Variably protease-sensitive prionopathy

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Abstract

Variably protease-sensitive prionopathy (VPSPr), originally identified in 2008, was further characterized and renamed in 2010. Thirty-seven cases of VPSPr have been reported to date, consistent with estimated prevalence of 0.7–1.7% of all sporadic prion diseases. The lack of gene mutations establishes VPSPr as a sporadic form of human prion diseases, along with sporadic Creutzfeldt–Jakob disease (sCJD) and sporadic fatal insomnia. Like sCJD, VPSPr affects patients harboring any of the three genotypes, MM, MV, and VV at the prion protein (PrP) gene polymorphic codon 129, with VPSPr VV accounting for 65% of all VPSPr cases. Distinguishing clinical features include a median 2-year duration and presentation with psychiatric signs, speech/language impairment, or cognitive decline. Neuropathology comprises moderate spongiform degeneration, PrP amyloid miniplaques, and a target-like or plaque-like PrP deposition. The abnormal PrP associated with VPSPr typically forms an electrophoretic profile of five to seven bands (according to the antibody) presenting variable protease resistance depending on the 129 genotype. The familial prion disease associated with the V180I PrP gene mutation which harbors an abnormal PrP with similar electrophoretic profile might serve as a model for VPSPr. Transmission to animals has definitively established VPSPr as a prion disease. Because of its recent identification, rarity, and the elusiveness of its abnormal PrP, VPSPr remains largely understudied.

INTRODUCTION

Variably protease-sensitive prionopathy (VPSPr) was originally described in 2008 based on a study of 11 US subjects (Gambetti et al., 2008). The identification of this rare and clinically rather indistinct novel condition, and its prompt independent validation that followed, was made possible by the existence of many national prion surveillance centers worldwide dedicated to the examination and tissue banking of prion diseases. During regular examination of acquired cases at the US National Prion Disease Pathology Surveillance Center (NPDPSC), it was noted that in rare but recurring cases, brain tissues tested positive for the presence of abnormal, disease-related prion protein (PrP¹TSE) by immunohistochemical techniques. However, they were negative following biochemical analysis by gel electrophoresis and immunoblotting of PrP¹TSE, a test that is typically performed following treatment with proteinase K (PK) given the common PrP¹TSE protease resistance in prion diseases. Enhanced analyses demonstrated the presence of a peculiar form of PrP¹TSE that was particularly sensitive to PK digestion and displayed a distinctive multiple-band profile upon immunoblotting.

Genetic analysis established that all subjects were homozygotes for the codon valine (V) at position 129 of the prion protein (PrP) gene, the site of a common and nonpathogenic methionine polymorphism, M129V (Parchi et al., 1999; Gambetti et al., 2003, 2011). Moreover, no mutation was found in and adjacent to the coding...
region of the PrP gene where genetic mutations reside in inherited prion diseases, thus defining VPSPr as a sporadic prion disease (Gambetti et al., 2003, 2008; Kong et al., 2004). A subsequent study (Zou et al., 2010) reporting 15 additional cases from the United States and Italy established that all three M129V genotypes, MM, MV, and VV, not just VV, were affected in VPSPr. This property makes VPSPr the only sporadic human prion disease that affects the three 129 genotypes besides sporadic Creutzfeldt–Jakob disease (sCJD) (Chapter 9). This second study also revealed that PrP^{TSE} resistance to PK treatment varied according to the 129 genotype. This finding led to the amendment of the disease label from the original protease-sensitive prionopathy (PSPr) in the first 2008 publication to the current VPSPr (Gambetti et al., 2008; Zou et al., 2010).

Two additional VPSPr features emerged. First, VPSPr prevalence in the three 129 genotypes was found to be MM 11%, MV 24%, VV 65% (Table 10.1), which is almost the opposite of that associated with sCJD (MM 68%, MV 16%, VV 16%) (Collins et al., 2006), indicating that the role played by the 129 genotype is different in the two diseases. Second, several VPSPr patients have a family history of cognitive impairment, which raises the possibility of a genetic component in VPSPr. Unfortunately, no genetic or neuropathologic examination has been performed on cognitively impaired relatives of VPSPr patients.

**EPIDEMIOLOGY**

To date, 37 cases of VPSPr have been published. They include 25 from the United States (Gambetti et al., 2008; Zou et al., 2010; Cannon et al., 2014), five from the United Kingdom (Head et al., 2009, 2010, 2013); two each from Italy and Spain (Giaccone et al., 2007; Rodriguez-Martinez et al., 2010, 2012; Zou et al., 2010), and one each from Austria, Germany, and the Netherlands (Krebs et al., 2007; Jansen et al., 2010; Assar et al., 2015). One of the US cases was diagnosed incidentally at autopsy of a 93-year-old neurologically asymptomatic individual (Ghoshal et al., 2015). As for the M129V polymorphic genotype, the 37 cases include 24 VV homozygotes, nine MV heterozygotes, and four MM homozygotes. In addition, the US NPDPSC lists a total of 57 cases of VPSPr (inclusive of the 25 published) from a total of 3279 sporadic prion disease cases, which would estimate the VPSPr prevalence at 1.7% among all sporadic prion diseases (US National Prion Disease Pathology Surveillance Center, 2017). Thirteen VPSPr cases are stored by the National CJD Surveillance and Research Unit in the United Kingdom, which by the same calculation would result in a 0.7% prevalence of VPSPr there (UK National CJD Research and Surveillance Unit, 2017). Head et al. (2013) have estimated that VPSPr accounts for one death per 4 years in the ~60 million UK population. Adding together the unpublished cases stored by the US and UK prion surveillance centers brings the total number of known VPSPr cases worldwide to at least 77. However, it is likely that VPSPr is underreported, given that in most cases it is initially misdiagnosed as one of the non-Alzheimer dementias, conditions in which autopsies are not regularly performed (Puoti et al., 2012).

