Figure 1 Top: CSF biomarker intercorrelation for principal components 1 and 2 from partial least squares-discriminant analysis. The discriminant regressor was Alzheimer's disease (filled circle) or healthy control (open square). Highest correlation was found for A β 42 with Alzheimer's disease. Tau and NFL also correlated with Alzheimer's disease; however, including some structure not related to the disease (loading in principal component 2). Bottom: Interindividual scores of included study objects for principal components 1 and 2 from partial least squares-DA. The principal components were derived by a projection of assessed CSF protein concentrations (included protein assessment as in the figure above). Filled circles=Alzheimer's disease; open squares=healthy controls.

$p=0.002$). There was also a significant decrease in CSF A β 42 in patients with Alzheimer's disease compared with controls (748 (297) v 1623 (429) pg/ml; $p<0.0001$) and a slight but significant decrease in CSF S-100 β (1.8 (0.9) v 2.5 (0.9) μ g/l; $p=0.014$). By contrast, there were no significant differences in CSF-NSE (7.4 (2.7) v 6.9 (2.1) μ g/l; $p=0.568$) or CSF GFAP (860 (297) v 717 (250) ng/l; $p=0.097$).

The combination of CSF tau and CSF A β 42 gave at best a sensitivity of 32/35 (91.4%) and a specificity of 17/19 (89.5%). A partial least squares analysis with all CSF biomarkers and clinical groups (Alzheimer's disease and controls), showed a relation between a diagnosis of Alzheimer's disease and high CSF tau, high CSF NFL, high CSF GFAP, and low CSF A β 42 concentrations (fig 1). The NSE and S-100 β in CSF showed no discriminative power so these additional biomarkers gave no further aid in the discrimination between Alzheimer's disease and controls. The sensitivity using all CSF biomarkers was 34/35 (97.1%) and the specificity was 17/19 (89.5%).

In agreement with previous findings, increased CSF tau and decreased CSF A β 42¹ was found in Alzheimer's disease, resulting in a good sensitivity and specificity for discriminating Alzheimer's disease from controls. As the ability of these CSF biomarkers to discriminate Alzheimer's disease from other dementia disorders is less than optimal, we tested whether the combined analysis of additional biomarkers for axonal degeneration (CSF NFL), neuronal degeneration (CSF NSE), and gliosis (CSF GFAP and CSF S-100 β) resulted in any further increase in the diagnostic sensitivity or specificity. However, there was only a marginal increase

in sensitivity (from 91.4% to 97.1%) whereas the specificity was unchanged (89.5%). Therefore we conclude that these biomarkers have little additional value as diagnostic biochemical markers for Alzheimer's disease.

We hypothesise that other biomarkers more specifically related to Alzheimer's disease pathogenesis, such as hyperphosphorylated tau, synapse specific proteins (for example, rab3a, synaptotagmin), or APP isoforms (for example, α -secretase or β -secretase cleaved APP), may have a larger potential as CSF biomarkers for Alzheimer's disease.

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Variant Creutzfeldt-Jakob disease is not associated with individual abilities to metabolise organophosphates

Since its identification as a distinct form of human prion disease, it has been demonstrated that vCJD is related to bovine spongiform encephalopathy (BSE),¹ thus providing evidence for transmission of the disease from cattle to humans. Despite widespread beef consumption, however, the number of cases of vCJD has been low and moreover, there is no history of unusual exposure to beef or its products among affected persons.¹ These findings may arise from a combination of factors, including the existence of environmental factors that may affect susceptibility, the long

incubation period for vCJD, uneven exposure to infected beef, and variations in individual genetic susceptibility to the transmission process. Of the known genetic factors, it has been established that polymorphisms of codon 129 of the prion protein gene confer individual susceptibility to vCJD.¹ However, this polymorphism is common in the normal population, suggesting that other genes contribute to genetic susceptibility to vCJD.

