Table 2 Clinical features of codon 129 homozygosity of methionine among V180I, sCJD-MM1 and sCJD-MM2

	V180I-MM n=139	sCJD-MM1 n=59	p Value (vs V180I-MM)	sCJD-MM2 n=8	p Value (vs V180I-MM)
Male/female	58/81	25/34		5/3	0.53
Codon 219	135 EE; 4 NA	54 EE; 5 NA		8 EE	
Age at onset (years)*	77.3±6.8 (78, 44–93, n=139)	68.9±9.1 (70, 40–89, n=59)	<0.001	60.3±11.9 (63, 43–74, n=8)	<0.001
Period from onset to death (months)*	23.1±15.1 (19, 5–70, n=75)	17.2±12.5 (15, 1–60, n=57)	0.032	22.3±12.0 (20, 10–50, n=8)	0.98
Myoclonus†	46/130 (35.4%)	52/59 (88.1%)		4/8 (50%)	<0.001
Period from onset to myoclonus (months)*	6.4±6.1 (5, 0–36, n=38)	2.0±2.4 (1, 0–13, n=49)	<0.001	7.3±4.0 (8, 3–11, n=3)	0.92
Cognitive impairment†	138/138 (100%)	59/59 (100%)		8/8 (100%)	1
Period from onset to cognitive impairment (months)*‡	0.5±1.4 (0, 0–7, n=121)	0.6±1.0 (0, 0–6, n=55)	1	15.6±40.3 (0, 0–115, n=8)	<0.001
Pyramidal signs†	66/132 (50%)	40/54 (74.1%)		2/7 (28.6%)	0.004
Period from onset to pyramidal sign (months)*	3.9±5.8 (2.5, 0–36, n=58)	2.9±4.4 (2, 0–24, n=38)	0.53	12 (n=1)	
Extrapyramidal signs†	71/133 (53.4%)	30/52 (57.7%)		2/8 (25.0%)	0.23
Period from onset to extrapyramidal signs (months)*	3.8±3.5 (3, 0–19, n=58)	2.2±4.3 (1, 0–24, n=29)	0.13	13.0±1.4 (13, 12–14, n=2)	0.002
Cerebellar dysfunction†	40/119 (33.6%)	32/45 (71.1%)		3/7 (42.9%)	<0.001
Period from onset to cerebellar dysfunction (months)*	2.9±2.7 (3, 0–9, n=33)	0.7±0.9 (1, 0–3, n=31)	<0.001	12.7±1.2 (12, 12–14, n=3)	<0.001
Visual disturbance†	10/109 (9.2%)	28/49 (57.1%)		2/7 (28.6%)	<0.001
Period from onset to visual disturbance (months)*	2.2±1.5 (2, 0–4, n=10)	0.7±1.7 (0, 0–7, n=26)	0.036	0 (n=2)	0.15
Psychiatric symptoms†	68/130 (52.3%)	32/51 (62.7%)		5/7 (71.4%)	0.36
Period from onset to psychiatric symptoms (months)*	1.6±3.0 (0, 0–19, n=62)	0.8±0.9 (1, 0–3, n=29)	0.32	5.3±7.1 (3, 0–15, n=4)	0.024
Akinetic mutism†	74/137 (54.0%)	44/57 (77.2%)		2/8 (25.0%)	0.001
Period from onset to akinetic mutism (months)*	9.8±6.6 (8, 1–27, n=64)	3.6±4.3 (2, 0–23, n=42)	<0.001	18 (n=2)	
PSWCs on EEG†	10/131 (7.6%)	55/59 (93.2%)		2/7 (28.6%)	<0.001
Hyperintensities on MRI	135/136 (99.3%)	57/57 (100%)	<0.001	5/8 (62.5%)	0.092
Positive rate of 14-3-3 protein in CSF†	46/53 (86.8%)	27/31 (87.1%)	1	NA	
Positive rate of t-τ protein in CSF†	48/53 (90.6%)	27/31 (87.1%)	0.72	NA	
Amount of t-τ protein in CSF (pg/mL) *	2965±1712 (2400, 146.0–9940.0, n=53)	7950±8423 (5450, 150.0–40120.0, n=29)	<0.001	NA	
Positive rate of PrP^{Sc} in CSF†	36/53 (67.9%)	27/30 (90.0%)	0.032	NA	

