Author’s response to reviews

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

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Author’s response to reviews:

To the Editor BMC Medical Genetics

Dear dr. Pasini,

On behalf of all authors I am submitting the revised version of our manuscript entitled “An unexpected, mild phenotype of glucocorticoid resistance associated with glucocorticoid receptor gene mutation Case report and review of the literature” for consideration to BMC Medical Genetics.

Upon the reviewer’s questions and comments we have reframed our manuscript, novel data and novel panels have been included.
Please find enclosed our revised manuscript and the rebuttal letter in which we made all efforts to properly address the reviewer’s comments and remarks.

All changes made in the text are highlighted in yellow.

After careful proofreading of the revised manuscript and considering the useful suggestions of the academic editor and the reviewers, we hope that our manuscript will be considered suitable for publication in your prestigious journal.

On behalf of the authors,
Sincerely,

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Answers to the reviewers' comments:
We thank the reviewers for their careful reading of our manuscript and their useful comments. We have revised the manuscript based on the comments and suggestions. The modified parts are highlighted in yellow.

Please find below our point-by-point reply to the reviewers’ comments.

Technical Comments:

1. Manuscript sections

Please reformat the manuscript to adhere to our submission guidelines (http://bmcmedgenet.biomedcentral.com/submission-guidelines/preparing-your-manuscript/case-report). The main manuscript text should therefore have the following sections: Background, Case presentation, Discussion and Conclusions.

Answer: Accordingly, the manuscript was reformat to adhere to the Journal’s guidelines.

2. Abbreviations

Please add this section at the end of the main manuscript text, defining all abbreviations used throughout the text.

Answer: This section was accordingly completed and added at the end of the main manuscript text.

3. Declarations

Please move the Declarations section after the Abbreviations.

Answer: The Declarations section was moved after the Abbreviations.
Reviewer #1

General:

Molnar and colleagues present a case report describing a rare mutation in the glucocorticoid receptor, a nuclear receptor which mediates a broad range of actions important to development, metabolism and immunity. The introduction is thorough, and well referenced, and the results are well described. However I have a few specific concerns, that need to be addressed before this manuscript is suitable for publication.

Answer: We thank Reviewer 1 for her/his positive comments. Accordingly, we made all modifications requested in order to clarify these concerns.

1) The figure presented is not of sufficient quality and is not informative to the reader in its current form. I include links to similar published studies for reference.

https://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-017-0413-8
https://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-017-0404-9
https://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-017-0412-9
https://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-017-0407-6

My suggestions:

(a) the existing figure needs additional detail, with labels,

(b) a schematic of the the exon structure of the GR gene,

(c) a schematic of the protein structure of GR, with functional domains labelled, and the site of the mutation indicated.

This information is detailed in the introduction and the presence of a diagram would better support the text.
Answer: We thank the Reviewer 1 for these suggestions and accordingly a new figure was made and included in the revised version. We put labels showing the codon and amino acid numbers both for the wild type and for the mutation. In addition, we included the chromatogram of the clinically healthy sister of the index patient, who also carries the c.2141G/A mutation in exon 8 of the GR gene. We made two novel panels containing the schematic 3D structure of the Arg (wild type) and the mutant Gln at the position 714. All relevant data and conclusions have been included into the revised manuscript.

2) It seems from the text that the sister of the index consented to biochemical tests and genetic screening. Can this data also be included?

Answer: We thank Reviewer 1 for this suggestion. The measurement of the baseline (morning) cortisol, ACTH, DHEAS, testosterone and prolactin were carried out in the sister of the index patient. Genetic testing of the Arg714Gln variant was also performed. In the revised version of the manuscript we included these results in the Table 1, while the result of the sequencing was included in the Figure 1.

3) Some of the studies described in table 2 are not included in the reference list. Table 2 needs to be referenced properly.

Answer: We thank Reviewer 1 for this remark. All studies mentioned in Table 2 were included in the reference list.

