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Supplementary Data

Clinical history summaries

Patient 1

Male, in the fourth decade of life presented with unsteadiness of gait around the time of a chest infection. By six months he was falling frequently and required a frame to walk. At a similar time he began to have problems with his memory of recent events and reading. Shortly thereafter he began to slur his speech and became incontinent of urine and faeces. Family history was censored. He was treated as a child with growth hormone for approximately 5 years for panhypopituitarism, with the last injection 23 years before the onset of symptoms. On examination at 9 months following onset he had spontaneous myoclonus, a cerebellar dysarthria, and finger-nose and heel-shin ataxia. He was able to walk only with the assistance of two persons. MMSE was 20/30. Limited MRI brain did not show any abnormalities. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. He died approximately 10 months after symptom onset.

Patient 2

Female, in the fifth decade of life presented with abnormal gait and balance. This progressed and she began to fall frequently. She developed abnormal sensory symptoms in the feet and jerkiness to movements. Family history was censored. She was treated as a child with growth hormone for approximately 10 years for panhypopituitarism, mental retardation and microcephaly, with the last injection 21 years before the onset of symptoms. On examination 10 months following onset she was unable to complete the MMSE. There was finger-nose, heel-shin and gait ataxia but no increased limb tone or altered reflexes. Some motor agitation was noted. Limited MRI brain showed high signal abnormalities of the putamen, caudate and thalamic nuclei. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. She died approximately 20 months after symptom onset.

Patient 3

Male, in the fifth decade of life presented with progressive unsteadiness of gait, muscle twitching and slurred speech. By 12 months he needed a stick to walk. By 20 months he was using a wheelchair and there were new problems with personality change becoming more irritable, abnormal memory for recent events and visual hallucinations. There was no family history of any neurodegenerative disorder. He was treated as a child with growth hormone for approximately 5 years for short stature, with the last injection 29 years before the onset of symptoms. On examination at 20 months following onset he was unable to walk due to ataxia. He had a cerebellar dysarthria, some nystagmus on lateral gaze, and ataxia measure by the finger-nose test and diadochokinesis. There was mild pyramidal pattern weakness and mildly increased muscle tone. MRI brain showed increased signal on T2 and

FLAIR images in the thalamus, caudate and putamen bilaterally. Minimental State Examination (MMSE) was 26/30. Cerebrospinal fluid (CSF) examination was positive for 14-3-3 protein. Electroencephalogram (EEG) examination showed non-specific slowing. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. His symptoms gradually progressed. He developed chest infections and died approximately 2 years after symptom onset.

Patient 4

Male, in the fifth decade of life received pituitary sourced growth hormone for short stature from the age of 7 for approximately twelve years receiving the last dose about 27 years prior to his current illness. He presented with a two month history of unsteadiness and heaviness of the legs followed by myoclonic jerks in the limbs and progressive loss of speech. By the time he was examined three months after the onset of symptoms speech had declined, he needed support of one person to walk and whilst sitting tended to fall backwards. On examination three months after the first symptoms he was mute and did not consistently respond to commands. There was rigidity in all the limbs with marked myoclonus. Tendon reflexes were brisk and the plantar responses probably extensor (withdrew). MRI showed restricted diffusion in the basal ganglia, thalamus, hippocampus and cortical ribboning in multiple areas and in the cerebellum. The EEG was generally slow and 14-3-3 was detected in the CSF. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine homozygous. Over the next two months the patient rapidly deteriorated entering an akinetic mute state and died 4 months after the first symptoms.

Patient 5

Female, in the fifth decade of life had surgery for a craniopharyngioma aged 10 years and from age 11 years received pituitary derived growth hormone for approximately 8 years; the last injection was approximately 28 years before the onset of her current illness. She presented to hospital with a six month history of irritability and unsteadiness of gait. Examination revealed mildly impaired cognitive function (MMSE 28/30), mild ataxia of limbs, greater in the legs, and markedly ataxic gait. MRI at that time showed equivocal diffusion restriction in the basal ganglia, thalamus and cerebellum, EEG and CSF analyses were normal. Over the next two months her gait ataxia worsened such that she needed the support of two people to ambulate and cognitive function declined scoring 22/30 on MMSE. Two months later she was confined to a chair and needed hoisting to get to bed. Over the next five months she gradually lost the ability to speak, became increasingly sleepy, developed myoclonus and spasticity of the legs and had increasing difficulty with swallowing. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. She died approximately 15 months after the onset of her illness.

