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OPEN PEER REVIEW

A Multicentric Study of ACE2 rs2285666 Polymorphism: The Association with COVID-19 in the Iranian Population

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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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Contents

1 **Original submission** Referee 1, Associate Editor 2
2 **Revision 1** 3
Authors' reply to reviewers 4
Reviewer and editor comments 19

1 Original submission

2 comments · 25 Sep 2024 – 28 Sep 2024

Referee 1 Reviewer

MINOR REVISION 25 Sep 2024

Dear Editor
In this study, the authors investigated the genetic diversity of the ACE-2 gene, which is the main cell receptor of the SARS-CoV-2 virus, and its relationship with the disease. The manuscript has been written well overall but needs some corrections in the English language. In my view, the manuscript can be accepted for publication after the following corrections:
1- Revise the manuscript for language errors and make it more fluent.
2- In the method section, part 4.3, for which genotypes were the differences statistically significant: GG, GA, or AA? The percentages in both groups are very close.
3- In the method section, part 4.3, it is not possible to claim a significant relationship in the distribution of genotypes among different ethnicities because the participants from different ethnicities included in the study differ in number (based on the number of participants), and the case and control groups were not matched by ethnicity. Please remove any claim about a significant relationship between ethnicities and genotype diversity.
4- Please transfer lines 198 and 199 to the result section.
5- Please rephrase this sentence in the discussion section, line 216: "Thus, be noted that our findings are representative of the general population since it has not consisted of a selected and highly limited study". The sentence is confusing.
6- In the conclusion section, lines 229 and 230, you cannot discuss disease severity because, in this study, the patients were not separated and grouped based on the severity of the disease, so you cannot make such a claim.

Associate Editor

MAJOR REVISION 28 Sep 2024

Dear Dr Zarei
The manuscript entitled “A Multicentric study of ACE-2 G8790A (rs2285666) polymorphism: Presence of Association with COVID-19 clinical phenotypes” was reviewed. Based on the reviewer’s comments, it needs MAJOR revision. The authors should revise the manuscript properly and clarify all issues raised by reviewers point by point. If the revised version satisfies the referees, the article will be accepted for publication.

2

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.

Reviewers' Comments:

Reviewer 1:
Dear authors:
Title and Abstract:
• Title: The current title is somewhat long and repetitive ("ACE-2" is repeated twice in keywords), and it could benefit from more concise phrasing that includes the study's key findings or focus on the Iranian population.

Response: The title of manuscript and the keywords were revised as recommended.

• Abstract: The abstract lacks sufficient detail regarding statistical significance, and the methodology is briefly mentioned without specifying key experimental controls or confounding factors. The conclusion is generalized, with no specific statistical outcomes presented.
Provide specific findings (e.g., percentages or p-values for significant associations) and clarify the contribution of this study compared to previous ones.
Introduction

Response: The detailed statistical data has been added to the abstract conforming to the reviewer’s comments.

• Rationale: The rationale for focusing solely on the G8790A polymorphism is not well justified. The introduction mentions other ACE-2 polymorphisms but doesn’t explain why G8790A was prioritized for this study.

Response: A sentence has been inserted to the abstract to mention the priority of G8790A polymorphism.

• Literature Review: The introduction does not sufficiently address the broader landscape of research on ACE-2 polymorphisms and COVID-19, particularly key findings from large-scale genetic studies that have been published recently. This weakens the positioning of this study's significance.

Response: In the last section of the introduction the literature review segment has been inserted.

• Expand the literature review to include more recent studies on ACE-2 and COVID-19. Justify why this particular polymorphism (G8790A) was selected for investigation and how this study fills gaps in existing research.

Response: In the last section of the introduction the importance of this research for filling the gaps of study has been inserted.

• Strengthen the introduction with a clearer explanation of the study's novelty, particularly in the context of global COVID-19 research.
Response: The novelty of the current study is the diverse population of Iranian people from different ethnicities which is mentioned in the last section of the introduction.

Methodology

• Confounding Factors: The methodology does not adequately control for confounding factors (e.g., co-morbidities, socioeconomic status, treatments received). It is unclear how such factors were accounted for in the analysis. using medical staff for the control group may introduce bias, as healthcare workers may have different levels of exposure or inherent protection (e.g., early vaccination, protective behaviors).

Response: The required data is not available. This issue has been be mentioned in the limitations of the study at the end of the part “3.1. Population study “.

• Address the potential bias in sample selection by ensuring that control participants are comparable to the case group.

