Author’s response to reviews

Title: Rare variant of HSPG2 is not involved in the development of adolescent idiopathic scoliosis: evidence from a large-scale replication study

Authors:

Chao Xia (190540905@qq.com)
Leilei Xu (peterxu_drumtower@163.com)
Bingchuan Xue (bingchuan_xue@163.com)
Fei Sheng (418500046@qq.com)
Yong Qiu (scoliosis2003@163.com)
Zezhang Zhu (zezhangzhu@126.com)

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Author’s response to reviews:

Dear editor

Thank you so much for the comments on our paper titled "Rare variant of HSPG2 is not involved in the development of adolescent idiopathic scoliosis: evidence from a large-scale replication study” (BMSD-D-18-00462). We thank the reviewers for their comments and appreciate their valuable suggestions, and have now revised the manuscript according to the concerns raised in the reviews. A detailed copy of the authors “point-by-point response to the reviewer’s comments” is attached, and specific changes addressing reviewers' comments are marked.

Editor comments :

Q1: Please include the email addresses of all authors on the title page.

A1: Thank you for your valuable suggestion. We have included the email addresses of all authors on the title page. Please refer to Pg1 for the modifications.
Q2: Please remove the Acknowledgement section from the title page.

A2: Thank you for your valuable suggestion. We have removed the Acknowledgement section from the title page. Please refer to Pg1 for the modifications.

Q3: Please change the heading “Introduction” to “Background”.

A3: Thank you for your valuable suggestion. We have changed the heading “Introduction” to “Background”. Please refer to Ln3 of Pg3 for the modifications.

Q4: In the methods, please remove the word “normal” before controls.

A4: Thank you for your valuable suggestion. We have removed the word “normal” before controls. Please refer to Ln13 of Pg5 for the modifications.

Q5: In the results section, it is not clear when you are comparing results between two groups, which group each result refers to, for example, “These two groups were found to have comparable HSPG2 expression (0.0015 ± 0.0011 vs. 0.0017 ± 0.0014, p = 0.57).”

A5: Thank you for your valuable suggestion. We have changed the words “two groups” to “two groups classified by curve severity”. Please refer to Ln7 of Pg8 for the modifications.

Q6: Please move the list of abbreviations to after the conclusion section.

A6: Thank you for your valuable suggestion. We have moved the list of abbreviations to after the conclusion section. Please refer to Ln4-10 of Pg11 for the modifications.

Q7: After the list of abbreviations, insert the heading Declarations and please reorder your Declarations section to match what is outlined in our Submission Guidelines and include all sections.

A7: Thank you for your valuable suggestion. We have inserted the heading Declarations and reordered the Declarations section to match the Submission Guidelines. Please refer to Ln13-24 of Pg11 and Ln1-15 of Pg12 for the modifications.
Q8: In the section “Ethics approval and consent to participate”, please ensure that you include the full name of the ethics committee and include a consent to participate statement, including whether written or verbal informed consent was obtained from the parents of the participants in this study that are under 18 years old.

A8: Thank you for your valuable suggestion. We have included the full name of the ethics committee and included a consent to participate statement. Please refer to Ln14-20 of Pg11 for the modifications.

Q9: Please describe the role of the funding bodies in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

A9: Thank you for your valuable suggestion. We have described the role of the funding bodies. Please refer to Ln1-6 of Pg12 for the modifications.

Q10: Please include a statement in the competing interests section that Zezhang Zhu is a member of the Editorial Board of BMC Musculoskeletal Disorders.

A10: Thank you for your valuable suggestion. We have included a statement in the competing interests section that Zezhang Zhu is a member of the Editorial Board of BMC Musculoskeletal Disorders. Please refer to Ln13-15 of Pg12 for the modifications.

Reviewer #1:

Q1: This is a good study with a clear message. I am pleased that a study with negative results is also being published. This is quite refreshing.

A1: Thank you for the favorable comments.

Reviewer #2:

Q1: I would ask were the standard criteria used to rule out AIS by the senior spine surgeon (less than 7 degrees of axial rotation on Adams forward bend)? They mention they used Adams forward bend just not the criteria they used.
A1: Thank you for your valuable suggestion. The standard criteria used to rule out AIS by the senior spine surgeon was “less than 7 degrees of axial rotation on adams forward bend”. And we have revised the methods part. Please refer to Ln15-16 of Pg5 for the modifications.

Q2: I see a cursory description of HSPG2 in scoliosis as far as single versus double curves. Is there any specific Lenke pattern associated, the numbers are so big, I would think there would be enough data to assess this.

A2: Thank you for your valuable suggestion. Due to the limitation of retrospective study, we failed to analyze the relationship between the HSPG2 gene and curve pattern. Further studies may be needed to investigate the association between them.

Q3: I think this study is important and I think there is something here. The discussion is good. Can the authors frame the discussion for the common surgeon in terms of what they should take away from the article? I think the discussion is almost there but I was confused by a lot of the genetic detail in reading it.

A3: Thank you for your valuable suggestion. The suggestive genes of AIS may play a important role in the diagnosis and therapy of AIS in the future. Our findings suggested that the target variants of HSPG2 gene may be not involved in the etiology of AIS. We believe that the role of HSPG2 in the development of AIS still needs further investigation. We have revised the discussion part. Please refer to Ln13-14 of Pg10 for the modifications.

