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Webb, TEF
Poulter, M
Beck, J
et al.

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Phenotypic heterogeneity and genetic modification of P102L inherited prion disease in an international series

T. E. F. Webb,[1,2] M. Poulter,[1] J. Beck,[1] J. Uphill,[1] G. Adamson,[1] T. Campbell,[1] J. Linehan,[1] C. Powell,[1] S. Brandner,[1,2] S. Pal,[1,2] D. Siddique,[1,2] J. D. Wadsworth,[1] S. Joiner,[1] K. Alner,[2] C. Petersen,[2] S. Hampson,[2] C. Rhymes,[2] C. Treacy,[2] E. Storey,[3] M. D. Geschwind,[4] A. H. Nemeth,[5] S. Wroe,[1,2] J. Collinge[1,2] and S. Mead[1,2]

[1]MRC Prion Unit and Department of Neurodegenerative Disease, UCL Institute of Neurology, [2]National Prion Clinic and National Hospital for Neurology & Neurosurgery, Queen Square, London, WCIN 3BG, [3]Department of Medicine (Neuroscience), Monash University, Melbourne, Australia, [4]Department of Neurology, University of California, San Francisco (UCSF), San Francisco, CA, USA and [5]Department of Clinical Genetics, Churchill Hospital and Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, UK

Correspondence to: Prof. John Collinge, Department of Neurodegenerative Disease and MRC Prion Unit, UCL Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WCIN 3BG, UK
E-mail: j.collinge@prion.ucl.ac.uk

The largest kindred with inherited prion disease P102L, historically Gerstmann-Sträussler-Scheinker syndrome, originates from central England, with émigrés now resident in various parts of the English-speaking world. We have collected data from 84 patients in the large UK kindred and numerous small unrelated pedigrees to investigate phenotypic heterogeneity and modifying factors. This collection represents by far the largest series of P102L patients so far reported. Microsatellite and genealogical analyses of eight separate European kindreds support multiple distinct mutational events at a cytosine-phosphate diester-guanidine dinucleotide mutation hot spot. All of the smaller P102L kindreds were linked to polymorphic human prion protein gene codon 129M and were not connected by genealogy or microsatellite haplotype background to the large kindred or each other. While many present with classical Gerstmann-Sträussler-Scheinker syndrome, a slowly progressive cerebellar ataxia with later onset cognitive impairment, there is remarkable heterogeneity. A subset of patients present with prominent cognitive and psychiatric features and some have met diagnostic criteria for sporadic Creutzfeldt-Jakob disease. We show that polymorphic human prion protein gene codon 129 modifies age at onset: the earliest eight clinical onsets were all MM homozygotes and overall age at onset was 7 years earlier for MM compared with MV heterozygotes ($P = 0.02$). Unexpectedly, apolipoprotein E4 carriers have a delayed age of onset by 10 years ($P = 0.02$). We found a preponderance of female patients compared with males (54 females versus 30 males, $P = 0.01$), which probably relates to ascertainment bias. However, these modifiers had no impact on a semi-quantitative pathological phenotype in 10 autopsied patients. These data allow an appreciation of the range of clinical phenotype, modern imaging and molecular investigation and should inform genetic counselling of at-risk individuals, with the identification of two genetic modifiers.

Keywords: P102L; sCJD; early-onset dementia; Gerstmann-Sträussler-Scheinker syndrome; prion disease

Abbreviations: *APOE* = apolipoprotein E; GSS = Gerstmann-Sträussler-Scheinker syndrome; IPD = inherited prion disease; NCS = nerve conduction studies; OPRI = octapeptide repeat insertion; *PRNP* = human prion protein gene; PrP^{c} = prion protein (cellular isoform); PrP^{Sc} = prion protein (scrapie isoform); sCJD = sporadic Creutzfeldt-Jakob disease

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Introduction

Prion diseases occur uniquely in sporadic, acquired and inherited forms. Inherited prion disease (IPD) accounts for around 15% of all human prion disease (Collinge, 2005). IPD also forms a significant proportion of familial young onset dementia (Finckh *et al*., 2000). The longer average duration of IPD, and need for genetic counselling of at-risk family members, results in a larger impact on health service

and social care providers when compared with other forms of prion disease. IPD is associated with point mutations in the human prion protein gene (*PRNP*), premature stop codon mutations and alteration in the number of octapeptide repeat insertions (OPRI) in the unstructured region of the N-terminal domain (Mead, 2006).

The P102L mutation has classically been associated with Gerstmann-Sträussler-Scheinker syndrome (GSS). This is characterized clinicopathologically by onset of cerebellar ataxia with dementia occurring much later and is associated with multicentric PrP amyloid plaques in the cerebellum and cerebral cortex. There is considerable heterogeneity, however, even between first-degree relatives, which has led to delayed diagnosis in those not presenting with typical GSS. The heterogeneity in age at onset, clinical symptoms and pathological findings raises questions about modifying factors that may play a role in other aetiologic categories of prion diseases and other forms of neurodegenerative disease. Phenotypic variability even within the same IPD P102L family does not always reflect the classical symptom pattern or pathological changes (Hainfellner *et al.*, 1995; Piccardo *et al.*, 1998; Arata *et al.*, 2006). It is for this reason that recent descriptions have favoured the use of a molecular genetic classification rather than the traditional clinicopathological ones (Collinge *et al.*, 1992, 2005). Explanations for this heterogeneity have begun to emerge, although the reasons for it remain largely unexplained (Parchi *et al.*, 1998; Wadsworth *et al.*, 2006).

The large family from central England, originally referred to as the 'W' kindred, was first reported 30 years ago with an unusual inherited neurodegenerative disease (Cameron and Crawford, 1974). While autosomal dominant transmission was recognized early on, an atypical form of Huntington's disease was suspected to be responsible. The observation that a member of the family presented with the classical clinical and neuropathological features of sporadic Creutzfeldt-Jakob disease (sCJD) was confused when other affected family members fitted neither clinical nor pathological criteria for this disease (Rosenthal *et al.*, 1976). An inherited susceptibility to neurological disease of different types including sCJD was therefore postulated (Rosenthal *et al.*, 1976; Adam *et al.*, 1982). Following the demonstration that these heritable disorders may also be transmissible to experimental animals (Masters *et al.*, 1981), the possibility of an agent originating in the genome, and not requiring exogenous aetiological factors for its development, was proposed (Baker *et al.*, 1985). In 1989, linkage of the P102L *PRNP* mutation to GSS in members of this kindred was reported (Hsiao *et al.*, 1989), only the second reported mutation in a neurodegenerative disease [the earlier being the report of a six OPRI *PRNP* mutation in another kindred (Owen *et al.*, 1989)].

In this article, we present genetic and genealogical investigation of multiple international P102L pedigrees that we have identified over 17 years of *PRNP* diagnostic testing in London. Microsatellite and genealogical analyses have allowed us to build an updated and expanded family tree for the 'W' kindred, the largest P102L kindred reported to date. Additionally, we present genealogical and molecular evidence of multiple independent P102L mutations from the United Kingdom and elsewhere in the English-speaking world. This large collection of a rare inherited disease documents the modern neurological and molecular investigations and marked phenotypic variability of P102L prion disease. A genetic correlation with clinical and pathological parameters has also been made, identifying two previously uncharacterized modifiers of the phenotype of this mutation.

Materials and Methods

Clinical information

For recent patients, a standardized history and examination was performed either in hospital, where additional investigations such as MRI and EEG were carried out, or on domiciliary visits. Peripheral neurophysiological examinations were performed if clinically indicated by dysaesthesiae or lower motor neuron signs. Neuropsychological assessments were also carried out where patients consented to them. Routine investigations and investigations into other potential causes of neurological deterioration had often been carried out before diagnosis was reached and the results of these were sought. For historical patients, clinical histories and examination details were obtained where possible from medical notes completed at the time of clinical review. In some patients, notes had been destroyed prior to the beginning of this study and information on presentation and cause of death was sought from relatives and death certificates. Historical patients in whose cases tissue did not survive for DNA analysis have been considered affected if there is evidence of a neurological disease causing paralysis, cerebellar features and/or dementia occurring before the age of 65 years without an alternative cause being established. Patients were classified based on current neurological assessment or medical records, according to their presenting features. Individuals who presented with prominent cognitive or psychiatric features were contrasted with those without such features, who tended to present with unsteadiness and weakness in the absence of frank cognitive or psychiatric features.

