Author’s response to reviews

Title: Vitamin D receptor and binding protein polymorphisms in women with polycystic ovary syndrome: a case control study

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Author’s response to reviews:

Thank you for reviewing our study.

We attached the responses to reviewer as an supplemental material.

Please find the files.

BEND-D-19-00323

Vitamin D receptor and binding protein polymorphisms in women with polycystic ovary syndrome: a case control study

Do Kyeong Song; Hyejin Lee; Young Sun Hong; Yeon-Ah Sung

BMC Endocrine Disorders

Dear Dr. Song,

Your manuscript "Vitamin D receptor and binding protein polymorphisms in women with polycystic ovary syndrome: a case control study" (BEND-D-19-00323) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Endocrine Disorders.

Their reports, together with any other comments, are below. Please also take a moment to check our website at
https://www.editorialmanager.com/bend/ for any additional comments that were saved as attachments. Please note that as BMC Endocrine Disorders has a policy of open peer review, you will be able to see the names of the reviewers.

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Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Submission Guidelines on the journal homepage.

A decision will be made once we have received your revised manuscript, which we expect by 28 Sep 2019.

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I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

Giovanna Muscogiuri

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Technical Comments:

Editor Comments:

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Reviewer reports:

Flavio Cadegiani, MD, MSc, PhD (Reviewer 1):

Overall comments:

This is an interesting study trying to correlate and understand the correlations and roles of vitamin D in the pathogenesis of PCOS and possibly its severity.

Overall, the manuscript has a very high quality, and is potentially publishable.

However, I have some important concerns, that are raised throughout this review, that need to be addressed prior to acceptance.

References should be healthy revised, as many do not demonstrate or report for what they were reported by the authors to show.

Also, several more recent studies could provide better substantiation for the affirmations and statements provided in the manuscript.

-> We revised the manuscript with several more recent studies.
The lack of serum vitamin D levels is an important limitation that should be highlighted (it was mentioned only in the very end of the discussion), since VDBP levels and its levels of activity may largely depend on their binding status with vitamin D, and different genotypes may be correlated with strength of link with vitamin D, which would eventually lead to differences in metabolic patterns. Also, VDR gene expression also depends indirectly on vitamin D status, which could compromise the consequences of the variants of these genes.

- We emphasized this limitation in the revised manuscript (Discussion section, line 297-304, page 14).

Abstract:

Line 32 "A 75-g oral…" fits better than "The 75-g oral…"

- We changed the sentence as you recommended (Abstract section, line 31, page 2).

Line 42 - The Cdx2 polymorphism was NOT significantly associated with increased insulin and insulin sensitivity after multiple linear regression, so this must be made explicit even in the abstract.

- We inserted this sentence as you commented (Abstract section, line 43-44, page 3).

Lines 62-67

- In the way it is described, it seems that all affected women has all the features of POCS. Please allow a sentence describing the Rotterdam criteria, i.e., that women do not need all, but 2 out of 3 criteria for the diagnosis of PCOS.
- I would split the features in those three that are the fundamental ones, from the others.
- Since you are presenting basic information of PCOS, which is absolutely write, once readers may not always be familiar with this disease, a slight more precise information should be provided.
- My suggestion would be something like this:

"Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age, characterized by three fundamental criteria: clinical and/or biochemical hyperandrogenism, oligo/amenorrhea, and polycystic ovary morphology, among which women suspected for PCOS need to present two out of these three criteria for the diagnosis of OTS, and also insulin resistance (IR) and compensatory hyperinsulinemia [1]."

- We revised the first sentence as you suggested (Background section, line 63-68, page 4).
Line 67 - Studies are not so recent. The affirmation of these associations is correct, but may create an idea of proven causality relationship, which is not. Indeed, causality relationship between vitamin D and metabolic effects has not been established, and longitudinal studies to evaluate the metabolic effects of vitamin D increase in vitamin D deficient PCOS women are yet to be conducted.

-> As you recommended we inserted the sentences in the Background section (line 70-74, page 4).

Line 70 - Vitamin D is inversely associated with obesity, irrespective of underlying conditions. However, it failed to demonstrate causal correlation. Perhaps, capacity of fat storages of vitamin D may be a potential reason for these associations. I recommend to join this sentence with the previous one.

-> As you recommended we joined the sentence in the Background section (line 77, page 4).

Line 72 - Vitamin-D-related genes do not seem to have meaningful or clinically relevant influence on insulin secretion


-> We deleted the contents containing the association of vitamin-D-related genes and insulin secretion.

