Author's response to reviews

Title: A combination of Ascorbic acid and Alpha-tocopherol to test the effectiveness and safety in the Fragile X syndrome: study protocol for a phase II randomized placebo-controlled trial.

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Author's response to reviews:

We accept all the comments of the reviewers and have changed the text accordingly to their corrections and suggestions. As requested, we give a point-by-point response to the reviewer's concerns:

Reviewer 1:

#1: I found the background too long and complex. I would suggest to the authors to simplify the message.

We have eliminated some paragraphs to simplify the message of the introduction.

#2: In the Discussion section the authors mention the treatment with arbaclofen. This therapy has currently been blocked. For more details, please check the paper « de Esch CE, Zeidler S, Willemsen R. Neurosci Biobehav Rev. 2013 Oct 30. pii: S0149-7634(13)00236-4. doi: 10.1016/j.neubiorev.2013.10.012. «

We have included this new reference.

#3: A table summarizing and comparing the different trials for FXS would be very useful for readers.

We have included a new table indicating the trials.

Reviewer 2:
#1: paying attention to the gene names (that should be indicated in italics).

We have corrected the gene names following your advice.

#2: I am looking forward to the outcome of this study, although I am afraid that the outcome measures (that however largely correspond to those employed by all the other major studies performed on FXS individuals) may not be sensitive enough to capture an improvement in the patients' behavior.

We have chosen these tests following the bibliography and we expect to have good sensitiveness.

Reviewer 3:

(1) Since the examinations will be performed in short interval, how the learning effects in tests will be avoided?

Following the instructions indicated in the learning test, a learning effect will be avoided subtracting 6 points to the second and third evaluation, if it is repeated before 6 months. We will reduce 6 points to all the learning measurements in the second and third evaluation.

(2) Does alpha-tocopherol accumulate in the body?

It is quite an interesting consideration. It is known that tocopherol is a lipophilic vitamin, and although it accumulates in lipid reservoirs in the body, 90% of total body mass of alpha-tocopherol is recovered in the liver, skeletal muscle and adipose tissue (J Nutr. 1990 Mar;120(3):233-42).

We have used a low dose of 10mg/kg/day (max 600mg/day) in the study due to safety reasons; there are studies where they showed safety of this dose in neonates. Eur J Clin Pharmacol. Feb 2010; 66(2): 109–118.

There are a number of studies indicating the safety of tocopherol. There is clinical trial evidence that vitamin E supplements appear safe in amounts $\leq 1600$ IU (1073 mg RRR-alpha-tocopherol or the molar equivalent of its esters) and that vitamin C supplements of $\leq 2000$ mg/d are safe for most adults (Am J clin Nutr. 2005 Apr;81(4):736-45.).

A meta-analysis of 57 randomized controlled trials found that vitamin E supplementation, up to doses of 5,500 IU/day, had no effect on all-cause mortality (Lancet. 2003;361(9374):2017-2023). Furthermore, a meta-analysis of 68 randomized trials found that supplemental vitamin E, singly or in combination with other antioxidant supplements, did not significantly alter risk of all-cause mortality ( JAMA. 2007;297(8):842-857).
(3) There are many trials of individuals with fragile X syndrome on-going and they should be introduced based on the action of drugs used in the trial in Discussion. We have included a new table including all the trials and drug targets.

(4) There are some special concerns in Introduction:
- Martin and Bell described the first families which clearly demonstrated the X-linked inheritance of mental retardation caused by Fragile X syndrome. It should be noticed that although some female individual showed intellectual disability it was milder than in males and the boys with severe intellectual disability were born to normally intelligent mothers.
  We have corrected this concern in the introduction.
- Reference #9 is incorrect. References #7 and 8 should be together.
  We have corrected this mistake.
- The neuropathological findings should be based on references from human studies (references #10-13 are all mouse studies).
  We have corrected this mistake.
- References #20 and 21 should be replaced by a review or up-dated references describing better the properties of FMRP as a RNA binding protein.
  We have corrected this mistake.
- GSH is introduced with an abbreviation many times.
  We have reduced the times we used GSH.
- Reference #81 is primarily for alpha-linolenic acid supplementation and not for vitamin C.
  We have eliminated this reference.

Discretionary Revisions:
- What are the urine and blood tests to be performed?
We have included a relation of measurements that will be done in blood and urine.