Reviewer’s report

Title: Hypotonia and intellectual disability without dysmorphic features in a patient with PIGN-related disease

Version: 1 Date: 09 Mar 2017

Reviewer: Romain Peanne

Reviewer’s report:

Abstract (page 02):

1. Line 32: use the GPI abbreviation for glycosylphosphatidylinositol. You should be consistent in using either the abbreviation or the full name throughout the manuscript.

Your answer: the abstract have limited word count. We feel that the explaining in the first sentence that GPI stands for glycosylphosphatidylinositol is enough.

My comment: I agree. If you read my comment, I just mentioned you should be consistent in using abbreviations in the manuscript. Once you introduced the mutation, stick to it.

Case presentation:

2. Page 06: it might be useful to add a table, describing the retained variants in the patients, including the mutations at both the transcript and protein levels, their inheritance pattern, and the scoring of their pathogenicity according to in silico software, e.g. Polyphen2, Alamut, SIFT, Mutation Taster. In addition, the authors could add the sequencing results of both the proband and his parents for both SCNA1 and PIGN genes.

Your Answer: We have made the appropriate changes. Supplementary Table was added to the manuscript, as well as the IGV screen shots of the Variant as Supplementary Figure 2, which we believe does not bring anything to the manuscript.

My comment: when your whole report is about the identification of new mutations responsible for PIGN deficiency and that you also found an additional mutation in SCN1A, I think that the table brings more information. For the sequencing results, you could have try to make a decent figure instead of screenshots. Feel free to remove the supplementary figure 1 if you want.

3. Page 06 line 56: "(…) the missense variant was interpreted as a variant of unknown significance". I agree that just on the basis of the given scores of in silico programs and the fact this variant has not been reported before you first considered classify it as VUS. Nevertheless, you decided to proceed to functional studies with flow cytometry to gain more insight into its pathogenicity as written on the following page, line 07 "(…) the pathogenicity of the missense variant called for functional studies to prove causality before interpreting this genotype as
pathogenic". I would reformulate this paragraph, since I find it confusing. The reader indeed really gets the idea that your decision is to exclude this variant from your analysis.

Your Answer: We have made appropriate changes to clarify the paragraph, however, it is clear (in clinical setting or research) that the genotype was suggestive of a diagnosis of PIGN-related diseases: 1) one variant is a loss-of function 2) the two variants were rare and in trans, 4) PIGN p.Arg95Gln is located in the GPI ethanolamine phosphate domain, 4) functional studies confirmed the pathogenicity of the genotype by showing GPI-defects. We seriously have no doubt that the diagnosis right.

My comment: I don't doubt your diagnosis either. I just asked to reformulate your explanation, since it is poorly written. In addition, there is absolutely not point in insisting on this dichotomy for diagnosis/clinical vs. research settings. Both are necessary nowadays, especially in the context of inborn errors of metabolism.

4. Page 07 lines 9-12: "in order to examine the effect of the p.Arg95Gln variant on the function of PIGN (...)". I totally disagree with this statement. This flow cytometry experiment allows you to look at the global function/dysfunction of PIGN in the patient's cells, e.g. the impact of BOTH identified mutations. Please reformulate. If you want to look at the pathogenicity of the p.Arg95Gln variant alone, you also should have analyze blood cells of the father too, since you report it is paternally-inherited. Even though a single heterozygous variant for PIGN can't lead to disease of course. Please reformulate.

Your answer: We have made appropriate changes to clarify the paragraph, however, we don't agree with the reviewers. No other variants are deletion duplications were reported in PIGN gene, in this patient. It is clear (in clinical setting or research) that the genotype was suggestive of a diagnosis of PIGN-related diseases: 1) one variant is a loss-of function 2) the two variants were rare and in trans, 4) PIGN p.Arg95Gln is located in the GPI ethanolamine phosphate domain, 4) functional studies confirmed the pathogenicity of "the genotype" by showing GPI-defects. We seriously have no doubt that the diagnosis right. All publications on GPI defects, test affected proband only not unaffected parents by flow cytometry. Our patient granulocytes (cmh1157) showed a half log reduction in fluorescence intensity following staining with anti-human CD59 and FLAER compared to the carrier mother (cmh1158) and healthy controls, indeed healthy carriers, such as the mother (with the LOF variant), do not showed GPI deficiency. In this particular case, the father was not available for studies for many complex reasons.

My comment: Again, I don't doubt the diagnosis, but your explanation was there as well very poorly written and confusing. You should pay more attention when describing mutations or assessing their pathogenicity. I understand that your don't have cells of the father for specific reasons, but nevertheless I didn't ask to repeat the FC experiment. Just to bring clarity in your manuscript.

