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Changed membrane integration and catalytic site conformation are two mechanisms behind the increased Aβ42/Aβ40 ratio by presenilin 1 familial Alzheimer-linked mutations

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ABSTRACT

The enzyme complex γ-secretase generates amyloid β-peptide (Aβ), a 37–43-residue peptide associated with Alzheimer disease (AD). Mutations in presenilin 1 (PS1), the catalytical subunit of γ-secretase, result in familial AD (FAD). A unifying theme among FAD mutations is an alteration in the ratio Aβ species produced (the Aβ42/Aβ40 ratio), but the molecular mechanisms responsible remain elusive. In this report we have studied the impact of several different PS1 FAD mutations on the integration of selected PS1 transmembrane domains and on PS1 active site conformation, and whether any effects translate to a particular amyloid precursor protein (APP) processing phenotype. Most mutations studied caused an increase in the Aβ42/Aβ40 ratio, but via different mechanisms. The mutations that caused a particular large increase in the Aβ42/Aβ40 ratio did also display an impaired APP intracellular domain (AICD) formation and a lower total Aβ production. Interestingly, seven mutations close to the catalytic site caused a severely impaired integration of proximal transmembrane/hydrophobic sequences into the membrane. This structural defect did not correlate to a particular APP processing phenotype. Six selected FAD mutations, all of which exhibited different APP processing profiles and impact on PS1 transmembrane domain integration, were found to display an altered active site conformation. Combined, our data suggest that FAD mutations affect the PS1 structure and active site differently, resulting in several complex APP processing phenotypes, where the most aggressive mutations in terms of increased Aβ42/Aβ40 ratio are associated with a decrease in total γ-secretase activity.

1. Introduction

The presence of senile plaques and neurofibrillary tangles in cerebral cortex and hippocampus are the main neuropathological hallmarks of Alzheimer disease. Processes leading to plaque and tangle formation result in neuronal loss in the affected brain areas. The senile plaques are composed of extracellular aggregates of the small secreted amyloid β-peptide (Aβ) [1] that can range from 37–43 amino acids in length [2]. Aβ is derived from the amyloid precursor protein (APP), which is sequentially cleaved by the membrane bound β- and γ-secretases [3]. Simultaneously, the APP intracellular domain (AICD) is released into the cytosol. Aβ40 and Aβ42 are the most common forms of Aβ, where the Aβ42 peptide is more prone to aggregate and form soluble dimers,

Abbreviations: APP, amyloid precursor protein; Aβ, amyloid-β peptide; AICD, amyloid precursor protein intracellular domain; AD, Alzheimer disease; FAD, familial AD; TMD, transmembrane domains; PS, presenilin; NTF, N-terminal fragment; CTF, C-terminal fragment; ER, endoplasmic reticulum; Lep, leader peptidase; BD8, blastocyst-derived embryonic stem cells; GVP, Gal4VP16; GCB, γ-secretase inhibitor coupled to biotin; WT, wild type; FLIM/FRET, Fluorescence Lifetime Imaging/ Fluorescence Resonance Energy Transfer; CHAPSO, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonic acid; Bis-Tris, 2-(bis(2-hydroxyethyl)amino)-2-(hydroxymethyl)propane-1,3-diol; MSD, Meso Scale Discovery; RM, rough microsomes; CRM, column-washed dog pancreas rough microsomes; Endo H, endoglycosidase H; MGD, minimal glycosylation distance

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oligomers, protofibrills and eventually insoluble plaques [4–6]. The soluble forms are implied to be the toxic species that causes synaptic and neuronal damage as well as impaired memory and long-term potentiation in the rodent brain [7–9].

The multiprotein complex, γ-secretase is an unusual intramembrane-cleaving aspartyl protease composed of presenilin (PS), nicastrin, Pen-2 and Aph-1 [10–14]. PS is a highly conserved membrane protein with nine transmembrane domains (TMDs) and it harbours the catalytic site with the two conserved aspartate residues that are located in TMD6 and 7 [13,15–18]. Once all components are assembled, the PS molecule becomes activated by endoproteolysis, generating an N-terminal and a C-terminal fragment (NTF and CTF). γ-Secretase hydrolyzes its substrate within the hydrophobic TMDs, which require access to water molecules in the catalytic site [19].

Currently, more than 185 autosomal dominantly inherited familial AD (FAD) causing mutations have been identified in PS1, 13 in PS2 and 33 in APP, strongly suggesting that an altered Aβ metabolism plays a pivotal role in AD pathogenesis. Most PS FAD mutations are situated within or flanking the conserved hydrophobic TMDs and are, except for the Δexon9 mutation, missense mutations resulting in single amino acid changes or deletion of two amino acid residues. In general, FAD mutations in PS1 and 2 cause an increase in Aβ42/Aβ40 ratio, either by decreasing the production of Aβ40 or by increasing the Aβ42 generation [20–24]. Previously, Takami et al presented an Aβ product line-model where APP is sequentially cleaved after every third or fourth amino acid by γ-secretase, depending on the initial ε-cleavage site [25]. Recently, Cháves-Gutiérraz et al studied six FAD PS1 mutations' impact on ε- and γ-cleavage sites and reported that they impaired the fourth cleavage site in both Aβ40 ($AICD_{50–99}$ + Aβ49 > Aβ46 > Aβ43 > Aβ40) and Aβ42 ($AICD_{49–99}$ + Aβ48 > Aβ45 > Aβ42 > Aβ38) product lines and thus gave increased Aβ42/Aβ40 ratio [26]. The altered Aβ42/Aβ40 ratio appears to be a very important determinant; both for the process for amyloidosis and for disease onset [21,27]. In this report, we have performed an even more extensive study and examined 13 different PS1 FAD mutations and their effect on the production of five APP processing products: Aβ38, Aβ40, Aβ42, Aβ43 and AICD.

