No founder effect in three novel Alzheimer's disease families with APP 717 Val→Ile mutation

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Abstract

We sequenced exons 16 and 17 of the APP (amyloid precursor protein) gene in 18 unrelated French Alzheimer's disease (AD) patients. These patients had an onset before the age of 60 and belonged to families with autosomal dominant transmission of the disease. We detected the APP 717 Val→Ile mutation in three out of 18 (16.6%) families. In these three families, all affected subjects had the APOE 3/3 genotype, but their ages of onset ranged from 38 to 60 years, indicating that factors other than the APOE genotype influence age of onset. Analysis of two polymorphic loci adjacent to the APP gene showed that at least two independent mutational events had occurred within these pedigrees, in spite of their origin in the same region of France.

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The genetic basis of Alzheimer's disease (AD) is heterogeneous. Autosomal dominant forms of the disease involve the APP gene located on chromosome 21¹ and the presenilin 1 and 2 genes²⁻⁵ located on chromosome 14 and chromosome 1, respectively. Besides these major genes, the apolipoprotein E (APOE) gene, located on chromosome 19, is also implicated in the diathesis of the disease. A strong association has been described between AD and the APOE ε4 allele.⁶ Patients bearing the 4 allele develop the disease earlier⁷ and have more deposition of amyloid β peptide in the brain than those without the ε4 allele.⁸

To date, four types of pathogenic APP mutations have been found in AD⁹⁻¹¹ and a further two in hereditary cerebral haemorrhage with amyloidosis - Dutch type.^{12,13} Although direct sequencing of the entire coding region of the APP gene has been performed by several groups,¹⁴⁻¹⁶ all pathogenic mutations identified so far have been found to cluster within exons 16 and 17. In vitro studies have shown that these mutations cause AD by altering the APP processing in a way that is amyloidogenic.^{17,18} APP mutations have been detected in a few families world wide. The most common mutation results in a valine to isoleucine substitution at codon 717. This specific mutation has

been identified in 13 independent pedigrees (five of Japanese origin, four of British origin, three of Italian origin, and one of Australian origin).¹⁹⁻²⁵ Reviewing published reports on APP mutations, Van Broeckhoven²⁶ has stated that the APP 717 Val→Ile mutation accounts for about 5.5% of early onset AD families. However, the frequency of this mutation greatly differs among studies. Such variations may reflect either variability in mutation frequency in different populations or differences in ascertainment criteria of families. Using three polymorphic loci linked to the APP gene, Tanaka *et al*²⁷ did not observe any significant linkage disequilibrium in five Japanese pedigrees harbouring the APP 717 Val→Ile mutation. They concluded that these families do not derive from a common founder patient.

The mean age of onset among chromosome 21 linked families is in the fifties whereas in pedigrees linked to chromosome 14, the mean age of onset is in the forties.²⁸ However, in these two types of families, large variations in the age of onset are observed individually. Recently, St George-Hyslop *et al*²⁹ provided circumstantial evidence that, in patients with the APP 717 Val→Ile mutation, the APOE genotype could influence the age of onset. This suggestion has been strengthened by the report of a delayed age of onset in APOE ε2 patients from families harbouring the APP 717 Val→Ile mutation.^{23,30,31}

Over the past four years, we have ascertained families with autosomal dominant, early onset AD, some of which had already been negatively tested for the APP 717 Val→Ile mutation.³² In the present study we report the detection of APP 717 Val→Ile mutations in three early onset AD families and show that these three novel AD families do not result from a founder effect.

Subjects and methods

AD FAMILIES

Ninety two EOAD probands (onset before the age of 60), fulfilling the NINCDS-ADRDA criteria for probable AD,³³ were ascertained from consecutive admissions to several French hospitals. A segregation analysis showed that, in 18 pedigrees, the pattern of familial aggregation was consistent with autosomal dominant inheritance of a pathological gene fully penetrant by the age of 60.³⁴ These pedigrees were screened for mutations in exons 16 and 17 of the APP gene.

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Figure 1 Detection of the APP 717 Val→Ile mutation in family FAD RO3. Exon 17 of the APP gene was PCR amplified and directly sequenced (see Methods). The sequences observed in the affected proband (bottom) and in an unaffected subject (top) are shown. The N, indicated by an arrow, corresponds to the heterozygous mutation. The highest and lowest peaks correspond to the mutant (A) and wild type nucleotide (G), respectively. The predicted amino acid change is indicated under the nucleotide sequence.

SCREENING FOR APP EXONS 16 AND 17 MUTATIONS

After informed consent was obtained, blood samples were collected from the 18 affected probands. Exon 16 of the APP gene was PCR amplified from total blood genomic DNA using 16Fb (5'-AACTAATTGGTTGTCC-TGC-3') as sense primer and 16R (5'-GGGGTTTTCACCATATTGGC-3') as antisense primer. To amplify exon 17, we used the intronic primers described by Fidani *et al.*²¹ To facilitate sequencing, we added an M13 reverse sequence (5'-CAGGAAACAGCTATGACC-3') to the 5' end of the sense primers and an M13-21 sequence (5'-TGTAACGACG-GCCAGT-3') to the 5' end of the antisense primers, respectively. PCR reactions were performed in a final volume of 50 µl containing 0.5 µmol/l of each primer and 1.25 U of *Taq* DNA polymerase from Stratagene (La Jolla,

