The Journal of Pathology: Clinical Research

Supplementary Figures

Rapid amplification of prions from variant Creutzfeldt-Jakob disease cerebrospinal fluid

Marcelo A Barria, Andrew Lee, Alison Green, Richard Knight, Mark Head

National CJD Research & Surveillance Unit, Centre for Clinical Brain Sciences, Deanery of Clinical Medicine, The University of Edinburgh, The United Kingdom.

Author for correspondence: Dr Marcelo A Barria, Bryan Matthews Building, Western General Hospital, Edinburgh, EH4 2XU, United Kingdom. Tel: +44 131 537 3483. E-mail: Marcelo.Barria@ed.ac.uk

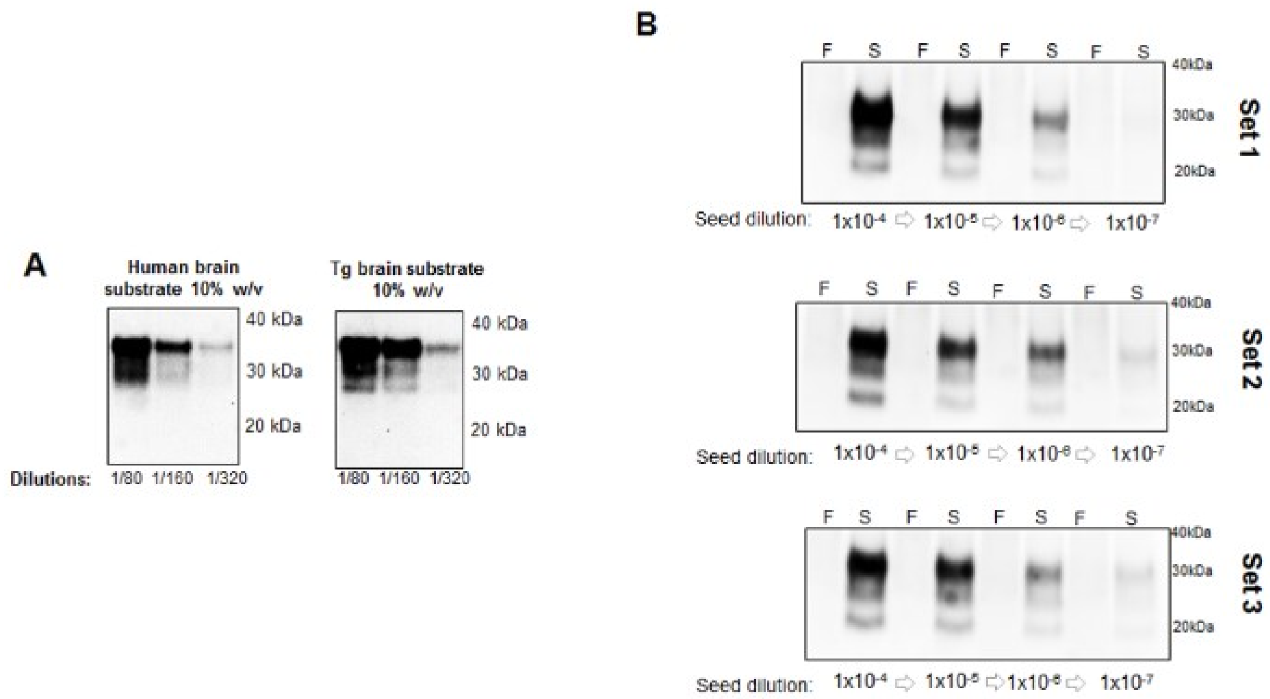

Figure S1: Evaluation of human PrP expression levels in the humanised transgenic mouse model used for substrate and the ability to support *in vitro* conversion by PMCA. (**A**) Brain material derived from transgenic mouse containing the human form of the prion protein homozygous for methionine at codon 129 (Tg brain substrate), and non-CJD human post-mortem brain tissue with an equivalent codon 129 polymorphism (Human brain substrate), were homogenised at 10% weight/volume (10% w/v). The samples were further diluted in conversion buffer to compare the total PrP expression levels (dilutions: 1/80, 1/160, 1/320). Detection of human PrP was assessed by Western blotting using 3F4 mAb. The human PrP was detected in the form of two major bands in the range of 20–40-kDa molecular mass. (**B**) Amplification of vCJD prions using the standard protocol of PMCA. Serial dilutions of 10% w/v vCJD human brain homogenate ($1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$) were diluted in substrate and amplified for 96 cycles (48 hrs). The amplification of the serially diluted vCJD samples were performed in triplicate (Set1, Set 2 and Set 3). Aliquots of the seeded amplification mixture were retained (Frozen samples “F”) for comparison to the samples subject to amplification (Sonicate samples “S”). Samples were treated with proteinase K, and PrP^{res} was evaluated by Western blotting using 3F4 mAb. The amplified sample showed a type 2B banding pattern typically observed in vCJD. Reference molecular marker have been included. Molecular mass of electrophoretic markers is given in kilodaltons (kDa). At least three technical repeats were performed with similar results and a representative Western blot presented.

