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A novel alternative splice variant of nicastrin and its implication in Alzheimer disease

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Abstract

Nicastrin interacts with γ-secretase complex components predominantly via the N-terminal third of the transmembrane domain. The authentic transmembrane domain is critically required for the interaction with γ-secretase complex components and for formation of an active γ-secretase complex. In this study, we have identified a novel alternatively spliced transcript of nicastrin in human brain tissue. This transcript (NCSTN-ΔE16) lacks exon 16 of nicastrin mRNA, which leads to deletion of 71 amino acids just upstream of its transmembrane domain. Its expression pattern was analyzed in the hippocampus of patients with pathologically diagnosed Alzheimer disease (cases) and non-Alzheimer dementia (controls). In patients with the APOE-ε4 allele, the frequency of Alzheimer disease appeared to be increased in the NCSTN-ΔE16-positive group, but the association was not statistically significant. In conclusion, the expression of NCSTN-ΔE16 transcript may confer some additional risk for developing Alzheimer disease beyond the risk due to ApoE-ε4 allele. Further investigation in larger scale population would be necessary to address its potential implication in Alzheimer disease.

Keywords: Alzheimer disease; Nicastrin; Apolipoprotein E; Alternative splicing

Introduction

Accumulation of amyloid plaques in the brain is a key component of the pathology of Alzheimer disease (AD). Amyloid β-peptide (Aβ), the main component of amyloid plaques, is released from the β-amyloid precursor protein by β- and γ-secretases (Hardy and Selkoe, 2002). Recent studies revealed that nicastrin is a component of γ-secretase complex, which also contains presenilin-1/presenilin-2, APH-1 and PEN-2 (Takasugi et al., 2003).

Yu et al. first reported that artificial deletion mutants of the conserved hydrophilic DYIGS domain in nicastrin decreased Aβ production, whereas a double-missense mutation (D336A+Y337A) increased Aβ production (Yu et al., 2000). Capel et al. reported that a decrease of nicastrin expression by RNAi in HEK293 cells was accompanied by reduced expression of presenilin-1, APH-1aL, and PEN-2 and reduced Aβ generation. Overexpression of wild-type nicastrin restored their reductions, while expression of nicastrin lacking the transmembrane domain did not (Capell et al., 2003). These results suggest that nicastrin plays an important role in activation of γ-secretase complex, production of Aβ peptide and onset of Alzheimer disease.

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Materials and methods

Subjects

All subjects were Japanese ($n=23$, 74% female, all clinically demented, age range at death 69–98 years). They were inpatients at Fukushimura Hospital (Toyohashi, Aichi, Japan), and were cognitively evaluated by neuropsychological tests such as the Mini-Mental State Examination during hospitalization.

Treatment of autopsied brain

When they died, autopsy and pathological diagnosis were carried out according to the criteria of the Consortium to Establish a Registry for Alzheimer's disease (Mirra et al., 1991). Written consent of the patients' guardians for diagnosis and biochemical, molecular biological and genomic research was obtained. The autopsied brain was weighed, and cut midsagitally. One half of the brain was divided into several portions (frontal, temporal, parietal, occipital cortex, hippocampus, etc.), snapped frozen in liquid nitrogen, and stored at −80 °C. The other half was fixed and used for pathological diagnosis, as described previously (Akatsu et al., 2002). Based on this pathological diagnosis, subjects were divided into AD group and non-Alzheimer dementia (non-AD) group.

Genotyping

APOE genotyping was performed using DNA samples extracted from dissected brain tissues, according to the procedure described previously (Yoshiiwa et al., 1997).

Screening for novel splicing variants and sequencing

Total RNA was extracted from the frozen hippocampus using Trizol (Invitrogen, Carlsbad, CA, USA.), according to the manufacturer's protocol, and first strand cDNAs were synthesized from 5 μg total RNA with an oligo$(dT)_{12-18}$ primer using 50 units superscript II RNase H^- reverse transcriptase (Invitrogen) in a total volume of 20 μl, according to the manufacturer's protocol. The cDNAs were diluted at 1:5 with distilled water, and then 2 μl was used as a template for PCR with Platinum Taq DNA polymerase (Invitrogen) and the sense and anti-sense primers listed in Table 1. Sequencing was performed by direct sequencing method with a dye terminator cycle sequencing FS kit (PE Biosystems) following the manufacturer's protocol.

