Reviewer's report

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

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Reviewer: Flavio Cadegiani

Reviewer's report:

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.

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.

Abstract:

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

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.

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]."

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.

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.

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


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).
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.

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.

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

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

Would this be a post-hoc study then?

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

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?

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).

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

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.

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

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.

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.

- 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.

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

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

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

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".

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.
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)

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.

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

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.

Table 1:

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

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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