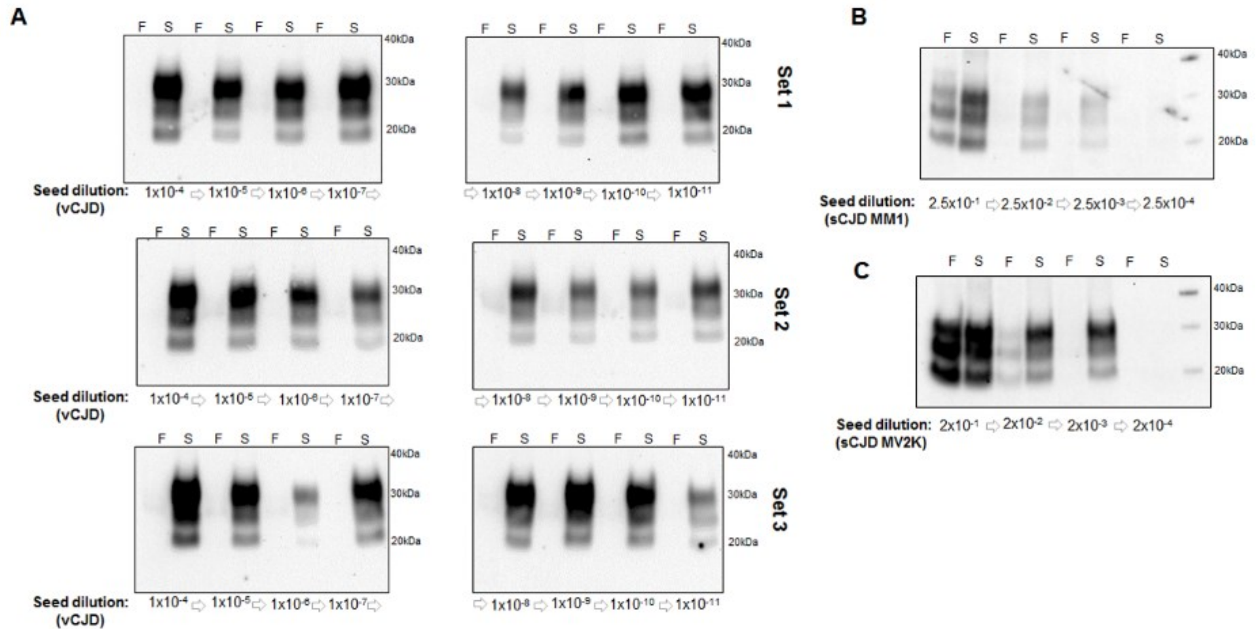

Figure S2: Amplification of CJD prions by hsPMCA. (**A**) Serial dilutions of 10% w/v vCJD human brain homogenate diluted in codon 129MM transgenic mouse substrate ($1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ $1x10^{-8}$, $1x10^{-9}$, $1x10^{-10}$, and $1x10^{-11}$) were amplified by hsPMCA for 96 cycles (48 hrs). Amplification of the serially diluted vCJD samples by hsPMCA was performed in triplicate (Set1, Set 2 and Set 3). Samples retained as frozen ("F") or subjected to amplification by sonication ("S") were proteinase K digested and PrP^{res} was evaluated by Western blotting using 3F4 mAb. The amplified sample showed a type 2B banding pattern typically observed in vCJD. (**B**) Amplification of sCJD MM1 prions derived from 10% w/v human brain homogenate diluted in codon 129MM transgenic mouse substrate ($2.5x10^{-1}$, $2.5x10^{-2}$, $2.5x10^{-3}$ and $2.5x10^{-4}$) and subjected to hsPMCA for 96 cycles (**C**) Amplification of sCJD MV2K prions derived from 10% w/v human brain homogenate diluted in codon 129MM transgenic mouse substrate ($2x10^{-1}$, $2x10^{-2}$, $2x10^{-3}$ and $2x10^{-4}$) and subjected to hsPMCA for 96 cycles. Reference molecular marker have been included. Molecular mass of electrophoretic markers is given in kilodaltons (kDa). At least three technical repeats for vCJD and 3 confirmed sCJD MM1 and MV2K cases were evaluated with similar results. A representative Western blot is presented.