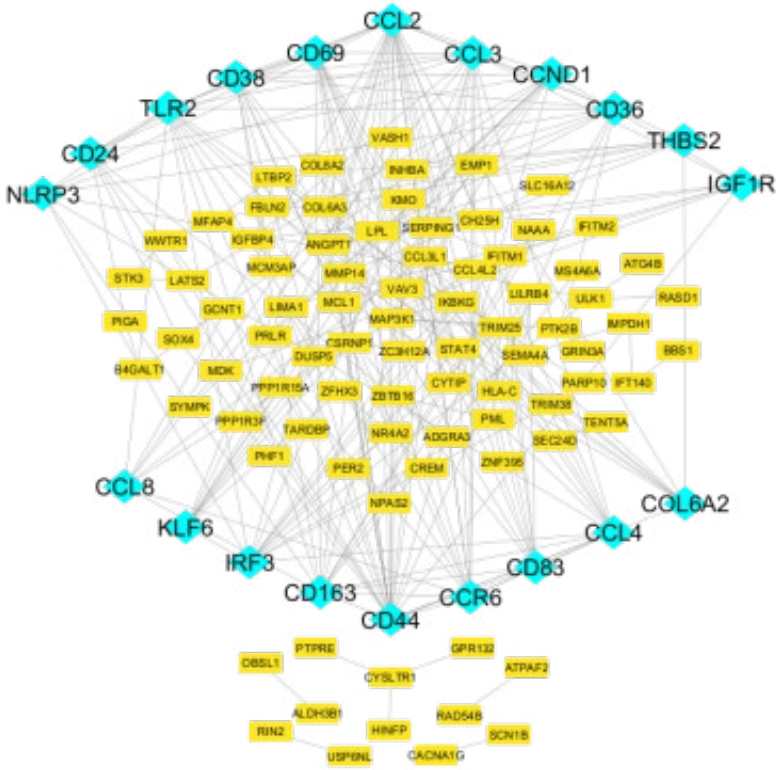

Figure 3. Network of protein-to-protein interaction and detected hub genes (from genes common among CMV and EP

	Betweenness	Closeness	Degree
1	CCL2	CCL2	CD44
2	CD44	CD44	CCL2
3	CD69	CCL3	CCL3
4	KLF6	TLR2	CD163
5	IRF3	CD69	TLR2
6	CD163	CD163	CCR6
7	CCND1	CCR6	CD69
8	LILRB4	CD38	IRF3
9	NR4A2	CCL4	CCND1
10	CD24	CCND1	CD38

Table 2: The Top 20 Genes from The Biological Network (Common Genes of CMV And Ep) Were Analyzed Using Three Different Topological Methods Via the Cyto Hubba Plugin

3.3 Functional Enrichment Analysis

According to functional enrichment analysis, the hub genes are involved in several biological processes and molecular functions according to Gene Ontology (GO) analysis. We plotted the significantly enriched terms based on the p value, as illustrated in Figure 4. Responding to interferon-gamma was the top enriched biological process, while CCR chemokine receptor binding was the top molecular function. This analysis clearly revealed that most hub genes are involved in biological processes that might be closely associated with crosstalk between CMV and EP.

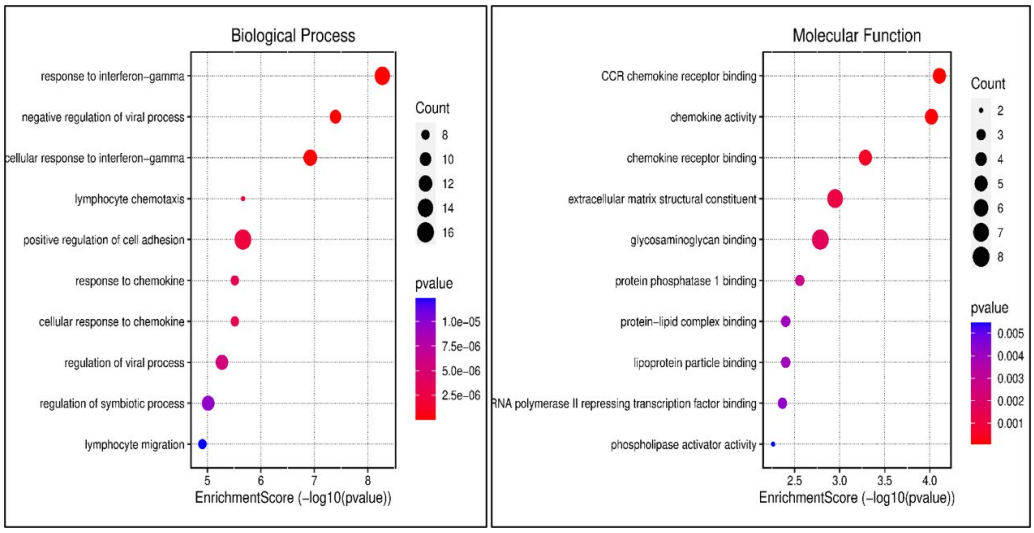

Figure 4: 10 Gene Ontology Terms of Hub Genes Shared between CMV and EP

4. Discussion

Using transcriptomics data integrated with network biology analysis has been commonly utilized to identify and reveal potential biomarkers shared between multiple diseases. This study focused on using this approach to determine the potential molecular crosstalk between CMV and EP. We retrieved public transcriptomics datasets with strict inclusion and exclusion criteria to achieve optimal representation of conditions. The significant genes common to the two conditions were determined via DEG analysis. Subsequently, the common genes in the protein-to-protein network were analyzed. This robust approach revealed the CCL2, CD44 and CCL3 genes as the top three genes. The CCL2 gene has been previously reported to be a proinflammatory cytokine that is highly expressed in monocytes in response to viral infection, cancer, and autoimmune diseases [25]. Additionally, increased expression of CCL2 was reported previously in patients with EP [26,27]. Furthermore, CD44 is a glycoprotein receptor on the surface of blood cells. It works as a cell–cell interaction molecule for processes such as adhesion and migration [28]. CD44 was found to be involved in neuron synopsis in EP individuals [29]. Activated CMV appears to express CD44 in all T memory cells [30]. CCL3 is a cytokine inflammatory mediator for homeostatic and pathological conditions [31]. All the downregulated genes identified in this study are strongly associated with the inflammatory process, as suggested by enrichment analysis. These findings shed light on the inflammatory process and immune response as potential crosstalk mechanisms between CMV and EP. Moreover, the results revealed the important roles of these chemokine molecules. Therefore, further investigations of the roles of the CCL2, CD44, and CCL3 genes as crosstalk partners between these two conditions are recommended. The power of utilizing a network for assessing crosstalk between diseases has previously been demonstrated by several studies. Specifically, networks were constructed to determine the molecular crosstalk between COVID-19 and Alzheimer's disease using microarray and RNA-seq datasets. This study demonstrated the potential of identifying hub gene-drug interactions. However, it is also important to pinpoint some limitations of this study. 1) The lack of wet laboratory experiments showed the need for additional investigations, and 2) revealing an unconnected network might impact the overall topology analysis. In future work, we suggest increasing the level of interaction between the nodes of the network rather than having physical interactions among the proteins.

5. Conclusion

In conclusion, we provide evidence for crosstalk between CMV and EP through inflammatory and immune responses. The CCL2, CD44, and CCL3 genes have been identified as crosstalk molecules [32-55].

Acknowledgments: The authors would like to acknowledge the Medical Laboratory Sciences Department at Oman College of Health Science for providing guidelines.

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