

High Throughput Screening of Differentially Expressed Cytokines of Cerebrospinal Fluid to Predict the Progression of Hand, Foot and Mouth Disease

Junjie Chen

Guangzhou Women and Children's Medical Center <https://orcid.org/0000-0001-6947-3453>

Zijing Xiao

Guangzhou Women and Children's Medical Center

Peiqing Li

Guangzhou Women and Children's Medical Center

Xiaoqi Yang

Guangzhou Women and Children's Medical Center

Jing Wang

Guangzhou Women and Children's Medical Center

Ru Wei

Guangzhou Women and Children's Medical Center

Sida Yang

Guangzhou Women and Children's Medical Center

Fangfang Qi

Sun Yat-sen University Zhongshan School of Medicine

Dandan Hu (✉ guohdd@126.com)

Guangzhou Women and Children's Medical Center

Research

Keywords: protein array, bioinformatics analysis, potential biomarker, inflammatory cytokines

Posted Date: May 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-527714/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Hand, foot and mouth disease (HFMD) usually has a good prognosis, but in a small number of serious neurological complications can also occur especially to those younger than 3 years old children. Therefore, early diagnosis of hand, foot and mouth disease is very important to improve the cure rate of children with severe HFMD. In this study, we determined to find new potential disease-related indicators in the cerebrospinal fluid (CSF) to predict the progression and prognosis of HFMD. We collected CSF samples from mild or severe HFMD in the acute phase. The 1000 cytokines in CSF samples were analyzed using the Raybiotec Cytokine array, then Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were carried out to evaluate the related biological process and potential signaling pathways. We successfully screened 9 DEPs, including CCL5, IL17c, EDAR, TACI, Nectin1, VCAM1, SIGLEC1, CD69 and ROBO4 in the CSF, which may be potential biomarkers for predicting disease progression and prognosis. CD69 and ROBO4 might be a specific indicator suggesting severe HFMD, while elevated expression of NECTIN1 might suggest disease improve, highlighting the potential to predict remission.

Introduction

Hand foot and mouth disease (HFMD) is a common infectious disease in children caused by enterovirus (EV) -the main serotypes include Coxsackie virus (CV) group A type 4–7, 9, 10 and 16, and group B type 1–3 and 5, some Echo-virus serotypes and Enterovirus A71 (EV-A71)] [1]. It is mainly affects pediatric populations less than 5 years old, and the most common types are CV-A10, 16 and EV-A71, the latter accounting for the majority of severe cases[1, 2]. Pediatric populations are the main source of infection, and the infection may be dominant, subclinical or recessive[3]. Enterovirus can survive in hydrothermal conditions and can be transmitted through feces, throat secretions, saliva and other body fluids of infected individuals, therefore, HFMD has a high latent infection rate[3].

HFMD a common viral infection in children, that has been a severe public health concern worldwide [1].From 2008 to 2018, 20,537,199 cases of HFMD were reported in China, among which 157,065 were severe cases. The average annual rate of severe cases was 1.05/100,000, with a severe rate of 0.76%. There were 3,668 deaths, and the fatality rate of severe cases was 2.34%[4]. HFMD can be divided into common type and severe type: common cases show acute onset, fever, scattered herpes in oral mucosa, macular papules and herpes on hands, feet and buttocks, inflammatory erythema around herpes and less fluid in blisters. It can be accompanied by cough, runny nose, loss of appetite and other symptoms. Some cases only showed rash or herpetic angina. Most of patients were cured within one week. However, a few cases (especially those under 3 years old) developed rapidly meningitis, brainstem encephalitis, encephalomyelitis, pulmonary edema, circulatory disorders and so on appeared in about 1–5 days after the onset of the disease [4]. Very few cases were critically ill and could cause death, and the remaining cases could have sequelae.

Although the underlying molecular mechanism of nervous system symptoms in HFMD remains unclear, the involvement of inflammatory cytokines and chemokines in inflammatory response has been proved by previous studies[5–7]. Compared with the healthy volunteers, cytokine fluctuate were observed in mild cases, and severe EV71-infected HFMD with complications, indicating that cytokines play an important role in the progression of EV71 infection and may be targeted for diagnosis and treatment. Therefore, exploring these inflammatory factors and/or chemokines involved in the progression of HFMD will help to find potential indicators of disease progression; especially to the server HFMD accompanied by neurological complications. It will be valuable to carry out clinical intervention in the early stage of the disease in future. However, there are now only a few indicators of peripheral blood, rather than CSF to assess the disease progress[2, 3][8, 9].

Therefore, we will explore the CSF inflammatory cytokines and chemokines, and further find potential indicators that can help to judge the progress and severity of the disease in the present study. We collected the CSF samples from 4 cases of HMFd and 2 cases of common pneumonia to analyzed their differential proteins by GSH-CAA-X00 kit technology.

Materials And Methods

We collected clinical data and CSF of 6 patients who were hospitalized in Guangzhou Women and children's Medical center in Guangzhou, China, from 2017 to 2019. According to the clinical standard of the guidelines for diagnosis and treatment of hand foot mouth disease (2018 Edition)[3] issued by the Ministry of health and the consensus of experts, four patients with enterovirus detected in throat swabs or feces and with serious neurological symptoms or not were divided into severe group (SEV) and mild group (MIL; $n = 2$ each group). The two patients who did not detect enterovirus in throat swabs and feces and did not meet the diagnosis of HFMD were classified as control group (CTR). The CSF of these 6 patients were separated and stored at $-80\text{ }^{\circ}\text{C}$. This study was approved by the ethics committee of Guangzhou women and children medical center (36700). MRI images obtained of 5 patients (out of 6) from SEV (2 patients), MIL (2 patients) and CTR (1 patient) groups to observe the pathology, especial for the SEV patients. The informed consent of the child's parents or guardians was obtained before the experiment. The clinical data and laboratory results of these children are shown in Table 1.