Codon 219 is presented with total cases of that polymorphism type. EE means glutamic acid homozygous. NA means data not available.
Medians are compared using analysis of variance with Dunnett's post hoc test for age of onset, the period from disease onset to death or the appearance of each symptom and sign, the two-tailed Mann-Whitney U test for the period from onset to akinetic mutism and the CSF biomarker level. Frequencies of positive cases are compared using the two-tailed Fisher's exact test.
*Age of onset, period of time from disease onset to death or the appearance of each symptom and sign and CSF biomarker level are presented as mean±SD (median, range, cases).
†Frequencies of positive cases are presented as positive cases/total cases (percentage).
‡These were zero-inflated.
CSF, cerebrospinal fluid; PSWCs, periodic sharp wave complexes; sCJD, sporadic Creutzfeldt-Jakob disease; t-τ, total τ.

Table 3 Clinical features of codon 129 heterozygosity of methionine/valine between V180I and sCJD-MV

	V180I-MV n=45	sCJD-MV n=7	p Value
Type 1 or 2		5 type 2; 2 NA	
Male/female	20/25	3/4	1
Codon 219	44 EE; 1 EK	7 EE	
Age at onset (years)*	76.7±7.6 (78, 57–92, n=43)	62.0±7.0 (62, 51–73, n=7)	<0.001
Period from onset to death (months)*	27.8±16.3 (25, 7–64, n=23)	26.2±12.9 (21, 12–43, n=6)	0.98
Myoclonus†	21/43 (48.8%)	5/7 (71.4%)	0.42
Period from onset to myoclonus (months)*	9.2±7.2 (7, 2–30, n=18)	8.5±4.7 (7.5, 4–15, n=4)	0.86
Cognitive impairment†	43/44 (97.7%)	7/7 (100%)	1
Period from onset to cognitive impairment (months)*‡	0.6±1.4 (0, 0–5, n=38)	3.0±4.5 (0, 0–10, n=5)	0.26
Pyramidal signs†	14/42 (33.3%)	2/6 (33.3%)	1
Period from onset to pyramidal sign (months)*	5.2±4.2 (5, 0–14, n=11)	12 (n=1)	
Extrapyramidal signs†	23/40 (57.5%)	5/6 (83.3%)	0.23
Period from onset to extrapyramidal signs (months)*	3.8±4.5 (2, 0–16, n=18)	5.5±6.6 (4, 0–15, n=4)	0.58
Cerebellar dysfunction†	12/38 (31.6%)	6/6 (100%)	0.003
Period from onset to cerebellar dysfunction (months)*	3.4±4.1 (3, 0–12, n=8)	5.8±5.4 (4, 0–14, n=5)	0.50
Visual disturbance†	1/34 (2.9%)	1/5 (20%)	0.24
Period from onset to visual disturbance (months)*	(n=0)	(n=0)	
Psychiatric symptoms†	16/38 (42.1%)	3/7 (42.9%)	1
Period from onset to psychiatric symptoms (months)*	2.0±2.6 (0, 0–7, n=13)	4.5±2.1 (5, 3–6, n=2)	0.24
Akinetic mutism†	30/44 (68.2%)	3/7 (42.9%)	0.23
Period from onset to akinetic mutism (months)*	13.2±10.9 (9, 0–49, n=23)	12.5±5.0 (13, 9–16, n=2)	
PSWCs on EEG†	5/39 (12.8%)	2/6 (33.3%)	0.23
Hyperintensities on MRI	44/44 (100%)	7/7 (100%)	1
Positive rate of 14-3-3 protein in CSF†	11/18 (61.1%)	NA	
Positive rate of t-τ protein in CSF†	12/18 (66.7%)	NA	
Amount of t-τ protein in CSF (pg/mL)*	2025±1441 (1689, 170.0–6430.0, n=18)	NA	
Positive rate of PrP^{Sc} in CSF†	7/18 (38.9%)	NA	

Codon 219 is presented with total cases of that polymorphism type. EE and EK mean glutamic acid and glycine homozygous, respectively. NA means data not available.
Medians are compared using the two-tailed Mann-Whitney U test for age of onset, the period from disease onset to death or the appearance of each symptom and sign and the CSF biomarker level. Frequencies of positive cases are compared using the two-tailed Fisher's exact test.
*Age at onset, period of time from disease onset to death or the appearance of each symptom and sign and CSF biomarker level are presented as mean±SD (median, range, cases).
†Frequencies of positive cases are presented as positive cases/total cases (percentage).
‡These were zero-inflated.
CSF, cerebrospinal fluid; PSWCs, periodic sharp wave complexes; sCJD, sporadic Creutzfeldt-Jakob disease; t-τ, total τ.

