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Differential processing of amyloid precursor protein in brain and in peripheral blood leukocytes

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Abstract

Since Amyloid precursor protein (APP) fragments exist in many tissues throughout the body, including the fluid compartments of blood, they have been the focus of numerous investigations into their potential as a biomarker of Alzheimer's disease (AD). Using immunohistochemistry, immuno-electron microscopy, Western blotting and quantitative RT-PCR we examined whether APP processing in leukocytes is analogous to APP processing in the brain. We show APP immunoreactivity at light and electron microscopic levels in the cytoplasm and nucleus of peripheral blood leukocytes (PBL) yet our Western blotting data demonstrated that brain and PBL contain different APP fragments and differentially expressed APP processing enzymes. ADAM10, nicastrin and BACE2 were present in brain but were undetected in PBL. PS1 and BACE1 were detected in both tissues but showed different patterns in Western blots. Quantitative PCR results identified Nephilysin as the only processing enzyme we interrogated where Western and qPCR data coincided. Although our data on differential processing of APP in brain and PBL point to exercising caution when generalizing between blood and brain with regard to mechanisms, they have no implications regarding utility as biomarkers.

1. Introduction

Molecules resulting from the posttranslational processing of amyloid precursor protein (APP) play a central role in the pathophysiology of Alzheimer's disease (Hardy and Selkoe, 2002; Walsh et al., 2005; Haass and Selkoe, 2007; Shankar et al., 2007; Palop and Mucke 2010; Lublin and Gandy, 2010; Shimada et al., 2011; Jonsson et al., 2012), and are also ubiquitously present in many tissues throughout the body (e.g. Roher et al., 2009). In the search for biomarkers of AD, these two facts have provided a rationale for examining easily

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5 Conflict of interest

To the best of their knowledge, the authors do not have an actual or potential conflict of interest with regard to the present research.

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obtained peripheral tissues for biomarkers of AD, and APP fragments in blood have received special attention in these studies (Borroni et al., 2010; Takeda et al., 2010; Hampel et al., 2010; Mukaetova-Ladinska, et al., 2012). The APP products circulating in the fluid compartment(s) of blood have a number of potential sources, including from brain, endothelial cells, platelets and leukocytes (e.g. Konig et al., 1992; Sandbrink et al., 1994). To minimize such multiplicity of sources, we restricted our investigation to the leukocyte compartment of blood. Since it has been shown that leukocytes express APP (e.g. Konig et al., 1992; Li et al., 1999) and that peptides produced from APP may serve as AD biomarkers (e.g. Holtzman, 2011; Mayeux and Schupf, 2011), we hypothesized that APP processing in leukocytes may be similar to APP processing in the brain. In view of data showing transport of a C-terminal fragment of APP to translocate to neuronal nuclei (Kimberly et al., 2001) and to modulate transcription (Cao and Sudhof, 2001, 2004; Muller et al., 2008; Buoso et al., 2010), we used immunohistochemistry, immuno-electron microscopy, Western blotting and quantitative RT-PCR to examine APP in cytoplasm and nucleus of peripheral blood leukocytes (PBL) as well as the expression of selected enzymes involved in the processing of APP. Selected leukocyte data were compared to brain. We conclude that although APP fragments appear to distribute similarly in both neurons and PBL, the APP fragment *species* in these two tissues are not completely comparable. Our data suggest this lack of comparability may be related to differential expression of APP processing enzymes in brain and in leukocytes. Previously demonstrated differential splicing of APP message in these two tissues (Konig et al., 1992) may also contribute to the observed lack of comparability. This lack of comparability does not signify leukocyte gene products cannot provide useful biomarkers. It does, however, suggest a need for caution in generalizing between blood and brain with regard to mechanisms.

2. Materials and methods

2.1 Cases

Samples were obtained from a total of twenty-six individuals. All specimens were collected with informed consent and under IRB-approved protocols.

For Western blots, immunohistochemistry (IHC) and immunoelectron microscopy, blood samples were obtained from four Alzheimer's disease, three age matched non-demented controls, three young controls (in their 20's), and one Parkinson's disease. The AD, PD and matched controls came from the Alzheimer's Disease Center at the University of Rochester Medical Center and the Cleo Roberts Clinic at Banner Sun Health Research Institute. Young controls were laboratory personnel. Blood samples were drawn by an experienced phlebotomist and processed for IHC, immuno-electron microscopy and Western blotting. Postmortem human superior frontal gyrus was obtained from two Alzheimer's and two age matched controls from the brain bank at the University of Rochester Alzheimer's Disease Center.

