Peer Review File

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Reviewer A

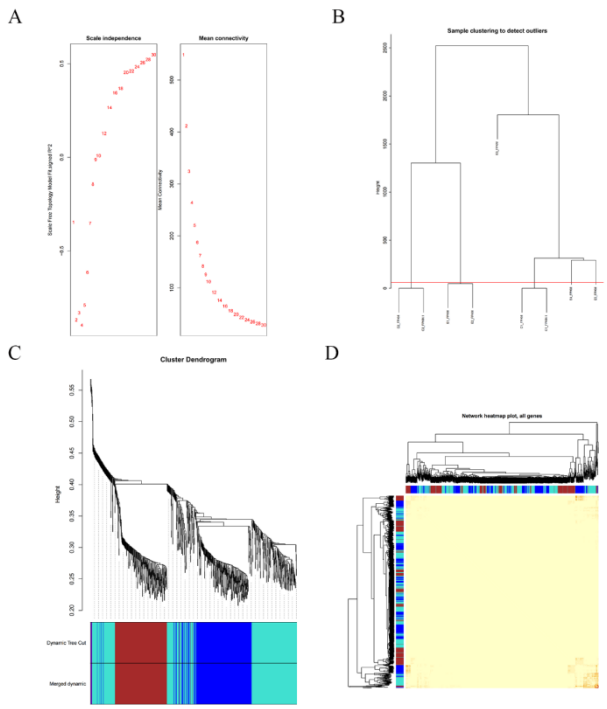
The paper titled "Identification of epidermal growth factor receptor as an immune-related biomarker in epilepsy using multi-transcriptome data" is interesting. The pathophysiology of epilepsy was correlated with EGFR. Thus, EGFR could be a novel biomarker of juvenile focal epilepsies, and the findings provide promising therapeutic targets for epilepsy. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.
Reply: Thank you so much for your valuable advice. Currently several studies have revealed the immune-related biomarkers in epilepsy (PMID: 35822432, 32532251). These studies have explored the expression of these molecules in epilepsy, but lack in-depth exploration of immunity. Our research strived to develop an immune-related ceRNA network and examine the Immune cell infiltration wdetermine the abundance and quantity of the immune cells in epilepsy. An epilepsy mouse model was employed to validate the manifestation of the key gene. Our findings will provide new insights into the mechanism of epilepsy development and highlight a promising prognostic and treatment marker for epilepsy patients. We revised the manuscript accordingly. Thank you very much!
Changes in the text: line 100-102, Page 3-4; line 134-136, Page 4-5.
line 100-102, Page 3-4;
Immune response and inflammatory mediators contribute to epileptogenesis while reducing the seizure threshold in individuals (13,14). **Currently several studies have revealed the immune-related biomarkers in epilepsy (15,16). These studies have explored the expression of these molecules in epilepsy, but lack in-depth exploration of immunity.**
line 134-136, Page 4-5:
Our findings will provide new insights into the mechanism of epilepsy development and highlight a promising prognostic and treatment marker for epilepsy patients.

2) The description of Figure 7A is mentioned in the results of this study, but 7A is not shown in the figure. Please carefully check and make corrections.

Reply: We are very sorry for the mistake. We have revised the manuscript accordingly. Thank you very much!
Changes in the text: line 599, Page 22

3) The bioinformatics analysis of this study is too simplistic, and it is recommended to add weighted gene co expression network analysis to identify immune related genes that contribute to the development of epilepsy.
Reply: Thank you for your valuable feedback on our manuscript. We appreciate your comments regarding the need to add weighted gene co expression network analysis (WGNCA) to identify immune related genes in our study. Our study has included 7 samples including 5 epilepsies and 2 controls, and the WGCNA website does not recommend conducting WGCNA analysis on cases with less than 15 samples (https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/). We apologize for not clearly describing the sample size in the article. In addition, we completed the WGCNA analysis based on your suggestion (as shown in the figure below), but did not achieve satisfactory results. We clearly described our sample size in the revised manuscript. Please refer to line 609-621, Table S1: Clinical characteristics and related information of the control group and epilepsy groups, Page 24. Thank you very much!



4) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply: We are very sorry for the mistake. We have revised the figures accordingly. We sent all the figures with the compressed attachments and modified them in the original text. Thank you very much!
Changes in the text: line 555-602, Page 17-22

5) How to analyze the immune infiltration patterns of epilepsy based on the ceRNA network related to epilepsy in this study? It is recommended to add relevant content.
Reply: We thank the reviewer for the comments and the reviewer's point is well taken. In the research, we employed CIBERSORT and ssGSEA to estimate immune cell infiltration between the control and epileptic samples. A significant difference in the degree of immune infiltration was observed in the control and epileptic samples. Based on this, we evaluated the biological activities and signaling pathways especially immune response of mRNA in the ceRNA networks using GO and KEGG analysis. The findings established that immunoregulation is significantly involved in the pathogenesis of epilepsy. Due to the limitations of bioinformatics analysis, we cannot analyze the immune infiltration patterns of epilepsy based on the ceRNA network related to epilepsy. We apologize for any confusion caused by the lack of clarity in this section. We have revised the relevant content accordingly. Thank you very much!
Changes in the text: line 290-294, Page 9.
The results demonstrated that the key ceRNAs may be involved in endocrine resistance, prolactin signaling pathway, and MAPK cascade (Figure 6E). Darker colors signified higher scores, and EGFR was selected as the core IRG for follow-up studies. A total of 38 lncRNAs, one miRNA (MIR27A), and one mRNA (EGFR) formed the immune-related core ceRNA network **(Figure 6F)**.

6) It is recommended to add research on the progression of EGFR hypersensitivity response to epileptic seizures in the discussion.
Reply: We appreciate your suggestion to add research on the progression of EGFR hypersensitivity response to epileptic seizures in the discussion. We have conducted a thorough literature review and identified several relevant articles. It was reported that overexpression of EGFR caused by focal copy number gains was identified in epilepsy (PMID: 33274363). Furthermore, EGFR was significantly associated with the risk of epilepsy occurrence (PMID: 33878595), and EGFR inhibitor was identified as a novel antiepileptic choice (PMID: 30235116). We have revised the manuscript accordingly. Thank you very much!
Changes in the text: line 380-387, Page 12
Further, it was reported that EGFR was significantly associated with the risk of epilepsy occurrence (48), and overexpression of EGFR caused by focal copy number gains was identified in epilepsy (49). EGFR belongs to the receptor tyrosine

kinase family. DNA methyltransferase 3 alpha (DNMT3) may regulate EGFR involved in cortical development, neuronal plasticity, and epileptogenesis in patients with type II FCD (50). Mice that underwent brain-specific EGFR ablation were found to be sensitive to KA-induced seizures and showed signs of neurodegeneration (50,51). **EGFR inhibitor was identified as a novel antiepileptic choice (52).**

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Narrative review of epilepsy: getting the most out of your neuroimaging, ransl Pediatr, PMID: 34012857". It is recommended to quote the article.

Reply: We appreciate the valuable advice from the reviewer. We noticed that the introduction part of this paper is not comprehensive enough. The key role of brain imaging in the diagnosis, follow-up, and preoperative evaluation of epilepsy patients requires the attention of neuroradiologists and trainees approaching pediatric brain imaging (PMID: 34012857). We have revised the introduction section of our manuscript to incorporate the article and provide a more comprehensive overview of the current state of the field. We believe that these revisions have improved the manuscript and provided valuable context for readers. Thank you very much!

Changes in the text: line 87-89, Page 3.

During the last decades, due to the increasing use of MRI in epilepsy, cortical malformations have been "reconsidered" as one of the most frequent etiologies of focal epileptic seizures in both pediatric and young adults' population (1).

8) It may be more meaningful to add functional research on key ceRNAs.

Reply: Thanks a lot for your advice. In response to your comments, we employed Metascape (http://metascape.org) to revealed the underlying mechanism of key ceRNAs (EGFR, GRB2, KRAS, FOS, ESR1, MAPK1, MAPK14, MAPK8, and PPARG). The results demonstrated that the key ceRNAs may be involved in endocrine resistance, prolactin signaling pathway, and MAPK cascade (as shown in the figure below). We revised the manuscript accordingly. We hope that these revisions have addressed your concerns, and we thank you again for your thoughtful feedback.

