Reviewer’s report

Title: Next generation sequencing with copy-number-variant detection expands the phenotypic spectrum of HSD17B4-deficiency

Version: 3
Date: 27 December 2013

Reviewer: Jose E Abdenur

Reviewer’s report:

The authors present an important expansion of the phenotype of HSD17B4 defects. Abnormalities in this gene have been associated with a severe-infantile onset peroxisomal disease (bi-functional protein defect) as well as with Perrault syndrome, a disease presenting in adolescence with ovarian dysgenesis, hearing loss and ataxia.

The patient in this report is a male, with cerebellar ataxia, peripheral neuropathy, hearing loss and azoospermia. The molecular diagnosis was obtained after whole exome sequencing data was re-analyzed with computational algorithms designed to infer copy number variants from targeted sequencing data, which revealed a 12kb deletion, compounded with a novel missense variant.

This report adds important information into the literature of this rare genetic defect and raises some interesting questions about the interaction between the peroxisomal and the mitochondrial functions. The publication of two new variants in the HSD17B4 gene will be helpful for clinicians and laboratories alike struggling with the interpretation of previously unreported next generation sequencing findings. The use of the copy number variant algorithm, CONIFER, stresses the importance of mining next generation sequencing data with additional computational strategies. The paper reads very well, and although the information is concisely presented, the discussion could be enhanced.

MAJOR COMPULSORY REVISIONS:

Case presentation:

1) Line 99-102: Please provide information about the laboratory and method used for the long-and very long-chain fatty acids testing and include the actual results (values for each marker and ratios, units and controls). If available, please also include results for other important markers of peroxisomal function like C26:0, C26/C22, C24/C22, di- and tri-hydroxycholestanolic acids and plasmalogen levels. Even if the above results were within normal limits, the information will be important to highlight that normal values of the most commonly used markers of peroxisomal function cannot exclude a mild DBP defect.

2) The potential link between this peroxisomal abnormality and a mitochondrial disease deserves more attention. Line 105: Please provide information about the laboratory and methodology used to measure ETC activity in muscle. Please
clarify what was considered a “normal activity” for complex I and how was that “normal” determined (ie. control run with the patient’s sample, the laboratory’s own historical data, literature, etc). A table including all the results of ETC testing will be useful and can be added into the supplemental information section.

MINOR ESSENTIAL REVISIONS:

Please add “Peroxisomal defects” to the list of key words.

DISCRETIONARY REVISIONS:

Case presentation:

1) Line 87: the Statement “he had an average IQ” appears contradictory with the information presented in the first paragraph of the case presentation, where the authors mention that the patient had cognitive impairment and needed help at school. Therefore, if available, please provide the actual IQ score and the testing methodology used.

Additionally, the information about the developmental delays presented in the “supplementary clinical information” is brief, and highlights the fact that motor impairments were more severe than the intellectual disabilities in this patient. I would suggest incorporating that information in the main text.

2) Line 99: Please clarify that the “small amounts of lactate” was found through urine organic acid analysis. I also suggest mentioning in the text that the elevated lactate was the only abnormality found in the urine organic acid analysis. (The additional information regarding the urine organic acids included in the supplementary clinical information can be omitted. Small amounts of succinic and 2-keto-glutaric acids are frequently found in urine and are unlikely to be relevant in this case).

3) The potential link between this peroxisomal abnormality and a mitochondrial disease is important. It would be interesting to assess whether sequence interpretation of the 5’UTR of the HSD17B4 gene would reveal a mitochondrial targeting sequence which could be generated by alternate splicing.

Conclusions:

1) The finding of low testosterone and azoospermia in this patient is interesting. Have the authors performed a more comprehensive steroid profile analysis?

It will be useful to the readers to have a more in depth discussion as what is the suspected role of HSD17B4 gene in steroid synthesis, and speculation as to why this particular patient may suffer from azoospermia and low testosterone levels.

Some additional information added to the paper may stimulate this discussion. For example, authors may want to mention that DBP was discovered as the steroid converting enzyme 17-#-hydroxysteroid dehydrogenase type IV (Ferdinandusse et al, Ann Neurol 2006; 59:92-104). Although until now the role type IV 17#-HSD in steroid metabolism is not completely clear, this enzyme is thought to play a role in testosterone metabolism (Penning T. Human

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests