Author's response to reviews

Title: Genome-wide expression profiling and functional characterization of SCA28 lymphoblastoid cell lines reveal impairment in cell growth and activation of apoptotic pathways.

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Author's response to reviews:

Dear Editor, BMC Medical Genomics,

Thank you for your interest in our paper. We have followed your comments as follows:

1) The sub panels of Figure 3 with not significant results were removed as requested (panels 3A, 3C, 3D, and 3E), and legend changed accordingly.

2) Minor points raised by referee 2 have been edited. In particular:

Q: Fig. 3F. Place the loading control below TFAM (as in Fig.5G).
A: Changed as requested
3) Extend the time of the proliferation assay.

Concerning this point, we changed figure 2A adding one time-point at 6 hours. We initially tested the number of lymphoblasts to be used in the proliferation assay, and verified that the minimal was 30,000 cells per well. This number is already below the working concentration for LCLs of 250,000 cells/ml; therefore we do not think it is possible to start with less cells.

Following this experiment, we performed a pilot study measuring proliferation at 24, 48 and 96 hours: control cell lines were dying at 96 hours due to overgrowth (see supplementary image for the editor). Based on these data, we decided to limit proliferation assay to 48 hours.

To add further time-points, we need to prepare 12 cell lines (6 patients and 6
controls), and the experiment need to be repeated three times. We have estimated these experiments will take between 30 and 45 days.

Based on our pilot study, we think that in the best hypothesis, we will be able to add one day to the proliferation curve (3 days instead of 2). However, extending the proliferation assay of one day does not seem to add further information: patients and controls, in fact, showed proliferation growth rates significantly different already at 3 hours. It is therefore unclear to the authors the need to extend the time points.

In any case, we are ready to fulfil your request, if you think it is necessary.

Sincerely,

Alfredo Brusco, Ph.D.