Reviewer's report

Title: Novel FOXL2 mutations in two Chinese families with blepharophimosis-ptosis-epicanthus inversus syndrome

Version: 1 Date: 26 May 2015

Reviewer: Laura Crisponi

Reviewer's report:

This paper reports two novel FOXL2 mutations, an indel mutation (c.675_690delinsT; p.A226_A230del), which would result in the deletion of 5 alanine residues from the poly-Alanine tract in the protein, and a missense mutation (c.223C>T; p.L75F) in two Chinese families with Blepharophimosis/ptosis/epicanthus inversus syndrome (BPES). Both mutations appear to be associated with Type II BPES. These findings expand the spectrum of FOXL2 mutations, and considering this should be the first report of a poly-Alanine contraction associated to BPES, this is of particular interest. Generally, this is a descriptive study, and there are a number of concerns that need to be addressed.

- Major Compulsory Revisions

1. The exact role of the polyalanine tract in FOXL2 has not yet been elucidated. It has been reported to be a mutational hotspot for BPES: 30% of FOXL2 mutations lead to polyalanine expansions, and 13% are novel out-of-frame duplications (De Baere et al., 2001; De Baere et al., 2003; Beysen et al., 2008). De Baere et al. (2001) show that there is still no clear cut genotype–phenotype correlation with regard to BPES type, although predicted truncation before the poly A tract usually leads to type I (with POF) and expansion of the poly A to type II (without POF).

This should be the second FOXL2 poly-Ala contraction reported so far. The first was a partial deletion of the poly-Ala tract, removing 10 out of 14 alanines (A221_A230del), with the rest of the protein intact, found only in one POF patient with no eye defects (Harris et al, 2002). For this reason, the data presented here must be better presented and debated in the discussion and emphasized in the conclusion.

2. For the same reason the clinical details of the female patients should be detailed, i.e.; at what age the menopause occurred? How many pregnancies and at what age? Any hormone levels data available?

3. The background and the references list should be implemented with a comprehensive review of all the mutations found so far in FOXL2 associated to both BPES and POF patients.

4. Results from pathogenicity prediction software should be reported into a table.
5. The sequencing result reported in Figure 4 for the indel mutation is not clear. Could you add the mutant electropherogram without alignment with the normal sequence?

6. Needs some language corrections before being published. An English mother tongue should revise it.

- Minor Essential Revisions
1. Please adjust the gene name FOXL2 in italic throughout the text.
2. Keywords, add polyalanine tract.
3. Sometimes it is type II BPES and sometimes BPES type II or BPES II. Please adjust to be always reported in the same way.
4. Line 42, leave out “The” and starts the sentence with “Two”.
5. Line 61, add “helix” to the “winged/forkhead transcription factor”.
6. Line 62, expression studies revealed that FOXL2 (without specifying whether protein or mRNA) and add reference 7 to this sentence.
7. Line 68, substitute deletion/insertion with indel and leave a space after the second bracket.
8. Lines 81-83, please revise this sentence and specify who gave the informed consent.
9. Line 90, since the four primers used for mutational analysis are the same of the ref. 7 cited, please remove Table 1.
10. In methods, I'd leave only two sections: Patients and Mutational analysis (comprising PCR/PCR product cloning and Sequencing and Mutation Analysis)
11. In the section patients, please specify some details on the probands, in particular the age. In the section Mutational analysis, please specify on how many patients from the two families you performed the analysis on the entire FOXL2 gene.
12. Line 114, I guess both variations were further evaluated in family members and 100 normal controls, not only the missense. Please adjust accordingly.
14. Pathogenicity prediction analysis should be done also with mutation taster who considers also splice-site changes, loss of protein features and changes that might affect the amount of mRNA.
15. Conclusions must be a separated section.
16. Line 139, change granulose in granulosa.
18. Lines 163,177, correct poly-phenylalanine with poly-alanine or better with poly-Ala.
19. Abbreviations, add poly-Ala tract for poly-alanine and adjust the text accordingly.


21. Revise well all the spaces throughout the manuscript, figures and figure legends.

- Discretionary Revisions

1. Figure 2 legend, line 266, please change with “…two fragments of 304 and 289 bp using 6% agarose gel electrophoresis.”

2. Figure 3 legend, line 268, please change “Pictures representing the ocular defects of BPES patients from two Chinese families”.

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

**Quality of written English:** Needs some language corrections before being published

**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.