Author’s response to reviews

Title: Case report of whole genome sequencing in the XY Female: identification of a novel SRY mutation and revision of a misdiagnosis of androgen insensitivity syndrome

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Author’s response to reviews:

Dear Dr Karaca,

Re: BMC Endocrine Disorders manuscript revision (BEND-D-16-00093R1)

We thank the reviewers for their comments and constructive feedback. Our responses to specific suggestions follow below.

Reviewer #1:

1. It would be helpful for the authors to comment on WGS results from other known disease-associated genes: DHH, SF1, DAX1, AMH, and AMH-R2 -- were they completely normal?

- No, there were some variants in other genes associated with DSDs. The manuscript has been modified (shown below) to highlight this and explain why they were excluded.
Case Presentation, Pg 7, line 70: Variants found on WGS were filtered based on mutational consequence (snpEff impact high or moderate) and phenotypic correlation. WGS revealed variants in eight genes associated with disorders of sexual development, namely: AKR1C4, DMRT1, MAP2K1, MAP3K1, NR0B1, NR5A1, POR and SRY. All but the NR0B1 and SRY variants were excluded based on variant population frequencies >1% in the 1000 Genomes (1K), Exome Sequencing Project (ESP) and Exome Aggregation Consortium (ExAC) databases. The NR0B1 variant was then excluded because of a population frequency >1% amongst individuals in the ExAC database of the same ethnicity as the patient.

The remaining hemizygous missense a hemizygous missense variant in the SRY gene...

2. Is it possible that the expression of these genes could have been altered in a way that would not have been detected by WGS, for instance, altered methylation status leading to decreased transcription of the gene?

- Yes, we agree that the presented method of WGS is unable to detect methylation status, and methylation can rarely underlie disorders of sexual development as described by Sandbacka et al (Fertil Steril 2011; 95(8):2703-6). The newer technology of single-molecule real-time bisulfite sequencing enables detection of methylation, however this was not pursued as the SRY variant explained the molecular cause of the patient’s DSD. In the interest of brevity and simplicity, this has not been discussed in the manuscript though this could be included if the reviewers and editorial staff felt that it was additive.

3. The authors state "With the increasing availability, cost-effectiveness, speed and understanding of WGS, we support its use in the clinical evaluation of the XY female..." Could the authors briefly comment on the current affordability of such an assay outside of a research setting?

- Yes, the manuscript has been adjusted below:
Conclusion, Pg 8, line 206: “In current clinical practice, the cost of WGS is approximately US$2600 with the exact price depending on laboratory throughput and the technologies employed. The cost is generally less in research settings and further decreases are expected with improving efficiency in the sequencing and bioinformatic processes. With the increasing availability, cost-effectiveness...”

Reviewer #2:

1. Was there any evidence of microscopic gonadoblastoma or dysgerminoma in the streak gonads given the presence of a Y chromosome?

   - Yes, she had evidence of both. See below.

Case Presentation, Pg 5, line 126: “Histopathology demonstrated bilateral gonadoblastoma, and dysgerminoma in the right gonad.”

2. The Background section is very comprehensive and the appropriate description of the differences between CAIS and CGD might be better served in a Discussion after the Case Presentation.

   - We agree with this comment but the CAIS vs CGD comparison remains in the Background section as per the BMC Endocrine guidelines for case report formatting. We would be happy to create a 'Discussion' section and move this to there if the editorial staff preferred.

Other than the above changes, minor changes have been made to the manuscript to match the formatting of BMC Endocrine and to reflect the current affiliations of the authors. Both tracked and clean versions of the revised manuscript have been uploaded.
Thank you for the opportunity to submit a revised manuscript. We look forward to hearing the final outcome.

Many thanks,

Sunita De Sousa (on behalf of all authors)