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OPEN PEER REVIEW

Genetic and Clinical Study of Children with Familial Mediterranean Fever in Northeastern Iran

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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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1 Original submission

4 comments · 6 Sep 2023 – 9 Oct 2023

Referee 1 Reviewer

MAJOR REVISION 6 Sep 2023

Dear Author
Thank you for your submission.
This article needs English language editing.
Abstract
1- Please check that the word count does not exceed 250.
2- Please check the keywords against MeSH.
3- Please don't start sentences with therefore.
4- Please give the frequency of patients with fever.

Material and Methods
1- What are the criteria for diagnosing FMF in your patients? Please give the reference.
Results
1- Please describe the cases of arthritis involving the patella. Is this kind of involvement common in FMF?
2- What are VUS variants and a pathogenic mutation?
3- The author didn't give the results of the statistical analysis of all the variables mentioned in the materials and methods section, such as height and weight…
Discussion
1- Please give the results for the other variables in the results section and check the literature for similar articles.
2- Please give the advantages and disadvantages of your study in the discussion section without a specific title.
3- Please omit the suggestion section.
Conclusion
1- This section should give the main concept of your study and can recommend other studies in this field based on your results. Please revise this section.

Best Wishes

Referee 2 Reviewer

REJECT 8 Oct 2023

The abstract could be slightly expanded to include a brief summary of the key findings.
The section mentions "Data Reproducibility," but it doesn't provide any information about the reproducibility of the study's data. If data sharing or reproducibility was a part of the study, it should be elaborated upon.
While the article mentions the statistical analysis conducted, it does not present any statistical results or p-values. Providing some statistical results could enhance the comprehensibility of the findings. It mentions Mean ± SD, but there is no corresponding table or data to support these values. The article lacks information about the specific genetic data analysis techniques applied. Genetic data analysis often involves steps such as variant calling, quality filtering, and statistical analysis. Describing these steps is crucial for transparency. If statistical methods were used in the genetic analysis, describe the specific tests or models applied. Include details about the statistical software used and significance thresholds.
The article mentions some limitations of the study, such as incomplete family history data. It would be helpful to discuss the potential impact of these limitations on the study's results and conclusions.

The suggestions section briefly mentions the need for further investigations in other regions. You could elaborate on why such investigations are important and what specific research questions they should address.
The text contains various grammar and punctuation errors, such as missing commas, inconsistent capitalization, and sentence fragments. Thorough proofreading for language issues is needed.
Lack of Detail: It's important to specify the techniques, equipment, and reagents employed for genetic testing. This should include the names of specific tests, PCR protocols, sequencing platforms, and software tools used for data analysis.
There is no mention of how the sample size for genetic testing was determined. Providing information on sample size calculation or justification for the sample size is essential in research articles.
The section should include details on quality control measures for genetic data, including how data quality was assessed, how low-quality data points were handled, and any exclusions made based on data quality.
It's important to specify the genetic markers or regions that were investigated in the study. Provide details about the genes, loci, or variants of interest and how they were selected.
Mention if any validation steps were performed to confirm the results of genetic testing. Validation of genetic findings is crucial to ensure the reliability of the data.
Provide a timeline for the genetic testing process, including when samples were collected, processed, and analyzed. This can help readers understand the study's workflow.
If applicable, mention whether the genetic data will be made available to other researchers or deposited in a public database. Data sharing is important for reproducibility and transparency.
While it's mentioned that the study was approved by the ethics committee, there is no information about obtaining informed consent from participants. Ethical considerations related to genetic testing, such as consent for genetic analysis and data storage, should be described.

Associate Editor

MAJOR REVISION 9 Oct 2023

Thanks to the authors and appreciation to the researchers. The manuscript presents valuable research. But some revisions are needed.
ABSTRACT:
1- The study design should be mentioned in the first line of the methods section of the ABSTRACT.
2- A summary of the statistical analysis results should be mentioned in the results section of the ABSTRACT.

Editor-in-Chief

MAJOR REVISION 9 Oct 2023

Dear Author,
Thank you for submitting the article. It needs some revision. Please revise it based on the reviewers' and AE's comments.

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.

Responses to reviewers' comments

We would like to sincerely thank you for all your constructive comments, which significantly improved the quality of this article. According to the raised comments, we made some changes to the article step-by-step, highlighted in blue. Please consider during the revision, we updated the genetic data with Varsam and Clinvar, and according to ACMG guidelines, we conducted the statistical analysis again. In the following, we respond to comments.

