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Novel APP mutation V715A associated with presenile Alzheimer's disease in a German family

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Sirs: Missense mutations in exons 16 or 17 of the amyloid precursor protein gene (*APP*) cause presenile Alzheimer's disease (AD), typically with mean onset ages below 55 years. Sixteen different *APP* mutations have been identified in 43 autosomal dominant AD families [2]. All mutations occur at or near the α-, β- or γ-secretase cleavage sites involved in the production of the amyloid β peptide (Aβ) that is deposited in the amyloid plaques and blood vessel walls in AD brains. Here, we describe a novel *APP* V715A mutation near the γ-secretase cleavage site at the C-terminal of Aβ.

A 50-year-old German woman was referred to the neurological center at the University of Münster, Germany, with memory problems. The patient had a family history of dementia, consistent with autosomal dominant inheritance (Fig. 1). The mean onset age in the family was 52 years (n = 4, range 48–55 years). According to her husband, the patient had progressive worsening of memory since the age of 48 years, which made it impossible for her to continue her career as a bank employee. During standardized clinical, neurological and neuropsychiatric examinations she met the NINCDS-ADRDA criteria for probable AD [4]. She had a moderately reduced verbal learning ability and memory performance, reduced orientation in time and reduced attention. In addition, she had retarded, although correct, intellectual capacity. Positron emission tomography (PET) showed parieto-occipital hypoperfusion of the brain and electro-encephalography showed moderate, nonspecific general changes. Autopsy confirmation of AD had not been obtained in deceased members of the family.

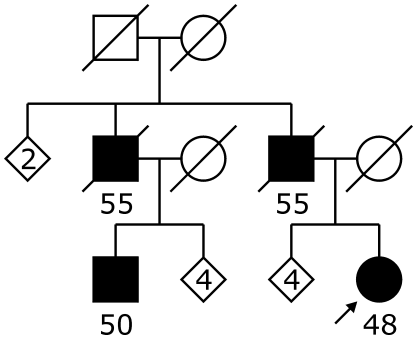

Fig. 1 Pedigree of German AD family. Numbers below symbols indicate onset ages of AD. Numbers inside symbols represent number of offspring. The proband is indicated with an arrow

Sequencing of PCR amplified fragments of exons 16 and 17 of *APP* and all coding exons of presenilin 1 (*PSEN1*) and 2 (*PSEN2*) was performed by the VIB Genetic Service Facility (http://www.vibgeneticservicefacility.be/). In *PSEN1* and *PSEN2* mutations were excluded. In *APP* exon 17, we identified a T > C transition at nucleotide 2087 of the *APP* cDNA sequence (GenBank accession number X06989), predicting a valine to alanine substitution at codon 715 (V715A, German *APP* mutation). The mutation creates a *Pvu*I recognition site and its presence in the patient's DNA was confirmed by PCR-RFLP analysis. No additional patients or at risk family members could be tested for the mutation, since they were afraid of the psychological burden and refused cooperation in the genetic study. However, we did not identify the *APP* V715A mutation in 100 German control chromosomes using the PCR-*Pvu*I assay. Also, at the same codon, a V715M mutation was previously reported to co-segregate with presenile AD in an Italian family [1], illustrating that a mutation at this codon can cause autosomal dominant AD.

We introduced the V715A mutation in APP_{695} cDNA cloned in vector pCDNA3 using the Quick Change Site-Directed Mutagenesis system (Stratagene). Human embryonic kidney cells (HEK293) were transiently transfected with *APP* V715A or wild type (WT) *APP* cDNA using Lipofectamine (Gibco BRL) according to the manufacturer's procedures. After incubation of the transfected cells in OPTIMEM serum-free medium for 24 hrs, the medium was collected, concentrated four times, and $A\beta_{42}$ and $A\beta_{40}$ levels were measured using the INNOTEST β-amyloid$_{1-42}$ ELISA (Innogenetics) and the human β-amyloid$_{1-40}$ ELISA (Biosource) assays respectively. A two-tailed unpaired Student's *t* test was used to compare the $A\beta_{42}/A\beta_{40}$ ratio for *APP* WT and mutant transfectants. Cells transfected with the APP_{695} V715A cDNA demonstrated a significantly ($p < 0.01$) increased $A\beta_{42}/A\beta_{40}$ ratio by 4.12 times compared with cells transfected with WT *APP* cDNA. These results are comparable to a 3.12 times elevation of the $A\beta_{42}/A\beta_{40}$ ratio in mice primary neuronal cells transfected with the same mutation [3] and are consistent with the inverse correlation between onset age and secreted $A\beta_{42}/A\beta_{40}$ ratios. Elevated $A\beta_{42}/A\beta_{40}$ ratios were observed for all C-terminal pathogenic *APP* mutations suggesting that they cause AD through similar agents in similar pathways.

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