For quantitative RT-PCR, blood samples were obtained from two Alzheimer's, two aged matched non-demented controls, two young controls (in their 20's) and one Parkinson's disease case. The AD, PD and matched controls for qPCR were from the Cleo Roberts Clinic at Banner Sun Health Research Institute. Young controls were laboratory personnel. Postmortem human superior frontal gyrus (SFG) for qPCR was obtained from two Alzheimer's and two aged matched controls from the brain bank at Banner Sun Health Research Institute.

2.2 Immunohistochemistry

For IHC, blood was drawn into EDTA tubes. Leukocytes were then isolated by lysing RBC with 1.5M ammonium chloride, 0.1M sodium bicarbonate and 10mM EDTA, centrifuged at

2000 rpm for 5 minutes and after a series of washes in 1X PBS, resuspended in 1X PBS and counted on a hemocytometer. $1.5\text{--}2.0 \times 10^5$ cells were applied onto poly-L-lysine coated glass slides and allowed to air dry and stored at RT until use. Slides were washed with PBS then fixed for 15 minutes with 2% paraformaldehyde in PBS. After rinsing with PBS and PBS + 0.1% Triton X-100 (Sigma) and blocking with normal goat serum or normal horse serum, the slides were incubated overnight in a moist chamber at 4°C in primary antibodies targeted at the C-terminus of APP (rabbit polyclonal- Calbiochem) 1:500. After washing, bound primary antibodies were detected by Alexa 488-conjugated secondary antibodies (Molecular Probes). Slides were also treated with Hoechst Nuclear stain (1:10,000) and Sudan Black (0.3 %) to suppress autofluorescence.

2.3 Confocal Microscopy

Leukocytes were isolated as stated above and then applied onto Superfrost Plus glass slides (VWR) and allowed to air dry and stored at RT until use. Slides were washed with PBS then fixed for 10 minutes with 2% paraformaldehyde in PBS. After rinsing with PBS and PBS + 0.1% Triton X-100 (Sigma) and blocking with 1% BSA (Sigma), the slides were incubated overnight in a moist chamber at 4°C in primary antibodies targeted at the C-terminus of APP (rabbit polyclonal- Calbiochem) 1:500. After washing, bound primary antibodies were detected by Alexa 488-conjugated secondary antibodies (Molecular Probes). Slides were also treated with red-fluorescent 7-aminoactinomycin D (7-AAD) nuclear stain (Molecular Probes) at 1:50 and Sudan Black (0.3 %) to suppress autofluorescence. The microscope used was an Olympus IX70 with argon and krypton lasers. Imaging done with Fluoview (Olympus) software.

2.4 Immunoelectron microscopy

Buffy coats were fixed in 0.1M sodium phosphate buffered 4.0% paraformaldehyde for 3 hours, rinsed in phosphate buffer, pelleted into 2.0% agarose (low temperature melting), dehydrated in a graded series of methanol to 100% at -20°C, transitioned into Lowicryl K4M resin at -20°C and polymerized in capsules for 5 days under ultraviolet light at -20°C. Thin sections (70–80nm) were placed onto formvar coated nickel grids, blocked with a 1/10 dilution of PowerBlock (Biogenex, San Ramon, CA), rinsed (5 minutes $\times$ 4) in distilled H₂O and incubated in a 1:100 dilution of rabbit polyclonal anti-Amyloid Precursor Protein, C-terminal (Calbiochem) overnight at 4°C. The grids were rinsed in phosphate buffered saline (PBS) (5 minutes $\times$ 5) and incubated 30 minutes in a 1/20 dilution of Protein A conjugated to 15nm gold. Negative controls substituted the primary with PBS only and were incubated in a 1/20 dilution of Protein A 15nm gold. The grids were rinsed in PBS (5 minutes $\times$ 4 times) and incubated 10 minutes in 2.5% glutaraldehyde to stabilize the gold labeled antigen. The grids were rinsed in distilled H₂O (5 minutes $\times$ 4), stained for 8 minutes in aqueous uranyl acetate and 10 minutes in lead citrate. The labeled sections on the grids were photographed using a Hitachi 7100 transmission electron microscope with an attached MegaView III (Soft-Imaging System, Lakewood, CO) digital camera.

