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BRAIN RESEARCH

Research Report

Expression of APP pathway mRNAs and proteins in Alzheimer's disease

Toshifumi Matsui[a], *Martin Ingelsson*[b], *Hiroaki Fukumoto*[a], *Karunya Ramasamy*[a], *Hisatomo Kowa*[a], *Matthew P. Frosch*[c], *Michael C. Irizarry*[a], *Bradley T. Hyman*[a,*]

[a]*Alzheimer Disease Research Unit, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Charlestown, MA 02129, USA*

[b]*Public Health and Caring Sciences, Uppsala Univ., Uppsala, Sweden*

[c]*C.S. Kubik Laboratory for Neuropathology, Massachusetts General Hospital, Boston, MA 02114, USA*

ARTICLE INFO

Article history:
Accepted 31 May 2007
Available online 5 June 2007

Keywords:
Alzheimer's disease
APP
Aβ
Kunitz
Microglia

ABSTRACT

In both trisomy 21 and rare cases of triplication of amyloid precursor protein (APP) Alzheimer's disease (AD) pathological changes are believed to be secondary to increased expression of APP. We hypothesized that sporadic AD may also be associated with changes in transcription of APP or its metabolic partners. To address this issue, temporal neocortex of 27 AD and 21 non-demented control brains was examined to assess mRNA levels of APP isoforms (total APP, APP containing the Kunitz protease inhibitor domain [APP-KPI] and APP770) and APP metabolic enzymatic partners (the APP cleaving enzymes β-secretase [BACE] and presenilin-1 [PS-1], and putative clearance molecules, low-density lipoprotein receptor protein [LRP] and apolipoprotein E [apoE]). Furthermore, we evaluated how changes in APP at the mRNA level affect the amount of Tris buffer extractable APP protein and Aβ40 and 42 peptides in AD and control brains. As assessed by quantitative PCR, APP-KPI ($p=0.007$), APP770 ($p=0.004$), PS-1 ($p=0.004$), LRP ($p=0.003$), apoE ($p=0.0002$) and GFAP ($p<0.0001$) mRNA levels all increased in AD, and there was a shift from APP695 (a neuronal isoform) towards KPI containing isoforms that are present in glia as well. APP-KPI mRNA levels correlated with soluble APPα-KPI protein (sAPPα-KPI) levels measured by ELISA ($\tau=0.33$, $p=0.015$ by Kendall's rank correlation); in turn, soluble APPα-KPI protein levels positively correlated with Tris-extractable, soluble Aβ40 ($p=0.046$) and 42 levels ($p=0.007$). The ratio of soluble APPα-KPI protein levels to total APP protein increased in AD, and also correlated with GFAP protein levels in AD. These results suggest that altered transcription of APP in AD is proportionately associated with Aβ peptide, may occur in the context of gliosis, and may contribute to Aβ deposition in sporadic AD.

1. Introduction

The 40 or 42 amino acid amyloid β protein (Aβ40, Aβ42), a product of amyloid precursor protein (APP) metabolism, accumulates in the AD brain (Selkoe, 1999). APP gene is alternatively spliced to produce three major APP mRNA species. APP751 and APP770 contain a Kunitz-type serine protease inhibitor domain (APP-KPI), and APP695 lacks this domain. APP-KPI isoforms are present in both neurons and glia, whereas the APP695 isoform is expressed primarily in neurons.

* *Corresponding author*. Fax: +617 724 1480.
E-mail address: bhyman@partners.org (B.T. Hyman).

0006-8993/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.brainres.2007.05.050

Increased expression of APP due to trisomy 21 (Rumble et al., 1989) or to triplication of the APP gene itself (Rovelet-Lecrux et al., 2006) leads to increased amyloid deposition. Although earlier studies did not demonstrate a change in APP mRNA levels in AD, an increase in KPI containing isoforms of APP protein levels has been reported (Harrison et al., 1996; Moir et al., 1998; Preece et al., 2004). We therefore re-examined the relationship of APP mRNA levels, APP protein, and the expression of metabolically related molecules in sporadic AD using sensitive quantitative polymerase chain reaction (qPCR) methods and ELISA assays.

Generation of Aβ depends to a great extent on a sequential co-ordinated pathway (Haass et al., 1992) (Koo and Squazzo, 1994) (Soriano et al., 1999) in which APP interacts in the Golgi, endoplasmic reticulum, cell surface and/or endocytic compartments with the APP cleaving enzyme β-secretase (BACE), which cleaves the N-terminal domain of APP (Huse et al., 2000) (Vassar et al., 1999) (Sinha et al., 1999). The truncated stub, referred to as C99, includes the transmembrane domain which then interacts with γ-secretase (including the genetically implicated molecule presenilin-1 [PS-1]) to release Aβ from the membrane (Selkoe, 2001). Trafficking partners that mediate APP subcellular interactions include low-density lipoprotein receptor protein (LRP) (Kang et al., 2000) (Van Uden et al., 2000a), which co-traffics with APP (Kinoshita et al., 2001). The LRP is a neuronal receptor for apolipoprotein E (apoE) and α2-macrogloblin, also implicated in the pathogenesis of AD (Blacker and Tanzi, 1998) (Strittmatter et al., 1993) (Rebeck et al., 1993) (Van Uden et al., 2000b).