4) The discussion lacks sufficient reference to, and description of other GR mutations reported in the literature. Several, additional references should be included, and discussed (although, I think some of these refer to studies described in table 2). If not already included, these should be summarised in Table 2.
Kino et al 2001 Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. J Clin Endocrinol Metab 86:5600-5608

Treble et al 2010 Familial Glucocorticoid Resistance Caused by a Novel Frameshift Glucocorticoid Receptor Mutation J Clin Endocrinol Metab 95: E490-E499

Charmandari et al 2008 A novel point mutation in the amino terminal domain of the human glucocorticoid receptor (hGR) gene enhancing hGR-mediated gene expression. J Clin Endocrinol Metab 93:4963-4968

Charmandari et al 2006 Functional characterization of the natural human glucocorticoid receptor (hGR) mutants hGR R477H and hGR G679S associated with generalized glucocorticoid resistance. J Clin Endocrinol Metab 91:1535-1543


Charmandari et al 2005 A novel point mutation in the ligand-binding domain (LBD) of the human glucocorticoid receptor (hGR) causing generalized glucocorticoid resistance: the importance of the C terminus of hGR LBD in conferring transactivational activity. J Clin Endocrinol Metab 90:3696-3705

McMahon et al 2010 Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). J Clin Endocrinol Metab 95:297-302


Answer: We thank Reviewer 1 for this comment. Both the Discussion and Table 2 were completed with all these references mentioned.

Finally, we would like to thank the Reviewer for his/her comments and suggestions and we hope that the revised manuscript will meet your acceptance.

Reviewer #2

General:

The paper describes the expert evaluation of a patient with infertility which resulted in the discovery of glucocorticoid resistance. A glucocorticoid receptor mutation was discovered which may plausibly explain the phenotype. The mutation has been described before. A sibling also carries the mutation, but has no phenotype, therefore, it is possible that the mutation is not causative, but a polymorphism. The function of the mutant receptor was not tested in this paper.

Answer: We thank Reviewer 2 for this comment. We completely agree with the Reviewer regarding the questionable pathogenicity of this GR variant. However, this variant was previously described and functionally characterized (Nader et al.[1]). From that work it looks convincing that in homozygote form this variant has a pathogenic role. However, our cases might suggest that this variant in heterozygote form might be clinically insignificant. This is the main reason why we decided to publish our case. In the revised version we included novel data and additional comments about our cases suggesting the role of GR variants may associate with highly variable phenotypes.
1) A mild/negligible phenotype with GR allele loss was reported before...Trebble et al JCEM, and this should be referenced.

Answer: We thank the Reviewer 2 for this comment. The reference list and Table 2 were completed with the suggested references further confirming that GR variants may associate with clinically very heterogeneous phenotypes.

2) The introduction contains very many errors of grammar, and science, especially in relation to the GR structure and function.

Answer: We thank Reviewer 2 for this remark. We completely rewrote the introduction section containing information about the GR structure and function. A native, English spoken scientist edited our manuscript.

Finally, we would like to thank the Reviewer for his/her comments and suggestions and we hope that the revised manuscript will meet your acceptance

Reviewer #3

General:

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.
Answer: We thank Reviewer 3 for her/his positive comments. Regarding to the pathogenicity of this variant we relied on the previously published work by Nader et al. where the functional consequences were experimentally demonstrated. In addition, based on our experience with sequencing of the GR (NR3C1) gene in more than 60 patient and controls we did not find this variant in any of them suggesting at least that this is a rare variant. In addition this variant was not present in many genetic databases including ExaCT, Exome Variant Server, HGMD.: 

However, we agree with the Reviewer that our cases (showing both the unexpected mild phenotype in our Index case and a completely insignificant phenotype for her sister) may suggest that indeed this variant might have only a moderate level of pathogenicity. Accordingly, we included all these data and comments in the revised version.

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.

Answer: We thank the Reviewer for this suggestion. In the revised version we included a sentence about the rarity of this variant in our population. “In addition, this variant was not detected in more than 60 patients and controls tested either for glucocorticoid resistance or Cushing’s syndrome in our Laboratory. Moreover it was not present in commonly used genetic databases including Exome Variant Server (evs.gs.washington.edu/EVS), Exact (exac.broadinstitute.org) and SNPeff (http://snpefffect.switchlab.org)

Minor comments:

Abstract

The manuscript needs some further English language editing to eliminate some inconsistencies in the text.
Answer: We thank Reviewer 3 for this suggestion, and according to the following remarks, some modifications and corrections were performed.

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.

Answer: These sentences were corrected to “local, tissue-specific or generalized symptoms due to partial insensitivity to glucocorticoids.”.

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.

Answer: As was suggested we rephrased this paragraph.

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

Answer: It was included.

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

Answer: As suggested, we included 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).