The average age of onset in V180I-MM and V180I-MV was about 10 years older than those of sCJD-MM1 and sCJD-MM2, and MV2, respectively. The period from disease onset to death was longer in V180I-MM than in sCJD-MM1, as previously reported,[4] but was almost the same as in sCJD-MM2 (table 2). Among the 16 total autopsied patients with V180I in this cohort, few patients had additional neuropathological alterations such as Alzheimer's disease.[6 7] There was no difference between definite and probable or possible cases with V180I mutation. The periods from onset to the occurrence of myoclonus, cerebellar dysfunction, visual disturbance and akinetic mutism in V180I-MM were significantly longer than those in sCJD-MM1. However, except for visual disturbance, the length of onset to the occurrence of all other signs was shorter than those in sCJD-MM2 (table 2). As for the clinical features of 129MV, there was no significant difference between V180I-MV and sCJD-MV, except for age of onset (table 3).

The analysis of the probability of occurrence of neurological symptoms and signs similarly demonstrated reduced severity in patients with V180I-MM compared to those with sCJD-MM1. While 88.1% of patients with sCJD-MM1 developed myoclonus, only 35.4% of patients with V180I developed myoclonus. Pyramidal signs, cerebellar dysfunction, visual disturbance and akinetic mutism were also less frequent in patients with V180I-MM1 than in patients with sCJD-MM1. However, as previously reported,[9] cerebellar and visual systems were not completely spared in patients with V180I.

EEG and MRI findings

PSWCs were observed in only 7.3% of patients with V180I-MM, but in over 90% of patients with sCJD-MM1 (table 2). MRI revealed hyperintensities with a similar positive rate in V180I-MM and sCJD-MM1, but was observed less frequently in patients with sCJD-MM2. The

Table 4 Effects of the codon 129 polymorphism on the clinical features of V180I

	129MM n=139	129MV n=45	p Value
Male/female	58/81	20/25	0.862
Age at onset (years)*	77.3±6.8 (78, 44–93, n=139)	76.7±7.6 (78, 57–92, n=45)	0.701
Period from onset to death (months)*	23.1±15.1 (19, 5–70, n=75)	27.8±16.3 (25, 7–64, n=23)	0.159
Myoclonus†	46/130 (35.4%)	21/43 (48.8%)	0.149
Period from onset to myoclonus (months)*	6.4±6.1 (5, 0–36, n=38)	9.2±7.2 (7, 2–30, n=18)	0.154
Cognitive impairment†	138/138 (100.0%)	43/44 (97.7%)	0.242
Period from onset to cognitive impairment (months)*	0.5±1.4 (0, 0–7, n=121)	0.6±1.4 (0, 0–5, n=38)	0.456
Pyramidal signs†	66/132 (50.0%)	14/42 (33.3%)	0.075
Period from onset to pyramidal signs (months)*	3.9±5.8 (3, 0–36, n=58)	5.2±4.2 (5, 0–14, n=11)	0.136
Extrapyramidal signs†	71/133 (53.4%)	23/40 (57.5%)	0.719
Period from onset to extrapyramidal signs (months)*	3.8±3.5 (3, 0–19, n=58)	3.8±4.5 (2, 0–16, n=18)	0.460
Cerebellar dysfunction†	40/119 (33.6%)	12/38 (31.6%)	1.000
Period from onset to cerebellar dysfunction (months)*	2.9±2.7 (3, 0–9, n=33)	3.4±4.1 (3, 0–12, n=8)	0.973
Visual disturbance†	10/109 (9.2%)	1/34 (2.9%)	0.460
Period from onset to visual disturbance (months)*	2.2±1.5 (2, 0–4, n=10)	(n=0)	NA
Psychiatric symptoms†	68/130 (52.3%)	16/38 (42.1%)	0.357
Period from onset to psychiatric symptoms (months)*	1.6±3.0 (0, 0–19, n=62)	2.0±2.6 (0, 0–7, n=13)	0.576
Akinetic mutism†	74/137 (54.0%)	30/44 (68.2%)	0.116
Period from onset to akinetic mutism (months)*	9.8±6.6 (8, 1–27, n=64)	13.2±10.9 (9, 0–49, n=23)	0.190
PSWCs on EEG†	10/131 (7.6%)	5/39 (12.8%)	0.339
Hyperintensities on MRI†	135/136 (99.3%)	44/44 (100.0%)	1.000
Positive rate of 14-3-3 protein in CSF†	46/53 (86.8%)	11/18 (61.1%)	0.035
Positive rate of t-τ protein in CSF†	48/53 (90.6%)	12/18 (66.7%)	0.014
Amount of t-τ protein in CSF (pg/mL)*	2965±1712 (2400, 146.0–9940.0, n=53)	2025±1441 (1689, 170.0–6430.0, n=18)	0.022
Positive rate of PrP^{Sc} in CSF†	36/53 (67.9%)	7/18 (38.9%)	0.049

