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# Change in the molecular properties of CH1641 prions after transmission to wild-type mice: Evidence for a single strain

Lucien J. M. van Keulen<sup>1</sup>  | Corry H. Dolstra<sup>1</sup> | Ruth Bossers-de Vries<sup>1</sup> |  
Alex Bossers<sup>1</sup> | Jorg G. Jacobs<sup>1†</sup> | Thierry Baron<sup>2</sup>  | Juan Maria Torres<sup>3</sup> |  
Jan P. M. Langeveld<sup>1</sup>

<sup>1</sup>Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, the Netherlands

<sup>2</sup>Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail (Anses), Laboratoire de Lyon, Unité Maladies Neurodégénératives, Lyon, France

<sup>3</sup>Centro de Investigación en Sanidad Animal (CISA), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Valdeolmos, Spain

## Correspondence

Lucien J.M. van Keulen, Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands.  
Email: [luccien.vankeulen@wur.nl](mailto:luccien.vankeulen@wur.nl)

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## Abstract

**Aim:** CH1641 was discovered in 1970 as a scrapie isolate that was unlike all other classical strains of scrapie isolated so far. We performed bio-assays of CH1641 in mice in order to further characterise this specific isolate.

**Methods:** We inoculated the original CH1641 isolate into ovine and bovine prion protein (PrP) transgenic mice as well as wild-type mice. In addition, we performed cross- and back passages between the various mouse lines to examine if one identical prion strain was isolated in all mouse lines or whether multiple prion strains exist in CH1641.

**Results:** We report the first successful transmission of CH1641 to wild-type RIII mice and via RIII mice to wild-type VM mice. Unexpectedly, analysis of the protease-resistant prion protein (PrP<sup>res</sup>) in wild-type mice showed a classical scrapie banding pattern differing from the banding pattern of the original CH1641 isolate. Cross- and back passages of CH1641 between the various mouse lines confirmed that the same prion strain had been isolated in all mouse lines.

**Conclusions:** The CH1641 isolate consists of a single prion strain but its molecular banding pattern of PrP<sup>res</sup> differs between wild-type mice and PrP transgenic mice. Consequently, molecular banding patterns of PrP<sup>res</sup> should be used with caution in strain typing since they do not solely depend on the properties of the prion strain but also on the host prion protein.

## KEYWORDS

classical scrapie, protease resistant prion, PrP protein, PrP<sup>Sc</sup>, strain typing, transgenic mice

**Abbreviations:** AR, attack rate; IP, incubation period; Prnp, prion protein gene (mice); PRNP, prion protein gene (sheep); PrP, prion protein; PrP<sup>C</sup>, cellular prion protein; PrP<sup>res</sup>, protease-resistant prion protein; PrP<sup>Sc</sup>, scrapie-associated prion protein; Sinc, scrapie incubation period gene (mice); Sip, scrapie incubation period gene (sheep).

Jan P.M. Langeveld, Retired.

† Deceased in 2017.

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## INTRODUCTION

CH1641 was originally isolated in 1970 in the United Kingdom from a natural case of scrapie in a Cheviot sheep (ear tag number 16x41) [1]. This animal originated from the so-called positive line of Cheviot sheep, carrying the scrapie incubation period (Sip) gene sA/sA, which is associated with a short incubation period and high susceptibility to natural scrapie. The sA/sA genotype is now known to be mainly linked to the VRQ allele of the ovine prion protein gene (PRNP) with valine, arginine and glutamine coded at codons 136, 154 and 171, respectively [2]. The isolate was further passaged in the negative line of Cheviot sheep with the Sip pA/pA genotype closely linked to the ARQ, AHQ and ARR alleles of the PRNP gene [2, 3]. Sheep from this negative line typically showed a prolonged incubation period compared with sheep of the Sip sA/sA line when challenged with most classical scrapie strains. However, when infected with CH1641 the reverse occurred: a short incubation period was seen in sheep of the Sip pA/pA genotype while the incubation period in sheep of the Sip sA/sA genotype was prolonged. This showed that CH1641 was clearly different from previously isolated strains of scrapie although at the time, it was unknown whether CH1641 was a single strain of scrapie or consisted of a mixture of scrapie strains. Attempts to transmit CH1641 to C57Bl and VM mice failed, and it was therefore concluded that CH1641 also did not transmit to wild-type mice in contrast to most other classical strains of scrapie [4].

In prion-infected animals or humans, the conversion of the normal cellular form of the prion protein (PrP<sup>C</sup>) into a protease-resistant form results in the accumulation of aberrant forms of prion protein called scrapie-associated PrP (PrP<sup>Sc</sup>) in nervous and sometimes lymphoid tissues. In vitro proteolysis of PrP<sup>Sc</sup> leads to variably N-terminally shortened fragments of protease-resistant PrP (PrP<sup>res</sup>). Western blot analysis of tissue homogenates under experimental proteolytic conditions can reveal variations in molecular PrP<sup>res</sup> patterns that have been associated with specific prion strains. Both in CH1641 and BSE-infected sheep and goats, proteolysis of PrP<sup>res</sup> results in a ~19 kDa unglycosylated PrP<sup>res</sup> band compared with a ~21 kDa band with most other classical scrapie cases. This extended N-terminal proteolysis of PrP<sup>res</sup> in CH1641 and BSE results in the loss of the PrP epitope region 80–99, which can be demonstrated with monoclonal antibodies directed to this specific region using Western blotting or immunohistochemistry (IHC) [3, 5–9]. CH1641 can be discriminated from BSE in sheep by the existence of a second population of PrP<sup>res</sup> (PrP<sup>res</sup>#2) consisting of a C-terminal fragment with an approximate size of the unglycosylated PrP<sup>res</sup> band of ~10–14 kDa [6, 10, 11]. This fragment can be demonstrated only with antibodies that recognise epitopes in the C-terminal region ~165–234. Despite these specific differences, some scrapie field isolates remain that cannot unequivocally be identified because they show intermediate characteristics between CH1641 and BSE [11–13]. In these cases, strain typing in mice is often the last resort to definitively characterise prion strains. However, only limited attempts to strain type the original CH1641 strain

### Key Points

- For the first time, the original CH1641 isolate has been transmitted to wild-type mice.
- The resulting molecular profile of CH641 in wild-type mice is different from the original CH1641 homogenate and its transmissions to (b)ovinizied mice.
- This is not caused by the selection of a different prion strain from a mixture of strains that could be present in CH1641, as we demonstrate that CH1641 is composed of a single strain.

have been undertaken in transgenic mice and transmission to wild-type mice has been reported to be unsuccessful so far [1, 4, 14]. There have been reports of transmission of ‘CH1641-like’ field cases to transgenic and wild-type mice but it remains unclear whether the strain isolated in wild-type mice was identical to CH1641 or if another strain had been isolated from a mixture of scrapie strains often present in scrapie field cases [13, 15–17]. We report here the successful transmission of the original CH1641 isolate to wild-type mice together with a detailed description of the molecular banding pattern of PrP<sup>res</sup> and the immunohistochemical profile of PrP<sup>Sc</sup> in both wild-type and transgenic mice. To ensure that the same strain had been isolated in each mouse line, cross passages were undertaken between the various mouse lines. We show that upon transmission to wild-type mice, CH1641 loses its specific molecular characteristics while preserving its unique strain properties.

## MATERIALS AND METHODS

### Mouse lines

All mouse lines were bred in-house and were originally founded by breeding couples that were kindly donated by colleagues from other research institutes (see Acknowledgements). Non-transgenic mice comprised the RIII mice carrying the scrapie incubation period (Sinc)/prion protein (Prnp) gene Sinc<sup>s7</sup>/Prnp<sup>a</sup> encoding 108L and 189T on the mouse PrP gene, and the VM mice (Sinc<sup>p7</sup> or Prnp<sup>b</sup>) encoding 108F and 189V on the mouse PrP gene [18, 19].

Transgenic mice expressing the ovine or bovine PrP allele were established under a murine Prnp<sup>0/0</sup> genetic background by random integration. Tg338 mice express ~8-fold the V<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele of the ovine PrP [20], Tg59 mice (or Tg59) express ~3-fold the A<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele of the ovine PrP [21] and Tg110 express ~8-fold the bovine PrP allele [22] compared with PrP<sup>C</sup> levels in the natural host.

## CH1641 inoculum

CH1641 was kindly donated by Dr Jim Foster of the former Neuro-pathogenesis Unit (NPU), currently part of the Roslin Institute and R(D)SVS, University of Edinburgh, UK. This strain was originally isolated from a natural case of scrapie in the NPU positive line (Sip sA/sA) Cheviot flock and had been experimentally passaged in sheep of the negative line (Sip pA/pA). The fourth passage of CH1641 was used as the inoculum for the mouse inoculations in our experiments and consisted of a pool of five AXQ/AXQ sheep brains (X indicating that either arginine (R) or histidine (H) is present at codon 154). In addition, two wild-type sheep and one wild-type goat were inoculated intracerebrally in order to obtain larger quantities of ovine and caprine CH1641 brain tissue as reference material.