This study aimed to establish whether polymorphisms in the paraoxonase (PON) family of genes are associated with incidence of vCJD and was based on the hypothesis that exposure to OPs, widely used as insecticides in the United Kingdom, was causally related to the BSE epidemic.² PON1 and PON2 have a major role in the detoxification of many organophosphate pesticides: PON1 allelic variants confer fast or slow abilities to detoxify these xenobiotics.³ PON1 is also known to protect against accumulation of potentially harmful oxidised lipids: this scavenging role of PON1 has been used to provide a rationale for the association of both PON1 and PON2 polymorphic variants with predisposition to heart disease.⁴

The rationale for our study is also supported by the finding that, in cultured cells, the organophosphate pesticide phosmet, widely used at high doses in the United Kingdom to eradicate warble fly, upregulates cell surface levels of normal prion protein in human neuronal cells⁵; high levels of PrP expression are themselves known to be associated with increased ease of transmission of prion diseases.¹ Although it has been shown that vCJD does not seem to be associated with exposure to organophosphates present in head lice treatments,⁶ our study aimed to establish whether persons affected by vCJD are more genetically susceptible to organophosphate exposure than the normal population.

Using the polymerase chain reaction and restriction analysis, we genotyped 26 patients with vCJD, 19 patients with sporadic CJD, and 10 neurological controls for both codon 54 and 192 of PON1 and codon 311 of PON2 polymorphisms.^{3,4} In addition, we genotyped 93, 117, and 95 normal persons, respectively for codon 54 and 192 of PON1 and codon 311 of PON2 polymorphisms.

All patients were clinically diagnosed and neuropathologically confirmed. None of the patients with vCJD that we studied belonged to the cluster recently found in Leicestershire.⁷

Statistical analysis of the data was performed using the Pearson's χ^2 test ($p<0.05$).

The distribution of PON1 and PON2 genotypes and allele frequencies in patients and controls is shown in table 1. All genotype frequencies did not deviate significantly from the predicted Hardy-Weinberg equilibrium (data not shown). The frequencies of alleles L(Leu) and M(Met) at codon 54 of PON1 were respectively 0.672 and 0.328 in the control population ($n=93$), 0.654 and 0.346 in vCJD, 0.684 and 0.316 in sporadic CJD, and 0.700 and 0.300 in neurological controls. The frequencies for alleles A(Gln) and B(Arg) at codon 192 of PON1 were respectively 0.726 and 0.274 ($n=117$) in the controls, 0.731 and 0.269 in vCJD, 0.737 and 0.263 in sporadic CJD, and 0.700 and 0.300 in neurological controls. Finally, the frequencies for alleles S(Ser) and C(Cys) at codon 311 of PON 2 were respectively 0.774 and 0.226 in controls ($n=95$), 0.769 and 0.231 in vCJD, 0.763 and 0.237 in sporadic CJD, and

Table 1 Distribution of PON1 and PON2 genotypes and allele frequencies in cases and controls*

	Controls	Neurological controls	Sporadic CJD	Variant CJD
PON1:				
Codon 54				
Genotype				
LL	38 (40.9)	4 (40)	7 (36.8)	8 (30.8)
LM	49 (52.7)	6 (60)	12 (63.2)	18 (69.2)
MM	6 (6.4)	—	—	—
n	93	10	19	26
Allele				
L(Leu)	0.672	0.700	0.684	0.654
M(Met)	0.328	0.300	0.316	0.346
PON2:				
Codon 311				
Genotype				
AA	63 (53.8)	5 (50)	10 (52.6)	14 (53.8)
AB	44 (37.6)	4 (40)	8 (42.1)	10 (38.5)
BB	10 (8.5)	1 (10)	1 (5.3)	2 (7.7)
n	117	10	19	26
Allele				
A(Gln)	0.726	0.700	0.737	0.731
B(Arg)	0.274	0.300	0.263	0.269
PON2:				
Codon 311				
Genotype				
SS	57 (60)	5 (50)	12 (63.2)	15 (57.7)
CS	33 (34.7)	4 (40)	5 (26.3)	10 (38.5)
CC	5 (5.3)	1 (10)	2 (10.5)	1 (3.8)
n	95	10	19	26
Allele				
S(Ser)	0.774	0.700	0.763	0.769
C(Cys)	0.226	0.300	0.237	0.231

Values in parentheses are percentages. All data were analysed using Pearson's χ^2 test (significance taken as $p < 0.05$). There were no significant differences between the cases and controls.

0.700 and 0.300 in neurological controls. There was no significant association between any of the PON polymorphisms studied and vCJD, sporadic CJD, or the other neurological disorders (table 1). Our data show that PON polymorphic variants are not associated with vCJD. These data, together with the data of Churchill *et al.*,⁶ indicate that exposure to organophosphates is unlikely to contribute to the incidence of vCJD.

