Author’s response to reviews

Title: Gaucher disease: Single gene molecular characterization of one-hundred Indian patients reveals novel variants and the most prevalent mutation.

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Author’s response to reviews:

Cover letter

30th December 2018
To,
The Editor
BioMed Central, UK

Sub.: Resubmission of the manuscript (ID: MGTC-D-18-00440:)

Dear Matteo Pasini,

Enclosed is the manuscript entitled, ‘Gaucher disease: Single gene molecular characterization of one-hundred Indian patients reveals novel variants and the most prevalent mutation.’ for publication in your esteemed journal if found suitable.

I thank the editor and all the reviewers for providing valuable inputs in our manuscript. I have provided point-by-point response to the reviewers. Should you have any query feel free to contact us. On behalf of all the authors, I confirm that all the authors have gone through the revised manuscript and none has any objection in getting it published in the journal BMC Medical Genetics. The authors declare that they have no competing interests (financial or non-financial) in the present study.

Thanking you,

Yours sincerely,

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Response to reviewers

On behalf of all the authors I thank the reviewers for providing the valuable inputs. This will surely help us improve the manuscript quality. Following is the point by point response to the reviewers’ comments.

Mary Anne D. Chiong (Reviewer 1):
Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors. Very informative results and commending the comprehensiveness of the study.

1. Grammar, sentence construction and spelling should be improved/checked in the entire manuscript.
Response: We have screened the manuscript for any grammatical and/or spelling errors.

2. Why was the Romanian study on mutations specified in the introduction? why not other Asian population or other studies from Indian ethnic groups?
Response: The Romanian study was mentioned to establish the link for the list of common mutation identified in Gaucher disease. Studies for Asian population, Indian ethnic group and other population are mentioned in discussion part (for example reference number 18 onwards).

3. In the methodology, when you say suspected, what were the inclusion criteria? just one manifestation like thrombocytopenia is already eligible for the study? please specifically enumerate your inclusion criteria- i.e. whether they will still be included even after a negative enzyme or other biochemical assays? or are they included even with normal assays but with Gaucher cells in the bone marrow? because you have patients whom you said had normal beta glucosidase activities.
Response: The inclusion criteria included the age of onset, presence of common clinical phenotypes like unexplained hepatomegaly, splenomegaly, anemia, and thrombocytopenia with or without any bone abnormality. The patients were included under the following circumstances:
   i. Patients having above mentioned symptoms even after a normal enzyme study from leucocytes and presence of Gaucher cell in Bone marrow and whose Acid sphingomyelinase study was found to be normal as there is an overlapping phenotype in both the condition like hepatosplenomegaly and anemia, thrombocytopenia and raised plasma chitotriosidase.

4. You should place the ages and male female ratio not in the methods but in the results.
Response: The suggested changes have been made in the manuscript.

5. In the results you said moderate splenomegaly, how would you define that? what is mild and severe splenomegaly then?
Response: Splenomegaly can be classified into two types based on the size and weight of the spleen. The normal size of the spleen is around 11 cm. When the spleen size increases between 11-20 cm it is considered as mild splenomegaly. When the spleen size increases more than 20 cm it is considered as severe splenomegaly. The above criteria are established by Poulin et al. Splenic artery embolization before laparoscopic splenectomy. An update. Surg Endosc. 1998 Jun;12(6):870-5. We followed the same criteria for the classification of the splenomegaly in the
patients.

6. In the molecular analysis, sorry for my ignorance but is there a specific change for the complex C mutation? if yes, what is it?
Response: There is no specific change for the complex C mutation in the molecular analysis.

7. In the discussion how were you able to classify that there 77 type 1, 12 type 2, etc. was it based on clinical manifestations or mutations or both?
Response: The classification of the Gaucher disease types were made on the bases of clinical manifestations and the age of onset. We have not observed any mutation that was found to be specific with the type of Gaucher diseases

8. In the discussion you said that there was a low prevalence of bone diseases in the patients investigated. how low? because this was one of your clinical inclusion criteria -WITH or Without bone abnormality. so, what were the predominant mutations you saw in those with bone abnormality if its not 84GG?
Response: As can be seen from the data, most of our patients were diagnosed at an early age with massive splenomegaly and hepatomegaly. Bone involvement was mainly observed in less than 5 patients from the series of 100 patients especially in those with type I GD. We could not find any mutation specific Bone abnormality and 84GG mutation was not observed in any of our patients with GD till date. Most common mutation observed in those with bone abnormality was also L444P as like other cases with GD.