**DEFINITION**

VPSPr is a novel sporadic prion disease, which may account for almost 2% of all sporadic prion diseases. Similar to sporadic Creutzfeldt–Jakob disease (sCJD), VPSPr affects individuals harboring each of the three genotypes at codon 129 of the PrP gene, thus segregating VPSPr into three subtypes: MM, MV, and VV. The M129V genotype slightly affects disease phenotype and PrP^{TSE} features. Overall, mean age at onset is 70 and duration 2 years. Clinical presentation often includes one or more of three sets of signs: psychiatric abnormalities, speech/language impairment, and cognitive decline (Table 10.1). Histopathologic features include moderate cortical and subcortical spongiform degeneration (SD) and miniplaques in the cerebellum, while PrP immunostaining often reveals a distinctive target-like granular pattern. Depending on the antibody used, PrP^{TSE} forms a five- or seven-band ladder-like electrophoretic profile with increasing protease resistance from VV to MM subtypes.

**CLINICAL FEATURES**

**Demographics**

VPSPr is unique given its departure from the clinical aspects typically observed in other human prion diseases. The clinical diagnosis of VPSPr is difficult because of its nonspecific clinical presentation and noncontributory diagnostic tests that are typically suggestive of prion disease.

The polymorphism at codon 129 of the PrP gene affects many aspects of this prion disease, including susceptibility to the disease, age at onset, illness duration, clinical symptoms, and diagnostic test results (Table 10.2). Contrary to sCJD, the majority of VPSPr cases are homozygous for valine at codon 129 (65%), and the minority are homozygous for methionine (11%) (Table 10.2). VPSPr is typically a mid- to late-life illness with a mean age at onset of 70 years (range 48–87 years) (Puoti et al., 2012). The presence of valine at codon 129 seemingly has a dose–effect relationship toward a younger age at onset (mean age at onset: VV = 67, MV = 74, MM = 78 years) (Table 10.2).
### Table 10.1

Classification of variably protease-sensitive prionopathy

<table>
<thead>
<tr>
<th>129 subtype</th>
<th>N</th>
<th>Onset (years)</th>
<th>Course (months)</th>
<th>Type of presentation</th>
<th>Histopathology SD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PrP immunostaining pattern&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. cort</td>
<td>BG</td>
</tr>
<tr>
<td>MM&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3</td>
<td>66 (55–78)</td>
<td>56 (41–78)</td>
<td>Parkinsonian, ataxic, cognitive</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>MV</td>
<td>9</td>
<td>73 ± (65–81)</td>
<td>37 (7–72)</td>
<td>Psychiatric, parkinsonian, cognitive behavioral, aphasic</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>VV&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>64 ± (48–77)</td>
<td>30 (10–78)</td>
<td>Psychiatric, ataxic, cognitive, behavioral, spastic gate, gaze palsy</td>
<td>As above but forming target-like structures</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Typical spongiform degeneration (SD) severity, rated relatively to the three genotypes, and typical immunostaining patterns.

<sup>b</sup>Often clinical presentation included two types coexisting or in rapid succession.

<sup>3</sup>MM group includes four cases, but the fourth was asymptomatic.

<sup>d</sup>Includes one case treated with doxycycline for 4 years.

BG, basal ganglia; C. cort, cerebral cortex; Cereb, cerebellum.
### Table 10.2
Clinical characteristics of variable protease-sensitive prionopathy (VPSPr)

<table>
<thead>
<tr>
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<th>PRNP codon 129 polymorphism</th>
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<tbody>
<tr>
<td></td>
<td>Met-Met</td>
</tr>
<tr>
<td>Mean age at onset</td>
<td>78 (64–87)</td>
</tr>
<tr>
<td>(years, range)</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>41 (10–73)</td>
</tr>
<tr>
<td>(months, range)</td>
<td></td>
</tr>
<tr>
<td>Family history of</td>
<td>33%</td>
</tr>
<tr>
<td>dementia</td>
<td></td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Psychiatric symptoms, aphasia, parkinsonism, ataxia</td>
</tr>
<tr>
<td>Advanced clinical</td>
<td>Cognitive impairment, aphasia, myoclonus</td>
</tr>
<tr>
<td>symptoms</td>
<td></td>
</tr>
<tr>
<td>EEG suggestive of prion disease</td>
<td>50%</td>
</tr>
<tr>
<td>(14-3-3 and tau)</td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td>50%</td>
</tr>
<tr>
<td>Brain MRI suggestive of</td>
<td>0%</td>
</tr>
<tr>
<td>prion disease</td>
<td></td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid; EEG, electroencephalogram; MRI, magnetic resonance imaging.
An important difference between VPSPr and other sporadic prion diseases is its prolonged illness duration, which likely affects clinical ascertainment of cases. The duration of VPSPr is best measured in years as opposed to months, with a mean disease duration of 2 years (Puoti et al., 2012). Cases with durations over 6 and 7 years have been described in the literature (Appleby et al., 2009; Head et al., 2013). The codon 129 polymorphism affects disease duration, with the presence of valine resulting in a shorter duration, which is the opposite of what is observed in sCJD (Table 10.2)( Parchi et al., 1999; Puoti et al., 2012). Because of the insidious onset and slower progression of clinical symptoms compared to sCJD, it is likely more difficult to accurately determine the time of disease onset in VPSPr cases.

Clinical course

The clinical presentation and course in VPSPr can best be described as an atypical dementia and differ from the phenotype observed in most cases of sCJD. One or more of a triad of symptoms characterizes the clinical presentation of VPSPr: psychiatric symptoms, speech/language impairment, and cognitive decline (Puoti et al., 2012). Most VV and MV cases present with psychiatric symptoms, cognitive decline, and/or aphasia. Cases homozygous for methionine have initial symptoms more similar to classic sCJD with psychiatric symptoms, aphasia, parkinsonism, and ataxia. Psychiatric symptoms often include disinhibition, euphoria, impulsivity, and apathy (Zou et al., 2010). Speech and language impairments include anomic or semantic aphasia with or without dysarthria. Cognitive impairment is most often associated with frontal lobe dysfunction (e.g., dysexecutive syndrome).

Whereas the initial presentation of VPSPr is mostly characterized by cortical impairments, subcortical and cerebellar symptoms arise later in the disease course. Cognitive decline is observed in all cases at some point in the disease (Puoti et al., 2012). Parkinsonism, ataxia, and myoclonus often develop later in the disease course. Additionally, certain codon 129 polymorphisms are marked by a paucity of specific symptoms. For example, aphasia is rare in MV cases and psychiatric symptoms are rare in MM cases (Zou et al., 2010). Clinical criteria including symptoms are proposed in Table 10.3.

**Table 10.3** Proposed clinical criteria for variable protease-sensitive prionopathy

<table>
<thead>
<tr>
<th>A. Symptoms (both 1 and 2)</th>
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<tbody>
<tr>
<td>1. Cognitive impairment</td>
</tr>
<tr>
<td>2. Two or more of the following:</td>
</tr>
<tr>
<td>(a) Psychiatric symptoms</td>
</tr>
<tr>
<td>(b) Parkinsonism or aphasia</td>
</tr>
<tr>
<td>(c) Ataxia or myoclonus</td>
</tr>
</tbody>
</table>

| B. Duration < 8 years |

| C. Lack of alternative etiology or phenotype divergence from other neurodegenerative atypical dementias |

Tests used to diagnose prion disease are often unhelpful in the diagnosis of VPSPr. Hence, the ability to successfully diagnose VPSPr relies on the clinical phenotype and exclusion of other etiologies.

**Electroencephalogram (EEG)**

Periodic sharp-wave complexes (PSWCs) on EEG are suggestive of prion disease and are part of the World Health Organization’s diagnostic criteria for sCJD (World Health Organization, 1998). The presence of PSWCs is rare in VPSPr, with an estimated overall sensitivity of 9% (Puoti et al., 2012). PSWCs have been detected in 25% of MV and 50% of MM cases. No reported VV cases have demonstrated PSWCs. Typically, EEGs are normal or demonstrate generalized slowing (Gambetti et al., 2008). In some cases, EEGs are initially normal and then progress to generalized slowing later in the disease course (Head et al., 2009; Rodriguez-Martinez et al., 2012).

**Cerebrospinal fluid**

The increase of certain cerebrospinal fluid (CSF) proteins can be suggestive of prion disease. Rise of 14-3-3 in CSF with a clinical duration less than 2 years is part of the World Health Organization diagnostic criteria for sCJD (World Health Organization, 1998). Unfortunately, CSF 14-3-3 protein is typically negative or weakly positive (e.g., nondiagnostic) in VPSPr (Gambetti et al., 2008; Head et al., 2009; Jansen et al., 2010; Rodriguez-Martinez et al., 2012; Assar et al., 2015). However, a study detected positive CSF 14-3-3 protein in 50% of all UK VPSPr cases (Head et al., 2013). When CSF 14-3-3 is combined with tau, diagnostic sensitivity is 21% in all VPSPr cases with differing sensitivities by codon 129 genotype (Puoti et al., 2012). No MV cases demonstrated diagnostic 14-3-3/tau levels, whereas 50% of MM and 37% of VV cases...
had CSF results suggestive of prion disease. In the UK series, CSF S100b was positive in 75% of cases (Head et al., 2013), though this is not a diagnostic test that is routinely used in the diagnosis of prion disease. Two VV cases of VPSPr were reported to have elevated CSF protein levels (175 mg/dL and 126 mg/dL), the significance of which is unknown (Gambetti et al., 2008). CSF from one case of VPSPr was tested with real-time quaking-induced conversion (RT-QuIC) and found to be negative (Lattanzio et al., 2017). However, using the second generation RT-QuIC (IQ-CSF) 3 out of 3 cases of VPSPr were positive (Franceschini et al., 2017).

**Neuroimaging**

Brain magnetic resonance imaging (MRI) is a sensitive and specific diagnostic tool for sCJD. Hyperintensity on diffusion-weighted imaging (DWI) is typically seen in the basal ganglia and/or in a gyral pattern of the cortex in sCJD (Zerr et al., 2009); however; these brain MRI findings suggestive of prion disease are typically not present in VPSPr. Only two cases to date have demonstrated brain MRI findings suggestive of prion disease, both of which were VV at codon 129 (Zou et al., 2010; Assar et al., 2015). Brain MRIs of VPSPr cases typically demonstrate diffuse cortical atrophy, with some cases showing preferential atrophy of the frontal and/or parietal lobes and the cerebellum (Gambetti et al., 2008; Rodriguez-Martinez et al., 2012; Head et al., 2013). Overall, diagnostic sensitivity of brain MRI is 3% (Puoti et al., 2012). Single-photon emission computed tomography (SPECT) has been performed in three cases of VPSPr. All cases demonstrated hypoperfusion, which was global in one case and asymmetric in two (Gambetti et al., 2008; Rodriguez-Martinez et al., 2012).

**DIFFERENTIAL DIAGNOSIS**

Because the clinical features of VPSPr resemble atypical dementias, these cases are most often initially misdiagnosed as non-Alzheimer disease dementias. The most common clinical misdiagnoses of VPSPr include normal-pressure hydrocephalus, dementia with Lewy bodies, and frontotemporal lobar degeneration (Gambetti et al., 2011). One can certainly understand how VPSPr may be initially misdiagnosed as an atypical dementia. The prominent symptoms of aphasia, parkinsonism, and frontal lobe syndrome are suggestive of several frontotemporal lobar degeneration subtypes, including frontotemporal dementia, primary progressive aphasia, semantic dementia, progressive supranuclear palsy, and corticobasal syndrome. The prominent parkinsonian symptoms with cognitive impairment may also be suggestive of dementia with Lewy bodies or Parkinson disease dementia. Because of the prominence of psychiatric symptoms, some cases may initially be diagnosed as having a mood disorder (Gambetti et al., 2008; Assar et al., 2015).