*Age of onset, period from disease onset to death or the appearance of each symptom and sign and CSF biomarker level are presented as mean±SD (median, range, case number).
†Frequencies of positive cases are presented as positive cases/total cases (percentage).
Medians are compared using the two-tailed Mann-Whitney U test for age of onset, the period from disease onset to death or the appearance of each symptom and sign and the CSF biomarker level. Frequencies of positive cases are compared using the two-tailed Fisher's exact test.
CSF, cerebrospinal fluid; PSWCs, periodic sharp wave complexes; t-τ, total τ.

pattern of hyperintensities in patients with V180I was uniquely distributed in the cerebral cortex.

Effect of *PRNP* polymorphism on clinical symptoms and signs of V180I

We also analysed the effect of the codon 129 polymorphism on the clinical symptoms of patients with V180I (table 4).

In a total of 184 patients with V180I, 139 patients (75.5%) were 129MM, while the remaining 45 (24.5%) were 129MV. We detected only three patients with the V180I mutation on the same allele as valine. In this case, the clinical features were no different from those with the mutation on the same allele as methionine. We also analysed the codon 219 polymorphism in 179 patients with V180I and 54 patients with sCJD-MM1, all of whom showed glutamic acid homozygosity (table 2). When analysing the influence of the codon 129 polymorphism on clinical symptoms and signs in patients with V180I, no symptoms or signs, in terms of the occurrence rate and the speed to develop them after disease onset, were affected by codon 129MV.

CSF biomarkers

Positive tests of the CSF 14-3-3 protein and t-τ proteins in patients with V180I-MM were similar to those of patients with sCJD-MM1 (table 2). However, the median value of t-τ proteins in the CSF of patients with V180I-MM was significantly lower than that of patients with sCJD-MM1. In patients with V180I-MM, we found fewer positive tests for PrP^{Sc} in the CSF than in patients with sCJD-MM1. Patients with V180I-MV, in particular, showed significantly fewer positive tests for 14-3-3, t-τ protein and PrP^{Sc} in the CSF, as well as in the amount of CSF t-τ (p=0.034, 0.023, 0.001 and <0.001, respectively; multiple comparison using Fisher's exact and Kruskal-Wallis tests).

Effect of age

In order to exclude whether any variables were age dependent, we compared some laboratory and CSF findings in patients with V180I and with sCJD older than 75 years (table 5). We found that positive rates of PrP^{Sc} were comparable, although whether the greater percentage of older patients with PrP^{Sc} is age dependent or due to the small sample size is currently unclear.

Table 5 Laboratory and CSF findings of gPrD-V180I compared to sCJD older than 75 years

	V180I n=186	sCJD (>75 years) n=11	p Value
Male/female	78/108	25/34	1.00
Age at onset (years)*	77.2±6.9 (78, 44–93)	80.9±4.2 (80, 76–89)	0.056
PSWCs on EEG†	16/172 (9.3%)	10/11 (90.9%)	<0.001
Positive rate of 14-3-3 protein in CSF†	57/71 (80.2%)	7/8 (87.5%)	1.00
Positive rate of t-τ protein in CSF†	61/71 (85.9%)	6/8 (75.0%)	0.60
Amount of t-τ protein in CSF (pg/mL)*	2727±1688 (2400, 146.0–9940.0, n=71)	6569.8±4270.3 (8995.0, 150.0–10290.0, n=8)	0.035
Positive rate of PrP^{Sc} in CSF†	44/71 (62.0%)	7/8 (87.5%)	0.246

Medians are compared using the two-tailed Mann-Whitney U test for age at onset, the period from disease onset to death or the appearance of each symptom and sign and the CSF biomarker level. Frequencies of positive cases are compared using the two-tailed Fisher's exact test.
*Age at onset and the appearance of CSF biomarker level are presented with mean±SD (median, range, case number).
†Frequencies of positive cases are presented with positive case number/total case number (percentage).
CSF, cerebrospinal fluid; gPrD, genetic form of prion disease; PSWCs, periodic sharp wave complexes; sCJD, sporadic Creutzfeldt-Jakob disease; t-τ, total τ.