9. You mentioned about modifier genes in the discussion re Leu483Pro manifesting as type 1. have there been any studies before that elucidated these modifier genes for this mutation in relation to the diversity of the clinical manifestations? because it has always been thought as a severe mutation. if studies are existing maybe you should include that in your discussion.
Response: We could not find any literature describing specific modifier genes responsible for the phenotypic variation due to Leu483Pro mutation. However, studies are conducted to identify the modifier genes in Gaucher Disease. Following is a brief description incorporated in the manuscript.

'A Genome-wide Association Study by Zhang et al. identified CLN8 as a potential modifier gene responsible for the phenotypic variation in the type 1 GD patients with p.Asn409Ser homozygotes [34]. However, the impact of modifier genes on GD is a grey area.'

Irene Paradisi (Reviewer 2):
Comments on the manuscript MGTC-D-18-00440: "Gaucher disease: Single gene molecular characterization of one-hundred Indian patients reveals novel variants and founder mutation"

General comments: The manuscript is well written, and all the important details that were suggested to the authors for their previous publication were taken into account. The text is easy to read and understand. There are some minor and major comments:

Abstract:

1. Using the word "variant" to denote pathogenic mutations is not recommended. "Variants" can be used in both normal and abnormal DNA changes; the herein reported changes are clearly damaging.
Response: The term ‘variant’ is replaced by the term ‘mutation’ in the abstract.

Background section:

2. Page 6, line 123: the cytogenetic location of the gene according to OMIM; NCBI Gene ID: 2629, GeneCards, etc. is 1q22
   Response: The cytogenetic location has been changed as per the suggestion.

Methods section:

3. Page 7, line 155; page 15, line 357: correct 4-methylumbeliferryl by 4-methylumbeliferyl
   Response: The spelling is corrected as per the suggestion.

Results section:

4. Page 9: What is the average inbreeding coefficient in the biological related families? Or, what is the most frequent consanguineous relationship? First degree relatives?
   In the Abstract, line 75, patients are described as "unrelated". But 26% of them are indeed related. A more specific description of this issue should be included.
   Response: The parents of total 26 patients had consanguineous marriage with their first-degree relatives. Hence the average inbreeding coefficient of these patients is 3.1% [(1/2)5 = 1/32 = 0.031 = 3.1%]. However, the patients i.e. the index cases are not related to each other. No two patients descended from the common ancestor. Hence, the term ‘unrelated’ was applied for the patients. The average inbreeding coefficient of the 26 patients is mentioned in the text.

5. Page 9-10, lines 218-220: Since India is a very large country, I would suggest to give more specific data (in a summarized way) about the geographic origin of the patients (perhaps mentioning the States, plus the Regional Area: West, North, etc.)
   Response: The states have been added in the manuscripts. However, the patients are distributed across each state hence the regional distribution is not possible. But we can say that maximum number of patients were from western region of the country. Though this cannot be used as the geographical preponderance because most prominent hepatologists in the country are from western region, hence it could be a selection bias. Even you see that there is very little presentation from the eastern part of the country but that cannot be said that GD is not there but it is mainly due to lack of awareness in those part of the country.

6. Page 10, line 236: "prevalence in 62% patients affected with GD"…this prevalence figure corresponds to the identified mutations in the patients sample i.e. 93 patients. It might be better described as…its frequency was 62% of the mutations detected…" or something like this.
   Response: The above changes are incorporated on page 12, line 282, 283.

7. Page 10, lines 241-242: mutations included in the complex alleles should be mentioned in the text, in addition to the Table 2 legend.
   Response: Mutations included in the complex alleles have been incorporated in the text.