5. Page 07 lines 14-16: I'm not sure I understand your experiment. You mention that "granulocytes were gated after staining with mouse anti-human CD59 and FLAER". Can you explain your experimental protocol please? Normally you should first gate your different cell
populations to isolate them and consequently analyze the fluorescence of your staining of interest, e.g. in your case CD59 and FLAER. I'm skeptical about your results…. Which markers were used for population isolation? Which gating parameters?

Your answer: As this is a case report with limited word count, not a research article, and the method has been previously published in the details by our co-authors, DR Xuan Yuan and DR. Robert A. Brodsky from Johns Hopkins Division of Hematology, we do not feel the need to add more details. See reference 24) Brodsky RA, Mukhina GL, Li S, Nelson KL, Chiurazzi PL, Buckley JT, Borowitz MJ: Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. American journal of clinical pathology 2000, 114(3):459-466.

My comment: The fact this is a case report and not a research article doesn't mean you don't have to be accurate in (1) the analysis your perform and (2) the assessment of your results.

6. Page 07 line 19: what do you mean by "performed on a research basis"? Furthermore, you mention that the experiment was performed on blood granulocytes but in figure 02 are presented the results for both erythrocytes and granulocytes… Please update those results/comments.

Your answer: The expression of GPI anchors was determined by staining granulocytes with flow cytometry is not a clinical test, it's done on research basis to assess GPI defects.

My comment: please remove the comment 'performed on research basis'. Flow cytometry analysis is part of the diagnostic flowchart for PIG-related defects, and on top of that this discrimination between clinics and research is futile and doesn't bring any added value to the manuscript. Please correct.

7. Page 07 line 21: "demonstrated a shift in this patient's granulocytes, indicating (...)". Which staining are you talking about? FLAER, CD59? As I already mentioned it before, the two stainings are used to visualize two different things, either the abundance of GPI-anchor at the cell surface, or the abundance of a specific kind of GPI-anchored protein. Please be more accurate in the description of your results. In addition, did you consider staining with another marker, specific for granulocytes, such as CD24? If not, why? CD59 is actually a marker present at the cell surface of both erythrocytes and granulocytes.

Your answer: CD24 on granulocytes, CD157 on monocytes were similar as that in reference controls.

My comment: please mention it in the manuscript.

Figure 02:

8. The figure is very confusing and of poor resolution. My initial comment is for the legend which is absolutely not clear. Why to not call the different plots control, patient, mother, negative control?
Your answer: The figure with better resolution will be submitted after manuscript acceptance. The plots are presented accordingly to our co-authors expertise.

My comment: Why are you enable to provide a figure with a higher resolution now? In addition, please correct the identification of the plots by control, patient, mother, negative control. You mentioned repetitively thought the manuscript that this is a case report and not a research article. I agree with that and therefore think that you should try to clarify the figure for readers not familiar with FC analysis. Minimum you can hence do is simplify the identification of the plots.

9. Moreover, you report that you observe the fluorescence levels of CD59 in both the left (erythrocytes) and the right panel (granulocytes). However, while you look at anti-CD59 for red cells, you look at APC-A for granulocytes. Can you explain? APC is a fluorescent molecule, but coupled to which protein? What are you looking at in this panel?

Your answer: As this is a case report with limited word count, not a research article, and the method has been previously published in the details by our co-authors, DR Xuan Yuan and DR. Robert A. Brodsky from Johns Hopkins Division of Hematology, we do not feel the need to add more details. See reference 24) Brodsky RA, Mukhina GL, Li S, Nelson KL, Chiurazzi PL, Buckley JT, Borowitz MJ: Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. American journal of clinical pathology 2000, 114(3):459-466.

My comment: please answer the question. Eventually ask your coauthors for more information.

10. Still in the right panel, while you observe a shift towards the left in patient's cells compared to control cells, you observe a positive shift of the same amplitude for mother's cells. Once again, why no comments about that? This is assuming that the central plot in dark green in the one of the control cells (and hence should be blue), since the colors are not very clear on this panel.

Your answer: The figure with better resolution will be submitted after manuscript acceptance. The plots are presented accordingly to our co-authors expertise.

My comment: You're validating your diagnosis on the FC experiments. However, the data you're presenting are far from be clear, and this for two reasons: (1) the very poor quality of the figure and (2) the badly and confusing writing used to described you results. Please be clear in the description of your results and please upload a nicer figure, both for resolution and readiness. See comment 09.