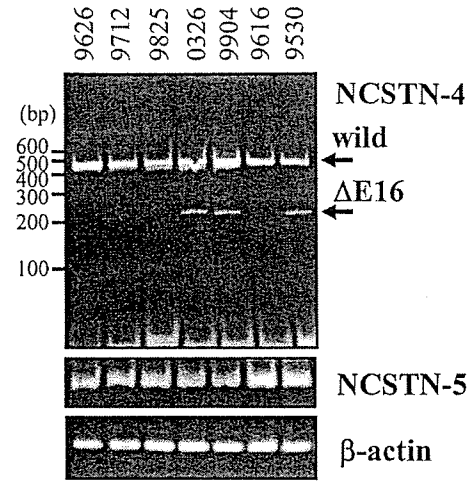

Fig. 1. Identification of a novel alternatively spliced variant of NCSTN. Upper panel; RT-PCR from human hippocampus with primers, NCSTN4-F and NCSTN4-B. Note the 427 bp band (wild-type) exists in all patients, while the 214 bp band (ΔE16) exists in some patients (0326, 9904, and 9530). Middle panel; RT-PCR from hippocampus with primers, NCSTN5-F and NCSTN5-B (see Table 1) as intra-molecular control. Lower panel; RT-PCR from hippocampus with β-actin primers as external control.

Reverse-transcription PCR (RT-PCR)

RT-PCR was performed with the cDNAs from the hippocampus, the primers; NCSTN4-F and NCSTN4-B, and

Table 1
Primers used for screening for splicing variants of nicastrin

	Name	Sequences	Position
Sense primer	NCSTN1-F	GCTAACAGACAGGAGCCGAACG	94–115
	NCSTN2-F	TGGGCAATGGTTTGGCTTATG	642–662
	NCSTN3-F	GAGAAGAGTGGTGCTGGTGTCC	1346–1367
	NCSTN4-F	GCCCCACCAACACCACTTATG	1818–1838
	NCSTN5-F	TGGACTGAGAGCCGCTGGAAAG	2084–2105
	NCSTN6-F	GGGTTCCTGATTAAAGCCAACAAC	1712–1735
	NCSTN7-F	TCATGGTTCCAGTCTATCCTCAGG	1736–1759
	NCSTN8-F	GCCTTGTCTCCTGCCTTTGAAC	2030–2051
Anti-sense primer	NCSTN1-B	CTTCATAAGCCAAACCATTGCC	665–644
	NCSTN2-B	TGAGGATGACAGCAGGGACACC	1382–1361
	NCSTN3-B	AAGTGGTGTTGGTGGGGCTGGAGAC	1835–1811
	NCSTN4-B	GGAGCAATGAAAAGGACATCAGC	2244–2222
	NCSTN5-B	AGCACGCCCACCCTAATGTG	2806–2787
	NCSTN6-B	GCATTGATGCAGTAGGTGACGATG	2217–2194
	NCSTN7-B	CAGTGGGACAGATGCTCTAGGAAG	2333–2310
	NCSTN8-B	CTGAAGGGCAAATTAGGGTGG	2584–2564
	NCSTN9-B	AAAAGTAGAAGGGTCCTGAAGGG	2600–2577

The number depicts the position of the sequence in NCSTN cDNA (Genbank Accession # AF240468).

Platinum Taq DNA polymerase at 95 °C for 0.5 min, 55 °C for 1 min, at 72 °C for 1 min, for 40 cycles.

Statistical analysis

AD group (cases) and non-AD group (controls) were further divided by the presence of APOE-ε4 allele into APOE-ε4-positive and APOE-ε4-negative groups. The frequency of NCSTN-ΔE16 transcript was compared between AD and non-AD groups by χ^2 analysis. Differences with p values of <0.05 were considered significant.

Results

With the primers, NCSTN4-F and NCSTN4-B, two major bands were detected in some brain samples (Fig. 1). The most frequent was a 427 bp band (wild-type), followed by a 214 bp band. Sequencing analysis revealed that this latter transcript was an in-frame splicing variant that lacks exon 16, and it was designated "NCSTN-ΔE16". The exact result of sequencing analysis is described in Fig. 2A. The schematic structure of NCSTN-ΔE16 is illustrated in Fig. 2B.