Reviewer #3

Q1: To write more about the phenotypic relationship that may exist between Rare variant of HSPG2 and AIS. What are the possible clinical manifestations of this rare variant?

A1: Thank you for your valuable suggestion. Rare variants of HSPG2 have recently been reported to function as a potential contributor to the susceptibility of AIS in the Caucasians. Previous studies also reported that HSPG2 gene was associated with progressive IS. To our knowledge, clinical manifestation of this rare variant remains unknown. The function of this rare variant should be investigated through in-vivo cellular experiment and animal model to clarify its relationship with phenotypes of AIS.
Q2 : I would like the authors to better specify the location of the muscle where the expression of the HSPG2 gene was studied. Were the multifidus? In patients with AIS, was the muscle removed from the concavity or convexity? Was it withdrawn at the apex of the curve?

A2: Thank you for your valuable suggestion. We choose the multifidus for expression analysis. For AIS patients, we collected the muscle sample from both the concave side and convex side at the apex of the curve, and no significant difference were found between the two sides. We have clarified this information in the methods. Please refer to Ln14-15 of Pg6 for the modifications.

Reviewer #4

Q1: The investigators should indicate the number of exons in HSPG2 (94). HSPG2 should be italicized.

A1: Thank you for your valuable suggestion. We have revised the discussion part to indicate the number of exons in HSPG2. Please refer to Ln24 of Pg9 for the modifications. Following your suggestion, the gene name has been italicized throughout the manuscript.

Q2: There is a known function for HSPG2 which the authors do not discuss as the gene which has 94 exons encodes perlecan which binds to basement membrane proteins such as collagen and laminin and to cell surface receptors. Mutations in this gene are associated with Schwartz-Jampel syndrome Type 1 and Dyssegmental Dysplasia, Silverman-Handmaker Type. Schwartz-Jampel syndrome is associated with kyphoscoliosis.

A2: Thank you for your valuable suggestion. We have revised the discussion part to discuss the function of HSPG2. Please refer to Ln24 of Pg9, Ln1-5 of Pg10 and Ln5-16 of Pg19 for the modifications.

Q3: Is there a better control population that could be used? The control population used for this study was from a group of patients with lumbar disc herniation. I am concerned this may not be the most "clean" phenotype with respect to spine, since the controls are also affected with a phenotype that affects the spine. In doing so, it is possible that the hypothesis of there being differences in HSPG2 coding variants between patients with severe scoliosis curves and the control population used may be falsely rejected.

A3: Thank you for your valuable suggestion. The best kind of controls may be adolescent patients with spine fracture. However, these cases were rare. So we choose the patients with
LDH as controls. Samples of adolescent patients with spine fracture will be collected and analyzed as controls in the future studies.

Q4: I am not clear about the breakdown of the 90 patients used in the mRNA expression analysis. The authors state that 42 had a curve magnitude greater than 60 degrees and 58 had a curve magnitude less than 60 degrees. This equals 100 patients. Additionally they state that 8 patients and 2 controls were excluded from the analysis due to degradation of total RNA. It would be helpful if the final number of cases and controls analyzed for mRNA could be indicated.

A4: Thank you for your valuable suggestion. We have revised the results part. A total of 98 patients and 28 controls were included for expression analysis. 8 patients and 2 controls were excluded from the analysis due to degradation of total RNA. 42 had a curve magnitude greater than 60 degrees and the other 48 had a curve magnitude less than 60 degrees. So a total of 90 patients and 26 controls were included for expression analysis. Please refer to Ln20-23 of Pg7 and Ln5-6 of Pg8 for the modifications.

Q5: A flaw of this study is, as pointed out by the authors, the fact that all exons of HSPG2 were not sequenced. It is possible that there are rare variants for HSPG2 in their population which were not identified.

A5: Thank you for your valuable suggestion. The number of exons of HSPG2 is 94. Considering the high cost of whole-exome sequencing for this large-scale study, we didn’t sequence all the exons of HSPG2. Further whole-exome sequencing for familial idiopathic scoliosis may be needed to investigate the potential role of rare variants of HSPG2 in AIS.

Q6: Page 3, line 12: Please change Hormones to hormones.

A6: Thank you for your valuable suggestion. We have revised it. Please refer to Ln11 of Pg3 for the modifications.

Q7: Discussion-first sentence: I would say: Genetic findings of AIS have been greatly hindered by its clinical and genetic heterogeneity. I do not think "incomplete penetrance" is an applicable term as penetrance relates to a specific gene and whether or not there is a clinical effect with a particular mutation in that gene
A7: Thank you for your valuable suggestion. We have change the words “incomplete penetrance, unknown mode of inheritance, and population heterogeneity” to “its clinical and genetic heterogeneity.” Please refer to Ln17-18 of Pg8 for the modifications.

Q8: On page 10, line 8, change exome should be changed to exon.

A8: Thank you for your valuable suggestion. We have changed “exomes” to “exons”. Please refer to Ln16 of Pg10 for the modifications.

Again, we would like to thank you for taking the time to review our manuscript and believe that our findings are of high significance. We would be most grateful for your kind consideration of this revised manuscript for potential publication in BMC Musculoskeletal Disorders. We look forward to hearing from you at your earliest convenience.