Lines 74-75 - Th reference 8 did not perform a multivariate regression analysis to adjust for vitamin D levels for the analysis of metabolic parameters. Differences in vitamin D status can easily up- or -downregulate VDR density and level of activity, which confound these findings.

Lines 75-77 - References 8 and 9 failed to find the correlations stated by the authors. Hence, this affirmation is at least controversial. Reference 10 has poor quality of assessment. Reference 11 did not adjust the CC genotype for serum vitamin D levels to evaluate whether susceptibility for PCOS still remains significant (likely not, as p was close to 0.05).

Lines 83-84 - Reference 17 did not adjust the insulin levels for the serum absolute levels of VDBP/DBP, serum 25(OH)D and 1,25(OH)D levels, which avoids accurate conclusions, since level of activity of DBPs highly depend on the availability of vitamin D, while its concentrations may also have metabolic effects, regardless of the polymorphisms.

-> As you commented we changed the references to recent studies (Backgroud section, line 80-83, page 4).

Methods -
Overall comments: It is UNCLEAR whether the study was retrospectively using post-hoc data from 2010-2011, or if this was performed for this study.

-> We recruited the participants and collected blood samples between December 2008 and October 2010.

Was the genotypification performed back in 2010-2011? Or stored blood was analyzed?

-> We genotyped the VDR gene and VDBP gene polymorphisms from the stored blood (Methods section, line 158, page 8).

Were these women recruited again, or the full process was performed in 2010-2011 data?

-> We recruited the participants and collected blood samples between December 2008 and October 2010. Later, we genotyped the VDR gene and VDBP gene polymorphisms from the stored blood.

Would this be a post-hoc study then?

-> Yes, we genotype the gene polymorphisms from the stored blood.

Lines 100-102 - This information is repeated in lines 126-127. One of them should be excluded.

-> Yes, we excluded the second sentence in lines 126-127.

Lines 105-107: the most accepted criteria for PCOS is the Rotterdam criteria. The NIH criteria has been known to underdiagnose PCOS, since it requires both disturbance of menstrual cycles and hyperandrogenism, while up to 10-15% of PCOS women have regular periods, and that not all express hyperandrogenism. Did the authors also perform the diagnosis using any other criteria?

-> As you commented, definition and diagnosis of PCOS are still controversial. Because women with hypothalamic amenorrhea can be classified as PCOS according to Rotterdam criteria, we defined PCOS according to NIH criteria.

Lines 127-129: please highlight in any place of the manuscript that total testosterone levels evaluated by any other method than tandem mass spectrometry is highly imprecise (when T levels are below 100 ng/dL).

-> We already highlighted the sentence in the discussion section (line 304-306, page 14). We added the contents in the method section (line 138, page 7).

Lines 139-140: why 90 and 120 minutes after baseline? And not only 120, or the whole curve (30, 60, 90 and 120)?

-> We revised the sentence in the method section (line 147-148, page 7).
Results:

Overall comments:

- Specific comments of the AA variants of the VDG genes and those of the VDBP gene variants were not performed in the results section (in a same way that comments were performed on the other VDR gene variants).

- Gene variants distributions are clearly similar between PCOS and non-PCOS women, and this should be made explicit.

- I missed tables for VDBP gene variants similar to those performed for VDR gene polymorphisms in Tables 4 and 5. It seems that a full job was made for VDR gene but only half job was made for the analysis of the VDBP gene polymorphisms. Regardless of the results, please provide tables with the parameters according to the genotypes of VDBP, similarly to those performed with the VDR gene and shown in Tables 4 and 5.

- As you commented we inserted the sentence about the similarity of genetic variants distribution between two groups (Results section, line 193-194, page 9). There were no differences of parameters according to the genotypes of VDBP. So we did not show the tables with the parameters according to the genotypes of VDBP. We attached the tables in the supplemental materials.

Lines 189-194 - Information is repeated twice. I would keep with the second sentence.

- As you recommended, we deleted the results about table 6.

Lines 195-199 - The AA polymorphism in controls was associated with higher fasting insulin and HOMA-IR, whereas this specific genotype was not correlated with the same differences in PCOS. It means that the higher insulin and HOMA-IR associated with the AA polymorphism in controls was not found under the presence of PCOS. This should be at least mentioned.

- As you suggested we mentioned the comments of the AA variants of the VDG genes and those of the VDBP gene variants (Results section, line 211-214, page 10).

