Network: Systematic Gene Identification and Functional Analyses in Common CNS Disorders

Project: Identification of Genes that Regulate Aβ42 Levels

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Introduction

Aß42 production is genetically linked to AD, but Aß40 is not. The ratio of Aß42 to Aß40 is tightly controlled in-between narrow margins. Already small deviations in the Aß42 levels from the normal situation significantly affect the relative risk and disease age of onset. The identification of genes that specifically affect Aß42 production or Aß42/Aß40 ratio is of major interest for the molecular understanding of AD and these genes are prime targets for therapeutic intervention

Aß42 level regulation is sensitive to a number of cellular processes in protease regulation, protein trafficking and membrane composition. Presenilin mutation dependent alterations are the most common identified cause of familial AD. There are currently >130 different PS1 mutations known all of which result in increased Ab42 production and an earlier onset of the disease. In other forms of familial AD and the vast majority of sporadic AD alternative genes appear to be involved as a number of independent studies report a genetic influence on the disease in a ranges between 50-75%.

Our aim is highly focused on a single major goal – the identification of Aß42 levels regulating genes. For no other molecule such a wealth of data, spanning every scientific field involved from chemistry to clinical science, is available showing a clear-cut major impact on Alzheimer's disease generation. It is therefore only reasonable to concentrate efforts on this aspect.

Anticipated results/perspective: Identification of genes with potential therapeutic relevance involved in the initiation and progression of AD. We expect that several candidates will have a major role in other diseases.

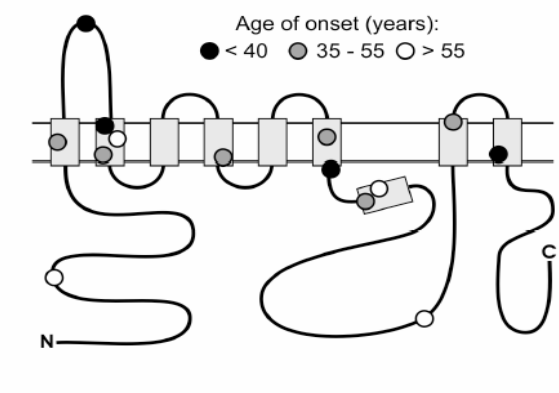

Fig 1: *Distribution of PS-FAD mutations selected for study. Duering, 2005.*

Results

In order to assess the weight of Aß42 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 Aß40/42-ratio and mean age of disease onset indicating a substantial extent of Aß42 contribution to FAD pathology. Our data strongly suggest that Aß42 is the decisive factor for age of onset in FAD.

Using clinical databases (http://molgen-www.uia.ac.be/ADMutations/ http://www.alzforum.org/res/com/mut/pre/table1.asp) on PS1 mutations and mean AOO, 14 PS1 point mutations were selected at random and cloned. 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 over-expression with SDS page and Western blotting, cell culture supernatants of three to five independent clones were collected, and Aß levels were analyzed by a novel ELISA using antibodies developed in our laboratory (G2-10 for detection of A 40, G2-13 for Aß42 and W0-2 for total Aß). The Aß quantifications were performed as described in the manufacturer's instructions. The accuracy of ELISA was verified by Western blotting as described Ida, 1996. Aß levels were expressed as ratio between the most common, 40 residue form of Aß40 and the pathogenic, 42 residue form Aß42. The data were normalized by defining the ratio of PS1 wild-type overexpressing cells (which is equal to vector control) as 1.

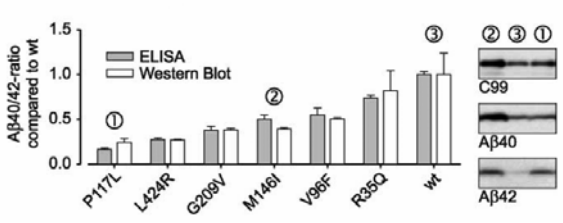

Fig 2: *Comparison of Western blot analysis and ELISA results of a randomized subset of clones exhibits nearly identical results. Bars indicate mean S.D. of three independent clones per mutation, wild-type was set to 1, sorting from lowest to highest age of disease onset. A representative Western blot of three cell lines (indicated by numbers in the diagram) shows that A 40 levels in conditioned media, but not Aß42 levels, correspond to C99 levels in cell lysates.*

Through regression analysis we discovered a highly significant correlation (p < 0.001) between known mean AOO and observed Aß 40/42 ratio. Noteworthy, the R-square is 96% for the evaluated range from 30 to 65 years. Interestingly, this correlation is linear allowing extrapolation of our data. In case of a PS1 mutation producing infinite levels of Aß 42, lowering the A 40/42 ratio in our assay towards zero, the predicted AOO would hypothetically be as early as 18 years (95% PI, {13;23}). This would be the earliest possible AOO caused by a heterozygous PS1 mutation. Similarly, the absence of Aß 42 would lead to an infinitely late AOO. Using the Aß ratio of wild-type PS1, which is genetically present in patients with sporadic dementia, extrapolation predicts a mean AOO of 79 years (95% PI, {74; 85}).