DISCUSSION

With a very rare incidence in Europe,[3] the V180I mutation was geoepidemiologically discovered mainly in Japan, and has turned out to be the most common cause of gPrD in Japan.[5] The reason for this geographical distribution difference is currently unclear, but racial and/or environmental factors are most likely involved.

Of the patients with V180I gPrD, 78 cases were of male patients and 108 cases were female, indicating a possible gender influence on the susceptibility of this mutation in the disease. Similar to other mutations in *PRNP*, women appear more susceptible.[3] Patients with sCJD-MM1 were characterised by fast, severe progression of the disease, and neurological malfunctions resulting from extensive brain lesions appeared in a period of less than 3 months (table 2). However, V180I progressed relatively slowly. Myoclonus, cerebellar signs and visual dysfunctions occurred less frequently and with greater latency in patients with V180I (table 2). PSWCs in the EEG, a frequent finding in patients with sCJD-MM1, were rarely detected in patients with V180I (table 2). While a triad of dementia, myoclonus and PSWCs in the EEG is typical of sCJD, patients with V180I mainly presented with cognitive impairment and a very low rate of myoclonus in the early stages, along with rarely detectable PSWCs. Instead of possible CJD, these cases tended to be misdiagnosed as dementia due to Alzheimer's disease. MRI could facilitate the diagnosis of V180I when a specific pattern of ribbon hyperintensity lesions is detected.[8 13] However, it may still be difficult to distinguish patients with V180I from patients with sCJD-MM1 because hyperintensity was similarly detected in patients with sCJD-MM1, necessitating direct testing of the *PRNP* gene.

Previous reports suggested that there were no visual and cerebellar clinical symptoms in V180I, and neuroimaging of the medial occipital lobes posterior to the parieto-occipital sulcus and the cerebellum revealed that they were not involved until the terminal stage.[9] These data posit V180I as a comparative analogue of sCJD[14] or a cortical form of sCJD with type-2 PrP^{Sc} and methionine homozygosity at codon 129.[15] In our current study of 186 patients with V180I, we found that 34% demonstrated clinical cerebellar dysfunction, and 8.3% presented with visual disturbances (tables 2 and 3). Although no detailed description of the exact manifestations of cerebellar and visual symptoms was recorded, and a subjective bias in identifying the true origins of these symptoms should be taken into consideration, our finding indicates that in order to confirm whether the cerebellum is actually spared in patients with V180I, it is critical to analyse the pathological and immunohistochemical features including PrP^{Sc} deposition and spongiform changes in a topological manner.

The penetrance of V180I was very low. Only 11 out of 186 patients (5.9%) had a family history of dementia, while family member involvement in the case of other gPrD mutations, such as E200K, P102L and P105L, was frequently noted.[3 5] Within the 11 patients with V180I in the current study who had a recorded family history, 3 patients had one family member each diagnosed with CJD. The remaining eight cases had family members of one generation above, or the same generation, who had dementia due to an unknown cause. The low penetrance of V180I, specific clinical features and MRI findings was intriguing, and leads us to speculate whether the V180I mutation is causative for the disease or is actually a disease-associated factor accompanying other protective or toxic factors. The V180I mutation is reported to have significantly higher proportions of overall prion disease (n=881, both p<0.001),[4] compared with the genotypes of *PRNP* in the general Japanese population (n=466; isoleucine allele at codon 180 was not detected).[16] These findings indicate that the V180I mutation is not simply a polymorphism, but is indeed disease related.

Different PrP^{Sc} glycotypes might lead to differential distributions of PrP^{Sc} throughout the brain,[17 18] and may account for the disparate affects on brain regions

underlying cerebral cortical symptoms as opposed to cerebellar symptoms, for instance. In this cohort, western blotting of brain homogenates from patients with V180I indicated only weak bands of the monoglycosylated and unglycosylated fragments.[5] In the future, studies should examine the role of factors that influence lesion topology on the disease's clinical expression and progression. We hypothesise that the different pattern of clinical and pathological features with V180I may represent different, but still topologically defined, neuronal loss when compared with sCJD-MM1. Elucidating this mechanism would require systematic pathological, immunochemical and biochemical studies of PrP^{Sc}.