Conclusions:

11. Page 07 lines 55-59: "A recent genotype-phenotype correlation has been suggested, (...) our patient further supports this". This is maybe my major comment of this manuscript, why don't you actually discuss that? If your report is in line with the work of Fleming and coworkers, I don't understand that in a similar way than what they described, you don't compare: (i) the
clinical phenotype of your patient to the one of all PIGN-deficient patients described so far, similarly to what they did and (ii) the types of mutations. The two mutations you identified are both localized in the phosphodiesterase domain of PIGN. To my knowledge, only the patient reported by Ohba and coworkers (2014) reported such a case, with the combination of both a missense and a stop mutation in this domain. Please discuss that.

Your answer: We have not made the changes, No genotype-phenotype correlations can be assess between the cases.

My comment: yes there is! You wrote it yourself in the manuscript and refer to the publication by Fleming and coworkers. Page 08, lanes 157-159 of your manuscript: "A recent genotype-phenotype correlation has been suggested, where congenital anomalies are found only in association with biallelic truncating variants; our patient further supports this."

You're contradicting yourself… That was your initial analysis, and you now corrected a bit further - page 09, lanes 181-182: "[…] however, no clear 182 convincing genotype-phenotype correlations have been reported or can be drawn."

Why is that? Can you give a clear explanation? You're discrediting your own work. I maintain my initial comment, please discuss your case vs the previously identified cases.

12. Page 08 lines 04-51: This part is quite lengthy and doesn't bring much information to the manuscript. I think that by now the added value of WES in diagnostic is not to be proven anymore, especially for the identification of inborn errors of metabolism. Please shorten this part and focus your discussion more on the genotype-phenotype correlation.

Your answer: We have not made the changes, since no convincing genotype-phenotype correlations have been reported or can be drawn. The point is not the added value of WES in diagnostic setting but the our perspective is in the context of clinical testing, not research WES, where gene panels are often lacking novel genes, novel /atypical phenotypes and interpretation of genotype in clinical setting should be open to expended clinical spectrum or atypical clinical presentation.

My comment: I maintain my comment and completely disagree with your answer. WES is now largely implemented in diagnostic platforms and in the context of clinical testing, and gene panels are only used in more and more restricted situations. Instead, clinical exomes (mendeleiomes) are being more and more used for a few years now. Your report isn't bringing any new argument in favor of WES. Shorten this part and emphasize on the genotype-phenotype correlation.

13. Page 08 lines 53-58 and page 09: see comment #22. Please discuss the added value of the reported case and how the identified mutations can lead to such a mild phenotype. In addition, how is Fryns syndrome providing further evidence for the genetic heterogeneity of PIGN deficiency? Please develop.
Your answer: We have not made the changes, since no convincing genotype-phenotype correlations have been reported or can be drawn. Our perspective is in the context of clinical testing, not research WES, where gene panels are often lacking novel genes, novel phenotypes and the interpretation of genotype in clinical setting should be open to expended clinical spectrum.

My comment: See comment 11. You're dressing drastically opposite conclusions from one page to the other. Please correct that.

General conclusion: Thiffault and colleagues report the interesting case of a 2-year-old male patient presenting compound heterozygous mutations for PIGN. In addition, the patient doesn't present congenital anomalies nor obvious dysmorphic features, suggesting in correlation with recent reports that those might not be core features of PIGN deficiency.

In conclusion, the data presented in this report is interesting, but at this stage I think that the manuscript isn't ready for publication yet. Despite my constructive comments addressed after a first review, very few modifications have been retained by the authors. I am perfectly aware that this is a case report and not a research article, but that doesn't mean either that the presented data shouldn't be thoroughly analyzed. The key result to fully validate a PIGN defect is flow cytometry. However the presented data are barely readable because of the poor quality of the figure, and very confusing (lack of clarity of the legend of the figure and poor explanation in the manuscript). I'm familiar with FC experiments and the analyze of the generated data, but I think that much more clarity is necessary for readers without that knowledge to understand the results presented here.

Furthermore after reviewing it looks like the authors got lost in the conclusion of their case report. Indeed, while they initially suggested that their case was in accordance with the recent genotype-phenotype correlation described by Fleming and coworkers, they now argue that such correlation has never been established and they can't compare their case to any other. From one page to the other they even contradict themselves (see comment 11).

Until the authors addressed all the comments they skipped so far (comments 6 to 13) and carefully reconsider their data and the conclusions they draw, I can't unfortunately recommend this manuscript for publication.

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

Yes

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?
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No

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