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Gerstmann-Sträussler-Scheinker disease associated with a novel PRNP mutation (V176G) harbours a distinctive clinical and molecular-pathological profile.

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analysis; VL, VJ and GK performed the epitope mapping; QXL performed the tau ELISA; all authors contributed to writing the manuscript.

ABSTRACT

Objective: To define the phenotypic profile of a neuropathologically confirmed form of Gerstmann-Sträussler-Scheinker disease (GSS) associated with a novel prion protein gene (*PRNP*) mutation. **Design:** Case report. **Setting:** Australian National Creutzfeldt-Jakob Disease Registry. **Patient:** A 61-year-old British-born woman with no history of neurodegenerative disorder or prion disease in first-degree relatives. **Main outcome measure:** Delineation of a previously unreported *PRNP* mutation and its associated clinical and molecular-pathological phenotype. **Results:** Rapidly progressive dementia, altered behaviour and cerebellar ataxia were salient clinical features immediately following minor elective surgery, with death 1 month later in an akinetic-mute state. Brain histopathological examination revealed neuronal loss, scant foci of spongiform change and diffuse multi-centric amyloid plaques, selectively immunoreactive for prion protein, within cerebral cortex, deep grey matter and cerebellar cortex. Tau immune-reactive neurofibrillary tangles and neuritic threads were present in the cerebral cortex. *PRNP* sequencing demonstrated a mutation predicting a valine to glycine substitution at codon 176, with valine homozygosity at polymorphic codon 129. Western blot analysis, including epitope mapping, of frozen brain tissue displayed a non-classical protease-resistant prion protein (PrP^{res}) banding pattern, with a prominent approximately 8 kDa protease-resistant fragment. **Conclusions:** We report a novel *PRNP* mutation in a person with neuropathologically confirmed GSS displaying a somewhat unusual constellation of clinico-pathological features, which overall subserve to further broaden an already diverse phenotypic spectrum.

INTRODUCTION

Gerstmann-Sträussler-Scheinker disease (GSS) is a rare, almost exclusively, genetically determined prion disease, typically characterised clinically by progressive cerebellar ataxia and dementia and neuropathologically by diffuse, prion protein (PrP)-containing, amyloid deposits[1]. Although the P102L mutation in the prion protein gene (PRNP) is the most commonly observed in GSS[2], numerous insertion, missense and point mutations, including P105L, A117V, H187R, F198S, D202N, Q212P, Q217R and Y218N, have been detected, probably contributing to the notable clinical and pathological diversity[2,3]. Typically, GSS follows an autosomal dominant inheritance pattern[4] and manifests a prolonged clinical course, with median illness duration of 39 months reported in large patient series[5]. In addition to progressive cerebellar ataxia and dementia, clinical findings in GSS may include myoclonus, extrapyramidal features and evidence of pyramidal tract dysfunction[3]. Cognitive decline usually follows cerebellar ataxia as a later symptom in the clinical course, especially in P102L GSS[4], and may be absent in patients with certain mutations, such as Q212R[6]; however, early prominent dementia without cerebellar ataxia has been described in some less common mutations such as G131V[7,8]. Neuropathological findings are also variable but generally there is evidence in the cerebrum and cerebellum of widespread, PrP immuno-positive, uni-and/or multi-centric amyloid plaques, associated with differing degrees of spongiform change, neuronal loss and astrocytic gliosis; tau-positive neurofibrillary tangles may also be observed[6,9,10,11].

Herein, we report a patient with neuropathologically confirmed GSS associated with a novel *PRNP* mutation, displaying an unusual combination of clinical and molecular-

pathological features, which overall subserve to further broaden an already diverse phenotypic spectrum.

