Author's response to reviews

Title: Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5 alpha-Reductase type 2 Deficiency. Case report and review of the literature in a perspective of evaluation of potential fertility of these patients.

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Version: 3
Date: 3 April 2014

Author's response to reviews: see over
Dear Ms April Rada and Dr Amar Agha:

Please find enclosed the second re-revised version (R2) of the manuscript entitled “Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5α-Reductase type 2 Deficiency. Case report and review of the literature in a perspective of evaluation of potential fertility of these patients.” By Lavinia Vija, Sophie Ferlicot, Diana Paun, Hélène Bry-Gauillard, Gabriela Berdan, Issam Abd-Alsamad, Marc Lombès and Jacques Young, that we are re-submitting for consideration to BMC Endocrine Disorders.

As requested, the manuscript has been extensively revised in accordance with the reviewers’ questions and comments. The "conclusion" section have been significantly shortened and the number of references reduced as requested.

We hope this new version will be acceptable for publication in BMC endocrine disorders, and would be pleased to provide you with any further information you may require.
Sincerely yours,
Professor Jacques Young, MD, PhD

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RESPONSES TO REVIEWERS

REVIEWER 1
Reviewer's report
Title: Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5 alpha-Reductase type 2 Deficiency. Case report and review of the literature in a perspective
of evaluation of potential fertility of these patients.

Version:2 Date:18 February 2014

Reviewer: Mark Sherlock

Reviewer's report:

Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5 alpha-Reductase type 2 Deficiency. Case report and review of the literature in a perspective of evaluation of potential fertility of these patients.

Vija et al

Dear Editor,

Many thanks for giving me the opportunity to review this manuscript by Vija et al.

The authors report the histological appearances and immunohistochemical staining with antimullerian hormone, androgen receptor and 3 beta-HSD in 3 patients, one with 5 alpha reductase type 2 deficiency, one with complete androgen insensitivity syndrome and one described as a control who had a testicular biopsy in the setting of obstructive azospermia.

The paper is well written and explores a very important area in this rare condition, namely spermatogenesis and possibility of future fertility.

R: We thank the reviewer for his very positive comments.

Comments

Minor Essential revisions

1. In the abstract and a number of stages during the manuscript the authors use the word confront, do they mean compare?
R: We corrected and replaced the word confront by compare, as suggested.

Major compulsory Revisions

1. There should be more clinical information regarding the 3 patients to aid comparison of FSH/ LH/ Testosterone and DHT etc. Some of this data are included in the supplemental table for the patient with 5 alpha reductase deficiency but should be included for control and CAIS patient also. Also the table should be part of the main text and not supplemental as it is very informative.

R: We added the requested hormonal values for all the three patients in the “Case presentation” section (pp 4-5). We also inserted Table 1 in the Manuscript, after the References section, as requested by the reviewer and editor.

2. Is the control patient a valid normal control given his obstructive azoospermia–is there any impact on testicular function from obstruction?

R: As the reviewer must know, for obvious ethical reasons, it is not possible to perform testicular biopsies in healthy men. As previously reported in several published papers, testicular spermatogenesis, when histologically evaluated, (testicular biopsies performed for both diagnostic and therapeutic purposes), is normal in subjects with obstructive azoospermia. See among others, the paper written by Puhse G et al (Puhse G et al., Hum Reprod, 2011, 26, 2606-2612). We consider the choice of this type of normal control, therefore, legitimate. This comment is now added on page 5, in the last paragraph of the “Case presentation” section.

3. Were investigators blinded to underlying medical condition for interpretation of the biopsy results?

R: We thank the reviewer for raising this important issue. Yes, this was the case. Testicular
histology was examined by one pathologist (Sophie Ferlicot), who had no knowledge of the patients’ particular pathologies. This is now indicated in the "Histology and immunohistochemistry" section (page 6).

4. There is a significant amount of discussion regarding immunohistochemical assessment of AMH; however there is little discussion of serum AMH levels in these patients. Serum AMH is elevated in patients with androgen insensitivity syndromes. Could the authors comment on this and if it may have a role as a marker of potential spermatogenesis in these patients given the differences reported in this study?

R: We thank the reviewer for this important comment. In our patient with 5 alpha-Reductase type 2 Deficiency, serum AMH levels were within normal range for postpubertal males (65 pmol/L, normal range 15-89), whereas for the CAIS patient, serum AMH was high (170 pmol/L; normal range 15-89), as previously reported in the literature for CAIS (Rey, R et al, J Clin Endocrinol Metab, 1994). This difference in serum AMH indicates that testosterone conversion into dihydrotestosterone seems not essential for AMH repression. This aspect was partially discussed in the previous version of the “Conclusions”, notably with regards to histological data. We now mentioned in the revised version that serum AMH was concordant with the AMH expression in Sertoli cells in our postpubertal patient (see page 12).

