Reviewer's report

Title: Assessment of cellular cobalamin metabolism in Gaucher disease

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Reviewer: Helen Michelakakis

Reviewer's report:

Abstract

Line 39 ,40: Gaucher disease (GD), one of the most prevalent lysosomal diseases, is caused by biallelic pathogenic mutations in the GBA1 gene that encodes beta-glucosidase (GCa

This is not always so .In rare cases GD is caused by mutations in the Saposin C  gene.

Line 41-42: Clinically, GD manifests with heterogeneous multiorgan involvement mainly affecting hematological, hepatic and neurological axes

Mention of the different types in relation to clinical manifestations should be included

Line 48: Dermal fibroblasts isolated from healthy subjects

The correct term is Skin Fibroblasts  and they are cultured not isolated .The term should be changed in all places it appears

Background

Line 94 : In rare cases GD is caused by mutations in the Saposin C  gene. This should be included together with relevant reference.

Line 115 : Reference no.7 only refers to elevated holo-transcobalamin findings. Need to include references for reduced plasma Cbl.

Line 62 : intracellular expression of transcobalamin. The use of the term expression is not justified on the basis of their experimental protocol.

Methods

Line 138 : one individual heterozygous carrier

Line 142 : Healthy human should be changed to Control human
Preparation of whole cell lysates. Is this the lysate that was used both for enzymic studies as well as for transcobalamin studies? If yes what is the role of protease inhibitors in the former and the use of the detergents in the later dissolve any membranes?]

General comment small number of cell lines tested.

Lines 173-174: As a control of lysosomal integrity, we measured α-glucosidase enzymatic activity assay using the substrate 4-methylumbelliferyl……. There is no way that they could evaluate lysosomal integrity by measuring the activity of another lysosomal enzyme. What is achieved by this assay is to show that the β-glucosidase deficiency assayed is not due to the quality of the specimen. The method employed for assaying of α-glucosidase activity should be given

Line 178: Expression of transcobalamin in whole cell lysates.

I do not think that the term Expression reflects what they have done.

Line 187: Cultured fibroblasts were isolated by…

Cultured fibroblasts were harvested by

Results

Line 254: asymptomatic heterozygous carrier should be changed to asymptomatic carrier of GD

Lines 259-261: It is well known that carriers of GD and most of the metabolic diseases, cannot be diagnosed through assaying of enzyme activity since there is a great overlap between carrier and control values. Furthermore they should not draw any conclusions from one cell line.

Line 264: See comment lines 173-174

Lines 266-268: These results exclude the occurrence of pleiotropic effects induced by mutations in the GBA1 gene as well as unwanted damage of lysosomal components during the sample preparation protocols.

What do the authors mean, pleiotropic effects induced by mutations in GBA1? Is this all based on normal α-glucosidase activity? See comment Lines 173-174

Fig1: (b) All examined subjects presented comparable α-glucosidase activity (nmol/mLxh), suggesting preserved activity of lysosomal components not associated with the GBA1 mutation

See comments lines 173-174; give abbreviations
It appears at least in some cell lines α-glucosidase activity is either or lower when the medium was supplemented with α-glucosidase activity. Could they comment on that? It would be helpful to have the individual values for the enzyme activities.

Line 280: Expression of Transcobalamin ….

I do not think that the term Expression reflects what they have done. It should be corrected everywhere it appears.

Line 286-287: These data only refer to fibroblasts when the cell that really suffers in GD is the macrophage, the disturbed function of which underlies many of the pathologies of the disease. So the statement that: previously reported abnormalities in plasma holo-TC in GD patients [7] do not seem to arise from abnormal biosynthesis/turnover of this protein in GD cells is not valid and should be rephrased. Also the present work does not deal with nerve cells.

Line 292-293: Under our experimental conditions, no differences were identified between healthy and GD cells, suggesting normal expression of transport protein transcobalamin.

This is not the impression I get looking at Fig2. Did they scan their blots?

Some type of quantitative assessment of this figure is needed. Also the term expression not appropriate.

Discussion:

The investigators have used fibroblasts. They have neither looked at the plasma of GD patients nor at other types of cells including macrophages and neuronal cells. Since we are not dealing with patients with primary defects in cobalamin metabolism but, if at all, with secondary defects caused by the primary disorder it should be taken into account the physiology of different cell types can be affected differently. This should be taken into account when discussing the results. Furthermore small numbers of cell lines /type hinders any definite conclusions.

The discussion should be shorter and more precise. The conclusion that: 'The presence of a normal α-glucosidase activity confirmed that under our cell culture and sample preparation conditions, the lysosomal compartment is overall preserved in the presence of mutations in the GBA1 locus, is not valid.

Although this could be a potentially interesting study it should not be accepted in its present form.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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