2.5 Immunoblotting

For Western blotting, blood was drawn into EDTA tubes. Leukocytes were then isolated by lysing RBC with 1.5M ammonium chloride, 0.1M sodium bicarbonate and 10mM EDTA and a series of washes in 1X PBS and spun at 1600 rpm for 10 minutes. Leukocyte pellet was resuspended in lysis buffer containing 20mM Tris, pH 7.5, 0.5% Nonidet P40, 1mM EDTA, 0.1M NaCl, 1 mM PMSF and protease inhibitor cocktail (Sigma P8340) and phosphatase inhibitor cocktails (Sigma P2850, P5726). After resuspension, the tubes were placed on ice for 20 minutes and vortexed intermittently during the incubation then centrifuged at $15,000 \times g$ at 4°C for 5 minutes. (Jenkins et al., 2001). Total protein concentration of supernatant was determined by DC Protein Assay (Bio-Rad 500-0112)

based on the Lowry method. Brain tissue was homogenized in the same lysis buffer (as above) and total protein concentration determined as above.

Proteins were separated by SDS-PAGE on Tris-HCl, 10–20% gradient gels (Bio Rad) and transferred to PVDF membranes (Immuno-Blot; Bio Rad), incubated in primary antibodies* as detailed in Table 1, then in HRP-goat anti rabbit secondary (Jackson ImmunoResearch) and detected by enhanced chemiluminescence technique (GE Healthcare).

Each blot contained the same samples in the same order at the total protein concentrations listed (Table 1). Equal loading of gels (10 ug) was confirmed by Coomassie stain (data not shown). Table 1 also lists the antibodies and concentrations used. All Western protocols were tested and optimized for primary and secondary antibody concentrations and protein loading amounts.

2.6 Quantitative RT-PCR

2.5 mL of fresh blood was drawn into PAXgene™ Blood RNA Tubes (Qiagen, Valencia, CA, USA) and total RNA extracted using PAXgene™ Blood miRNA Kit (Qiagen). Total RNA was extracted from brain tissue using the RNeasy Mini Kit (Qiagen) according to manufacturers instructions. RNA integrity and concentration was determined by Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The average RIN for all samples used was 8.8. The quantitative RT-PCR procedure was as follows: 500 ng of total RNA was reverse transcribed with the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). 2ul of a 1:5 dilution of cDNA was combined with TaqMan® Gene Expression Master Mix (Applied Biosystems) and the TaqMan® Gene Expression Assay (Applied Biosystems) in a 30 ul reaction. The quantitative RT-PCR amplifications were run in triplicate on an iCycler iQ™ (Bio-Rad, Hercules, CA, USA). Thermal cycling conditions were: 2 minutes at 60 degrees C, 10 minutes at 95 degrees C and 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60 degrees C for 1 minute.

TaqMan® Assays were for APP (amyloid beta precursor protein), ADAM10, BACE1, BACE2, NCSTN (nicastrin), PSEN1 (presenilin 1), and NEP (neprilysin). GUSB (beta glucuronidase) was used as an endogenous control since its expression was found to be uniform across all samples. Normalized data were then determined as a ratio using the ($2^{-\Delta\Delta C_T}$) method (Livak and Schmittgen, 2001). Leukocyte gene expression fold change was calculated relative to gene expression in the SFG. Primers for the PCR reaction were selected to cover, as closely as possible, the same region as the immunogen used to generate each antibody (Table 2).

3. Results

3.1 Immunohistochemistry

Figure 1A shows localization of immunoreactivity for APP using Calbiochem antibody #171610 (Table 1) in AD peripheral blood leukocytes. This antibody will detect the soluble C-terminal fragments CTF γ CTF α and CTF β as well as full length of isoforms APP₆₉₅, APP₇₅₁ and APP₇₇₀. There is both diffuse and punctate immunoreactivity in the cytoplasm of most peripheral blood leukocytes. In addition, there appears to be punctate immunoreactivity in some leukocyte nuclei. It is, however, not possible to determine in these light level images whether this immunoreactivity was within the nucleus or above/below it.

3.2 Confocal Microscopy

To better localize APP immunoreactivity in PBL we examined a different AD sample with confocal microscopy using the same Calbiochem antibody as in the epifluorescence experiment. Figure 1B shows a similar pattern of immunoreactivity as in Figure 1A with diffuse and punctate immunoreactivity in the cytoplasm of most PBL and the apparent colocalization of APP within the nucleus, demonstrated by the yellow labeling.

3.3 Immuno electron microscopy

Since the apparent immunoreactivity in the nucleus at the epifluorescence and confocal microscopic level could only suggest but not prove nuclear localization, we undertook an immuno-electron microscopic examination of APP in AD peripheral blood leukocytes in order to establish whether or not the immunoreactivity at the light level within leukocyte nuclei was indeed within nuclei. We used the same Calbiochem antibody as in our immunohistochemistry experiments. Figure 2A shows ring-like immuno gold particles within the nucleus. Figure 2B shows immuno gold reactivity in association with a cytoplasmic organelle that probably corresponds to the cytoplasmic punctate immunoreactivity seen at the light level. We were not able to definitively identify the structure with which this cytoplasmic immunoreactivity is associated.