8. Page 11, line 247: the sentence "Each mutation was present in two patients" is confusing, since there would be five mutations in four chromosomes (two patients)?
   Response: Each mutation was identified in different sets of two patients. This has been more clearly mentioned in the text. Following is the formatted text.
The given study uncovered the known mutations like c.1504C>T (p.Arg502Cys), c.371T>G (p.Met124Arg), c.754T>A (p.Phe252Ile), c.827C>T (p.Ser276Phe), and c.254G>A (p.Gly85Glu); each in different sets of two patients. These mutations were comparatively less commonly observed.

Discussion section:

9. Page 13, line 296, please correct the mutation nomenclature.
Response: The mutation nomenclature is corrected to c.1263_1317del (55Del).

10. Page 13, line 306: "it is justifiable to consider p.Leu483Pro as the founder mutation in the Indian population". To propose that there is a founder effect in the population, it is necessary to study markers / haplotypes that inform on the common genetic origin (IBD= identical by descent) of the mutation in the country, or if it is an Identical by state (IBS) mutation in India. There are several examples in which the same mutation has different genetic origins in a population (see for example: J Genet. 2017, 96(4):583-589; Eur J Med Genet. 2015; 58(2):59-65).
Response: I agree with the reviewer’s suggestion about the haplotypes analysis. However, we, as a non-governmental organization, do not have additional fund to conduct such study on a large population. I am afraid, the suggested study might not be possible in the limited time with limited resources. But you can look at our recent paper on carrier frequency of p. Leu483Pro published in BMC Medical Genetics (Ref: Sheth et al. BMC Medical Genetics 2018,19:178). Which was found to be 1 in 600 healthy populations from a pool of 1200 randomly selected healthy people from the country. All these observations made us to conclude that the given mutation seems to be a founder mutation in India.

11. Page 13, line 314, please include the current nomenclature for the mutation 84GG
Response: The current nomenclature c.84dupG (84GG) is inserted in the manuscript.

12. Page 14, line335-336: "Long term follows up of our patients with type 1 GD will help to understand the heterogenic effect of the said genotype on the phenotype"…. There is no description along the text whether the patients are receiving enzyme replacement therapy. If so, it is difficult to establish a genotype-phenotype correlation and/or a long-term evolution of phenotypic manifestations, since enzyme activity is being provided to the patients; this should be mentioned.
Response: Two patients with type 1 GD have recently started receiving enzyme replacement therapy. This information has been incorporated in the manuscript. Following is the modified text.
‘Owing to the high cost of enzyme replacement therapy and poor financial background of the patients, only two patients with type 1 GD are provided the therapy. However, the long term follows up of our remaining patients with type 1 GD will help to understand the heterogenic effect of the said genotype on the phenotype.’

13. Table 2, page 24: check minor corrections highlighted in yellow.
Response: The highlighted mistakes are corrected in the table.

Major comments:

14. The research provides useful and valuable data on the epidemiological genetics of Gaucher disease in that country. It is a quite complete manuscript, in which the pathogenic effects of the new variants were evaluated. Nevertheless, some aspects regarding population genetics should be included in the discussion:

(a) The high frequency of the pan-ethnic mutation L444P (p.L483P) is an interesting finding, which
however could be discussed a little more. Its frequencies vary between geographical regions in the country? Is it more frequent in the 26% of consanguineous families?
Response: We have carried out separate study on the carrier frequency of the said mutation in normal 1200 people in the country and interestingly 1 in 600 people are carrying mutant allele p.L483P. There is no geographical variation observed nor consanguinity where we can say p.L483P mutation is more common. It is equally distributed throughout the country irrespective of the region, caste or community.

(b) As mentioned, proposing p.L483P as a founder mutation in India must be based in genetic evidence (haplotypes) and/or historical records documenting the classical genetic forces that contributes to a founder effect (isolation, bottleneck, genetic drift, gene frequency). This discussion is lacking in the text. Moreover, using "founder mutation" in the article title can suggest that this phenomenon was indeed demonstrated. Perhaps "founder" can be replaced by "prevalent".
Response: As mentioned, due to lack of additional funds we have not carried out the haplotype analysis and hence the term ‘founder’ is replaced by ‘prevalent’.