Genealogical investigations

Genealogical work was carried out on all P102L patients from the English-speaking world with tissue samples and/or clinical information donated to the MRC Prion Unit, London. This was initially limited to family tree information from the informant (patient or relative). Further work was carried out where a geographical proximity to a known patient from the 'W' kindred was established and later where microsatellite haplotyping suggested a common haplotype to an extant patient. Information was gathered from census returns; birth, marriage and death certification as well as parish records; and work-house, asylum and hospital records, where these could be traced. The results of clinical investigations were sought where hospital notes survived. Pathological reports have been sought where no tissue survives for examination using modern techniques.

Molecular analysis and investigations

DNA was extracted from peripheral blood lymphocytes, and in some patients from frozen post-mortem brain tissue, using Nucleon Blood DNA kit or a modified phenol–chloroform extraction. The open reading frame was screened for the presence of deletions or insertions in *PRNP* using size fractionation of a PCR amplicon. The *PRNP* open reading frame was sequenced using an ABI-377 automated DNA sequencer. All previously identified P102L patients with DNA stored in the MRC Prion Unit where research consent had been given underwent *PRNP* locus haplotype analysis using 12 microsatellites on chromosome 20, 3–6 Mb (NCBI Entrez-gene mapviewer, http://www.ncbi.nlm.nih.gov/sites/entrez). PCR using fluorescent primers specific for the microsatellite regions were carried out followed by size discrimination on an acrylamide gel using MegaBace sequence analyser 3.0 software (Amersham Biosciences, 2001). Microsatellite haplotypes were determined empirically from pedigree information. Statistical work was carried out using SPSS version 12.0.1 for Windows (SPSS.com) and Excel software (Microsoft). Estimates of effect size were made by linear regression analysis. Statistical genetic analysis used PLINK software (version 0.99, http://pngu.mgh.harvard.edu/purcell/plink/).

Neuropathology

Five of the 10 patients described here have had neuropathology reported previously, primarily before the availability of PrP immunohistochemistry (Rosenthal *et al.*, 1976; Adam *et al.*, 1982). Samples were taken from formalin-fixed brain, where available. Cortex (frontal and occipital), basal ganglia and cerebellum were sampled. Tissue was fixed in 10% buffered formal saline, followed by incubation in 98% formic acid for 1 h. Following further washing for 24 h in 10% buffered formal saline, tissue samples were processed and embedded in paraffin wax. Sections were cut at a nominal thickness of 4 μm, treated with 98% formic acid for 5 min and then boiled in a low ionic strength buffer (2.1 mM Tris, 1.3 mM EDTA, 1.1 mM sodium citrate, pH 7.8) for 20 min. All blocks were stained with haematoxylin and eosin and with immunohistochemistry using anti-PrP monoclonal antibodies ICSM18 (1:10 000 dilution of a 1 mg/ml solution) which labels mutant and wild-type PrP and ICSM35 (1:3000 dilution of a 3 mg/ml solution) which labels only wild-type PrP (D-Gen, London) and were incubated at 42°C for 32 min. Haematoxylin was used as the counterstain. Haematoxylin and eosin staining of serial sections was done using conventional methods. Appropriate controls were used throughout.

Samples were examined by the same pathologist (S.B.), blinded to clinical details, and comparisons made between them. A semi-quantitative scale was used to grade spongiform change and PrP deposition (Fig. 1). Diffuse and plaque-related PrP deposition was documented and contrasted. Clinicopathological comparisons were made following this, with note being made of phenotype, *PRNP* codon 129 and apolipoprotein E (*APOE*) genotypes.

Results

Genealogy and genetic studies of shared ancestry

Eighty-four patients were identified from what were initially considered to be 15 separate kindreds. Sixty of these patients presented for the first time, while six are patients who were reported previously but in whose cases significant additional information has been obtained and is presented here. Twenty-two of the total 84 patients, including members of 14 kindreds, had been seen at the National Prion Clinic or on domiciliary visits by clinic staff. Eight were seen and examined by T.W. Of the remaining 62 patients, 5 are contemporary patients seen at other neurological centres and where DNA or tissue samples and clinical data has been supplied with patients' consent and ethics committee approval. Fifty-seven patients are historical, typically being the ancestors of contemporarily identified patients, in whom a diagnosis of young onset neurological disease had been made or had been reported by immediate descendents (see Materials and methods section). Among the historical patients, a variety of clinical diagnoses had been applied and were obtained from hospital notes and death certificates (Table 1). While diagnostic labels have changed over time, those used still provide information about predominant symptoms and clinical impressions of physicians at the time.

A mutation-associated microsatellite haplotype for the UK IPD P102L 'W' kindred was determined (Fig. 2). Five smaller unrelated kindreds shared this haplotype, supporting the existence of a recent common ancestor for all of these patients (shown as kindred 1 in Fig. 3). Further, IPD P102L patients did not share a haplotype with either the large kindred or with each other, suggesting that separate mutational events had been responsible for their occurrence (kindreds 2–9, Fig. 4). A final kindred where no DNA was obtainable for haplotyping is not included in Fig. 4.

Following the identification of a common mutation-associated haplotype, genealogical work in three patients directly established links by common ancestor with the 'W' kindred. Two other families, which shared a haplotype, could not be linked directly to the 'W' kindred. In both patients, ancestors were identified in the same or neighbouring villages to the original 'W' kindred. In one family, an illegitimate ancestor provides a likely link; his mother was living adjacent to a known member of the 'W' kindred, according to contemporary census entries. The inability to link in the final family genealogically may relate to illegitimacy or alternatively may be explained by a common ancestor prior to the end of the 18th century, when the earliest identified shared relatives for the 'W' kindred were living. Emigration by family members accounts for the identification of related individuals in other parts of the English-speaking world: Canada, the United States and Australia. Migration to other parts of the United Kingdom explains the lack of geographical proximity to known patients in recent generations and the failure up to now to recognize the common ancestry without supportive genetic evidence of common microsatellite haplotype. The genealogical work performed suggests an earliest common ancestor for the presented patients born in the mid-eighteenth century. However, the mutation itself may have originated before this time.

Clinical findings

Following microsatellite and genealogical studies, 40 new patients were identified from the 'W' kindred, likely to be associated with the P102L *PRNP* mutation. Twenty-eight further patients from nine separate European kindreds apparently not related to each other or to the 'W' kindred are also presented. A full list of the major clinical findings is provided in Table 2. Details on each patient are also provided in Appendix 1 of Supplementary data, except in cases of patients previously reported or where patients are still living and details have not been included for the sake of confidentiality.

The most commonly occurring primary presenting features were unsteadiness (present in 72% of patients), cognitive symptoms (28%) and leg weakness (22%). Less common were leg pains or dysaesthesiae (16%) and severe psychiatric features (10%). New onset deafness, epileptic seizures, parkinsonism and diplopia occurred as presenting features in individual patients (2% of patients where data were available) (Fig. 5).

The commonest clinical syndrome was progressive cerebellar ataxia associated with leg weakness and areflexia, similar, but not identical to, the classical GSS phenotype. Indeed, cerebellar signs were present in nearly all of the patients where information could be obtained at some point in the illness (98%). Cognitive features were usually mild initially and developed later in the illness course. Cognitive features were eventually present, however, in the large majority of patients at some point during the disease where information was available (81%), although in many