3.4 Western blotting

In order to characterize the nature of the leukocyte immunoreactivity seen at the light and e.m. levels, we conducted Western blotting of total protein extracted from peripheral blood leukocytes. Since immunoreactivity of APP peptides has also been described in brain (e.g. Cao and Sudhof, 2001; Gouras et al., 2005; Mohamed and Posse de Chaves, 2011), we included brain samples in our Western blots.

Figures 3A and 3B show that Western blots of brain and blood samples, utilizing antibodies directed at the C-terminus of APP, produce different patterns depending on the specific antibody used. The Calbiochem antibody suggests a basic similarity in APP fragments in both brain and blood, although there are some differences between these two tissue types at higher molecular weights. The intense band at approximately 80 kDa found in both brain and blood is undetermined. However, the landmark study on APP by Selkoe et al., (1988), demonstrated strong, specific staining in immunoblots of human cortical homogenates at ~72 kDa from their antibody derived from the C-terminus of APP. Also, in monocytes and lymphocytes, Jung et al., (1999) found a reactive band at ~82 kDa using an antibody to the C-terminus of APP. Finally, it has been shown that caspase mediated cleavage in the C-terminus of APP produces a polypeptide of ~85 kDa (Gervais et al., 1999; Zhao et al., 2003). These studies demonstrate it may not be unexpected to see immunoreactivity at ~75–85 kDa with an antibody directed against the C-terminus of APP, just as we saw, but further examination is needed to determine its identity or why it was undetectable with the Sigma antibody. The higher molecular weight (~110 kDa) doublet is believed to be the glycosylated form of APP₆₉₅ and the upper band (~120) is APP₇₇₀ (Buxbaum et al., 1998).

On the other hand, the Sigma antibody shows major differences in both amount and isoforms of APP in brain and in PBL that suggest differences in processing of APP in these two tissue types. Although the higher molecular weight pattern is the same with both Sigma and Calbiochem antibodies, the undefined ~80 kDa band was virtually undetectable in brain or blood with the Sigma antibody. The band seen at ~60 kDa is believed to be a degradation product. The faint, somewhat diffuse bands below the 17.2 kDa marker are consistent with the expected sizes for CTF α (~9 kDa) and CTF β (~11 kDa).

Figure 3 demonstrates that there are different patterns of immunoreactivity in brain and in peripheral blood leukocytes from control, AD and PD cases (the latter in Figure 3B), which may be attributed to differential posttranslational processing. In order to investigate tissue differences in APP processing, we examined expression of selected proteins involved in processing APP by Western blotting of brain and PBL protein extracts (Figure 4). We also examined expression of these genes at the transcript level by quantitative PCR (Figure 5). The Western data show that some of the APP processing proteins examined were detected in brain but appear undetectable in protein extracted from AD leukocytes. These were: ADAM10, nicastrin and BACE2 (the latter is, however, present in PD leukocytes). Other enzymes that were detected in protein extracted from both brain and AD leukocytes, but showed different patterns in leukocytes, are neprilysin, PS1 and BACE1, which was undetected in PD leukocytes.

These data are consistent with the suggestion that APP is differentially processed in brain and blood cells in AD.

3.5 Quantitative RT-PCR

In order to determine whether the differential appearance of APP processing enzymes in leukocytes and brain was a function of posttranslational processing or differential expression at the transcript level, we quantified transcript expression by qPCR. These data show that message levels of ADAM10, BACE2, nicastrin and PS1 are similar in both brain and leukocytes. BACE1 message was much reduced in leukocytes compared to brain, while neprilysin message was much increased in leukocytes compared to brain (Figure 5).

4 Discussion

Our data demonstrate APP immunoreactivity at light and electron microscopic levels in both cytoplasm and nucleus of peripheral blood leukocytes. The apparent nuclear localization of some APP peptide(s) seen at the light level in fluorescence and confocal microscopy was confirmed by immunoelectron microscopy which showed gold particles labeled with an antibody targeting the C-terminus of APP as a ring like structure. We suggest that these structures may correspond to the “spherical nuclear spots” described by von Rotz et al. (2004) in both HEK293 and SH-SY5Y cells co-expressing AICD, Fe65 and Tip60.

