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A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of Aβ42(43)

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We report a novel mutation in the amyloid precursor protein gene (APP I716V) which probably leads to familial early onset Alzheimer's disease with an onset age in the mid 50s. Cells transfected with cDNAs bearing this mutation produce more Aβ1–42(43) than those transfected with wild-type APP and this effect is additive with that of the previously reported APP V717I mutation thus providing a novel approach for further increasing Aβ1–42(43) in model systems.

INTRODUCTION

Mutations in the amyloid precursor protein (APP) that cause early onset familial Alzheimer's disease (FAD) have previously been identified (1). Those which lead solely to AD have been reported at two sites: at codon 717 and a double mutation at codons 670/1 (K/M670/1N/L) (for review see ref. 1). These mutations, and the presenilin mutations which also lead to FAD, have in common the feature that they increase the concentration of Aβ ending at Aβ42(43) (2–8). Here we report the identification of a novel mutation in APP which is associated with an Alzheimer phenotype and show that it too results in an increased extracellular concentration of Aβ42(43).

RESULTS AND DISCUSSION

Here we report the identification of a new mutation in the APP gene (I716V) and demonstrate that this mutation also increases the relative concentration of Aβ1–42(43) in both transfected cells and in plasma, providing additional support for the hypothesis that alterations in Aβ concentration are a critical and early feature in the development of AD. Further, we demonstrate an additive increase in the relative concentration of Aβ1–42(43) from cells transfected with constructs containing both the I716V and the V717I mutations. This finding provides a novel approach for further increasing Aβ1–42(43) concentration in model systems.

All the families with AD caused by APP mutations have disease onset ages from 45–60 years: most typically, with an onset age in the mid-50s. In this context, the family we report here, in which the mean documented onset age was ~53 years, was a candidate for having an APP mutation. Sequencing revealed a mutation changing the predicted amino acid at codon 716 from isoleucine to valine. This mutation is likely to be pathogenic due to its proximity to the previously identified V717I mutation and it has not previously been observed in any of the hundreds of normal samples that we and others have previously sequenced. The only other living affected family member did not wish to be sampled and this precluded segregation analysis of the mutation.

A common feature of early onset FAD-linked mutations is to increase the concentration of Aβ ending at Aβ42(43). To determine whether the I716V mutation also affected the extracellular concentration of Aβ1–42(43), we performed transient transfection analysis in both CHO and human embryonic kidney (HEK) 293 cells. The results obtained from a representative experiment are shown in Table 1. Relative to wild-type and the vector control, the I716V mutant cDNA caused a 30–60% increase in the proportion of Aβ1–42(43) in both of the cell lines examined. This increase was similar to the effects of the V717I mutation analyzed in parallel in these series of experiments and as reported previously (3,8). Importantly, the increase in the absolute concentration of Aβ1–42(43) produced by I716V transfected cells was observed even when the absolute concentrations of Aβ1–40 were essentially identical. In order to both increase the '*n*' number for statistical analysis and account for variance in transfection efficiency between experiments we focused our subsequent analysis on the ratio of Aβ1–42(43) to Aβ1–40 as this has previously been shown to be elevated invariably in both the APP (V717I) and the presenilin mutations (2–8). The ratios obtained from three additional independent transient transfections in both HEK 293 and CHO cells have been combined with the ratios obtained from the experiment in Table 1 to produce Figure 1. These results demonstrate an ~2-fold increase in the ratio of Aβ1–42(43) to Aβ1–40 when compared with the wild-type control (P = 0.0026, Mann-Whitney).

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Table 1. Effects of APP mutations on Aβ concentration