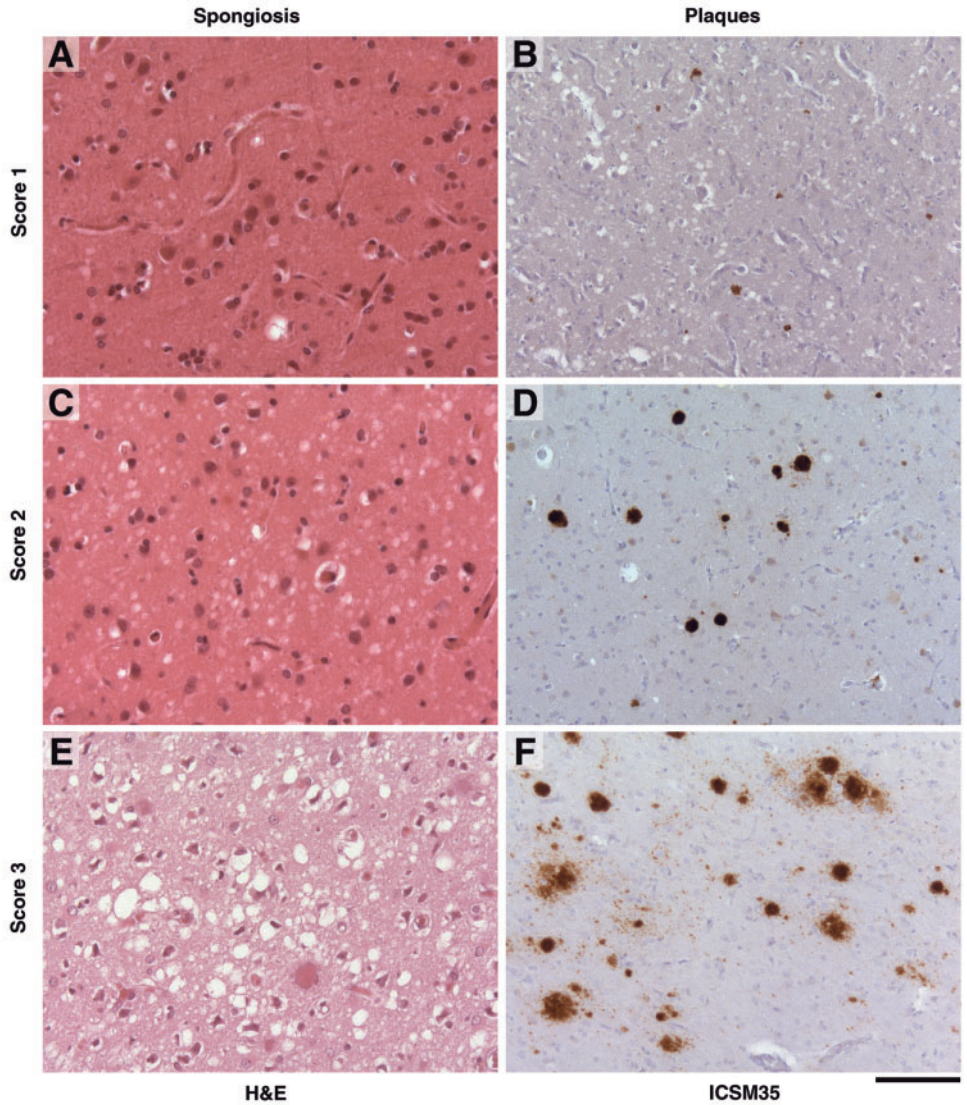

Fig. 1 Representative histology from P102L patients showing appearances graded semi-quantitatively. (**A**, **C** and **E**) show progressively worsening degrees of spongiform change on haematoxylin and eosin stain of brain slices. (**B**, **D** and **F**) show increasing levels of plaque deposition, as demonstrated by immunohistochemistry with anti-PrP antibody ICSM 35. Views in the left-hand column do not necessarily correspond to the same region as the right column. **A**, **C** and **D** are from Patient 2.VII.2. **B** and **E** are from Patient VII.4, while panel F is from Patient 3.VIII.1. The scaling bar shown is 100 μM.

Table 1 Listed causes of death from certificates for historical patients of P102L IPD

Diagnosis	Number of patients
Disseminated sclerosis	6
Cerebellar degeneration	4
Paralysis	3
Cerebral degeneration	1
Cerebral and cerebellar cortical atrophy	1
Spinal cerebellar atrophy	1
Progressive cerebellar ataxia	1
Hereditary progressive ataxia	1
Olivo-ponto-cerebellar degeneration	1
Huntington's chorea	1
Heredo-familial muscular dystrophy	1
Neurofibromatosis	1
Intracranial haemorrhage	1
Binswanger's encephalitis	1
Multiple cerebrovascular accidents	1
Senile dementia	1
Schizophrenia	1

These are listed by frequency. For the many examples with only one individual each given this diagnosis, they have been listed loosely by type in the following order: cerebellar lesion implicated, genetic causes, vascular causes, cognitive/psychiatric and other.

patients these were mild. Lower motor neuron pattern leg signs with areflexia, often associated with objective evidence of muscle weakness and myopathic gait, was present in the majority of patients (79%) (Fig. 6). In one patient (4.VII.1), frank lower limb fasiculations were observed. Sensory symptoms in the legs were also common, with dysaesthesiae and hyperaesthesiae being frequently described by affected patients (69%). Extrapyramidal features, especially parkinsonism and severe psychiatric symptoms such as personality change, delusions, paranoia and visual hallucinations, occurred in around half of patients (48 and 47% of patients, respectively). Apraxia, however, in contrast with patients with *PRNP* insertional mutations (Collinge *et al.*, 1992, 2005; Mead *et al.*, 2006), was recorded in only a single individual. Myoclonus and seizures occurred in a minority of patients, although still relatively common (36 and 7%, respectively). One patient (VII.2) presented with a prominent generalized dystonia in addition to a cerebellar ataxia. This has not been reported previously in association with IPD P102L and the unusual phenotype in this patient delayed diagnosis until post-mortem examination of brain tissue. No reports of choreic movement disorders or of the dyspraxia and premorbid personality changes commonly reported in some other forms of IPD were obtained (Collinge *et al.*, 1992; Mead *et al.*, 2006).

	D20S181 -1494556		D20S193 -1353575		D20S473 -1201628		D20S889 -720087		D20S116 -613627		D20S482 -160790		D20S97 -142854		D20S895 419565		D20S849 527025		D20S873 929772		D20S95 1049310		D20S194 1475648	
VI.14	**160**	164	**142**	148	**181**	178	**277**	279	**108**	110	**156**	156	**267**	285	**215**	221	**230**	230	**191**	199	**88**	86	**208**	204
VII.1	-	-	**142**	152	**181**	181	**277**	283	**108**	110	**156**	160	**267**	267	**215**	215	**230**	230	-	-	**88**	86	**208**	196
VIII.1	**160**	156	-	-	-	-	**277**	275	-	-	**156**	160	**267**	267	-	-	-	-	**191**	191	**88**	88	-	-
VI.15	**160**	166	**142**	152	-	-	**277**	275	**108**	106	**156**	160	**267**	267	**215**	215	**230**	230	**191**	199	**88**	92	**208**	202
VIII.2	**160**	160	**142**	148	-	-	**277**	279	**108**	110	**156**	156	**267**	271	**215**	223	**230**	230	**191**	201	**88**	88	**208**	190
VIII.5	**160**	160	**142**	148	-	-	**277**	275	**108**	112	**156**	168	**267**	273	**215**	223	**230**	214	**191**	199	**88**	88	**208**	220
2.VII.2	160	164	148	148	181	181	275	275	110	112	160	164	273	289	223	225	230	230	191	191	92	92	208	216
3.VIII.1	164	168	134	152	181	181	279	289	112	114	160	164	267	273	215	219	214	230	197	199	88	92	196	200
4.VII.1	164	164	148	154	181	184	273	295	106	114	160	164	267	273	215	215	214	230	191	201	88	92	204	216
5.VIII.2	156	160	142	152	178	181	-	-	110	112	156	156	267	267	221	223	-	-	199	199	84	92	190	212
6.VIII.1	160	164	148	154	-	-	279	283	108	110	160	168	267	267	215	221	214	230	191	199	92	92	228	232
7.VIII.1	160	164	134	152	169	181	273	285	112	118	164	168	267	285	215	223	214	214	197	199	80	92	190	212
8.VIII.1	164	166	152	150	178	181	275	285	110	110	158	162	265	271	217	217	212	228	-	-	84	88	194	198
9.VIII.2	160	164	134	148	178	178	285	293	106	110	152	160	267	267	215	215	214	230	191	201	80	88	196	226

Fig. 2 Microsatellite genotypes for P102L IPD patients at 13 loci linked to codon 129. Shown left to right 5′–3′ with their physical distance from codon 129 below. *PRNP* is situated between D20S97 and D20S895. VI.14 is a member of the original 'W' kindred. Below are representative individuals from the five kindreds sharing a microsatellite background. The disease-associated haplotype is shown in bold. The *bottom half of the diagram* shows the microsatellite genotypes of representatives of the other eight P102L kindreds. Although it has not been possible to derive a mutation-associated haplotype for these, none appear to share a haplotype with the 'W' kindred or with each other.