METHODS & RESULTS

Clinical History and Investigations

The proband was a Caucasian, British-born woman whose first definite neurological symptoms occurred at age 61 years, around the time of elective surgery for removal of a dislodged breast implant. Over a period of years prior to surgery however, the patient had become increasingly estranged from all relatives. Infrequent, irregular involvement with family precluded confident delineation of any specific neurological or psychiatric symptoms and their chronological evolution but a sister maintained some contact, describing six-months of "low mood" and possible memory problems. In addition, posthumously, the sister of the proband became aware of some apparently unusual behaviour, such as buying inappropriately sized underwear and the likely hoarding of large supplies of cleaning products, although in contrast, financial affairs and housework had been maintained in good order. In the immediate post-operative period, the patient developed what was thought to be an acute confusional state with delusional ideation, for example claiming that she had swallowed a five cent coin and that staff members had been forcing her to swallow objects of jewellery. The proband's sister affirmed that the post-operative demeanour was completely uncharacteristic.

Following surgery, gait unsteadiness was also noteworthy and two falls occurred while in hospital. Mental status assessment in the post-operative period confirmed cognitive impairment (MMSE 20/30), especially defective short-term recall and mild

expressive and nominal dysphasia. Neurological examination revealed cerebellar ataxia with bilateral intention tremor, generalised hyper-reflexia and myoclonus. The patient never left institutional care following her surgery, experiencing rapid, steady cognitive and gross motor decline, dying in an akinetic-mute state around 1 month following surgery.

There was no known family history of neurodegenerative disease in first-degree relatives (figure 1), with the proband's mother dying aged 88 years from cancer and the father aged 92 years as a consequence of a head injury sustained from a fall. Scant, imprecise information existed for non-first degree relatives, especially in the patrilineage, but there was a history of a maternal aunt dying (age unknown) in a "mental hospital" after an illness of only a few months duration. The patient had migrated to Australia at the age of 37 years, around the time of onset of the bovine spongiform encephalopathy (BSE) epidemic. There was no history of recognised risk factors for iatrogenic prion disease.

Routine blood tests were unremarkable. CSF was normal aside from the presence of 14-3-3 proteins. MRI of the brain showed mild generalised atrophic changes (consistent with age) and a few, non-specific, deep white matter hyperintensities in the cerebral hemispheres, but there were no findings more typical of sporadic Creutzfeldt-Jakob disease (CJD) such as areas of gray matter restricted diffusion, nor increased signal on fluid attenuated inversion recovery (FLAIR) sequences (Figure 2). EEG showed attenuated alpha rhythms with frequent diffuse rhythmic delta activity but no periodic complexes.

Neuropathological Findings

Routine hematoxylin and eosin stains of frontal, temporal and occipital cortical sections showed numerous (densely eosinophilic) amyloid plaques (figure 3A), neuronal loss and astrocytic gliosis. The amyloid plaques, sometimes appearing multi-centric, were extensively deposited, most marked in the lower laminae, with immune-peroxidase studies showing reactivity with both 3F4 and 12F10 anti-PrP monoclonal antibodies (figure 3B); plaques did not demonstrate Aβ immuno-reactivity. Many of the amyloid plaques showed an associated microglial response but did not resemble florid plaques (figure 3A). The basal ganglia showed a similar prominent deposition of closely packed, granular deposits, again immuno-reactive with 3F4 and 12F10 anti-PrP monoclonal antibodies. Scant spongiform change was seen focally in the cerebral cortex, basal ganglia and cerebellar cortex. Hippocampal sections displayed numerous, tau immuno-reactive neurofibrillary tangles (NFT), in association with extensive neuropil thread formation and areas of neuritic plaque formation (figure 3C). In the cerebellum, there was extensive immuno-positive prion protein deposition within the molecular layer, patchy Purkinje cell loss and a moderately intense microglial response within the cortex. This extensive PrP-positive amyloid deposition in association with prominent tau pathology was felt to be most consistent with a neuropathological diagnosis of GSS.

***PRNP* Genotyping**

Appropriate consent for genetic examination was obtained. Genomic DNA was extracted from brain, and the *PRNP* open reading frame was sequenced in the forward and reverse orientations with overlapping primers (PrP106 and PrP46 sequences described previously[12]; PrPF2 5'-CCGAGTAAGCCAAAAACCAAC -3'; PrPR2 5'-

TCACTGCCGAAATGTATGATG -3'). Sequencing revealed the patient to be homozygous for valine at the polymorphic codon 129. A guanine (G) to adenine (A) nucleotide substitution was found in a 5' non-coding region, at position -21 relative to the start codon, and a synonymous A to G substitution was seen at the third position of codon 117, transitions both previously described as *PRNP* polymorphisms[13]. A thymine (T) to G transversion was detected at the second position of codon 176, resulting in a predicted amino acid change from a valine to glycine. This mutation has not previously been reported. A diagrammatic representation of these sequence variations is shown in Figure 4A.