	293 Cells			CHO cells		
	Aβ1–40	Aβ1–42(43)	Ratio (%)	Aβ1–40	Aβ1–42(43)	Ratio (%)
pcDNA3	31.1 ± 1.1	2.9 ± 0.6	9.3 ± 1.5	35.1 ± 0.1	3.0 ± 0.1	8.6 ± 0.4
APP695 wt	90.6 ± 6.8	8.1 ± 0.1	9.0 ± 0.5	59.1 ± 0.2	6.4 ± 0.2	10.9 ± 0.3
APP695 I716V	92.5 ± 3.0	13.5 ± 1.1	14.6 ± 0.7	138.7 ± 1.3	19.8 ± 1.5	14.3 ± 1.0
APP695 V717I	68.6 ± 1.9	11.4 ± 1.9	16.6 ± 2.4	54.1 ± 1.3	9.5 ± 0.5	17.5 ± 0.5
APP695 I/V716/7V/I	75.3 ± 0.1	20.3 ± 0.9	27.0 ± 1.1	63.0 ± 0.2	16.1 ± 1.1	25.6 ± 1.7
I716V plasma (n = 1)	87.2	12.4	14.2			
Control plasma (n = 5)	116.7 ± 11.4	10.7 ± 0.7	9.3 ± 0.4			

Each data point is the mean of two wells each analyzed in duplicate and expressed as pM ± standard deviation. Plasma concentration is reported as pM ± SE. These data show that the I716V and the V717I mutations increase the proportion of Aβ1–42(43) and that the double mutant I716V/V717I increases the proportion of Aβ1–42(43) more than each mutant individually. The ratio (%) shows [Aβ1–42(43)]/[Aβ1–40] as a percentage.

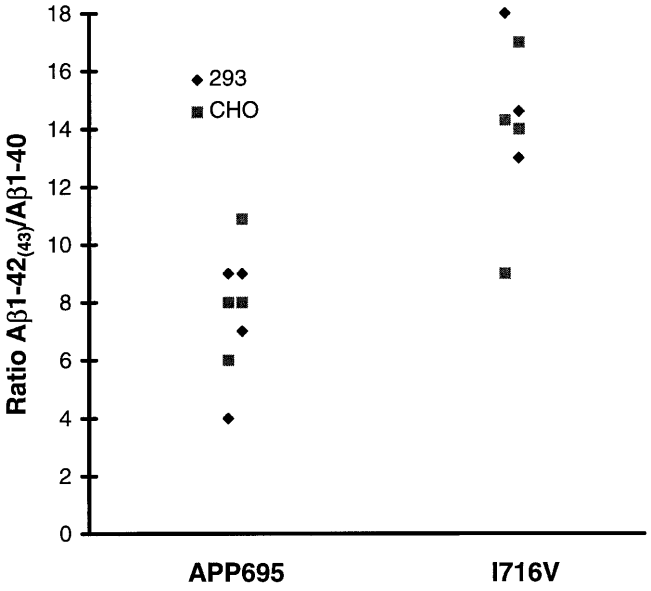

Figure 1. The effect of I716V on the ratio of Aβ1–42(43)/Ab1–40 compiled from four independent experiments. The ratio represents the [Aβ1–42(43)]/[Aβ1–40] expressed as a percentage.

We have previously reported elevations in plasma Aβ42(43) concentration in patients with FAD-linked mutations (3). The availability of only a single living identified carrier of the I716V mutation precluded an in depth analysis of plasma Aβ concentration in this kindred. An increase in the absolute concentration of Aβ42(43) was however observed in the plasma from this patient when compared with the average concentration of five non-demented, control subjects analyzed in parallel (Table 1). In fact, this individual had the highest concentration of Aβ42(43) in this series despite having the second lowest concentration of Aβ1–40. Thus, this mutation causes a clear increase in the absolute concentration of Aβ42(43) *in vivo*.

Thus, mutations at adjacent residues in APP, I716V and V717I, result in similar increases in the relative concentration of Aβ1–42(43). Due to the proximity of these mutations and the nature of the amino acid changes we were interested in exploring the effect of the combination of these mutations on the Aβ42 phenotype. To address this question we constructed a cDNA containing both the I716V and V717I mutations in the pcDNA3 vector. Our analysis of cells transiently transfected with this artificial double mutant construct demonstrated an increase in the ratio of Aβ1–42(43) to Aβ1–40 when compared with each individual mutation either separately (P = 0.02) or pooled (P = 0.0066) (Table 1). This feature may be useful in designing constructs which maximize Aβ1–42(43) concentration for modeling the disease process.

In conclusion, the results reported here extend the sites at which pathogenic APP mutations have been observed to five. Importantly, these pathogenic mutations continue to follow the established pattern that they increase the extracellular concentration of Aβ1–42(43). Thus, these results provide further support for the hypothesis that alterations in the concentration of Aβ play an early and critical role in the pathogenesis of AD.

