Jundishapur Journal of Microbiology
Volume 16 · Issue 9 · e137970

OPEN PEER REVIEW

# A New Duplication in 668 to 672 Sites of *UL54* Gene in *Cytomegalovirus* Ganciclovir Resistant Liver Transplanted Patients

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| SUBMITTED | REVISED | ACCEPTED | DOI |
| --- | --- | --- | --- |
| 30 May 2023 | 9 Oct 2023 | 9 Oct 2023 | 10.5812/jjm-137970 |

## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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## 1 Original submission

1 comment · 13 Jun 2023

### Referee 1 Reviewer

MINOR REVISION 13 Jun 2023

Dear Editor
The author of this manuscript evaluates possible mutations in the CMV UL54 gene, which is responsible for drug resistance in liver transplant recipients.
Reviewer's comments are as follows:
1- The whole manuscript needs to be revised for English language usage. The manuscript is full of confusing sentences. Some of them were mentioned in the revised Word file.
2- Please clearly state the inclusion criteria in the methods section.
3- Why did you choose a 50% cutoff for the bootstrap test?
It seems low. In some other studies, the minimum acceptable BS test value is stated as 70%.
4- How do the authors establish the relationship between the newly found mutation (AK124703.1:p.V668-G672dup) and drug resistance?
5- There was no phylogenetic tree figure in the manuscript; please add it.

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# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**Answer to reviewers' comments:**

**Dear Editor and reviewers,**

Thank you for your careful consideration, we have changed every mentioned point in the text and highlighted. Any further changes will be done if it is needed.

Sincerely yours,

Ramin Yaghobi

**Answer to reviewer 1:**

**Reviewer 1:**
Dear authors,

Thank you for submitting your valuable manuscript to the Journal of Medical Microbiology (JJM).

Below, I will provide some tips about your manuscript in different sections, which I hope will help you improve your manuscript. Please consider the issues raised and make any necessary revisions.

**ABSTARCT:**

QI. Please review the manuscript for grammar and English usage. Below are some of the writing problems that I encountered just by reading the abstract of the article.

RI. The English and grammar of the manuscript is revised completely.

The abstract appears to be a scientific research article on the topic of cytomegalovirus (CMV) infection in liver transplant recipients and the association of UL54 mutations with ganciclovir resistance. However, there are a few problems with the English language and content of the abstract:

QII. The sentence "Drug-resistant CMV infections might be a cause of high-dose treatment patients" is grammatically incorrect. It should be rephrased to something like "Drug-resistant CMV infections may require high-dose treatment in patients."

RII. The sentence is corrected.

QIII. The sentence "Using PCR real-time, 223 samples from 70 patients were positive for CMV selected for simple and nested PCRs" is poorly worded. It would be clearer to say something like "Real-time PCR was used to identify CMV-positive samples from 70 patients, and 52 samples from 23 patients were selected for analysis of UL54 gene mutations using simple and nested PCRs."

RIII. The sentence is corrected.

QIV. The sentence "The drug of choice for the treatment of systemic infections is ganciclovir" is a bit clunky. It would be better to say "Ganciclovir is the drug of choice for the treatment of systemic CMV infections."

RIV. The sentence is corrected.

QV. The sentence "Mutations in the UL54 gene encoding DNA polymerase, can lead to resistance" is a bit confusing. It would be better to say "Mutations in the UL54 gene, which encodes DNA polymerase, can lead to resistance."

RV. The sentence is corrected.

QVI. The sentence "After sequencing from 23 patients (52 samples), 25 mutations of UL54 gene were detected in 9 patients (15 samples) of CMV-infected patients at a median of 32 days posttransplant" is a bit wordy. It would be better to say "After sequencing 52 samples from 23 patients, we found 25 mutations in the UL54 gene in 9 patients who were CMV-infected at a median of 32 days posttransplant."

RVI. The sentence is corrected.

QVII. Therefore, it is generally better to have the manuscript reviewed by a native.

RVII. The whole manuscript reviewed for English editing.

**• There are also a few problems with the content of the abstract.**

QI. The abstract does not mention the significance of the findings. For example, it is not clear how the findings of this study could impact the treatment of CMV infection in liver transplant recipients.

RI. The result section of abstract changed.

QII. The abstract does not provide enough detail about the methods used in the study. For example, it is not clear how the mutations in the UL54 gene were detected.

RII. This point is corrected in the abstract.

QIII. The conclusion of the abstract is not very clear and could be improved. It should summarize the key findings of the study and provide a clear recommendation for clinical practice. The current conclusion mentions viral load testing and changing therapy, if necessary, but it is not clear how this relates to the UL54 mutations or ganciclovir resistance.

RIII. This point is considered in the text.

QIV**.** Overall, the abstract is a bit rough around the edges. However, the findings of the study are interesting and could potentially lead to new treatments for CMV infection in liver transplant recipients. With some polishing, the abstract could be a valuable resource for other researchers.

RIV. All the mentioned points are included in the abstract section.