Reviewer 1:

Dear Author, Thank you for your submission. This article needs English edition.

Abstract

Comment: 1-pls check the words to 250

Response: The required adjustment is done.

Comment: 2- Pls check the keywords with Mesh

Response: Corrected.

Comment: 3- Pls don't start the sentences with therefore

Response: Corrected.

Comment: 4- pls give the frequency of patients with fever

Response: It is added to Table 1. All patients had at least three bouts of fever before entering the study.

Material and Methods

Comment: 1- what is the criteria for diagnosis of FMF in your patients, Pls give the reference?

Response: Thank you. Necessary details are added to section 2.1.

Results

Comment: 1- pls describe the cases of arthritis involving patella. Is this kind of involvement common in FMF?

Response: Thank you for this comment. It was a typo in the earlier version, and it has been corrected to knee arthritis.

Comment: 2- What are VUS variants and pathogenic mutation?

Response: Please refer to the newly added Table 2.

Comment: 3- The author didn't give the results of statistical analysis of all the variables mentioned in material and method part, such as height and weight and….

Response: Thank you for this comment. We added details of the significance test. The following added to section 3 "For a significance statistical analysis, we compared means of age, age.of.onset, disease.period, rate.of.hospitalization, weight, and height using ANOVA at the level of significance $\alpha=0.05$. Suppose μ_{benign}, μ_{vus}, and $\mu_{pathogene}$, respectively denote the mean of any variables mentioned above for the three groups benigh, vus, and pathogen. Using p-values, we observed for all variables, except rate.of.hospitalization with p-value equal to 0.037, the null hypothesis $H_o: \mu_{benign}=\mu_{vus}=\mu_{pathogene}$ accepted at the level of 0.05, resembling no significant difference between these groups. For the rate.of.hospitalization, the null hypothesis H_o accepted and consequently a post hoc test ran to check which groups had different mean for this variable. Accordingly, the two groups, benign and pathogen, have other mean using the p-value equal to 0.039 in the multiple comparisons based on the Tukey HSD".

Discussion

Comment: 1- Plz give the results of other variables in result part and check the literature for similar articles

Response: Details added as suggested.

Comment: 2- Pls give the advantages and disadvantages of your study in discussion part without specific title

Response: Thank you. Pros and cons are added to the discussion section.

Comment: 3- Pls omit the suggestion part

Response: Done as suggested.

Conclusion

Comment: 1- This part should give the main concept of your study and can recommend other studies in this field based on your result. Pls revise this part.

Response: The following paragraph is added to the conclusion section:

The most common genotype in our study was E148Q which was reported to be benign according to the latest reports. M680I and M694V were the most frequent pathogenic mutations with 5 cases, respectively. which shows that FMF disease is rare in North-Eastern Iran and the number of pathogenic mutations is lower compared to North-West Iran and other studies. As a result, it is necessary to carry out genetic examination and treatment of the disease in order to control the

course of the disease and its complications (amyloidosis) in people who are clinically diagnosed with this disease.

Reviewer 2:

Comment: The abstract could be slightly expanded to include a brief summary of the key findings.

Response: Done as suggested.

Comment: The section mentions "Data Reproducibility," but it doesn't provide any information about the reproducibility of the study's data. If data sharing or reproducibility was a part of the study, it should be elaborated upon.

Response: We have not referred to data reproducibility in the article.

Comment: While the article mentions the statistical analysis conducted, it does not present any statistical results or p-values. Providing some statistical results could enhance the comprehensibility of the findings. it mentions Mean ± SD, but there is no corresponding table or data to support these values.

Response: Thank you for this comment. We added details of the significance test. The following added to section 3 "For a significance statistical analysis, we compared means of age, age.of.onset, disease.period, rate.of.hospitalization, weight, and height using ANOVA at the level of significance $\alpha=0.05$. Suppose μ_{benign}, μ_{vus}, and $\mu_{pathogene}$, respectively denote the mean of any variables mentioned above for the three groups benigh, vus, and pathogen. Using p-values, we observed for all variables, except rate.of.hospitalization with p-value equal to 0.037, the null hypothesis $H_o: \mu_{benign}=\mu_{vus}=\mu_{pathogene}$ accepted at the level of 0.05, resembling no significant difference between these groups. For the rate.of.hospitalization, the null hypothesis H_o accepted and consequently a post hoc test ran to check which groups had different mean for this variable. Accordingly, the two groups benign and pathogen, have other mean using the p-value equal to 0.039 in the multiple comparisons based on the Tukey HSD".