Changes in the text: line 196-198, Page 6.; line 586-592, Figure 6, Page 21.

line 196-198, Page 6:

plugin in Cytoscape.**Metascape (http://metascape.org) revealed the underlying mechanism of key ceRNAs (EGFR, GRB2, KRAS, FOS, ESR1, MAPK1, MAPK14, MAPK8, and PPARG).**

line 586-592, Figure 6, Page 21.

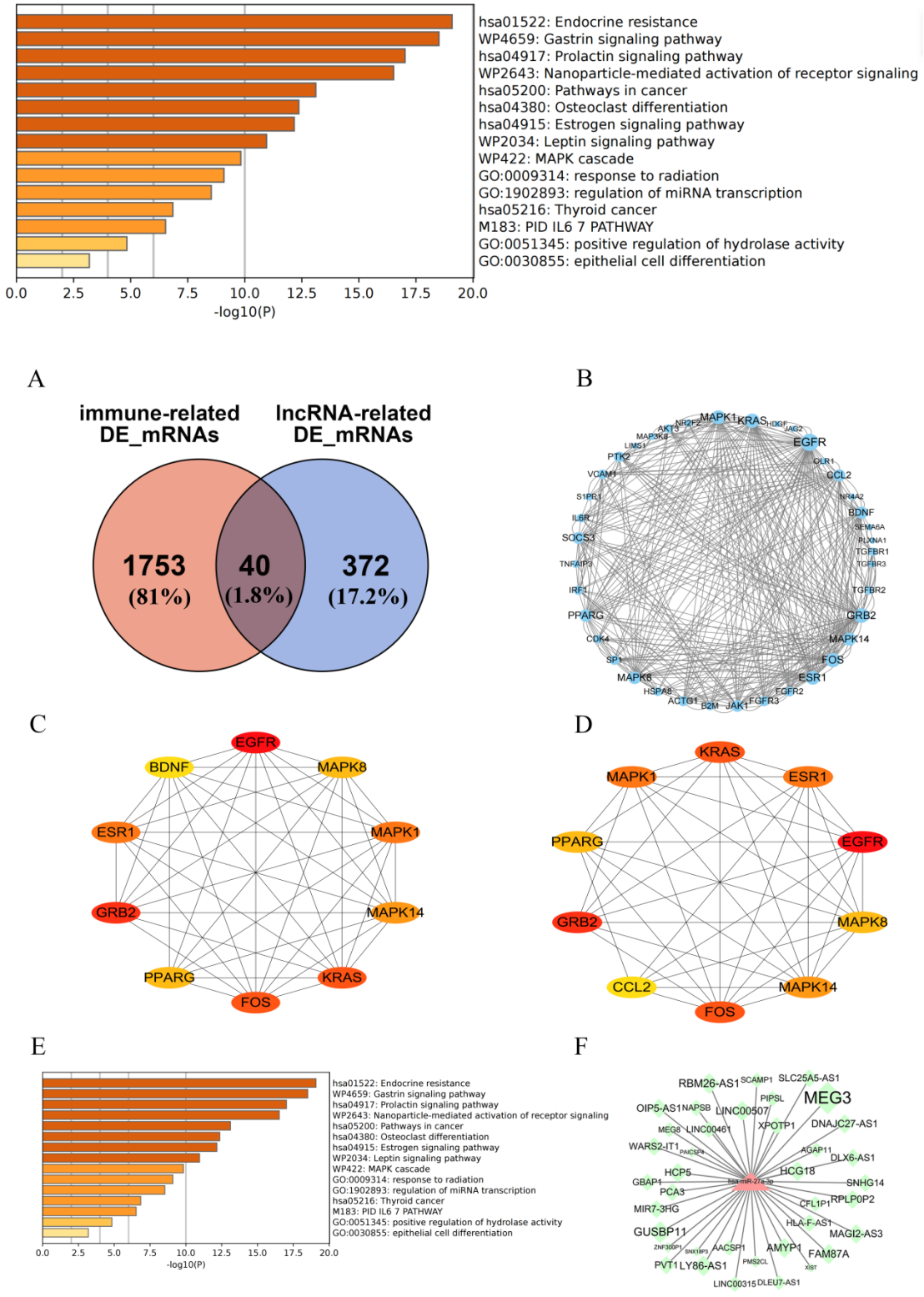


Figure 6 Immune-related core genes and PPI network construction. (A) Overlapped Venn of 40 immune-related DEmRNAs. (B) PPI network analysis, revealing 40 nodes and 375 edges in Cytoscape. Node size was positively correlated with degree value. The top 10 proteins in the PPI network ranked by (C) degree and (D) maximum neighborhood component methods. **(E)Metascape of key ceRNAs (EGFR, GRB2,**

KRAS, FOS, ESR1, MAPK1, MAPK14, MAPK8, and PPARG). (F) Sub-ceRNA network included 38 lncRNAs, one miRNA (hsa-miR-27a-3p), and one mRNA (EGFR). PPI, Protein-protein interaction; DEmRNAs, differentially expressed mRNAs; EGFR, epidermal growth factor receptor.

Reviewer B

1. **Figure 1**

The figure 1 was damaged and cannot be opened, please send the latest version in an editable format (DOC/PPT).

Reply: We are very sorry for the mistake. We have revised the manuscript accordingly.We provided a folder in the attachment to the mail, named "Figure 1-revised". It contains Figure 1 in PPT, PDF, DOC,TIF and PNG. Since we converted the DOC format but had poor picture quality, we tried to provide multiple formats for editing. Thank you very much!

2. Please double check figure 7. There's ONLY image A in the figure, so please remove the letter "A" in the legend, and revise figure 7A to figure 7 in the main text.

Figure 7 (A) Correlation between EGFR and infiltrating immune cells. Dot size is

18

296 (Figure 7). The final results showed that type 1 T helper cells were considered to be
297 important immune cells associated with epilepsy formation and that the expression of
298 the EGFR gene was positively correlated with EGFR as well as immature dendritic
