Author's response to reviews

Title: The significance of Notch ligand expression in the peripheral blood of children with hand, foot and mouth disease (HFMD)

Authors:

Zhen Jiang Bai (67316713@qq.com)
Yi Ping Li (ray8302880@163.com)
Jie Huang (j.shuang@163.com)
Yong Jun Xiang (415061394@qq.com)
Chun Yu Lu (luchunyu002334@sina.com)
Xiao Xing Kong (kxx670405@sina.cn)
Jian Mei Tian (Jianmeitian@yahoo.com.cn)
Jiang Huai Wang (jh.wang@ucc.ie)
Jian Wang (wj196312@gmail.com)

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Author's response to reviews: see over
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Philippa Harris, PhD
Executive Editor
BMC Infectious Diseases

Re: The significance of Notch ligand expression in the peripheral blood of children with hand, foot and mouth disease (HFMD) (MS: 1731217181069303)

Dear Dr. Harris:

On behalf of all the authors, I would like to thank you for providing us such a comprehensive review with both positive and constructive comments on our manuscript that has submitted for publication in BMC Infectious Diseases.

We have carefully considered the comments from the two reviewers, and are largely in agreement with their concerns. Specific points raised by the reviewers are dealt with in detail in the attached document named “Reply to the reviewers’ comments”, and all changes are highlighted in blue font color in the revised manuscript.

We are grateful for the opportunity to address the reviewers’ concerns and feel this has helped us to greatly improve the manuscript. We respectfully submit the revised manuscript for your consideration, and hope that the revision and responses meet the requirements of BMC Infectious Diseases.

Yours sincerely,

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Jiang Huai Wang, MD, PhD
Department of Academic Surgery
University College Cork
Cork
Ireland

Tel: 00353 21 4901275
Fax: 00353 21 4901240
Email: jh.wang@ucc.ie
Reply to the Reviewers’ Comments

Reply to the first reviewer’s comments

The first reviewer has raised a number of concerns for major compulsory revisions and several additional comments for minor and discretionary revisions, which we will endeavor to address below.

Major Compulsory Revisions

1. Footnotes of Tables 1, 2 and 3. Please clarify in footnotes (or in Methods) how the authors calculated the means and SD. Are the numbers the geometric means or arithmetic means of individuals in each group? Authors should show how many cells were acquired for FACS assays so that the readers could be able to compare the data with those from other publications.

The reviewer has raised an interesting point regarding “how the authors calculated the means and SD, and are the numbers the geometric means or arithmetic means of individuals in each group?”. In the present study, the lymphocyte subsets were expressed as the percentage and obtained from % gated events of each individual in different groups by FACScan analysis of the peripheral blood dual- or triple-stained with specific mAbs for different lymphocyte subsets. The numbers are the geometric means of individuals in each group. In addition, we have added the acquired events (cells) for FACScan analysis in the revised version (Methods, page 9, paragraph 1; Table 1 and Table 2).

2. Figure legends (or Methods) should be rewritten. The authors should explain in detail that, for example, each spot represents a case in all figures and the line represents the average level of the expression of each protein in Figures 1 and 2.

We thank the reviewer for this comment and have rewritten all figure legends for Figure 1 to Figure 4 in the revised version (Figure legends, page 22 and 23, paragraph 1 to 4).

3. Also, it is better to clarify if the expression levels of DIII1 and DII4, jaggad 1 and jagged 2 were ratios of normalized mean of GAPDH and how the relative quantification were performed for the genes in each group in legends of Figure 1 and 2 or in Methods section.

We have expanded the Methods section and figure legends to clarify the mRNA expression levels of Notch ligands Dll1, Dll4, Jagged1 and Jagged2 in Figure 1 and Figure 2, as requested by the reviewer (Methods, page 8, paragraph 2; Figure legends, page 22, paragraph 1 and 2).