As diagnosis of VPSPr currently stands, it is very difficult to discern the above atypical dementias from VPSPr in the initial stages of the disease. In fact, diagnosis of prion disease is often not considered until later in the disease course (Gambetti et al., 2008). One item that may signal a diagnosis of VPSPr, as opposed to one of the above atypical dementias, is a divergence from the expected clinical course of a previously diagnosed atypical dementia. For example, ataxia would not be expected in any of the above conditions and should prompt the consideration of VPSPr as the etiology. Additionally, a faster than expected disease progression may also be suggestive of VPSPr. Aside from disease-specific modalities (e.g., amyloid imaging), neuroimaging may be misleading and lead to an initial diagnosis of frontotemporal lobar degeneration due to frontal atrophy or asymmetric hypoperfusion on SPECT.

**NEUROPATHOLOGY**

The common histopathologic feature to all codon 129 genotypic subtypes of VPSPr is a moderate degree of SD, which similarly affects cerebral neocortex, entorhinal cortex, basal ganglia, and thalamus, and to a lesser degree the molecular layer of the cerebellum, while typically the hippocampus is spared (Fig. 10.1) (Jansen et al., 2010; Zou et al., 2010; Head et al., 2013). The SD brain distribution is similar to, but less severe than, that of sCJDMM1 (sCJD associated with PrP MM genotype and PrP^{TSE} type 1), the most common subtype of sCJD. However, the SD vacuoles of VPSPr differ in size since they are on average 50% larger than those of sCJDMM1 (9 ± 3 vs. 6 ± 1) and spread over a larger range of sizes (Parchi et al., 1999; Zou et al., 2010); however they are smaller and not as closely clustered or confluent as the vacuoles in the sCJDMM2 subtype (Parchi et al., 1999; Zou et al., 2010).

A distinctive feature is the presence of very small prion plaques in the molecular layer of the cerebellum and, rarely, the hippocampal region that are often irregular in shape and may comprise several cores (Fig. 10.2) (Gambetti et al., 2008; Zou et al., 2010; Head et al., 2013; Cannon et al., 2014). Immunoelectron microscopic examination of cerebellar and hippocampal miniplaques demonstrated poorly demarcated structures containing clusters of PrP-labeled fibrils embedded in an amorphous matrix. These features have been interpreted as consistent with poorly formed or immature amyloid plaques (Gambetti et al., 2008; Cannon et al., 2014). In VV cases, thioflavin S and FSB (bis-styrylbenzenes) staining procedures confirmed the presence of amyloid in many cerebellar miniplaques, while the cerebral...
neocortex remained seemingly negative (Flaherty et al., 2007; Puoti et al., unpublished data).

PrP immunostaining generally codistributes with SD and typically shows coarse granules in a background of a fine granular or “synaptic” pattern, often shaped as target-like formations with rounded clusters of granules surrounding a larger central one. A peculiar selective immune staining of the hippocampal molecular layer has also been noted in VV cases (Gambetti et al., 2008). In the cerebellum the pattern consists of small rounded deposits, which often show the tinctorial characteristics of real plaques and correspond to the

Fig. 10.1. Spongiform degeneration and prion protein (PrP) immunohistochemical pattern in the three genotypes of variably protease-sensitive prionopathy (VPSPr). Top row: mild to moderate spongiform degeneration in the three genotypes comprised of approximately 60% larger vacuoles than sporadic CJDMM1, the most common subtype of sporadic Creutzfeldt–Jakob disease. Middle and lower rows: PrP immunohistochemistry: fine granular deposits sometimes distributed in a tigroid pattern in VPSPr-129VV (VV, middle row); at higher magnification (lowest row) occasionally finer granules surround bigger ones in a target-like formation. Similar granular immunostaining in VPSPr-129MV but with less noticeable tigroid and target-like patterns (MV, middle and lowest rows). Plaque-like immunostaining in a fine granular background in VPSPr-129MM. Antibody to PrP 3F4. (Reproduced from Zou WQ, Puoti G, Xiao X, et al. (2010) Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. Ann Neurol 68: 162–172, with permission from John Wiley.)
miniplaques described above in the cerebellar molecular layer. Other cerebellar regions are not immunostained. Miniplaques and plaque-like deposits are also seen in the dorsal parts of the midbrain (Gambetti et al., 2008).

Separating neuropathologic features of the individual subtypes of VPSPr according to the 129 genotype is challenging because the variations are not substantial and the MM and MV subtype cohorts are still relatively small. Overall, VV and MV cases seem to share more similarities between them than with the MM subtype. While all subtypes share similar SD distribution profiles and similar vacuole sizes, the severity appears to be comparably higher in the VV and MV subsets than in the MM (Fig. 10.1) (Zou et al., 2010). The immunostaining patterns in the cerebral cortex of the VV subset most often consist of the aforementioned target-like formations. This pattern seems to become less well organized and detectable in MV heterozygotes and is virtually lost in MM homozygotes, where the immunostaining pattern is primarily plaque-like (Fig. 10.1) (Giaccone et al., 2007; Rodriguez-Martinez et al., 2010; Zou et al., 2010; Head et al., 2013). Similarly, presence of PrP amyloid miniplaques, while well documented in VV and MV subsets, has not been reported in MM homozygotes (Zou et al., 2010; Head et al., 2013). Combined, these findings seem to outline a trend running from the 129VV to the 129MM subset toward the formation of PrP TSE that shows an increasing propensity to aggregate and less to form amyloid. This trend would be inversely related to codon V dosage.