Answer: Thank you very much for this comment. Please see our answer to Reviewer 1’s question as well regarding the role of this variant.
“We completely agree with the Reviewer regarding the questionable pathogenicity of this GR variant. However, this variant was previously described and functionally characterized (Nader et al.[1]). From that work it looks convincing that in homozygote form this variant has a pathogenic role. However, our cases might suggest that this variant in heterozygote form might be clinically insignificant. This is the main reason why we decided to publish this case. In the revised version we included novel data and additional comments about the pathogenic role of this variant”.

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…

Answer: We thank the Reviewer for his comments. The complete paragraph was rephrased.

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.

Answer: The sentence was rephrased.

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.

Answer: It was explained.

Line 98-100: It would help to mention what are they expecting for the 24-h UFC after "low dose" DT.
Thank you for this comment. The whole paragraph was rephrased.

Line 104: Add references to statement.
Answer: Table 2 contains all the references.

Line 109: …life threatening such as…
Answer: It was corrected.

Line 109-112: Rephrase first sentence and same comment as for Line 64-65
Answer: The sentence was rephrased.

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.
Answer: These acronyms were explained.

Line 146: Mention the assays for these two hormones as well.
Answer: It was included

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?
Answer: This section was significantly shorten as the Reviewer suggested
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.

Answer: A new column was inserted for the reference ranges, and the Table was remade, and the proband sister’s data were also included.

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

Answer: All significant values specific for disease were highlighted in bold.

Use μ for micro.

Answer: Corrected.

Whether the parameter was measured from serum or plasma could be listed under the table.

Answer: These information were included in the footnote of the table.

It is not absolutely necessary but for non-endocrinologist readers explaining PEG, TSH, fT4 here as well could be helpful.

Answer: They were listed and explained under the table.

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.

Answer. In the revised version we included that this variant was not present in all databases mentioned. We found only the Arg714Gln variant in our cases.

Line 185: Please specify the steroid hormones which were measured and were within the reference ranges. Correct patients to patient.

Answer: Table 2 was remade, all reference ranges and all hormone values of the sister of the index patient were included.
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.

Answer: It was completed.

It would be worthwhile to explore if they might be related to the other published family.

Answer: Hungary is a small country and our Institute and Laboratory is the centre for endocrine genetics, hence we would be aware whether this cases would have been consulted abroad.

Discussion

Lines 190-196: This part could be shortened and just to highlight that the hyperprolactinaemia was excluded as a cause of infertility.

Answer: This part was shortened.

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.

Answer: Unfortunately no adrenal imaging was performed previously, and we have no possibility to do it until the submission deadline.

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

Answer: It is removed from the sentence.

Line 212 / Table 2: Add reference numbers from this paper to the table.

Answer: Table 2 was completed with the reference numbers.
Age as "young" should be replaced with newborn/infant/childhood age/teenager etc. if no specific information is available in the original reference.

Answer: It was corrected.

In the GR mutation column the HGVS nomenclature should be used consistently for the cDNA and the protein change description.

Answer: Corrected.

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.

Answer: In Table 2 we listed all the clinical phenotype and genotype data of all patients reported. We do not think that our cases should be in this table because this table contains only the previously reported cases.

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.

Answer: In the revised version we included all available data obtained from the unaffected sister. By doing this we wanted to further emphasize the heterogeneous phenotypes associated with GR variants.

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.
Thank you very much for this comment. We agree with the Reviewer and accordingly we included a sentence about the need for genetic counseling and also deleted our previous speculative comment.

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.

Answer: As we mentioned earlier, the complete manuscript was rewritten in order to emphasize that the genotype-phenotype association observed in our family might underline that the pathogenicity of this variant, especial in heterozygote form, is questionable.

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.

Answer: Accordingly, it was mentioned that other, not yet identified, modifiers might be involved in determination of phenotypes observed.

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

Answer: Corrected.

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

Answer: Heterozygous was added to the figure legend, and a new figure was made based on suggestions of Reviewer 1.

Finally, we would like to thank the Reviewer for his comments and questions. We believe that our manuscript significantly improved including all these clarifications.
Reviewer #4

General:

The manuscript "An unexpected, mild phenotype associated with glucocorticoid receptor gene mutation, Case report and review of the literature" describes the presentation of a patient with infertility. In the workup for the patient, it was concluded that she had partial resistance to glucocorticoids. As a result, the authors sequenced the glucocorticoid receptor and identified a mutation within the ligand binding domain, and specifically AF2: R714Q. This paper provides a valuable example of how GR mutations have the potential to manifest as different pathologies.