**INTRODUCTION:**

QI. the introduction does not clearly state the research question or hypothesis that the study aims to address. Despite mentioning that the purpose of the project is to determine the pattern of mutations associated with anti-ganciclovir drug resistance in Iranian CMV infected liver transplant patients, this statement is buried in the middle of a long paragraph. A clearer and more concise statement of the research question and its significance would help readers understand the purpose of the study and its potential impact.

RI. This point is corrected in the text.

QII. Finally, while the introduction references several sources to support the points made, there is no clear indication of the methodology or study design that was used to conduct the research. This information is essential to understanding the validity and reliability of the study, and should be included in the introduction or methods section of the article.

RII. This point is added to the text.

**MATERIALS AND METHODS:**

• The content of the Materials and Methods section of the article seems to be quite detailed and well-organized. However, there are a few potential issues that could be improved:

QI. Sample size selection: The article does not provide a clear justification for why only 23 out of the original 70 patients were selected for further studies. The criteria for selecting patients

with the "minimum CMV result" should be clearly defined to ensure that the sample is representative and unbiased.

RI. The sample size explanation modified.

QII. Lack of detail on immunosuppressive and antiviral drug regimens: While the article mentions the drugs used for immunosuppression and antiviral therapy, it does not provide any detailed information about dosages, durations, or any adjustments made to the regimens during the study. This information is important to help readers understand the context of the results and any potential confounding factors.
RII. The detail of immunosuppressive and antiviral drug regimens added to this section.

QIII. Insufficient detail on statistical analysis: While the article mentions the statistical tests used to analyze the data, it does not provide any details on the specific variables analyzed, or the methods used to control for potential confounding factors. More information on the statistical analysis would be helpful to ensure that the results are reliable and meaningful.
RIII. The needed detail on statistical analysis added.

QIV. Explain why you use four primers for simple PCR and four primers for nested PCR sets were used specifically for a highly conserved region?

RIV. Several primers were used to increase the specificity and sensitivity of simple PCR and nested PCR tests.

QV. Overall, the Materials and Methods section provides a good overview of the study design and methods used, but could benefit from more detail and clarity in some areas.
RV. It is tried to add more details and clarify this section.
**RESULTS:**
Qa. Lack of clarity on the purpose of the phylogenetic tree: The article does not provide a clear explanation of why the phylogenetic tree was constructed or how it relates to the research question. A more detailed description of the purpose and methods of the phylogenetic analysis would help readers understand the significance of the results.
Ra. The purpose of drawing a phylogenetic tree is not only a clinical view, but the purpose is to determine the location of circulating cytomegalovirus in liver transplant recipients from the perspective of the phylogenetic tree.

Qb. Unclear terminology: The article uses the term "Intra-species proximity" and "Intra-species differences" without providing clear definitions or explanations. It is unclear whether these terms refer to genetic distance, phylogenetic relationships, or some other measure of similarity or

difference. Clarifying the terminology would help readers understand the results more clearly.
Rb. All of the terminologies explained.

Qc. Lack of detail on the strains used for comparison: The article mentions several strains that were used as a reference for the phylogenetic analysis, but does not provide any information on how these strains were selected or what their significance is. More information on the selection and characteristics of the reference strains would help readers assess the validity and significance of the results.
Rc. These points are corrected in the text.

Qd. Fig3. The caption is vague and the quality of the photo is very poor.
Rd. The caption edited and the quality of photo improved.
Qe. Overall, while the section provides some information on the results of the study, it could benefit from more clarity and detail to help readers understand the significance of the findings.
Qe. We have added more details and clarified the result section.
**DISCUTION:**
QI. The discussion is not well organized. The authors jump from topic to topic without providing a clear connection between each point. This makes it difficult for the reader to follow the authors' train of thought.
RI. We reorganized the discussion.

QII. The discussion is not comprehensive. The authors only discuss a few of the findings of their study. They do not discuss the implications of their findings or how they relate to the existing body of research on CMV infection and ganciclovir resistance.
RII. The discussion is rewritten in order to be more comprehensive.

QIII. The discussion is not objective. The authors make several subjective statements, such as "Our results are important because they provide new insights into the development of ganciclovir resistance in CMV-infected liver transplant recipients." These statements are not supported by evidence and can be misleading to the reader.
RIII. The discussion is rewritten in order to be more objective.

QIV. Lack of context: Another issue with the discussion content is that it does not provide much context or background information to help readers understand why the topic is important. While the article briefly mentions that CMV infection is a common complication of liver transplantation, it does not explain why this is significant for patient outcomes or healthcare more broadly.
RIV. More background information is added.

QV. Limitations and future research: Finally, the discussion content of the article could be improved by more explicit statements about the limitations of the study and the implications for

future research. While the article provides some interesting findings about the incidence of ganciclovir resistance in liver transplant recipients, it is clear that there is more work to be done to fully understand the mechanisms of drug resistance and to develop more effective treatment strategies. By acknowledging these limitations and pointing to areas for future research, the discussion could be more engaging and informative for readers.

RV. The limitations and future research added to the end of discussion.