Another reported but little explored issue is the occurrence of co-pathologies in VPSPr. The majority of cases belonging to the three 129 genotypes harbor tau pathology preferentially in the lower medial temporal cortex, especially entorhinal and transitional cortices and hippocampal gyrus, which has been rated Braak stage 2 or higher, while the neocortex is minimally involved. Aβ plaques may also co-occur (Braak and Braak, 1995; Giaccone et al., 2007; Head et al., 2010; Rodriguez-Martinez et al., 2012). Ballooned neurons have been reported in two cases and pretangle formations have been observed in pigmented nuclei of one case (Giaccone et al., 2007; Rodriguez-Martinez et al., 2010; Cannon...
et al., 2014). Five cases harbored comorbidities of VPSPR and neurodegenerative diseases that included diffuse Lewy body disease (Head et al., 2010; Assar et al., 2015), argyrophilic grain disease (Giaccone et al., 2007; Rodríguez-Martínez et al., 2010), and amyotrophic lateral sclerosis (Cannon et al., 2014).

DISEASE-ASSOCIATED PRION PROTEIN

The PrP^TSE associated with VPSPR displays a panoply of peculiar features (Gambetti et al., 2008; Zou et al., 2010; Puoti et al., 2012). Following appropriate immunoblot procedures, PK-resistant PrP^TSE (resPrP^TSE) associated with VPSPR forms a distinctive ladder profile that comprises five or seven fragments (depending on the use of antibodies to the PrP N-terminus or to the C-terminus) (Fig. 10.3). The resPrP^TSE fragments originate exclusively from the monoglycosylated and unglycosylated isoforms, which results in the lack of diglycosylated isoform in the resPrP^TSE immunoblot profile even though this glycoform is present in the PK-untreated total PrP preparations (Gambetti et al., 2008; Zou et al., 2010).

The five PrP^TSE immunoblot bands demonstrated by N-terminus antibodies comprise fragments of 26, 23, 20, 17, and 7 kDa (Fig. 10.3) (Zou et al., 2010). Based on molecular weight and experiments with hydrofluoric acid to assess the presence of the glycolipid anchor, the five resPrP^TSE fragments have been tentatively identified (Notari et al., 2008; Dagdanova et al., 2010; Notari et al., unpublished data). Fragments 26 and 20 kDa originate from the monoglycosylated and unglycosylated isoforms, following N-terminus truncation by PK treatment; the 23- and 17-kDa fragments are the anchorless counterpart of the above two fragments, and the 7 kDa is a fragment that lacks a large portion of both N- and C-termini and is unglycosylated (Fig. 10.3) (Zou et al., 2010; Pirisinu et al., 2013). These conclusions are consistent with the result of enzymatic deglycosylation, which, as expected, reduces the original five fragments to three: the 20-kDa unglycosylated fragment, its 17-kDa anchorless counterpart, and the 7-kDa internal fragment (Fig. 10.3) (Zou et al., 2010).

Antibodies to the PrP C-terminus demonstrate the presence of two additional bands: one with an electrophoretic mobility of 12–13 kDa that matches the CTF12/13, two C-terminal fragments described in all sCJD subtypes (Zou et al., 2003), and one migrating at 7 kDa. The latter, despite the similar molecular weight, must be quite different from the 7-kDa fragment detected by the N-terminal antibody and rather may be an additional C-terminal fragment extensively truncated at the N-terminus.

The peculiar lack of the PrP^TSE diglycosylated form has been shown to result from the presence of only the isoform monoglycosylated at residue 197 among the resPrP^TSE fragments, while neither the isoform monoglycosylated at residue 181 nor the 181 and 197 diglycosylated isoform is converted to resPrP^TSE (Xiao et al., 2013).

The N- and C-termini of the 7-kDa fragment have been characterized by epitope mapping (Pirisinu et al., 2013). The N-terminus appears ragged with alternative termini at residues 97, 99, and 103, while the C-terminus has been identified at residue 152. This amino acid sequence would predict a relative mass of 5–6 kDa. No similar data are available for the N-termini of the other fragments.

Fig. 10.3. Immunoblot of proteinase K-treated prion protein (resPrP^TSE) from VPSPR-129MV and sCJDVV2 subtype demonstrated by an N-terminus antibody and diagrammatic representation of all bands detected by antibodies to N- and C-terminus. (A) The immunoblot compares the five-band ladder profile formed by resPrP^TSE associated with VPSPR with the three-band profile of a sporadic Creutzfeldt–Jakob disease subtype. Antibody 1E4. (B) In black: four fragments detected by antibodies to the N-terminus; In light gray: the fragment recognized by antibodies to C-terminus, the so-called CTF-12/13 (Zou et al., 2003); in deep gray: the two different fragments as for N- and C-terminus but comigrating 7-kDa fragments, one detected by antibodies to the N-terminus and the other by antibodies to C-terminus. Diglyc: diglycosylated isoform; Monoglyc: monoglycosylated; M.anchor: presumed monoglycosylated anchorless; U.anchor: presumed unglycosylated anchorless; Unglyc: unglycosylated.
Two other distinctive features of VPSPr PrP^TSE are amount and sensitivity to protease digestion. Analyses of the representation of total PrP^TSE (comprising both PK-sensitive and resistant components) carried out in VPSPr-VV have shown that total PrP^TSE accounts for approximately 3.5% of PrP preparations, including normal or cellular PrP (PrPC) and PrP^TSE, about 16 times less than in the sCJDMM1 subtype (Gambetti et al., 2008). Furthermore, according to this study, 24% of the total PrP^TSE is PK-resistant, compared to nearly 90% in the sCJDMM1 subtype. These data explain the low amount of resPrP^TSE in VPSPr (Gambetti et al., 2008).