Table 1
Clinic characteristics of patients

	SEV (n = 2)	MIL (n = 2)	CTR (n = 2)
Age/month	82,18	12,10	2,144
Female/Male	Male, Male	Male, Female	Male, Male
Symptom			
Spasm	yes, yes	no, no	no, no
Fever	yes, yes	yes, yes	yes, yes
Rash	yes, yes	yes, yes	no, no
Cough	no, no	no, no	yes, yes
Laboratory Data			
CSF CRP(mg/l)	0.03,0.06	0.02,0.3	0.05/0.04
CSF WBC(*10 ⁶ /L)	4,2	2,1	5,5
CSF MP(g/l)	0.17,0.49	0.20,0.33	0.47,0.22
Serum WBC(*10 ⁹ /L)	12.9,10.9	9.2,14	7.8,8.2
N%	92,74	33,30	35,73
L%	2,17	52,54	49,20
Imaging Characteristics			
Abnormal of Brain MRI	yes, yes	yes, no	no, no
Abnormal of EEG	yes, no	no, no	no, no

2.2 Data Analysis

The original data obtained by kit scanning is processed by Raybiotech software for kit background removal and inter kit normalization. The differential proteins were screened by fold change, and the selection conditions were as follows: (1) fold change = < 0.5 or fold change > = 2; (2) it is recommended to select the average (fluorescence) signal value > 150 for each group. The horizontal and vertical coordinates respectively represent the average expression of AveExp (the logarithm average value of each sample value). Red indicates up-regulated protein, blue represents down regulated protein, and gray indicates no significant difference.

Results

3.1 MIL-CTR GO analysis and KEGG pathway analysis.

We identified 215 DEPs between the MIL and CTR, including 204 (94.9%) down-regulated proteins and 11 (5.1%) up-regulated proteins (Fig. 1). According to the GO enrichment results, regulation of lysophocyte proliferation, regulation of leucocyte cell proliferation, chemoattractant activity, virus receptor activity, hijacked molecular function were the enriched GO terms.

The KEGG analysis results showed that DEPs were involved in a total of 29 pathways, including Malaria, Melanoma, Viral protein interaction with cytokine and cytokine receptor. Cytokine cytokine receptor interaction and Cell adhesion molecules (CAMs).

3.2 SEV-CTR GO analysis and KEGG pathway analysis

There were 187 DEPs between the SEV and CTR, including 172 (92%) down regulated proteins and 15(8.0%) up-regulated proteins (Fig. 3). Regulation of lymphocyte promotion, regulation of monocyte cell promotion, regulation of leucocyte promotion, regulation of leucocyte cell adhesion, the transmembrane-ephrin receptor activity, the virus get receptor activity, hijacked molecular function were the enriched GO terms.

DEPs participated in 30 pathways. Among these, malaria, Melanoma, ECM receptor interaction, cytokine cytokine receptor interaction, cell adhesion molecules (CAMs) have more DEPs involved.

3.3 SEV-MIL GO analysis and KEGG pathway analysis

According to the overall proteins expression of all samples, there are 58 DEPs between SEV and MIL, including 22 (37.9%) down-regulated proteins and 36 (62.1%) up-regulated proteins (Fig. 1). Leukocyte migration, carbohydrate activity, virus receptor activity, hijacked molecular function, cytokine activity were the enriched GO terms.

The KEGG analysis results showed that DEPs were involved a total of 5 pathways- nitrogen metabolism, cytokine cytochrome receptor interaction, cell adhesion molecules (CAMs), IL-17 signaling pathway, NF kappa B signaling pathway.

3.4 Gradually changing differential expression of proteins

We focused on proteins that were up-regulated or down-regulated as the disease progressed, and found that the expression of the following proteins increased gradually (Fig. 4): ROBO4, CD69; The expression of the following proteins decreased gradually: CD48, CRTAM, Glypican 1, Nectin-1, Desmocollin-3, BAFF. These proteins will need to be discussed in detail and more attention will be paid to them in future studies.

3.5 The Magnetic Resonance Imaging (MRI) result of these cases

We collected brain MR images of 5 of the 6 children, and consulted two experienced radiologists to read the images. Surprisingly, the MR images of these five children showed no obvious abnormal imaging findings in the sub cortex white substance (WS), thalamus (TH), mesencephalon (MES), pons, medulla oblongata (MO), and no characteristic changes in the red nucleus (RN) and substantia nigra (SN), which were associated with serious neurological symptoms in severe HFMD patients (Fig. 5 and Fig. 6). These results suggested that MRI data might not be the best diagnostic criteria for HFMD patients.

Table 2

Group	Cytokine-cytokine receptor interaction	Cell adhesion molecules
Sever-MIL	TNFSF13B/CCL5/CXCL8/IL17RB/CXCL10/IL17C/IL27/EDAR	PVR/Nectin1/Nectin2/VCAM1/Siglec1
MIL-CTR	PPBP/IL31RA/EDAR/IL7R/PF4/IL17C/BMP8A/IL23R/TNFRSF13B/CCL5/IL27/CXCL14/CXCL10/IL18/TNFRSF9/AMHR2/CCL15/PRL/IL3/IL16/IL1RAP/IL22RA1/GDF15/IL20RB/PRLR/ACVRL1/GHR/CD40LG/CCL16/TNFSF9/IFNGR1/CCL18/TNFSF13B/TNFRSF14	Siglec1/VCAM1/SDC4/ICAM1/SELL/ICAM2/ITGAV/VTCN1/ICOSL1/Nectin1/Nectin3/CD28/CD40LG/CD58/CDH5
SEVER-CTR	PPBP/IL31RA/IL7R/TNFSF13B/BMP8A/IL23R/PF4/TNFRSF13B/CXCL14/CCL5/IL17C/EDAR/TNFRSF9/AMHR2/IL18/IL16/IL3/RELT/IL1RAP/PRL/TNFSF9/PRLR/IL20RB/TNFRSF10C/IL12B/IL17RB	PVR/Nectin1/SDC4/ITGAV/ICAM1/VCAM1/VTCN1/Siglec1/SELE1/Nectin3/SELL/PDCD1/CDH5