Out of the 23 patients examined in this study, 10 were diagnosed pathologically with AD (mean age at onset of dementia: 72.2±7.4 years, mean age at death: 81.7±8.2 years, mean brain weight at death: 1024±105 g) and 13 were diagnosed as non-AD (mean age at onset of dementia: 83.2±8.4 years, mean age at death: 89.2±7.5 years, mean brain weight at death: 1099±109 g). Non-AD included normal physiological aging, multiple infarctions, diffuse Lewy-body disease, Parkinson disease, etc. (data not shown). 13 patients carried the APOE-ε4 allele (APOE-ε4-positive group), while

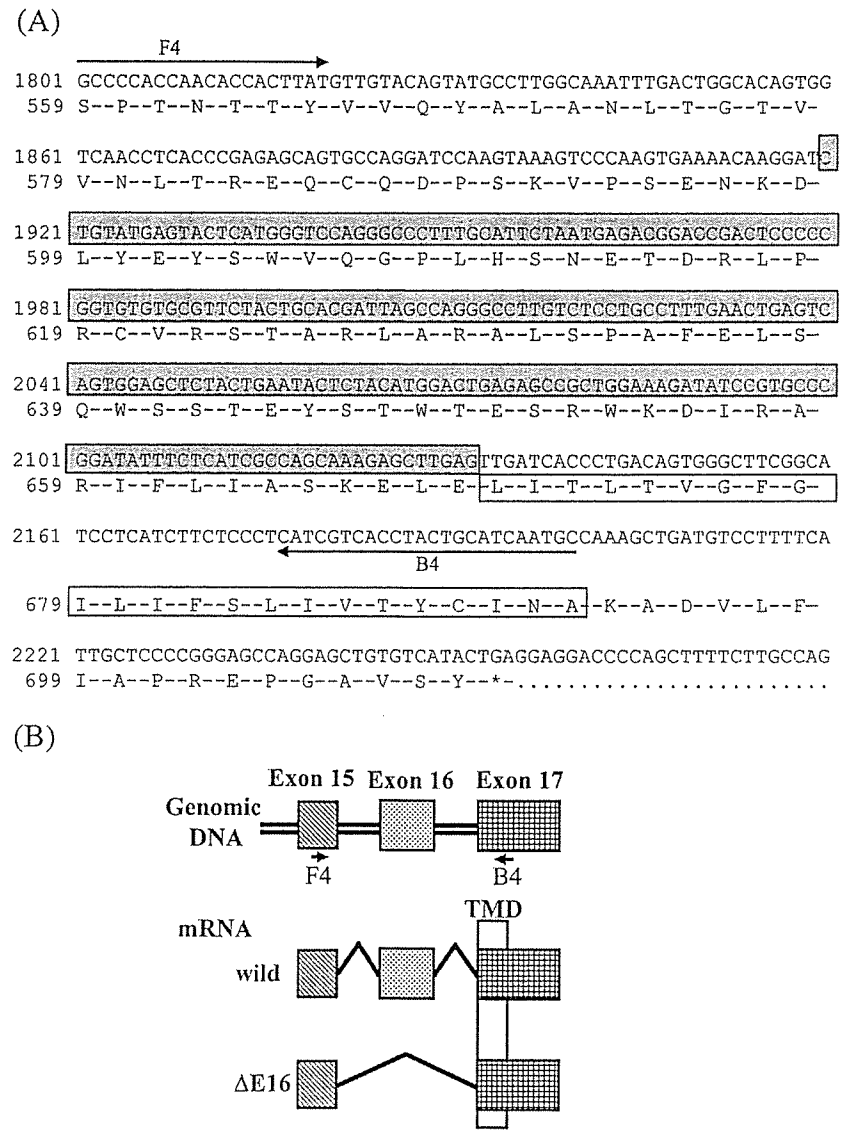

Fig. 2. Structure of the splicing variant of NCSTN. (A) Sequencing analysis revealed that NCSTN-ΔE16 is an in-frame splicing variant lacking exon 16. Open box corresponds to amino acid sequence of the transmembrane domain. Gray box corresponds to exon 16 sequence, which is deleted in NCSTN-ΔE16. F4 (NCSTN4-F) and B4 (NCSTN4-B) are the primers used to detect NCSTN-ΔE16. (B) Schematic representation of NCSTN gene and its wild-type (wild) and alternatively spliced transcript (ΔE16).

