De novo presenilin 1 mutations are rare in clinically sporadic, early onset Alzheimer's disease cases

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Abstract

The presenilin 1 (PS1) gene, located on chromosome 14, is the major gene involved in the autosomal dominant forms of early onset Alzheimer's disease (AD). In order to estimate the frequency of de novo PS1 mutations, we have sequenced the PS1 open reading frame in 13 clinically diagnosed patients with no affected relatives, who had developed AD before the age of 50. In one case with onset at 37 years, we identified a missense mutation resulting in a methionine to lysine substitution at codon 139 of the PS1 gene. This substitution is the fourth identified at the same codon. This study, in agreement with previous reports, suggests that de novo PS1 mutations can occur but at a low frequency.

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Alzheimer's disease, the most common cause of dementia in the elderly, is inherited in an autosomal dominant fashion in a subset of patients with early onset of the disease. Three genes involved in these autosomal dominant forms of early onset Alzheimer's disease (EOAD) have been identified so far: the amyloid precursor protein (APP) gene located on chromosome 21q21,¹ the presenilin 1 (PS1) gene located on chromosome 14q24.3,² and the presenilin 2 (PS2) gene located on chromosome 1q42.1.³⁻⁵ The PS1 gene is the major locus for familial EOAD, since PS1 mutations have been identified in approximately 30-50% of the families.⁶ Except for one splicing mutation,⁷ all the PS1 alterations documented so far have been missense mutations. These mutations show remarkable allelic

heterogeneity since approximately 40 PS1 amino acid substitutions have been documented in at least 70 families with EOAD,⁶ and we have identified 13 different missense mutations in 15 French families^{8,9} (unpublished data). In two families, in which we had identified a PS1 mutation, the absence of affected relatives two generations ago led us to speculate that the mutation had occurred de novo. In order to assess the frequency of de novo PS1 mutations, we performed sequencing analysis of the PS1 gene in EOAD patients with no affected relatives.

Subjects and methods

PATIENTS

This study was performed in 13 unrelated French patients fulfilling the NINCDS-ADRDA criteria for probable AD.¹⁰ These patients were selected according to two criteria: (1) onset of AD before the age of 50 years and (2) being isolated cases as defined by absence of affected sibs, parents, and uncles/aunts. After informed consent was obtained, peripheral blood lymphocytes were collected and Epstein-Barr virus transformed lymphoblastoid cell lines were established. The APOE genotyping of these patients was performed by *HhaI* restriction fragment analysis.¹¹

SCREENING FOR PS1 MUTATIONS

The coding region of the PS1 gene was analysed by reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing, as previously described,⁸ except that the regions corresponding to codons 72-187 and 164-287 were separately amplified using the 901F,² 901R primers (GTC CAC AGC AAC GTT ATA GG) and the 1111F (GTG CTA TAA GTT AGT CCA TG) and 1111R² primers, respectively. To perform dye primer sequencing, we added a M13 reverse sequence (5'-CAGGAAACAGCTATGACC-3') to the 5' end of the sense primers (901F and 1111F) and a M13-21 sequence (5'-TGTAACAC-GACGGCCAGT-3') to the 5' end of the antisense primers (901R and 1111R), respectively.

Results

Among the 13 patients with clinically sporadic EOAD (table 1), we identified in one subject (ALZ034) a heterozygous mutation (ATG→AAG) at codon 139, which is predicted to result in a methionine to lysine substitution (data not shown). This mutation, Met139Lys, removes a *BclI* restriction site which allowed us to test by restriction fragment analysis, as previously described,⁸ the mother

Table 1 Characteristics of the EOAD patients

Patient	Sex	Age of onset (y)	PS1 mutation	ApoE genotype
ALZ 004	M	50	—*	ε3/ε4
ALZ 021	F	45	—	ε3/ε3
LIL 002	F	50	—	ε3/ε3
SAL 599	F	45	—	ε3/ε3
SAL 590	F	49	—	ε3/ε4
CRN 008	M	43	—	ε3/ε3
CRN 001	F	41	—	ε3/ε3
REN 001	F	50	—	ε3/ε3
ROU 004	M	41	—	ε3/ε3
ROU 060	M	50	—	ε3/ε4
ALZ 034	F	37	Met139Lys	ε3/ε3
CAE 016	F	50	—	ε3/ε3
SAL 008	F	50	—	ε3/ε4

*No mutation was detected.

Figure 1 Pedigree of family ALZ034. Filled symbol, affected subject; open symbols, asymptomatic subjects; numbers under the symbols are ages at examination or death. The number in brackets is age of onset of AD; +/+, wild type genotype; +/139, heterozygous Met139Lys mutation. The pedigree has been partially encrypted to protect confidentiality of family members.

and one brother of the proband. No mutation was detected among these relatives. In this family, the father was dead and was unaffected by the age of 74 (fig 1). Among the 13 patients, the majority ($n=9$) had the APOE $\epsilon 3/\epsilon 3$ genotype, four had the $\epsilon 3/\epsilon 4$ genotype, and none had the $\epsilon 4/\epsilon 4$ genotype (table 1).

Discussion

We have analysed the PS1 gene in a sample of 13 clinically diagnosed EOAD patients (range of onset ages 37 to 50 years) with no affected relatives. Using a RT-PCR approach, we identified, in one patient who had developed AD by the age of 37, a new missense mutation of the PS1 gene. RT-PCR has been shown to be an efficient method for detecting PS1 mutations,⁸ but we cannot exclude that in the other patients we have missed mutations located outside the coding region or resulting in mRNA decay. Although we cannot exclude that the Met139Lys substitution corresponds to a rare variation without any biological consequence for PS1 function, we consider that this substitution is probably pathogenic; we did not detect this substitution in more than 50 unrelated subjects, indicating that it is not a common polymorphism. Furthermore, codon 139 corresponds to a hot spot of mutation since three different substitutions (Met139Ile, Met139Thr, Met139Val) have already been described in EOAD families.⁶ Codon 139, in exon 5, is located in one of the two PS1 regions in which most mutations are found.⁶ Restriction

analysis of two unaffected first degree relatives of the ALZ034 proband, aged 75 and 50 years, showed that they do not carry the mutation. The father was dead, unaffected by the age of 74, and had no family history of AD among his sibs or parents (fig 1). Although one PS1 point mutation with incomplete penetrance by the age of 68 has recently been described,¹² it seems very unlikely from the examination of the pedigree that non-penetrance is involved in our family. The alternative explanation, assuming that paternity is correct, is that this substitution has occurred de novo. Recently, studies^{13 14} have incidentally reported the occurrence of de novo PS1 mutations in isolated EOAD patients. Our study shows that a low proportion of clinically sporadic EOAD cases could be explained by such de novo mutations. We suggest that isolated cases with onset below the age of 50 should be screened for PS1 mutations.

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