The codon 129 polymorphism in *PRNP* plays an important role in determining the disease phenotype and the type of PrP^{Sc} present in sCJD.[14 19 20] It was also reported that the codon 129 polymorphism affects the phenotype in gPrD.[21–23] In our study, while 75.5% had methionine in the normal allele (MM homozygous), 24.5% had valine in the normal allele (MV heterozygous). We observed that when the codon 129 polymorphism occurred in the allele opposite to the V180I mutation, its influence on the clinical symptoms and signs were similar to the wild type MV polymorphism (table 4). However, the MV polymorphism in codon 129 significantly lowered the positive test rate and amount of CSF biomarkers such as the 14-3-3 protein, τ protein and PrP^{Sc} positivity, suggesting that the codon 129 polymorphism may contribute to the severity and/or speed of neurological degeneration. Moreover, there might be other unknown disease modifying factors that contribute to the clinical features and course of genetic prion disease. In addition, the codon 129 and 219 polymorphisms have been reported to be risk and protective factors, respectively, for sCJD.[24–27] In our study, similar to the study of patients with sCJD-MM1, all patients with V180I tested for the codon 219 polymorphism were glutamic acid homozygous (table 2). This result further suggests that codon 219 heterozygosity would be a protective factor in resisting prion disease onset. Interestingly, the frequency of codon 129 in MV heterozygous patients with the V180I mutation is greater than that in the general Japanese population, creating a discrepancy in the hypothesis that codon 129 homozygosity increases the susceptibility to prion disease.

Although there are several reports describing V180I in terms of its clinical features, imaging characteristics, pathology, immunohistochemistry and biochemistry, most were either case reports or analyses of a small number of cases.[6–8 28–31] To the best of our knowledge, the current study is the first large cohort clinical study of V180I. From this study, we conclude the clinical features of V180I to be as follows: (1) a late age of onset and slow progression; (2) a relatively low occurrence rate, and slow development of symptoms such as myoclonus, cerebellar abnormalities and visual disturbances; (3) a low detectable rate of PSWCs in EEGs, and a high detectable rate of hyperintensity in diffusion-weighted or fluid-attenuated inversion recovery imaging; (4) lower t-τ protein levels in the CSF versus sCJD-MM1 and (5) an extremely low likelihood of a family history of V180I.

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Acknowledgements The authors are grateful to all the members of the Prion Disease Surveillance Committee, Japan, for collecting data and offering advice. They also thank the patients with CJD and their families for providing clinical information about the patients. Our manuscript has been edited by professional native English-speaking editors (http://www.editage.jp).

Contributors TQ drafted the manuscript and analysed the data. NS and M Hizume revised the manuscript, designed the study and analysed the data. NS and MT performed the statistical analysis and obtained funding. RA and KS analysed the CSF samples. M Higuma, IN, TH, SM and AK coordinated the study. TK analysed the prion protein gene and supervised the study. YN collected patient data. MY and HM supervised this study.

Funding The contributions of NS, YN, TK, MY and HM to this study were supported in part by a Health and Labour Sciences Research Grant for Research on Measures for Intractable Diseases (Prion Disease and Slow Virus Infections, the Research Committee on Surveillance and Infection Control of Prion Disease) from the Ministry of Health, Labour and Welfare of the Japanese government.

Competing interests None.

Patient consent Obtained.

Ethics approval Institutional Ethics Committee of Tokyo Medical and Dental University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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BMJ Open

Clinical features of genetic Creutzfeldt-Jakob disease with V180I mutation in the prion protein gene

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BMJ Open 2014 4:
doi: 10.1136/bmjopen-2014-004968

doi:10.1111/ncn3.159

CASE REPORT

Elevation of 8-hydroxy-2′-deoxyguanosine in the cerebrospinal fluid of three patients with superficial siderosis

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Key words
8-hydroxy-2′-deoxyguanosine, biomarker, cerebrospinal fluid, oxidative stress, superficial siderosis.

Accepted for publication 21 November 2014.

