Author’s response to reviews

Title: Association of IL10 and TGFB Single Nucleotide Polymorphisms with Intervertebral Disc Degeneration in Iranian Population: A case control study

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Author’s response to reviews:

Dear Dr. Pasini,

Thank you for your consideration of the manuscript “Association of Single Nucleotide Polymorphisms of IL10 and TGFB with Intervertebral Disc Degeneration” by the BMC Medical Genetics.

Our responses to the reviewers’ queries are discussed below. Meanwhile, a revision-marked draft of the revised manuscript is respectfully enclosed accordingly to the instructions.

Please address all correspondence concerning this manuscript to me at rezaei_nima@tums.ac.ir.

Thank you for your consideration of this manuscript.

Sincerely,

Nima Rezaei
Reviewer reports:

Georg Omlor (Reviewer 1): General comment:

Q1: The authors present a well conducted important study on a large to midsize population group. The results seem important for further basic science studies dealing with the etiology of disc degeneration. Before publication, I recommend some language editing by a native speaker.

A1: The whole manuscript was reviewed in order to simplify the sentences and make it more easy-to-read. Accordingly, the difficult phrases were substituted with simple ones in the meantime of keeping the main content intact. It was also checked for any typo or grammatical error.

Specific comments:

Q2: Was the control group adjusted to age and sex of the patients-group? If not, possible effects might be further discussed.

A2: The controls consisted of 70 females and 70 males, which was not remarkably different from the cases. This was stated in the methods (page 5, line 13) and also the results (page 7, lines 8-9).

Actually, as we were looking for inherited mutations and SNP variants (not the somatic ones), the age was not considered as a causative factor for SNP variation. However, this was mentioned as a limitation in the discussion as well (page 11, lines 4-5)

Bianca Bianco (Reviewer 2): Overall it is an interesting study, but I have the following concerns and comments:

1. In the abstract section

Q3: The abstract is confusing and needs to be rewritten. The objective of the study needs to be clear. In addition, inform the rs of the SNPs studied.
A3: The abstract has been revised and the objective is written more clearly (page 2, lines 2-9). The studied SNPs and their rs numbers are added in the abstract as well (page 2, lines 11-13)

2. Patients and Methods

Q4: The sample size was calculated?

A4: PASS 11 software was used to calculate sample size. Accordingly, and in order to have a power of 80% and significance of 0.05, a sample size of at least 50 in each group was calculated. This was added to the methods page 6, lines 17-18 as:

“Using the PASS 11 software, a sample size of 50 would achieve 81% power to detect an effect size (W) of 0.4000 using a 1 degree of freedom Chi-Square Test with a significance level (alpha) of 0.05.”

Q5: And Hardy-Weinberg equilibrium? Please, this information must be added to the text. Besides, although the methodology of polymorphisms genotyping has already been described it is essential to describe it briefly.

A5: The Hardy-Weinberg equilibrium was calculated using Online Encyclopedia for Genetic Epidemiology studies, which was added to the methods (page 6, lines 24-25). The HWE for each investigated SNP is added to the table 2 as well. The brief description of polymorphism genotyping is added to both methods (page 6, lines 9-12) and abstract (page 2, lines 13-15)

Q6: There is no approval by ethics committee.

A6: This study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS). This was added to methods page 5, lines 18-19.
3. Results

Q7: The tables are presented as charts.

A7: The information of Tables 2 and 3 are summarized as charts as well. We may include these results in the format of either tables or charts (or both). Actually, the charts might be more easy-to-understand, but the tables include more quantitative values (P-value, OR, 95% CI, and HWE) which makes the paper more suitable for future systematic review and meta-analysis studies. This would be helpful to have more citations as those researchers who are interested in systematic reviews and meta-analysis would cite the paper as well. Therefore, we may suggest keeping both tables and charts in this manuscript. However, the reviewer’s comments are highly respected as well.

The charts are added as figures 1-3, which are cited in the respective parts of results as well.

Q8: The patients age range from 19 to 62-year-old. Is this adequate? Would not disc degeneration be expected as an aging process?

A8: Yes, of course, the critic is right; disc degeneration is an aging process and the incident rate would increase in elderly. For the current investigation, we added another analysis comparing the average of age in different genotypes and alleles of each SNP. The description is added to the results (page 8, lines 11-17) and also Table 4.

However, the main purpose of current study was to investigate the association of inherited SNPs (not somatic mutations) with presence of disease. In case of significant results, we would conduct another study in order to investigate the SNP distributions in different age groups.

4. Discussion

Q9: The criticisms with the current manuscript are the small sample. The criteria for performing genetic studies include homogeneous population, robust methodology and sample size with statistical power. Besides, the strengths and weaknesses of the study must be discussed.
A9: The consideration of criteria for performing genetic studies and also the strengths and weaknesses of the current study were added to the end of discussion (page 10, lines 26-28; and page 11, lines 1-12)