A compounding issue is that antibody 3F4, which is widely used to probe human PrP^TSE species, does not react well with some fragments of VPSPr-associated PrP^TSE, particularly in the VV subtype (Gambetti et al., 2008). As for the PK sensitivity of PrP^TSE, it is highest in the VV subtype and lower in subtypes MV and MM (Fig. 10.4). In fact, PK titration experiments showed that VPSPr MM and MV have a low PK sensitivity even compared with those of sCJD subtypes (Saverioni et al., 2013). However, PK sensitivity also varies among the five resPrP^TSE fragments recognized by antibodies to the N-terminus. The 7-kDa fragment is the most PK-resistant across the three subtypes; it is the only highly PK-resistant fragment in VPSPr VV while two more fragments, 23 kDa and 17 kDa, show significant and similar PK resistance in the MV and MM subtypes (Fig. 10.4) (Zou et al., 2010). Notably, all these three fragments are anchorless.

Several tests performed to assess the conformational characteristics of VPSPr total PrP^TSE have underlined divergences with PrP^TSE species associated with sCJD subtypes (Gambetti et al., 2008; Pirisinu et al., 2013; Saverioni et al., 2013; Cali et al., unpublished data). First, sedimentation in sucrose gradients and fractionation by gel filtration, both of which separate PrP^TSE aggregates according to size (Rhodes et al., 2009), have shown that VPSPr PrP^TSE forms far fewer large aggregates than the PrP^TSE of sCJDVV1 (30% vs. 58% of total PrP^TSE), with few aggregates exceeding 2000 kDa. Similar data have been obtained by Saverioni and colleagues (2013) in a study comparing VPSPr with other sCJD. However, VPSPr data differ much less from those of Gerstmann–Sträussler–Scheinker (GSS) subtype carrying the A117V PrP gene mutation (GSS A117V) (Gambetti et al., 2008). Moreover, the banding pattern formed on immunoblot by PrP^TSE collected from the fractions that contain large aggregates differed in VPSPr from that of sCJDVV1 (the sCJD subtype used in this comparative study) and, to a lesser extent, from that of GSSA117V.

Second, PrP^TSE conformational stability has been tested with the conformational stability and solubility assay, which derives stability data by measuring the amount of the denaturing agent guanidine hydrochloride needed to solubilize 50% of native total PrP^TSE (GdnHCL1/2) (Pirisinu et al., 2013). The conformational stability and solubility assay has demonstrated that PrP^TSE conformational stability is higher in VPSPr VV (GdnHCL1/2 2.18) than in the VPSPr MV and MM subtypes that have similar GdnHCL1/2 values (1.63 and 1.67, respectively) (Pirisinu et al., 2013). Similar results have been obtained with the comparable conformational stability assay (Peretz et al., 2001; Cali et al., 2009; Cali et al., unpublished data).

The properties of the resPrP^TSE associated with VPSPr invite two considerations. First, the lack of the 181 glycoform in VPSPr resPrP^TSE is likely due to the incompetence of the normal 181 glycoform to adopt...
the resPrPTSE conformation characteristics of VPSPr and, therefore, to acquire PK resistance. Alternatively, it has been suggested that the 181 glycan is anomalous and hinders the conversion of the 181 glycoform to resPrPTSE (Nishina et al., 2006; Xiao et al., 2013). Second, the presence of both anchor-linked and anchorless conformers of the mono- and unglycosylated isoforms (fragments 26 and 23, as well as 20 and 17 kDa, respectively) exemplifies the remarkable heterogeneity of PrPTSE in VPSPr, which must reflect multiple conformations. As a result of this heterogeneity, a fraction of these two isoforms is PK-resistant at the C-terminus while the other is not and becomes anchorless. Furthermore, the size of the C-terminus region that remains PK-sensitive in the two anchorless fragments differs from that of the 7-kDa fragment. Based on the relative mass and detectability with the antibody to the C-terminus, the PK-sensitive C-terminal region of the 23- and 17-kDa anchorless fragments must be small once the mass reduction due to loss of the anchor is taken into account. In contrast, according to epitope mapping data, the 7-kDa fragment is generated by PK digestion of nearly 80 C-terminus residues.

Finally, the original report of VPSPr also described the limited presence of the three-band resPrPTSE characteristic of sCJD (Gambetti et al., 2008). Significant to minimal amounts of three-band resPrPTSE were detected in putamen, thalamus, and substantia nigra of three out of eight cases tested (Gambetti et al., 2008). This finding indicates that the three-band resPrPTSE characteristic of sCJD may occasionally occur in subcortical regions of VPSPr subjects. More recently, Peden et al. (2014) and Rodriguez-Martinez et al. (2012) and their colleagues have reported the presence of sCJD resPrPTSE in the cerebellum of three cases of VPSPr VV. In the latter report, sCJD resPrPTSE type 2, but the PrP immunostaining pattern of the cerebellum was apparently consistent with VPSPr rather than sCJDVv2, as would be expected by the presence of resPrPTSE type 2 in a subject harboring the VV genotype. All of these features, but especially the number and heterogeneity of the resPrPTSE fragments that imply distinct conformations, currently establish resPrPTSE as a unique resPrPTSE isof orm or strain among sporadic human prion diseases.

**EXPERIMENTAL TRANSMISSION AND IN VITRO AMPLIFICATION**

**Experimental transmission**

VPSPr has been experimentally transmitted to transgenic (Tg) mice in two concurrent studies (Diack et al., 2014; Notari et al., 2014a). Notari and colleagues (2014a) inoculated four lines of Tg mice expressing PrPC with 129 residues M or V at levels ranging from one- to eight-fold normal human brain levels on a murine PrPC null background. Brain homogenates (BH) from 12 VPSPr subjects that comprised six VV, four MV, and two MM subtypes were used as inocula. Mice of the same lines served as negative and positive controls. None of the 112 mice challenged with VPSPr BH developed clinical signs. 54% of the mice challenged with matching (i.e., having the same M or V genotype) BH from VPSPr VV or MM, showed histologic lesions that immunoreacted with PrP antibodies and 34% had positive immunoblots, further confirming the presence of PrPTSE (Fig. 10.5). There appeared to be no significant variation in attack rate between high (8×) and intermediate (3×) PrP expression mice. Five of six VPSPr VV BH and one of two MM BH inoculated into 129 matching mice transmitted the disease. The non-transmissible MM BH was from a clinically asymptomatic subject diagnosed at autopsy.