Patient 6

Male, in the fifth decade of life received pituitary sourced growth hormone for short stature from the age of 9 years for approximately 5 years, the last injection being given 28 years before the onset of

current symptoms. He presented with a 6 month history of progressive unsteadiness of gait and recent falls. Examination at that time revealed no cognitive dysfunction, mild limb ataxia and gross ataxia/apraxia of gait. MRI showed restricted diffusion in the basal ganglia, thalamus, several cortical areas and the cerebellum. The EEG had an excess of slow activity and the CSF was normal. Over the next five months he lost the ability to walk, developed myoclonus and incontinence of urine and his cognitive function declined (MMSE 24/30). Three month later he developed increasing dysarthria, dysphagia, myoclonus and cognitive decline (MMSE 9/30) and then increasing rigidity of the limbs. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. He died 13 months after the initial symptoms.

Patient 7

Male, in the fifth decade of life presented with excessive tiredness and headaches. He began to fall without explanation. By 10 months he had an obvious balance disorder with motor agitation and began to develop cognitive problems. His personality changed, he made increasing mistakes at work and became lost in familiar places. There was no family history of any neurodegenerative disorder. He was treated as an adolescent with growth hormone for approximately 3 years for panhypopituitarism, with the last injection 28 years before the onset of symptoms. On examination at 19 months following onset he had spontaneous myoclonus and reduced speech output. There was increased limb tone, brisk tendon reflexes and poor coordination. Limited MRI brain showed high signal abnormalities of the putamen, caudate and thalamic nuclei, with additional signal change in the frontal cortex. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine-valine heterozygous. He died approximately 18 months after symptom onset.

Patient 8

Male, in the sixth decade of life had received pituitary sourced growth hormone for restricted growth from the age of 16 for approximately 2 years. The last injection was approximately 32 years prior to his current illness. He presented with pain in legs and progressive unsteadiness and over the next 6 months developed increasing social isolation, visual hallucinations and paranoia. Eleven months after the onset of symptoms he developed progressive cognitive decline and myoclonus. On admission to hospital approximately twelve months after onset of symptoms he uttered occasional words but could not follow commands. On neurological examination he had rigidity of all limbs with wasting, had myoclonus and was markedly ataxic. Power was normal in the arms but could not be tested in the legs because of pain. All the tendon reflexes were present apart from at the ankle tendon reflexes. Plantar responses were flexor. Full sensory examination was not possible. Brain MRI revealed restricted diffusion in the basal ganglia, thalamus, cingulate gyrus and cerebellum, the EEG had excess of slow rhythms and CSF 14-3-3 was not detected. *PRNP* analysis showed no mutations, the codon 129 genotype was methionine homozygous. He progressively declined entering an akinetic mute state and died seventeen month after the onset of symptoms.

Supplementary Table 1:

P-values comparing iCJD vs other CJD on study outcomes using various analysis methods

	CAA Score	Topographical Aβ score	CERAD Score	Semiquantitative Aβ Score	Digital Aβ quantification
Ranksum test restricted to the strata aged 36-51 years (n=27)	0.005	0.02	0.001	0.02	0.34*
t-test restricted to the strata aged 36-51 years (n=27)	-	-	-	-	0.04
Complete cases multivariable regression adjusting for *APOE*ε4 status and age as main effects (N=115)	<0.001	<0.001	<0.001	<0.001	0.01
Complete cases multivariable regression adjusting for *APOE*ε4 status and age as main effects, using 1000 bootstrap replicates to assess the replicability given the small numbers with iCJD	0.11	0.02	0.07	0.01	0.049
Multiple imputation multivariable regression including all individuals adjusting for *APOE*ε4 status and age as main effects (N=124)	<0.001	<0.001	<0.001	<0.001	0.01
*The ranksum test is shown here for completeness: however it is underpowered, and is a less appropriate outcome for this truly continuous outcome than the t-test					

