Mean age of onset in familial Alzheimer’s disease is determined by amyloid beta 42

Marco Duering a,1, Marcus O.W. Grimm a,1, Heike S. Grimm a, Johannes Schröder b, Tobias Hartmann a,∗

a Center for Molecular Biology Heidelberg (ZMBH), Im Neuenheimer Feld 282, 69120 Heidelberg, Germany
b Section of Gerontopsychiatry at the Psychiatric Clinic, University of Heidelberg, Vöhringerstr 4, 69115 Heidelberg, Germany

Received 16 March 2004; received in revised form 27 July 2004; accepted 9 August 2004

Abstract

More than 130 known mutations in the presenilin-1 (PS1) gene result in familial Alzheimer’s disease (FAD) with a mutation specific age of disease onset. These mutations increase amyloid beta 42 (Aβ42) levels, and this increase has been validated in recent years as one pathogenic factor in FAD. However, further malfunctions of mutant presenilin-1 are discussed as well. In order to assess the weight of Aβ42 regarding the pathogenesis of FAD, we expressed mutant forms of PS1 (30–65 years onset age) in COS-7 cells and analyzed amyloid beta levels by a novel ELISA. We found a strong correlation (r=0.98, p<0.001) between the Aβ40/42-ratio and mean age of disease onset indicating a substantial extent of Aβ42 contribution to FAD pathology. Our data strongly suggest that Aβ42 is the decisive factor for age of onset in FAD.

© 2004 Published by Elsevier Inc.

Keywords: Alzheimer’s disease; Beta amyloid-protein; Presenilin-1; Age of onset; Pathogenesis

1. Introduction

It is currently not possible to predict the onset of Alzheimer’s disease (AD) before the first signs of cognitive decline appear. Otherwise, more than 150 genetic mutations and an autosomal-dominant pattern of inheritance are known, that inevitably result in familial AD (FAD) at a mutation specific age of onset (AOO). One hundred and thirty of these mutations occur in presenilin-1 (PS1), increasing the production of amyloid beta 42 (Aβ42) [17], which is proteolytically derived from the amyloid precursor protein (APP) by cleavage of β- and γ-secretase. Over the past years it has been established that this increase is indeed a pathogenic factor in FAD [19]. Aβ42 levels correlate with amyloid plaque onset in transgenic mouse AD models [1,10]. In contrast, correlations between disease onset and Aβ42 overproduction were weak or not significant in previous studies [13,14]. Therefore, it was concluded that besides amyloid pathology there have to be other, independently acting factors contributing to pathogenesis. Indeed several other malfunctions of PS1 mutations have been identified, such as the disturbance of calcium homeostasis [6,8], increased vulnerability of neurons to excitotoxic necrosis [5], altered glycoprotein processing [3], interference with CREB binding protein proteolysis [16] and the impairment of kinesin-based axonal transport [15]. However, it is currently not possible to estimate the weightiness of these or other dysfunctions regarding the pathogenesis of FAD or AD.
point mutations were selected at random and cloned into pcDNA3.1/Zeo(+) (Invitrogen, USA). These mutations were transfected (Lipofectamine 2000, Invitrogen) in COS-7 cells (DSMZ, Germany) together with SP-C99 (DA) [4], cloned in pIREShyg3 (BD Clontech, USA). This construct, a truncated form of the amyloid precursor protein, does not necessitate β-secretase cleavage and yields higher Aβ levels for measurement than APP. Presenilin-1 wild-type cDNA (wt) and empty pcDNA3.1 vector (MOCK) served as controls. Cells were cultured in DMEM (Sigma, USA) with 10% FCS (PAN Systems, Germany) at 37 °C and 5% CO2. After simultaneous selection in 250 μg/ml zeocin (Invitrogen) and 400 μg/ml hygromycin (PAA Laboratories, Austria) independent cell clones (approximately five for each PS1 mutation), with unavoidable variations in expression levels of PS1 and SPA4CT, were isolated and expanded. After verification of PS1 overexpression with SDS page and Western blotting (data not shown), cell culture supernatants of three to five independent clones were collected, and Aβ levels were analyzed by a novel ELISA (hAmyloid-β ELISA High Sensitive, The Genetics Company, Zürich, Switzerland) using antibodies developed in our laboratory (G2-10 for detection of Aβ40, G2-13 for Aβ42 and W0-2 for total Aβ). The Aβ quantifications were performed as described in the manufacturer’s instructions. The accuracy of ELISA was verified by Western blotting as described [9]. Aβ levels were expressed as ratio between the most common, 40 residue form of Aβ40 and the pathogenic, 42 residue form Aβ42. The data were normalized by defining the ratio of PS1 wild-type overexpressing cells (which is equal to vector control) as 1.

