Reviewer's report

Title: Detection of the GPI-anchorless prion protein fragment PrP226* in human brain

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Reviewer: Stanley B. Prusiner

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The manuscript by Dvorakova et al. describes the presence of a C-terminally truncated fragment (at position 226, denoted PrP226*) of PrP in brain tissue of patients with prion diseases and healthy controls. Moreover, the paper shows that PrP226* participates in the formation of alternative folded, PK-resistant aggregates in several CJD cases. The analysis was performed by DELFIA measurements of total PrP226* in native or denatured samples. This follows the conformational dependent immunoassay (CDI); the comparison of CDI (total PrP) and PrP226* DELFIA (specific for PrP226*) data established a correlation between these two assays. Furthermore, it adds to the general knowledge of PrP isoforms present in detectable amounts in human brain tissue. This study raises the general question about the generation of PrP226* and its role in physiological processes or disease.

The manuscript can be considered for publication in BMC Neurology after clarification and addition of the following points:

1.) PK-resistant PrP226* was detected with a highly specific antibody. The authors should add immunoblots of patient tissue using this particular antibody to compare glycosylation patterns and size of PK-resistant PrP226* to the GPI-anchored full-length PrP isoforms usually visualized with antibodies detecting all isoforms.

2.) Error bars are presented in Figure 1E but not in Figure 1A and C. Please add.

3.) The authors need to discuss the abnormality in PrP226* DELFIA measurements from healthy patients. I would have expected that the ratio of D/N measurements to be ~1 in a healthy individual. Do the authors see the same effect with recombinant PrP226* or is this exclusive to PrP226* derived from human tissue?

4.) The authors should display the genotype of the GSS patient because the denatured PrP226* DELFIA value is highest from this patient compared to all other examined samples. It would be important to know if the GSS mutation generates this C-terminally truncated PrP without the GPI anchor attached, e.g., the Y226X mutation as reported by Jansen et al.

Level of interest: An article whose findings are important to those with closely
related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.