No transmission occurred when the 129 genotype was mismatched (i.e., BH VV was inoculated to 129M or MM into 129V mice). Surprisingly, there was also no transmission when BH MV was inoculated to 129V or 129M mice. These findings clearly point to an amino acid sequence barrier introduced by the M129V polymorphism.

Histologic lesions of two types were observed. The first featured poorly structured and often clustered plaques, most often located at the border of the hippocampus and the overlying white matter, and occasionally in periventricular regions (Fig. 10.5). Plaques appeared to be more prevalent in mice expressing PrPC 8 fold than 3 fold and to be more compact and better formed following inoculation with BH from VPSPr VV than MM (Fig. 10.5). The lesion of the second type consisted of focal SD located in the lacunosum-moleculare layer of the hippocampal formation (Fig. 10.5). Both plaques and SD areas were intensely reactive with PrP antibodies. Widespread PrP deposition was not detected in any of the mice challenged with any VPSPr BH. Immunoblot demonstrated small amounts of resPrPTSE forming a ladder resembling (Fig. 10.6), but apparently more PK-resistant than that associated with VPSPr, which was exclusively observed in the 8× PrP expression 129V Tg mice. Deglycosylation reduced the bands to three that matched the electrophoretic mobility of the corresponding VPSPr bands (Fig. 10.6). No PrPTSE was recovered in negative controls while all sCJD subtypes transmitted to positive controls with efficiency comparable to that reported in other studies. Surprisingly, none of the positive mice transmitted the disease on second passage.

Very similar results were obtained by Diack and coworkers (2014), following inoculation of BH from
**Fig. 10.5.** Immunohistochemistry and histopathology brain lesions in Tg mice inoculated with variably protease-sensitive prionopathy (VPSPr) extracts. (A) Clusters of prion plaques typically observed at the border of hippocampus and overlying white matter (Tg(HuPrP129V)×8); box marks area shown in (B). (B) At higher magnification, plaques appear often confluent or fragmented and are intensely prion protein (PrP)-immunoreactive; at hematoxylin and eosin stain, plaques show an amorphous core lacking the spiny appearance of typical kuru plaques (inset). (C) In (Tg(HuPrP129M)×2) mice inoculated with VPSPr-129MM, PrP aggregates generally appear looser and form fewer well-bounded plaques. (D–F) Second type of lesion consisting of PrP granular deposits in the lacunosum-moleculare layer of the hippocampal formation (D and E) that codistribute with spongiform degeneration (F) (box in D marks area shown in E). Antibody 3F4. (Reproduced from Notari S, Xiao X, Espinosa JC, et al. (2014a) Transmission characteristics of variably protease-sensitive prionopathy. Emerg Infect Dis 20: 2006–2014.)

**Fig. 10.6.** Immunoblot of abnormal prion protein (PrP\textsuperscript{TSE}) recovered from brains of variably protease-sensitive prionopathy (VPSPr)-inoculated Tg mice and controls. (A) Extract from brains of VPSPr-inoculated mice (VPSPr+), treated with increasing amounts of proteinase K (PK) show PK-resistant PrP\textsuperscript{TSE} (resPrP\textsuperscript{TSE}) fragments with a ladder-like electrophoretic profile even at high concentrations of PK (50 μg/mL). Nonspecific bands are seen in negative control mice (VPSPr−). The banding pattern in VPSPr+ roughly recapitulates that of resPrP\textsuperscript{TSE} from the VPSPr inoculum (VPSPr Inoc). (B) VPSPr+ extracts treated with PK (25 μg/mL) and PNGase F show three resPrP\textsuperscript{TSE} bands migrating at ∼20 kDa, ∼17 kDa, and ∼7 kDa that replicate those of similarly treated VPSPr inoculum (VPSPr Inoc). No bands can be detected in the negative Tg mice (VPSPr−). All preparations were run on the same gel, but unnecessary lanes were removed. Antibody 1E4. (Reproduced from Notari S, Xiao X, Espinosa JC, et al. (2014a) Transmission characteristics of variably protease-sensitive prionopathy. Emerg Infect Dis 20: 2006–2014.)
two subjects with VPSPr-VV and one with VPSPr-MV to Tg mice that were produced by gene targeting and expressed human PrP MM, MV and VV at physiologic levels. Transmission succeeded only with VPSPr-VV. Challenge of Tg mice with BH mismatched for the 129 residue also seems to support the presence of a primary structure barrier, as in the study of Notari and colleagues (2014a), although perhaps not as strict, since VPSPr VV was transmitted to 129M Tg mice, albeit to only one of 15 inoculated mice. Histologic lesions and patterns of PrP deposition were virtually identical, although lesions apparently were less frequent, presumably due to the lower PrP expression levels of the mice used in this study. Plaques were shown to contain amyloid by thioflavin S staining. No PrP TSE was demonstrated by immunoblot analyses. No data were reported on second passage.