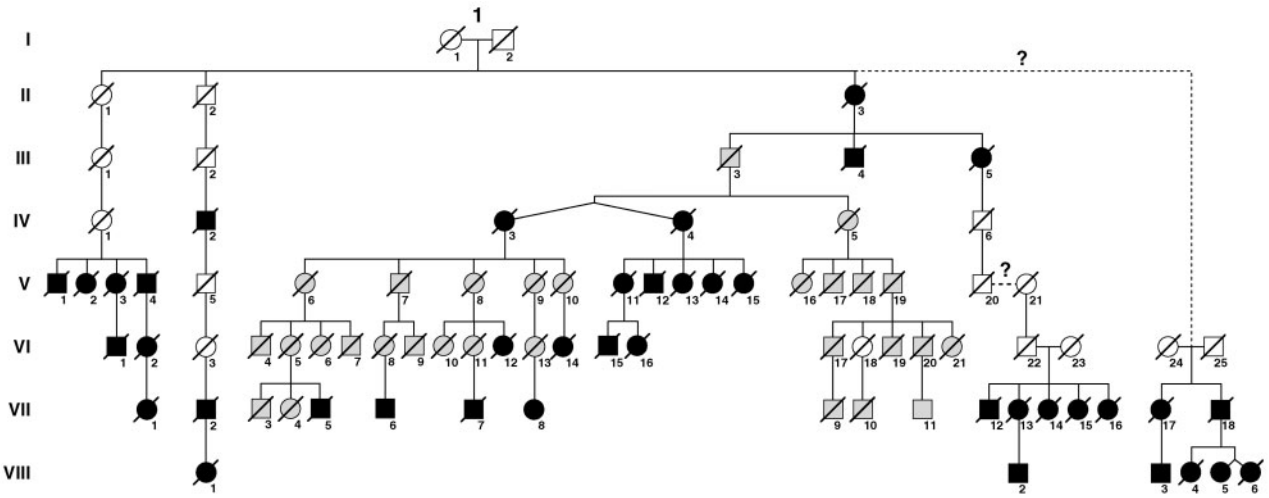

Fig. 3 Pedigree of the original 'W' kindred (in grey) with newly identified and linked kindreds (in black). Only presumed affected patients (filled symbols) or those thought to have transmitted the mutation but not known to be affected (empty symbols) have been included for the sake of simplicity and confidentiality. All of the new patients have been linked genealogically to the original family with the exception of the two small pedigrees on the right of the diagram where possible links are represented with a question mark. Members of these kindreds both share a microsatellite background with the 'W' kindred and have ancestors closely located geographically to them. Possible reasons for the inability to uncover an exact link genealogically are discussed in the text.

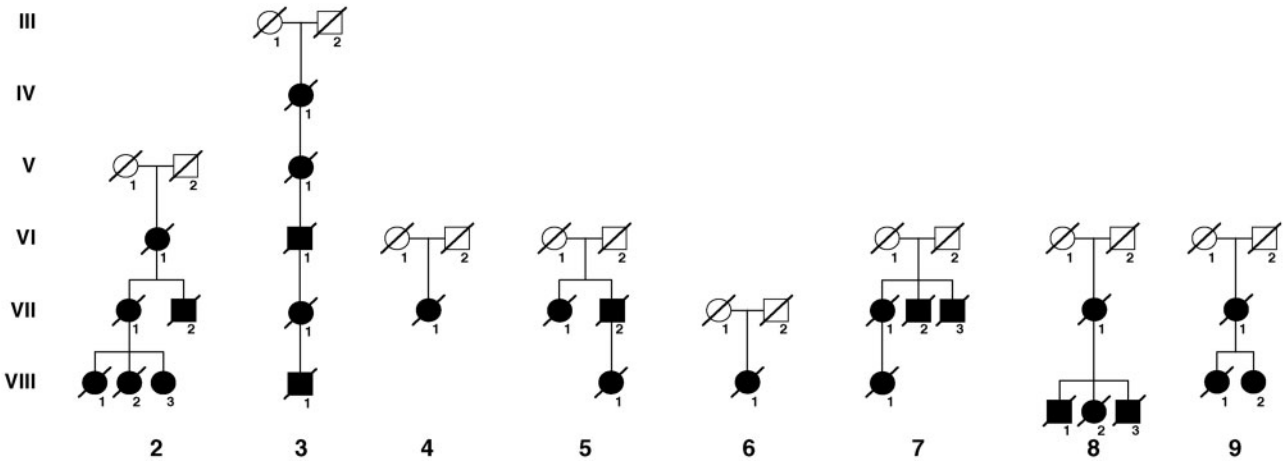

Fig. 4 Eight further family trees (six from the UK, two from elsewhere in Europe). Microsatellite haplotype analysis from these patients suggests that none is related to the 'W' kindred or to each other. Separate mutational events are therefore likely to have been responsible.

There was a considerable heterogeneity regarding clinical features. Although a majority of patients presented with the phenotype of progressive ataxia with a later onset of cognitive involvement, a significant subset presented with predominantly psychiatric and cognitive features that often remained the major clinical feature, in spite of the later development of cerebellar and other physical signs. A still smaller subset presented with a dementing illness having a rapid and more global pattern of deficits reminiscent of sCJD. In five of the patients presented here, where rapid onset and uncertain or absent family history complicated the clinical picture (VI.15, VI.9, VII.7, 4.VII.1 and 6.VIII.1), the diagnosis of sCJD was considered; indeed, one patient (VI.9) met World Health Organisation's diagnostic criteria for probable and the others possible sCJD.

Mean age at onset was 50 years (range 27–66 years, SD 9.4) while mean age at death was 55 years (range 33–69 years, SD 8.7). Mean duration of illness was 49 months but the range was very wide (7–132 months, SD 26.1). The mutation appears to have occurred in all the patients presented, linked to the commoner *PRNP* codon 129 methionine allele. As a result, all the patients where genotyping was performed were methionine homozygotic (MM) or methionine/valine heterozygotic (MV). Evidence from some other forms of IPD (but not previously for P102L) shows that codon 129 homozygotes have an earlier age at onset and death than 129 heterozygotes (Collinge *et al.*, 1992; Dlouhy *et al.*, 1992; Mead *et al.*, 2006). Among the P102L patients reported here, MM homozygotes have an earlier age at onset compared with MV heterozygotes (47.3 ± 10.2 versus 54 ± 5.7, $P = 0.02$, *t*-test

Table 2 Clinical features from PI02L IPD patients reported

Pedigree number	Sex	Sex of affected parent	Age at onset (years)	Age at death (years)	Disease duration (months)	Codon 129 genotype	Presenting features	Personality/psychiatric changes	Progressive cortical dementia	Myoclonus	Pyramidal signs	Cerebellar ataxia/dysarthria	Extra pyramidal features	Lower motor neuron signs in lower limbs	Sensory symptoms/signs	Seizures	Chorea
II.3	F		40	40	7			++	(+)								
III.3	M	F		53					++								
III.4	M	F		52			Severe depression	++	(+)								
III.5	F	F		68										(+)			
IV.2	M	(M)		64					(+)								
IV.3	F	M		55					+								
IV.4	F	M		55													
V.1	M			54													
V.2	F	(F)															
V.3	F	(F)		58			Fall, psychiatric changes	+	(+)								
V.4	M	(F)		69			Slurred speech, difficulty walking					(+)		(+)			
V.6	F	F		58			Slurred speech, difficulty walking		–			+					
V.7	M	F	47	52	60		Unsteadiness		+			+			+		
V.8	F	F	46	49	36				+		+	+					
V.9	F	F	66	69	36		Pain in legs, difficulty walking		+			+			+		
V.10	F	F		56							+						
V.11	F	F	61	61	7		Leg weakness	–	–	–	–	+	–	–	–	–	–
V.12	M	F		60								+					
V.13	F	F		61													
V.14	F	F		60													
V.15	F	F		62			Unsteadiness, weakness in legs										
VI.1	M	F	49	54	72	MV	Seizure, personality change	++	+	–	+	+	+	–		+	
VI.2	F	M	63	66	58	MM	Unsteadiness, falls	+	+	–	+	++	–	+	–	–	–
VI.4	M	F	66	67	36		Unsteadiness, deafness				+	+	+	+	–		
VI.5	F	F	54	65	132		Leg weakness and numbness		+		++	+		–	++		
VI.6	F	F	57	60	36		Leg weakness		+		++	+	++	++	+		
VI.7	M	F	60	63	36		Forgetfulness		+		+						
VI.8	F	M	48	53	60		Unsteadiness				++	+		++	+		
VI.9	M	M	42	43	13		Unsteadiness	++	+	+	++	+		+		–	–
VI.10	F	F	50	53	25		Walking problems, leg weakness		+		++	+		++			
VI.11	F	F	39	45	61		Depression, memory loss	–	+		+	+					
VI.12	F	F	48	51	26	MV	Left arm tremor, cognitive decline	–	++	–	+	(–)		–			
VI.13	F	F	57	63	72	MM	Unsteadiness, difficulty running	+	+	–	+	+	–	+	–	–	–
VI.14	F	F	65	69	48		Gait and writing problems	–	+	++	+	+	++	+	+	–	–
VI.15	M	F	58	58	8	MV	Irritability, cognitive decline		+	–	+	+	+	+	–	–	–
VI.16	F	F	58	62				–	–	–	–	+	–	–	–	–	–
VII.1	F	F	27	33	60	MM	Unsteadiness		+	+	+	++	++	+	+		
VII.2	M	(F)		47					++								
VII.3	M	F	43	47	48		Unsteadiness				+	+		+	++		
VII.4	F	F	40	46	72	MM	Sensory symptoms, gait	–	+	–	++	++	–	+	++	–	–
VII.5	M	F	63	64	12	MM	Writing and gait difficulties	–	–	+	–	+	+	+	+	–	–
VII.6	M	F	57			MM	Unsteadiness		–	–	++	+	–	+	+	–	–
VII.7	M	F	37	39	25	MM	Irritability, unsteadiness	++	+	–	++	++	–	–	–	–	–
VII.8	F	F	58			MM	Cognitive decline	+	++	–	–	+	–		–	–	–
VII.10	M	(F)	56	61	60	MV	Unsteadiness, cognitive decline	+	+	–		+		++			