Discussion:

Overall comments:

- Authors should highlight that even being younger, PCOS women had worse metabolic features than controls. These differences would likely be more prominent if age was similar between groups.
We inserted the sentences in the Discussion section (line 308-310, page 14).

- Hyperandrogenic states and aspects of metabolic syndrome are both commonly associated with lower SHBG levels, which may help explain lower SHBG in PCOS. Please highlight this.

We inserted the sentences in the Results section (line 187-188, page 9).

- Higher BMI could influence the activity of certain gene polymorphisms.

We inserted the sentence in the Discussion section (line 256, page 12).

- Please highlight that gene variants are UNLIKELY to influence the occurrence of PCOS, as variants distributions were similar between PCOS and non-PCOS women.

We highlighted the sentence in the Discussion section (line 241-242, page 12).

Lines 2017-208: The reference 24 does not evaluate the effects of vitamin D/calcium supplementation according to the initial vitamin D status.

We switched the previous reference 24 to other recent study entitled as “The Role of Vitamin D Oral Supplementation in Insulin Resistance in Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.” (Reference 24)

Lines 209-210: It is INCORRECT that several studies demonstrated that low vitamin D LEADS to insulin resistance. First, the study mentioned in reference 26 associated, but does not confirm any sort of causality relationship, as this was not an interventional-longitudinal study. Second, there are no other studies that confirms the causality relationship proposed by the authors. Hence, two corrections to be made: 1. Change from "vitamin D deficiency leads to IR" to "vitamin D deficiency may be correlated with IR", and 2. Add that this was observed in PCOS: "vitamin D deficiency may be correlated with IR, at least in PCOS".

As you suggested we changed the sentence in the Discussion section (line 223-224, page 11).

Lines 210-212: Reference 27 has a major flaw for not adjusting for body weight along the interventional study. Did these women lose weight? Were they controlled for eating behaviors? Because all mentioned improvements may also occur with weight loss, regardless of the vitamin D status.

We switched the previous reference 27 to other recent study entitled as “The effect of vitamin D supplementation on insulin resistance, visceral fat and adiponectin in vitamin D deficient women with polycystic ovary syndrome: a randomized placebo-controlled trial.” (Reference 27)

Lines 217-219: Please specify whether this works for 1,25(OH)D, 25(OH)D, or both (or which type of vitamin D leads to the mentioned actions)
-> As you mentioned we revised the manuscript (Discussion section, line 231, page 11).

Lines 249-251: Your study showed HIGHER insulin and HOMA-IR in the VDR Cdx2 AA genotype in controls, which is INCONSISTENT and OPPOSITE to the affirmation of lower fasting insulin and HOMA-IR in both PCOS and controls in Australia. Please revise.

-> We revised the sentence as you commented (Discussion section, line 260-262, page 12)

Line 283: "First study to evaluate the impacts" instead of "First study to evaluation the impact"

-> As you recommended we revised the sentence (Discussion section, line 294, page 14).

Conclusions:

Lines 299-300: After multiple linear regression analysis, none between fasting insulin or HOMA-IR remained significantly different according to genotype. Also, the power of the study is below 80%, required to allow any conclusions.

Hence, I disagree that from the present study the genotyping of VDR gene to predict women with PCOS for IR. Also, there is not such a practical application of genotyping for the assessment of risk of IR, as although these may exist differences, these are not different enough to predict IR in a way that no other model or assessment could not predict.

-> As you commented we revised the conclusion (line 318-321, page 15).

Table 1:

Is the difference in fasting glucose really significant (p = 0.006 ?)? It is not, apparently.

-> Yes, using SPSS program, the difference was significant.

Abdul Basit (Reviewer 2): Overall, the manuscript is good, but English language need to be improved.

-> We received already English language editing service. We further revised the manuscript.

Following are few comments as;

* In background, line 80 should be added at the end in line 88 after "phenotypes".
As you recommended we added the sentence after “phenotypes’ (Background section, line 93-94, page 5).

Methodology

* Some more details of project should be added.

-> We added the contents in the Methods section (line 102-103, page 5).

* Inclusion criteria of control is missing.

-> We included these contents in the methods section. “Control subjects had no evidence of ovulatory dysfunction or hyperandrogenism. Subjects were excluded if they had taken medication (e.g., steroids, oral contraceptives, metformin, or thiazide diuretics) within the past 3 months.” (line 122-124, page 6)

* Briefly discuss how non pcos females were identified only on menstrual regularity.