PrP^{res} Analysis

Frozen post-mortem brain specimens from the cerebellum (Ce) and occipital pole (OP) were homogenized to 10% (wt/vol) in phosphate buffered saline (PBS). Aliquots were analyzed by Western blot with or without proteinase K (PK) digestion as previously described[12]. Briefly, samples were digested with 100 µg/mL PK for 1 hour at 37°C, before mixing with the appropriate PAGE sample buffer. Samples (utilizing double the volume of PK digested compared to undigested) were resolved on 16% Tris-glycine or 12% NuPAGE (Invitrogen) gels and transferred to PVDF membrane (Millipore) before incubation with the appropriate primary and secondary antibody and visualization using enhanced chemiluminescence (ECL Plus; Amersham).

Initially, before PRNP sequencing results were known, samples were subjected to SDS-PAGE and Western blot analysis of PrP following PK digestion (PrP^{res}) alongside glycotype 1-4 controls, employing the commonly used 3F4 antibody as previously described[14]. As seen in Figure 4B, the characteristic di-, mono- and un-

glycosylated triple banding pattern of PrP^{res} was not present in the proband. Rather, a prominent immuno-reactive, PK-resistant fragment of less than 15kDa was observed. Numerous additional minor bands, creating a "ladder" spanning from >15 to at least 50kDa, often superimposed on subtle lane smearing, were also observed.

Epitope mapping using antibodies spanning the length of the prion protein (see Figure 4C and Table 1) was carried out to further characterise the small fragment. As seen in Figure 4C, the PrP^{res} fragment, resolving at approximately 8kDa, was not immuno-reactive with antibodies to the far N-terminus (8B4) or those with epitopes beyond codon 143 (ICSM 18) towards the C-terminus of the prion protein, leading to the conclusion that the 8kDa fragment resulted from combined N- and C-terminal truncation of PrP^{res}. Based on its apparent molecular weight and the known antibody epitopes, we predict the 8kDa fragment results from PrP cleavage around residues 60 and 140. Further, the higher molecular weight bands are thought to represent aggregation of the 8kDa fragment into highly stable, higher order species.

Tau Analysis

CSF was examined by ELISA (INNOTEST hTau Ag and Phospho-Tau (181P), Innogenetics N.V. Belgium), according to manufacturer's instructions, and showed both total tau and tau phosphorylated at threonine 181 were elevated: total tau 1409 (normal <433pg/ml), with phospho-tau 126 (normal <93pg/ml). Reference ranges were determined from 59 well characterized, healthy elderly volunteers participating in the Australian Imaging Biomarker Lifestyle (AIBL) study[15].

Cerebellum and hippocampus were homogenised (10% [wt/vol] in PBS) and analyzed by western blotting for the different tau isoforms using 4-12% NuPAGE gels and probed with a rabbit anti-Human Tau antibody (1:3000; Dako). In the cerebellum, (absence of tau pathology) there was no difference in the tau isoform profile when comparing the proband with age-matched control and Alzheimer's disease (AD) brains. In the hippocampus (abundant tau pathology), the tau isoform profile of the proband did not resemble that seen in either the control or AD brain (data not shown).

DISCUSSION

Characteristically, GSS is inherited in an autosomal dominant manner[4], and therefore usually occurs in the setting of a known family history of similar neurodegenerative disorder. No history of similar neurological disease was known amongst first-degree relatives; however, absence of family history can occur in up to 30% of GSS pedigrees[2]. Awareness of a maternal aunt dying in a mental hospital from a poorly characterised illness of apparently only months in duration is of uncertain but possible relevance.

