Author’s response to reviews

Title: Polymorphic variation of Hypoxia Inducible Factor-1 A (HIF1A) gene might contribute to the development of knee osteoarthritis

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MS: 1013579374160006 - Polymorphic variation of Hypoxia Inducible Factor-1A (HIF1A) gene might contribute to the development of knee osteoarthritis.

COVER LETTER FOR ANSWERS

We read the reviewers reports and we modified the text according to the comments. We think that in the present version the paper is ready to be re-reviewed.

Thank you for the attention you are paying to our paper.

Best regards

REVIEWER 1

We thank the reviewer for the positive criticism and suggestions provided.

Reviewer: W den Hollander

Reviewer’s report:

In this manuscript the authors have performed a gene targeted association analysis for OA in 4 genetic variants that are located in either the HIF1a or WISP1 gene. Apart from the rather poor written English throughout the manuscript, I have got the following major points which should be addressed:

1) For a genetic association study the sample size (70 patients vs. 66 controls) is fairly small, the authors should elaborate on this in the discussion.
The reviewer is right. We agree with the reviewer that it is advisable to increase the sample size. Additionally, a paragraph was included in the discussion section in which it manifest the need to increase the number of study subjects in order to strengthen the statistical power of results in this study.

Additionally the manuscript was sent to an English native speaker to perform a complete revision.

2) There is a significant difference in BMI between the two groups, as is uric acid for that matter. Could the authors explain why they believe their significant HIF1a SNP would be involved in OA, rather than in BMI or uric acid biology.

Interesting point raised by the reviewer. In order to verify this aspect we performed a lineal regression analysis that showed no correlation between BMI and uric acid with the HIF-1# polymorphism was found. We reported these findings in the results section.

3) The discussion about the hypothesized HIF1a polymorphism consequences is hard to follow. People carrying the risk allele were shown to express HIF1a to higher extent, but it is nowhere mentioned in the paper whether this would be good or bad for cartilage homeostasis.

In order to improve the reading we modified the sentence as follows:

“Our results show that the presence of the CC homozygous variant or C allele represent potential risk factors for development of knee OA; contrarily, we detected that the heterozygous variant of CT or T allele of the rs11549465 polymorphism of the HIF1A gene (in comparison with the homozygous carriers) play a protector role against the disease. This phenomenon may be explained by the fact that the presence of this polymorphism confers greater stability to the HIF-1# protein, as demonstrated by Tanimoto et al [22]. In their study it has been demonstrated that the substitution of proline by serine in the 582 (P582S) position enhances its transcriptional activity. This permit supposes a beneficial effect in maintaining cartilage homeostasis and therefore avoiding joint damage. A similar phenomenon was also obtained in other immunological disease such as type 2 diabetes mellitus [23].”

4) Also, the authors state that heterozygous carriers are protected for OA. In what comparison? Compared with homozygous carriers of the risk allele? Please clarify this and point 4 in the main text.

The reviewer is right. The comparison was made with the homozygous carriers. We clarify this point in the previous answer. It has been also included in the discussion section.

And as a minor point:

1) Please mention the rs identifiers as opposed to the amino acid changes. While the amino acid change is of course of interest for biological interpretation, from the point of view of the reader it is easier to look up rs identifiers in online
databases.

We agree with the comment of the reviewer. We have included the rs identification of the polymorphisms analyzed in the main text when appropriate.

REVIEWER 2

We thank the reviewer for the comments aimed to improve the manuscript

Reviewer: Won Sang S Park
Reviewer's report:

General Comments:

The authors have described a clinical study evaluating the relationship between the polymorphisms of the HIF1A and WISP1 genes and development of osteoarthritis in a Mexican population and found that P582S SNP of the HIF1A is associated with knee osteoarthritis. The biggest problem with this study is that it does not have much power. In particular, the authors should genotype a larger group of case and controls. In addition, detailed and careful criteria of the selection of patients and control should be provided.

We are aware that a larger cohort of patients is needed in order to strength our results. We included this aspect as the main limitation of our study in the discussion. However we believe that our preliminary results could be of interest to the international literature since that there are no papers focused in this topic, especially involving a population with particular genotype such as Mexican population.

Additionally we provided more detailed and careful inclusion and exclusion criteria in the text.

Inclusion criteria were:
1) Diagnosis of knee OA according the international criteria (40) and corroborate by the X-ray using the Kellgren and Lawrence score (grade #2 was included).
2) Patients > 40 years
3) All participants declared to be native to the central region of Mexico, and to have parents and grandparents born in the same geographical region.

Whereas the exclusion criteria were:
Other concomitant inflammatory diseases such as: rheumatoid arthritis, crystal-related arthritis, septic arthritis, and knee surgery reported.

Asymptomatic healthy subjects sex and age matched were include as control group.

Comments

1) They analyzed HIF1A and WISP1 polymorphisms in 70 patients with knee OA and 66 healthy controls. However, they found zero frequency genotype in SNPs of HIF1A, suggesting that the number of the patients is not big enough. Making
an analysis of the power of the study is completely necessary. The number of both the patients and healthy control must be increased.

We agree with the observation of the reviewer. We are planning to continue with the study in order to increase the number of patients. However, as mentioned in the previous answer, we believe that there are points of interest that deserve to be considered. In particular, it is of relevance that our study shows how the variant rs11549465 in the HIF1A gene verified in the Mexican Population could differ from that typically reported in other populations.

We additionally performed an analysis to estimate statistical power of the Odds ratio obtained (with confidence level of 95%) for the sample size of our study, and showed that the statistical power for the protection genotype CT is 0.87; and for the risk genotype CC is 0.99).