## Mouse inoculations

For mouse passage, a 10% w/v (primary passage) or 1% w/v (second and higher passages) brain homogenate in physiological saline containing 1.4 mg/ml ampicillin was produced with an Ultra-Turrax® T25 homogeniser (IKA, Germany). The dispersing rotors of the homogeniser had been decontaminated twice (once in an assembled state and once in a disassembled state) with each decontamination cycle consisting of at least 24 h immersion in 2 M sodium hydroxide followed by porous-load autoclaving at 134°C and 3 bar for 30 min to ensure absolute prion sterility. After the homogenate had been routinely checked for bacteriological sterility, 0.02 ml was inoculated under general anaesthesia into the left cerebral hemisphere of groups of 5–20 RIII, VM, Tg338, Tg59 or Tg110 mice. For cross passage between the various mouse lines, a mouse brain from at least the second or higher passage in the donor mouse line was used in order to ensure that no detectable remnants of the original CH1641 inoculum were present (dilution original inoculum in the third passage  $> 10^5$ ).

## Calculation of incubation period and attack rate

Mice were monitored daily for clinical disease and euthanised at terminal disease. The incubation period (IP) was measured as the number of days between inoculation and terminal end-point/death. Mean IP  $\pm$  sd (in days) were calculated for all groups of mice. Attack rates (AR) were calculated as the number of mice that were clinically positive and PrP<sup>Sc</sup> positive (cl+/ PrP<sup>Sc</sup>+) divided by the number of injected mice still alive at the time of the first cl+/ PrP<sup>Sc</sup> + mouse. Animals that were cl-/PrP<sup>Sc</sup> + were considered to be pre-clinically infected and were excluded from the calculations of the mean IP and AR. In case of a negative transmission, the survival time of the last surviving mouse is shown as an indication of the maximum life expectancy and the AR shown as 0/total number of mice injected. Secondary (blind) transmissions from pooled mouse brains from the primary passage were undertaken to distinguish between true negative, sub-clinical and non-adaptive amplification in the primary transmission.

Transmissions were considered to be stabilised when the IP of the last passage did not statistically differ from the IP of the previous passage in the same mouse line (Welch's *t*-test,  $p > 0.05$ ).

## Necropsy and histological procedures

Mice that were found dead were decapitated and the heads frozen at  $-20^{\circ}\text{C}$  until further processing. The frozen brain was collected using TSE sterile instruments and cut sagittally. One half of the brain was fixed in 10% neutral buffered formalin for 24 h while the other half was stored at  $-20^{\circ}\text{C}$  for sub-passage or Western blot analysis.

Mice that were at the endpoint of terminal disease were euthanised by cervical dislocation and decapitated. The top of the skull was carefully removed and the head was either frozen at  $-20^{\circ}\text{C}$  and processed as described above for the mice that were found dead or fixed in 10% phosphate-buffered formalin for at least 24 h. After formalin fixation, the brain was removed from the skull and cut into the following coronal levels: A. septal nuclei, corpus striatum and anterior cortex; B. diencephalon (thalamus and hypothalamus), hippocampus, lateral and entorhinal cortex; C. mesencephalon, pons and occipital cerebral cortex; D. pons and anterior cerebellum.

Formalin-fixed coronal and sagittal brain halves were fixed for another 24 h before routine processing into paraffin blocks. Paraffin blocks were cut into 4  $\mu\text{m}$  thick sections that were collected on aminopropyltri-ethoxysilane (Sigma, USA) coated glass slides and dried for at least 48 h in a 37°C incubator.

## Antibodies

Purified PrP-specific monoclonal antibodies that were used included: 12B2 (<sub>93</sub>WGQGG<sub>97</sub>) and 6C2 (<sub>114</sub>HVAGAAA<sub>120</sub>) (WBVR, Lelystad, the Netherlands), Sha31 (<sub>148</sub>YEDRYRE<sub>155</sub>) and SAF84 (<sub>166</sub>YRPVDQY<sub>172</sub>) (Bertin Pharma, France) and R145 (<sub>213</sub>VEQMCIT-QYQR<sub>223</sub>) (rat monoclonal, APHA, UK). Corresponding epitopes are based on sheep PrP amino acid numbering as mapped by Pepscan analysis using 15-mer solid phase synthetic peptides [23]. Only the C-terminal antibody SAF84 also recognises PrP<sup>res</sup>#2 in CH1641 scrapie [6, 10].

## SDS-page and Western blot analysis

Longitudinally cut mouse brain halves were homogenised with disposable polypropylene pestles to 10% (w/v) homogenates in lysis buffer and digested with proteinase K as described previously [24]. Duplicate samples were either deglycosylated with PNGase F (P0704S kit, New England Biolabs) according to the manufacturer's protocol or treated similarly without adding PNGase F. The PNGase F digested sample and non-digested sample were supplemented with PBS13 to a final volume of 100  $\mu\text{l}$  and dialysed (Slide-A-Lyzer™ MINI Dialysis Device, ThermoFisher Scientific 69,560). After dialysis, the samples were

precipitated with three volumes of ice-cold acetone and incubated for 2 h at 20°C. Samples were centrifuged for 10 min at 21,000 ×g and 4°C and the pellets resuspended in NuPAGE LDS sample buffer. Prior to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), samples were denatured by heating for 10 min at 96°C under disulfide-bond-reducing conditions. For electrophoresis, 17 well NuPAGE 12% Bis Tris 1.0 mm precast gels were run in triplicate in two morpholinoethanesulfonic acid buffer at 100 V for 80 min. Per lane, 0.9 mg tissue equivalents (TE) were applied in 10 µl loading buffer. Rec-ovPrP ARQ and molecular mass See Blue markers (Invitrogen) were included as references. The three gels were electro-transferred simultaneously onto PVDF membranes (Immobilon-FL, Millipore) with 20% methanol transfer buffer (Novex) for 30 min at 50 V followed by 45 min at 150 V. After transfer the three blots were blocked overnight at 4°C with 5% casein in TRIS-buffered solution with Tween 20 (TBST, VWR). The three blots were separately developed with one of three different monoclonal antibodies: 12B2, Sha31, and SAF84 at respective concentrations of 0.2, 0.25, and 0.5 µg IgG/ml in TBST with 5% casein for 1 h at room temperature. After incubation the membranes were washed three times for 5 min with TBST and the secondary antibody Rabbit anti-mouse-AP (Dako/Agilent) was applied at a concentration of 0.46 µg IgG/ml in TBST with 5% casein for 1 h. After washing three times for 5 min with TBST and an additional 5 min with UltraPure distilled water, colour was developed with the substrate BCIP/NBT (Sigma) for 5 min. Blots were scanned with the Amersham Imager 600 and molecular weights were determined with ImageQuant 1D gel analysis.

### Immunohistochemistry and PrP<sup>Sc</sup> profiling

Sections were deparaffinised in xylene, rehydrated in graded alcohols and pre-treated by immersion in formic acid for 30 min followed by autoclaving at 121°C for 5 min in citrate buffer pH 6.0 (Antigen Unmasking Solution, Vector Laboratories, UK). After cooling down with running tap water, sections were incubated for 60 min with the mouse monoclonal antibody 6C2 for the wild-type mice or the rat monoclonal antibody R145 for the transgenic mice (both at 0.4 µg

IgG/ml). An HRP anti-mouse IgG polymer or anti-rat IgG polymer (ImmPRESS, Vector Laboratories) was used respectively as the secondary antibody, and colour was developed with diaminobenzidine (DAB+) substrate (Dako, Agilent, USA). DAB colour was intensified by 10 min of immersion in 0.5% CuSO<sub>4</sub> solution (Merck, USA). Sections were briefly counterstained with haematoxylin, dehydrated in graded alcohols and mounted permanently.

Stained sections were scanned with a BX51 microscope equipped with a motorised stage and Cell Sense<sup>®</sup> imaging software (Olympus, Germany) as described previously [25]. Each brain section was photographed using overlapping images, which were aligned to give a high-resolution overall image of the whole brain section. True colour IHC images were phase colour-coded according to the intensity of the DAB immunolabelling with dark brown pixels (intensity 1–60) displayed in dark red, middle brown pixels (intensity 61–130) in red, and light brown pixels (intensity 131–190) in yellow.

### Statistical analysis

Statistical analyses of incubation periods were performed using GraphPad Prism9 (GraphPad Software Inc., USA). One-way analysis of variance was used to establish whether variations between groups of data were greater than expected. Differences between pairs of groups were considered statistically significant if the probability for equality was <0.05 in multiple comparison tests (Welch's *t*-test).

## RESULTS

### Homologous passage of ovine CH1641

#### Transmission

CH1641 transmitted to all mouse lines with a 100% attack rate except for the VM mice in which transmission did not result in clinical disease at first or second passage although some mice in the primary passage showed small PrP<sup>Sc</sup> deposits in the brain at necropsy (Table 1). In the

**TABLE 1** Incubation periods and attack rates of the homologous passages of ovine CH1641 in the various mouse lines

Passage	R111	VM	Tg338	Tg59	Tg110
1	794 ± 149 (18/18)	>845 (0/20) <sup>a</sup>	160 ± 15 (19/19)	283 ± 46 (12/12)	243 ± 29 (12/12)
2	737 ± 62 (12/12)	>828 (0/15) <sup>b</sup>	158 ± 3 (15/15)	255 ± 11 (13/13)	214 ± 19 (4/4)
3	622 ± 69 (9/9)		153 ± 3 (4/4)	262 ± 15 (14/14)	210 ± 14 (5/5)
4	510 ± 13 (10/10)		154 ± 5 (5/5)	258 ± 14 (8/9)	218 ± 8 (5/5)
5	491 ± 9 (10/10)				
6	477 ± 6 (10/10)				

Incubation periods are defined as the number of days between inoculation and terminal disease/death expressed as the mean ± standard deviation. The attack rates are shown in brackets.