Our proband manifested rapid cognitive and gross motor decline following her minor elective surgery, dying approximately 1 month after the procedure. The proband's limited contact with her family prior to surgery rendered precise determination of presenting symptoms and their duration impossible. Notwithstanding the longstanding estrangement from her family, information available, however suggests a relatively brief duration of cognitive and behavioural changes for up to 6 months prior to hospital admission. Although limited survival after first symptoms is reported in GSS, it is uncommon that durations of this length occur in GSS patients[5,6,10,11]. Typically,

GSS follows a prolonged clinical course, evincing a median duration of over three years[5]. Our patient aligned more to the contrasting relatively short median survival observed in sporadic CJD[5]. In addition, molecular pathological evaluation of our propositus using the 3F4 antibody demonstrated prominent, low molecular weight, protease-resistant PrP^{res} bands on Western blots of brain, a feature more usually associated with much longer survival[6,10]. Similarly, although valine homozygosity at codon 129, as seen in our patient, has been reported in GSS, it is most unusual and further, also appears to usually correlate with prolonged symptomatic illness[4,6,10,11].

Predominance of shorter (<15kDa) PK-resistant PrP^{res} fragments on Western blots has been observed in GSS in association with numerous mutations, including those clustered towards the C-terminus of the prion protein (H187R, F198S, D202N, Q212P, Q217R and Y218N), as well as those situated more in the middle of the protein (P102L, P105L, A117V, G131V and S132I)[6,10,16]. It remains to be determined how PrP containing mutations within the C-terminus, including V176G, engender processing such that the core of amyloid deposits appear to be constituted by truncated mutant species that do not actually harbour the mutation[10].

Aligned to previous reports for the G131V, S132l, Y160X, H187R, F198S, D202N, Q217R, Y218M and Q227X mutations[6,9,10,16], the presence of shorter, non-classical PrP^{res} fragments on Western blots in our proband was associated with the neuropathological profile of high amyloid plaque burden, neurofibrillary degeneration and relative paucity of spongiform change in the brain; however, some mutations, such as P102L, P105L and A117V, despite the presence of shorter PrP^{res} fragments on Western blots and considerable amyloid deposition in the brain do not consistently

display neurofibrillary degeneration[16]. It is noteworthy that those GSS patients displaying predominance of longer 21-30kDa PrP^{res} fragments on Western blots, as exemplified by some P102L carriers, generally have more prominent neuropil vacuolation, usually associated with "synaptic" type PrP immunostaining rather than mainly amyloid plaque burden[10]. The presence of tau-positive neurofibrillary tangles and neuropil threads as frequently observed in GSS, including our proband, underscores the likelihood that accumulation of aberrant tau in the brain may be a relatively non-specific secondary consequence to certain primary cerebral amyloidoses, as typified by Alzheimer's disease.

In summary, in addition to expanding the range of *PRNP* mutations found in neuropathologically confirmed prion disease, our case is of interest and informative through displaying a constellation of clinical, genetic, molecular and pathological features, which although individually recognised across the spectrum of GSS, are unusual in their co-occurrence in a single person. Consequently, our proband serves to further broaden the already diverse genetic and phenotypic spectrum recognised for GSS.

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FIGURE LEGENDS

Figure 1 Family tree depicting the proband (solid fill) and first degree relatives with current ages or ages at death (in years). No first-degree relative was reported to suffer from any neurological disease with the exception of one son (line fill) with schizophrenia. O = female; □ = male; strikethrough = deceased.

Figure2. Representative, proband, brain magnetic resonance images. Fluid attenuated inversion recovery (A), diffusion weighted (B) and apparent diffusion coefficient map (C) images demonstrate mild age-appropriate cerebral atrophy, associated with some non-specific T2 hyperintensities, but absence of changes typically found in human prion disease, especially sporadic Creutzfeldt-Jakob disease.

Figure 3. Neuropathological findings.

A) Temporal cortex showing numerous plaques (encircled) in combination with neuronal loss and astrocytic gliosis (arrowheads) (haematoxylin and eosin x 200) with B) the plaques immunoreactive with the anti-PrP monoclonal antibody 12F10 and associated with C) tau immunoreactive neurofibrillary tangles and prominent neuropil threads.