Comment: The article lacks information about the specific genetic data analysis techniques applied. Genetic data analysis often involves steps such as variant calling, quality filtering, and statistical analysis. Describing these steps is crucial for transparency. **: If statistical methods were used in the genetic analysis, describe the specific tests or models applied. Include details about statistical software used and significance thresholds.

Lack of Detail: It's important to specify the techniques, equipment, and reagents employed for genetic testing. This should include the names of specific tests, PCR protocols, sequencing platforms, and software tools used for data analysis.

Response: Thank you for your comment. The following explanation is added to the methodology section "DNA extracted from 3 ml of whole blood according to salting out DNA extraction

procedure and all exons of MEFV gene were amplified using specific primers for each exon (table…). Sanger sequencing was performed by ABI 3130xl sequencer according to manufacture; data was analyzed by sequencer software and the pathogenicity of the candidate variant was evaluated according to ACMG guidelines and using bioinformatics prediction tools, such as Mutation Taster (http://www.mutationtaster.org), SIFT (sift.jcvi.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), PolyPhen-2 (http://genet ics.bwh.harvard.edu/pph2)."

Comment: The section should include details on quality control measures for genetic data, including how data quality was assessed, how low-quality data points were handled, and any exclusions made based on data quality.

Response: We added, "Sequencing results must be evaluated for quality. Along with the software analysis accomplished internally by the sequencing equipment, a quality analysis review of the sequencing data was performed by visually inspecting both strands. The data used in our study is obtained from the golden standard repositories mentioned above that shows the data quality is already assessed." to section 2.2.

Comment: The article mentions some limitations of the study, such as incomplete family history data. It would be helpful to discuss the potential impact of these limitations on the study's results and conclusions.

Response: One of these limitations is the lack of reliable and complete family history to form the genealogy of patients, such as separation of parents or death of a family member, which hinders accurate genetic analysis. Necessary details are added to the Discussion.

Comment: The suggestions section briefly mentions the need for further investigations in other regions. You could elaborate on why such investigations are important and what specific research questions they should address.

Response: We added more details to the Discussion.

Comment: The text contains various grammar and punctuation errors, such as missing commas, inconsistent capitalization, and sentence fragments. A thorough proofreading for language issues is needed.

Response: The English text was carefully checked and all grammatical, spelling and punctuation errors were corrected. We used premium accounts of Grammarly and sider.ai.

Comment: There is no mention of how the sample size for genetic testing was determined. Providing information on sample size calculation or justification for the sample size is essential in research articles.

Response: Thank you for this comment. Please consider our study was a case series based. Indeed, a rheumatologist diagnosed FMF disease for those who visited the clinic and prescribed a genetic test. The total number of 29 patients, thus included in our study with MEFV genome mutation.

Comment: It's important to specify the genetic markers or regions that were investigated in the study. Provide details about the genes, loci, or variants of interest and how they were selected.

Response: We added the following paragraph to section 3

Clinical diagnosis proposed familial Mediterranean fever since mutations in MEFV gene are related to this disease, to identify causative mutation, we sequenced all 10 exons of the MEFV gene with specific primers using the sanger sequencing method that was performed by ABI 3130xl sequencer according to manufacture, data was analysis by sequencer software and pathogenicity of candidate variant was evaluated by VarSome tool according to ACMG method. The results are summarized in Table 6.

Comment: Mention if any validation steps were performed to confirm the results of genetic testing. Validation of genetic findings is crucial to ensure the reliability of the data.

Response: Thank you for this comment. To confirm the detected mutations, both forward and reverse sequencing were performed. We added to section 2.2.

Comment: Provide a timeline for the genetic testing process, including when samples were collected, processed, and analyzed. This can help readers understand the study's workflow.

Response: Please consider the genetic testing is done by patients and there is no specific timeline to be tabulated.

Comment: If applicable, mention whether the genetic data will be made available to other researchers or deposited in a public database. Data sharing is important for reproducibility and transparency.

Response: Thank you for this comment. The statement "The mutations found in the patients under our study were previously reported and are publicly available in dbSNP with a specific rs number" added under the title "Data Availability" at the end of the paper.