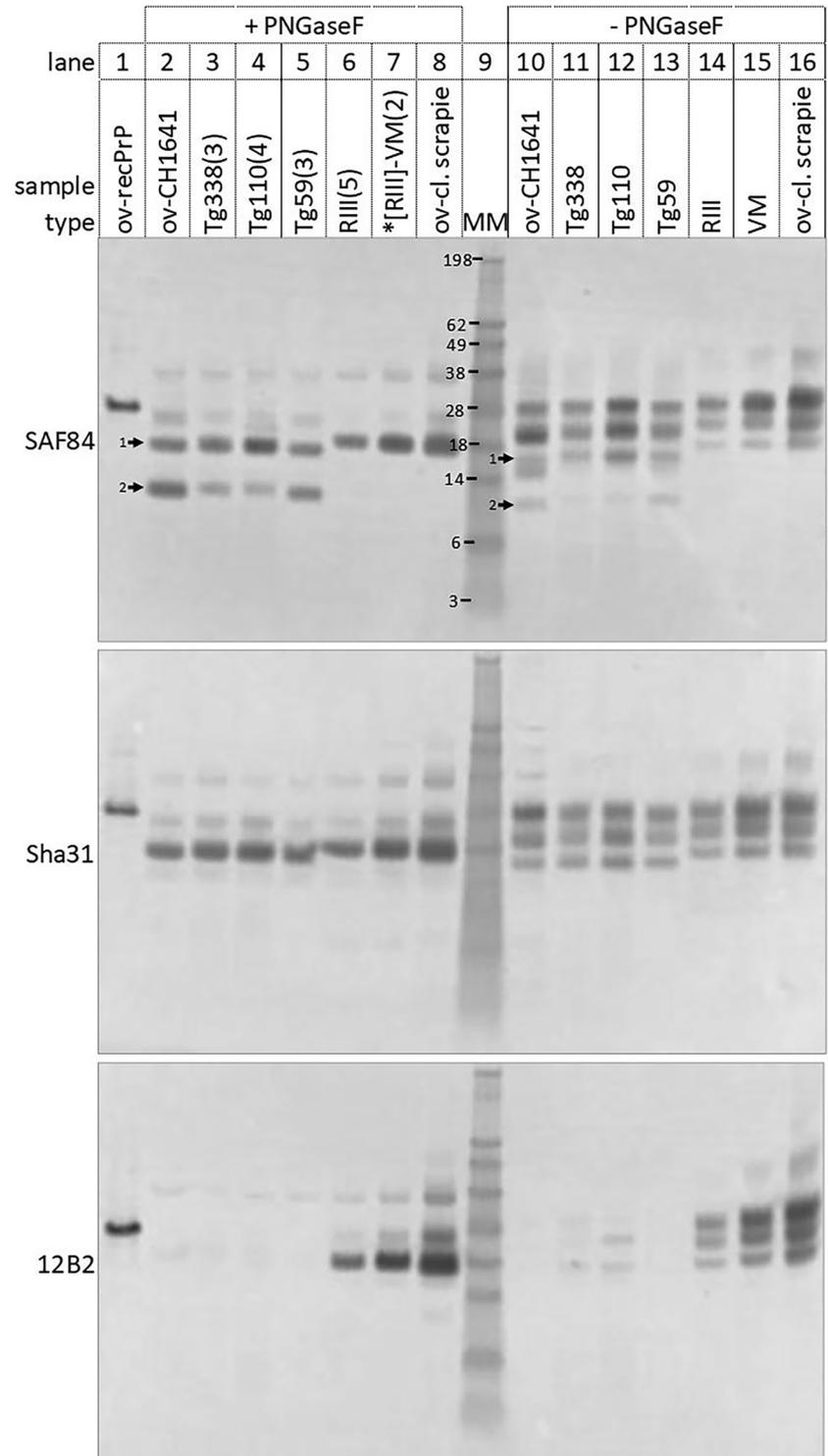
<sup>a</sup>No clinical disease but 3/20 mice showed PrP<sup>Sc</sup> deposits in the brain at necropsy.

<sup>b</sup>Blind passage of a 10% homogenate of pooled mouse brains from the primary passage.

R111 mice, the incubation period to terminal disease at primary passage was quite long ( $794 \pm 149$  days) and decreased with subsequent passages to  $477 \pm 6$  days at the sixth passage. It remains to be seen whether stabilisation has been reached as the 7th passage is still running. In the transgenic mice, incubation periods at primary passage were much shorter and stabilised already at the second passage (Table 1).

### PrP<sup>res</sup> Western blot analysis

With western blotting, the passage of CH1641 into the three transgenic mouse lines yielded PrP<sup>res</sup> patterns which were comparable with the original sheep CH1641 homogenate showing the two triplet PrP<sup>res</sup> populations #1 and #2 (Figure 1, upper blot). This was most obvious when the glycosidic groups had been removed by enzymatic



**FIGURE 1** Homologous passage of ovine CH1641 in the various mouse lines. Parallel runs of western blotting with brain homogenates from the homologues passage of CH1641 in the various mouse lines, immunoblotted with three different PrP-specific monoclonal antibodies. Passage to the transgenic mouse lines yields a similar dual triplet pattern as the original sheep CH1641 homogenate whereas passage into R111 mice yields a single triplet pattern comparable with classical scrapie. The position of the unglycosylated PrP<sup>res</sup> bands 1 and 2 as detected in the SAF84 immunoblots is indicated by arrows. Lane 1: full-length ovine recPrP (ARQ). Lane 2: original ovCH1641 homogenate. Lanes 3–6 homologous passage of ovCH1641 in Tg338, Tg110, Tg59 and R111 mice respectively. Lane 7: passage of CH1641 from R111 mouse into VM mice. Lane 8: classical ChCH1641sheep scrapie. Lane 9: molecular mass SeeBlue standards in kDa. The passage number is shown in brackets. Lanes 10–16 are identical to lanes 2–8 but not treated with PNGaseF. PrP<sup>res</sup> samples per lane applied correspond to 0.9 mg equivalents of brain tissue. \*After the initial passage in R111.

digestion with PNGase F (Figure 1, lanes 3–5). This treatment yielded two bands of unglycosylated PrP<sup>res</sup> with molecular masses similar to sheep CH1641 scrapie: band 1 with a molecular weight of  $\sim 15.8 \pm 0.2$  kDa and band 2 with a molecular weight of  $\sim 11.0 \pm 0.3$  kDa (Figure S1.A). Band 1 was only seen with SAF84 and Sha31 and not with monoclonal 12B2 indicating the absence of the 12B2 epitope (<sub>93</sub>WGQGG<sub>97</sub>) located in the N terminal region of PrP<sup>res</sup> (Figure 1, lower blot). Band 2 appeared reactive only with the monoclonal antibody SAF84 but not with Sha31 and 12B2 indicating a C-terminal fragment. In wild-type RIII mice, however, a western blot pattern similar to classical sheep scrapie was observed with only one PrP<sup>res</sup> band of  $\sim 18.0 \pm 0.3$  kDa after deglycosylation and a significant level of the 12B2 epitope (Figure 1, lanes 6 and 8, Figure S1.A).

### PrP<sup>Sc</sup> profiling

PrP<sup>Sc</sup> immunohistochemical profiles of the homologous passage of CH1641 in each mouse line are shown in Figures S2–S5 together with a specification of the neuro-anatomical areas affected (Table S2). In the brain of RIII mice, PrP<sup>Sc</sup> depositions were much heavier and involved different neuro-anatomical sites compared with transgenic mice (Figure S2 vs Figures S3–S5). Especially in the stratum granulosum of the cerebellum and in the sensory and motor nuclei of the brainstem, heavy PrP<sup>Sc</sup> depositions were seen in the RIII mice, which

were absent in the transgenic mice. In the transgenic mice, PrP<sup>Sc</sup> depositions were generally much finer, often intracellular and more diffusely spread throughout the brain (Figures S3–S5).

## Cross passage of mouse passaged CH1641

### Cross passage to wild-type mice

To verify that the same prion strain had been isolated in each mouse line, the homologous passages of CH1641 were cross-passaged between all mouse lines with the VM mice serving only as recipient mice since the passage of ovCH1641 to VM mice had been unsuccessful. Surprisingly, CH1641 passaged through RIII did transmit to VM mice with a 100% attack rate and an incubation period that stabilised around 330–340 days at the third passage. Transmission of CH1641 from transgenic mice to VM mice did not result in clinical disease although PrP<sup>Sc</sup> deposits were detected in some mice at necropsy on primary passage but not on secondary passage (Table 2). Western blotting of the brains of CH1641-infected VM mice yielded a classical scrapie PrP<sup>res</sup> pattern similar to the homologous passage of CH1641 in RIII mice (Figure 1, lanes 7 and 15). The immunohistochemical PrP<sup>Sc</sup> profile of CH1641 in VM mice showed heavy accumulations of PrP<sup>Sc</sup> and a distinctive neuroanatomical pattern (Figure S6).