QVI. Overall, the discussion of the article is not well-written and does not add much value to the study. The authors should revise the discussion to make it more organized, comprehensive, and objective.

RVI. The discussion is rewritten in many parts in order to become more organized, and objective.

QVII. Here are some suggestions for how the discussion could be improved:

The authors could organize the discussion by topic. For example, they could start by discussing the incidence of ganciclovir resistance in CMV-infected liver transplant recipients. They could then discuss the different types of ganciclovir resistance mutations and their impact on treatment. Finally, they could discuss the implications of their findings for the management of CMV infection in liver transplant recipients.

The authors could expand the discussion to include more of the findings of their study. For example, they could discuss the relationship between ganciclovir resistance and viral load. They could also discuss the impact of ganciclovir resistance on patient survival.

The authors could make the discussion more objective by avoiding subjective statements. For example, instead of saying "Our results are important," the authors could say "Our results provide new information about the development of ganciclovir resistance in CMV-infected liver transplant recipients."

RVII. The suggestions are considered in the discussion.

**CONCLUSION:**

• Based on this text, here are a few potential issues with the conclusion content of the article:

QI. The conclusion is not well organized. The authors jump from topic to topic without providing a clear connection between each point. This makes it difficult for the reader to follow the authors' train of thought.

RI. The conclusion is rewritten totally to be more organized.

QII. The conclusion is not comprehensive. The authors only mention a few of the findings of their study. They do not discuss the implications of their findings or how they relate to the existing body of research on CMV infection and ganciclovir resistance.

RII. The conclusion is rewritten totally to be more comprehensive.

QIII. The conclusion is not objective. The authors make several subjective statements, such as "We hope that the extent and effects of resistance in the clinical setting help optimize treatment instruction." These statements are not supported by evidence and can be misleading to the reader.

RIII. The conclusion is rewritten totally to be more objective.

QIV. Lack of clarity: The conclusion is somewhat unclear and difficult to follow. It jumps between different topics, including the observation of a novel mutation, the importance of viral load monitoring, and the need for continuous monitoring of antiviral resistance. It could benefit from clearer organization and structure to help readers understand the main points.

RIV. It is tried to clear the conclusion.

QV. Limited scope: The conclusion does not provide a comprehensive summary of the study's findings or their implications. While it mentions the incidence of UL54 ganciclovir resistance mutations and the importance of monitoring viral load and antiviral resistance, it does not provide much detail about how these factors were measured or what the results mean for patient outcomes.

RV. The conclusion is rewritten in order to provide adequate scopes.

QVI. Lack of context: The conclusion does not provide much context or background information to help readers understand why the study is important or how it fits into the broader field of liver transplantation and CMV infection. It would be helpful to provide more information about the prevalence of CMV infection in SOT recipients and the challenges of treating drug-resistant strains.

RVI. The conclusion is rewritten in order to be more context.

QVII. Limited recommendations: The conclusion provides some general recommendations for optimizing treatment instruction, but it could benefit from more specific and actionable advice based on the study's findings. For example, the conclusion could provide guidance on how often viral load and antiviral resistance should be monitored and what thresholds should be used to guide treatment decisions.

RVII. The whole conclusion section is rewritten and recommendations are added.

QVIII. Overall, while the conclusion touches on some important topics, it could benefit from clearer organization, more comprehensive summary of the study's findings, more context, and more specific recommendations for clinical practice. The conclusion of the article is not well written and does not add much value to the study. The authors should revise the conclusion to make it more organized, comprehensive, and objective.

RVIII. The conclusion is rewritten in order to be more organized, and comprehensive.

**REFERENCES**

QI. And as the last thing, 49 references for an original article seems a lot. Reduce it if possible

RI. The old and unnecessary references are deleted.

**Answer to reviewer 2:**

**Reviewer 2:**

Dear Editor

The author in this manuscript evaluates possible mutations in CMV UL54 gene which is responsible for drug resistance in liver transplant recipients.

Reviewer's comments are as follow:

Q1- Whole the manuscript needs to be revised based on English language. The manuscript is full of confusing sentences. Some of them were mentioned in the revised word file.

R1. This point is considered in the text.

Q2- Please mention to inclusion criteria in method section clearly.

R2. The inclusion criteria is added to the method section.

Q3- Why did you choose 50% cut off for boot strap test? It seems low. In some other studies the minimum acceptable BS test mentioned as 70%.

R3. In this way, thus, based on the default made by the MEGA X software after drawing the phylogenetic tree, the branches that were matching to less than 50% bootstrap replicates were distorted and the primary trees were gained by using Neighbor-Join and BioNJ algorithms automatically by using Maximum Composite Likelihood (MCL) approach.

Q4- How the authors approve the relationship between new found mutation (AK124703.1:p.V668-G672dup) and drug resistance?

R4. In this study, we have no claim on the certainty of drug resistance due to the creation of a new mutation (AK124703.1:p.V668-G672dup), but there is a possibility that this mutation may lead to resistance.

Q5- There was no phylogenetic tree figure in the manuscript please add it.

R5. The photo is added.

## 2 Revision 1

0 comments

*No public comments in this round.*