-> We included these contents in the methods section. “Control subjects had no evidence of ovulatory dysfunction or hyperandrogenism. Subjects were excluded if they had taken medication (e.g., steroids, oral contraceptives, metformin, or thiazide diuretics) within the past 3 months.” (line 122-124, page 6)

* Local study reference 21, better to give following reference as mentioned study based on this consensus report


-> We switched the reference as you suggested (Reference 21).

* In line 112, to exclude participants what criteria was used, either Known cases? or biochemical and hormonal testings were done to exclude them?

-> Exclusion of 21-hydroxylase-deficient nonclassic adrenal hyperplasia was performed using a basal morning 17-hydroxyprogesterone >2 ng/ml.

* How many times cases and control visited hospitals?
Once they visited the hospital on the date of blood sampling.

* first biochemical and hormonal assays were done to exclude PCOS and then controls are invited to come on third day of cycle for OGTT and insulin tests?

-> On the same day, sampling for testosterone levels and OGTT were done.

* Their fertility status and family history of PCOS was taken?

-> We did not get their fertility status and family history of PCOS.

* Vitamin D levels were done in both groups?

-> Because we did not measure vitamin D levels, we could not compare vitamin D levels between groups. We highlighted this limitation in the Discussion section (line 298-304, page 14).

Discussion

* It's too lengthy with grammatical errors.

Conclusion

* Line 300; IR may be considered as a core of PCOS

* then what benefit will be expected by doing genotyping of VDR gene? as it will be more expensive?

-> Yes. As you commented, IR is an important metabolic feature of PCOS. However, as not all women with PCOS have IR, predicting IR in women with PCOS would be useful. Further studies are needed to evaluate whether genotyping of the VDR gene may be useful in young women with PCOS for prediction of IR in large populations.

Pavlina Andreeva-Gateva (Reviewer 3): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.
This is a very interesting paper evaluating genotypes and allele frequency of vitamin D receptor and vitamin D protein binding protein in patients with PCOS vs. control. Authors revealed that genetic variations in VDR and VDBP were not associated with increased risk for PCOS. In contrast, the VDR Fok-I polymorphism was associated with testosterone level and the Cdx2 polymorphism with insulin sensitivity in PCOS.

Comments:

1. How the Control group was recruited? Why the size of the Control group is twice as the PCOS group?

- We recruited volunteers by newspaper and online advertisements to evaluate the menstrual health of Korean women under 40 years of age living in Seoul. Subjects were excluded if they had taken medication (e.g., steroids, oral contraceptives, metformin, or thiazide diuretics) within the past 3 months. Control subjects had no evidence of ovulatory dysfunction or hyperandrogenism. The diagnosis of PCOS was based on the National Institutes of Health criteria. We included these contents in the method section.

2. There is missing description of the workflow of the genetical evaluations. Please, provide.

- Of 2950 women who voluntarily participated in this study, who were recruited by newspaper and online advertisements, 1030 subjects were excluded as they had taken medication (e.g., steroids, oral contraceptives, metformin, or thiazide diuretics) within the past 3 months. Among 1920 subjects, 432 women were diagnosed as PCOS. And 661 women with ovulatory dysfunction or hyperandrogenism were excluded, eventually 927 women were classified as control subjects.

3. In the Introduction please specify why you decided to evaluate Fok-I, Cdx2, Apa-I, and Bsm-I.

- In the previous studies, VDR Apa-I and Bsm-I polymorphisms are correlated with susceptibility to PCOS in the Asian population (Reference 10, 11). So we decided to confirm the correlation of VDR genes including Apa-I and Bsm-I polymorphisms with susceptibility to PCOS in Korean women.

4. In the Discussion - please comment Fok-I, Cdx2, Apa-I, and Bsm-I.

- “VDR genetic variants (VDR Fok-I (rs10735810), Cdx2 (rs10875695), Apa-I (rs7967152), and Bsm-I (rs1544410)) are unlikely to influence the occurrence of PCOS, as variants distributions were similar between PCOS and non-PCOS women”. We inserted this sentence in the Discussion section, line 234-235, page 11.
Among VDR genes, “the VDR Fok-I polymorphism was associated with testosterone levels, and the Cdx2 polymorphism was associated with insulin sensitivity in women with PCOS.” We inserted this sentence in the Discussion section, line 211-213, page 10.

If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

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