Author's response to reviews

Title: Associations between genetic polymorphisms and temporomandibular joint disorder

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Version: 2 Date: 30 December 2010

Author's response to reviews: see over
Reply Reviewer 1

Reviewer's report
Title: Associations between genetic polymorphisms and temporomandibular joint disorder

Version: 1 Date: 29 November 2010
Reviewer: KeWei Wang

Reviewer's report:
This manuscript describes the analysis of genetic polymorphisms located on 17 genes of oral mucosa samples from 86 TMD patients. Authors performed the genotyping of over 220 individuals on 17 genes. They identified five polymorphisms that involve folate deficiency, psycho-physical stress and oxidative metabolism, and showed their strong association with TMD. Although those identified 5 polymorphisms need to be further confirmed, the overall findings of this study are interesting. I have a few comments/questions:
1. What was the OR value for the DRD4 gene analyzed?
Reply
We have modified table 3 and add OR of this gene

2. First paragraph of Method, page 5, at least needs rewording (including grammar) and requires careful revision.
Reply
Methods has been reedited

3. Table 1 includes 26 SNPs studies, not 27 SNPs?
Reply
we have counted 20 SNP and 7 length polymorphism

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Reply Reviewer 2

Title: Associations between genetic polymorphisms and temporomandibular joint disorder

Version: 1 Date: 23 November 2010
Reviewer: Susan Armijo-Olivo

Reviewer's report:
Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)
Title:
the term: “temporomandibular joint disorder” should be replaced for
“temporomandibular disorders”. The term “temporomandibular joint disorder” is an old term and has been replaced by the term suggested.

Reply

We have replaced all of the terms "temporomandibular joint disorder," although many articles of 2009 and 2010 also used the term TMJ

Also I would suggest creating a title that is reflecting the results of the study and more attractive.

Reply

The title has been changed by specifying the genes whose polymorphisms have been found more prevalent in the TMD

A. Abstract:
Background and objective: No clearly stated which the objectives and the need for the study were. Please clarify.

Reply

We changed the text of the abstract clarifying the objectives of the study

Methods: “After obtaining the data, a chi-square test was performed to evaluate the different frequencies of the alleles and genotypes in the TMD patients and healthy controls.” Do you want to say that a chi-square test was used to determine the differences in proportions of subjects exhibiting alleles and genotypes between TMD patients and healthy subjects? If yes, statistical methods should be reworded. It does not read very well.

Reply

we have rewritten the section on statistical methods in the abstract

Results: six of the polymorphisms (......) showed “significant?” associations with TMD. It is not clear to me when the authors stated the following: “exhibiting significant changes in their allele and genotype frequencies”. Could you please clarify this sentence?

Reply

We tried to clarify the sentence as suggested by the referee

I would delete the sentence: “However, in other studies, other polymorphisms, such as ESR1 (rs9340799/rs2234693) and SLC6A4 (5-HTTLPR), did not show associations.” This is part of discussion. Generally, it is not used as part of abstract.

Reply

The sentence has been deleted

Conclusions: The results should make a link with the conclusions regarding the polymorphisms. It seems that conclusion is not connected with the results and it looks odd the way it is right now. Maybe in the results part you should add a little bit that these polymorphisms are related to inflammatory oxidative stress and neurotransmission responses to pain for example.

Reply

We have rewritten the conclusion and added more explicit data to results

B. INTRODUCTION:
1. I would add that TMD is commonly associated with other related-symptoms in the head and neck. Please add more information specifically to TMD and their importance.

Reply

we added a sentence and a reference on the possible association of the TMD to the head and neck pathology

2. Regarding the causes of TMD, there is a long list of factors that are questionably linked with TMD. I would suggest to authors adding that information since the causes of TMD are still a matter of a lot of debate and also to point out that a multifactorial origin might be plausible.

Reply

we added a sentence about the controversy and difficulty of clarifying the etiology of TMD but we think that is difficult to dismiss the multifactorial origin of TMD, because of the prevalence of sexual readiness and variety of clinical symptoms, but it is true that if scientific community does not find the exact etiology, any coherent hypothesis could be plausible.

3. Regarding the statement: “it is assumed that a combination of factors related to environmental and/or nutritional stress”. Could you please provide examples of environmental and nutritional stress? Also this statement needs references.

Reply

The sentence, is a general evaluation of the enviromental importance and in particular of the nutritional factor of any process of tissue growth or differentiation as factor of the disease. In the paper there are two references, refered to this sentence, although not in the place that you refered but we add two more in the cited place

4. When talking about genetics, could you please expand more on that since this is the essence of the paper and explain what type of genetics variants are present in TMD?