Discussion

Hand foot mouth disease (HFMD) is an acute infectious disease caused by enterovirus, which occurs mostly in preschool children[4]. Due to the rapid progress of a few cases (especially those under 3 years old), meningitis, encephalitis (brainstem encephalitis is the most dangerous), encephalomyelitis, pulmonary edema, circulatory disorders and so on appear in about 1–5 days after the onset of the disease. Very few cases are critically and may lead to death. The survival cases may have sequelae. It is worth mentioning that the locations of lesions of EV71 HFMD-associated brainstem encephalitis are mainly on the dorsal and medulla oblongata[10]. However, no significant abnormalities were observed on MRI in this study. There are high levels of cytokines and chemokines in serum or cerebrospinal fluid of HFMD patients infected by enterovirus. Especially in severe patients, immune response and inflammatory response are imbalance, which leads to complications and even death [11]. More and more attention attached to the potential mechanism of abnormal regulation of immune and inflammatory state induced by enterovirus. A large number of studies have suggested that immune inflammatory response is closely related to enterovirus infection related hand, foot and mouth disease[12–17], but the specific pathway of action is not completely clear. In this study, we screened the DEPs among SEV-MIL, SEV-CTR and MIL-CTR from 1000 kinds of proteins use GSH-CAA-X00 kits. The potential indicators for predicting the progression of HFMD were determined by GO enrichment analysis and KEGG pathway enrichment analysis.

Based on KEGG database, we found that cytokine cytokine receptor interaction (CCRI), cell adhesion molecules (CAMs) pathways are involved in the occurrence and progression of HFMD. Four DPEs (CCL5, IL17c, EDAR and TACI) on CCRI pathway participate in the three stages of changes, and the three DEPs (Nectin1, VCAM-1 and Siglec1) in CAMs pathway participate in the changes of three stages (Table 3). CCL5 has chemotactic effect on a variety of leukocytes: 1) chemotactic monocytes; 2) chemotactic to non-stimulated CD4 + CD45RO + memory T cells and activated CD4 + and CD4 + T cells; 3) chemotaxis eosinophils and stimulates their degranulation; 4) Pretreatment of T cells can increase their adhesion to endothelial cells stimulated by IL-1. These properties make CCL5 play an important role in immune regulation, autoimmune disorder and inflammatory process[18]. It has been reported that high levels of CCL5 in serum can be used as a biomarker to judge the severity of HFMD and may be involved in immunopathological changes[19]. This study also confirmed the possibility of CCL5 as a potential biomarker. Interestingly, in the present study, we found that IL-17c levels in obvious increased in CSF sample of SEV group relative to MIL group. Although IL17c in the progression of HFMD has not been clearly reported, IL-17c is a neurotrophic cytokine that protects peripheral nerve systems during HSV reactivation. Therefore, IL17C may be a potential target to improve the prognosis of patients with severe HFMD.

Nectin1 promotes cell-cell contact through the formation of homotype or heteromorphic trans dimer[20]. Heterophilic interaction has been detected between Nectin1 and Nectin3, as well as between Nectin1 and Nectin4. It has the activity of promoting neurite growth, and is the receptor of herpes simplex virus 1 / HHV-1, herpes simplex virus 2 / HHV-2 and pseudorabies virus / PRV[21]. Some studies have shown that the differential expression of Nectin1 induced by EV-71 and CV-A16 infection can lead to the disruption of cell junction and eventually the disruption of cell barrier function.[22] On the one hand, Siglec1 promotes pathogen infection; on the other hand, Siglec1 can also regulate innate and adaptive immune responses[23]. Since inflammation can induce Siglec1 expression, its expression is related to the disease process, it can be used as an index of disease evaluation and curative effect observation.

EDAR, as a part of a signaling pathway, which plays an important role in the the interaction between two embryonic cell layers, called ectoderm and mesoderm[24]. Some studies have shown that EDAR is associated with Sjogren's syndrome, which may suggest that EDAR participates in the inflammatory process to a certain extent[25]. TNFRSF13B provides instructions for the production of a protein called TACI, which is present on the surface of B cells in the immune system. These special white blood cells help protect the body from foreign invaders such as bacteria and viruses. When B cells mature, they produce a special protein called antibodies (also known as immunoglobulins). Antibodies attach to specific foreign invaders, marking that they will be eliminated. TACI

promotes cell signal transduction by interacting with other proteins, plays a role in B cell survival and maturation, and participates in antibody production. VCAM-1 plays a pathophysiological role in immune response and leukocyte migration to inflammatory sites[26].The role of EDAR, TNFRSF13B and VCAM-1 in the progression of HFMD needs to be further explored.

The results further verified that CD69 and ROBO4 expression gradually increased in CSF with HFMD progression (From CTR to MIL to SEV). Roundabout4 (Robo4) is a transmembrane receptor that belongs to the Roundabout (Robo) family of axon guidance molecules. Robo4 is an endothelial-specific receptor that participates in endothelial cell migration, proliferation, and angiogenesis and the maintenance of vasculature homeostasis[27].Subcutaneous injection of LPS into Robo4-knockout mice has been shown to reduce circulating IL-6 levels and the production of inflammatory cytokines[28],therefore, ROBO4 can be a potential therapeutic targets.CD69 is a membrane-bound, type II C-lectin receptor. It is a classical early marker of lymphocyte activation due to its rapid appearance on the surface of the plasma membrane after stimulation[29]. CD69 could be a possible therapeutic target for arthritis and asthma in human patients. We need more experiments to verify the role of CD69 in the progression of HFMD.

In addition, recent reports say that positive MRI findings demonstrated lesion in midbrain, medulla oblongata and thalamus in EV-71 infected HFMD patients with neurological symptoms included myoclonus and dysphagia, and facial palsy [30–32]. However, our data showed no obvious abnormalities in several related brain areas, including sub cortex white substance, thalamus, mesencephalon, pons, medulla oblongata, red nucleus and substantia nigra, implying that MRI lesion signaling might not be positive associated with clinical characteristics, even in the sever patient. The inconsistent results may be due to the patients without EV-71 infection, which is a neurotropic virus, in the present study[33]. Therefore, screening the underlying biomarkers of CSF to predict early HFMD and HFMD progress is a valuable strategy in clinical application.

