Reviewer’s report

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

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Reviewer: Philip F. Giampietro

Reviewer's report:

In this study Xia et al. genotyped 1752 Chinese female patients with severe scoliosis curves and 1584 normal controls for the SNV p.Asn786Ser (rs143736974) in HSPG2. They also genotyped common variants in HSPG2. They examined mRNA expression in the paraspinal muscles of 90 patients (which were further subdivided into moderate and severe curves) and 26 controls.

The investigators did not find any patients in their cohort with the SNV p.Asn786Ser HPSG2 mutation. No statistically significant differences between cases and controls were noted in 16 SNPs covering HPSG2. No significant differences in mRNA expression between cases and controls were observed.

I think this paper does contribute to the literature regarding HSPG2 and its possible relationship to scoliosis. I have several concerns/questions regarding the experimental approach.

1- The investigators should indicate the number of exons in HSPG2 (94). HSPG2 should be italicized.
2- 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.
3- 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.

4- 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.

5- 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.

Minor points:

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

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

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

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Please indicate the quality of language in the manuscript:

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