Author’s response to reviews

Title: FSH may be a useful tool to allow early diagnosis of Turner syndrome

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Answers to Reviewer’s comments (Ewa Malecka-Tendera, Reviewer 2):

Comment: The study was performed in relatively small group of girls with TS although they all have gonadotropin levels measured in a certain point of their life. However the size of group and different age at which gonadotropins were measured do not allow to draw definite conclusions about their level fluctuations.

Answer: This is indeed a small group of girls with TS. This is due to the fact that this is a long-term follow-up study (mean: 10.7 years; range: 3.0 to 16.5 years). Gonadotropins measurements were not done just at specific points of their lives, but as a routine instead. The number of gonadotropins measurements per patient ranged from two to 21 (median: 10). As mentioned in Methods, follow-up visits were scheduled every 6 months, and measurements were available both in early childhood (< 5 years) and in the following years. Most (12/15) were followed until adolescence. Thus, conclusions were based on a longitudinal study, rather than a transversal study.

Comment: I cannot agree with the final statements that FSH measurement can replace in underdeveloped countries karyotyping the girls with short stature. The final diagnosis of TS has to be made on chromosomal analysis and it would be even not ethical to tell the parents that their daughter has TS basing on FSH level.

Answer: The authors totally agree with the reviewer that it would be inconceivable to replace karyotyping for a FSH measurement.
In the Discussion section, the authors stated that “our results indicate that many girls with TS could be diagnosed earlier if FSH measurements were routinely done in girls with unexplained short stature.”; that “It has already been proposed that elevated FSH levels in childhood should prompt cytogenetic evaluation.”; and finally that “this widely available and highly specific test to detect gonadal dysgenesis may lead to early diagnosis and prompt treatment in a significant number of patients when karyotype is not accessible to screen all girls with growth deficiency.”

However, for the authors “to diagnose” always meant to make a cytogenetic diagnosis (karyotyping). Maybe it was not clear enough in the text (and we apologize for that) that when an elevated level of FSH is found, this girl must have a karyotype performed and that despite its low availability in underdeveloped countries these cases must be prioritized.

Thus, in the Discussion section, page 8, line 221, the authors now state that “Even though FSH measurement is not always sensitive for the diagnosis of TS in girls with unexplained short stature, this widely available and highly specific test to detect gonadal dysgenesis may lead to early diagnosis and prompt treatment in a significant number of patients by giving priority to performing their karyotype when this test is not accessible to screen all girls with growth deficiency.”

In the Conclusions, page 9, line 239, the authors now state that “These results indicate that inclusion of this test in the guidelines for the evaluation of girls with unexplained short stature would allow early diagnosis and treatment in a significant number of patients by prioritizing their cytogenetic evaluation, particularly when resources for chromosomal studies of all girls with growth deficiency are limited.”

Comment: In the abstract this conclusion is even more confusing as there is a contradiction between indication for karyotyping and statement " when resources for chromosome study are limited".

Answer: In the conclusion which appears in the abstract the authors now state that “Karyotyping of girls with short stature and high FSH levels would allow early diagnosis of Turner syndrome in a significant number of patients, particularly when resources for chromosome study of all girls with growth deficiency are limited.”

Comment: Authors did not perform, in fact, the statistical analysis to prove whether the differences in gonadotropin levels were significantly different than in healthy girls as there is no control group age matched to the girls with TS.

Answer: The prepubertal levels in the general population determined by the manufacturer of FSH and LH kits (Roche Elecsys®) were used as controls.
Comment: Elevated FSH which is dominating LH is a well known phenomenon in girls with gonadal dysgenesis due not only to TS. They may be even elevated to some extent in girls with benign premature thelarche.

Answer: The authors agree with the reviewer. As it already appeared in the Discussion section, page 9, line 223, “Even though gonadal dysgenesis is also a feature of other disorders of sex development, these conditions are usually not associated with short stature. This is the case of complete gonadal dysgenesis, either 46,XY (which may be due to mutations in SRY gene, among others ref) or 46,XX (which may be due to mutations in FSH receptor gene, among others). Thus, in some cases a normal karyotype may be found and lead to further investigations on the origin of high FSH levels.” It is also possible that in some girls with short stature the finding of elevated FSH may be a false-positive, as in the example of benign premature thelarche; however, even though it may not be 100% specific the authors do believe the test to be useful.

Comment: Other comments of minor significance - the introduction is certainly too long describing well known facts about TS.

Answer: The authors agree with the reviewer, and the first three paragraphs were shortened.