Reviewer's report

**Title:** An unexpected, mild phenotype of glucocorticoid resistance associated with glucocorticoid receptor gene mutation Case report and review of the literature

**Version:** 1 Date: 07 Nov 2017

**Reviewer:** Peter Gergics

**Reviewer's report:**

It was my pleasure to review the work of Molnar and coworkers who provided a detailed description of a patient with glucocorticoid resistance.

Glucocorticoid resistance is a very rare disease and there are less than 20 cases reported to date. They make a compelling case with their clinical findings for glucocorticoid resistance in a female patient evaluated for infertility. They support their hypothesis with Sanger sequencing showing a known glucocorticoid receptor gene variant in a heterozygous form. Although the NR3C1 p.R714Q has been tested in vitro previously (Ref 14 Nader et al 2010) caution should be exercised when calling it pathogenic. Based on the ACMG criteria (https://www.nature.com/gim/journal/v17/n5/full/gim201530a.html) they only have proof of moderate level pathogenicity at best and they solely rely on the previously published work. I have one major concern and a number of minor concerns/comments they should improve in their manuscript.

**Major concern:**

They have to add something more to our knowledge about glucocorticoid resistance than there is another case with the same NR3C1 p.R714Q variant by at least showing that the same variant is not present in the same population. This was not shown previously and would actually establish the moderate level of pathogenicity. If the authors could add further information by stating that the variant wasn't found by sequencing >100 alleles then that should be mentioned in the abstract.

**Minor comments:**

Abstract

The manuscript needs some further English language editing to eliminate some inconsistencies in the text.
Line 50: …partial or generalized… Do they mean partial, generalized? It can only be partial deficiency of the receptor and in a generalized fashion. Complete deficiency would be lethal and they don't show data for tissue specific expression. Same statement appears in Line 89.

Lines 52-55: There is too much information in one sentence which makes "normal" not interpretable. In a healthy individual in the light of mentioning the LDDT one would expect a statement what happened to the morning serum cortisol. Are they suggesting whether there is or isn't a circadian rhythm present? Rephrase sentence and/or split it into two sentences. Consider adding the information about the normal Bone Mineral Density to the abstract as this is typically part of glucocorticoid resistance.

Line 57: Instead of "mutation analysis" please state that it was Sanger sequencing of the coding region...

Line 62: There may be little benefit to use the named syndrome phrasing. Maybe they should mention glucocorticoid resistance in the title as well.

Lines 64-65: The authors mention the variant positive, heterozygous, healthy sister previously and in the light of this the phenotype seems to range from unaffected through mild to severe disease. Please address this issue here and in the manuscript later. This reviewer is puzzled about the fact that the sister doesn't seem to have a fertility problem (as line 131 suggests).

Background

Line 78: In the traditional point of view they are right but there is a large array of research with plasmamembrane GRs. Please rephrase sentence.

Line 81: It might be worth mentioning GRs homo and heterodimerization here.

Line 83: …binds to at least two…

Line 89-91: Rephrase sentence. The logical order would be to mention GR leading to inadequate negative feedback in the pituitary on ACTH production resulting in increased ACTH and then in increased cortisol production.
Line 91-92: Only acute high levels of ACTH stimulate mineralocorticoid production and secretion while chronic, high ACTH doesn't. They should be clear about that they mean the overstimulated steroid biosynthesis to produce more glucocorticoids triggers the escape mechanisms to make more androgens and mineralocorticoids.

Line 98-100: It would help to mention what are they expecting for the 24-h UFC after "low dose" DT.

Line 104: Add references to statement.

Line 109: …life threatening such as…

Line 109-112: Rephrase first sentence and same comment as for Line 64-65

Patients and methods:

Line 124: For the non-endocrinologist readers please explain all acronyms such as PEG when used the first time in the text. Same applies to ACTH in the abstract.

Line 146: Mention the assays for these two hormones as well.

Line 152-164: Is this different from what they did in their cited reference Koper et al? Could they just cite one of their previous works instead and shorten the description?

Line 168 / Table one: In the table the first column contains too much information at once. Please insert a new column for the reference ranges. Be consistent with the number of significant figures when specifying the reference range and providing the value measured. There are many of these but for example the TSH reference range has two significant figures while the value presented has three but sometimes two. Use μ for micro. Whether the parameter was measured from serum or plasma could be listed under the table. It is not absolutely necessary but for non-endocrinologist readers explaining PEG, TSH, fT4 here as well could be helpful.

Line 184: For all gene variants the reference transcript ID as well as the Minor Allele Frequency should be listed from the most frequently used databases such as ExAc, Gnomad, 1000G, EVS etc. or if they are not present then that information. The same codon is affected by known SNPs (rs759445158, rs771583164) and this information for the c.2141G>A is relevant.
Line 185: Please specify the steroid hormones which were measured and were within the reference ranges. Correct patients to patient.

Line 186: It would be important to know any information about the parents of the index patients and it is unfortunate that they declined testing or sharing information about their health record, vital signs such as height, weight, blood pressure. For the completeness please mention that clinical screening was also denied. It would be worthwhile to explore if they might be related to the other published family.

Discussion

Lines 190-196: This part could be shortened and just to highlight that the hyperprolactinaemia was excluded as a cause of infertility. What would be interesting though is to know whether imaging of the adrenal glands has shown any abnormality? This could help for future clinicians if they would encounter another case of glucocorticoid resistance.

Line 198-200: Consider revising "therefore" in the sentence.

Line 212 / Table 2: Add reference numbers from this paper to the table. Age as "young" should be replaced with newborn/infant/childhood age/teenager etc. if no specific information is available in the original reference. In the GR mutation column the HGVS nomenclature should be used consistently for the cDNA and the protein change description. A matrix type listing of the clinical signs / phenotype would greatly help the readers to compare the cases and oversee the similarities and differences. Consider adding the current index patient as well. If in the judgement of the current authors there is a similarity in the phenotypes / affected protein domain / age of diagnosis etc. that would make this comparison more useful than the current listing by the year of the publication.

Line 213: Some opinion should be provided about the unaffected sister carrying the same variant.

Lines 220-224: Their explanation is highly speculative and should be omitted. They might be right but there is another 50% chance of having a fetus with a homozygous wild type genotype for NR3C1 codon 714. In that case it is even more difficult to contemplate what would be the effect on the fetus by maternal administration of high dose glucocorticoids.
Line 226: The term "causation" can be used when the level of evidence for pathogenicity is in the "very strong" category of the ACMG criteria. If they show evidence for the same variant not present in the same population then they can describe that they present the complete evidence for moderately strong pathogenicity by the ACMG criteria.

Line 229: As their clinical and steroid hormone analysis includes the healthy sibling they have a case for a healthy individual as well. This reviewer wonders if there could be a modifier gene - maybe in the same complex with the glucocorticoid receptor.

Lines 262 and 265: References have the non-abbreviated journal names.

Line 282: Please add heterozygous to the figure legend. Traditionally in such figures the peak-on-peak is positioned in the middle of the figure and the reference allele is mentioned first and then the variant such as G/A

**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.

No

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

Yes

**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.

Not relevant to this manuscript

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published
Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal