Author's response to reviews

Title: Ovarian dysfunction and FMR1 alleles in a large Italian family with POF and FRAXA disorders: Case report

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Author's response to reviews: see over
Dear Prof. Liz Hoffman
Assistant Editor BMC Medical Genetics,

I wish to thank you for your letter concerning the submission of manuscript MS: 5769245361176476. “Ovarian dysfunction and FMR1 alleles in a large Italian family with POF and FRAXA disorders: Case report” by Miano et al..

As requested, I have adjusted the paper according to the Reviewer’s comments, and I have submitted the revised version using the online system. All the corrections were reported in bold in the revised manuscript.

Herein, I reported the point-by-point responses to the Reviewers 1 and 2.

After the acceptance of the revised paper, I will send the copies of the patient’s consent by fax.

I'm looking forward to hearing from you soon,

Yours Sincerely,
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Comments and answers to the Reviewer 1.
Major Compulsory Revisions

Point 1.
The reviewer stated that it is not clear which patient is the proband because in Results & Discussion section it seems to be the male with fragile X syndrome. According to this observation, in Results & Discussion paragraph 2, we replaced the sentence: “Because the family showed a young-boy with FRAXA syndrome (IV:15), we analyzed him to reveal FMR1 expanded alleles” with

“The proband IV:7, is a young woman with severe POF disease as described above. Because of the presence in the family of a young-boy (IV:15) with Martin-Bell syndrome (MIM300624) we started our analysis establishing the segregation of FMR1 expanded alleles in the family. As expected, IV:15 had a FRAXA syndrome with an FMR1....”

Point 2.
In the last paragraph of Results & Discussion, according to the suggestion’s of the reviewer, we replaced the sentence “In particular, we believe that the association of
POF with expanded FMR1 alleles could be verified through the generations allowing the prediction of premature menopause and the implementation of strategies to advance conception”

with

“In particular, we believe that the association of POF with expanded FMR1 alleles must be verified through the generations allowing the clinical counseling of familial POF and eventually the implementation of strategies to advance conception”.

Minor Essential Revisions

Point 1. According to the reviewer’s suggestions, we improved the description of the family relationship using the ID numbers and replaced the sentence (at page 4, 3rd paragraph) “The mother (III:3; age 49) of the case study, ……………The aunt III:10 (age 46), daughter and sister of the two POF females, respectively, had no symptoms of premature menopause. Her son (IV:15), a young-boy of 6 years old, had Fragile X syndrome…..”

with

“The female III:3 (age 49), mother of the case study, who entered menopause at 45 years old, had no symptoms of POF disorder. She had a karyotype with 46, XX inv(2)(p12q13), the same inversion observed in the proband IV:7. The female IV:5 (age 25), the sister of the proband IV:7, had regular menses and a normal karyotype; while their maternal grandmother (II:2, age 75) had five daughters and underwent a hysterectomy at age 47 because she was affected by diffuse fibromatosis of the uterus. The female II:3 (age 65) had two sons (III:6 and III:7) and similarly to her sister (II:2) underwent at hysterectomy at age 46. Both II:6 (age 60) and III:9 (age 47), that are respectively the great-aunt and the aunt of the proband, suffered from a POF syndrome with amenorrhoea at age 35. The female III:10 (age 46), daughter and sister of the two POF females (II:6 and III:9, respectively) had no symptoms of premature menopause. She had two children, a 11-year-old daughter, IV:13 ….”

Point 2. According to the reviewer corrections, we replaced the word “pre-puberal” with “pre-pubertal”.

Point 3. In the description of Materials and Methods, we added one reference for DNA extraction method, as suggested by the reviewer. It appeared listed in References as “17. Sambrook JF, EF, Maniatis T: Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Laboratory Press. 1989”.

Point 4. In Results and Discussion, paragraph 2, according to the reviewer’s suggestions, we improved the discussion about the transmission from individual II-6. Given that, we added the sentence “In the mother-daughter transmission (II:6-III:10), the presence in III:10 of the FMR1 allele with 100 CGG repeat could be the result of a backward mutation or contraction upon transmission from the mother with 200 CGG allele. This phenomenon is the product of the high instability of the FMR1 CGG repeats that was found to increase with increasing of the repeat length and that, generally, occurs with higher frequency in the paternal transmission than in maternal transmission [12,20, 21]. The female IV:7 (the proband) had a POF diagnosis and she was the second cousin of the FRAXA male. Therefore, we extended…..”.

In addition to this, we included two new references, listed as “20. Sullivan AK, Crawford DC, Scott EH, Leslie ML, Sherman SL: Paternally transmitted FMR1

**Point 5.** We eliminated the term “the POF girl”.

**Point 6.** We have corrected the discrepancies between the text and the figure. In the corrected figure, it is the individual II:2 with the genotype 31/100 and the individual III:5 with the genotype 24/200.

**Point 7.** The reference Berry-Kravis *et al* appeared in the reference’s list as 21.

**Point 8.** We improved the English writing of the entire manuscript accordingly to the corrections of an English-speaking scientist.

**Comments and answers to the Reviewer 2.**

**Major Compulsory Revisions**

**Point 1.** Regarding the miscarriages showed in the pedigree, we have no evidences that the spontaneous abortions presenting by the females III:2, III:3, III:6 are related to the POF condition. This point is discussed at page 5 in Case presentation Section. On the other hand, some reports (Levi *et al* 2001; Burton *et al* 2000; et) described the recurrence of infertility and spontaneous abortion in POF families.

**Point 2.** Regarding the age of the women in the generation II and III that showed no history of POF but regular menses, this point is described at page 5 in Case presentation section. They are the risk women: III:4 38 years old, III:5 35 years old, and III:6 36 years old; and the others: II:3 65 years old, II:4 64 years old, III:1 57 years old III:2 50 years old, III:3 49 years old, III:4 38 years old, III:5 35 years old, III:6 36 years old, III:9 47 years old and III:10 46 years old.

We added in the text the age information for the females II: 2, II:3, II:6, and III:9 at page 4 in Case presentation section.

**Point 3.** About the females III:1 and III:2, both had an apparently regular fertility life and entered menoapuse at 51 and 50 age, respectively. Their state of health is good and apparently they cannot add clinical data to improve familial POF counseling and/or predictive diagnosis in the family.

**Point 4.** We agreed with the reviewer’s suggestion that a more detailed analysis of the family and of the affected women will make the family more informative.

Nevertheless, we believe that our report will be of great interest to those studies closely related to POF research and enrich the spectrum of the POF families with complex presentation that is likely to derive from the additive effect of different factors.

To underline this point, we modified the sentence in the last paragraph (page 6-7) of Result and Discussion section adding “…In our opinion and accordingly to several reports [1-6, 21], …” and the sentence “In conclusion, our case report represents an original observation on the coexistence within one family of different factors involved in two different ovarian failure conditions, one- *FMR1* related and one not- *FMR1* related. On the other hand, it can be considered an important warning for the genetic counseling of familial POF. In particular, we believe that the association of POF with expanded *FMR1* alleles must be verified through the
generations allowing the clinical counseling of familial POF and eventually the implementation of strategies to advance conception.”