(continued)

Table 2 Continued

Pedigree number	Sex	Sex of affected parent	Age at onset (years)	Age at death (years)	Disease duration (months)	Codon 129 genotype	Presenting features	Personality/psychiatric changes	Progressive cortical dementia	Myoclonus	Pyramidal signs	Cerebellar ataxia/dysarthria	Extra pyramidal features	Lower motor neuron signs in lower limbs	Sensory symptoms/signs	Seizures	Chorea
VII.12	M	(M)		65													
VII.13	F	(M)	59	66	84		Unsteadiness, falls, pain in legs	+		–	++	+	++	–	++	–	–
VII.14	F	(M)		61													
VII.15	F	(M)		55								(++)					
VII.16	F	(M)		37			Eccentric behaviour	(++)									
VII.17	F	(M)	47	53	72		Unsteadiness, cognitive decline	–	+	–	(+)	+	(+)			–	–
VII.18	M	(M)		62								+					
VIII.1	F	M	44	49	60	MV	Cognitive decline		+	(–)		(–)					
VIII.2	M	F	52			MM	Unsteadiness, leg weakness	–	–	–	++	+	+	++	++	–	–
VIII.3	M	F	56			MV	Unsteadiness, leg numbness	–	–	+	++	++	+	++	++	–	–
VIII.4	F	M	63	67	48	MV	Cognitive decline, unsteadiness	++	++	+	–	+	+	++	–	+	–
VIII.5	F	M	60			MV	Difficulty walking, leg weakness	–	–	–	+	+	–		+	–	–
VIII.6	F	M	53	60	85	MV	Diplopia, tinnitus, leg weakness	–	+	+	+	+		+		–	
2.VI.1	F	–	50	55	60												
2.VII.1	F	F	41	49	96	MM	Unsteadiness, dysphagia		+			+					
2.VII.2	M	F	52	58	72	MM	Unsteadiness and dysarthria	–	+	–	+	++	–	++	+	–	–
2.VIII.1	F	F	41	45	48	MM						+					
2.VIII.2	F	F	34	39	56	MM											
2.VIII.3	F	F	35			MM											
3.IV.1	F		55	57	24						(+)						
3.V.1	F	F	55	57	24						(+)						
3.VI.1	M	F		42										(++)			
3.VII.1	F		36	39	36			–	–	–	–	–	–	–	–	–	–
3.VIII.1	M	F	48	53	60	MV	Unsteadiness, leg pain	+	++	++	++	++	+	++	–	–	–
4.VII.1	F	F	57	59	28	MV	Unsteadiness, cognitive decline	++	+	+	+	++	–	++	+	–	–
5.VII.1	F	(F)		42								(+)					
5.VII.2	M			47			Unsteadiness and memory loss		+			+					
5.VIII.1	F	M	38	45	43	MM	Memory loss, unsteadiness		+		+	+	+		+	–	–
6.VIII.1	F		45	45	7	MM	Pain in limbs, blurred vision	+	+	+	+	+	+	+	++	–	–
7.VIII.1	F		42	49	89	MM	Unsteadiness	–	+	+	+	++	–	+	++	–	–
8.VII.1	F																
8.VIII.1	M	F	57	61	46	MV	Unsteadiness and memory loss	–	–	+	–	++	–	+	+	–	–
8.VIII.2	F	F		54								(+)					
8.VIII.3	M	F		57								(+)					
9.VII.1	F			58			Memory loss with pain in legs		(+)			(+)			(+)		
9.VIII.1	F	F		54			Unsteadiness					(+)					
9.VIII.2	F	F	53			MM	Unsteadiness, falls, dysarthria	–	+	–	+	++	–	++	++	–	–
10.1	M	F	37	43	64		Unsteadiness					++		+	+		
10.2	M	M	47	50	42	MM	Unsteadiness	–	+	–	+	++	–	+	++	–	–

'+' denotes record of a feature being present. '++' is used where clinicians have recorded findings as 'severe' or 'marked' or where deficit has interfered with independence. '–' denotes a feature mentioned as being absent by clinicians. Missing data is represented by a blank. Data relying on inference from death certificate details or family memories are surrounded by brackets.

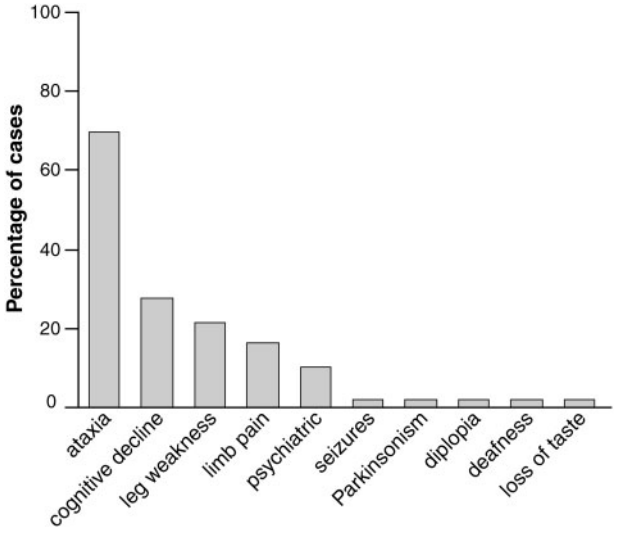

Fig. 5 Clinical features on presentation in PI02L IPD. The relative frequency of cognitive symptoms and signs on presentation is notable, along with the commonly occurring peripheral limb symptoms and psychiatric features.

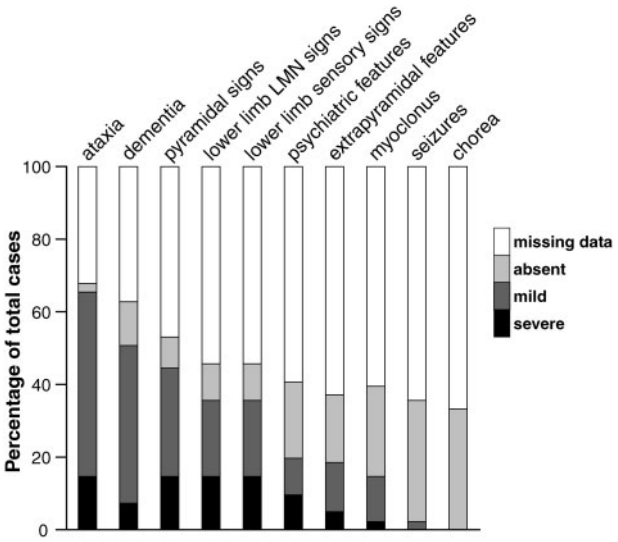

Fig. 6 Clinical features reported during course of PI02L IPD. The high percentage of patients where cognitive deficits were apparent, even if only in later stages, is notable as is the frequency of sensory signs and lower motor neuron signs. Chorea was not reported or observed in any of the patients presented.

heteroscedastic). When individuals with known codon 129 genotype were ranked by age at onset, the youngest eight ($n = 31$) were codon 129MM. In addition, the standard deviation in age at onset and death for codon 129MM was much wider than that for codon 129MV. There was no significant difference in duration of illness between the two codon 129 genotype groups.