The reviewer also raised another important issue, related to serum AMH levels in patients with 5 alpha-Reductase type 2 Deficiency. There are only few data in the literature. As far as we know, there is only one Brazilian group (Stuchi-Perez EG, et al. J Pediatr Endocrinol Metab. 2005 Dec;18(12):1383-9) that reported serum AMH levels in 14 patients (both prepubertal and postpubertal). These data suggest that AMH levels were normal in postpubertal patients, consistent with our findings. We added these new comments in the “Conclusion” section of the revised version.
Unfortunately, to the best of our knowledge, there are no published studies comparing serum AMH with testicular histology (with respect to spermatogenesis) in patients with 5 alpha-Reductase type 2 Deficiency. In our reported case, there was no histological evidence for spermatogenesis, despite serum AMH within normal range. This issue remains an open question; however, if this could be confirmed in other patients, it is unlikely that AMH levels may constitute a suitable predictive value as a marker for spermatogenesis.

**REVIEWER 2**

Reviewer's report
Title:Testicular histological and immunohistochemical aspects in a post-pubertal patient with 5 alpha-Reductase type 2 Deficiency. Case report and review of the literature in a perspective of evaluation of potential fertility of these patients.
Version:2 Date:27 February 2014
Reviewer:Tríona O'Shea
Reviewer's report:
Minor Reviews:
1) In abstract (conclusions) consider replacing "confront" with either "compare" or "contrast"
R: We thank the reviewer for this suggestion. Please find the corrections in the revised manuscript.

2) Are differing levels of spermatogenesis in different patients with 5 alpha reductase deficiency related to position of testes or to differing levels of DHT?

R:
 a) We thank the reviewer for this important question. Analysis of the available literature as well as the histological analysis for the reported case, as presented in Table 1, indicates a strong percentage of impaired spermatogenesis (7/8, 87%) in cryptorchid 5 alpha-reductase type 2 deficiency patients. These findings suggest that testicular ectopic/cryptorchid position is probably deleterious for spermatogenesis, as we previously specified in the “Background” section of the paper (pages 3-4).
b) We also thank the reviewer for the comments. We compared serum dihydrotestosterone (DHT) levels in subjects with histologically normal spermatogenesis with those measured in patients presenting with impaired spermatogenesis (spermatogenic arrest at the level of spermatogonia-SGA, or Sertoli cell only-SCO) (see Table 1). Within the first group of patients (n=4), mean DHT levels were of 0.26 ± 0.05 ng/mL whereas in the second group mean DHT levels were 0.12± 0.07 ng/mL. We noticed a trend toward reduction in serum DHT levels in patients with impaired spermatogenesis; however, the comparison between the two groups was not significant (p<0.09) because of the small number of cases in each group.

3) Is TESE feasible in patients with cryptorchidism?

R: TESE feasibility in cryptorchid patients with 5-alpha reductase type 2 deficiency is an important therapeutic concern. Previous papers on 5-alpha reductase type 2 deficient patients did not help much to answer to this question, even though some patients did achieve paternity. Nevertheless, TESE might be a potential promising method for spermatozoids retrieval in patients with azoospermia related to 5 alpha-Reductase type 2 Deficiency, as suggested, indirectly, by some publications. Indeed, Matsubara et al (Matsubara K et al, Fertil Steril,2010) reported an oligozoospermic 29 year old patient with 5-alpha reductase type 2 deficiency, presenting with few normal and motile spermatozoa on semen analysis, with paternity achieved by ICSI. Moreover, two brothers with a history of cryptoorchidism were reported to achieve naturally paternity (Nordenskjold A, Ivarsson, SA. J Clin Endocrinol. Metab, 1998). These observations suggest that cryptoorchidism does not always completely impede spermatogenesis. We could, therefore, speculate that some normal spermatozoa might be recovered by TESE in 5-alpha reductase type 2 deficient patients. TESE might be thus a successful strategy for such cryptoorchid patients, as previously reported for cryptoorchid patients with nonobstructive azoospermia of various origins (Raman JD, Schlegel PN. J. Urol.,2003), including AIS (Massin N et al. Clin Endocrinol.(Oxford), 2012). This issue is now discussed in the “Conclusion” section of the revised paper

REVIEWER 3

Reviewer's report

Reviewer: Michael O'Reilly
Reviewer's report:

SRD5A2 deficiency – testicular histology

Vija and colleagues present an interesting case report on testicular histology and immunohistochemistry in a patient with SRD5A2 deficiency.

These limited results may provide further clues on fertility options in such patients, particularly with regard to utilization of appropriate assisted conception techniques.

The commendable features of this paper are its thorough histological and immunohistochemical techniques, clear images and a reasonable discussion around potential implications for fertility.