Conclusion

To sum up, this study screened differentially expressed proteins among patients with severe HFMD, mild HFMD and non-HFMD patients, and carried out GO analysis and bioinformatics analysis of KEGG signaling pathway according to the results, and analyzed the complex biological processes involved in DEPs, the distribution and molecular functions of the proteins were discussed. The multiple signaling pathways involved in the differential expression of the proteins were obtained, and potential biomarkers were screened out as indicators for the diagnosis and differentiation of HFMD with different severity. However, in HFMD patients, CCL5, IL17c, EDAR, TNFSF13B, Nectin1, VCAM1, Siglec1, CD69 and ROBO4 may participate in different clinical progress. These potential indicators may be useful for clinical intervention, such as early treatment of mild patients or close monitoring of patients before the disease further develops into severe or critical state, to improve the prognosis of severe patients.

Abbreviations

HFMD:Hand-foot-mouth disease;

CSF:cerebrospinal fluid;

GO:Gene Ontology;

KEGG:Kyoto Encyclopedia of Genes and Genomes;

DEPs:differentially expressed proteins;

CCRI:Cytokine-Cytokine Receptor Interaction;

CAMs:Cell Adhesion Molecules;

MR:magnetic resonance

Declarations

Acknowledgments

We are indebted to patients and their families, clinical staff for technical assistance, patient care, sampling and tissue data acquisition.

Funding

This work was supported by: National Natural Science Foundation of China (No. 81801206) Science and Technology Key Project for People's Livelihood of Guangzhou, China (No. 201803010026); Natural Science Foundation of Guangdong Province, China (No. 2018A030313756 No.2020A1515010014); Medical Science and Technology Research Foundation of Guangdong, China [No. A2019373].

Availability of data and materials

The data analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

Dandan Hu and Fangfang Qi designed the experiment, Junjie Chen and Zijing Xiao wrote the paper and approved the final manuscript, All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Center for Disease Control and Prevention of Guangdong Province. All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The samples used in the study were all provided with the informed written consent of patients' family members.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH: **Virology, epidemiology, pathogenesis, and control of enterovirus 71**. *The Lancet infectious diseases* 2010, **10**(11):778-790.
2. Puenpa J, Wanlapakorn N, Vongpunsawad S, Poovorawan Y: **The History of Enterovirus A71 Outbreaks and Molecular Epidemiology in the Asia-Pacific Region**. *J BIOMED SCI* 2019, **26**(1):75.
3. Li X, Ni X, Qian S, Wang Q, Jiang R, Xu W, Zhang Y, Yu G, Chen Q, Shang Y *et al*: **Chinese guidelines for the diagnosis and treatment of hand, foot and mouth disease (2018 edition)**. *WORLD J PEDIATR* 2018, **14**(5):437-447.
4. Cui JZ, Nie TR, Ren MR, Liu FF, Li Y, Wang LP, Tan JB, Chang ZR, Li ZJ: **[Epidemiological characteristics of fatal cases of hand, foot, and mouth disease in children under 5 years old in China, 2008-2018]**. *Zhonghua Liu Xing Bing Xue Za Zhi* 2020, **41**(7):1041-1046.
5. Shang W, Qian S, Fang L, Han Y, Zheng C: **Association study of inflammatory cytokine and chemokine expression in hand foot and mouth disease**. *Oncotarget* 2017, **8**(45):79425-79432.
6. Griffiths MJ, Ooi MH, Wong SC, Mohan A, Podin Y, Perera D, Chieng CH, Tio PH, Cardosa MJ, Solomon T: **In Enterovirus 71 Encephalitis With Cardio-Respiratory Compromise, Elevated Interleukin 1 , Interleukin 1 Receptor Antagonist, and Granulocyte Colony-Stimulating Factor Levels Are Markers of Poor Prognosis**. *J INFECT DIS* 2012, **206**(6):881-892.
7. He Y, Feng Z, Wang W, Chen Y, Cheng J, Meng J, Yang H, Wang Y, Yao X, Feng Q *et al*: **Global cytokine/chemokine profile identifies potential progression prediction indicators in hand-foot-and-mouth disease patients with Enterovirus A71 infections**. *CYTOKINE* 2019, **123**:154765.
8. Zhang Y, Liu H, Wang L, Yang F, Hu Y, Ren X, Li G, Yang Y, Sun S, Li Y *et al*: **Comparative study of the cytokine/chemokine response in children with differing disease severity in enterovirus 71-induced hand, foot, and mouth disease**. *PLOS ONE* 2013, **8**(6):e67430.
9. Pei X, Fan X, Zhang H, Duan H, Xu C, Xie B, Wang L, Li X, Peng Y, Shen T: **Low frequency, weak MCP-1 secretion and exhausted immune status of peripheral monocytes were associated with progression of severe enterovirus A71-infected hand, foot and mouth disease**. *CLIN EXP IMMUNOL* 2019, **196**(3):353-363.
10. Zeng H, Wen F, Gan Y, Huang W: **MRI and associated clinical characteristics of EV71-induced brainstem encephalitis in children with hand-foot-mouth disease**. *NEURORADIOLOGY* 2012, **54**(6):623-630.
11. Yi L, Lu J, Kung HF, He ML: **The virology and developments toward control of human enterovirus 71**. *CRIT REV MICROBIOL* 2011, **37**(4):313-327.
12. He Y, Feng Z, Wang W, Chen Y, Cheng J, Meng J, Yang H, Wang Y, Yao X, Feng Q *et al*: **Global cytokine/chemokine profile identifies potential progression prediction indicators in hand-foot-and-mouth disease patients with Enterovirus A71 infections**. *CYTOKINE* 2019, **123**:154765.
13. Yu L, He J, Wang L, Yi H: **Inflammatory profiles revealed the dysregulation of cytokines in adult patients of HFMD**. *INT J INFECT DIS* 2019, **79**:12-20.
14. Zhang W, Huang Z, Huang M, Zeng J: **Predicting Severe Enterovirus 71-Infected Hand, Foot, and Mouth Disease: Cytokines and Chemokines**. *Mediators Inflamm* 2020, **2020**:9273241.
15. Rasti M, Khanbabaee H, Teimoori A: **An update on enterovirus 71 infection and interferon type I response**. *REV MED VIROL* 2019, **29**(1):e2016.
16. Hsia SH, Lin JJ, Chan OW, Lin TY: **Cardiopulmonary failure in children infected with Enterovirus A71**. *J BIOMED SCI* 2020, **27**(1):53.
17. Luo Z, Su R, Wang W, Liang Y, Zeng X, Shereen MA, Bashir N, Zhang Q, Zhao L, Wu K *et al*: **EV71 infection induces neurodegeneration via activating TLR7 signaling and IL-6 production**. *PLOS PATHOG* 2019, **15**(11):e1008142.
18. Marques RE, Guabiraba R, Russo RC, Teixeira MM: **Targeting CCL5 in inflammation**. *EXPERT OPIN THER TAR* 2013, **17**(12):1439-1460.
19. Yu P, Bao L, Xu L, Li F, Lv Q, Deng W, Xu Y, Qin C: **Neurotropism In Vitro and Mouse Models of Severe and Mild Infection with Clinical Strains of Enterovirus 71**. *Viruses* 2017, **9**(11):351.
20. Samanta D, Almo SC: **Nectin family of cell-adhesion molecules: structural and molecular aspects of function and specificity**. *CELL MOL LIFE SCI* 2015, **72**(4):645-658.
21. Martinez WM, Spear PG: **Amino acid substitutions in the V domain of nectin-1 (HvC) that impair entry activity for herpes simplex virus types 1 and 2 but not for Pseudorabies virus or bovine herpesvirus 1**. *J VIROL* 2002, **76**(14):7255-7262.