Apparently similar localization have been previously described in the cytoplasm and nucleus of neurons (e.g. Cao and Sudhof, 2001; Muresan and Muresan, 2004; Ohkawara et al., 2011). However, our Western blotting data reveal that brain and PBL contain different APP fragments, suggesting that immunohistochemical demonstration of similar locations be viewed with caution, depending on properties of specific antibodies used. We further demonstrate differential expression of APP processing enzymes in brain and in PBL which is consistent with the appearance of differential APP isoforms in these two tissues. This differential of processing enzymes suggests, but does not prove, that the different fragments of APP in brain and in blood cells may be related to differences between these two tissues in posttranslational processing attributable to differential presence of APP processing enzymes in these two tissues. An additional contributor to the difference between brain and leukocyte APP products may also be derived from differential splice isoforms of APP mRNA in leukocytes and neurons (e.g. Sandbrink et al., 1996; König et al., 1992). Whether the differential processing we have described here results in different APP fragments localizing to the nucleus in brain and in leukocytes is not clarified by our data. If this were the case, it could have significant consequences for gene expression profiles in these two tissues.

Although there have been studies of specific subclasses of PBL in AD, particularly lymphocytes (e.g. Sultana et al., 2011; Stieler et al., 2012), we did not distinguish among

subclasses. However, we argue our data are not the product of any small subclass of leukocytes because the vast majority of cells were immunopositive (Fig. 1) and the Western blot signals were strong (Figs. 3 and 4). In addition, we did not examine the entire family of molecules that contribute to posttranslational processing of APP. However, the molecules we did examine (Fig 4), in concert with the Western blotting of APP (Figure 3), serve to establish the principle of differential posttranslational processing of APP in two different tissues.

The discordance between our Western blot and our qPCR data is notable, with Western blots showing undetectable expression of ADAM 10, BACE 2 and nicastrin in PBL relative to brain, while qPCR data show approximately similar message expression of these molecules in brain and PBL. The apparent increased signal for neprilysin in leukocytes relative to brain seen in Figure 4 is consistent with increased signal for neprilysin relative to brain seen in quantitative PCR data (Figure 5). However, although our Western blots show decreased signal for ADAM10, BACE1, BACE2 and nicastrin in leukocytes compared to the signal in brain, our quantitative PCR data only show reduced message for BACE1 in leukocytes compared to brain. ADAM10, BACE2 and nicastrin show virtually equivalent levels of message expression in leukocytes and brain. This raises the question of whether our Western and qPCR data suggest differential stability of these enzymes in brain and in leukocytes, as has been shown for the differential half life of interferon regulatory factor 7 (IRF-7) in different cell types (e.g. Prakash and Levy, 2006). Additionally, it has long been recognized that message and protein expression need not always be in a 1:1 stoichiometric relationship (e.g. Gygi et al., 1999). We conclude that the apparent quantitative discrepancies between our Western data and our qPCR data may be related to a number of factors including differential protein stability as a function of cellular environment, enzyme activity, antibody sensitivity, regulation of translation, etc.

Although the major point of the data presented here was to compare APP processing in brain and in blood, it may be useful to comment that there were no detectable qualitative differences between AD and control leukocytes in expression of APP processing enzymes in Western blotting (Figure 4). Preliminary qRT-PCR data show similar results with a slight increase of BACE2 expression (data not shown). We also draw attention to the differences between the one PD case and AD and control cases in expression of BACE1, BACE2 and neprilysin seen in the Western blots.

Our data showing differential processing of APP in brain and in peripheral blood leukocytes emphasizes the potential variety of sources of APP products in the fluid compartment of blood. The quantitative contributions of differentially processed APP from vascular cells, including leukocytes, and that due to passage of blood through the brain in the composition of the fluid compartments of blood is unknown. From the point of view of blood compartments as providing biomarkers this is irrelevant. If a biomarker works, that is sufficient. However, the extent to which cellular or fluid compartments of blood may be a reflection of events in the brain must be considered with caution.