Figure 4. Molecular-genetic characterisation. **A.** Schematic representation of the prion protein open reading frame and protein, with the nucleotide changes, codon positions and resulting amino acid changes detected in the proband as indicated. **B.** Proteinase K (PK) digest and PAGE/Western blot analysis (using 3F4 antibody) of frozen brain tissue from two brain regions of the proband (Ce – cerebellum; OP – occipital pole) compared to glyotype controls (T1, T2, T3, T4 – PrP^{res} glycotypes 1, 2,

3, 4[17,18]. **C.** Characterisation of the small amyloidogenic PrP^{res} fragment by PAGE/Western blot epitope mapping, utilising anti-prion protein antibodies spanning the length of the protein as specified (see also Table 1). Schematic representations of the small fragment and oligomeric species, and of the potential N- and C-terminal truncation sites based on antibody epitopes, are highlighted on the right and below, respectively.

Table 1. Panel of anti-PrP antibodies used for epitope mapping.

1° Antibody	*1° dilution*	*2° antibody (HRP conjugated)*	*2° dilution*	*Supplier*
8B4 (monoclonal)	1:10,000	Anti-mouse	1:10,000	Alicon
SAF32 (monoclonal)	1:3,000	Anti-mouse	1:10,000	Caymen Chemical
03R19 (polyclonal)	1:5,000	Anti-rabbit	1:10,000	V Lawson[18]
3F4 (monoclonal)	1:5,000	Anti-mouse	1:10,000	Covance
ICSM18 (monoclonal)	1:40,000	Anti-mouse	1:10,000	D-Gen
5B9-biotin (monoclonal)	1:50,000	Streptavidin	1:40,000	Abcam
EP1802Y (monoclonal)	1:10,000	Anti-rabbit	1:5000	Abcam

