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February 15, 2015

Dear Editor,

Please find enclosed our revised manuscript in a Word format, as requested (file name: 15727-review.doc).

Title: Modeling diseases of noncoding unstable repeat expansions using mutant pluripotent stem cells

Author: Shira Yanovsky-Dagan, Hagar Mor-Shaked, Rachel Eiges

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 15727

The manuscript has been improved according to the concerns of the reviewers. Our point-by-point responses to these comments are as follows:

1. Minor corrections of typos were made according to the concerns of all the reviewers. Revisions were made according to the suggestions of reviewers #2 and #3:

Reviewer #2:

(1) **The review will greatly benefit if the authors briefly summarize their thoughts on future direction of research in this area**.

Our thoughts regarding future directions are listed at the end of each section of the review. For each mechanism we mentioned the questions that are still needed to be answered and the possible contribution of the human based model system of mutant stem cells. In addition, we now added a general paragraph summarizing all these ideas in the "Concluding Remarks" (page 24 line 18- page 25 line 6).

Reviewer #3:

(1) **While conveniently obtaining rich data from the ESCs or iPSCs, a criticism is that these cells undergoing unknown number of repeated duplications which would likely confound the incidence of unstable repeats artificially beyond natural occurrence. Subsequently, data derived from these cells line may bias the potential pathological mechanism. This should be discussed during their analyses in correlation to the diseases related to the large unstable non-coding expansions, and again indicted in their Concluding Remarks section.**

Although it is by now established that ESCs and iPSCs tend to carry typical chromosomal aberrations that favor their propagation in culture, there is no evidence for further repeat amplification beyond the range observed in somatic cell of patients (added to the text in page 20, line 17). Nevertheless, the reviewer is correct in pointing that ESCs and iPSCs do carry specific genetic and epigenetic aberrations, and this is now referred to in the text (page 23, line 23).

(2) **It will be helpful to include an illustration on example of unstable non-coding expansions and show how the transcriptions are disrupted in one of the mechanism e.g. Reaped-mediated epigenetic modifications through changes of methylation and histone code.**

Aberrant epigenetic modifications can change local gene transcription and their effect may vary from one locus to another, depending on the location of the expansion in the gene. While in some genes it abolishes the activity of a promoter (as in FXS and C9orf72) or an enhancer (as in DM1), in other genes it interferes with RNA transcription elongation (as in FRDA) or termination (as in FSHD). This is why we chose to present an illustration that summarizes all four mechanisms described in the text, in the context of pluripotent stem cells research.

2. References and typesetting were corrected.

3. Reference to Figure 1 was inserted into the text.

4. We now submit an audio file describing our core tip.

Thank you again for accepting our review for publication in the *World Journal of Stem Cells*.

With kind regards,

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