In the present study, we examined temporal neocortex to examine whether mRNA levels of APP and close APP metabolic enzymatic partner molecules including BACE, PS-1, LRP and apoE are altered in AD, and to examine whether there is any evidence of co-regulation of these genes in AD or control brain. Furthermore, we reasoned that possible alterations in APP isoforms could be related to pathological brain features such as neuronal cell loss or gliosis; we therefore assessed mRNA levels of neuron-specific enolase (NSE) or synaptophysin, reflecting neuronal density or synapses, and glial fibrillary acidic protein (GFAP) as a marker for astrocytosis (Gutala and Reddy, 2004; Ingelsson et al., 2004; Kim et al., 2000). The total amount of APP mRNA in AD and control brain did not differ, but there was a shift in AD brain away from the APP695 isoform towards KPI containing isoforms, which correlated with NSE mRNA levels. By contrast, APP-KPI mRNA levels increased about two-fold in AD and correlated well with glial fibrillary acidic protein (GFAP) mRNA levels. APP-KPI mRNA levels correlated with soluble APPα-KPI protein levels, which also, in turn, correlated with Tris extractable (soluble) Aβ40 and 42 in AD brain.

2. Results

2.1. mRNA levels of total APP, APP-KPI and APP metabolic enzymatic partners molecules in AD and control brains

mRNA levels measured by the quantitative PCR method are summarized in Table 1. As expected, NSE mRNA levels decreased in AD, while GFAP mRNA levels increased in AD (Table 1 and Fig. 1). Differences in total APP mRNA levels between AD and Control brains were not statistically significant. By contrast, APP-KPI, APP-KPI ratio, APP770, PS-1, LRP and apoE mRNA levels were increased in AD brain. No significant difference was noticed in BACE mRNA levels between disease and control brains. ApoE4 genotyping was determined on 25 of the AD brains and on 10 of the control brains. The presence or absence of an apoE ε4 allele did not impact the expression of any of the mRNA levels studied here (not shown).

Table 1 – mRNA levels of APP metabolic partners

mRNA levels	AD (n=27)	Controls (n=21)	p-value*
Total APP	1.45±1.37	1.03±0.46	0.410
APP-KPI	0.94±0.79	0.54±0.27	0.007
APP-KPI ratio**	0.75±0.30	0.56±0.27	0.0146
APP770	0.20±0.21	0.08±0.05	0.004
PS-1	0.12±0.08	0.08±0.07	0.004
LRP	0.20±0.14	0.09±0.04	0.003
ApoE	1.76±1.79	0.53±0.39	0.0002
BACE	0.42±0.22	0.36±0.23	0.241
GFAP	5.00±5.67	1.00±1.33	<0.0001
NSE	0.10±0.05	0.17±0.10	0.004
Synaptophysin	0.17±0.24	0.38±0.42	0.037

Values are mean±S.D. Each value was standardized by GAPDH. *Mann–Whitney U test; **APP-KPI ratio was calculated by dividing APP-KPI by total APP.

To understand whether the mRNA changes reflected gliosis or neuronal loss, we next examined whether mRNA levels correlated with either GFAP mRNA levels (reflecting gliosis), or NSE or synaptophysin mRNA levels, reflecting neuronal depopulation. As shown in Table 2, in AD brain APP770, PS-1, and LRP mRNA levels positively correlated with GFAP mRNA levels. These correlations were not observed in control brains. By contrast, in control brain total APP and APP-KPI mRNA levels positively correlated with NSE mRNA levels. These results support a shift in APP mRNA, especially KPI and APP770 isoforms, from neuronal to glial populations in AD.

2.2. Soluble APPα protein levels by ELISA and their correlations with APP mRNA levels in AD brains

Soluble sAPPα protein levels were assessed in AD and control brain (Table 3). Soluble APPα-KPI protein levels correlated significantly with APP-KPI mRNA levels in AD and control brain. The elevated APP-KPI mRNA in AD brain was mirrored by 14% higher mean soluble APPα and sAPPα-KPI protein levels in AD, although the increase was not significant (Fig. 2).

2.3. Soluble sAPPα-KPI protein levels correlate with Tris extractable Aβ40 and Aβ42 in AD brain

In AD brain, soluble APPα-KPI protein levels positively correlate with their downstream metabolites, Tris extractable soluble Aβ40 and 42 (Fig. 3). Soluble sAPPα-KPI protein levels also correlate with GFAP protein levels (τ=0.26, p=0.009), but not with synaptophysin protein levels (τ=0.09, p=0.18), supporting the glial contribution to Tris soluble sAPPα-KPI protein as well as mRNA levels.