Author’s response to reviews

Title: Genetic Diversity and Epidemiology of Genogroup II Noroviruses in Children with Acute Sporadic Gastroenteritis in Shanghai, China, 2012-2017

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BMC Infectious Diseases

Dear Editor in chief,

Thank you for the opportunity to revise this paper for resubmission. We appreciate the comments and suggestions from the reviewers and believe that their input has improved the quality of our manuscript. We revised the manuscript in accordance with the reviewers’ comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors.

Please see below our detailed responses to individual comments made by the reviewers. We hope you will find these changes acceptable. Thank you for considering this revised manuscript for publication in BMC Infectious Diseases. We welcome further suggestions to improve the clarity of our manuscript for publication in your journal.
Sincerely yours,

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Response to Reviewers

Editor:

Critique 1: Figure 1: Avoid plotting bars in 3D. X- and Y-axis titles are missing.
Response: We apologize for our slip of the pen. We have modified Figure 1 according to your advice in part of Figures.

Critique 2: Figure 2: X- and Y-axis titles are missing.
Response: We beg your pardon for our negligence. We have modified Figure 2 as your advice in part of Figures.

Critique 3: Referring to Reviewer 2's comment on Figure 2, one may refer to this UK norovirus report (Figure 1) on how to visualize data by months: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/800503/Norovirus_update_2019_weeks_16_to_17.pdf
Response: Thank you for the good suggestion. We have modified the Figure 2 as your and Reviewer 2's comment recommended in part of Figures. Moreover, we also rephrased the results and discussion on Page 2, Line 40-41, Page 7, Line 144-146 and Page 10, Line 206-208.
Critique 4: Figure 3: The legend of the phylogenetic tree is too brief.

Response: Thank you for the comment. We have revised the legend of the phylogenetic tree in part of Figure Legends on Page 22, Line 476-482.

Reviewer 1 (Jing Lu):

Critique 1: In this study, the sequences of RdRp and VP1 gene were sequenced separately. This could not provide the solid evidence on the combination of RdRp/VP1 NoV genotype. Are there any multiple genotypes infections identified in a single case? If there is, the combination of RdRp/VP1 genotype could be wrong due to PCR bias.

Response: Thank you for your comments. For the first question, we used combination of RdRp/VP1 NoV genotypes instead of recombinant NoV genotypes because the sequences of RdRp and VP1 gene were sequenced separately like other studies (For Example: BMC Infect Dis. 2018 May 30;18(1):246. doi: 10.1186/s12879-018-3157-y, Biomed Res Int. 2015;2015:468304. doi: 10.1155/2015/468304, Viruses. 2019 May 29;11(5). pii: E400. doi: 10.3390/v11050400.). If we used recombinant NoV genotypes, the sequences of ORF1/ORF2 overlap should be also amplified to confirm the recombination event using SimPlot or RDP4 analysis. For the second question, the combination of RdRp/VP1 genotypes were reliable because no multiple genotypes infections were identified in a single case according to our results.

Critique 2: Short sequences of RdRp and VP1 may be difficult to define the sub genotypes of NoVs. In the Figure 3, the GII.4_2006b and GII.4_Sydney_2012 phylogenetic clusters should be clearly marked if author wanted to mention the dynamic change of these two GII.4 sub genotypes in AGS cases. The full length of RdRp and VP1 genes of representative isolates are recommended used in phylogenetic analysis.

Response: Thank you for the good suggestions. For the first question, longer sequences of RdRp and VP1 for defining the sub genotypes of NoVs may be more accurate. However, short sequences of RdRp and VP1 used for defining genotypes and the subgenotypes of NoVs were reliable and more operational in practical work. Moreover, most of the literatures used short sequences of RdRp and VP1 for determining the genotypes and the subgenotypes of NoVs (For example: PLoS One. 2017 Dec 13;12(12):e0189504. doi: 10.1371/journal.pone.0189504, Biomed Res Int. 2015;2015:468304. doi: 10.1155/2015/468304 and J Trop Med. 2016;2016:2707121. doi: 10.1155/2016/2707121). For the second question, because GII.4 subgenotypes were analyzed using the Norovirus Genotyping Tool v.2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/ ) in this study, the GII.4_2006b and
GII.4_Sydney_2012 phylogenetic clusters were not marked in the phylogenetic tree. For the last question, the main purpose of this study was to investigate the molecular epidemiology of norovirus in outpatient children. The full length of RdRp and VP1 genes is usually needed for analyzing the evolution and genetic variation characteristics of NoVs. We will adopt your good advice in our future research.

Critique 3: Why did author choose Kimura two-parameter model for ML analysis? Was it based on the model test result?

Response: Thank you for your comment. For the first question, Kimura two-parameter model is recommended for phylogenetic analysis of nucleic acid sequence using the ML method and Neighbour-joining method. But the ML analysis is more accurate than the Neighbour-joining method for analyzing high similarity nucleic acid sequence. Thus, we chose Kimura two-parameter model for ML analysis in this study. For the second question, this model is recommended for phylogenetic analysis of nucleic acid sequence using the ML method. Some studies have successfully adopted this model for NoVs’ phylogenetic analysis (For example: Med Microbiol Immunol. 2018 Aug;207(3-4):201-210. doi: 10.1007/s00430-018-0541-6 and Infect Genet Evol. 2015 Apr;31:48-52. doi: 10.1016/j.meegid.2015.01.008. ). Thus, we used this model directly.

