Author's response to reviews

Title: Diagnosis of Noonan syndrome and related disorders using Target Next Generation Sequencing.

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Version: 4  Date: 23 December 2013

Author's response to reviews: see over
Dear Editor,

We are submitting to your attention the revised version of the paper entitled “Diagnosis of Noonan syndrome and related disorders using Target Next Generation Sequencing”.

The text has been modified according to reviewer’s requests.

In particular

**Reviewer n.1**

**Minor comments**

1) Under Discussion: Page 10: (paragraph 3 line 8) the authors stated that "In one patient (caseno.16) we identified two unpublished mutations affecting two consecutive of PTPN11 aminoacids, D395Y and Y396H. Both mutations were inherited from an affected father. Variability of clinical expression in this family was quite obvious, since only the son manifested NS facial anomalies and developmental delay while the father had congenital total alopecia." This part deserves more elaboration and discussion.

We agree that this point was not very clear so we modify this sentence and insert a comment about this case in Discussion pag.10 line n.14-18.

**Reviewer n.2**

**Major Compulsory Revisions**

1) Authors characterized 11 mutations initially, however they were not described later in results and discussion

In discussion we do not described the 10 previously characterized cases (6 positive and 4 negative) because we used them to assess the analytical sensitivity and specificity of our panel as we discuss on page n.7 in the Results paragraph “Validation set”.

2) The methods are not completely addressed. For example, page 1: How authors selected the gene list?

In Methods page n.5 line n.18 we insert a sentence to explain how we select the genes and insert the corresponding reference

3) They mentioned to apply NGS to 92% of region for the listed target genes, how threshold was selected?

On methods page n.6 line n.21 we explain that we choose the threshold on the basis of the Guidelines of the American College and insert the reference.

4) The mutations in these genes were already reported?

On table n.3 we insert a column with the reference. If the mutation is described in this study for the first time we reported p.s (present study).

5) There must be a detailed figure how the analysis was carried out especially describing the chromosomal locations of genes listed along with their individual contribution in disease.

We prepare a figure as requested (figure n.2). This figure is mentioned at the end of Methods, page n.7 line n.4
6) In figure 2, only single mutation has been shown, more examples are required to show Reproducibility. We insert two other mutations in this figure and the figure number have been changed from number 2 to number 3.

Minor Essential Revisions:

7) Language: The language is not appropriate for publication. Many sentences are incomplete and/or incorrect. For example, page 7: TSCA approach reduced up to 12 the number of exons, page 9: while in the other case clinical evaluation was unable, page 9: with a significantly reduction of time to reach...
Typing errors: page 1: ass be after excluded, page 6: As a SNP database, inappropriate usage of adverbs (a, an, and the) at many places. Please ask a native English speaker for corrections.

The paper have been corrected by a native English speaker and the error above mentioned have been corrected.

8) All the gene names must be in italic.
Bibliography: Formatting no ok, For example: references 9 and 12: look year. References 2 and 3: check author names formatting: These errors have been corrected.

Supplemental from Reviewer n.2 (not mentioned on Major and Minor Revision)

9) Page 5: Authors mentioned that they performed analysis on 57,932 bp target region: Which they used to design probe? It is not described in detail. What were the average sizes of amplicons?
We do not described the algorithm because the design of the probe have been done with the on line software Design studio (Illumina) and the algorithm is property of Illumina, to clarify this point we specify on page 5 line 10.

10) How they calculated coverage? The coverage have been calculated on Design Studio, we specify it on page n.8 line n.2

11) Which SNP data base authors used? Whether it contained all the known SNPs in target genes?
We used the dbSNP database that contain all the known SNPs in target genes, we insert this information on page n.6 line 15.

12) Particularly, looking in Table 3, most of the mutations were described in PTPN11 and SOS1 genes; while authors characterized 11 genes, please explain. Moreover, in page 7, validation data clearly indicates that out of 38 mutations, frequency in most of genes of RAS pathways is 5% or less. In discussion, major focus of authors was PTPN1 gene; other mutations have not been discussed properly. Conclusion is also not matching to reported data.
As we described in discussion this data overlap with data from literature, to better explain and underline this data we expand and improve the conclusion.
Thank you very much for your attention.

With best regards

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