Both studies prompt several considerations. VPSPr MV failed to transmit and at present remains the only VPSPr subtype with no evidence of transmission. The lack of clinical signs in PrP TSE -positive mice is likely due to the focality of the lesions and their location in relatively silent brain regions. Prion plaques similar in type and location to those described in these two studies have been observed previously in Tg and wild-type mice challenged with human BH or in vitro preparations that share the property of harboring plaques or seeding plaque formation (Piccardo et al., 2007; Asante et al., 2013; Pirisinu et al., 2016). The failure to transmit the disease upon second passage is puzzling but not unprecedented (Wadsworth et al., 2004; Piccardo et al., 2007, 2013). Based on the low amount of resPrP TSE in VPSPr, which indicates a low conversion efficiency, it has been proposed that due to limited amplification and propagation of the seed at first passage, along with the relative short lifespan of the mouse, the actual resPrP TSE concentration in the whole brain of the inoculated mouse is lower than that of the inoculum (Notari et al., 2014a). Furthermore, the failure of the second passage also suggests that there is no significant adaptation of the seed to the host during the first passage in VPSPr transmission despite the long incubation. Future studies of VPSPr transmission to more receptive hosts, such as the bank vole (Nonno et al., 2012), as well as experiments of PrP TSE rescue from brains of mice used in these two studies may clarify this issue.

Nonetheless, both these studies definitively establish that VPSPr is a transmissible prion disease and further highlight the unique properties of VPSPr PrP TSE among sporadic prion diseases.

**In vitro amplification**

The propensity of VPSPr PrP TSE to convert PrP C to its misfolded form was analyzed with protein misfolding cyclic amplification (PMCA) by two groups. Successful, albeit weak, amplification of VPSPr-VV PrP TSE has been reported by Peden et al. (2014), using two kinds of substrate: “human PrP expressing Tg mice” and human brain. The stronger amplification was obtained with Tg mice as substrate. Additionally, in this study, a PrP TSE species, described as similar to the sCJD type 2 and found in the cerebellum of two VV cases, was positively amplified with PMCA. Notari and coworkers, in a preliminary study (Gambetti et al., 2012; Notari et al., 2014b), succeeded in amplifying VPSPr PrP TSE using human PrP C from different Tg mice as substrate. The highest amplification was obtained using PrP C 129V from Tg152 mice expressing four to eight times normal levels of PrP C as substrate (Telling et al., 1995). With Tg mice expressing PrP129M or 129V at the same lower level (2 × ) (Kong et al., 2005 and unpublished data), amplification was sensitive to the M129V polymorphism and occurred only when 129 residues matched; however these mice supported a less robust amplification. Following PK treatment, the amplified resPrP TSE reproduced the same electrophoretic ladder-like profile as the seed (Gambetti et al., 2012; Notari et al., 2014b).

Peden et al. (2014) also demonstrated the capacity of VPSPr to convert recPrP to its misfolded form by real-time quaking-induced conversion (RT-QuIC), another amplification technique.

These studies definitively establish the propensity of VPSPr to replicate in vitro.

**ANIMAL MODELS**

It has recently been found that resPrP TSE associated with in a familial form of CJD harboring the V180I PrP gene mutation (fCJD V180I) displays an electrophoretic profile and antibody immunoreactivity that mimic those of VPSPr (Fig. 10.7) (Xiao et al., 2013). The electrophoretic profile, when probed with N-terminus antibodies, includes five bands that co-migrate with those of VPSPr, although show some difference in the ratios. Remarkably, as in VPSPr, both monoglycosylated at residue 181 and diglycosylated isoforms fail to convert to resPrP TSE; thus, of the three glycoforms only the 197 monoglycosylated is converted even though total PrP from fCJD V180I contains both monoglycosylated and diglycosylated isoforms like VPSPr. The virtually identical mobility of the five bands supports the notion that the resPrP TSE fragments have common properties in the two diseases, and therefore anchorless fragments may also be present in fCJD V180I. These striking similarities indicate that the resPrP TSE isoforms associated with VPSPr and fCJD V180I share similar conformations in spite of the different etiologies of the two diseases (genetic in fCJD V180I, sporadic in VPSPr). Because it is
a genetic disease, fCJDV180I would lend itself better to the study of pathogenetic mechanisms and might be a valid model to shed light on VPSPr.

**CONSIDERATIONS ON ETIOLOGY AND PATHOGENESIS**

Because VPSPr is rare, has been identified recently, and is associated with a particularly complex PrP^TSE isoform, it is especially challenging to speculate on its etiology and pathogenesis. According to the current hypothesis of multiple strain formation and competitive selection of the most efficient strain (Surewicz and Apostol, 2011; Weissman et al., 2011; Gambetti, 2013), VPSPr-associated PrP^TSE might result from the aberrant selection of an incompetent strain. This hypothesis would be consistent with rarity, as well as inefficient conversion and propagation of the PrP^TSE associated with VPSPr. The selection of the VPSPr PrP^TSE is likely to be strongly favored by 129V allele dosage, since VV homozygotes account for 65% of all affected subjects, despite the 12% frequency of this genotype in a normal Caucasian population (Zimmermann et al., 1999). This distribution is different from that of sCJD, where MM subjects account for 68% of cases and the VV for 16% (Collins et al., 2006). This divergence implies distinct roles for the 129 genotypes in the two diseases.

VPSPr-affected subjects frequently have a positive family history that cannot be ignored. Cognitive impairment with prevalence as high as 60% has been reported in the original cohort of 11 VPSPr VV patients (Gambetti et al., 2008). Two additional VV patients had a family history of a condition similar to amyotrophic lateral sclerosis (or amyotrophic lateral sclerosis was a comorbidity (Jansen et al., 2010; Cannon et al., 2014). Since no mutation has been detected in the coding region of the PrP gene in any of VPSPr-affected subjects, the presence of a variation involving other regions of the PrP gene or other parts of the genome needs to be investigated to assess the role of a genetic component in VPSPr.

VPSPr-associated PrP^TSE also has unique features, including the inability to acquire resistance to proteases by the PrP glycosylated at residue 181, regardless of whether it is the mono- or diglycosylated isoform, and by part of the C-terminus. Furthermore, data from experimental transmission also indicate that, compared to most subtypes of sCJD, conversion of the host PrP^C and propagation of newly formed PrP^TSE are slow and very limited. These features need to be clarified to begin understanding the pathogenetic mechanisms that underlie VPSPr.

**REFERENCES**


