Author's response to reviews

Title: A novel MANBA mutation resulting in residual beta-mannosidase activity associated with severe leukoencephalopathy: a pseudodeficiency variant?

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

Attached please find a revised version of our manuscript, entitled ‘A MANBA mutation resulting in residual beta-mannosidase activity associated with severe leukoencephalopathy: a pseudodeficiency variant?’, by F. Sabourdy, P. Labauge, M. Nieto, V. Latorre, H. M. Frostad Riise Stensland, D. Renard, G. Castelnovo, N. de Champfleur, and myself, to be submitted as an Original Article to the Editorial Board of *BMC Medical Genetics*.

The manuscript has been amended according to all Reviewers’ suggestions and comments, as follows:

**Reviewer A. Zlotogorski**

We agree with the Reviewer’s remarks. The fact that the MANBA mutation we identified might be a pseudodeficiency was already discussed in the text. The Reviewer underlines that only 57 Norwegian and 15 Palestinians controls were tested. This represents more than 100 alleles (which is the usual number tested to exclude a polymorphism) and none of them contained the mutation. While we had no access to a sufficient number of Algerian individuals, we preferred to include Palestinian controls to have individuals from a comparable ancestry (North Africa – Middle East).

Minor point: page 4, the correction has been made.

**Reviewer C. Sa Miranda**

1. Additional experiments have been performed to further characterize the mutation. Western blot analysis using an antibody against beta-mannosidase was carried out both on cultured cells from the patient (to analyse the endogenous protein) and on HEK293 cells that overexpressed either the wt or mutant enzyme. These data are now presented in
2. As pointed out by the Reviewer, pseudodeficient enzymes can present with higher residual activity than that described in the present study (and, indeed, in some diseases, some disease-causing mutations may be responsible for 10-15% residual activity). However, there are reports for pseudodeficiencies of lysosomal enzymes resulting in very low (<10%) residual activity (see Ref. 26 by G.H. Thomas for a review). Thus, it appears very difficult to draw definite conclusions as to a pseudodeficiency based only on the level of residual activity.

A sentence has been added in the Discussion to discuss this point.

3. As requested, we specify in the text (page 7) that the mutation has already been identified (but not characterized) by our group.

4. The title has been changed for: «A MANBA mutation resulting in residual beta-mannosidase activity associated with severe leukoencephalopathy: a pseudodeficiency variant?» (the word 'novel' has been deleted in the Title, Abstract, Results and Discussion).

5. The mutant protein has been further characterized by Western blot. However, since no cultured skin fibroblasts were available, we used the patient’s lymphoblasts. We also analyzed HEK cells transiently transfected with wild-type or mutant cDNA.

6. The fact that no oligosacchariduria was evidenced for this patient is mentioned in the original description of the case (Ref. 22). However, as suggested by the Reviewer, this has been added in the Discussion. No morphological studies of the skin have been performed since the patient’s condition has now considerably deteriorated, and skin biopsy would not be acceptable in this context.

Hoping that our revised manuscript can be considered for publication.

Sincerely yours,

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