**TABLE 2** Incubation periods and attack rates of the cross passages of mouse passaged CH1641.

		CH1641-donor mouse line (passage number)				
		CH1641-RIII(3)	CH1641-Tg338(3)	CH1641-Tg59(2)	CH1641-Tg110(2)	
Recipient mouse line and passage number	VM	1	590 ± 65 (13/13)	>766 (0/15)	>856 (0/15) <sup>a</sup>	>697 (0/15) <sup>b</sup>
		2	343 ± 24 (8/8)	>797 (0/15)	>802 (0/15)	>704 (0/15)
		3	333 ± 8 (9/9)			
	RIII	1		883 ± 112 (5/12)	743 ± 139 (9/12)	852 ± 130 (5/7)
		2		530 ± 54 (7/7)	751 ± 33 (8/8)	494 ± 45 (9/9)
		3				
	Tg338	1	196 ± 7 (10/10)		152 ± 3 (15/15)	164 ± 23 (10/10)
		2	181 ± 5 (4/4)		158 ± 4 <sup>c</sup> (10/10)	150 ± 2 <sup>c</sup> (10/10)
		3	154 ± 4 <sup>c</sup> (5/5)			
Tg59	1	344 ± 70 (11/11)	232 ± 25 (12/12)		256 ± 15 (14/14)	
	2	268 ± 14 (9/9)	261 ± 13 <sup>d</sup> (10/10)		256 ± 8 <sup>d</sup> (10/10)	
	3	254 ± 20 <sup>d</sup> (8/8)				
Tg110	1	458 ± 66 (7/8)	201 ± 3 (5/5)	219 ± 20 (14/14)		
	2	221 ± 4 (5/5)	216 ± 3 <sup>e</sup> (5/5)	214 ± 3 <sup>e</sup> (9/9)		
	3	209 ± 11 <sup>e</sup> (5/5)				

Incubation periods are defined as the time between inoculation and terminal disease/ death and are expressed as the mean ± standard deviation in days. The attack rates are shown in brackets.

<sup>a</sup>No clinical disease but 6/15 mice showed PrP<sup>Sc</sup> deposits in the locus coeruleus and/ or superior colliculus at necropsy.

<sup>b</sup>No clinical disease but 1/15 mice showed PrP<sup>Sc</sup> deposition in the locus coeruleus at necropsy.

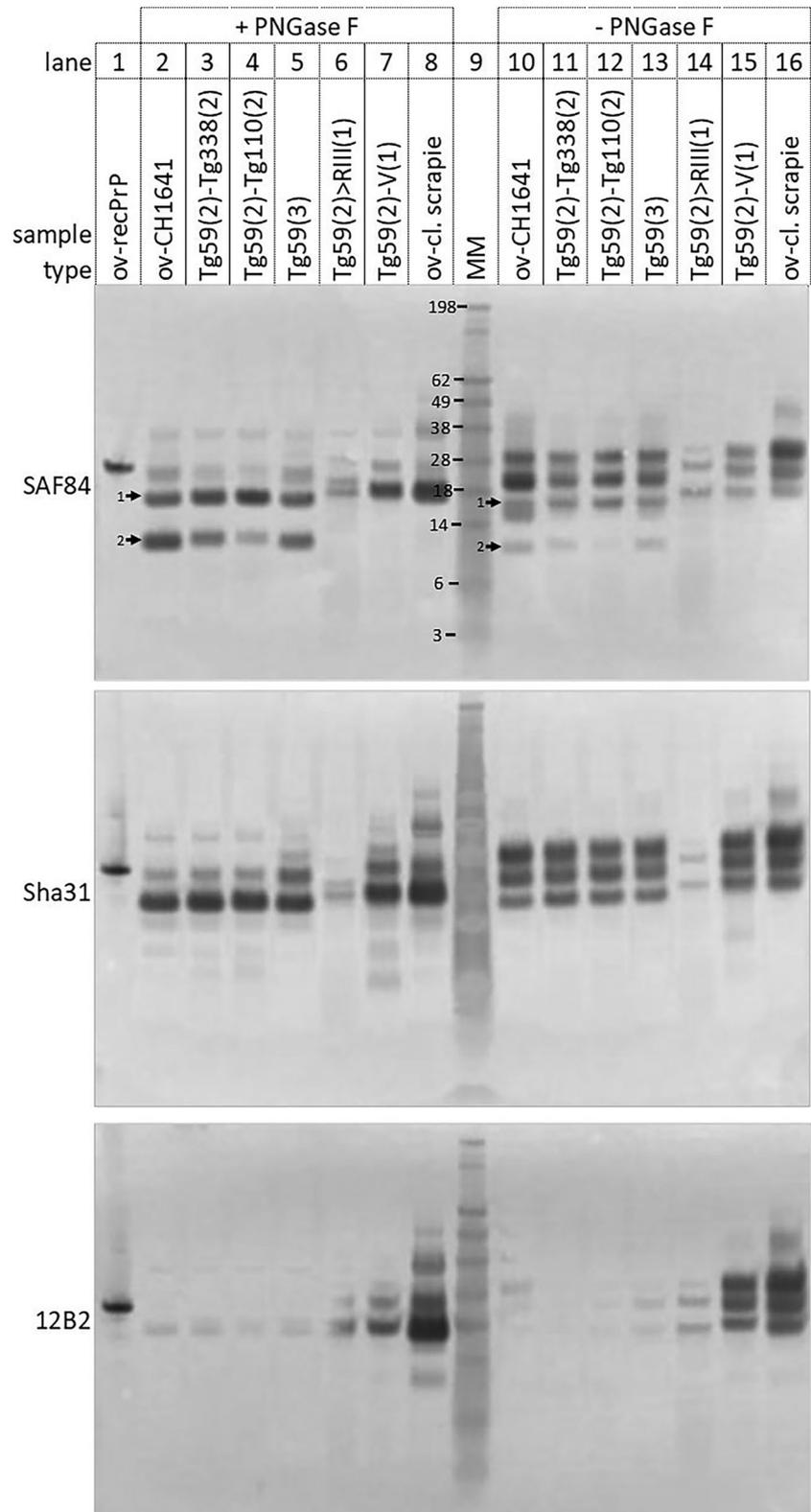
<sup>c</sup>not statistically different ( $p > 0.05$ , Welch's  $t$ -test) from the homologous passage of CH1641 in Tg338 mice.

<sup>d</sup>Not statistically different ( $p > 0.05$ , Welch's  $t$ -test) from the homologous passage of CH1641 in Tg59 mice.

<sup>e</sup>Not statistically different ( $p > 0.05$ , Welch's  $t$ -test) from the homologous passage of CH1641 in Tg110 mice.

The transmission of mouse-passaged CH1641 from transgenic mice into RIII mice resulted in long incubation periods with slow stabilisation as was also seen in the homologous passage of CH1641 in RIII (Table 2). Western blotting resulted in the classical scrapie PrP<sup>res</sup>

banding pattern with a single PrP<sup>res</sup> population and high 12B2 signal (Figure 2 lanes 6 and 14, Figure S1.B) whereas transmission between the transgenic mouse lines retained the dual population pattern and minimal 12B2 signal (Figure 2, lanes 3,11 and 4,12, Figure S1.B). The



**FIGURE 2** Cross passage of CH1641 from Tg59 mice into the various mouse lines. Parallel runs of western blotting with brain homogenates from the cross passage of CH1641 from Tg59 mice into the various mouse lines, immunoblotted with three different PrP-specific monoclonal antibodies. Passage from Tg59 mice into the transgenic mouse lines retains the CH1641 specific dual triplet PrP<sup>res</sup> pattern and decreased 12B2 signal but passage from Tg59 into wild-type mice RIII and VM results in the classical scrapie pattern with a single triplet band and preservation of the 12B2 epitope. Position of the unglycosylated PrP<sup>res</sup> bands 1 and 2 as detected in the SAF84 immunoblots are indicated by arrows. Lane 1: full-length ovine recPrP (ARQ). Lane 2: original ovCH1641 homogenate. Lanes 3–7 passage of CH1641 from Tg59 into Tg338, Tg110, Tg59, RIII and VM mice resp. Lane 8: classical sheep scrapie. Lane 9: molecular mass SeeBlue standards in kDa. The passage number is shown in brackets. Lanes 10–16 are identical to lanes 2–8 but not treated with PNGaseF. PrP<sup>res</sup> samples per lane applied correspond to 0.9 mg equivalents of brain tissue, except for lane 14 where 4.5 mg (TE) had been loaded because of the weak signal.

PrP<sup>Sc</sup> profiles in the RIII mice after passage through transgenic mice were similar to the PrP<sup>Sc</sup> profile after homologous passage in RIII mice with heavy PrP<sup>Sc</sup> depositions in the colliculus superior, stratum granulosum of the cerebellum and motor and sensory nuclei in the brainstem (Figure 3).

### Cross passage to transgenic mice

Cross-passage of CH1641 from RIII and VM (after initial passage through RIII mice) into the transgenic mouse lines resulted in 100% attack rates with short incubation periods that stabilised quickly as in the homologous challenges in the transgenic mice. Incubation periods at stabilisation were not significantly different from the incubation periods of the homologous passage of CH1641 in each transgenic mouse line (Table 2 and Table S1). Western blot analysis of transgenic mouse brains showed the typical dual population pattern that was indistinguishable from the pattern after homologous transmission in the transgenic mice indicating that the CH1641 specific western blot pattern had been restored after transmission from the wild-type mice to transgenic mice (Figure 4 and Figure S1.C). In addition, the transmission of CH1641 from RIII mice to transgenic mice showed PrP<sup>Sc</sup> profiles in the transgenic mice that were identical to the homologous passage in the transgenic mouse line (shown for the Tg59 mice in Figure 5).