3. Results

We analyzed 14 Presenilin-1 mutations (Fig. 1A) in COS-7 cells (Table 1). In this analysis standard deviations of the measured Aβ40/42 ratios were low (average S.D. 8%), and mutant ratios were significantly different from wild-type ratio (p < 0.005, Mann–Whitney test). Western blotting of a subset of clones confirmed ELISA results and showed that as expected, expression levels of the precursor protein SP-C99 differed among subclones and matched Aβ40 levels (Fig. 1B).

Through regression analysis we discovered a highly significant correlation (p < 0.001) between known mean AOO and observed Aβ40/42 ratio (Fig. 1C). Noteworthy, the R-square is 96% for the evaluated range from 30 to 65 years.

Fig. 1. (A) Position of the randomly selected mutations in the PS1 molecule. (B) Comparison of Western blot analysis and ELISA results of a randomised subset of clones exhibits nearly identical results. Bars indicate mean ± S.D. of three independent clones per mutation, wild-type was set to 1, sorting from lowest to highest age of disease onset. A representative Western blot of three cell lines (indicated by numbers in the diagram) shows that Aβ40 levels in conditioned media, but not Aβ42 levels, correspond in C99 levels in cell lysates. (C) Correlation between mean age of onset and Aβ40/42 ratio, including 95% confidence interval (dashed line) and 95% prediction interval (dotted line). Dots show mean ± S.D. of three to five independent clones per mutation, wild-type was set to 1. (D) Correlation with Aβ40 and Aβ42 levels, dots show mean levels, wild-type was again set to 1. According to the Aβ40/42 ratio, reciprocal correlation was used for Aβ42 levels.
bearing cell lines. For the most part this is due to the advances of Aβ detection technology, allowing us to detect Aβ levels with high precision and very small standard deviations, which was previously not possible [13,14].

Our results were obtained using mutations in the protein most commonly affected in FAD, the PS1. Occasionally, mutations in the APP gene have been observed that similarly increase Aβ42 production. Interestingly, the APP Swedish mutation, also causing FAD, exhibits an increase in both, the Aβ42 and Aβ40 levels, resulting in an unaltered Aβ40/Aβ42 ratio compared to the wild-type. This leads to the assumption that Aβ42 levels, not the ratio between Aβ40 and Aβ42 levels, affect the AOO. Hence, in our experiments Aβ40 presumably served as an internal normalization standard. This statement is supported by several observations: isolated Aβ42 levels, but not isolated Aβ40 levels, already strongly correlate with the AOO. Recent findings in transgenic mice show, that PS1 mutations increase Aβ42 levels, whereas Aβ40 levels are not altered [10]. And our Western blot data show corresponding levels for the precursor SP-99 and Aβ40, but not Aβ42. Therefore, in our experimental setting, Aβ40 can be considered as an indicator for the amount of expressed precursor protein, which differs among subclones.

Correlation is not causality. But causality between Aβ42 and the pathogenesis has already been pointed out over the past years [18,19]. For this reason, our data allow an estimate to be made regarding the extent to which Aβ42 contributes to FAD pathogenesis. As >96% of the variance in AOO can be explained by the variance in Aβ40/Aβ42-ratio (as stated by an R² value of 0.96), it has to be concluded that Aβ42 is the major pathogenic factor for PS1-mediated FAD. Consistent with this, ApoE-ε4, the other established major genetic factor in AD, reduces the mean AOO only slightly to 75.5 years [2]. Our results do not exclude the possibility of considerable deviations from the predicted AOO for individual cases or individual FAD families. We addressed here the potency of PS1 mutations to shift the mean age of disease onset towards younger ages. Spreading around this mean is present to a variable degree for individual FAD families. The extent of this spreading is likely due to other factors [11], that may contribute to the Aβ effect.

Extrapolating our data, we noticed a striking match of the calculated AOO for wild-type PS1 and the mean AOO for non-familial dementia. Although wild-type PS1 is also present in healthy people and therefore additional factors must be present for the development of the disease, this close match shows that such extrapolations are reasonable. It also indicates a similar role for Aβ42 in sporadic AD, which is in line with recent longitudinal studies showing increased Aβ42 levels in blood plasma to be a possible predictor for later AD development and mortality [12].

Aβ42 was the favorite AD culprit for a long time [19], but until now it was not possible to address quantitatively its impact. The surprisingly strong impact of Aβ42 overproduction on FAD onset age highlights the neurodegenerative potential of this molecule. Moreover, with a solid basis at hand to address the major factor in FAD onset age, evalua-
References


