Presenilin and the UPR

To the editor — Disruption of calcium homeostasis, inhibition of protein glycosylation, and various cellular stresses provoke the accumulation of unfolded protein in the endoplasmic reticulum (ER); this phenomenon is called 'ER stress'. In response to ER stress, eukaryotic cells increase transcription of genes encoding ER-resident chaperones such as BiP/GRP78 and GRP94; this induction system is termed the unfolded protein response (UPR)[1]. The mediators of ER stress signalling are known to be ER-resident transmembrane kinases such as Ire1 and PERK. These proteins sense the perturbed environment in the ER, leading to downstream signalling by a process that depends on oligomerization and autophosphorylation.

Previously, we reported[2] that the familial Alzheimer's disease (FAD)-linked presenilin1 (PS1) mutation perturbs the signalling of the UPR, involving a decrease in the transcriptional induction of BiP/GRP78 mRNA and the inhibition of the activation of Ire1α. Recently, Thinakaran and colleagues[3] have refuted our previous results, claiming that FAD-linked PS1 mutants do not have a discernible impact on the UPR. In our original paper we did not emphasize that the defect in the UPR caused by the FAD PS1 mutation is of a quantitative nature and exhibits strict dosage and time-course sensitivity. Thus, it is important to compare wild-type and mutant cells across time, dose and cell types to substantiate our explanation for the discrepancies between the results obtained by the two laboratories.

As shown in Fig. 1, FAD-linked PS1 mutants delay the activation of stress transducers such as Ire1α and PERK during ER stress. However, the effects of PS1 mutations could be masked by treatment with excessive doses of ER stress inducers or by prolonged stimulation. Thus, one cause of the inability of Thinakaran and colleagues to reproduce our results might have been their use of time points that were too late (the activation of Ire1α and PERK in their study was observed 5 h after ER stress). Another possibility might be that they used cells (HEK 293 and Neuro2a) that are less sensitive to ER stress. Although HEK 293 cells were also used in our paper, they were transiently transfected and overexpressed FAD PS1 mutants. Although HEK 293 cells overexpressing PS1 mutants show the downregulation of the UPR signalling, it is difficult to detect in the stable transfectants of HEK 293 cells as used in their paper.

Last, preincubation for 1 h with fresh medium before treatment with agents is most important to obtain constant data for the ER stress response because the induction levels of BiP/GRP78 mRNA are considerably altered if this change of media is not conducted. The decrease in BiP/GRP78 mRNA induction in cells expressing FAD PS1 mutations was ~30% and 50% of controls in PS1 mutation knock-in fibroblasts and in stably transfected SK-N-SH cells, respectively. To detect these subtle defects in the UPR in PS1 mutation-expressing cells, the cells should be carefully handled under the same experimental conditions.

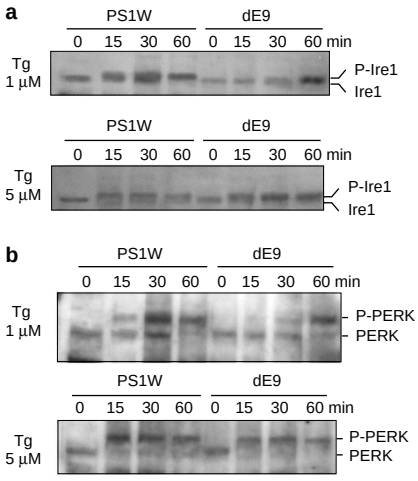

Figure 1. **Inhibited activation of endogenous ER stress transducers. a**, SK-N-SH cells stably transfected with each PS1 construct were stimulated with thapsigargin (Tg, 1 and 5 μM) for the indicated durations, and Ire1α was detected by western blotting. When cells were treated with 1 μM Tg (upper panel), the Ire1α bands were completely shifted (phosphorylated) in cells expressing wild-type PS1 (PS1W) within 15 min. In contrast, no shift of Ire1α-immunoreactive bands was seen in PS1ΔE9 (dE9)-expressing cells that were treated with the same dose of Tg within 30 min. At 60 min after ER stress, Ire1α was phosphorylated even in mutant cells. When cells were treated with 5 μM Tg (lower panel), Ire1α was phosphorylated within 15 min in both cell lines. **b**, SK-N-SH cells stably expressing PS1 constructs were treated with 1 μM (upper) and 5 μM (lower) Tg, and the changes in the levels of PERK phosphorylation were examined. The PS1 mutation inhibited or delayed the phosphorylation of PERK after ER stress when cells were treated with 1 μM Tg, but at 60 min after ER stress, PERK was almost completely phosphorylated in both cell lines. The differences in phosphorylation kinetics of PERK between wild-type and mutant cells disappeared when cells were treated with 5 μM Tg.

K. Imaizumi*, T. Katayama†‡, M. Tohyama†‡

**Division of Structural Cell Biology, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-0101, Japan; †Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; ‡CREST of Japan Science and Technology Corporation (JST), 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
e-mail: imaizumi@bs.aist-nara.ac.jp

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More light on *Decoding Darkness*

To the editor — This letter concerns my recent review of the book, *Decoding Darkness: The Search for the Genetic Causes of Alzheimer's Disease* by Rudolph Tanzi and Ann Parson[1]. Several errors have been pointed out to me and I would like to alert readers to these. First, the 'errors in fact' mentioned in the review were few and of a minor nature. Second, an allusion was made regarding the confusion of the original linkage of the Massachusetts General Hospital families with familial Alzheimer's disease (AD) to chromosome 21, which was subsequently shown to contain a presenilin 1 mutation. I thought the text might have explained the reassignment of the gene locus from chromosome 21 to 14 in a way that could confuse the casual reader. In fact, the book clearly explains that lod (logarithm of the odds) scores are statistical results that are prone to error, as in the initial assignment of chromosome 21. The statement that the readers might be left hanging on some of these issues was therefore inappropriate.

The achievements in AD research by Rudy Tanzi's laboratory within the past two decades have been outstanding. The presentation of these achievements, and those of others, in a book such as *Decoding Darkness* provides an invaluable reference for AD researchers and much information and inspiration for the general public. I hope that readers will share my overall excitement about the book and its many positive aspects and not be distracted by the minor concerns that were expressed in the review.

E. Koo

Neurosciences, 0691, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0691, USA
e-mail: edkoo@ucsd.edu

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