Comment: While it's mentioned that the study was approved by the ethics committee, there is no information about obtaining informed consent from participants. Ethical considerations related to genetic testing, such as consent for genetic analysis and data storage, should be described.

Response: All suspected FMF patients should undergo genetic testing for mutations. This problem was explained to the parents and then the patient came for the test. These issues were registered in the university's ethics committee with number IR.MUMS.MEDICAL.REC.1401.112. Please consider we did not mention this consent in the body of the paper; however, we added the above number when we submitted the form as the journal requirements of ethics.

Reviewer 3:

Thanks for valuable article

Comment: Line 113 before the age of 20 year added

Response: Corrected.

Comment: References about genetic study in FMF are not new(about 2011), if newest journals are available added.

Response: We used all available related references in the paper.

2 Revision 1

2 comments · 15 Nov 2023 – 15 Nov 2023

Associate Editor

MINOR REVISION 15 Nov 2023

The great effort in improving the manuscript is highly appreciated. However, there is room for further improvement, which the authors should consider.

Editor-in-Chief

MINOR REVISION 15 Nov 2023

Dear Author,
Your manuscript still needs some revision. Please revise it carefully based on the reviewers' and AE's comments.

3

Revision 2

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.

Responses to reviewers' comments

We would like to sincerely thank you again for all your constructive comments, which led us to put more details to this article and clarify outstanding terms. According to the raised comments, we made some changes to the article step-by-step, highlighted in blue. In the following, we respond to comments.

Comment: There are some dictation mistakes in the article especially in tables

Response: Some minor changes made to the headings.

Abstract

Comment: Please give the abbreviations for VUS**.**

Response: VUS stands for Variant of Uncertain Significance. Added as suggested.

Material and Method

Comment: What is the name of your center? Is it a referral center? Do you have a separate rheumatologic ward?

Response: Thank you for this important comment. Some more details added to section 2.1.

Comment: What is the ethical code?

Response: we added a subsection under the title “Ethical Considerations” with the following details: “This study was approved by the Vice-Chancellor of Research at Mashhad University of Medical Sciences (Code: IR.MUMS.MEDICAL.REC.1401.112). The written informed consent letter was obtained from all parents/guardians”.

Comment: Dose clinical follow up done only by phone calling? Did you have any visit?

Response: Details added to section 2.2.

Result

Comment: Please give the statistical information in material and Method part.

Response: Some details about statistical information are added as suggested.

Comment: the results of height, parental history, ESR, CRP, complication, and drug and dose variables are not found in the result part**.**

Response: Statistical details of the variable mentioned above added in section 3.

Discussion

Comment: In this sentence “Our study showed that 44.8% of FMF cases had arthritis, and 6.9% had chest pain, similar to other studies.” What is you reference

Response: Corrected as suggested.

Comment: In this sentence “The average number of white blood cells, ESR, and CRP were increased in all patients in our study. These results are consistent with other reports” there isn’t any result of ESR and CRP in the result part**.**

Response: Thank you for this comment. We added Table 8 with the necessary descriptive analysis. Further explanations are also added to the text.

Conclusion

Comment: This part is very long. Please give the main and important result of your study and give a comprehensive recommendation about the result and for other researches in this field.

Response: Thank you. We have add the following to the conclusion section: “Our findings show that the most common genotype in our study was E148Q, which was reported to be benign, M680I and M694V were the most frequent pathogenic mutations, with 5 cases, respectively, which shows that FMF disease is rare in Northeastern Iran and the number of pathogenic mutations is lower compared to Northwest Iran and other studies. Further studies with a larger number of patients and examination of the MEFV gene are necessary for the relationship between FMF symptoms and genotypes in order to treat the disease to control its course and amyloidosis”.

3 Revision 2

3 comments · 21 Dec 2023 – 22 Dec 2023

Referee 1 Reviewer

ACCEPT 21 Dec 2023

Dear author
Thank you for the corrections.
The article is accepted after these corrections.
Please check these items:
1- Dose of colchicine in adults and children
2- Please don't give information about data analysis in the results section. It can be moved to the methods section.
3- Please give the results of all studies conducted in Iran on this issue.

Associate Editor

ACCEPT 22 Dec 2023

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

Editor-in-Chief

ACCEPT 22 Dec 2023

Dear Authors,
Thank you for paying attention and responding to the reviewer's comments.