### Back passage into transgenic mice

To complete a full transmission cycle, CH1641 was passaged to Tg338 mice, then to RIII mice and was finally re-isolated in Tg338 mice. Passage of ovCH1641 into Tg338 mice retained the CH1641 specific dual triplet PrP<sup>res</sup> pattern and decreased 12B2 signal but cross passage from Tg338 into RIII mice resulted in the classical scrapie pattern with a single triplet band and preservation of the 12B2 epitope. The cross-passage back into the Tg338 mice restored the specific dual triplet pattern and decreased the 12B2 signal. Thus, the CH1641 pattern was lost upon transmission to RIII mice but was fully restored when re-isolated in Tg338 mice (Figure 6).

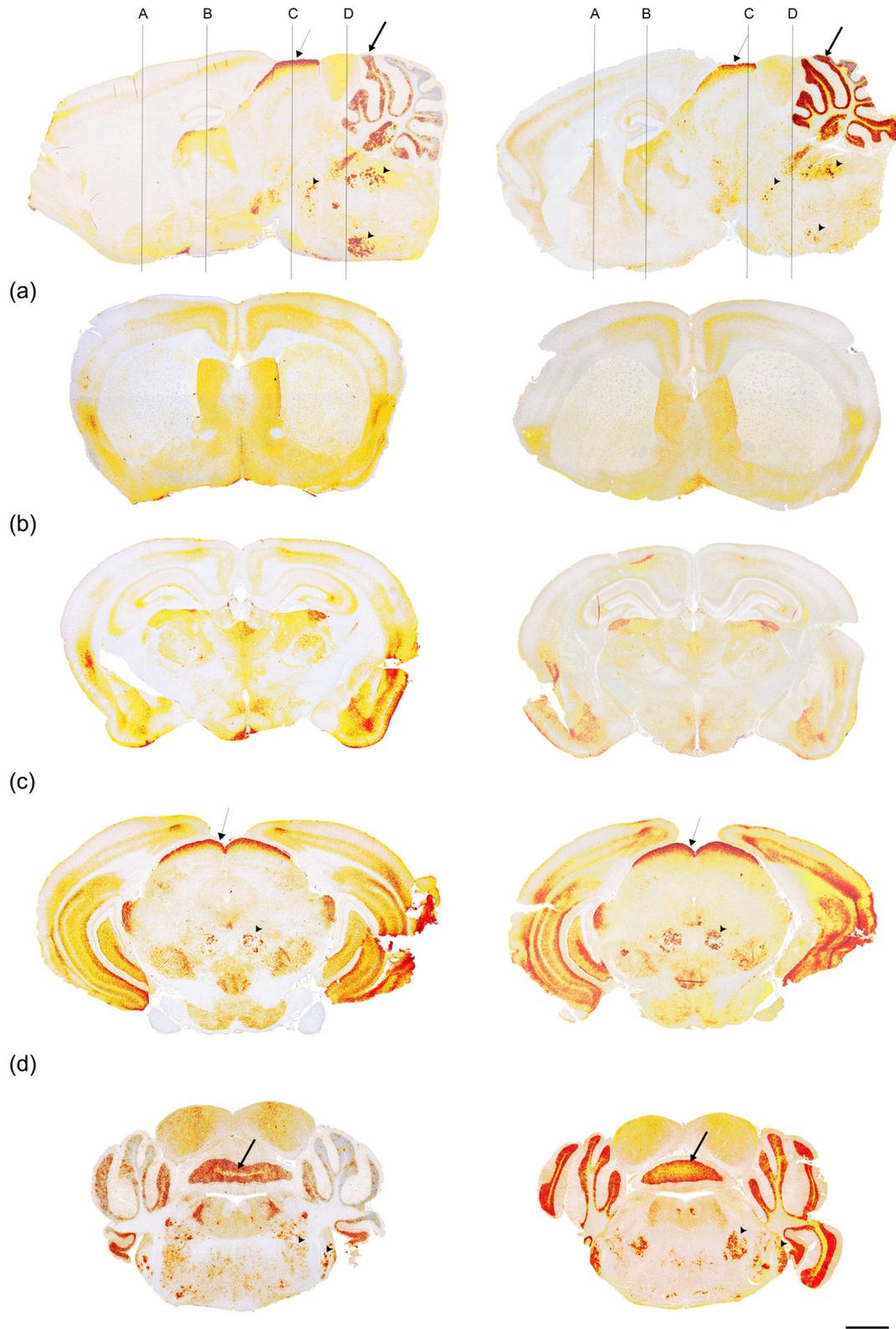
## DISCUSSION

Our study shows that the original CH1641 isolate can be transmitted to wild-type mice and that it behaves as a single and unique strain in both wild-type and transgenic mice. CH1641 was considered for a long time to be non-transmissible to wild-type mice as previous attempts to transmit the original CH1641 isolate to C57BL/6 mice and VM mice had been unsuccessful [4, 14]. While we report similar non-transmission results in the Sinc<sup>p7/p7</sup> VM mice, our results in the Sinc<sup>s7/s7</sup> RIII mice differ with a 100% attack rate on primary transmission. This was not the result of a conformational mutation or the selection of a different prion strain from a possible mixture of strains

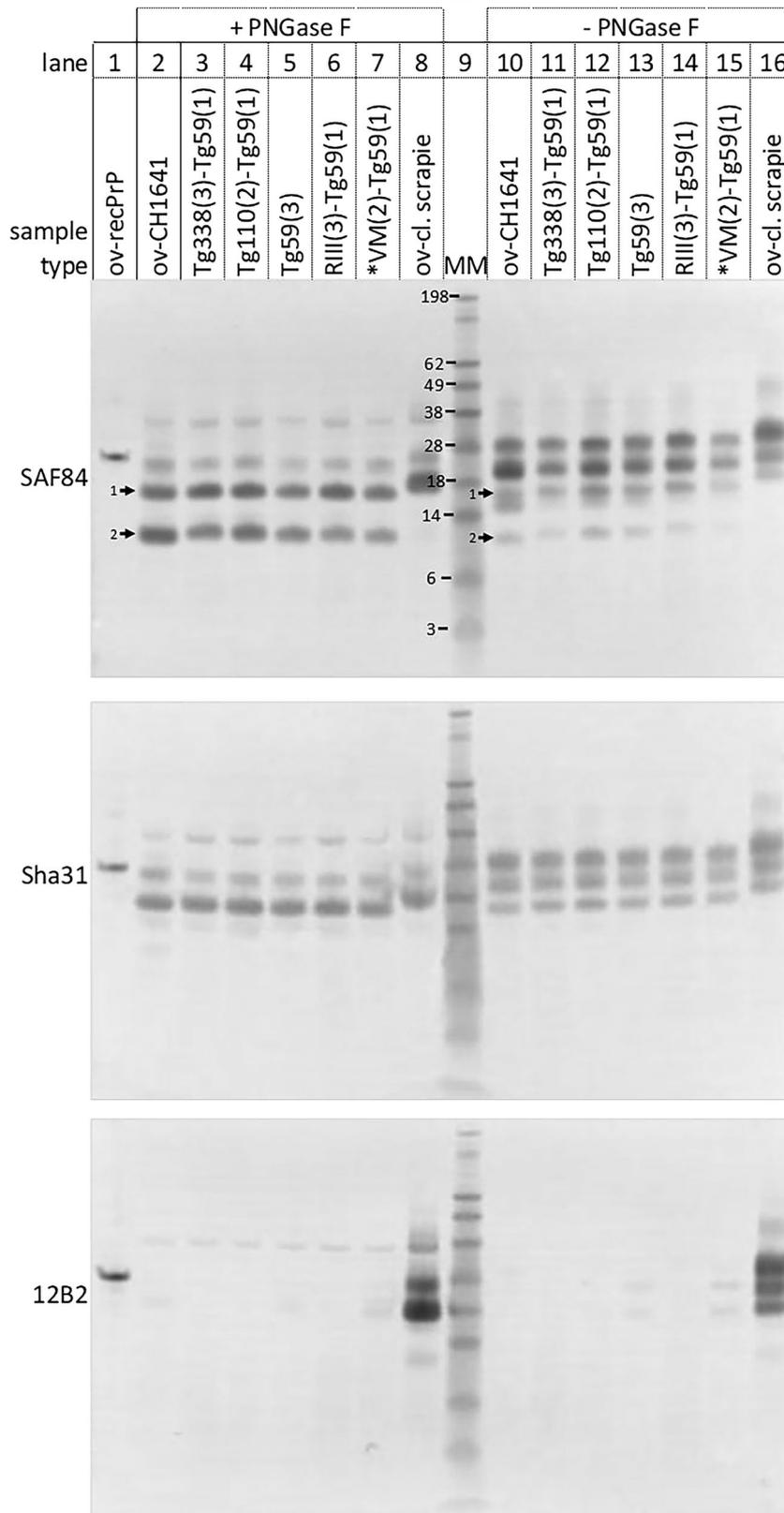
in the original CH1641 isolate, as cross passages clearly showed CH1641 to be an identical single prion strain in all mouse lines. Transmission efficiency of a prion strain between species has been shown to depend on the degree of conformational homology between donor PrP<sup>Sc</sup> and receptor PrP<sup>C</sup> with single amino acid polymorphisms in the PrP molecule of both donor and host having a profound impact on transmission efficiency [26]. It is unlikely that the difference in transmission efficiency of CH1641 to wild-type mice in our study could have been attributable to the polymorphisms in the host PrP as both C57BL/6 and RIII mice share the same PrP genotype and have never shown strong differences in susceptibility to various prion strains. It is, however, possible, that the origin of the CH1641 inoculum could be the cause of the difference in susceptibility as the sheep genotypes from which the original CH1641 inoculum was prepared might have been different. We used a fourth passage of CH1641 in sheep comprising three different genotypes: ARQ/ARQ, ARQ/AHQ and AHQ/AHQ (personal communications Dr Jim Foster). Although the genotype composition of the other CH1641 inocula cannot be identified with 100% certainty anymore, single polymorphism differences in the PrP genotypes of the donors could have resulted in varying transmission efficiency as the donor PrP sequence has been shown to influence transmission efficiency between species [27–29].