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Abstract

Superficial siderosis is a progressive disorder of the central nervous system in which chronic intrathecal bleeding leads to hemosiderin deposition in the brain and spinal cord. Although it is hypothesized that oxidative stress caused by deposited iron contributes to the pathomechanism of superficial siderosis, there is a paucity of research supporting this hypothesis. We examined the cerebrospinal fluid of three patients with superficial siderosis for the oxidative stress marker 8-hydroxy-2′-deoxyguanosine. The origin of bleeding was identified in two of the three patients, who subsequently underwent surgical treatment. We detected elevated 8-hydroxy-2′-deoxyguanosine levels in the cerebrospinal fluid of all three patients that remained high in two patients, even after surgical treatment. Elevated 8-hydroxy-2′-deoxyguanosine levels suggest that oxidative stress is involved in the pathomechanism of superficial siderosis.

Introduction

Superficial siderosis is a chronic disorder characterized by hemosiderin deposition in the central nervous system that causes slowly progressing sensorineural hearing disturbances, cerebellar ataxia and myelopathy. Hemosiderin deposition occurs in the leptomeninges and parenchyma beneath the surfaces of the brain and spinal cord. The eighth cranial nerves, cerebellum, brainstem, spinal cord and entorhinal cortex are preferentially involved. In most cases, the causes and origins of continuous or intermittent bleeding into the cerebrospinal fluid (CSF) can be identified.[1] In magnetic resonance imaging (MRI), a hypointense rim on T2-weighted images typically can be observed in the brain and spinal cord.

In superficial siderosis, chronic bleeding into the CSF leads to entry of heme, one of the blood breakdown products, across the surfaces of the central nervous system.[2,3] Heme is then converted into ferritin, hemosiderin and free iron. It is thought that the deposition of iron, including hemosiderin, results in the production of reactive oxygen species (ROS), which in turn causes damage to proteins, lipids and deoxyribonucleic acid (DNA), leading to neural dysfunction and ultimately cell death.[4] Despite this hypothesis, there is a paucity of biochemical evidence that implicates oxidative stress in superficial siderosis. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), a well-known oxidative stress marker, is produced by oxidation of deoxyguanosine, a component of DNA.[5] 8-OHdG can be detected at high levels in various disorders. For example, one report showed that elevated levels of 8-OHdG were found in CSF of Alzheimer disease and in correlation to percentage of oxidized Coenzyme Q-10, which is a mitochondrial oxidative damage indicator.[6] In another study, 8-OHdG was elevated in CSF of Parkinson's disease and multiple system atrophy patients.[7] Therefore, we hypothesized that 8-OHdG in CSF would be increased reflecting the ROS produced by hemosiderin deposits in superficial siderosis. Here, we report three patients with superficial siderosis who showed elevated CSF levels of 8-OHdG.

Case reports

Case 1. A 70-year-old man experienced chronic progressive hearing loss, ataxic gait and urinary dysfunction over several decades. On examination, he showed severe bilateral hearing loss, dysarthria, limb and truncal ataxia, and pyramidal tract signs. He used a walker because of gait ataxia. Brain and spine MRI showed a hypointense rim on T2- and T2-star-weighted images. Lumbar puncture showed red blood cells, and a CSF analysis showed elevated levels of total tau protein (t-tau) and 8-OHdG 12 months before

Table 1 Clinical evaluation and 8-hydroxy-2′-deoxyguanosine and tau levels in cerebrospinal fluid

Case	Age (years)/sex	Disease duration (years)	Time of CSF evaluation	Evaluation of ataxia	Hearing thresholds by four-frequency pure tone average (0.5/1/2/4 kHz) in right/left ear (dB)	CSF 8-OHdG (ng/mL)	CSF total tau (pg/mL)
1	70/male	31	12 months before surgery	13	(110.0)/77.5 (5 months before surgery)	0.69	1608
			26 months after surgery	22	(110.0)/77.5	0.88	211
2	53/male	3	12 months before surgery	11 (immediately before surgery)	37.5/40.0	0.21	1839
			22 months after surgery	13	48.8/43.8	0.36	1210
3	66/female	12	On diagnosis	9	(110.0)/45.0	0.39	329

Four-frequency pure tone average (0.5/1/2/4 kHz) was calculated as an average of thresholds in 0.5/1/2/4 kHz for each ear in decibel (dB). Parentheses signify hearing loss worse than the examined level denoted (See Fig. S1 for audiograms). Normal range for 8-hydroxy-2′-deoxyguanosine (8-OHdG) is <0.06 ng/mL, as determined by measurement of CSF from 11 control individuals who were aged over 40 years (59.8 ± 13.5 years, 5 males/6 females), with enzyme-linked immunosorbent assay (Japan Institute for the Control of Aging, Shizuoka, Japan).[8,9] Control individuals include seven without neurological disease, three with lumbar stenosis and one with migraine. The normal range for tau is <604 pg/mL, which was also measured by enzyme-linked immunosorbent assay. Tau level was measured in eight of the same 11 control individuals, including four without neurological disease, three with lumbar stenosis and one with migraine. Ataxia was evaluated by the scale for the assessment and rating of ataxia score 0 (normal) to 40 (most severe).

surgery (Table 1).[8,9] Diagnosis of superficial siderosis was given, and repeated MRI were carried out to search for a possible bleeding origin. Spinal MRI showed a ventral, fluid-filled collection and a dural defect at Th2/3, which was closed surgically. Thereafter, although the CSF repeatedly tested negative for red blood cells, the patient's symptoms continued to worsen, and the scale for the assessment and rating of ataxia score rose from 13 to 22. Hearing loss did not recover (Table 1 and Fig. S1), and CSF analysis 26 months after operation showed that the level of 8-OHdG remained high, whereas t-tau reduced to normal levels (Table 1).

Case 2. A 53-year-old man presented with a chronic progressive course of ataxic gait, bilateral hearing difficulty and dysarthria. On examination, he showed bilateral hearing loss, dysarthria, and limb and truncal ataxia. Brain and spine MRI showed a hypointense rim on T2- and T2 star-weighted images, a spinal ventral fluid-filled collection and a dural defect at Th1/2. Lumbar puncture showed red blood cells, and CSF analysis showed elevated levels of both t-tau and 8-OHdG 12 months before surgery (Table 1). The patient was diagnosed as superficial siderosis probably as a result of dural defect. The dural defect was sutured successfully through surgery. Although a lack of red blood cells in the CSF was repeatedly confirmed after surgery, the patient's symptoms continued to progress. Scale for the assessment and rating of ataxia score and hearing thresholds slightly exacerbated 22 months after operation (Table 1 and Fig. S1). CSF analysis showed that t-tau was reduced; however, the 8-OHdG level was elevated (Table 1).

Case 3. A 66-year-old woman presented with chronic hearing loss, dysarthria and gait ataxia. Neurological examination showed bilateral hearing loss, dysarthria, and limb and truncal ataxia. Lumbar puncture showed the presence of red blood cells. Although brain and spine MRI showed a typical hypointense rim on T2- and T2 star-weighted images, no bleeding origin could be identified. CSF analysis on diagnosis showed that 8-OHdG was elevated, whereas CSF t-tau was within normal levels (Table 1).

Discussion

This report provides the first evidence of oxidative stress in superficial siderosis by measuring CSF levels of 8-OHdG. Free iron derived from hemosiderin deposits in cells, such as microglia, astrocytes and Bergmann glia, can produce ROS; this is assumed to result in the elevated 8-OHdG levels in the CSF.[4] Thus, it is suggested that high levels of 8-OHdG arise from persisting hemosiderin deposits, which has been accumulated in the central nervous system over decades.

Although a decrease of CSF t-tau was observed after surgical repair of bleeding origin in our cases, as reported in a previous study, 8-OHdG levels did not decrease and symptoms continued to progress after surgical treatment.[10] As an interpretation, as surgical treatment stopped bleeding, but did not readily remove iron deposits in the central nervous system, continuing oxidative stress would produce 8-OHdG and might modestly damage the central nervous system as long as iron deposits persist. Although reduced CSF t-tau suggests that ongoing injury in neurons were mitigated to some extent after surgical treatment, the effect might be insufficient in the present cases. Evaluation of CSF 8-OHdG and t-tau in larger cohorts would be required to confirm our results, and study of the relationship between 8-OHdG levels and disease severity might be important. It would also be interesting to know whether 8-OHdG levels can reflect decreases in iron deposition if an iron chelation therapy could remove such deposits.

In conclusion, in the present cases we observed elevated levels of CSF 8-OHdG, a biomarker for oxidative stress, which is considered to play an important role in the pathomechanism of superficial siderosis.

Acknowledgments

The regional ethics committee of Tokyo Medical and Dental University approved the use of the clinical records for this study. All patients were enrolled after providing their informed consent. The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Audiograms in superficial siderosis patients.