LETTERS TO THE EDITOR

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The Glu318Gly Substitution in Presenilin 1 Is Not Causally Related to Alzheimer Disease

To the Editor:

With 49 different mutations in the coding region, presenilin 1 (the gene is denoted "PSEN1"; the protein is denoted "psen1") is the most frequently mutated gene in early onset (onset age <65 years) Alzheimer disease (AD [MIM 104300]) (Sherrington et al. 1995; Cruts and Van Broeckhoven 1998). PSEN1 missense mutations are generally considered fully penetrant mutations. Mostly they are found in patients with a positive family history of early-onset AD compatible with autosomal dominant inheritance. Patients carrying the same mutation usually display very similar onset ages (Van Broeckhoven 1995).

An A→G transition at codon 318 in exon 9 of PSEN1, resulting in the nonconserved Glu→Gly substitution, has been reported, by us (Cruts et al. 1998) and others (Sandbrink et al. 1996; Forsell et al. 1997), in familial AD cases with onset ages of 35–64 years (Cruts and Van Broeckhoven1998). However, segregation of Glu318Gly with AD could not be demonstrated, because either no or too few relatives were available for DNA testing. PSEN1 Glu318Gly involves the last codon of exon 9 and is located in the middle part of the sixth hydrophilic loop of psen1. Because of the high variability in onset age of AD and the mutation's location in a psen1 region that is less conserved between psen homologues in human and other species, we previously had hypothesized that the Glu318Gly could be either an incompletely penetrant mutation or a rare polymorphism (Cruts and Van Broeckhoven 1998).

To evaluate the frequency of Glu318Gly and its contribution to AD, we screened incident and prevalent demented cases and age- and sex-matched controls derived from the Rotterdam Study. This is a prospective single-center population-based study of elderly residents ⩾55 years of age who are from a Rotterdam suburb (Hofman et al. 1991). Cognitive functioning was assessed and diagnosis of dementia made on the basis of the DSM-III-R definition (American Psychiatric Association 1987). Possible and probable AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)–Alzheimer's Disease and Related Disorders Associations criteria (McKhann et al. 1984). Vascular dementia was diagnosed according to NINCDS–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria (Roman et al. 1993). At baseline, 474 prevalent demented cases were diagnosed (Ott et al. 1995). During follow-up, another 146 incident cases of dementia were detected (Ott et al. 1998). From 345 prevalent cases, 134 incident cases, and 256 controls, blood samples were available for DNA extraction. Controls were randomly selected among nondemented participants in the Rotterdam Study and were group matched on the basis of age (5-year intervals) and sex. To facilitate rapid screening for Glu318Gly, we developed a mismatch PCR assay that allows detection by *Bst*NI digestion of the mismatch PCR product. The forward mismatched primer was 5′-ATCCAAAAATTCCAAGTATAATCC-AG-3′ and the reverse primer was 5′-CTGGGCAT-TATCATAGTTCTCAAG-3′.

PSEN1 Glu318Gly was observed in 2 (1.5%) incident and 11 (3.4%) prevalent demented cases and in 9 (4.1%) controls. In contrast to previous reports, we detected Glu318Gly in individuals who were elderly. The frequencies in incident and prevalent cases versus those in controls were compared by the Fisher exact test and were found to be not significantly different ($P = .22$ and $P = .65$, respectively). Of 13 demented Glu318Gly carriers, 10 were diagnosed with AD (4 possible AD and 6 probable AD), 1 with dementia associated with Parkinson disease, 1 with vascular dementia, and 1 with dementia associated with multiple sclerosis. Mean age at onset in demented Glu318Gly carriers (83.4 ± 4.7 years, range 72–88 years) was similar to that in demented noncarriers (81.0 ± 7.7 years, range 52–97 years). Cognitive functioning measured by the mini-mental state examination in the control group was similar in Glu318Gly carriers (26.4 ± 2.9) and noncarriers (27.0 ± 2.0). Since the $\epsilon 4$ allele of apolipoprotein E (APOE) is known to increase risk for AD (Pericak-Vance and Haines 1995), we also examined the APOE genotypes in the demented cases. However, the APOE*$\epsilon 4$-allele frequency in the demented Glu318Gly cases (19%) was not different from that in the total group of de-

mented cases (21%), excluding a possible interaction between APOE*ϵ4 and Glu318Gly. Together, these findings demonstrate that Glu318Gly is a rare allele (22 carriers/676 individuals, allele frequency 1.6%) in the Dutch population analyzed and that its presence is not associated with AD or dementia in general.