Table 2
Summary of the characteristics of AD and non-AD subjects by detection of NCSTN-ΔE16 transcript

	Pathological diagnosis		
	Overall	AD	Non-AD
Age at onset of dementia			
NCSTN-ΔE16(−)	79.5±9.9(11)	71.5±9.1(4)	84.0±7.4(7)
NCSTN-ΔE16(+)	75.6±9.3(9)	72.7±7.0(6)	81.3±12.1(3)
P value	0.38	0.84	0.75
Age at death			
NCSTN-ΔE16(−)	85.8±9.5(12)	79.5±9.3(4)	88.9±8.4(8)
NCSTN-ΔE16(+)	86.2±7.8(11)	83.2±7.9(6)	89.8±6.7(5)
P value	0.90	0.54	0.83
Brain weight at death			
NCSTN-ΔE16(−)	1071±96(12)	1073±138(4)	1071±80(8)
NCSTN-ΔE16(+)	1060±131(11)	991±71.6(6)	1143±144(5)
P value	0.81	0.34	0.35

Values are means±S.D. (number of cases). No significant difference was detected between NCSTN-ΔE16(−) and NCSTN-ΔE16(+) groups. AD: Alzheimer disease, non-AD: non-Alzheimer dementia.

10 patients did not (APOE-ε4-negative group). RT-PCR analysis with the primers, NCSTN4-F and NCSTN4-B, detected the wild-type NCSTN transcript in the hippocampus in all 23 patients, while it also detected NCSTN-ΔE16 in 11 patients.

The age at onset of dementia, age at death and brain weight at death were not significantly different between NCSTN-ΔE16(−) group and NCSTN-ΔE16(+) group in overall, AD, and non-AD patients, as described in Table 2. NCSTN-ΔE16 transcript was detected in 6 out of 10 AD patients and 5 out of 13 non-AD patients. The difference in the frequency of NCSTN-ΔE16 transcript between AD cases and non-AD controls was not significant ($p=0.55$) (Table 3). When analysis was limited to APOE-ε4-negative patients, the NCSTN-ΔE16 transcript was detected in 1 out of 2 AD patients, and 4 out of 8 non-AD patients. The frequency of NCSTN-ΔE16 transcript was not significantly different between AD patients and non-AD patients, either ($p=1.00$). Likewise, when analysis was limited to APOE-ε4-positive patients, the NCSTN-ΔE16 transcript was detected in 5 out of 8 AD patients, and 1 out of 5 non-AD patients. The frequency of NCSTN-ΔE16 transcript was not significantly different between AD patients and non-AD patients, either ($p=0.35$).

Discussion

Assembly of nicastrin into γ-secretase complex is essential for activation of γ-secretase and generation of Aβ. In molecular and cellular biological studies, Capell et al. reported that nicastrin interacts with γ-secretase complex components predominantly via the N-terminal third of the transmembrane domain (670–692 amino acids). The authentic transmembrane domain of nicastrin is critically required for the interaction with γ-secretase complex components and for formation of an active γ-secretase complex (Capell et al., 2003).

In this study, in the human hippocampus, we identified a novel alternatively spliced transcript lacking exon 16, which encodes the 71 amino acid sequence just upstream of this functional transmembrane domain (see Fig. 2A). This transcript was detected in some patients, but not in others. The cause of this dissociation is unknown. It is not clear if this endogenous deletion may affect the function of nicastrin and the activity of γ-secretase in the human brain or even in vitro. Change in the activity of γ-secretase may influence the risk of AD. Accordingly, the implications of the expression of this transcript and AD pathology were examined here.

When we analyzed overall patients, the difference in the frequency of NCSTN-ΔE16 transcript between AD cases and non-AD controls was not significant. As described in most other studies, APOE-ε4 allele is a major risk factor for developing AD. It is estimated to account for about 40–50% of the genetic variation in late-onset AD (Roses, 1996). To examine the association between the existence of NCSTN-ΔE16 transcript and the development of AD independently of APOE genotype, we further categorized AD and non-AD patients by the presence of APOE-ε4 allele into APOE-ε4-negative and APOE-ε4-positive groups. In APOE-ε4-negative group, the difference between AD cases and non-AD controls was not significant, either. In APOE-ε4-positive group, the frequency of NCSTN-ΔE16 transcript appeared to be higher in AD cases than in non-AD controls. However, the association was not statistically significant because of the small population size. This suggests the possibility of interaction between NCSTN-ΔE16 and APOE-ε4, so that NCSTN-ΔE16 only influences risk if an individual carries APOE-ε4; however, statistical tests for interaction were not significant.

