DIFFERENTIAL EFFECTS OF MUTATIONS IN CODON 716 OF THE AMYLOID PRECURSOR PROTEIN ON APP PROCESSING: CLINICAL AND BIOCHEMICAL CORRELATES

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Introduction: Mutations in the APP gene have been implicated in the pathogenesis of familial Alzheimer disease (FAD). Our group recently described the I716F mutation, an aggressive FAD-associated mutation in the codon 716 of APP.

Aims: To characterise the known FAD mutations in codon 716 of APP: I716F, I716T and I716V.

Methods: Mutated cDNA constructs encoding I716F, I716T and I716V mutations were generated from human wild-type APP695 cDNA by site-directed mutagenesis. CHO cells were transfected with these constructs and cultured. Medium was collected 24 hours after transfection and human Amyloid-beta40 and Amyloid-beta42 were measured by ELISA. Amyloid-beta42/40 ratios were calculated for wild-type APP and the APP mutations. Results are expressed relative to wild-type. APP-C-terminal fragments and APP intracellular domain (AICD) were measured by western-blot.

Results: Amyloid-beta42/40 ratios were increased in cells transfected with mutant APP as follows (% of wild-type APP): I716F: 283%, I716T: 276%, I716V: 142%. The increase reached statistical significance ($p<0.05$; one-way-ANOVA) in I716F and I716T mutations. There was an inversely linear relationship ($R^2=0.97$) between the Amyloid-beta42/40 ratio and the mean age at onset of AD (I716F: 31yo, I716T: 36yo, I716V: 53yo). Some mutations led to an accumulation of APP-C-terminal fragments but only the APP I716F mutation reduced AICD generation.

Conclusions: Mutations in the codon 716 of APP differentially alter APP processing. They alter the amyloid-beta42/40 ratio and some disturb the overall protein processing. Furthermore, the increase in the Amyloid-beta42/40 ratio is inversely related with the mean age of onset of AD.