Reviewer 2 (Yuanyun Ao):

Major issues:

Critique 1: The authors described the epidemiology of norovirus throughout the study. however, the noroviruses were detected just using the primers targeting the GII norovirus combination of RdRp and capsid genes; the identification for another human norovirus genotypes such as GI and GIV were not conducted in this study. Thus, I am not sure if the true positive rate of norovirus that displayed, based on the current date. An enzyme immunoassay (EIA) or RT-PCR were firstly suggested to tested for the presence of all human norovirus genotypes, and then the norovirus positive samples were subjected to genotyping by RT-PCR.

Moreover, in view of the accuracy of the data, we changed the title from "Genetic Diversity and Epidemiology of Noroviruses in Children with Acute Sporadic Gastroenteritis in Shanghai, China, 2012-2017" to "Genetic Diversity and Epidemiology of Genogroup II Noroviruses in Children with Acute Sporadic Gastroenteritis in Shanghai, China, 2012-2017". We have also made precise changes to the corresponding contents in the article. For the second question, enzyme immunoassay (EIA) has lower sensitivity than RT-PCR in detecting NoVs. Thus, all the samples were subjected to both norovirus detection and genotypes identification using RT-PCR directly in this study. All norovirus positive samples detected by RdRp were consistent with that by capsid genes. But we will consider your suggestion in our next research.

Critique 2: Since the clinical data were collected, the symptoms characteristics of different norovirus in patients with acute gastroenteritis could be suggested to analyze in this study, except for age, season and genotype characteristics of norovirus.

Response: Thank you very much for your propose. Because all the patients came from outpatient, the clinical characteristics that we could obtain were limited. But we will modestly accept your comment in our following work.

Critique 3: During 2012 to 2017, diverse genotypes of noroviruses has been found to be circulated in Shanghai. However, there is no substantial date showing the systematically analyses of norovirus sequences and evolution, especially GII.2, GII.4, GII.17 viruses that had been associated with epidemics worldwide.

Response: Thank you for the comment. The main purpose of this study was to investigate the distribution and epidemiology of different GII norovirus genotypes by targeting RdRp and capsid genes in outpatient children from Children’s Hospital of Fudan University in Shanghai. Thus, we did not systematically analyze NoVs evolution in this study. But your advice is very useful and we will make more analysis in our next study.

Critique 4: Table 3: I would suggest to use a figure not a table, as it seemed more clear to show the results.

Response: Thank you very much for your propose. We have modified Table 3 to Figure 4 and Figure 4 became Figure 5. Please see the Figures part. The quotes and figure legends were changed accordingly in the body of the article and Figure Legends on Page 8, Line 181, Page 9, Line 191, and Page 22, Line 484-488.
Critique 5: Figure 2: It is very not proper that the authors analyzed the seasons of norovirus by combining the months with six years. It may be correctable to describe and claim the relationship between seasons and noroviruses by divided months in each years. Please accordingly rephrase the results in the paper.

Response: Thank you for your guidance. We have modified Figure 2 as your advice and rephrased the results and discussion on Page 2, Line 40-41, Page 7, Line 144-146 and Page 10, Line 206-208.

Minor comments:

Critique 6: Line 29: replace "Noroviruses" with "Norviruses (NoVs)".

Response: Thank you for your suggestion. We have replaced "Noroviruses" with "Noroviruses (NoVs)" as your advice on Page 2, Line 29.

Critique 7: Line 30: delete "prevalence and"

Response: Thank you for your advice. We have delete "prevalence and" on Page 2, Line 30-31.

Critique 8: Line 33: add "1433" before "children".

Response: Thank you for your suggestion. We have added "1433" before "children" on Page 2, line 33-34.

Critique 9: Line 39 to 40: replace "With high detection" with "with the high detection rate".

Response: Thank you for your guidance. With the change an English native speaker expert made, we have replaced "With high detection" with "with the highest detection rate" on Page 2, Line 39-40.

Critique 10: Line 76: replace with "rotaviruses" for "rotavirus".

Response: Thank you for your recommendation. We have replaced "rotavirus" with "rotaviruses" on Page 4, Line 75.
Critique 11: Line 85 to 91: please rephrase the sentences. It's too wordy.
Response: Thank you for your proposal. We have modified the sentence on Page 4, Line 84-89.

Critique 12: Line 93 to 94: please delete "conducted the current study".
Response: Thank you for your advice. We have deleted "conducted the current study" on Page 4, Line 91-92.

Critique 13: Line 103: please add the definition of acute gastroenteritis or reference.
Response: Thank you for your suggestion. We have added the definition of acute gastroenteritis and cited a reference on Page 5, Line 102-106.

Critique 14: Line 122 to 123: replace "phylogenetic analysis on the nucleotide sequences obtained in our study and sequences data from GenBank" with "phylogenetic analysis on the sequences in our study and GenBank"
Response: Thank you for your guidance. With the change an English native speaker expert made, we have modified this sentence on Page 6, Line 124-125.

Critique 15: The writing style should be revised by a English native speaker scientist.
Response: Thank you for your comment. We have invited an English native speaker expert to review overall English. Certificate of English Amendment has been sent to the editor.