R: We sincerely thank the reviewer for his very positive comments.

The questionable features of this paper are as follows:

Major points:

1. The manuscript is too long and should be significantly shortened. A good starting point would be the discussion, which is almost five pages long and could be shortened to two or three without compromising the message of the article. In addition a maximum of 25 references should be more than sufficient.

R: We paid a particular attention to the reviewer’s comments. Not only we have shortened the length of the “Conclusion” section, but we have also removed 8 references from the previous version of the manuscript. The remaining references appear mandatory for a good comprehension of the manuscript.

2. No meaningful discussion of the potential role of cryptorchidism in the development of the irreversible spermatogenic defects of SRD5A2 deficiency. This needs to be discussed in the context of impaired androgen activation, and the potential relative contribution of each to the
observed findings. Although speculative, the authors need to discuss why one is more likely than the other in the underlying aetiology of failure of spermatogenesis in this condition.

R: We thank the reviewer for this interesting comment. Although we briefly discussed the potential role of cryptorchidism in the “Background” section of the previous version of the manuscript, we agree that it would be useful to further discuss this aspect in the “Conclusion”. This was also one of the suggestions of Reviewer 2. We included further discussion on the revised “Conclusion”.

3. No mention in the introduction of the various isoforms of 5-alphareductase, where they are expressed in humans or their various biological activities, eg androgen activation and glucocorticoid inactivation. This may be brief but is important in the context of 5alpha reductase metabolism.

R: As requested, a sentence concerning the three isoforms of 5 alpha reductase has been added (see Background section in the revised version).

4. Can the testicular histology of the control patient be truly categorized as ‘normal’ in the setting of obstructive azoospermia? The authors need to defend the choice of control patient.

R: This Reviewer’s comment is indeed relevant. As the reviewer may be aware of, for ethical reasons, it is not possible to perform testicular biopsies in healthy men. As already reported in several published papers, histological examination of testicular spermatogenesis, for both diagnostic and therapeutic purposes, was normal in subjects with obstructive azoospermia. See among others the paper written by Puhse G et al (Puhse G et al., Hum Reprod, 2011, 26, 2606-2612). The choice of this type of normal control is therefore legitimate. We added this comment in the Case presentation section, page 5.

Minor points:

1. Repeated use of the word ‘aspect’ is not entirely advisable and does not read well in most instances – for example, mature aspects might be better phrased as ‘mature features’, or ‘mature profile’ etc.
R: As requested, the word “aspect” has been replaced when appropriate in most parts of the manuscript.

2. Why did the authors choose to measure immunostaining of 3-beta HSD2 as opposed to another steroidogenic enzyme, eg 17betaHSD3? Please elaborate on this briefly.

R: As already known and mentioned by the reviewer, these two enzymes (3ßHSD2 and 17ßHSD3) are required for testosterone biosynthesis in Leydig cells, in relation to their expression in such cells (Kotula-Balak M et al, 2012, Hydroxysteroid Dehydrogenases – Localization, Function and Regulation in the Testis, pp 265-288). We did choose to assess 3ßHSD2immunostaining on paraffin sections because we previously obtained good immunohistochemical results. Unfortunately, we do not have access to17ßHSD3 antibody.

3. Page 4 – the statement ‘we also confront our findings to the analysis of the available literature’ reads poorly and should be rewritten.

R: We apologize for this statement. As requested, the sentence has been rephrased.

4. Androgen receptor expression is low in 8% of those seminiferous tubules with an immature profile. Please discuss the likelihood of this being a direct effect of androgen metabolism or simply related to seminiferous tubule immaturity.

R: We thank the reviewer for this very important comment. However, we cannot offer a clear answer, as the underlying factors and mechanisms involved in AR expression in Sertoli cells in the human testis are not known.

It is very unlikely that testosterone or its metabolite, dihydrotestosterone, initiate AR expression because AR is not expressed in Sertoli cells (SC) during both fœtal and neonatal periods despite the presence of testosterone synthesis and activity (Scott, HM et al, Endocr Rev, 2009, 30(7):883-925). Conversely, AR expression in SC progressively increases in prepubertal boys, while the testicular biosynthesis of testosterone remains inactive.

With respect to the potential relationship between seminiferous tubules maturity and AR expression in SC, a clear relation has not been established yet. Indeed, in newborn AR expression in SC is lacking, while seminiferous tubules are immature. In young prepubertal boys, AR expression in SC increases between 4 to 8 years of age, whereas seminiferous tubules immaturity persists. In conclusion, it seems that seminiferous tubules maturity does
not govern AR expression in SC. This point although very interesting, is beyond the scope of the present paper, keeping in mind that both Reviewers and Editor ask the length of the manuscript to be shortened. As a result, we prefer not to include this discussion in the revised version of the manuscript.