Response: The purpose of the current study was to compare the allele frequencies among the studied population and there was no bias in the sample selection which is mentioned at the end of the part “3.1. Population study “.

• Provide more information about how confounding variables were controlled or considered.
Response: As described before, such confounding variables were not controlled in the study which was mentioned as the limitations of the study.

Results:
Ethnic Group Differences: Although the study aimed to explore genetic variation among different Iranian ethnicities, the analysis does not provide a detailed breakdown of how the results may vary by group. The sample size for some groups (e.g., Baloch) is too small to draw meaningful conclusions.
Response: This issue refers to limitations of the study which includes low sample number for some ethnics. It is recommended for the future studies in the conclusion section.

Discussion
• Overstatement of Findings: The authors tend to overstate the clinical relevance of their findings without sufficiently discussing limitations. For instance, they conclude that the AA genotype is linked to severe COVID-19, but this should be tempered by acknowledging that the sample size for AA carriers was relatively small.

Response: A statement which declares the mentioned limitation of the study was inserted at the end of the 3rd paragraph of the discussion.

• Inadequate Discussion of Limitations: While the discussion mentions some limitations, such as sample size, it does not sufficiently address others (e.g., potential selection bias, methodological constraints, and lack of confounding control).

Response: Thanks to the valuable comment, the statement of the limitations has been inserted to the different parts of the article including methods, discussion and conclusion.

• Comparisons with Other Studies: The discussion could be enriched by a more thorough comparison with similar studies, including any conflicting results or alternative explanations for the findings.
Response: Some similar studies have been mentioned in the discussion and compared to our results.

Tables & Grammar:
• Statistical Clarity: Some tables lack clear indication of the statistical significance of differences between groups. For instance, while p-values are mentioned in the text, it is unclear from the tables alone whether the differences between genotypes are statistically significant.

Response: The tables have been revised and include the mentioned p-values.

• Table Titles: The titles of the tables are somewhat generic and do not provide enough information for readers to understand the content without referencing the main text.

Response: The titles of the tables have been revised.

• Grammar Issues: There are several awkward phrasings, grammatical errors, and sentence fragments that could be improved for readability and professionalism. For example, some sentences are overly complex or use passive voice unnecessarily.
Response: The title of manuscript and the keywords were revised as recommended.

• Flow Between Sections: There are abrupt transitions between some sections, particularly between the results and discussion. Clearer transitions would help the reader follow the narrative more easily.
Response: The valuable comment was considered to revise the confluency between results and discussion.

Table 1: Demographic, clinical manifestation, and underlying disease of the patients
• Statistical Significance: The table does not clearly indicate whether the differences between SARS-CoV-2 positive and negative groups are statistically

significant. For example, is the higher percentage of respiratory distress in the positive group statistically significant compared to the negative group?

Response: The table has been revised as recommended.

• P-values: There are no p-values provided in the table to confirm whether the observed differences are significant. Without p-values or confidence intervals, it is hard to judge the relevance of the differences.

Response: The table has been revised as recommended.

• Ethnicity Distribution: The distribution across different ethnic groups (Lur, Fars, Turkmen, Baloch) is mentioned, but the table doesn't provide insights into the statistical significance of differences in the clinical manifestations across these groups.

Response: The table has been revised as recommended.

• Sample Size Disparity: The Baloch group has a very small sample size (n = 12, or 3%). This limits the statistical power and raises concerns about how representative the findings are for this group.

Response: This issue was concluded in the limitations of the study.

Table 2: Distribution of G8790A genotypes in male and female individuals
• Missing Statistical Details: As in Table 1, there are no p-values or statistical tests provided to assess the significance of the differences between genders or ethnic groups. The differences between groups could be by chance, and without statistical testing, this remains unclear.

Response: The table has been revised as recommended.

• Sample Size for Ethnic Groups: The sample sizes for some ethnic groups (especially the Baloch) are very small, making it difficult to draw reliable conclusions about genotype distribution. Small sample sizes limit statistical power and increase the risk of Type I or Type II errors.

Response: This issue was concluded in the limitations of the study.

• Confounding Factors: There is no indication that the analysis has controlled for confounding factors like age, underlying health conditions, or exposure levels, which could influence genotype distribution.
Response: This issue was concluded in the limitations of the study.

Table 3: Distribution of G8790A genotypes in different ethnicities
• Statistical Power: The table shows a clear difference in genotype frequency across different ethnicities, but no p-values or statistical tests are provided to show whether these differences are significant. It is important to test whether the differences in genotype frequencies are statistically significant, particularly given the small sample sizes in some groups.