The importance of the donor PrP sequence on transmission efficiency is also shown in the cross passages of CH1641 to VM mice in our study. Transmission of Tg338-passaged CH1641 to VM mice was unsuccessful at primary passage. Blind secondary passage did also not result in transmission indicating an absolute species barrier. Transmission of ovCH1641 or Tg59-/Tg110-passaged CH1641 to VM mice did not result in clinical cases but did show several mice with PrP<sup>Sc</sup> deposits in the brain at necropsy. However, secondary transmission did not result in a productive transmission indicating non-adaptive prion amplification in the primary passage rather than sub-or preclinical infection [30]. Transmission of RIII-passaged CH1641 to VM mice resulted in a 100% transmission efficiency on both primary and secondary passage. This was clearly not the result of a conformational mutation of the prion strain as cross passage to transgenic mice resulted in re-isolation of the same CH1641 strain proving identical propagation had occurred in the RIII mice. Since both the prion strain (CH1641) and the host (VM mice) were identical in all these passages, this shows the impact of the donor PrP sequence on the species barrier and the transmission results.

Few studies have been undertaken on the transmission of the original CH1641 isolate. However, several groups have reported on the transmission of 'CH1641-like' sheep field cases with a similar banding pattern as the original CH1641 isolate. Béringue et al reported on the transmission of eleven 'CH1641-like' isolates to Tg338 mice and found a 19 K PrP<sup>res</sup> signature in the brains of all Tg338 mice [31]. Miyazawa et al used a 'CH1641-like' sheep field isolate that failed to transmit to wild-type mice and transmitted it to Tgbov mice resulting in a CH1641-like banding pattern of PrP<sup>res</sup> in the brains of these mice. After passage through Tgbov mice, they were able to transmit this isolate to wild-type mice with retention of the CH1641-like banding pattern [16, 22]. Baron et al reported



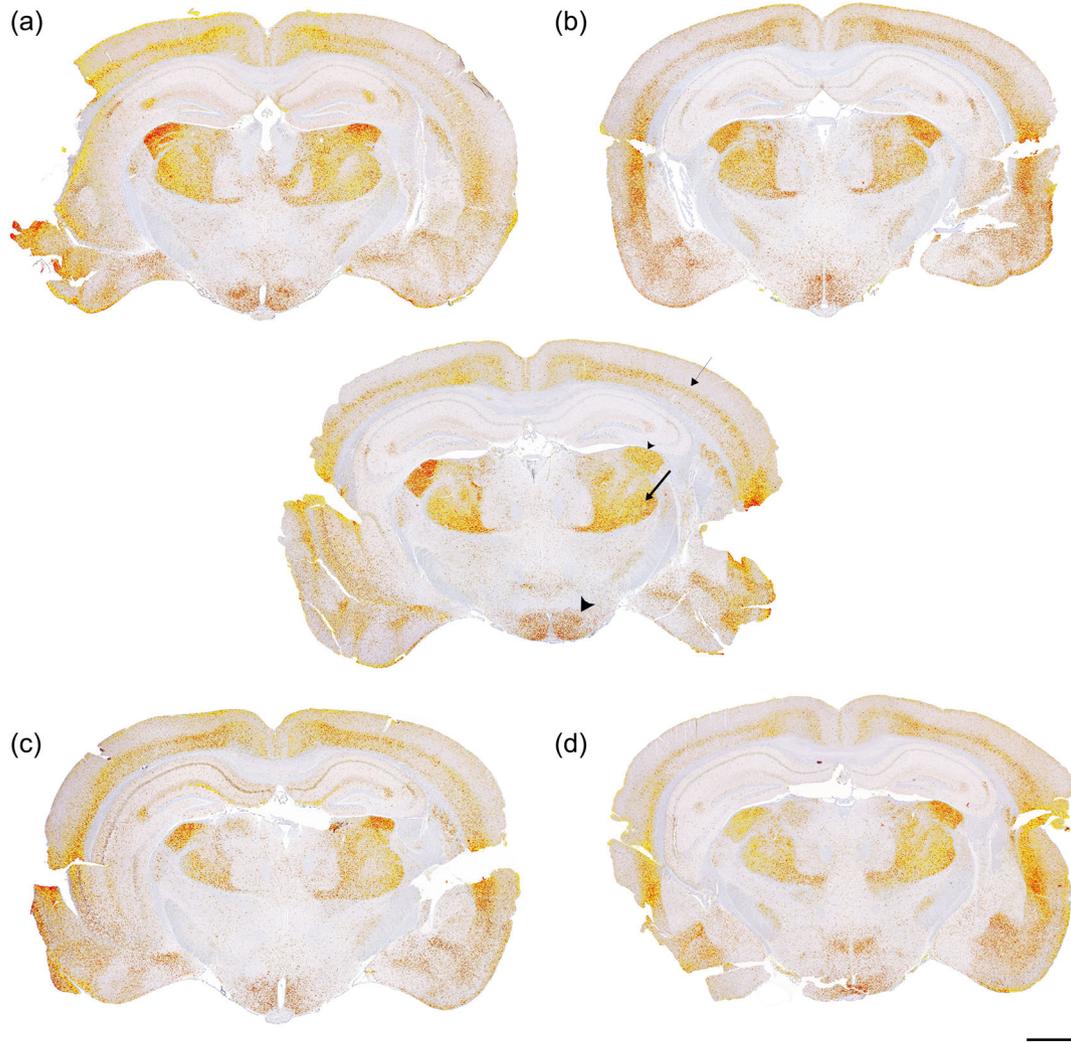
**FIGURE 3** Cross passage of CH1641 from Tg59 mice to RIII mice. PrP<sup>Sc</sup> profile of CH1641 passaged from Tg59 mice into RIII mice (CH1641-Tg59(2)-R(2), left column). At the right, the homologous passage of CH1641 in RIII is shown for comparison. Profiles are shown for the sagittal section (top) and the cross sections at levels A, B, C and D. Notice the similar heavy staining in the colliculus superior (thin arrow), stratum granulosum of the cerebellum (thick arrow) and the motor and sensory nuclei of the brainstem (arrowheads). Bar = 1000  $\mu$ m.



**FIGURE 4** Cross passage of CH1641 from the various mouse lines to Tg59 mice. Parallel runs of western blotting with brain homogenates from the cross passage of CH1641 from the various mouse lines into Tg59 mice, immunoblotted with 3 different PrP-specific antibodies. Passage into the Tg59 mice yields a dual triplet PrP<sup>res</sup> pattern irrespective of the donor mouse of the inoculum. Position of the unglycosylated PrP<sup>res</sup> bands 1 and 2 as detected in the SAF84 immunoblots are indicated by arrows. Notice the loss of signal in the blot immunostained with 12B2. Lane 1: full-length ovine recPrP (ARQ). Lane 2: original ovCH1641 homogenate. Lanes 3–7 passage of CH1641 from Tg338, Tg110, Tg59, RIII and VM mice resp. into Tg59 mice. Lane 8: classical sheep scrapie. Lane 9: molecular mass SeeBlue standards in kDa. The passage number is shown in brackets. Lanes 10–16 are identical to lanes 2–8 but not treated with PNGaseF. PrP<sup>res</sup> samples per lane applied correspond to 0.9 mg equivalents of brain tissue. \*After initial passage in RIII mice.

transmission of ‘CH1641-like’ isolates from sheep to Tg59 mice and C57Bl/6 mice [6, 17]. The resulting PrP<sup>res</sup> molecular pattern in the brains of the Tg59 mice showed a CH1641-like banding pattern but in the C57Bl/6 mice, a classical scrapie pattern was seen similar to our