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References

- Borroni B, Agosti C, Marcello E, Di Luca M, Padovani A. Blood cell markers in Alzheimer Disease: Amyloid Precursor Protein form ratio in platelets. *Exp Gerontol.* 2010; 45:53–56. [PubMed: 19699790]
- Buoso E, Lanni C, Schettini G, Govoni S, Racchi M. Beta-Amyloid precursor protein metabolism: focus on the functions and degradation of its intracellular domain. *Pharmacol Res.* 2010; 62:308–317. [PubMed: 20561999]
- Buxbaum JD, Thinakaran G, Koliatsos V, O'Callahan J, Slunt HH, Price DL, Sisodia SS. Alzheimer amyloid protein precursor in the rat hippocampus: transport and processing through the perforant path. *J Neurosci.* 1998; 18:9629–9637. [PubMed: 9822724]
- Cao X, Sudhof TC. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60 [Erratum appears in *Science* 2001 Aug 24;293(5534): 1436]. *Science.* 2001; 293:115–120. [PubMed: 11441186]
- Cao X, Sudhof TC. Dissection of amyloid-beta precursor protein-dependent transcriptional transactivation. *J Biol Chem.* 2004; 279:24601–24611. [PubMed: 15044485]
- Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang J, LeBlanc A, Smith D, Rigby M, Shearman MS, Clarke EE, Zheng H, Van Der Ploeg LHT, Ruffolo SC, Thornberry NA, Xanthoudakis S, Zamboni RJ, Roy S, Nicholson DW. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid- β precursor protein and amyloidogenic A β peptide formation. *Cell.* 1999; 97:395–406. [PubMed: 10319819]
- Gouras GK, Almeida CG, Takahashi RH. Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. *Neurobiol Aging.* 2005; 26:1235–1244. [PubMed: 16023263]
- Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol.* 1999; 19:1720–1730. [PubMed: 10022859]
- Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Bio.* 2007; 8:101–112. [PubMed: 17245412]
- Hampel H, Shen Y, Walsh DM, Aisen P, Shaw LM, Zetterberg H, Trojanowski JQ, Blennow K. Biological markers of amyloid beta-related mechanisms in Alzheimer's disease. *Exp Neurol.* 2010; 223:334–346. [PubMed: 19815015]
- Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; 297:353–356. [PubMed: 12130773]
- Holtzman DM. CSF biomarkers for Alzheimer's disease: Current utility and potential future use. *Neurobiol Aging.* 2011; 32:S4–S9. [PubMed: 22078172]
- Jenkins JK, Suwannaroj S, Elbourne KB, Ndebele K, McMurray RW. 17- β -Estradiol alters Jurkat lymphocyte cell cycling and induces apoptosis through suppression of Bcl-2 and cyclin A. *Int Immunopharm.* 2001; 1:1897–1911.
- Johnsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham RR, Huttenlocher J, Bjornsdottir G, Andreassen OA, Jonsson EG, Palotie A, Behrens TW, Magnusson OT, Kong A, Thorsteinsdottir U, Watts RJ, Stefansson K. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature.* 2012 Jul 11. [Epub ahead of print].
- Jung SS, Gauthier S, Cashman NR. β -amyloid precursor protein is detectable on monocytes and is increased in Alzheimer's disease. *Neurobiol Aging.* 1999; 20:249–257. [PubMed: 10588572]
- Kimberly WT, Zheng JB, Guenette SY, Selkoe DJ. The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. *J Biol Chem.* 2001; 276:40288–40292. [PubMed: 11544248]

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- Konig G, Monning U, Czech C, Prior R, Banati R, Schreiter-Gasser U, Bauer J, Masters CL, Beyreuther K. Identification and differential expression of a novel alternative splice isoform of the beta A4 amyloid precursor protein (APP) mRNA in leukocytes and brain microglial cells. *J Biol Chem.* 1992; 267:10804–10809. [PubMed: 1587857]
- Li QX, Fuller SJ, Beyreuther K, Masters CL. The amyloid precursor protein of Alzheimer disease in human brain and blood. *J Leukocyte Biol.* 1999; 66:567–574. [PubMed: 10534110]
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{−(Delta Delta C(T))} Method. *Methods.* 2001; 25:402–408. [PubMed: 11846609]
- Lublin AL, Gandy S. Amyloid-beta oligomers: possible roles as key neurotoxins in Alzheimer's Disease. *Mt Sinai J Med.* 2010; 77:43–49. [PubMed: 20101723]
- Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's disease: plasma Aβ40 and Aβ42, and genetic variants. *Neurobiol Aging.* 2011; 32:S10–S19. [PubMed: 22078169]
- Mohamed A, Posse de Chaves E. Aβ internalization of neurons and glia. *Int J Alzheimer's Dis.* 2011;127984. [PubMed: 21350608]
- Mukaetova-Ladinska EB, Abdel-All Z, Dodds S, Andrade J, Alves da Silva J, Kalaria RN, O'Brien JT. Platelet immunoglobulin and amyloid precursor protein as potential peripheral biomarkers for Alzheimer's disease: findings from a pilot study. *Age Ageing.* 2012; 41:408–412. [PubMed: 22258116]
- Muller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog Neurobiol.* 2008; 85:393–406. [PubMed: 18603345]
- Muresan Z, Muresan V. A phosphorylated, carboxy-terminal fragment of beta-amyloid precursor protein localizes to the splicing factor compartment. *Hum Mol Genet.* 2004; 13:475–488. [PubMed: 14722157]
- Ohkawara T, Nagase H, Koh CS, Nakayama K. The amyloid precursor protein intracellular domain alters gene expression and induces neuron-specific apoptosis. *Gene.* 2011; 475:1–9. [PubMed: 21145952]
- Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci.* 2010; 13:812–818. [PubMed: 20581818]
- Prakash A, Levy DE. Regulation of IRF7 through cell type-specific protein stability. *Biochem Biophys Res Commun.* 2006; 342:50–56. [PubMed: 16472772]
- Roher AE, Esh CL, Kokjohn TA, Castano EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Daus ID, Kuo YM, Emmerling MR, Soares H, Quinn JF, Kaye J, Connor DJ, Silverberg NB, Adler CH, Seward JD, Beach TG, Sabbagh MN. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement.* 2009; 5:18–29. [PubMed: 19118806]
- Sandbrink R, Masters CL, Beyreuther K. Beta A4-amyloid protein precursor mRNA isoforms without exon 15 are ubiquitously expressed in rat tissues including brain, but not in neurons. *J Biol Chem.* 1994; 269:1510–1517. [PubMed: 8288617]
- Sandbrink R, Masters CL, Beyreuther K. APP gene family. Alternative splicing generates functionally related isoforms. *Ann NY Acad Sci.* 1996; 777:281–287. [PubMed: 8624099]
- Selkoe DJ, Podlisny MB, Joachim CL, Vickers EA, Lee G, Fritz LC, Oltersdorf T. β-amyloid precursor protein of Alzheimer disease occurs as 110-to 135-kilodalton membrane associated proteins in neural and nonneural tissues. *Proc Natl Acad Sci.* 1988; 85:7341–7345. [PubMed: 3140239]
- Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci.* 2007; 27:2866–2875. [PubMed: 17360908]
- Shimada H, Ataka S, Tomiyama T, Takechi H, Mori H, Miki T. Clinical course of patients with familial early-onset Alzheimer's disease potentially lacking senile plaques bearing the E693Δ mutation in amyloid precursor protein. *Dement Geriatr Cogn Disord.* 2011; 32:45–54. [PubMed: 21846988]