Several genetic studies have focused on the association between nicastrin polymorphisms and the onset of AD. Helisalmi et al. reported that one haplotype of nicastrin significantly increased the risk of AD in patients without an APOE-ε4 allele in the Finnish population (Helisalmi et al., 2004). Dermaut et al. reported that one SNP haplotype of nicastrin is increased in patients with familial early-onset AD without the APOE-ε4 allele in the Dutch population (Dermaut et al., 2002). On the contrary, Orlacchio et al. (2004) and Cousin et al. (2003) reported no such associations. Thus, there is disagreement in opinion, and further investigation of this matter is necessary.

In conclusion, the expression of NCSTN-ΔE16 transcript may confer some additional risk for developing Alzheimer disease beyond the risk due to ApoE-ε4 allele. Further

Table 3
The number of subjects with or without NCSTN-ΔE16 transcript in overall AD and non-AD patients, and by ApoE genotype subgroups

	Overall[a]		APOE-ε4-negative[b]		APOE-ε4-positive[c]	
	AD	Non-AD	AD	Non-AD	AD	Non-AD
	($n=10$)	($n=13$)	($n=2$)	($n=8$)	($n=8$)	($n=5$)
NCSTN-ΔE16(−)	4	8	1	4	3	4
NCSTN-ΔE16(+)	6	5	1	4	5	1

a: $\chi^2=0.36$, $p=0.55$, b: $\chi^2=0.00$, $p=1.00$, c: $\chi^2=0.85$, $p=0.35$.

investigation in larger scale population would be necessary to address its potential implication in AD.

Acknowledgements

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Original Article

Vascular complications in dementia with Lewy bodies: A postmortem study

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The effects of cerebrovascular lesions on DLB are not yet fully understood, whereas the development of Alzheimer's disease (AD) is known to be associated with cerebrovascular lesions. In this study, we investigated the frequency of concomitant cerebrovascular pathologies in autopsy-proven DLB cases ($n = 25$) in comparison with AD cases ($n = 63$). We also investigated the correlation between cerebrovascular pathologies and the clinical features of DLB cases. On gross inspection, five cases of DLB and seven cases of AD were complicated by cerebral hemorrhages and the difference was significant; most of the lesions in DLB were subdural hemorrhages, possibly related to trauma. Nine cases of DLB and 25 cases of AD had grossly identified infarctions, but no significant difference was observed. Three cases of DLB and four cases of AD had concomitant hemorrhages, while 10 cases of DLB and 43 cases of AD had infarcts on microscopic inspection. There was a significant difference in the frequency of microscopic infarcts between DLB and AD, whereas no significant difference was noted in the frequency of microscopic hemorrhages. In DLB cases without vascular complications, memory disturbance was common as the initial symptom, while parkinsonism was more common in those with vascular complications. However, no significant difference was observed between DLB cases with and without vascular complications with respect to the frequency of individual clinical symptoms over the whole clinical course. These findings suggest that grossly identified hemorrhages are more common in DLB because of trauma, while microinfarcts are less common in DLB than AD, although the reason remains unclear. Such vascular complications might affect the clinical manifestations, in particular, the initial symptom, of DLB.

Key words: Alzheimer's disease, cerebral hemorrhage, cerebral infarction, dementia with Lewy bodies, vascular complication.

INTRODUCTION

Although neuronal degeneration due to neurofibrillary tangle and senile plaque formation is considered to be central in the pathogenesis of Alzheimer's disease (AD), the development of the disease might also be associated with cerebrovascular lesions. Alzheimer's disease and vascular dementia share some of the risk factors for atherosclerosis, such as diabetes mellitus or hypertension,[1] and a history of cerebral infarction might increase the incidence of AD by as much as 50%.[2] Atherosclerosis, as indicated by vessel wall thickness and plaques in the carotid arteries, has been shown to be associated with the development of AD.[3] Cerebrovascular lesions also play an important role in determining the appearance and severity of the symptoms of AD.[4] These findings strongly support the hypothesis that cerebrovascular lesions are implicated in the development of AD, at least in part.

However, the effects of cerebrovascular lesions on other neurodegenerative diseases are not yet fully understood. Several studies have investigated the relationship between stroke and Parkinson's disease (PD), but the results are conflicting as some show a reduced risk of ischemic and hemorrhagic stroke, while others indicate an increased

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likelihood of stroke-related death.[5–11] Interestingly, Jellinger recently investigated the prevalence of vascular lesions in DLB, the second most frequent cause of degenerative dementia, and showed that the frequency was lower in DLB than in either PD or a control.[12]

In the present study, we investigated the frequency of concomitant cerebrovascular pathologies in autopsy-proven DLB cases in comparison with AD cases. We also compared the clinical features of DLB cases with and without vascular complications on the hypothesis that vascular complications might affect the clinical course of DLB.