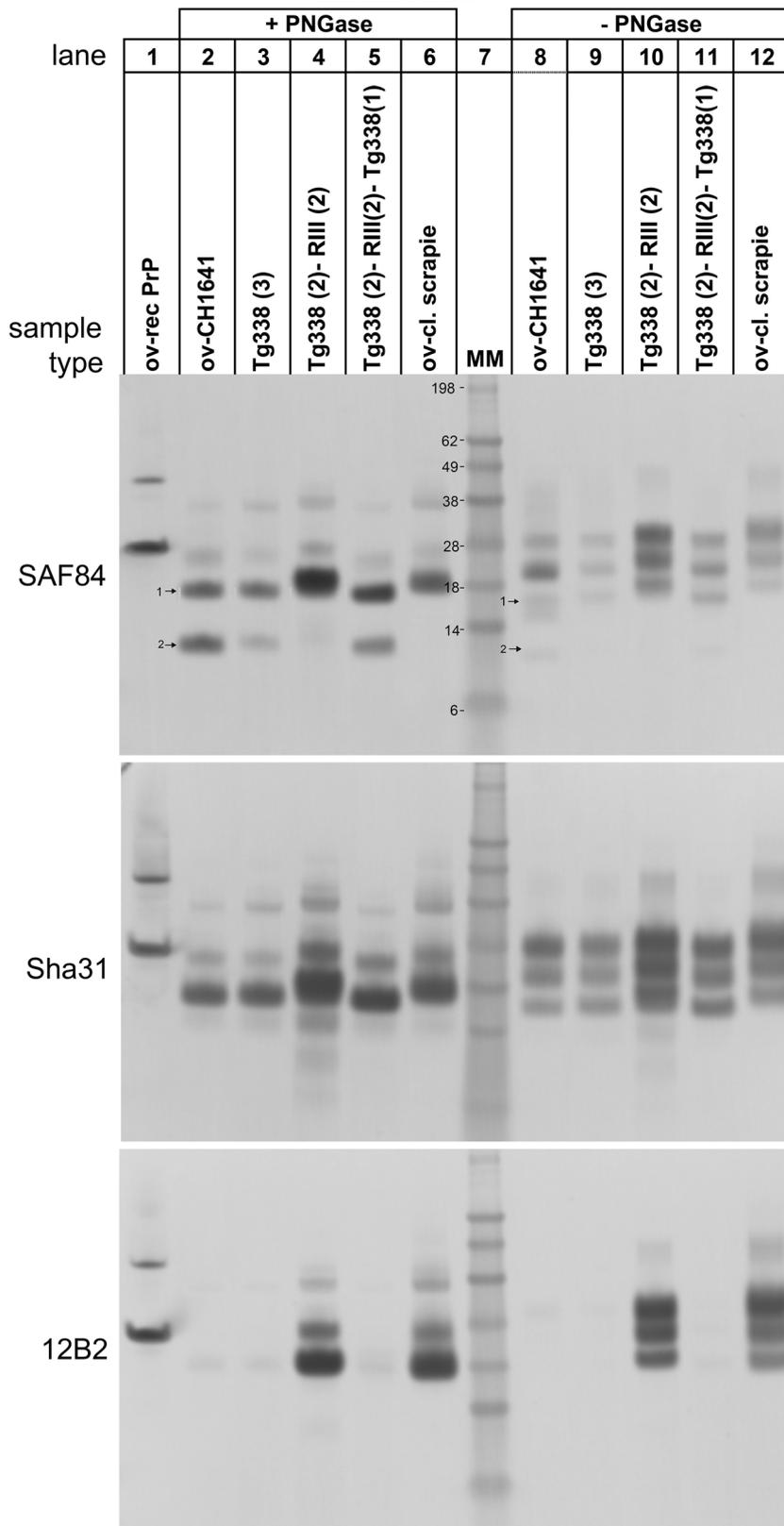
findings. However, since no cross passages were undertaken to transgenic mice it is unclear whether the scrapie strain isolated in the wild-type mice was indeed CH1641 or whether another classical scrapie strain had been isolated from a possible mixture of strains often



**FIGURE 5** Cross passage of CH1641 from the various mouse lines to Tg59 mice. PrP<sup>Sc</sup> profiles at the level of the (hypo)thalamus in Tg59 mice A. CH1641-Tg338(3)-Tg59(1); B. CH1641-Tg110(2)-Tg59(1); C. CH1641-R111(3)-Tg59(1); D. CH1641-R111(2)-VM(2)-Tg59(1). In the centre, the PrP<sup>Sc</sup> profile of the homologous passage of CH1641 in Tg59 mice for comparison. PrP<sup>Sc</sup> depositions are identical in localisation and include amongst others the cerebral cortical layer 5 (thin arrow), ventral posterior complex of the thalamus (thick arrow), dorsal part of the lateral geniculate complex (small arrowhead) and the periventricular hypothalamic nucleus (large arrowhead). Bar = 1000  $\mu$ m.

present in field cases. We show with immunohistochemistry that after the cross passage of CH1641 between the various mouse lines, a PrP<sup>Sc</sup> profile is obtained in each mouse line that is indistinguishable from the PrP<sup>Sc</sup> profile of the homologous passage of CH1641 in that mouse line. In addition, the stabilised incubation periods in transgenic mice after the cross passage of CH1641 were not statistically different from the incubation periods of the homologous passage in each transgenic mouse line. None of the PrP<sup>Sc</sup> profiles showed any similarities with previously described profiles of known scrapie and BSE reference strains [25]. The isolation of the same strain in each of the mouse lines indicates that the original CH1641 isolate likely consists of a single and unique scrapie strain rather than a mixture of scrapie strains. This particular strain shows a dual molecular banding pattern in transgenic mice like the original sheep CH1641 isolate but adopts a classical banding pattern in wild-type mice.

Theoretically, one could argue that CH1641 could be composed of a mixture of two substrains: a 19 K strain replicating preferably in Tg mice (with a minor 21 K component) and a 21 K substrain replicating preferably in wild-type mice (with a minor 19 K component). We consider this to be highly unlikely because (1) the 19 K substrain does not replicate in VM mice shown by the negative transmission of CH1641 from the Tg mice to VM mice. If CH1641 would have been a mixture of 19 K/21 K in the Tg mice you would expect the minor 21 K component to transmit to VM mice not being hampered by competition with the major 19 K strain. Yet this does not happen in the first passage and not even in the second (blind) passage from any of the three Tg mouse lines; (2) as VM mice do not support the replication of the 19 K strain, you would expect the transmission of the 21 K strain of CH1641 from VM mice to Tg mice to result in transmission of the 21 K, again not being completed by the 19 K strain. Yet this



**FIGURE 6** Re-isolation of CH1641 in Tg338 mice after intermediate passage through RIII mice. Parallel runs of western blotting with brain homogenates from the passage of CH1641 from Tg338 mice into RIII mice and then back into the Tg338 mice. The dual population pattern is lost upon transmission to RIII mice but is fully restored when re-isolated in Tg338 mice. Immunoblotted with three different PrP-specific monoclonal antibodies. Position of the unglycosylated PrP<sup>res</sup> bands 1 and 2 are indicated by arrows. Lane 1: full-length ovine recPrP (ARQ). Lane 2: original ovCH1641 homogenate. Lane 3: passage of CH1641 into Tg338 mice. Lane 4: passage of CH1641 from Tg338 into RIII mice. Lane 5: back passage of CH1641 passed through Tg338 and RIII mice and then back into Tg338 mice. Lane 6: classical sheep scrapie. Lane 7: molecular mass SeeBlue standards in kDa. The passage number is shown in brackets. Lanes 8–12 are identical to lanes 2–6 but not treated with PNGaseF. PrP<sup>res</sup> samples per lane applied correspond to 0.9 mg equivalents of brain tissue.

transmission yields the 19 K strain in all three Tg mouse lines arguing for the shift of 21 K into 19 K; (3) transmission of the 21 K strain to RIII is a very slow process with incubation periods close to the life expectancy of the mice. This means that if a slower 19 K strain were

present in the RIII mice, it would have an even longer incubation period well beyond the life expectancy of the mice. Thus, propagating the 21 K strain in RIII mice by homologous subpassage would almost certainly result in a very low titre of the 19 K strain (or even its

elimination). This would result in a decreased attack rate and very long incubation periods in the cross-passage to Tg mice. Yet the attack rates are 100% at first cross passage and incubation periods are not much longer than to be expected after crossing the species barrier.

The molecular pattern of PrP<sup>res</sup> in Western blots is often used in prion research as an alternative method for strain identification. Compared with the time-consuming and costly strain typing by bioassay, Western blotting is quick and relatively cheap to perform. In fact, WB has been shown to reliably differentiate between various TSE strains e.g. between atypical strains and classical BSE or scrapie and CH1641 [5, 6, 10, 24]. However, it cannot be used to differentiate between the various strains of classical scrapie since all these strains exhibit a nearly similar classical pattern in WB [9, 12]. In addition, we show here that the molecular pattern of a prion strain as determined by WB, is also dependent on the PrP genotype of the host. Therefore, strain typing by molecular analysis should be exerted with caution since it not only depends on the strain properties but also the host.

#### AUTHOR CONTRIBUTIONS

LK, JL and AB conceptualised and designed the study. LK performed all animal experiments, necropsies and brain collection. CD was in charge of the histopathological work and LK analysed the histopathological data. RV conducted all the biochemical work and analysed it together with JL. TB and JMT constructed and supplied the transgenic mouse lines. LK wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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#### CONFLICT OF INTEREST STATEMENT

None.

#### PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/nan.12963>.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### ETHICS STATEMENT

All animal experiments were performed in compliance with the institutional guidelines, Dutch national guidelines (Dutch Central Authority for Scientific Procedures on Animals, permit numbers AVD401002016522 and AVD40100202014038) and the European Community Council Directives 86/609/EEC and 2010/63/EU.