The topology of a membrane protein is largely dependent on the hydrophobicity of the TMDs and the charged residues flanking the TMDs play an important role during insertion into the membrane [28]. Most eukaryotic membrane proteins, including the human PS1, are co-translationally integrated into the endoplasmic reticulum (ER) membrane by the Sec61 translocon [29,30]. The translocon recognizes the hydrophobic TM sequences and allows them to partition into the membrane while hydrophilic loops are translocated into the ER lumen or retained in the cytosol [31]. However, in some cases the TM sequences of multi-spanning membrane proteins fail to be recognized (marginally hydrophobic TMs) and need assistance from other parts of the protein for efficient integration and folding [32–34]. Previously, by introducing glycosylation acceptor sites into the molecule and measuring the glycosylation status, it has been shown that PS1 contains nine TMDs [16]. Here we report how efficiently each PS1 wild type TM segment is recognized and inserted into the membrane by the translocon, by engineering them into the model protein leader peptidase (Lep) [35,36]. Moreover, we have selected 35 disease-related PS1 FAD mutations and studied whether the insertion process is affected in terms of efficiency in targeting and recognition in comparison to the wild type protein. Finally, we have analyzed how six out of 35 FAD mutants affected the conformation of the catalytic site of PS1, using an active site inhibitor pull-down method.

2. Results

2.1. PS FAD mutations differentially affect Aβ42/40 ratio, Aβ38, Aβ43 and AICD production

In order to get a deeper understanding of the mechanisms behind PS1 FAD mutations, we selected 13 different FAD mutations scattered throughout the protein, and transiently transfected them into cells deficient for PS1 and PS2 (BD8), to nullify the influence of endogenous PS. It has previously been reported that generally all PS FAD mutations have the same phenotype regarding elevation of the Aβ42/Aβ40 ratio [20–24,26]. Here we extend these studies by looking at more Aβ products and AICD generation in order to get a clearer understanding of the PS1 FAD mutations role in APP processing. The selected mutations were located in TM2, TM3, TM6, H7 and TM7; some have been studied previously by other research groups (L166P, A246E, Δexon9, G384A and F386S) and others have not been examined in this context before. All PS1 protein variants were expressed and endoproteolysed with the exception for the Δexon9 mutant which lacks the endoproteolytic site [37] (Fig. 1A). When measuring Aβ38, Aβ40 and Aβ42 generation by MSD technology, we observed that all mutations increased the Aβ42/Aβ40 ratio to different extent, except for the A246E mutant that did not affect the ratio compared to PS1 wild type (Fig. 1B). This is consistent with previously published results [20–24,26]. The highest ratios were generated by the L166P and G384A mutants that gave rise to substantially elevated levels compare to PS1 wild type, 7.3 and 6.0 times, respectively. In addition, I143T, L392P and Δexon9 increased the Aβ42/Aβ40 ratio, with 4.4, 3.6 and 3.2 times, respectively. Interestingly, the L166P, I143T, L392P and G384A mutations, also had a pronounced decrease in Aβ40, with a minimum of 50% reduction compared to wild type. Moreover, these mutations, except L166P, had elevated Aβ42 levels and especially G384A increased Aβ42 (four times compared to wild type) (Fig. 1C). The rest of the mutations were more homogeneous, displaying an increased Aβ42/Aβ40 ratio ranging from 1.2-2.6 in relation to wild type and had a similar pattern in the Aβ40 and Aβ42 distribution (Fig. 1B and C). However, the Aβ38 values were more similar between the FAD mutations. All, except A246E and L250S, showed a reduction in Aβ38 levels (39–79% of wild type) (Fig. 1C). Only six of all FAD mutations showed Aβ43 values, above the detection limit of 0.62pM (WT, E280A, P284L, Δexon9, G384A, F386S and S390I). All, except E280A and F386S, showed increased Aβ43/Aβ40 ratio compared to wild type. The most pronounced increase was for P284L and G384A (6.1 and 14 times increase compared to wild type, respectively) (Fig. 1D).

Next, we characterized the FAD mutations further by examining their overall effect on γ-secretase activity by monitoring AICD production from the ε-cleavage site by using a Luciferase-reporter gene assay [38]. In line with a recent report from Chávez-Gutiérrez et al. [26] and Bentahir et al. [22], we observed that not all FAD mutations affect AICD formation. Most mutations that we investigated showed no decrease in AICD formation, except I143T, L166P, Δexon9 and G384A, which all lowered the production (Fig. 1E). The most impaired production was observed for L166P that reduced AICD formation to 20% compared to wild type. In addition, I143T, Δexon9 and G384A decreased the AICD formation with 30–40% in comparison to PS1 wild type. Overall, the PS1 FAD mutations decreased the formation of the smaller Aβ peptides, Aβ38 and Aβ40, in favor to the production of the longer forms (Aβ42 and Aβ43), suggesting an impairment in the fourth cleavage sites of both Aβ product lines. However, the PS1 FAD mutations with a Aβ42/Aβ40 ratio above 3.2, (L392P, I143T, L166P, Δexon9, G384A,