In this article the authors sought to determine the genetic cause of hearing loss in 79 patients. They used a targeted gene panel that sequences all known deafness genes using massively parallel sequencing (MPS). They examined single nucleotide variants and indels and provide a novel method for determination of copy number variants (CNVs) in these genes. They validate their method for CNV detection with qPCR. CNVs were the apparent cause of deafness in 5 patients. Overall the diagnostic rate was at most 29/79 patients (37%).

Overall, this research is well-intentioned and the results could be valuable to the deafness research community and the broader field of human genetics. However, the study is hampered by an inadequate acknowledgement of previous studies with similar methods, a lack of data supporting genetic diagnoses, and by poor English. It was difficult to assess the results due to a lack of clarifying data on the genetic results for the patients. Insufficient description of analysis and methods would preclude reproducibility of this study as currently described.

MAJOR COMPULSORY REVISIONS

1. The authors describe a new method for CNV analysis in massively parallel sequencing data. Several other groups have created similar methods. There are more than 20 other methods described for determining CNVs from MPS data in Zhao, Min, et al. "Computational tools for copy number variation (CNV) detection using next-generation sequencing data: features and perspectives." BMC Bioinformatics 14.Suppl 11 (2013): S1. The authors should ideally compare their novel method to one of these methods or at least indicate for what reason they did not use a previously published method.

2. There is a lack of information regarding the patients, making the genetic mutations described difficult to evaluate. Please provide a table that lists each patient, clinical data (for example, age, onset of hearing loss, type of hearing loss, family history, etc), candidate variants, and causative variants.

3. The overall results were confusing. What was the overall diagnostic rate? The number seemed to vary between 21-29. Please also more clearly indicate the overall results of the study with either a table or a simple graph showing total number of patients, patients given a diagnosis, and by what type of variant (CNV, SNV, indel). Please compare the overall findings of this study to similar studies.
using targeted gene panels for deafness.

4. Please explain in the Methods section why 4 different analysis methods were used. This is highly atypical for MPS studies. Please describe how the analysis methods differed and what the overall differences were between the methods. In addition, the Galaxy platform described in the Methods section is a framework for analysis of MPS. What tools in Galaxy were used? In addition, on page 6 the authors refer to a “custom knowledge database.” Please describe what this is.

5. In the Methods section, line 133, the authors indicate that they used Sanger sequencing to validate the platform. What does N=8 refer to? Number of samples? Was this validation for CNVs or SNVs or both? The authors indicate that a “high percentage of detected sequence variations . . . agreed with each other.” A formal sensitivity, specificity, positive predictive value, and negative predictive value analysis should be performed along with data indicating which variants were screened for this analysis.

6. I am concerned that the DSPP variants the authors identified are platform-specific errors. I would find it incredibly surprising if not impossible that variants in DSPP are nearly as common a cause of deafness as mutations in GJB2. We and others have seen variants in DSPP, especially indels, as platform-specific errors. I think that these variants in DSPP should be examined more closely. Were they validated with Sanger sequencing? Do they segregate with deafness in families? DSPP is considered a dominant deafness gene. If these were sporadic cases do the authors assume that all of these DSPP mutations arose de novo? Please address this surprising finding.

7. The method for targeted capture is not described adequately on line 123. I understand that the authors have previously published it, but it would be helpful for clarity to provide a brief summary of the method. Also, on Line 146, the authors state “we normally hybridize 20 samples together.” Is this how many were done in this study? This is not formal scientific English and the sentence should be revised and clarified in order for the method to be reproducible.

MINOR ESSENTIAL REVISIONS

1. Please have the manuscript proofread by a native English speaker. Here are some of the grammatical/spelling errors I have identified:
   a. Title: should read something like “Combined examination of sequence and copy number variations in human deafness genes improves genetic diagnosis for genetic deafness”
   b. Line 54: “supplement” should be “supplemental”
   c. Line 68: “mega bps” should be “mega base pairs (MB)”
   d. line 73 and line 75: “in causing” should be “to” and “that cause” respectively
2. Please provide a reference for line 71
3. Line 119: InPE1.0 primer is used two times?
4. Please describe briefly your targeted capture method on line 123.
5. Line 135: “sequencing quality score,” do you mean Phred-like quality score?
6. Line 144: There is a formatting issue – is the square supposed to be a “<”? 
7. Line 162: “putatively” should be “putative” 
8. Line 215: “compare” should be “compared” 
9. Please revise the sentence on line 243-246; due to poor grammar it is confusing. 
10. Line 249: “structure” should be “structural” 
11. Line 253: “about how” is not formal scientific English, please revise 
12. Line 294: “investigated” should be “investigating” 
13. Please move the description of Figure 1 from the text to the Figure legend. 
14. How did the authors ensure that the qPCR primers used for STRC were specific? This gene is notoriously difficult to amplify without amplifying the pseudogene. This may be the reason for the false positive result.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I am part of the non-profit Molecular Otolaryngology & Renal Research Laboratories at the University of Iowa that offers a similar genetic testing panel for deafness. Our laboratory is non-profit and I do not have a conflict of interest in evaluating this manuscript.