Response: Table 3 has been totally removed and its data has been transferred to other tables. There are two new tables added instead of Table 3 which makes the results presentation more clearly.

• Sample Size for Subgroups: Once again, the Baloch ethnic group has a very small sample size (n = 12). This raises concerns about whether the findings are representative of this group, and whether the results can be generalized across the population.

Response: The low number of the Baloch ethnics is concluded in the limitations of the study. Table 3 has been totally removed and its data has been transferred to other tables. There are two new tables added instead of Table 3 which makes the results presentation more clearly.

• Genotype Frequency: The minor allele frequency (MAF) is included, which is helpful, but it could be compared to MAF data from other populations to contextualize whether the frequencies observed in this study are higher or lower than those found in other studies or populations.

Response: Table 3 has been totally removed and its data has been transferred to other tables. There are two new tables added instead of Table 3 which makes the results presentation more clearly.

Table 4: Association between the G8790A polymorphism and clinical symptoms
• Inconsistent Statistical Presentation: While p-values are provided for some clinical symptoms, they are missing for others (e.g., muscle pain). This inconsistency makes it difficult to assess the significance of some of the results.

Response: The table 4 is now numbered as table 5. The table has been revised completely and the mentioned comment is edited in the table.

• Sample Size Concerns: The small number of patients with the AA genotype raises concerns about statistical power. For example, only 16 AA patients are listed under "Female," and such a small sample size may not be sufficient to draw firm conclusions about the association between genotype and symptoms.

Response: This issue was concluded in the limitations of the study.

• Provide p-values for all clinical symptoms listed to ensure consistency in the reporting of statistical significance.

Response: The table 4 is now numbered as table 5. The table has been revised completely and the mentioned comment is edited in the table.

• Clarify the limitations of the small sample size for certain genotypes, especially the AA genotype.

Response: This issue was concluded in the limitations of the study.

• Consider performing a multivariate analysis that accounts for potential confounding factors (e.g., age, comorbidities) to strengthen the association between genotype and symptoms.
Response: This issue was concluded in the limitations of the study.

Table 5: Association between the G8790A polymorphism and laboratory variables
• Lack of Statistical Rigor: The table lacks p-values or confidence intervals for most of the laboratory variables, making it impossible to determine whether the differences between genotypes are statistically significant. For example, are the differences in WBC or lymphocyte count between GG, GA, and AA genotypes meaningful?

Response: Table 5 is now numbered as Table 6. The p-values are embedded in the table. The table has been revised completely and the mentioned comment is edited in the table.

• Confounding Factors: It is unclear whether the analysis has controlled for confounding factors that might influence laboratory values (e.g., age, medication use, severity of COVID-19).
Response This issue was concluded in the limitations of the study.

Overall, while the tables present valuable data, they lack sufficient statistical rigor and clarity. The primary weaknesses across all tables include the absence of statistical significance indicators (p-values, confidence intervals), the small sample sizes for certain subgroups (especially the Baloch ethnicity and AA genotype carriers), and the lack of control for confounding factors.

Response: The table has been revised completely and the mentioned comment is edited in the table.

Reviewer 2:
Dear Editor
In this study, the authors investigated the genetic diversity of the ACE-2 gene, which is the main cell receptor of the SARS-CoV-2 virus, and its relationship with the disease. the manuscript has been written well overall but needs some corrections in the English language. in my view, the manuscript can accepted for publication after the following corrections:
1- Revised for language errors and make it more fluent

Response: The manuscript was revised as recommended.

2- In the method section, part 4.3, Exactly the difference in which genotypes were statistically significant? GG, GA or AA. The percentages in both groups are very close.

Response: The results were revised as recommended.

3-In method section part 4.3, It is not possible to claim a significant relationship in the distribution of genotypes among different ethnicities. because the participants from different ethnicities included in the study are not the same (based on the number of participants) and matching was not considered between the case and control group based on ethnicity. Please remove any claim about a significant relationship between ethnicities and genotype diversity

Response: Thanks for the valuable comment, the mentioned data has been removed from the results.

4- Please transfer lines 198 and 199 to the result section

Response: The lines 198 and 199 to the result section were added to the result section.

5- Please rephrase this sentence in discussion section line 216. "Thus, be noted that our findings are representative of the general population since it has not consisted of a selected and highly limited study". the sentence is confusing.