Although nicely reported, I have a few issues with the paper as written:

Answer: We thank the Reviewer for her/his positive comment.

1) The authors claim that the R714Q mutation is in AF2. Most structures of the GR-LBD (Eric Xu and others) classify the cofactor binding pocket composed in which the LXXLL motif sits as AF2. The position of R714 is quite far from this pocket and might be better described as being within the LBD.

Answer: We thank Reviewer 4 for this comment. We included a novel figure about the three-dimensional structure of the wild type and the mutant 714 residue. In addition, a detailed explanation about the functional consequences have been added.

2) On Page 4, the authors refer to the LBD as containing both AF1 and AF2. Although AF2 is almost always associated with the LBD, most (if not all) publications identify AF1 as being a region surrounding residue 200 within the N-terminal domain of GR. There may be other surfaces that bind GRIP1 on the LBD, but they are not commonly referred to as AF1.

Answer: We thank Reviewer 4 for this remark. As the other Reviewers also suggested, this section was completely rewritten.

3) It is not clear how this mutation might decrease hormone binding. If this is known from other work, it should be discussed.

Answer: We thank Reviewer 4 for this comment. The substitution of arginine by glutamine in the 714 position causes conformational changes of the ligand-binding domain, resulting in a 2-
fold reduction in affinity to ligand, which was described by Nader et al.[1] The discussion was accordingly completed.

4) I was confused by how the pathology was classified as Chrousos syndrome. How is Chrousos syndrome defined? What is the hormonal and dex suppression profile of Cushings? Exactly how does this patient differ from the Cushings profile?

Answer: We thank Reviewer 4 for this important question. Both Chrousos and Cushing syndrome are characterized by elevated serum and urinary cortisol levels, which fail to suppress after administration of low dose dexamethasone. In case of (primer) Cushing syndrome, the ACTH level is decreased due to the negative feedback mechanism of the Hypothalamic-pituitary-adrenal axis. In case of Cushing disease, the elevated cortisol level is caused by the ACTH overproduction. The serum cortisol level has no circadian rhythm in both Cushing syndrome and Cushing’s disease.

In contrast, in Chrousos syndrome, the serum cortisol preserves its circadian rhythm, and the plasma ACTH is usually elevated due to the glucocorticoid resistance/partial insensitivity, which stimulates the ACTH production through the feedback mechanism of the HPA axis, but normal ACTH levels also occurred. The chronic excess of ACTH results an overstimulated steroid biosynthesis, therefore an increased production of adrenal steroids, even with androgenic and/or mineralocorticoid activity. Therefore hypertension, hypokalaemia, hirsutism and infertility may develop. In contrast to the Cushing syndrome, glucocorticoid resistance is characterized by hypercortisolism without the clinical stigmata of Cushing syndrome. The definition of Chrousos syndrome was accordingly corrected in the manuscript. The following table summarizes the similarities and differences between the two diseases.

In the revised version we included the main clinical and laboratory findings of Cushing’s disease and Chrousos syndrome

5) In the abstract, it says that administration of dex caused suppression of cortisol at midnight, however on page 8 it is not clear whether cortisol levels were normal at night with or without dex. Please clarify.

Answer: We thank Reviewer 4 for this remark. Without dexamethasone, the morning cortisol was elevated and midnight cortisol was within the reference range suggesting a preserved circadian rhythm. After the overnight dexamethasone suppression test, morning serum cortisol was not suppressed adequately. The sentence in the abstract and on page 8 in the manuscript were accordingly rephrased. All laboratory data were included in Table 1.
In short, although this paper provides a useful correlation between a GR mutation and glucocorticoid-related syndrome, how this syndrome differs from Cushings or other glucocorticoid insensitivity pathologies is not clear. Without clear definitions of Chrousos syndrome, Cushing syndrome, and how the patients profile matches one over the other, this work will not allow the community to confidently associate this mutation with a specific kind of glucocorticoid insensitivity.

Answer: We thank Reviewer 4 for this comment. In the revised version we tried to clarify the definition, pathology and symptoms of Chrousos syndrome and Cushing’s disease. The pathogenicity of this variant is very variable from severe disease to clinically insignificant state.

We would like to thank the Reviewer for his/her comments and questions.

Reference