Intriguingly, the data presented here relates to 54 female affected patients but only 30 males ($P = 0.01$, binomial test given a predicted proportion of 0.5). As a consequence of this discrepancy, individuals included here were much more likely to have inherited the mutation from their mothers than from their fathers, where such information was available (54 affected mothers, 19 affected fathers, $P = 1 \times 10^{-4}$). The 30 affected males transmitted the mutation to 4 sons and 13 daughters. The 54 affected females transmitted the mutation to 24 sons and 31 daughters. This sex distortion was not significant. A similar effect was also reported in 6-OPRI IPD (53 affected females versus only 33 males, $P = 0.008$, binomial test) (Mead *et al.*, 2006) and when combined with these data, the overall significance was more marked (109 affected females versus 65 males, $P = 0.001$). In addition, the proportion of females to males with both mutations was similar (1.6 : 1 in 6-OPRI, 1.9 : 1 in P102L). No differences were found in terms of age at onset or death when comparing male and female affected patients or indeed when comparing those who inherited the mutation from their mothers versus those who inherited it from their fathers.

Neither age at onset nor duration of illness was significantly different between patients with a predominantly cognitive or psychiatric versus those with predominantly cerebellar or motor onset. There was no correlation of codon 129 genotype with these subgroups of cognitive/psychiatric compared with the peripheral/cerebellar onset groups, although numbers were small and in many patients the codon 129 was unavailable ($n = 22/44$ with missing genotype data).

Electroencephalography

A total of 16 individuals had EEG performed after disease onset and 13 of these demonstrated non-specific abnormalities. All of these abnormal studies were in the context of significant cognitive impairment or psychiatric symptoms and were performed between 6 months and 3 years after onset. One of these individuals also had widespread epileptiform discharges with no apparent clinical correlates. Three further studies were reported as normal. These were 2–3 years after symptoms developed, although none of these patients demonstrated significant cognitive or psychiatric symptoms or signs at the time of the study, each having a predominantly cerebellar syndrome. In one patient (VII.8) an EEG was performed many years before symptom onset when the neurological examination was unremarkable. This was also a normal study, although a repeat 1 year after onset of predominantly cognitive symptoms was mildly, though non-specifically, abnormal, with no periodic sharp wave complexes. In a single patient already reported, periodic sharp wave complexes were found in the context of a sCJD-like clinical picture (Rosenthal *et al.*, 1976).

Electromyography and nerve conduction studies

Ten of the newly reported patients underwent peripheral neurophysiological studies, eight having nerve conduction studies (NCS) in combination with EMG, two having

NCS only. In all patients the investigations were indicated by painful sensory symptoms, sometimes accompanied by areflexia and leg weakness at the time of the study. Of the 10 patients who had NCS, 2 had evidence of a mild axonal sensorimotor neuropathy. In the other eight, no abnormalities were picked up on NCS in spite of symptoms and often signs suggestive of peripheral neuropathy. A single patient had EMG evidence of muscle denervation in combination with an axonal neuropathy. EMG was normal in seven others, at least two of whom had objective weakness of the lower limbs on clinical examination. A single patient without NCS or EMG abnormalities in spite of painful sensory symptoms and leg weakness underwent thermal threshold testing. This demonstrated a defect in the lower limbs but not in the upper, consistent with either small fibre neuropathy or central pathway involvement. The patient was cognitively intact at the time of the investigation and therefore able to report the sensations accurately. Overall, there was not a consistent peripheral nerve deficit to account for sensory symptoms or areflexia.

Neuroimaging

Sixteen of the newly presented patients had neuroimaging. Fifteen had MRI performed while a further two also had CT scans. One patient, before the era of routine MRI, had only a CT. Three of the MRIs demonstrated a degree of generalized cerebral atrophy out of keeping with the age of the patient. In one patient, localized cerebellar atrophy was reported. These were all performed in patients with significant cognitive and or psychiatric symptoms. Five had normal MRIs between 1 and 3 years after onset of symptoms. In all of these patients, either cognitive or psychiatric features were absent (four patients) or there were only subtle cognitive and psychiatric symptoms reported (one patient).

Interestingly, four patients who had MRI performed were found to have multiple white matter lesions. In one patient, the combination of clinical signs and MRI appearances led to a diagnosis of Binswanger's disease being made before the correct diagnosis was reached. All of the CT scans performed were within normal limits. None of the MRI scans reported showed any changes associated with prion disease of other subtypes: the 'pulvinar' sign or cortical or basal ganglia high signal (Fig. 7) (Macfarlane *et al.*, 2007).

Neuropsychology

A total of 11 patients not previously reported had formal neuropsychological assessment after symptom onset, exploring two or more cognitive domains. In nine of these, evidence for cognitive deficits with decline from predicted pre-morbid functioning was elicited, even where bedside assessment suggested normal cognition. Although two further patients had no deficits on assessment in spite of clearly symptomatic disease, no formal assessment of executive function or attention had been performed in one and full verbal and performance IQ had not been completed in the other. Deficits detected on neuropsychological assessment were widespread, including frontal, subcortical and attentional abnormalities. However, these deficits were patchy, with no clear consistent pattern emerging. Three patients with a predominantly psychiatric or cognitive onset (VII.8, VIII.2 and 5.VIII.1) demonstrated evidence of frontal dysfunction. Patients with a predominantly cerebellar onset performed better on these tests, providing objective evidence for the distinction.

Cerebrospinal fluid

Nine individuals had CSF taken for analysis. In one patient there was borderline elevation of protein. In this patient oligoclonal bands were reported although no record of a serum sample for comparison has been found. In all other patients where CSF was examined, the samples were acellular with normal protein and glucose levels. In only three patients was CSF examined for the presence of the 14-3-3 protein, a non-specific marker of neuronal cell death, which is nonetheless useful in the diagnosis of sCJD (Sanchez-Juan *et al.*, 2006). 14-3-3 was positive and s100b was elevated in two of these 14-3-3-positive patients, while in the third, s100b and neuron-specific enolase were

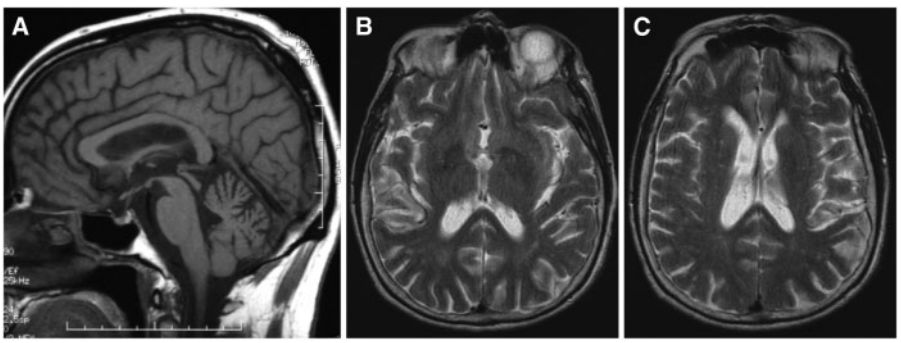

Fig. 7 MRI findings in P102L IPD. (**A**) Sagittal T_1-weighted image (of 2.VII.2) showing cerebellar atrophy. (**B**) and (**C**) Axial T_2-weighted images (of VI.2) showing multiple white matter lesions in the basal ganglia. Similar findings were found in two other patients leading in one to a diagnosis of Binswanger's disease being made in combination with the clinical picture. These findings are probably incidental but the possibility of a link to P102L IPD remains.

elevated but 14-3-3 protein was not detected. The P102L IPD patients with positive 14-3-3 were clinically heterogeneous, one being a rapid onset patient where sCJD was considered in the differential, the other being a slowly progressive cerebellar syndrome.

Non-*PRNP* genetic modifiers

The *APOE* genotype was ascertained in 22 patients. Surprisingly, this demonstrated a later onset of disease in individuals having at least one *E4* allele, compared with those with no *E4* (linear regression analysis $P = 0.015$). The *APOE* and *PRNP* codon 129 genotype modify phenotype independently of each other and together contribute to around 37% of the variability of P102L IPD age at onset (eta-squared of ANOVA).