Response: The mentioned sentence was re-written to be clearer.

6- In the conclusion section, lines 229 and 230, you cannot discuss disease severity because, in this study, the patients were not separated and grouped based on the severity of the disease, so you cannot make such a claim.

Response: It has been removed and revised in the manuscript.

Associate Editor's Comments:

Associate Editor 1:
Dear Dr Zarei
The manuscript entitled “A Multicentric study of ACE-2 G8790A (rs2285666) polymorphism: Presence of Association with COVID-19 clinical phenotypes” was reviewed. Based on the reviewer’s comments, it needs MAJOR revision. The authors should revised the manuscript properly and clarify all issues raised by reviewers point by point. If the revised version satisfies the referees, the article will be accepted for publication.

EIC Decision:

Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments.
Please see attached reviewer’s files.
1. Please answer point by point to the reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters.
2. Your answers to reviewers’ queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding. Moreover, add continuous line numbers for text and put suitable line numbers for each response.
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mentioned in the reviewers' comments, outline every change made a point by point, and provide suitable rebuttals for any comments not addressed.
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5. Please add the ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html

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Reviewer Assistant Comments:

These comments were generated by AI based on your manuscript. Please review them and consider incorporating the suggestions to enhance your article.

Based on the provided text regarding the study of ACE-2 G8790A (rs2285666) polymorphism and its association with COVID-19 clinical phenotypes, I will apply the relevant checklists for a comprehensive review. The study appears to be observational in nature, likely fitting into a cohort or case-control study framework.

Checklist Review

1. **STROBE Cohort Checklist**
- **Title and Abstract**: The title indicates a multicentric study, but it should explicitly mention the study design in the abstract.

Response: According to the comment, we explicitly mentioned the study design in the abstract.

- **Background/Rationale**: The background adequately explains the significance of genetic factors in COVID-19 outcomes.

- **Objectives**: The objectives are clearly stated, focusing on the relationship between the polymorphism and clinical outcomes.

- **Methods**:
- **Study Design**: The design is described, but more details on the setting and dates of recruitment would enhance clarity.

Response: It has been mentioned in the manuscript.

- **Eligibility Criteria**: The criteria for participant selection should be explicitly defined.

Response: It has been mentioned in the manuscript.

- **Variables**: Outcomes and potential confounders should be clearly defined.

Response: It has been mentioned in the manuscript.

- **Statistical Methods**: The statistical methods used for analysis should be detailed.

Response: It has been mentioned in the manuscript part 3.3. Statistical analysis.

- **Results**: The results section should include more detailed demographic data of participants and the handling of missing data.

Response: It has been mentioned in the manuscript.

- **Discussion**: Key results are summarized, but limitations and generalizability should be discussed more thoroughly.

Response: It has been mentioned in the manuscript.

2. **STROBE Case-Control Checklist**
- **Title and Abstract**: Similar to the cohort checklist, the design should be indicated in the title and abstract.
- **Background/Rationale**: The rationale is well-articulated.
- **Objectives**: Specific objectives are stated.
- **Methods**:
- **Eligibility Criteria**: Clear criteria for case ascertainment and control selection should be included.

Response: It has been mentioned in the manuscript.

- **Bias**: Any efforts to address potential sources of bias should be mentioned.

Response: No bias available. Mentioned in the limitations of the study.

- **Results**: The results should report the number of individuals at each stage of the study, including reasons for non-participation.

Response: Mentioned in the limitations of the study.

3. **STARD Checklist**
- This checklist is not directly applicable as the study does not focus on diagnostic accuracy but rather on genetic associations.

4. **PRISMA Checklist**
- This checklist is also not applicable as the study does not appear to be a systematic review or meta-analysis.

Summary of Recommendations
- **Clarify Study Design**: Ensure that the study design is clearly indicated in both the title and abstract.

Response: It has been mentioned in the manuscript.

- **Detail Methods**: Provide more information on eligibility criteria, statistical methods, and handling of missing data.

Response: It has been mentioned in the manuscript.

- **Expand Results**: Include detailed demographic characteristics and reasons for non-participation.

Response: It has been mentioned in the manuscript.

- **Discuss Limitations**: Address potential biases and limitations more thoroughly in the discussion section.

Response: It has been mentioned in the manuscript.

By addressing these points, the study can enhance its clarity and adherence to reporting guidelines, ultimately improving its contribution to the understanding of genetic factors in COVID-19 outcomes.

2 Revision 1

0 comments

No public comments in this round.