#### ORCID

Lucien J. M. van Keulen  <https://orcid.org/0000-0002-8645-5190>

Thierry Baron  <https://orcid.org/0000-0001-5396-7707>

#### REFERENCES

- Foster JD, Dickinson AG. The unusual properties of CH1641, a sheep-passaged isolate of scrapie. *Vet Rec.* 1988;123(1):5-8. doi:10.1136/vr.123.1.5
- Goldmann W, Hunter N, Benson G, Foster JD, Hope J. Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the sip gene. *J Gen Virol.* 1991;72(Pt 10):2411-2417. doi:10.1099/0022-1317-72-10-2411
- Jeffrey M, González L, Chong A, et al. Ovine infection with the agents of scrapie (CH1641 isolate) and bovine spongiform encephalopathy: immunochemical similarities can be resolved by immunohistochemistry. *J Comp Pathol.* 2006;134(1):17-29. doi:10.1016/j.jcpa.2005.06.005
- Dickinson AG, Bruce ME, Outram GW, Kimberlin RH. Scrapie strain differences: the implications of stability and mutation. In: *Proceedings of workshop on slow transmissible diseases. Takehashi, Okayama, Japan; 1984:105-117.*
- Stack MJ, Chaplin MJ, Clark J. Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies. *Acta Neuropathol.* 2002;104(3):279-286. doi:10.1007/s00401-002-0556-2
- Baron T, Bencsik A, Vulin J, et al. A C-terminal protease-resistant prion fragment distinguishes ovine "CH1641-like" scrapie from bovine classical and L-type BSE in ovine transgenic mice. *PLoS Pathog.* 2008;4(8):e1000137. doi:10.1371/journal.ppat.1000137
- Hope J, Wood SC, Birkett CR, et al. Molecular analysis of ovine prion protein identifies similarities between BSE and an experimental isolate of natural scrapie, CH1641. *J Gen Virol.* 1999;80(Pt 1):1-4. doi:10.1099/0022-1317-80-1-1
- Thuring CM, Erkens JH, Jacobs JG, et al. Discrimination between scrapie and bovine spongiform encephalopathy in sheep by molecular size, immunoreactivity, and glycoprofile of prion protein. *J Clin Microbiol.* 2004;42(3):972-980. doi:10.1128/JCM.42.3.972-980.2004
- Langeveld JPM, Pirisinu L, Jacobs JG, et al. Four types of scrapie in goats differentiated from each other and bovine spongiform encephalopathy by biochemical methods. *Vet Res.* 2019;50(1):97. doi:10.1186/s13567-019-0718-z
- Jacobs JG, Sauer M, van Keulen LJ, Tang Y, Bossers A, Langeveld JP. Differentiation of ruminant transmissible spongiform encephalopathy isolate types, including bovine spongiform encephalopathy and CH1641 scrapie. *J Gen Virol.* 2011;92(1):222-232. doi:10.1099/vir.0.026153-0
- Vulin J, Biacabe AG, Cazeau G, Calavas D, Baron T. Molecular typing of protease-resistant prion protein in transmissible spongiform encephalopathies of small ruminants, France, 2002-2009. *Emerg Infect Dis.* 2011;17(1):55-63. doi:10.3201/eid1701.100891
- Langeveld JP, Jacobs JG, Erkens JH, et al. Sheep prions with molecular properties intermediate between classical scrapie, BSE and

- CH1641-scrapie. *Prion*. 2014;8(4):296-305. doi:[10.4161/19336896.2014.983396](https://doi.org/10.4161/19336896.2014.983396)
13. Baron T, Biacabe AG. Molecular behaviors of “CH1641-like” sheep scrapie isolates in ovine transgenic mice (TgOvPrP4). *J Virol*. 2007;81(13):7230-7237. doi:[10.1128/JVI.02475-06](https://doi.org/10.1128/JVI.02475-06)
  14. Baron T, Crozet C, Biacabe AG, et al. Molecular analysis of the protease-resistant prion protein in scrapie and bovine spongiform encephalopathy transmitted to ovine transgenic and wild-type mice. *J Virol*. 2004;78(12):6243-6251. doi:[10.1128/JVI.78.12.6243-6251.2004](https://doi.org/10.1128/JVI.78.12.6243-6251.2004)
  15. Bencsik A, Baron T. Histopathological studies of “CH1641-like” scrapie sources versus classical scrapie and BSE transmitted to ovine transgenic mice (TgOvPrP4). *PLoS ONE*. 2011;6(7):e22105. doi:[10.1371/journal.pone.0022105](https://doi.org/10.1371/journal.pone.0022105)
  16. Miyazawa K, Masujin K, Matsuura Y, Iwamaru Y, Yokoyama T, Okada H. Interspecies transmission to bovinized transgenic mice uncovers new features of a CH1641-like scrapie isolate. *Vet Res*. 2018;49(1):116. doi:[10.1186/s13567-018-0611-1](https://doi.org/10.1186/s13567-018-0611-1)
  17. Vulin J, Beck KE, Bencsik A, Lakhdar L, Spiropoulos J, Baron T. Selection of distinct strain phenotypes in mice infected by ovine natural scrapie isolates similar to CH1641 experimental scrapie. *J Neuropathol Exp Neurol*. 2012;71(2):140-147. doi:[10.1097/NEN.0b013e3182439519](https://doi.org/10.1097/NEN.0b013e3182439519)
  18. Moore RC, Hope J, McBride PA, et al. Mice with gene targeted prion protein alterations show that Prnp, Sinc and Prni are congruent. *Nat Genet*. 1998;18(2):118-125. doi:[10.1038/ng0298-118](https://doi.org/10.1038/ng0298-118)
  19. Dickinson AG, Meikle VM, Fraser H. Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. *J Comp Pathol*. 1968;78(3):293-299. doi:[10.1016/0021-9975\(68\)90005-4](https://doi.org/10.1016/0021-9975(68)90005-4)
  20. Vilotte JL, Soulier S, Essalmani R, et al. Markedly increased susceptibility to natural sheep scrapie of transgenic mice expressing ovine prp. *J Virol*. 2001;75(13):5977-5984. doi:[10.1128/JVI.75.13.5977-5984.2001](https://doi.org/10.1128/JVI.75.13.5977-5984.2001)
  21. Cordier C, Bencsik A, Philippe S, et al. Transmission and characterization of bovine spongiform encephalopathy sources in two ovine transgenic mouse lines (TgOvPrP4 and TgOvPrP59). *J Gen Virol*. 2006;87(12):3763-3771. doi:[10.1099/vir.0.82062-0](https://doi.org/10.1099/vir.0.82062-0)
  22. Castilla J, Gutiérrez Adán A, Brun A, et al. Early detection of PrPres in BSE-infected bovine PrP transgenic mice. *Arch Virol*. 2003;148(4):677-691. doi:[10.1007/s00705-002-0958-4](https://doi.org/10.1007/s00705-002-0958-4)
  23. Slootstra JW, Puijk WC, Ligtoet GJ, Langeveld JP, Meloen RH. Structural aspects of antibody-antigen interaction revealed through small random peptide libraries. *Mol Divers*. 1996;1(2):87-96. doi:[10.1007/BF01721323](https://doi.org/10.1007/BF01721323)
  24. Jacobs JG, Langeveld JP, Biacabe AG, et al. Molecular discrimination of atypical bovine spongiform encephalopathy strains from a geographical region spanning a wide area in Europe. *J Clin Microbiol*. 2007;45(6):1821-1829. doi:[10.1128/JCM.00160-07](https://doi.org/10.1128/JCM.00160-07)
  25. van Keulen LJ, Langeveld JP, Dolstra CH, Jacobs J, Bossers A, van Zijderveld FG. TSE strain differentiation in mice by immunohistochemical PrP (Sc) profiles and triplex Western blot. *Neuropathol Appl Neurobiol*. 2015;41(6):756-779. doi:[10.1111/nan.12181](https://doi.org/10.1111/nan.12181)
  26. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. *Science*. 2007;318(5852):930-936. doi:[10.1126/science.1138718](https://doi.org/10.1126/science.1138718)
  27. Priem J, Langeveld JP, van Keulen LJ, van Zijderveld FG, Andreoletti O, Bossers A. Enhanced virulence of sheep-passaged bovine spongiform encephalopathy agent is revealed by decreased polymorphism barriers in prion protein conversion studies. *J Virol*. 2014;88(5):2903-2912. doi:[10.1128/JVI.02446-13](https://doi.org/10.1128/JVI.02446-13)
  28. Padilla D, Beringue V, Espinosa JC, et al. Sheep and goat BSE propagate more efficiently than cattle BSE in human PrP transgenic mice. *PLoS Pathog*. 2011;7(3):e1001319. doi:[10.1371/journal.ppat.1001319](https://doi.org/10.1371/journal.ppat.1001319)
  29. Igel A, Fornara B, Rezaei H, Beringue V. Prion assemblies: structural heterogeneity, mechanisms of formation, and role in species barrier. *Cell Tissue Res*. 2023;392(1):149-166. doi:[10.1007/s00441-022-03700-2](https://doi.org/10.1007/s00441-022-03700-2)
  30. Block AJ, Bartz JC. Prion strains: shining new light on old concepts. *Cell Tissue Res*. 2023;392(1):113-133. doi:[10.1007/s00441-022-03665-2](https://doi.org/10.1007/s00441-022-03665-2)
  31. Beringue V, Tixador P, Andreoletti O, et al. Host prion protein expression levels impact prion tropism for the spleen. *PLoS Pathog*. 2020;16(7):e1008283. doi:[10.1371/journal.ppat.1008283](https://doi.org/10.1371/journal.ppat.1008283)

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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