Two single nucleotide polymorphisms, known as 1368 and 34296, have been shown to be over-represented in sCJD patients compared with controls (Mead *et al.*, 2001; Vollmert *et al.*, 2006). These loci were genotyped in patients with available DNA to examine for a potential effect on phenotype in IPD P102L. No evidence for genotype modifications of age at onset was found.

Neuropathology

A total of 11 patients were available for neuropathological examination. Varying degrees of atrophy were observed macroscopically, with brain weights varying from 1200 to 1740 g. Histopathological examination demonstrated spongiform change, astroctyosis and PrP deposition. These three features, however, were very variable between individuals and brain regions. Spongiform change was present in some brain regions in the majority, but not all, of the patients examined (7 out of 10 patients) and varied from mild to severe. PrP deposition was most commonly found in the context of multicentric amyloid plaques. PrP antibodies ICSM 18 and ICSM 35 positively labelled these plaques. Multicentric plaques were commonly found in the molecular layer of the cerebellum but also less frequently in the granular layer. Similar plaques were found in the cortex in most patients examined (9 out of 10 patients) (Fig. 8). In a single patient (VI.12), the only definite abnormality seen was small numbers of plaques positively stained with ICSM 18 in the basal ganglia, although the tissue examined was 20 years old. More diffuse PrP deposition was demonstrated in the majority of patients available (7 out of 10). A single patient (VIII.1) showed very atypical diffuse staining that appeared to follow cortical tracts and was similar to other IPD patients but not to previously described P102L pathology.

Clinicopathological comparison demonstrated no clear association of clinical subtype (cerebellar onset or cognitive onset), disease duration or codon 129 genotype with severity of spongiform change and degree or type of plaque deposition. Six *PRNP* codon 129 MM homozygotes and four codon 129 MV heterozygotes were included. All the patients with available post-mortem tissue were of the common 3,3 *APOE* genotype. A single atypical patient (VII.1) with very young onset and prominent dystonia showed prominent PrP plaque deposition in all areas examined (including the basal ganglia), with no clear spongiform change. Findings were not dissimilar from other, later onset patients. Unfortunately, the atypical histological patient (VIII.1) had only limited clinical information available. Two muscle biopsies were also examined. The findings were non-specific on conventional stains and no PrP immunoreactivity was demonstrated.

Discussion

The patients presented here constitute by far the largest study of the classical GSS-associated *PRNP* mutation, P102L. Modern investigation, molecular genetic techniques and advances in pathological examination have allowed us to revisit a historical British pedigree and other smaller kindreds. We find evidence of a mutation hot spot at codon 102, a subgroup presenting with rapid cognitive decline and the significant modification of phenotype by two single nucleotide polymorphisms.

The microsatellite haplotyping presented here demonstrates the existence of multiple separate and worldwide P102L kindreds of UK origin, confirmed in most patients by established genealogical links that had not been identified previously. However, the identification of a further six UK P102L kindreds along with two others of European origin suggests that multiple mutational events have occurred in the recent past. This might be supported by the identification of at least one of these patients (6.VIII.1) in an individual without suggestive family history or of known P102L patients living nearby, suggesting that this might represent a novel mutation. Given the identification of IPD

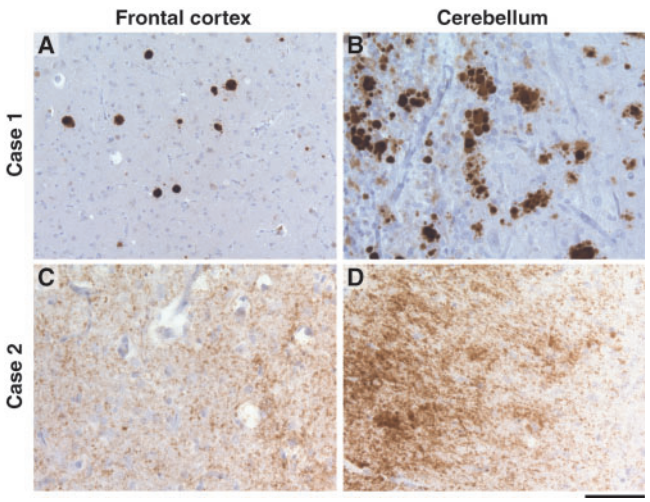

Fig. 8 Deposition of abnormal prion protein in cortex and cerebellum. The upper panels show characteristic multicentric PrP plaques, stained with the anti-PrP monoclonal antibody, ICSM35 (A+B from Patient 2. VII. 2). The lower panel shows synaptic deposition of abnormal PrP (C+D from Patient 7. VIII. 1), demonstrating significant pathological heterogeneity. The scaling bar shown in 100 μM.

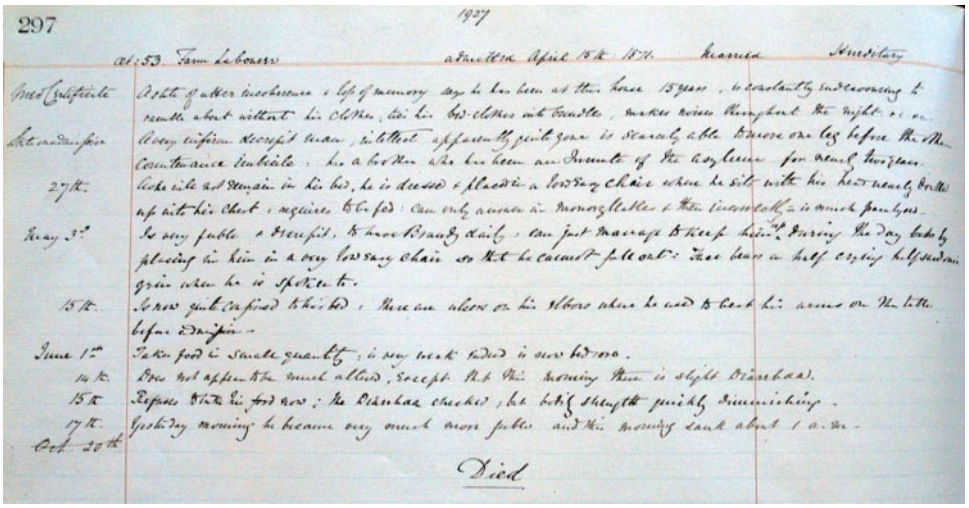

Fig. 9 Photograph of original 1871 asylum entry relating to admission and care of III.3 from Fig. 2. His condition is described as hereditary and reference is made to his relatives' care in the same institution. He is reported as being 'scarcely able to move one leg in front of another' and his countenance is described as 'imbecile'. He appears to have had dementia as well as weakness: 'Can only answer in monosyllables & then incoherently; is much paralysed'. He was treated with daily doses of brandy until he died. 'Paralysis' was listed as the cause on the death certificate.

patients in otherwise typical sCJD presentations, as well as in patients without a family history of neurodegeneration, novel mutational events may occur more commonly than previously thought. The commonest point mutations associated with IPD occur at cytosine-phosphate diester-guanidine dinucleotide sites hypothesized to be mutation 'hot spots' in human DNA (Vollmert *et al.*, 2006). These hot spots are related to the spontaneous demethylation of cytosine to thymidine. Cytosine-phosphate diester-guanidine dinucleotide sites are associated with E200K, D178N and P102L point mutations, which between them account for the bulk of IPD patients worldwide (Dagvadorj *et al.*, 2002; Mead, 2006). An alternative possibility to multiple separate mutational events being responsible for these apparently unconnected P102L IPD kindreds is that these small pedigrees do share a common ancestor with the large pedigree but that this was long enough in the past for linkage to the disease haplotype to have broken down. We estimate a probability of recombination of the 3MB microsatellite haplotype as about 10% per generation (assuming a genetic:physical ratio of 3.7).

The unexpected finding that significantly greater numbers of females were identified with P102L IPD and the consequently higher number of individuals having an affected mother rather than father as the parent from whom the mutation was inherited is puzzling. This discrepancy has been reported once previously in this family, albeit with smaller numbers of individuals and without available genetic techniques, allowing the linkage of kindreds not known to share ancestry (Baker *et al.*, 1985). It has also been observed in the large 6-OPRI IPD kindred from the South-East of England (Mead *et al.*, 2006). In the earlier study in P102L, the finding was explained by postulating that affected females had significantly more children than their unaffected siblings and that greater than half these were daughters. Such an explanation is intriguing, though hard to explain. Alternatively, because of possible illegitimacy, we suspected that genealogical research might more readily identify affected mothers than affected fathers. In order to consider the possibility of ascertainment bias, the gender of members of the kindred identified from parish records or census entries, but who have not been identified as having suffered from IPD P102L, were collected. An excess of males in these untraced individuals was indeed identified. When these were added to the presumed affected male and female individuals, an excess of females to males remained (82–68), although this was not significantly different from an expected proportion of 0.5. It seems likely that ascertainment bias is responsible for this gender difference, although the intriguing possibility of a biological explanation remains.