Reply

We put 18 references of genetic factors, 5 of them are directly related to TMD, by the way, as you can see in the references TMD it is also mentioned as TMJ even in papers of 2010, if you check pubmed genetics polymorphisms and TMD, as keywords, you find more o less 9 references directly related to TMD in the title, 4 of them are refered to estrogen receptor polymorphisms, 4 of them to serotonine transporter polymorphisms and 2 of them refered to COMPT polymorphisms.

We have added all new references to the Introduction

5. The following paragraph is odd and does not contribute in any aspect to the introduction. I would delete it or complemented with information linked to the previous topic: “The cells are influenced by genes and environmental conditions at the time they migrate, proliferate, differentiate, and synthesize the extracellular matrix in specific directions and magnitudes, finally resulting in macroscopic forms, such as the condylar aspect. Mechanical forces, therefore, modulate bone and cartilage growth”
Reply
We have deleted the paragraph but...
Perhaps the referee has not understood the paragraph because it was
separated by a period and apart from the next paragraph. We believe that by
bringing together two paragraphs the whole text makes sense and is also
important because the morphological development of any tissue, and more
than one joint may be exposed to any variable genetic and / or
environmental.

6. Is the question posed by the authors well defined?
1. The authors need to clearly state what is (are) the objective (s) of this study
and also why did the study these specific polymorphisms.
Reply 1
We have redefined the objective in paragraph summary.

There is no clear statement of why this study needs to be done. It is kind of
vague information; however, the link is not explicit
Reply 2
TMD is a pleiotropic syndrome involving multiple factors. We tested the
polymorphisms that have been previously reported and add the axis folate-
homocysteine-methionine metabolism related to all growing tissues, and
vitamin deficiency (folate) more prevalent in the Western world.
Furthermore, this cycle of folic acid is a pleiotropic metabolism that may be
related in two ways, genetics and epigenetics, with any multifactorial
syndrome. One of the objectives of the new genetics is to find any new
polymorphism influence in any disorder characterized, for the equation of a
multifactorial disease. On the other hand, many genetic studies are looking
for the new association not previously hypothesized (genearrays Genomics
and Genetics)

All this information is in the paper

C.METHODS
1. The recruitment and details of participants selection would be easier to
understand if a flowchart would be added.
2. There are some inconsistencies in the English grammar in the first part of the
methods section: For example: “14 because they were not come to the physical
examination and 33 because they were not confirmed clinical signs of TMD”. Do
you want to say that “14 subjects were excluded because they could not come
to the physical examination and 33 had not clinical signs of TMD”. Please revise
the grammar throughout the manuscript and correct the misspelling and these
odd sentences.
3. I recommend not using the expression: “responded positively or negatively”.
You should say: “subjects were diagnosed as having/ no having TMD based on
RDC/TMD criteria”
Reply
We have reprinted the text of methods taking into account all the
annotations of the referee

4. was there any sample size calculation to determine the sample size needed
prior data collection

Reply

Samples size was calculated in function of the prevalence of the TMD to obtain significance for the analysis of all individual polymorphism in function of its knowled genotypes frequencies in our population.

5. It is recommendable to add information regarding data collection and assessment:
   a. Who screened the subjects for inclusion? Could you please clarify that?
      Reply
      Dr Angel Aneiros MD from Malaga University and Dentist from University of London (The London Hospital, Medical College)

   b. Where subjects with TMD classified in different groups (i.e. myogenous, mixed, or according to the categories specified by the RDC/TMD?)
      Reply
      We think that the reprinted text of methods clarifies this question

   c. How was the evaluation performed?
      Reply
      We think that the reprinted text of methods clarifies this question

   d. Was any inclusion/exclusion criteria?
      Reply
      We think that the reprinted text of methods clarifies this question

6. The authors stated: “The Research Diagnostic Criteria for temporomandibular Disorders (RDC/TMD) outlined by the International RDC/TMD Consortium were used a diagnostic tool and the inclusion-exclusion criteria”. The RDC/TMD is a method to evaluate and diagnose TMD. I am not sure how the authors used as inclusion/exclusion criteria.
      Reply
      We add the inclusion/exclusion criteria

7. The authors presented the demographics as a text. I would prefer them to be presented in a table.
      Reply
      We think that with the reissue of demographic data is not necessary to include a flow chart

8. How control subjects were screened? What were the inclusion criteria for them?
      Reply
      We add the inclusion/exclusion criteria

9. Technique for genotyping: Please provide information regarding validity and reliability of the technique and software used to determine the outcomes for the study. Please provide references.
   reply
   We have extended methods, the whole process of incorporation of the
multiplex and bibliography

D. STATISTICS
1. I would recommend revising and rewording the statistics part of the manuscript. As a suggestion, please link the objectives of the study with the statistics performed.