2) The authors need to include Kellgren and Lawrence grade (KL grade) or joint space narrowing (JSN) grade of the patients with Knee OA and to analyze the relationship between these clinical grade and these polymorphisms.

We included patients with grade # 2 according the radiological scale of Kellgren and Lawrence (this is reported in the methods). We showed that the grade 2 maintain a relationship with the polymorphism, whereas the grade 4 did not showed any relationship. These results are reported as a supplementary file.

In the results section: “Additionally we investigate the relationship between the radiological grade of OA and the polymorphism showing that the grade 2 maintain a relationship with the polymorphism whereas the grade 4 did not showed any relationship (supplementary file).”

ADDITIONAL FILE
Gene and allele frequencies of the rs11549465 polymorphism in OA patients according the Kellgren-Lawrence scale

<table>
<thead>
<tr>
<th></th>
<th>OA group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>K&amp;L Grade 2, N=62</td>
<td>F n   F n</td>
<td>F n   F n</td>
</tr>
<tr>
<td>CC</td>
<td>58 0.93</td>
<td>49 0.74</td>
</tr>
<tr>
<td>CT</td>
<td>0 0.06</td>
<td>17 0.25</td>
</tr>
<tr>
<td>TT</td>
<td>0 0.00</td>
<td>0 0.00</td>
</tr>
<tr>
<td>C</td>
<td>120 0.97</td>
<td>115 0.87</td>
</tr>
<tr>
<td>T</td>
<td>4 0.03</td>
<td>17 0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>OA group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>K&amp;L Grade 4, N=8</td>
<td>F n   F n</td>
<td>F n   F n</td>
</tr>
<tr>
<td>CC</td>
<td>8 1.00</td>
<td>49 0.74</td>
</tr>
<tr>
<td>CT</td>
<td>0 0.17</td>
<td>0.25 NS</td>
</tr>
<tr>
<td>TT</td>
<td>0 0.00</td>
<td>0.00 NS</td>
</tr>
</tbody>
</table>
OA, Patients with knee osteoarthritis; K&L, Kellgren and Lawrence; OR, Odds ratio; CI, Confidence interval; F, gene and allele frequencies; *P value corrected by Bonferroni test, <0.05; NS, not significant; Significant P values and OR are reported in bold.

3) Clinical information including age, gender, BMI, glucose, cholesterol and uric acid were collected. Did they analyze the relationship between P582S polymorphism of the HIF1A and above clinical information?

We performed a regression statistical analysis in order to verify the correlation between the clinical variables above mentioned and the P582S polymorphism of the HIF1A.

We added these results in the results section. Briefly, no correlation between gender, BMI, glucose, cholesterol, uric acid, and P582S polymorphism of the HIF1A was found.

4) The p values that are reported in the results (Table 3 & 4) should be the p values adjusted by age and sex.

In the table 3, considering the genetic characteristics of our population, we consider the HWE test could support adequately the comparison of both study groups.

Regarding the table 4, a logistic multivariate regression adjusted for age and gender was performed, however, we found the same trend of association, but not was statistically significant (data no shown).

5) In Table 3 & 4, number of patients and control should be corrected. In addition, allele frequencies of rs2057482 and rs2929970 in control group are not correct. The number of both patients and controls has been corrected. Additionally the allele frequencies of rs2057482 and rs2929970 were also corrected.

6) In Table 2, they found a significant difference in BMI and uric acid level between patients and controls. Did they describe this difference in "Discussion" section?

As requested, we introduced a brief sentence in the discussion underlying the difference in BMI and uric acid level between patients and controls.

Our results show that the BMI in the patients with OA was higher with respect the healthy control group. This is in line with previous studies that reported how a higher BMI is a risk factor for developing knee OA, probably induced by the biomechanical joint stress [41].

Interestingly, our results showed a significant decreased level of uric acid in the OA patients with respect the healthy control. This is in line with the study of Mishra et al [42], who attributed this phenomenon to the antioxidant properties of
uric acid since they found an inverse correlation between uric acid and the presence of malondialdehyde (pro-oxidants agent). This induces to consider a possible link between the reduction of both the content and the antioxidant property of uric acid and the inflammatory process of OA.

REVIEWER 3
Reviewer: Panagiotis Lepetsos
Reviewer's report:

This is an interesting paper, by Torres et al, examining the association of P582S SNP (HIF1A) with knee osteoarthritis in a Mexican population. The authors have concluded that the presence of the specific polymorphism plays a protective role in the loss of articular cartilage. However, there are some problems that need to be solved.

1. The sample size of the population is quite small. In my opinion, 70 patients and 66 controls do not suffice to support the correlation of a SNP with knee OA.

We agree with the reviewer that it is advisable to increase the sample size. We included a paragraph in which it manifests the need to increase the number of study subjects in order to strengthen the statistical power of results in this study.

2. Patients and controls have different age and BMI. Thus, a multiple regression analysis is necessary to support any results. In my opinion, statistical analysis of this study is not sufficient.

In order to strengthen our results we perform a multiple regression analysis that showed the same trend of association, but not was statistically significant. These results have seen included in the text in the statistical analysis and results section.

3. The aforementioned problems and any other potential limitations are not even discussed in the discussion section.

We included a point of discussion of different points in the manuscript, and we added also a sentence reporting the limitation of our study.

In my opinion, this paper cannot be accepted for publication in the present form. I believe that the study should be redesigned, including a larger population number, and a multivariate approach in the interpretation of results.

We follow all the suggestions by the reviewer and a partial redesign was performed. We also included a multivariate approach in order to interpret better our results. We believe that in the present form the manuscript could be considered for a new revision.

The ongoing study, including a larger cohort of patients, will be useful to support more strongly our preliminary hypothesis.