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Monitoring an electroencephalogram for the safe application of therapeutic repetitive transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) has come to be widely used to evaluate the CNS since the first report on the use of TMS in humans by Barker *et al.*¹ in 1985. Depending on the frequency, intensity, and duration of stimulation, trains of repetitive TMS (rTMS) can transiently block or inhibit the function of a cortical region. It has been suggested that rTMS has therapeutic potential for the treatment of Parkinson's disease^{2,3} and psychiatric disorders.⁴ To apply rTMS as a clinical tool, an evaluation of the safety margins during the stimulation is required.

A 56 year old woman was admitted to Hokkaido University Medical Hospital on 6 October 1999 for treatment of involuntary movement of the trunk and lower limbs that had persisted for 8 years. In 1991, the patient had a spinal cord injury at thoracic and lumbar levels and a shearing fracture at the level of L1/L2. At that time, neurological examination disclosed paresis of the lower limbs, and the patient underwent a posterior fixation. Involuntary movement in her left thigh began a few months after the injury. In 1997, the involuntary movement became worse and had spread from the trunk to both limbs.

Examination using a surface EMG showed that the involuntary movement consisted of an elevation of the pelvis and a flexion and rotation of the trunk via the bilateral rectus abdominis, obliquus externus abdominis, and obliquus internus abdominis. Extension of the trunk also occurred during the involuntary movement via the bilateral quadratus lumborum and iliocostalis lumborum. In addition, an involuntary contraction of bilateral gluteus maximus muscles was seen in the hip joints. The occurrence of the involuntary movement was irregular and was not precipitated by any obvious conditions.

The patient was able to perform voluntary movement via the bilateral rectus abdominis,

obliquus externus abdominis, and obliquus internus abdominis muscles, but she was not able to perform voluntary movement using the hip flexors, which are under the control of L2, and lower level muscles. An EEG, recorded at rest, and MRI of the brain showed no significant abnormalities. In addition, the patient had no history of seizure.

Treatment was by means of drugs (clonazepam and haloperidol), transcutaneous electrical nerve stimulation of the lower intercostal nerve, which innervates the obliquus externus abdominis, and a lumbar extradural nerve block at the level of T10-12. However, these treatments had no effect on the involuntary movement.

A trial study of rTMS was designed to investigate whether it could improve the involuntary movement of the trunk and lower limbs. The study was performed using a commercially available stimulator (MagStim 200) and a round coil (13 cm in diameter) according to the following protocol: 50 stimuli of 0.25-Hz rTMS at the intensity of 110% of the motor threshold were delivered to the right prefrontal cortex in the counter-clockwise direction of the electrical current in the coil. In succession, 50 stimuli of 0.25-Hz rTMS at the intensity of 110% of the motor threshold were delivered to the left prefrontal cortex in the clockwise direction of the electrical current in the same coil. One session consisting of these 100 stimuli was delivered once a day and was repeated for 5 consecutive days.

The motor threshold was assessed by application of a single stimulation with inter-stimulus interval of more than 10 seconds to the presumed motor area⁵ for activation of the contralateral abductor pollicis brevis muscle.^{3,4} The directions of the electrical current of motor threshold measurement and rTMS of the ipsilateral prefrontal cortex were the same. This assessment was carried out an hour earlier than the first rTMS session. Motor threshold intensity was defined as the lowest stimulation intensity that induced five motor evoked potentials (MEPs) of 0.05 mV in peak to peak amplitude in 10 trials. Motor threshold intensities of the right and left abductor pollicis brevis muscles were 55% and 52% of the maximum stimulator output, respectively.

Left and right prefrontal cortex stimulations were defined as stimulations with the same coil centred over a point 5 cm anterior to the frontal scalp position for activation of the contralateral abductor pollicis brevis muscle.⁴ The patient agreed to participate in this trial before application of rTMS and gave informed consent to the study, which was approved by the local ethics committee.

During the application of rTMS, an EEG was recorded through F3, F4, C3, and C4, according to the International 10-20 system, in addition to monitoring MEPs on the bilateral obliquus externus abdominis muscles. Conventional EEGs recorded at rest before and after the rTMS trial did not show any abnormalities. Seizure was not seen during the measurement of motor threshold, although an EEG was not recorded. For the purpose of avoiding skin burn, radial notched electrodes were used while recording the EEG.

A focal slow wave (3-4 Hz) was recorded on C4 after the 4th stimulation of rTMS to the right prefrontal cortex on the first day of the trial. The slow wave disappeared at least 6 seconds later and reverted to an 8-10 Hz wave (fig 1). When rTMS to the right