Note: the digital score was log-transformed for normality and modelled using normal linear regression; other outcomes were modelled using ordinal logistic regression. Complete case main effects multivariable models excluded 9 patients with missing *APOE* status and included main effects for iCJD vs other, *APOE*ε4 and age. Multiple imputation models imputed missing *APOE* status using logistic regression, including iCJD vs other, age and interactions between age and *APOE*ε4/CJD category (which were then imputed using predictive mean matching). Results from 100 imputations were combined using Rubin's rules. With small numbers there was no evidence of non-linearity in the effect of age in any model ($p>0.05$).

In addition to the overall differences in log-transformed digital score between those with iCJD vs other types, there was also weak evidence that the rate of increase in digital score with age varied by iCJD vs other type (p=0.09). Similar results were obtained using multiple imputation (n=124) and complete case analysis (n=115). Results from multiple imputation below.

Factor	**Impact on log-transformed digital score**	**95% CI**	**P**	**Heterogeneity (interaction) p**
*APOE*ε4 positive vs negative	+0.79	(+0.45,+1.13)	<0.001	
iCJD vs other CJD (at age 60 years)	+2.30	(+0.31,+4.30)	0.02	
Per year older if iCJD (all *APOE*ε4 negative)	+0.12	(-0.00,+0.25)	0.06	0.09 vs other CJD
Per year older if other CJD, *APOE*ε4 negative	+0.01	(-0.00,+0.03)	0.11	
Per year older if other CJD, *APOE*ε4 positive	+0.16	(+0.03,0.30)	0.01	0.007 vs *APOE*ε4 neg.

Supplementary Table 2:

Next generation sequencing to exclude mutations known to be causal of amyloid-beta pathology

Sample Number	1	2	3	4	5	6	7	8
Mean Depth of Coverage	533	840	827	1014	719	711	829	646
% Coverage of Target (>10-fold)	99.86	99.86	99.96	99.96	99.96	99.94	99.96	99.86
Rare Variants Found?	None	None	None	1	None	1	None	3
APP	None	None	None	None	None	None	None	None
C9ORF72	None	None	None	None	None	None	None	None
CHMP2B	None	None	None	None	None	None	None	None
CSF1R	None	None	None	None	None	None	None	c.2132+5 C>T(3)
FUS	None	None	None	None	None	None	None	None
GRN	None	None	None	None	None	None	None	None
ITM2B	None	None	None	None	None	None	None	None
MAPT	None	None	None	Ala152Thr(1)	None	Ala178Thr(2)	None	None
NOTCH3	None	None	None	None	None	None	None	None
PRNP	None	None	None	None	None	None	None	None
PSEN1	None	None	None	None	None	None	None	None
PSEN2	None	None	None	None	None	None	None	None
SERPINI1	None	None	None	None	None	None	None	None
SQSTM1	None	None	None	None	None	None	None	None
TARDBP	None	None	None	None	None	None	None	None
TREM2	None	None	None	None	None	None	None	Thr96Lys(4)
TYROBP	None	None	None	None	None	None	None	None
VCP	None	None	None	None	None	None	None	c.18-5 T>C(5)

(1) rs143624519 is a possible risk factor for development of frontotemporal dementia and AD but is not a causative mutation[42]
(2) rs63750612 is thought to be a benign variant as it did not segregate with disease in a family with frontotemporal dementia and is present in controls at an allele frequency 0.0042 (ExAC)[43]
(3) rs17110908 is a splice region variant with an allele frequency of 0.00035 (ExAC). At the same nucleotide G>A (rather than G>T) is seen at a frequency of 0.015 in ExAC. Amyloid-beta pathology is not associated with published *CSF1R* mutations.
(4) rs2234253 has a frequency of 0.020 in ExAC. Reported as a risk factor for frontotemporal dementia but not for AD[44]. Not a causal mutation.
(5) rs114256093. Seen in ExAC at frequency of 0.046 in the African population. Amyloid-beta pathology is not associated with published *VCP* mutations.

References

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