- Stieler J, Grimes R, Weber D, Gartner W, Sabbagh M, Arendt T. Multivariate analysis of differential lymphocyte cell cycle activity in Alzheimer's disease. *Neurobiol Aging*. 2012; 33:234–241. [PubMed: 20395014]
- Sultana R, Mecocci P, Mangialasche F, Cecchetti R, Baglioni M, Butterfield DA. Increased Protein and Lipid Oxidative Damage in Mitochondria Isolated from Lymphocytes from Patients with Alzheimer's Disease: Insights into the Role of Oxidative Stress in Alzheimer's Disease and Initial Investigations into a Potential Biomarker for this Dementing Disorder. *J Alzheimers Dis*. 2011; 24:77–84. [PubMed: 21383494]
- Takeda S, Sato N, Rakugi H, Morishita R. Plasma beta-amyloid as potential biomarker of Alzheimer disease: possibility of diagnostic tool for Alzheimer disease. *Mol Biosyst*. 2010; 6:1760–1766. [PubMed: 20567751]
- von Rotz RC, Kohli BM, Bosset J, Meier M, Suzuki T, Nitsch RM, Konietzko U. The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. *J Cell Sci*. 2004; 117:4435–4448. [PubMed: 15331662]
- Walsh DM, Klyubin I, Shankar GM, Townsend M, Fadeeva JV, Betts V, Podlisny MB, Cleary JP, Ashe KH, Rowan MJ, Selkoe DJ. The role of cell-derived oligomers of Abeta in Alzheimer's disease and avenues for therapeutic intervention. *Biochem Soc T*. 2005; 33:1087–1090.
- Zhao M, Su J, Head E, Cotman C. Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease. *Neurobiol Dis*. 2003; 14:391–403. [PubMed: 14678756]

Figure 1.

(A) Representative micrograph of epifluorescence APP immunoreactivity in peripheral blood leukocytes of an AD case. Note punctate and diffuse immunoreactivity seen in the cytoplasm as well as punctate immunoreactivity that seems to be in some nuclei. APP immunoreactivity in green; nuclear marker Hoechst stain in blue (60X magnification). Visual comparison of images obtained from AD and age matched or young control samples (not shown) did not reveal any discernable difference. (B) High power confocal image of APP immunoreactivity in peripheral blood leukocytes of an AD case. Image is a merged APP (green) and nuclear (red) 0.15 μm slice; same field, same slice in the series for both. Again, punctate and diffuse immunoreactivity is seen in the cytoplasm. Colocalization of APP in the nucleus appears yellow. Arrow depicts cell that is magnified in the boxed inset.

Figure 2.