While the majority of P102L IPD patients here present with progressive ataxia accompanied by mild or absent cognitive symptoms and signs, a subset present with a predominantly cognitive and or psychiatric onset, with mild or absent cerebellar signs initially. Psychiatric involvement has been severe enough to necessitate antipsychotic medication in four patients (VII.1, VII.8, VIII.4 and 3.VIII.1) and inpatient treatment in two (VII.1 and VII.8). The existence of these distinct phenotypes (cognitive and psychiatric versus cerebellar onset) is supported by the finding of early prominent frontal executive impairment on neuropsychology in these patients not seen in other patients so tested. Neuropsychological assessment is now routine at the National Prion Clinic but in the past this was more usually performed only when clinical evidence of cognitive impairment existed. In addition to these neuropsychological differences, there is a suggestion that the cognitive and

psychiatric presentations have an earlier age at onset and death. Neuropsychology also highlights, however, that most if not all patients have cognitive deficits when psychology is performed, even if the findings are subtle, although selection bias may limit this conclusion. No pathological correlates of these two syndrome types have been identified, although numbers compared are small. Kuru and growth hormone-associated prion disease are notable for their onset with cerebellar symptoms (Collinge, 2001), which prompts speculation that cerebellar onset in P102L might result from the onset of prion replication outside the central nervous system. The range of tissue available did not permit the thorough testing of this hypothesis.

The high degree of clinical heterogeneity and the lack of characteristic findings on commonly available clinical neurological investigations make correct diagnosis of P102L IPD challenging. The finding of positive CSF 14-3-3 combined with the albeit unusual occurrence of periodic sharp wave complexes on EEG and an sCJD-like phenotype in some individuals raises the possibility of missed diagnosis. It also supports our clinical practice of advising *PRNP* analysis routinely in all those presenting with otherwise undiagnosed pre-senile dementing or ataxic illnesses.

Peripheral sensory symptoms and muscle weakness appear almost universal during the course of P102L IPD. The demonstration of muscle denervation and axonal sensorimotor neuropathy suggest peripheral neurological pathology is responsible. Experimental mice over-expressing wild-type PrP have shown demyelinating peripheral nerve pathology (Westaway *et al.*, 1994). However, evidence of peripheral neuropathy in P102L IPD was inconsistent and where present was axonal, a form that is not uncommon in the population from unrelated causes. No prion protein (scrapie isoform) positivity could be demonstrated on two muscle biopsies examined here and although an indirect pathological process could still be responsible, peripheral findings in P102L IPD may be incidental, while sensory symptoms could be centrally rather than peripherally driven.

Presented for the first time here is the finding that P102L IPD patients with methionine homozygosity at codon 129 present significantly earlier than codon 129 heterozygotes. Earlier age at onset is well recognized in codon 129 homozygotes in other IPD mutations (Collinge *et al.*, 1992; Poulter *et al.*, 1992; Mead *et al.*, 2006), although the association with IPD associated with point mutations rather than OPRIs is less clear (Dlouhy *et al.*, 1992). The less strong effect in P102L with respect to 6-OPRI may explain why this observation has not been made before where smaller numbers of patients have been examined (Hainfellner *et al.*, 1995; Barbanti *et al.*, 1996). There are several possible mechanisms of action of codon 129 genotype on clinical phenotype. It has long been known that the interaction between prion protein, scrapie isoform (PrP^{Sc}) and prion protein, normal cellular isoform (PrP^{c}) occurs most efficiently when the proteins have an identical primary structure (Palmer *et al.*, 1991); therefore, prion replication may occur more rapidly and clinical onset earlier in 129MM individuals. As a further complexity, the primary structure of PrP determines the permissible conformations of PrP^{Sc}. The extent to which a pathogenic 102L-129M PrP conformer is permitted by wild-type PrP^{c} with 129V or 129M may also be important [see Collinge, 2007 for a recent review (Collinge and Clarke, 2007)]. 102L-129M PrP may be able to adopt several different pathogenic conformations, which may be permissible to a greater or lesser extent by the wild-type protein (Parchi *et al.*, 1998; Hill *et al.*, 2006). The involvement of wild-type protein is known to be a variable phenomenon in P102L and a possible determinant of phenotype (Wadsworth *et al.*, 2006).

The identification of the P102L mutation on a methionine allele in all of the patients presented here with adequate haplotype data probably relates simply to the frequency of this allele in the background population. P102L patients existing on a codon 129 valine allele have been reported, apparently occurring in the context of a distinct phenotype with prominent psychiatric features and seizures, different from classical P102L codon 129 methionine patients (Young *et al.*, 1997; Bianca *et al.*, 2003). Such a phenotypic difference could relate to different prion strain propagation originating from the valine allele.

Genotype–phenotype correlations presented here, corrected for codon 129, demonstrate an effect of *APOE* genotype on age at onset, with individuals carrying the *E4* allele having a significantly later age at onset than those without. While this evidence appears in contrast to the strong association of *E4* with risk of Alzheimer's disease (Corder *et al.*, 1993) and to published work on the possible impact of *APOE* polymorphisms in sCJD showing an over-representation of the *E4* allele in sCJD patients (Amouyel *et al.*, 1994; Van Everbroeck *et al.*, 2001), such findings have not been replicated in all published studies (Nakagawa *et al.*, 1995; Zerr *et al.*, 1996) nor in our own observations (387 sCJD patients versus unaffected controls, unpublished data). Recent reports present a similar result in frontotemporalobar degeneration, with later disease onset in those with the *APOE E4* genotype in the context of progranulin mutations (Gass *et al.*, 2006; Beck *et al.*, 2008). *APOE* may thus have contrasting effects in the context of different neurodegenerative disease types.

Most of the P102L IPD patients shared common features on pathological analysis. Spongiform change, astrocytosis and PrP deposition, both as multicentric plaques and synaptic deposits, were seen in the majority. However, the degree of severity of spongiform change and plaque deposition were very variable. This did not seem to correlate with age at onset or duration of illness. Neither did predominantly cerebellar or cognitive clinical presentations seem to correlate with pathological findings. *PRNP* codon 129 MM homozygotes and MV heterozygotes were equally represented in the pathology series but no significant differences were seen between these two groups. However, the small

numbers in each subgroup and the age of some of the samples examined (with tissue up to 30 years old) may prevent correlations from being made. A single highly atypical clinical patient showed no significant histological differences from the rest of the series, while an atypical pathological patient has limited clinical data, although what is known about this individual is not obviously different from the other patients examined. These results suggest no clear clinicopathological correlations, but sample size was necessarily small.

Immunoblots of proteinase K-digested brain homogenate from P102L patients demonstrate a spectrum of involvement of protease-resistant wild-type PrP in P102L IPD (Wadsworth *et al.*, 2006). Four of the seven P102L IPD patients examined in this study were negative for prion protein (scrapie isoform), which most probably relates to sampling issues and the differing density of PrP deposition in tissue samples both within and between patients. Our large study therefore does not expand the existing data and we were unable to test whether protease-resistant PrP diversity might contribute to clinical heterogeneity. This remains a plausible concept in IPD that requires a large series of fresh frozen brain tissue and molecular analysis of PrP type following partial protease digestion.

The need for public health control measures, together with the evident diagnostic challenges that IPD heterogeneity causes, make a strong argument for including *PRNP* gene analysis in the list of investigations for suspected prion disease of any type and indeed of all undiagnosed familial dementia or cerebellar syndromes. Successful diagnosis allows clinicians to provide more accurate prognostic information to patients, to allow participation in the clinical trials and reduce the risk of iatrogenic transmission of disease. As a consequence of these geographically highly mobile ancestors, and the large number of untraced individuals in the nineteenth century who were clearly at risk of inheriting the P102L mutation, it remains likely that further patients and at-risk individuals exist who have yet to be identified. It is hoped that the data presented here will help to raise awareness of P102L IPD and its associated presentations.

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