4. The authors mentioned the counting of WBC and determination of total protein concentration in Methods and Discussion, but the results were not described in the Results section. The
readers may be interested to know what the differences were in these two indicators between the HFMD group and the control group without HFMD, and between the uncomplicated HFMD group and HFMD with encephalitis group.

We note the reviewer’s comment; however, we did mention total WBC counting and protein contents in CSF and their correlations with Dll4 expression levels in the peripheral blood from HFMD with encephalitis group in the Results section (Results, page 12, paragraph 2), but we did not describe the detection of total WBC and protein contents in CSF in the Methods section. A brief description of detecting CSF WBC numbers and protein contents has been added into the revised version (Methods, page 9, paragraph 2). In addition, in the present study CSF was only taken from subjects in HFMD with encephalitis group, but not from subjects in both control and uncomplicated HFMD groups, to measure total WBC and protein contents.

5. What is the volume used for WBC counts?

A 100 µl CSF sample from each subject in HFMD with encephalitis was used for total WBC counts (Methods, page 9, paragraph 2).

Minor Revisions


We have cited the latest data in 2012 and 2013 on HFMD from Chinese CDC (Background, page 5, paragraph 1; Reference 1, page 18), and included the paper entitled “Hand, foot, and mouth disease in China, 2008-2012: an epidemiological study” published in Lancet Infect Dis (Reference 2, page 18).

Discretionary Revisions

The virus identification should be included, if possible, by performing a quantitative real time PCR or conventional RT-PCR using samples from the patients. The readers may be interested in knowing how many cases were caused by EV71 or CVA16 or other enteroviruses (or unidentified viruses). Also, it is interesting to find out if there are any differences in these indicators investigated between cases caused by EV71 and CVA16.

We note the reviewer’s comment. In the present study, we did run PCR to identify EV71 and CoxA 16, but not other enteroviruses and unidentified viruses. In 82 HFMD cases, we detected
EV71 in 13 cases and CoxA 16 in 16 cases, with an EV71 positive rate at 15.9% (13/82), a CoxA 16 positive rate at 19.5% (16/82), and a totally positive rate at 35.4% (29/82). Due to the relative low positive rate, we did not include these data in the manuscript.


Reply to the second reviewer’s comments

The second reviewer has raised one comment for minor essential revisions and three comments for discretionary revisions, which we will endeavor to address below.

Minor Essential Revisions

It would be helpful if the authors included the best-fit line in the r squared analysis of correlations they state are positive.

We thank the reviewer for this comment, and in the revised version we have added the best-fit line in the R-square analysis of correlations with statistical significance (Figure 3 and Figure 4).

Discretionary Revisions

It may be beneficial to include a statement regarding the regulation of Dll1 by type 1 interferon, which was shown in an influenza infection model.

Based on the reviewer’s suggestion, the following sentences of “Furthermore, Notch ligands Dll1 and Dll4 are both involved in the initiation of an anti-viral response [9,10]. While type-I IFN-induced Dll1 expression on macrophages plays a critical role in preventing influenza A virus infection [9], Dll4 appears to limit physiologic and pathologic changes in the lung during respiratory syncytial virus infection by modulating the Th2 response [10].” have been included in the revised manuscript (Background, page 5, paragraph 2; References 9 and 10, page 19).

Is it possible to run a PCR to quantify the amount of virus in CSF and correlate this to Dll1/Dll4 expression in blood?

We thank the reviewer for this comment; however, we did not quantify the amount of virus in CSF, although we did run a PCR to identify the virus in the peripheral blood from children with HFMD.

It may also be useful to correlating dll1/dll4 with expression of specific genes known to be important downstream targets of Notch signaling such as CD25 or BCL-2.

We are grateful for this important comment made by the reviewer, regarding correlations of Dll1/Dll4 with the downstream target gene expression of Notch signalling, which will be certainly our next focus in an attempt to clarify the impact of Notch-mediated signalling pathway in the pathogenesis of HFMD.