Immuno electron microscopy for APP in Alzheimer peripheral blood leukocytes. The Calbiochem antibody against the C-terminus of APP (also shown in Figures 1 and 3) was used. (A) Arrow points to immuno gold particles in a ring-like configuration found within the nucleus. (B) Arrow points to immuno gold reactivity coupled with a cytoplasmic organelle. Boxed insets magnify the areas associated with immuno gold reactivity.

Figure 3.

Differential immunoreactivity of APP in brain and peripheral blood leukocytes. (A) Western blot with the Calbiochem antibody against the C-terminus of APP. This antibody will detect the soluble C-terminal fragments of APP as well as full length of isoforms APP₆₉₅, APP₇₅₁ and APP₇₇₀. (B) Western blot using Sigma antibody A8717 against the C-terminal fragment of APP. Specificity is equivalent to the Calbiochem antibody. Identical samples used for both blots at the protein loading amounts and antibody concentrations detailed in Table 1.

Figure 4.

Western blots demonstrating differential immunoreactivity of various APP processing enzymes in brain and peripheral blood leukocytes. Identical samples used for all blots at the protein loading amounts and antibody concentrations detailed in Table 1.

Figure 5.

Ratio of transcript expression of APP and APP processing enzymes in peripheral blood leukocytes relative to expression in brain. Values of 1 = similar expression level in brain and PBL. Values less than 1 = reduced expression in PBL relative to brain. Values greater than 1 = increased expression in PBL relative to brain.

Table 1

Antibodies and protein loading concentrations for Western blotting.

Antibody	Source	Catalogue #	Dilution	Brain, ug	Leukocytes, ug
APP, C-term	Calbiochem	171610	1:5000	20	20
APP, C-term	Sigma	A8717	1:1000	3.3	20
ADAM10	Calbiochem	PC528	1:1000	10	10
BACE1, C- term	Calbiochem	195111	1:1500	20	20
BACE2	Affinity Bioreagents	OPA1-01130	1:500	20	20
Nephrilysin	Chemicon	AB5458	1:1000	10	10
Nicastrin	Sigma	N 1660	1:2000	5	5
Presenilin-1, N-term	Zymed	71-1300	1:125	5	5

Table 2

Summary and comparison of TaqMan® assays used for qRT-PCR and antibodies for immunoblotting.

Target	TaqMan® Gene Expression Assay		Antibody		Both
	Assay ID	Context Sequence ^a	Location	Immunogen	
APP	Hs01552285_m1 ^b	TGCAGTATTGCCAAGAGTCTACCC	2–3 exon boundary near n-term	CKMQQNGYENPTYKFFEQMQM C-term (Calbiochem) KMQQNGYENPTYKFFEQMQM C-term (Sigma)	All should detect isoforms APP ⁷⁵¹ , APP ⁷⁷⁰ and APP ⁶⁹⁵
ADAM10	Hs00153853_m1	ACAGTGCAGTCCAAAGTCAAGGTCCT	11–12 exon boundary near C-term	PQRQRPRESYQMGHMR C-term	Only one known transcript
BACE1	Hs01121199_m1	CAGCGCTTGCCATGTGCACGATGAG	8–9 exon boundary C-term	CLRQHQHDDFADDISLLK C-term	Both should detect all four variants of BACE1
BACE2	Hs01048159_m1	CGAGCCCCCTGTGCAGAAATTGCAGG	7–8 exon boundary - iso C 8–9 exon boundary - iso A both C-term	CQRRPRDPEVVNDESS C-term	Both should detect isoforms A and C
Neprilysin	Hs01115447_m1	CTGAGGGGTCACGATTTTAGGTGAT	1–2 exon boundary Variant 1 only	Reference transcript variant 1	Both should detect variant 1
Nicastrin	Hs00950933_m1 ^c	TACGGAAACAGGTGGAGGATCTCCT	10–11 exon boundary near C-term	C-term; amino acids 693–709	Both should detect the only currently determined variant.
Presenilin-1	Hs00240518_m1	GCGGCGGGGAAAGCGTATACCTAAATC	1–2 exon boundary N-term; Variants 1 and 2	N-term; amino acids 1–79	Both should detect variants 1 and 2
Beta-glucuronidase	Hs99999908_m1	TGAACAGTCACCGACGAGAGTGCTG	11–12 exon boundary	N/A	N/A

^aOnly the context sequence is specified by Applied Biosystems. The probe sequence is found within (or is) the context sequence.

^bAPP: Chose the n-term TaqMan® Assay since the c-term assays detected two additional variants.

^cNicastrin: Alternatively spliced variants have been described but, as of yet, their full-length nature and sequence have not been determined.