The relatively high frequency of Glu318Gly in the Dutch population analyzed may be explained if all subjects are distantly related. To test this possibility, we genotyped several polymorphic DNA markers located within and near PSEN1 (Cruts et al. 1995, 1998). All Glu318Gly carriers (cases and controls) shared one allele for D14S77 (203 bp; frequency 8%), the PSEN1 promoter (allele T; frequency 12%), and intron 8 polymorphisms (allele A; frequency 54%). Allele sharing was also observed at D14S1028, with 20 of 22 Glu318Gly carriers sharing the same allele (239 bp; frequency 4%). No obvious sharing of alleles was observed at D14S1004. Frequencies of the shared alleles were calculated in 118 control individuals coming from the same Rotterdam suburb (C. M. van Duijn, unpublished data). The probability of detecting this allele combination independently in 22 cases (4×10^{-35}) strongly suggests that all Dutch Glu318Gly carriers have one common founder. Glu318Gly is also frequently observed in populations of different geographic and ethnic origins (Baker et al. 1998; Forsell et al. 1998; Helisalmi et al. 1998; Mattila et al. 1998; Reznik-Wolf et al. 1998; Torres et al. 1998).

The mechanism by which mutations in PSEN1 lead to AD remains largely unknown. However, an increasing amount of in vivo and in vitro evidence suggests that the mutated psen1 expresses its pathogenic effect by processing the amyloid precursor protein (APP) in such a way that increased levels of the 42-amino-acid form of the amyloid β peptide (Aβ42) are secreted (Hardy 1997). Aβ42 is believed to be pathogenic, since it is more prone to aggregation and therefore leads to accelerated amyloid β accumulation in the brain of patients with AD. To assess whether Glu318Gly also influences APP processing, we measured Aβ42 levels in conditioned media of HEK-293 cells stably transfected with the Glu318Gly PSEN1 cDNA, using an Aβ42-specific enzyme-linked immunosorbent assay (De Strooper et al. 1998). No increase in Aβ42 secretion was observed, compared with cell lines stably transfected with wild-type PSEN1 cDNA. The absence of increased APP processing into Aβ42 in vitro is consistent with our findings at the population level, which show no association of Glu318Gly with either AD or dementia.

A few possibilities remain unexplored. First, since we detected the Glu318Gly allele only in the heterozygous state, it cannot be excluded that Glu318Gly is associated with dementia in an autosomal recessive manner. However, there is no evidence supporting autosomal recessive inheritance in familial AD (Rao et al. 1994). The fact that neither none of 479 late-onset patients screened in the present study nor the 100 early-onset cases screened in earlier studies (Cruts et al. 1998) carried two copies of the allele makes it unlikely that homozygosity for Glu318Gly is a frequent cause of AD. Another as yet not excluded possibility is that all Glu318Gly carriers share a (disease) phenotype that is different from either AD or dementia and that has remained undetected in the present study. However, we are not aware of any nondementia phenotypes associated with genetic variations in PSEN1.

In conclusion, we provide evidence that PSEN1 Glu318Gly is not causally related to either AD or other types of dementia. The latter has important implications for genetic counseling, since Glu318Gly carriers are not at increased risk. Our observations also imply that care should be taken in assigning a pathological nature to mutations in PSEN1, when these mutations are reported in isolated cases or in familial cases but in the absence of conclusive evidence for cosegregation with the disease.

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