MATERIALS AND METHODS

Family ascertainment and clinical features

The family was identified through a telephone call from the professional caregiver to our research laboratory detailing the case and the family history. The proband is a 58 year old right handed woman. She had slowly progressive cognitive difficulty over a 5 year period. There was no history of significant head injury and she rarely drank alcohol.

On examination, the proband had preserved social graces, her Mini Mental Test score was 10 out of 30 (9), she had an impaired knowledge of current events and an anomia (scoring 29/60 on the Boston Naming Test; 10). Her neurological examination was otherwise normal. She scored 39/144 on the Dementia Rating Scale (11) and had difficulty with visual-spatial tasks (12). Her language was fluent but she made semantic substitutions and circumlocutions. She scored 11/44 on the Multilingual Aphasia Examination Token Test (13). Her comprehension of commands was impaired but her repetition of phrases was intact. On the Recognition Memory Test (14) she scored below the 5th percentile. Her MRI scan showed diffuse cortical atrophy, most prominent in the left anterior temporal lobe. The diagnosis was 'probable AD' (15).

Her father died of heart disease at age 80 years. Her mother died of probable AD in her early 60s and had cognitive problems from her early 50s. Her sister (the only sibling), age 55 years, has had progressive memory difficulty over a 2 year period. Her maternal

grandmother also had probable AD with an uncertain age of onset and death. The clinical features (age of onset, slow progression of disease and preservation of social graces) were reminiscent of the first family, F23, in which I717V was discovered (16): indeed the similarity was so pronounced that exon 17 of the APP gene was sequenced immediately.

Genetic analysis

Exon 17 of the APP gene was sequenced as previously described, except that a Pharmacia ALF automated sequencer was used. DNA sequencing revealed a heterozygote A→G transversion at codon 716 changing the predicted amino acid sequence from isoleucine (ATC) to valine (GTC). Resequencing in the opposite orientation revealed the corresponding change. This change destroys a *Sau*3A$_I$ enzyme cut site. PCR amplification of exon 17 with flanking intronic primers 17INF (5′-CCTCATCCAAAT-GTCCC-3′) and 17INR (5′-GGTAAGTTGCAATGAAT-3′) followed by restriction digestion with *Sau*3A$_I$ gives two fragments (256 and 243 bp) in normal individuals but in carriers of the mutation an additional fragment (499 bp) that corresponds to the uncut PCR product is observed. Screening for this change in the proband confirmed the alteration and failed to find this change in a further 50 normals. In addition, this exon of the APP gene has been sequenced by our group on the order of 500 times without finding this, or other changes except in cases where AD was segregating as an autosomal dominant disorder with an onset age in the 50s.

Mutagenesis, transfection and Aβ assay

The Transformer mutagenesis system (Clontech) was used to convert the wild-type APP695 sequence to mutants. To evaluate the effect of mutant APP cDNAs on Aβ concentrations we employed a transient transfection paradigm coupled with the well characterized BAN50/BA27 and BAN50/BC05 sandwich ELISA systems to determine the extracellular concentration of Aβ1–40 and Aβ1–42(43), respectively (Table 1). Cells were transfected with the pcDNA3 vector alone or with pcDNA3 containing APP695 wild-type, APP695 (I716V), APP695 (V717I), or APP695 (I716V,V717I) using DOTAP (Boehringer Mannheim) for 293 cells and Lipofectamine (Gibco BRL laboratories) for Chinese hamster ovary (CHO) cells at DNA concentrations previously determined to yield maximal transfection efficiency in these cell types (data not shown). At 6 h post-transfection the medium was changed to growth medium. The fresh medium was conditioned for 24–48 h to facilitate the measurement of Aβ by sandwich ELISA. Due to variation in transfection efficiency we focused our analysis on the ratio of Aβ1–42(43)to Aβ1–40. The absolute concentrations of Aβ1–40 and Aβ1–42(43) from a representative experiment are provided for comparison. Analysis of plasma Aβ concentration was performed essentially as described previously (3) except that the BAN50 capture was replaced with BNT77(raised against Aβ11-28) for the determination of Aβ ending at Aβ42(43) (7). This change obviates the need for pre-clearance with non-specific IgG1κ.

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