299 cells (R=0.857, P=0.024), mast cells (R=0.821, P=0.034), (Myeloid-derived suppressor
300 cells) MDSC (R=0.786, P=0.048), and plasmacytoid dendritic cells (R=0.857,
301 P=0.024), and negatively correlated with CD56dim natural killer cells (R=-0.929,
302 P=0.007)(Figure 7A).

Reply: We are very sorry for the mistake. We have revised the manuscript accordingly. Thank you very much!

Changes in the text: line 303 and 309, Page 10； line 605-606， Page 20

3. Reporting checklist

No related information was found in the main text, please check. If it is not applicable, please fill with N/A.

Experimental study design (statistics details)	Yes (indicate where provided: section/paragraph)	n/a
State whether and how the following have been done, or if they were not carried out.		
Sample size determination	Yes(BCA)	
Randomisation	Yes	
Blinding		N/A
Inclusion/exclusion criteria	Yes(at least one behavioral spontaneous recurrent motor seizure observed 28 d after injection considered a successful model of chronic epilepsy)	

Reply: We are very sorry for the mistake. We have revised the file accordingly. Thank you very much!

Change in the text：

Sample size determination	Yes(BCA)	
Randomisation		N/A

4. References/Citations

Please double-check if more studies should be cited as you mentioned “studies”. OR use “study” rather than “studies”.

115 (miRNAs) (23-25). Recent studies have shown that the lncRNA ZNF883 inhibits
116 NLRP3 ubiquitination and promotes epilepsy through upregulating USP47(26),and
117 lncRNA ZFAS1 might contribute to the progression of epilepsy by regulating the miR-
118 15a-5p/OXSR1/NF-κB pathway. However, this was only briefly examined in recent

Reply: We are very sorry for the mistake. We have revised the manuscript accordingly. Thank you very much!

Changes in the text: line 116-117， Page 4

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117 NLRP3 ubiquitination and promotes epilepsy through upregulating USP47(26),and