in 1.5% agarose gel. The apolipoprotein E gene (ApoE) was also genotyped as described previously.⁶ All analyses were confirmed by a repeat procedure. The remainder of the patient's family members did not consent to genetic examination.

Sequence analysis of PS1 disclosed a novel heterozygous T to A transition at the first position of codon 237 (fig 1). This mutation is predicted to result in the substitution of a phenylalanine for isoleucine (Phe237Ile). Restriction analysis confirmed the presence of a heterozygous mutation of Phe237Ile. There was no additional mutation in the whole coding exons of PS1 and PRNP, exon 16 and 17 of APP, or splice acceptor site of intron 8. Phe237Ile mutation was not found in 197 patients from the necropsy series. There was no deletion of the large segment of PS1 cDNA including exon 9, which was previously reported in familial Alzheimer's disease with spastic paraparesis.² The ApoE genotype of our patient was 3/3.

As there is no similar disease in his family and DNA samples from the remainder of the family members were not available, we cannot authenticate the relation between genetic abnormality and development of the disease. However, we suppose that the PS1 Phe237Ile is responsible for pathogenesis of our patient for five reasons.

Firstly, mutation in PS1 is the most popular genetic cause of familial Alzheimer's disease (FAD) and all mutations except Glu318Gly are responsible for early onset Alzheimer's disease.⁸ Glu318Gly is a frequent polymorphism which is found in 3.3% of the general population.⁸ To exclude the possibility that Phe237Ile is a polymorphism in a Japanese population, we screened for the presence of Phe237Ile in 197 patients from a necropsy series including non-demented controls and patients with sporadic Alzheimer's disease. The same mutation was not found in this population, suggesting that Phe237Ile is a rare mutation associated with FAD.

Secondly, two mutations in the transmembrane V domain produce very early onset Alzheimer's disease. The patients with Leu235Pro developed Alzheimer's disease at ages 29-35 and the patients with Met233Thr in their early 30s.^{1,9} Our patient also manifested his first neurological symptom at the age of 31.

Thirdly, three mutations of PS1, loss of exon 9 with or without mutation splice acceptor site mutation, and Arg278Thr are associated with Alzheimer's disease with spastic paraparesis.^{1,2}

Fourthly, codon 237 is well preserved in the related proteins such as the human PS1, human PS2, mouse PS1, and *Caenorhabditis elegans* Sel-12 protein. These data indicate that this region is important for the function of PS1 and mutation in codon 237 is certainly pathogenic.

Fifthly, PET examination showed hypometabolism in the temporoparietal lobes, which is a typical metabolic deficit of Alzheimer's disease. The similar pattern of hypometabolism was reported in the patients with variant Alzheimer's disease with spastic paraparesis.¹⁰ The result of the SPECT study was also compatible with diagnosis of Alzheimer's disease.

As the combination of five reasons as mentioned above is hardly explained by chance, we suppose that our clinical and genetic findings would be sufficient to diagnose our patient as having Alzheimer's disease with spastic paraparesis associated with the PS1

Phe237Ile mutation. We should examine genomic DNA and mRNA of PS1 from the patient with dementia and spastic paresis, even if it is an apparent sporadic case. Further collection of similar cases would establish clinical characteristics of Alzheimer's disease associated with the PS1 Phe237Ile mutation.

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Evaluation of CSF biomarkers for axonal and neuronal degeneration, gliosis, and β -amyloid metabolism in Alzheimer's disease

Although the accuracy rate of the clinical diagnosis of Alzheimer's disease is around 75%-90%, it is probably considerably lower

early in the disease course, when symptoms are vague. Therefore, in view of potential future disease modifying compounds there is a great need for reliable diagnostic biochemical markers for Alzheimer's disease in CSF.

Such markers should reflect the central pathogenic processes of the disease—that is, the disturbance in the metabolism of β -amyloid (A β) with subsequent A β deposition in senile plaques, the hyperphosphorylation of tau protein with subsequent formation of neurofibrillary tangles, neuronal degeneration, and gliosis.

Two promising biomarkers are tau protein (reflecting neuronal and axonal degeneration) and A β 42 (reflecting disturbances in A β metabolism and possibly A β deposition in senile plaques). The ability of the combination of CSF tau and CSF A β 42 to differentiate Alzheimer's disease from normal aging and depression is high, about 85%, also early in the course of the disease.¹ Similarly, most degenerative neurological disorders have normal concentrations. However, the specificity against other dementias is not optimal.¹ Thus, there is a need for additional CSF biomarkers for Alzheimer's disease, to further increase the diagnostic accuracy.

We therefore examined whether the addition of other CSF biomarkers (two neuronal and two glial proteins) would add further to the diagnostic ability to identify Alzheimer's disease. The neuronal proteins were neurofilament protein light subunit (NFL), the major protein component of neurofilaments (probably reflecting degeneration of myelinated axons) and neuron specific enolase (NSE), a neuronal glycolytic enzyme (probably reflecting degeneration of neuronal cell bodies). The glial proteins were glial fibrillary acidic protein (GFAP), an astrocyte specific protein considered to be the major component of glial filaments in reactive astrocytes, and S-100 β , a calcium binding protein also found in astrocytes (both reflecting gliosis).

From the longitudinal geriatric population study in Piteå, Sweden² we studied 35 patients with Alzheimer's disease, mean age 72.1 (SD 5.9) years, duration of disease of 48.9 (SD 32.0) months, and with MMSE scores of 23.5 (SD 4.7). The control group consisted of 19 subjects, mean age 71.2 (SD 7.3) years, without symptoms or signs of brain disorders, all with MMSE scores above 28.

The ethics committees at the universities of Umeå and Göteborg approved the study, conducted in accordance with the provisions of the Helsinki Declaration.

Analyses of CSF were performed using enzyme linked immunosorbent assays (ELISAs) as described previously in detail for total tau, A β 42,¹ NFL,³ GFAP,⁴ and S-100 β .⁵ The NSE in CSF was determined using a commercial ELISA from AB Sangtec Medical, Bromma, Sweden.

The Mann-Whitney *U* test was used for group comparisons and the Pearson correlation coefficient for correlations. The dataset was also investigated by principal component analysis using the SIMCA-S software (Umetri AB, Umeå, Sweden), and by partial least squares with cross validation as a validation tool for multivariate correlations between CSF biomarkers and diagnosis.

When comparing CSF biomarkers between patients with Alzheimer's disease and controls (values given as means (SD)), there was a significant increase in CSF tau (634 (288) v 375 (171) pg/ml; *p*<0.0001), and in CSF NFL (615 (456) v 295 (194) pg/ml;

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