22. Hu Y, Song J, Liu L, Zhang Y, Wang L, Li Q: **microRNA-4516 Contributes to Different Functions of Epithelial Permeability Barrier by Targeting Poliovirus Receptor Related Protein 1 in Enterovirus 71 and Coxsackievirus A16 Infections.** *FRONT CELL INFECT MI* 2018, **8**.
23. Siddiqui SS, Matar R, Merheb M, Hodeify R, Vazhappilly CG, Marton J, Shamsuddin SA, Al Zouabi H: **Siglecs in Brain Function and Neurological Disorders.** *CELLS-BASEL* 2019, **8**(10):1125.
24. Botchkarev VA, Fessing MY: **Edar signaling in the control of hair follicle development.** *J Investig Dermatol Symp Proc* 2005, **10**(3):247-251.
25. Sisto M, Barca A, Lofrumento DD, Lisi S: **Downstream activation of NF-kappaB in the EDA-A1/EDAR signalling in Sjogren's syndrome and its regulation by the ubiquitin-editing enzyme A20.** *CLIN EXP IMMUNOL* 2016, **184**(2):183-196.
26. **Emerging Roles of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Immunological Disorders and Cancer.** *INT J MOL SCI* 2018, **19**(4):1057.
27. Dai C, Gong Q, Cheng Y, Su G: **Regulatory mechanisms of Robo4 and their effects on angiogenesis.** *Biosci Rep* 2019, **39**(7).
28. Shirakura K, Ishiba R, Kashio T, Sakai M, Fukushima Y, Yamamoto N, Manabe S, Shigesada N, Tanaka T, Hino N *et al*: **Endothelial Robo4 regulates IL-6 production by endothelial cells and monocytes via a crosstalk mechanism in inflammation.** *Biochem Biophys Res Commun* 2018, **495**(1):801-806.
29. Cibrian D, Sanchez-Madrid F: **CD69: from activation marker to metabolic gatekeeper.** *EUR J IMMUNOL* 2017, **47**(6):946-953.
30. Lian ZY, Li HH, Zhang B, Dong YH, Deng WX, Liu J, Luo XN, Huang B, Liang CH, Zhang SX: **Neuro-Magnetic Resonance Imaging in Hand, Foot, and Mouth Disease: Finding in 412 Patients and Prognostic Features.** *J Comput Assist Tomogr* 2017, **41**(6):861-867.
31. Zeng H, Wen F, Gan Y, Huang W: **MRI and associated clinical characteristics of EV71-induced brainstem encephalitis in children with hand-foot-mouth disease.** *NEURORADIOLOGY* 2012, **54**(6):623-630.
32. Chen F, Liu T, Li J, Xing Z, Huang S, Wen G: **MRI characteristics and follow-up findings in patients with neurological complications of enterovirus 71-related hand, foot, and mouth disease.** *INT J CLIN EXP MED* 2014, **7**(9):2696-2704.
33. Wong KT, Munisamy B, Ong KC, Kojima H, Noriyo N, Chua KB, Ong BB, Nagashima K: **The distribution of inflammation and virus in human enterovirus 71 encephalomyelitis suggests possible viral spread by neural pathways.** *J Neuropathol Exp Neurol* 2008, **67**(2):162-169.