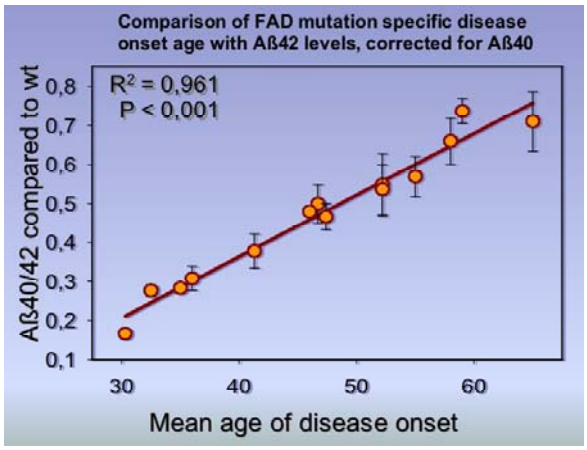

Fig 3: *Aß42 production, corrected for Aß40, predicts age of onset in PS1 familial AD. Correlation between mean age of onset and Aß40/42-ratio, Dots show mean +/- S.D. of three to five independent clones per mutation, wild-type was set to 1.*

This value is in the range of the mean AOO of approx. 82 years for non-familial dementia derived from current epidemiological data. In addition, the linear equation allows the effect of Aß 42 reducing treatments to be estimated. It predicts that already small changes in Aß levels strongly affect the AOO. At last, we addressed whether Aß 42 or Aß 40 levels alone correlate with the mean AOO. This was the case for Aß 42 (r = 0.75, p = 0.002), whereas, Aß 40 levels alone showed no significant correlation (r = 0.17, p = 0.55) with the AOO.

Aß42 responsive genes

In identifying Aß42 responsive genes, our microarray and other studies suggested an involvement of the lipid homeostasis pathway. By functional analysis we identified a direct interaction of human neutral sphingomyelinase, an enzyme which degrades sphingomyelin to ceramide (Grimm, Nat. Cel. Biol in press). Importantly this interaction occurs at physiological Aß levels and results in activation of this enzymes activity. This activation is absent in APP/APLP2 knock-out cells and reduced in APP knock-out mice (APP knock-out studies were in collaboration with Ulrike Müller, PI in the same network; PS knock-out studies were done in collaboration with Bart DeStrooper and PS knock-out studies in mice were done in collaboration with Jie Shen). Further, this activation is critically dependent on APP processing, which leads to Aß42 generation. Unlike Aß42, the main Aß peptide, Aß40, has only residual stimulating activity. There was no detectable involvement of ROS at physiological Aß concentrations.

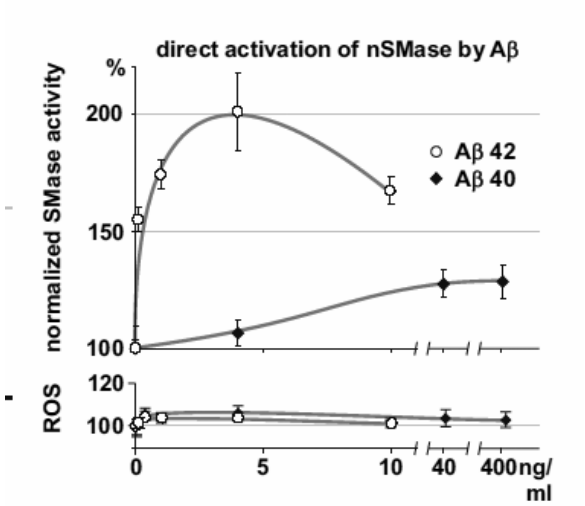

Fig 4: Aß function in lipid homeostasis. Dose response curve of purified human placenta nSMase activity to synthetic Aß40 or Aß42. ROS levels were determined in MEF PS1/2 -/- (by APF). Grimm, 2005 in press.

Outlook

Our studies revealed a number of Aß and presenilin responsive genes, validated for expression levels. Our studies further revealed a physiological and ubiquitous function for Aß. There are a number of very interesting candidates, most of them matching genomic loci previously identified for AD relevance. We are currently investigating their functional relevance to AD.

Lit.: ***1.*** *Duering et al., Mean age of onset in familial Alzheimer's disease is determined by amyloid beta 42, 2005, Neurobiol Aging, 6, 26, 785-8.* ***2.*** *Ida, et al, Analysis of heterogeneous ßA4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay, 1996, J. Biol. Chem., 37, 271, 22908-22914.* ***3.*** *Grimm et al. Regulation of Cholesterol and Sphingomyelin Metabolism by Amyloid ß and Presenilin Nat. Cel. Biol. in press.*