FIGURE 1

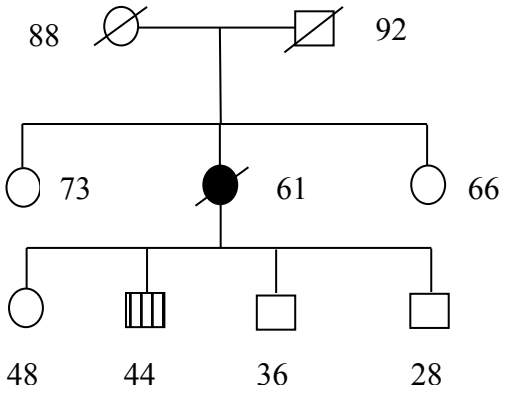


FIGURE 2

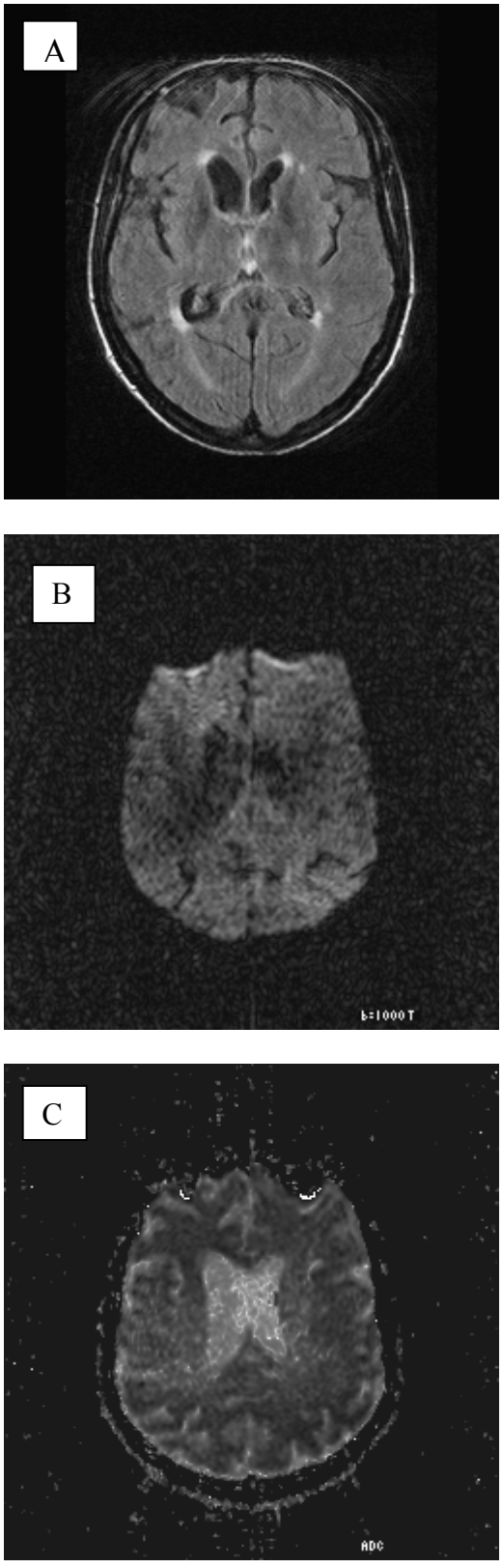


FIGURE 3

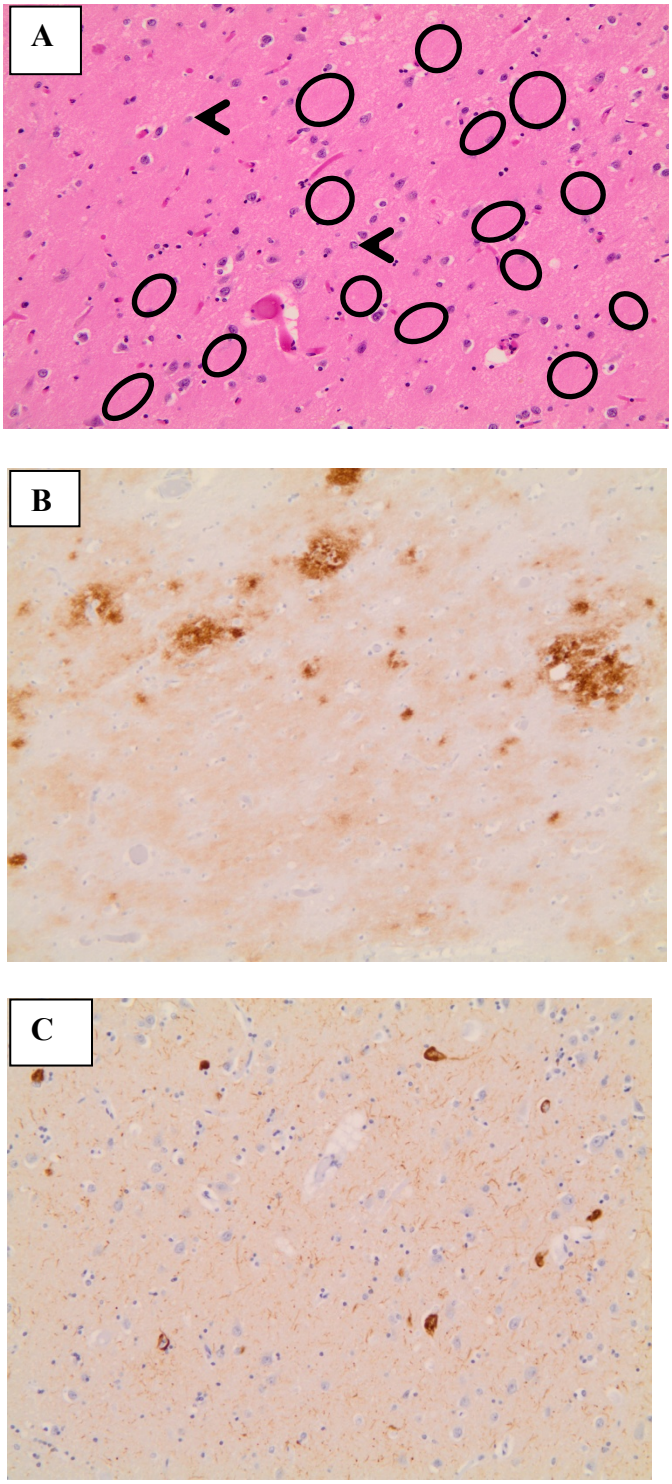
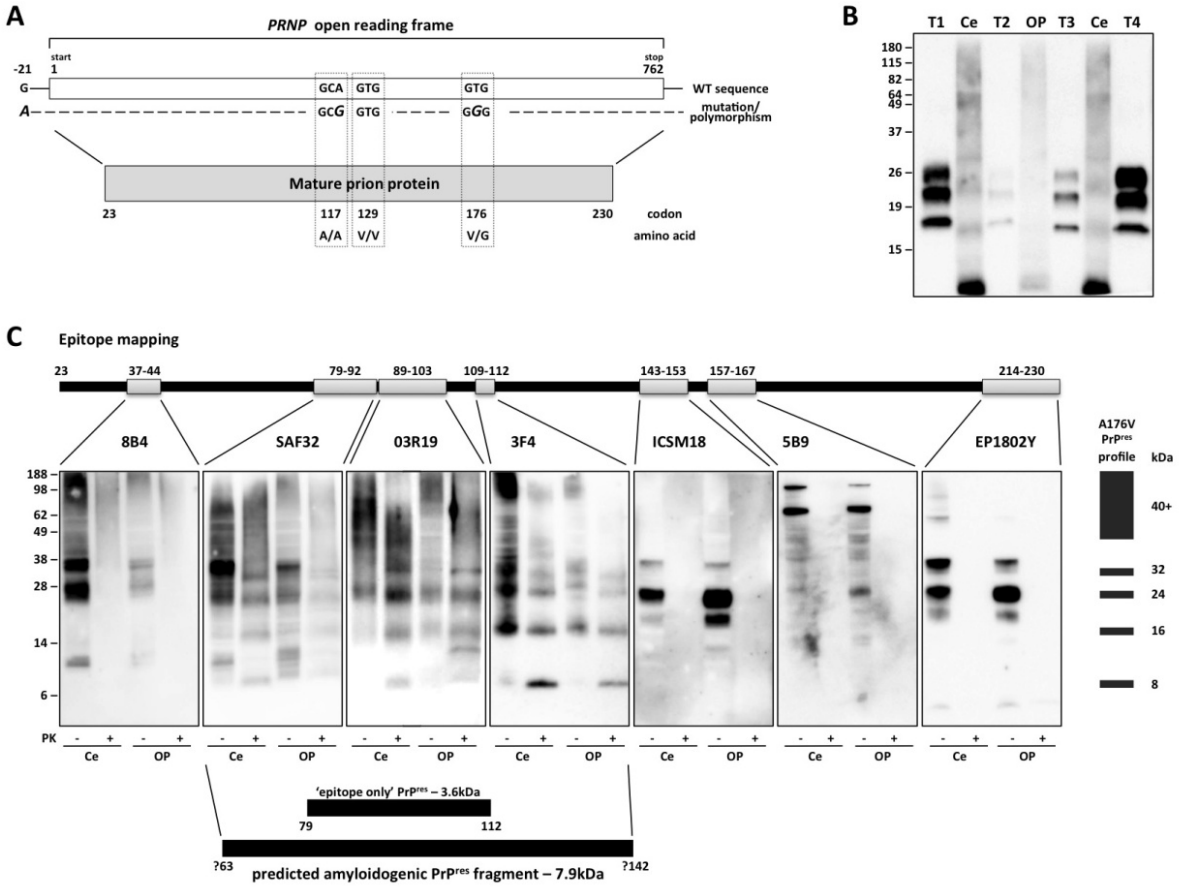


FIGURE 4


A
PRNP open reading frame
start
stop
-21
1
762
G
A
GCA
GTG
GTG
GCG
GTG
GGG
WT sequence
mutation/
polymorphism
Mature prion protein
23
117
129
176
230
codon
A/A
V/V
V/G
amino acid
B
T1
Ce
T2
OP
T3
Ce
T4
180
115
82
64
49
37
26
19
15
C
Epitope mapping
23
37-44
79-92
89-103
109-112
143-153
157-167
214-230
8B4
SAF32
03R19
3F4
ICSM18
5B9
EP1802Y
A176V
PrPres
profile
kDa
40+
32
24
16
8
188
98
62
49
38
28
14
6
PK
Ce
OP
'epitope only' PrPres – 3.6kDa
79
112
?63
?142
predicted amyloidogenic PrPres fragment – 7.9kDa