Figures

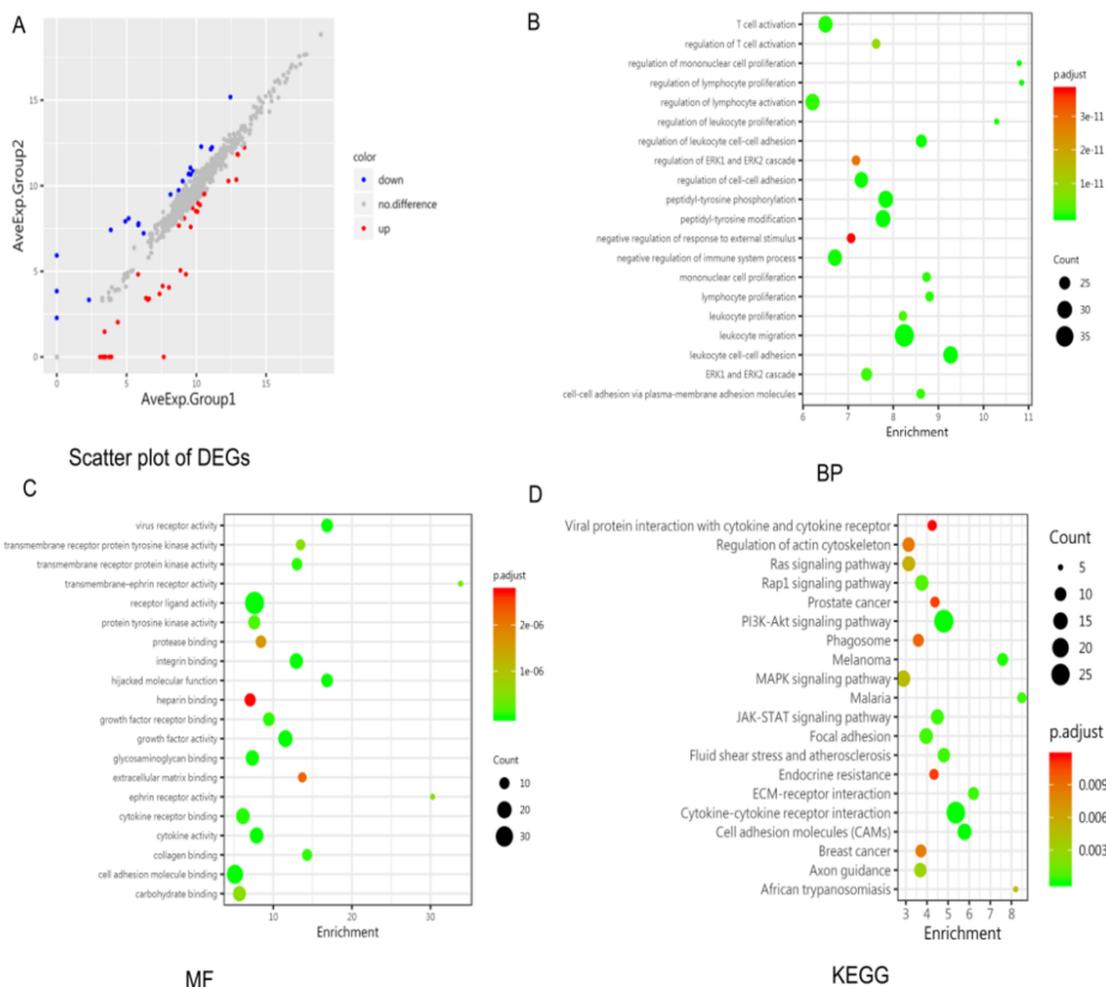


Figure 1

A caption was not provided with this version

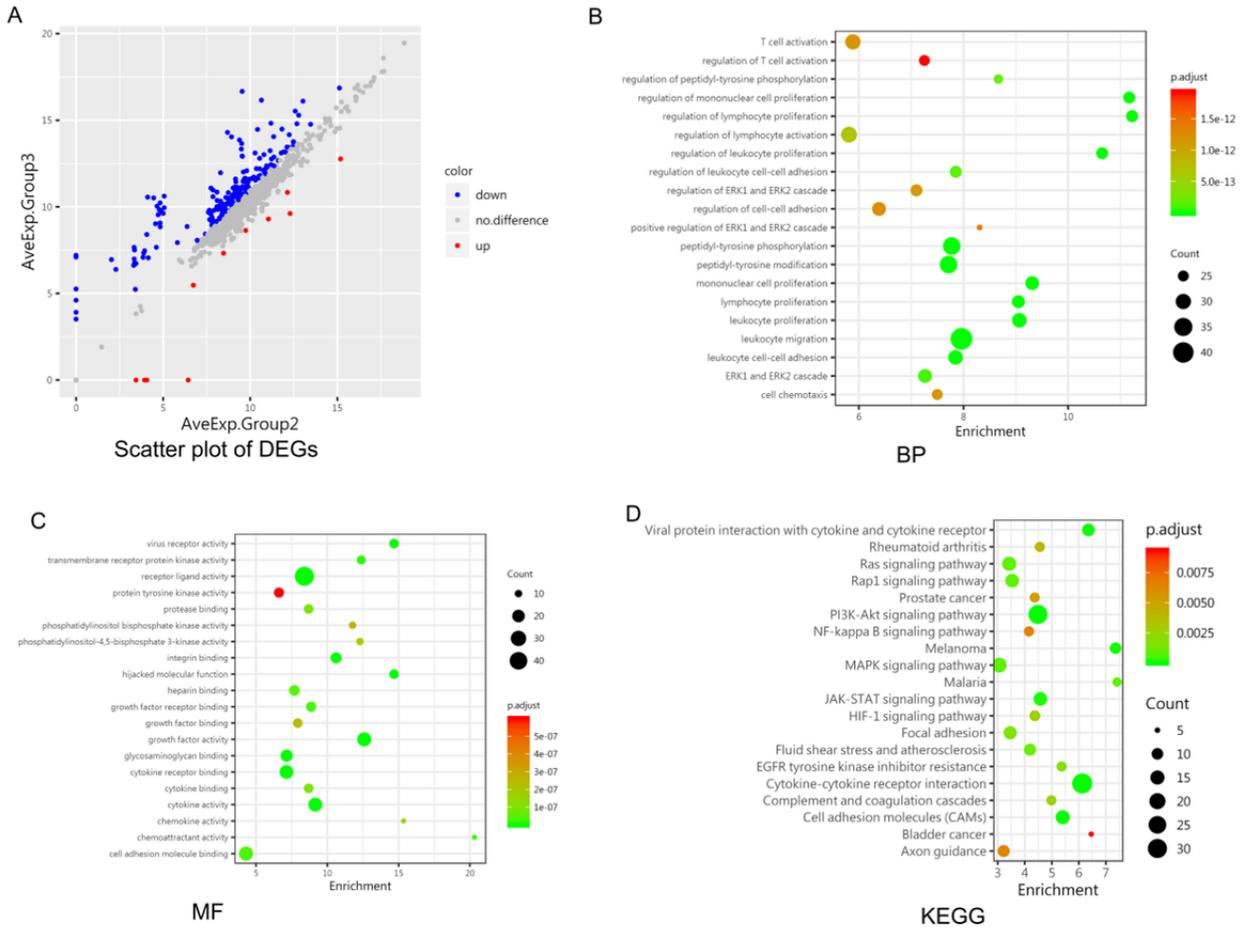


Figure 2

A caption was not provided with this version

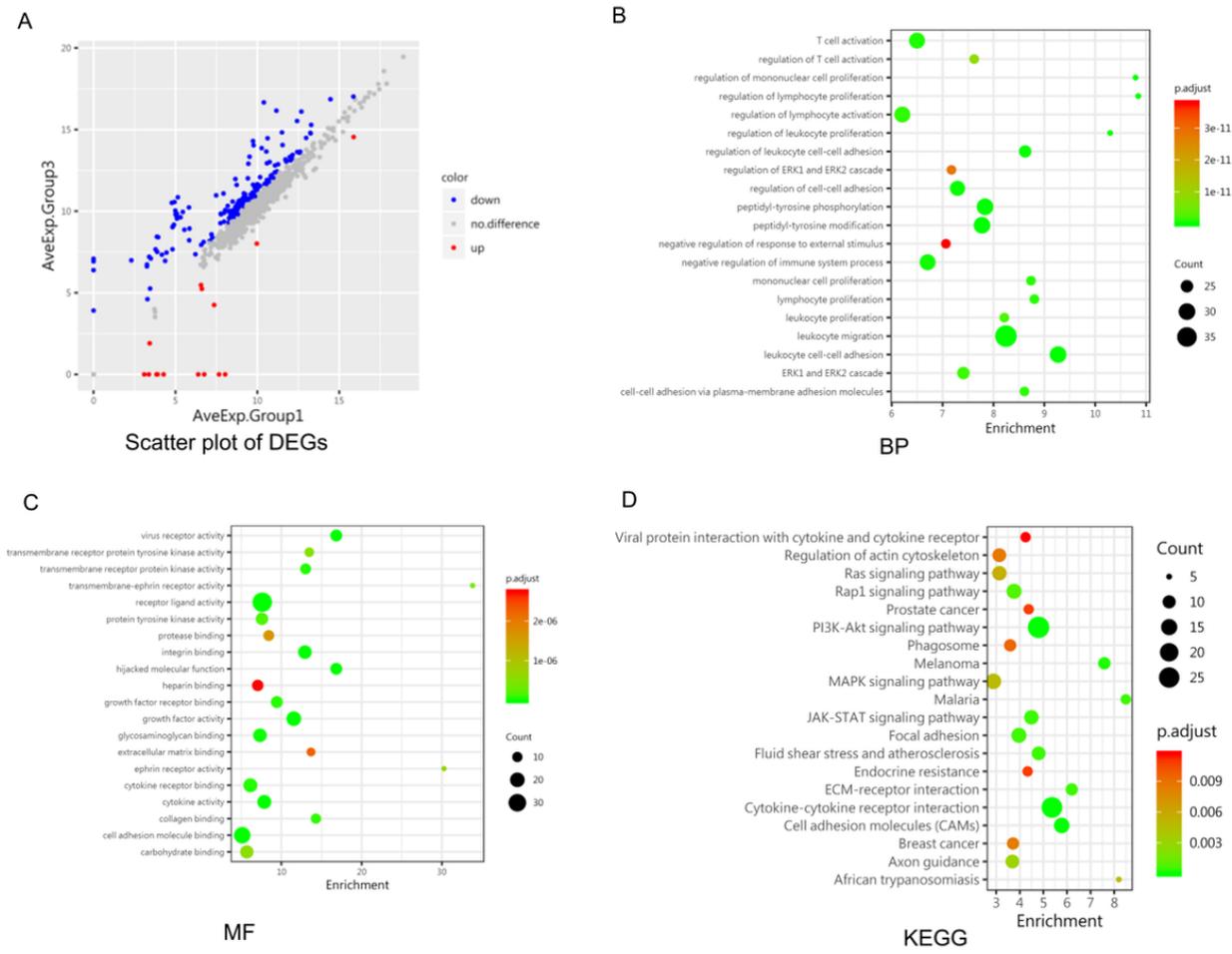


Figure 3

A caption was not provided with this version

A

Color Key

-1 0 1

Row Z-Score

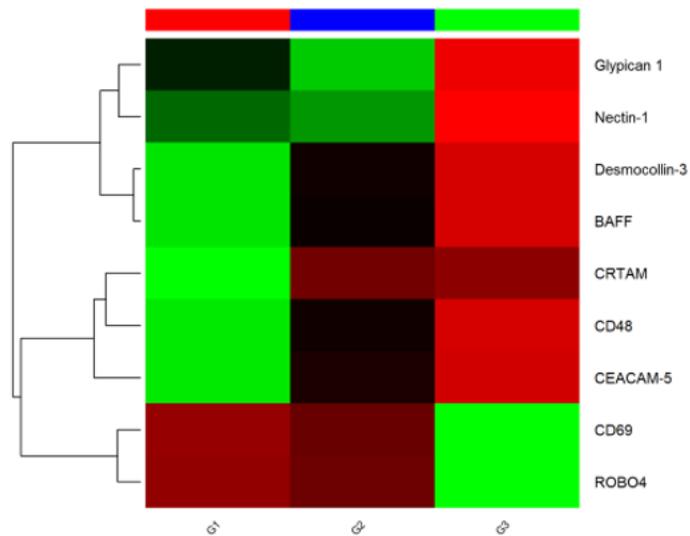


Figure 4

A caption was not provided with this version

