Author’s response to reviews

Title: Targeted/exome sequencing identified mutations in ten Chinese patients diagnosed with Noonan syndrome and related disorders

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Author’s response to reviews:

Dear editor,

Thanks for reviewing our manuscript. We have revised the manuscript based on the reviewer's suggestions and comments. Please see a point-to-point response below.

Your time and consideration is highly appreciated.

Best regards,

Yongguo Yu

Reviewer 1:

1. Introduction - remove results from this section

Results part has been removed.
2. Subjects - Just to confirm that only 10 individuals were sequenced, with 100% diagnostic rate, or are only diagnosed patients presented?

Only diagnosed patients with variants identified were presented in this study. This has been clarified in the "2.1 Subjects" section.

3. Methods 2.2, Whole exome sequencing - this section has numerous errors of fact and statements that do not make sense. This section requires revision by someone familiar with the process of whole exome sequencing. To highlight particular issue (not necessarily exhaustive): 1) The whole exome enrichment kit is not detailed, only the library preparation kit, which capture kit was utilised? 2) Variants are identified by the use of a genotype calling tool such as GATK or Samtools, not reference databases 3) You claim to annotate variants following filtering, how is the filtering information added? 4) Clarify and justify why variants whose frequencies (in what?) were >0.1% were filtered out.

4. Methods 2.2 - Tools used and reference databases need to be cited

5. Results 3.2 - Again, requires revision as per methods 2.2. Total reads, is this per person average, total across 10 samples? Also, for average coverage, what is your denominator for these coverages, capture kit baits, genes?

Response to point 3-5: We apologize for all the errors in the methods and results part involving sequencing. These parts have been rewritten. During the revision, we noticed that some of the patients went through targeted sequencing instead of whole exome sequencing. The relevant text has also been changed.

6. Novel variants - PTPN11 K70R has been previously reported (rs397516801) and BRAF F468C has been reported in leukemia (http://onlinelibrary.wiley.com/doi/10.1111/bjh.12735/full). These should be discussed more fully as this substantially adds to the evidence base for these variants and acknowledges prior work.

We thank the reviewer for pointing this out. PTPN11 K70R has not been published in the literature but was documented in Clinvar (identified in 5 affected individuals and segregates with symptoms of Noonan syndrome in one family). BRAF F468C has not been reported in NS or
related disorders previously, but was detected in a hairy cell leukemia (HCL) patient and a colorectal cancer patient. We corrected the information in the table and discussed those prior work in the "discussion" part.

Reviewer 2:

The abstract states that all mutations were shown to be de novo, however, there is no mention of parental testing in the manuscript. A description of the parental testing should be included in the methods and results.

A description had been added in the methods and results sections (the end of Methods 2.2; the end of the first paragraph of Results 3.2)

There are larger variant repositories than the NHLBI ESP such as Gnomad, the authors should report the variant frequencies from that resource. BRAF p.F468C is reported in gnomad in one individual and supports the pathogenicity of the variant.

Thanks for pointing this out. We have incorporated GnomAD frequency into Table 1. In Gnomad, p.F468S(rs397507473) is reported, but p.F468C has not been reported, though this variant has been presented in a leukemia patient in literature. We added this information to the discussion part.

The authors should state if they have received consent to publish the photos of the patient's faces. The photographs should be removed if specific consent to publish photographs has not been obtained.

We have got the consent from the parents for publication of photos, and we added a statement in the manuscript (Results 3.1). If needed, we could upload the consents as attachments.