CA). The PCR consisted of 35 cycles of 30 seconds at 94°C, 30 seconds at 50°C (exon 16) or 55°C (exon 17), and one minute at 72°C, preceded by three minutes at 95°C and followed by five minutes at 72°C. PCR products were purified by electrophoresis on low melt agarose gel and directly sequenced on both strands using the PRISM Ready Reaction Dye Primer sequencing kit (Applied Biosystems, Perkin Elmer/Cetus) and an Applied Biosystems model 373A automated sequencer. Screening for the APP 717 Val→Ile mutation in relatives was performed by restriction fragment analysis using *Bcl*I as described in Goate *et al.*¹

POLYMORPHISM ANALYSIS

The GT12 (D21S210) dinucleotide repeat marker and the dinucleotide repeat marker located within intron 1 of the APP gene were PCR amplified using the primers described in Warren *et al.*³⁵ and Zappata *et al.*³⁶ respectively. The sense primers were end labelled with the fluorescein dye C6-FAM (6-carboxyfluorescein) from Applied Biosystems and the size of the PCR products were determined using an internal lane size standard (GENESCAN-2500 ROX, Applied Biosystems) and a Model 672 Gene Scanner Fluorescent Fragment Analyzer (Applied Biosystems).

Results

Direct sequencing of exons 16 and 17 of the APP gene was performed in the 18 unrelated probands. In three cases, a G to A transition at base 2149 in exon 17 was detected, resulting in a valine to isoleucine substitution at codon 717 (fig 1). Since this substitution creates a *Bcl*I restriction site,¹ the occurrence of the mutation among relatives was studied by restriction fragment analysis. Fig 2 shows the cosegregation of the APP 717 Val→Ile mutation with the disease in family FAD RO3. The clinical characteristics of the three families are presented in table 1. Age at onset ranged from 38 to 60 years, with a mean of 51.7 (SD 7) years. Age at death varied from 49 to 68 years, with a mean of 58 (SD 5.2) years. All affected subjects from whom DNA was available had an ε3/ε3 APOE genotype. The three pedigrees originated from the same region (Normandy in the western part of France). Although genealogies were traced back to the beginning of the 18th century, we failed to find any common ancestors. To investigate the possibility of a founder effect, we looked for a potential linkage disequilibrium using a dinucleotide repeat sequence located in intron 1 of the APP gene³⁵ and the D21S210 marker,³⁶ which lies approximately 200 kb from the APP locus.²⁷ We found that only two of the three probands shared the same rare haplotype whose theoretical frequency in the general population, estimated from the frequency of the individual alleles,^{35, 36} can be estimated to be 7% (assuming equilibrium between the two loci) or 14% (assuming complete disequilibrium between the two loci). This haplotype segregated with the disease within the two families.

Table 1 Characteristics of the EOAD families with the APP 717 Val→Ile mutation*

Family	No of affected subjects	Age of onset†	Age of death†
FAD RO3	5	54 (5.1)	61.6 (3.8)
FAD RO4	5	54 (1.4)	54.5 (5.5)
FAD SAL1	9	47 (7.8)	57.5 (4.6)

* Duration of illness could not be precisely determined because most of the subjects with a known age of onset are still alive.

† Mean (SD) in years.

Figure 2 Segregation of the APP 717 Val→Ile mutation in family FAD RO3. (A) Pedigree of the family. Filled symbols, EOAD patients; open symbols, asymptomatic subjects; dots, obligate carriers, with age at death under 40 years. Clinical status of subjects I.1 and I.2 was unknown. (B) Detection of the APP 717 Val→Ile mutation by restriction fragment analysis. Exon 17 of the APP gene was PCR amplified and the 435 bp PCR product was digested with BclI. Restriction fragments were separated on a 1.5% agarose gel and visualised by ethidium bromide staining. The fragments sizes (in bp) of the molecular weight marker (HinfI digested Φ X174) are shown to the left of the gel. Lanes 1-8, subjects IV.4, 1, 2, 7, 8, 9, 10, and 3 as shown in fig 2A. Lane 9, affected proband of family FAD RO3 used as a control. Lane 10, PCR product before digestion. The wild type PCR product does not contain any BclI site whereas the APP 717 Val→Ile mutation, resulting in a BclI site, generates two fragments of 219 and 216 bp.

Discussion

Our results show that the APP 717 Val→Ile mutation is the most frequent APP mutation in the French AD pedigrees, as in other ethnic groups. The frequency of this specific mutation in our sample was three out of 18 (16.6%). This proportion can be compared to that found in similar samples (that is, early onset families with autosomal dominant transmission). In the Japanese, Italian, British, and Australian samples, the mutation has been detected in five out of 15 families (33.3%), three out of seven (43%), two out of 21 (9.5%), and one out of seven (14%) AD families, respectively.

In patients with the APP 717 Val→Ile mutation, we analysed the APOE genotype in order to investigate its influence on age of onset. All patients were homozygous $\epsilon 3/\epsilon 3$, thus precluding an analysis of the potential protective effect of the $\epsilon 2$ allele. However, it should be stressed that among these affected subjects a wide range of age of onset (38 to 60 years) was observed. This observation shows that factors other than

the APOE genotype influence the age of onset in patients with the APP 717 Val→Ile mutation.

To determine whether these three AD families result from a founder effect, we analysed two polymorphisms linked to the APP locus and we failed to find a common haplotype for the three probands. However, two out of the three probands are likely to have a common ancestor since, within these two families, affected subjects share a common haplotype. Assuming (1) absence of intragenic recombination and (2) stability of the two polymorphisms, our data suggest that at least two independent mutational events had occurred within the three pedigrees. This result, in agreement with the study of Tanaka *et al.*,²⁷ confirms that the APP 717 Val→Ile mutation has recurrently been generated in the past, probably by a mechanism of deamination of methylated cytosine, a common cause of C to T transition.

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