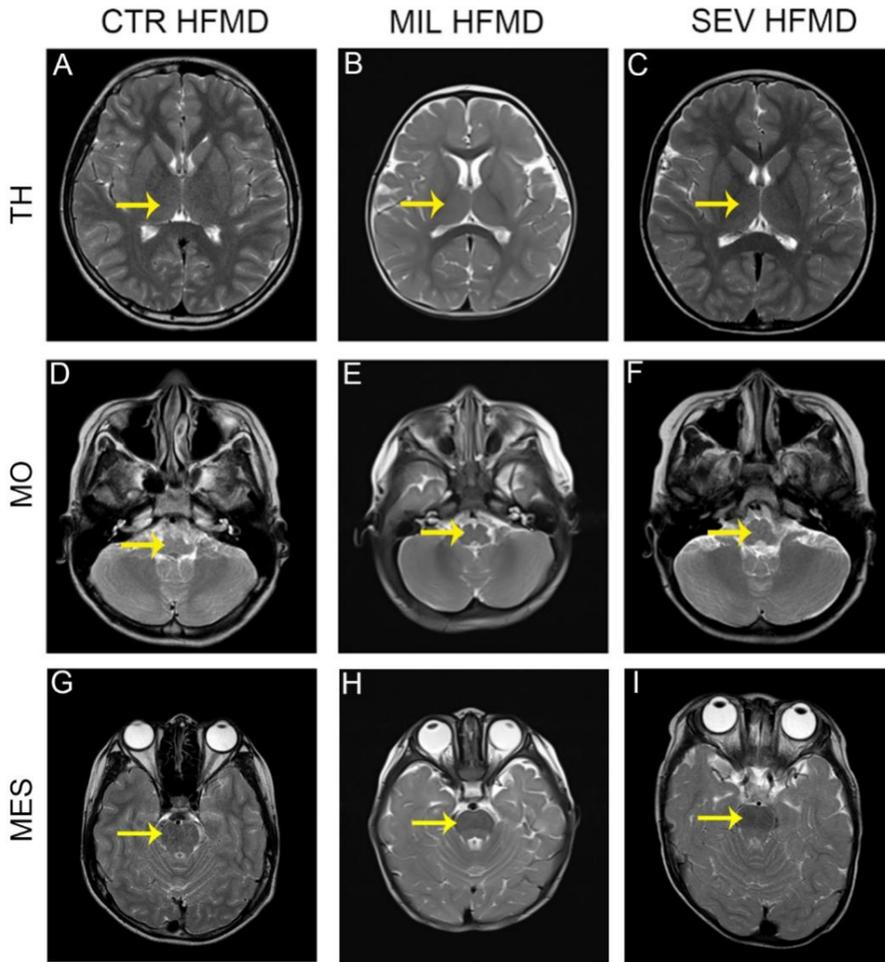


Figure 5

Brain MRI signal from a 144-month old boy without HFMD (CTR), a 10-month old girl with mild HFMD (MIL), an 82-month old boy with severe HFMD (SEV). Horizontal T1WI showed no abnormal signal at the TH, MO and MES among SEV patients, MIL patients and the CTR patients. TH: thalamus; MO: medulla oblongata; MES: mesencephalon.

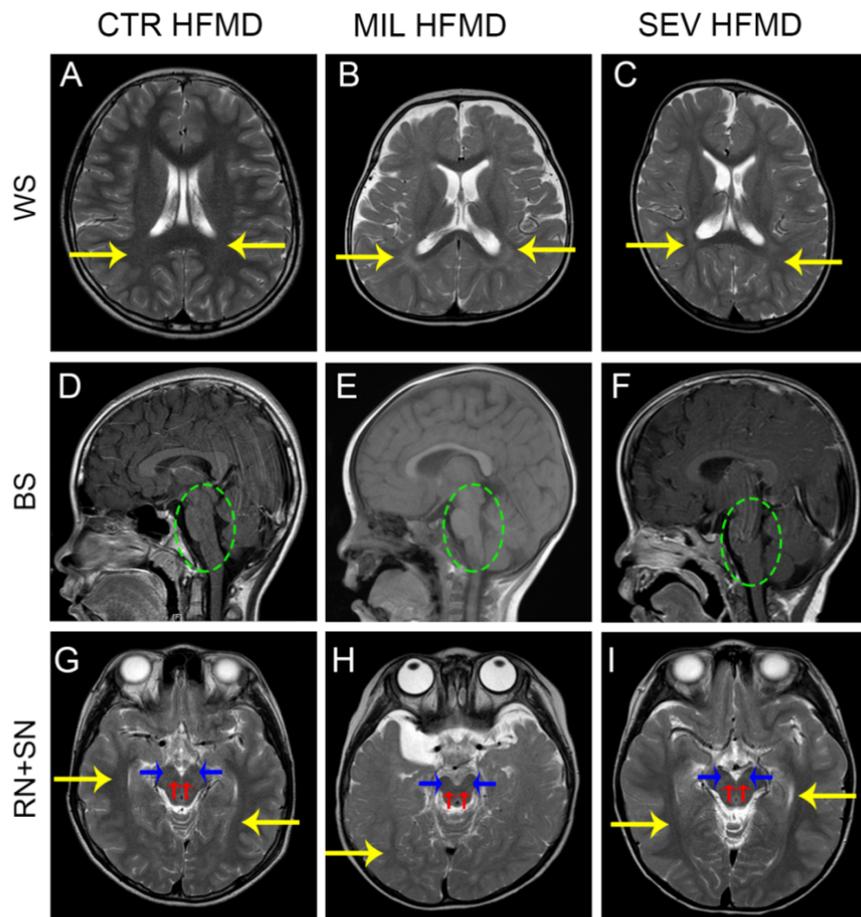


Figure 6

Brain MRI signal from a 144-month old boy without HFMD (CTR), a 10-month old girl with mild HFMD (MIL), an 82-month old boy with severe HFMD (SEV). Horizontal T2WI showed no abnormal signal at the WS (yellow arrows in A-C), BS (circles in D-F) and RN+SN (RN: blue arrows; SN: red arrows; WS: yellow arrows) among SEV patients, MIL patients and the CTR patients. WS: white substance; BS: brain stem; RN+SN: red nucleus and substantia nigra.