Reply
2. The statistics are very general and do not provide clear information of the analysis. Authors should be more precise in determining variables and different analyses.

3. How they calculated Odds ratios. Do they provide crude Odds ratio? What method was used?

Reply
The statistic has been rewritten taking into account the recommendations of the referee
4. Why Odds ratios were only calculated for only some comparisons?

Reply
Because we only put the significant Odd ratios to simplified the table
5. It seems that many associations were done by using chi-square test. Why the authors did not calculate the odds ratio adjusting for some other variables such as gender, age, etc

Reply
We calculate the different odd ratio by gender and no variation were obtained, Odd ratio by age have less sense because of the age homogeneity of the participants

6. I would recommend consulting with a statistician

Reply
We have consulted

E. RESULTS
1. I am not sure if Figure 1 is needed. There is no major explanation of it.

Reply
Figure 1 is cited in the results in the section of the SHMT gene. Besides, the discussion about the possible effects of polymorphisms are based on the interrelationships of the axis of folates, which is difficult to follow without the figure 1. We consider it important to keep Figure 1 in order to easily follow the discussion of the article.

2. Please revise the writing in results part. Results are reported too general and it is difficult to follow. Some sentences are cut or the idea is not clearly finished. For example: “From the four polymorphisms studied on the cytoplasmic Serine Hidroximetil Transferase 1 gene (SHMT1) in TMD patients, a significant increase (p<0.01; OR=3.99) was observed of the G allele (LEU) of the polymorphism rs1979277 (LEU435PHE)”. When compared with healthy subjects?

Reply
We have complete the sentence and we have revised the text adding more details to the text.
3. Please be more specific when reporting numbers in text. Please provide estimates and frequencies and 95%CI and exact p value. So, it is easier to understand where the results came from.

Replay
We put p exact values

4. Do not report “p” significance value in isolation. This is not helpful for readers.

Reply
We have done

F. DISCUSSION
1. I would add more information regarding the importance of genetics factors in TMD. Authors just mentioned it but they do not expand on it.

Reply
We have add more references of the genetics studies performed on TMD

2. The authors mentioned: “The folate-methionine axis takes part in both factors because in this axis”. Could you please clarify which factors are you referring to?

Reply
We have joined the paragraphs that were separated by a period which could lead to not understanding the connection of folate with the two factors, the genetic (polymorphisms) and epigenetic (environmental)

3. Please check the coherence of paragraph 2 of discussion with previous and following one. Not sure what they are trying to say

Reply
We have bond and corrected both paragraphs as it is said in the previous point

4. when talking about higher frequency ( please provide the number) 4rd paragraph discussion: “In our case, a higher frequency of the allele SHMT-rs1979277G was observed in TMD patients”

Reply
Done

5. There are many sentences in the discussion without references. I would recommend adding the backup for those statements.

Reply
We added new the references

6. Discussion, paragraph 8, “Regarding previously studied polymorphisms, in which associations with TMD...” please expand the results from other studies and controversies regarding genetic studies. This paragraph should be expanded to express better the idea.

Reply
We have expand it adding new references

7. Please address the limitations of this study

Reply
The limitations of the study are the same of the genetics studies for multifactorial diseases, each variable have a limited values and could be biased for circumstances that are unavoidable in any populational study. The
main limitations are the number of subjects and difficulties to analyse multiples genetic variables by statistical methods. Several softwares gives us posibilities but the limited population is the main limited factor.

8. Please provide a more refine discussion, addressing points that are directed to the objectives of the study. I find the discussion a little bit vague.
   Reply
   We don’t understand very well what the referee want at this point, the discussion is focused on the objectives and results found, which means the associations of the genetic polymorphisms and the possible explanation of the mechanism of each one. Folates polymorphism on the basis of the enzymes activity which produce more o less Homocysteine (Proinflamatory factor) and SAM (methylating factor, for imprinting and reimprinting of replicating tissues). Oxidation of mandibular tissues by GSTM1 polymorphisms as a marker of more o less oxidative predisposition. No association obtained by the most commun polymorphism studied for TMD which is the ESR1a, related to estrogen, and DRD4 as psiconeurologic factor, as a polymorphism associated with the pain perception and psicological factors.

G.CONCLUSIONS
The conclusion is not very clear. Maybe something is missing?
Please relate the conclusions to objectives
   Reply
   We have modified the conclusion

H. REFERENCES
There are some references that are not in a good format. Please check them again and follow the journal guidelines to format references.
   Reply
   We have checked the references

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)
1. Please locate references after punctuation
   Done
2. after a period, numbers should be written in letters, no numbers
   Done

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. Grammar and writing style should be revised
   Done
2. Table 2 needs to stand alone. What do the numbers in parenthesis mean?
   Corrected

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests