LETTERS TO THE EDITOR

Very early onset Alzheimer's disease with spastic paraparesis associated with a novel presenilin 1 mutation (Phe237Ile)

Mutations in the presenilin 1 (PS1) gene (PS1) are responsible for 30%–40% of early onset familial Alzheimer's disease and over 60 mutations have been found so far. There are phenotypic variations among mutations on PS1. Three PS1 mutations, deletion of exon 9 with and without splice acceptor site mutation, and Arg278Thr have been reported to be associated with Alzheimer's disease with spastic paraparesis.[1 2] We report clinical and genetic features of a man who developed very early onset Alzheimer's disease with spastic paraparesis, which was associated with a novel mutation of PS1, Phe237Ile.

A 35 year old Japanese man had graduated from a national university and had worked as a psychiatric counsellor for a local clinic. His first neurological symptom was gait disturbance at the age of 31. At the age of 32, mild memory impairment and decreased mental activity were noted. His neurological deficits progressed gradually. On neurological evaluation at the age of 33, diffuse hyperreflexia, ataxia in all limbs, bilateral Babinski's sign, and dementia (total IQ on the WAIS-R of 75) were noted. He gave up his job at this time. At the age 34, he could not live alone because of memory deficit and cognitive dysfunction (total IQ on the WAIS-R of 59). At the age of 35, he became bedridden due to deterioration of spastic paraparesis, and presented with partial or generalised seizures a few times. His parents (66 and 63 years old) and sibling (27 years old) had no neurological deficits. There was no similar disease in other members of his family. On admission, he was alert and oriented for place, but not for time. He had severe difficulties in immediate and delayed recall of presented materials. He could not answer his name and occupation, but could sometimes follow three step commands. He spoke only two word sentences and could not write any words. He also had difficulties in speech comprehension. His score on the mini mental state examination was 5. Cranial nerves were normal except for dysarthria. Deep tendon reflexes were hyperreactive and plantar responses were extensor bilaterally. Muscle tone was rigid and spastic in all limbs. He had neck dystonia. No apparent weakness was noted. He presented generalised bradykinesia. He had myoclonic involuntary movement in his face and arms. Sensation remained intact. There was no remarkable abnormality in coordination. He was incontinent of urine. The protein concentration in CSF was increased at 73 mg/l whereas the cell count was normal. The concentrations of neuron specific enolase (23.6 ng/ml) and tau protein (722 pg/ml) in CSF were increased. An EEG showed generalised slowing with background theta. Somatosensory evoked potential was normal. Brain MRI showed diffuse cerebral cortical atrophy. PET with 2-^{18}F-fluoro-2-deoxy-D-glucose as a ligand and a Tc-99m-ECD SPECT study demonstrated remarkable hypometabolism and hypoperfusion in the bilateral temporoparietal areas including the primary sensory and motor cortex, respectively.

Genomic DNA was extracted from blood. The whole coding exons of PS1 and prion protein gene (PRNP), exon 16 and 17 of the amyloid β protein precursor gene (APP), and splice acceptor site of intron 8 were amplified using a polymerase chain reaction (PCR) with primers previously described.[3–5] Sequencing of both the sense and complementary strand of the PCR product were performed by ABI PRISM model 310 using the ABI PRISM BigDye™ terminator cycle sequencing ready reaction kit (Perkin-Elmer, CA, USA). The novel mutation Phe237Ile in PS1 was confirmed by restriction fragment length polymorphism. The PCR product was digested with Hph I (Biolabs) and was resolved in 1.5% agarose gel. A normal allele was characterised by the single fragment of 369 bp and a mutated allele by two fragments of 248 and 121 bp. We also searched for this mutation in 197 Japanese patients from a necropsy series at a geriatric hospital in Tokyo (73 non-demented controls without CNS disorder, 59 sporadic patients with Alzheimer's disease, and 65 disease controls with various CNS disorders).[6] The possibility that large segments of PS1 were spliced out was also examined. RNA extracted from the blood was reverse transcribed and PCR was performed to produce cDNA from exon 3 to exon 12 of PS1 (sense primer: 5'-GTTACC TGCACCGTTGTCCTACT-3', antisense primer: 5'-GGAGATTGGAAGAGCTGGC AATG-3').[7] The PCR product was analyzed

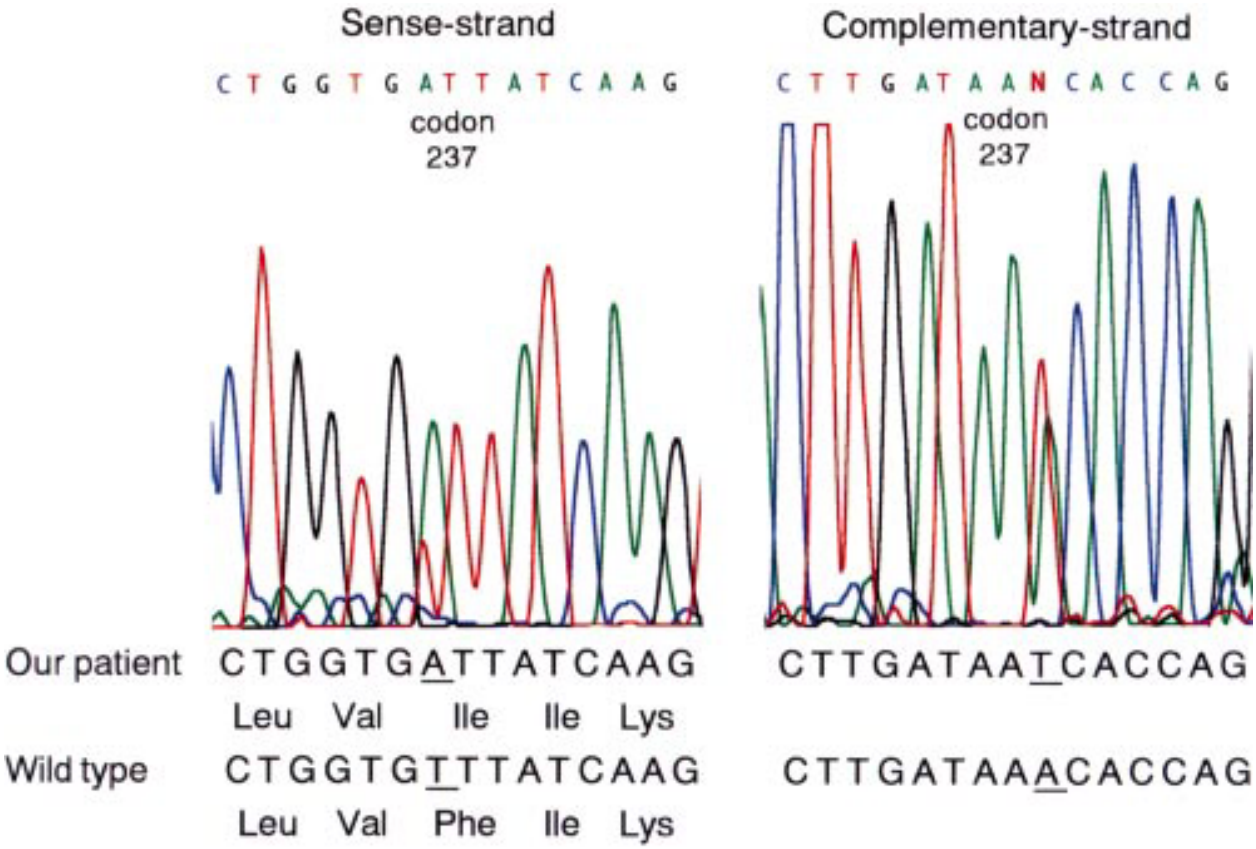

Figure 1 DNA sequence of exon 7 of PS1 of our patient and wild type. The patient has T to A transition at the first position of codon 237 leading to the Phe237Ile mutation.

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]

Forthly, 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 (Aβ) with subsequent Aβ 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 Aβ42 (reflecting disturbances in Aβ metabolism and possibly Aβ deposition in senile plaques). The ability of the combination of CSF tau and CSF Aβ42 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-100β, a calcium binding protein also found in astrocytes (both reflecting gliosis).

From the longitudinal geriatric population study in Piteå, Sweden[2] 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, Aβ42,[1] NFL,[3] GFAP,[4] and S-100β.[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;