Author’s response to reviews

Title: Premature ovarian insufficiency as a variable feature of blepharophimosis, ptosis, and epicanthus inversus syndrome associated with p.Leu75Phe FOXL2 mutation: a case report.

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Responses for the Reviewers:

Ad. Reviewer 1 (Petra Liskova):

Scientific language editing was introduced. The reviewer suggests to shorten the text, while Jeehyeon Bae (third reviewer) emphasizes the conciseness of the work as an advantage. The authors fulfilled the requirements concerning length of the paper introduced in the submission guidelines.

Reviewer comment: As Sanger sequencing of FOXL2 is more cost effective than WES, can the authors explain why WES was used as the primary approach?

The BPES syndrome is associated with mutations in the FOXL2 gene but the syndrome is rare and diagnosis was not obvious. While routinely studying rare diseases in our center we encounter many clinical cases which have been approached by Sanger with no results or results which were not satisfactory (possibility of additional variants contributing to phenotype). Thus, in our environment with high throughput WES facility and advanced bioinformatic pipeline we strongly prioritize early WES. Please note that in the present case, even if we had started with Sanger, due to atypical presentation, it is quite likely that WES would have to be performed anyway at some point.
Specific comments:

1. Secondary amenorrhea was an initial diagnosis, because before cessation of menses the patient had own menstrual cycles (since menarche, which occurred 5 years before POI). After additional tests suspicion of secondary amenorrhea was changed into premature ovarian failure. We changed the sentence: '18 years old nulliparous woman was diagnosed of secondary amenorrhea’ into ‘18 years old nulliparous woman suspected of secondary amenorrhea was referred to Endocrinology Outpatient Clinic’.

AMH abbreviation has been introduced.

2. Diagnosis of microphthalmia was supported by axial length measurements – the criteria for its recognition were introduced in the main text.

3. Abbreviations used in the main text are not the initials of the family members. MP = proband MS = proband’s sister, MF = proband’s father, MH = proband’s half-sister.

4. Changed.

5. Changed.

6. Explanation introduced.

7. Moreover, the pathogenicity estimation by Polyphen2 and Mutation Taster predicted this mutation as deleterious. More tools could be used for analysis, for example MutPred, SNP and GO.

The sentence: “Moreover, the pathogenicity estimation by Polyphen2 and Mutation Taster predicted this mutation as deleterious.” has been replaced by “Moreover, the pathogenicity estimation by MetaSVM which integrates scores from PolyPhen-2, MutationTaster, SIFT, GERP++, Mutation Assessor, LRT, FATHMM, SiPhy and PhyloP, and allele frequencies in 1KG database (Dong et al., 2015, Hum Mol Genet 24: 2125) predicted this mutation as deleterious.”


8. Changed.

9. Changed.
Ad. Reviewer 2 (Yueqiu Tan):

1. Reviewer’s comment: The authors should add the strategy of variant filtering in Whole exome sequencing.

The reference with strategy of variant filtering was added. We also added short description to the main text (pls. see lines 151-161).

2. Other causes of POI, such as endocrinopathies or autoimmune disorders were excluded. Researchers conducted numerous hormonal tests that allowed to exclude other endocrinopathies, including those with autoimmune background. Suspicion of BPES based on the characteristic phenotypic features. Due to the confirmation of the initial diagnosis, additional genetic tests were abandoned.

Minor points:

1. The structure of FOXL2 gene was explained in the manuscript.

2. Revised.

3. Revised.

Ad. Reviewer 3 (Jeehyeon Bae):

1. Accept without revision