# Discrimination of prion strain targeting in the CNS via reactive astrocyte heterogeneity in CD44 expression

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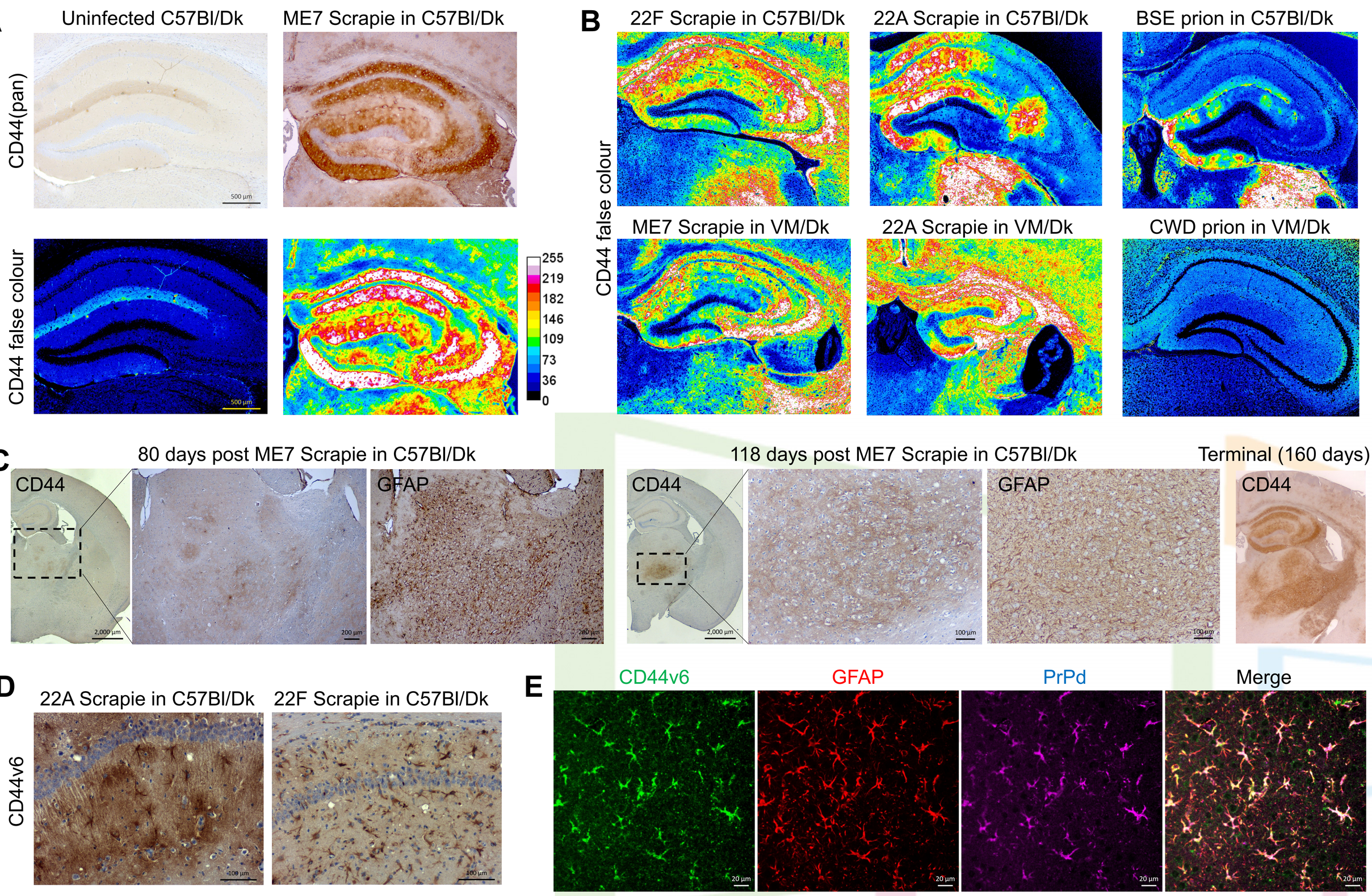
## Introduction

Prion diseases result in progressive accumulation of misfolded prion protein in the central nervous system, vacuolar spongiform change, neurodegeneration and reactive glial responses and are ultimately fatal. Many different prion agent strains exist which differ in disease duration, clinical signs and targeting of neuropathology. We identified that the transmembrane glycoprotein CD44 is highly upregulated in subsets of reactive astrocytes in regions of the brain specifically targeted by prions. The pattern of CD44 upregulation was unique for each combination of prion agent strain and host genotype and suggest an early and critical role for astrocytes in neurodegeneration.

## Methods

The distribution of CD44 was identified by immunohistochemistry in formalin fixed paraffin embedded terminal mouse brain samples from 15 distinct prion agent strains in both *Prnp*-a (C57Bl6/Dk) and *Prnp*-b (VM/Dk) genotype mice sourced from The Roslin Institute archive. For comparison and strain confirmation, spongiform vacuolation pattern (lesion profile), prion protein deposition ($PrP^d$), reactive microglial (AIF1) and astrocyte (GFAP) IHC were also performed on serial sections.

## Results




**A** Immunohistochemical analysis using N-terminal pan CD44 antibody clone IM7 reveals minimal staining in normal uninfected mouse brain but significant upregulation of CD44 following prion infection. DAB staining were false coloured using a 16 colour LUT following Haematoxylin-DAB deconvolution in Image J to represent staining intensity. **B** False-coloured panCD44 staining from a wide array of prion strains, including natural and experimental scrapie, Bovine spongiform encephalopathy (BSE) and Chronic wasting disease (CWD) in different Prnp genotype hosts reveals a unique pattern for every combination. **C** CD44 upregulation is observed at the earliest signs of neuropathological change in the primary target area (thalamus) and progresses throughout disease**. D** Immunohistochemical analysis using the splice variant 6 (CD44v6) antibody clone 9A4 confirms the astrocytic source of CD44 and upregulated expression of specific CD44 splice variants following prion infection. **E** Co-immunolocalisation of CD44v6, GFAP and disease-associated prion protein reveals CD44 expressing astrocytes act as reservoirs for prion replication in the central nervous system during prion infection.

## Conclusions

- Astrocyte heterogeneity revealed by differential expression of CD44 and CD44 splice variants during prion infection
- Early and critical role for astrocyte response in neuroinflammation and neurodegenerative diseases
- CD44+ astrocytes reveal targeting of prion strains allowing complete identification of both prion strain and host genotype
- CD44+ astrocytes reported in Alzheimer's, Amyotrophic lateral sclerosis and Alexander's disease patients and is a potential biomarker for defining differential targeting (sub-type diagnosis) in human neurodegenerative diseases

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