METHODS

Brains from 25 cases with DLB (Braak tangle stages ranging from 2–4,[13] 12 males and 13 females, age at death between 67 and 94 years, mean = 80.8 ± 6.6 years) and 63 age-matched and sex-matched cases with AD (Braak tangle stages 5 and 6, 22 males and 41 females, age at death between 67 and 94 years, mean = 83.2 ± 6.2 years) were employed in this study, which was approved by the Human Subjects Review Committees of both Yokohama City University and Fukushimura Hospital. For routine neuropathological evaluation, formalin-fixed, paraffin-embedded sections from each area of the brain were stained with HE, KB, and methenamin-silver stains. In addition, α-synuclein immunostaining was routinely used to detect Lewy bodies. All cases of DLB fulfilled the postmortem criteria for DLB:[14] 12 cases were the neocortical-type and 13 cases were the transitional-type. All AD cases fulfilled the criteria of definite AD, using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol,[15] with neurofibrillary changes ranging from Braak tangle stages 5–6.

One hemisphere of the brain was fixed in 4% paraformaldehyde in a 0.1 mol/L phosphate buffer after autopsy, while the other hemisphere was deep-frozen for biochemical analysis. Coronal slices of 1 cm thickness were cut through the hemisphere fixed in paraformaldehyde and transverse slices were cut through the brainstem and cerebellum. The brain slices were initially examined for the presence of vascular lesions, including cerebral hemorrhages and infarcts, by gross inspection. The slices were then embedded in paraffin and cut into 7-μm-thick sections for microscopic examination; sections were taken every 1 cm from that hemisphere. The presence of vascular lesions was microscopically investigated, mainly using sections stained with HE and by the KB method.

The clinical symptoms of all cases were assessed through an evaluation of clinical records with respect to the presence of vascular risk factors including hypertension, hyperlipidemia, heart disease, diabetes mellitus, and tobacco use. The presence of visual hallucinations, delusions, wandering, and parkinsonism also was investigated in the DLB cases. Cognitive fluctuation, one of the core features of DLB, was not evaluated in the present study because it was difficult to assess this symptom retrospectively from the clinical records.

The difference in brain weight between DLB and AD cases and the difference in the frequency of vascular complications between DLB cases with mild neurofibrillary pathology (Braak tangle stage 2 or 3) and those with relatively severe pathology (Braak tangle stage 4) were analyzed by two-sample t-tests or χ^2-tests. The difference in the frequency of clinical symptoms between DLB with and without vascular lesions also was evaluated by χ^2-tests. Logistic regression was used to evaluate the differences between DLB and AD in age, onset and duration of the disease, and frequency of hemorrhages, infarctions, and vascular risk factors. The data were analyzed using SPSS for Windows, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Concomitant vascular risk factors for dementia with Lewy bodies and Alzheimer's disease

As some reports have argued that the difference in frequency of vascular complications between PD and AD might be related to the difference in concomitant vascular risk factors,[5] the difference in the frequency of the risk factors for DLB and AD was assessed (Tables 1,2). In the present study, there was no difference in the frequency of the major vascular risk factors, including hypertension (odds ratio [OR] = 1.18, $P = 0.80$), hyperlipidemia (OR = 1.09, $P = 0.96$), diabetes mellitus (OR = 0.66, $P = 0.68$), and tobacco use (OR = 1.36, $P = 0.62$).

Frequency of vascular complications in dementia with Lewy bodies and Alzheimer's disease

On gross inspection, five cases (20%) of DLB and seven cases (11%) of AD were complicated by cerebral hemorrhages, and most of the lesions were subdural hemorrhages possibly related to trauma (Tables 1,2). Nine cases (36%) of DLB and 25 cases (40%) of AD had grossly identified infarctions. There was a significant difference between DLB and AD in the frequency of hemorrhages (OR = 6.44, $P = 0.03$), while the difference was not significant with respect to infarctions (OR = 1.96, $P = 0.39$). Three cases (12%) of DLB and four cases (6%) of AD had concomitant hemorrhages, while 10 cases (40%) of DLB and 43 cases (68%) of AD had infarcts on microscopic inspection. The cases with hemorrhages on gross inspection outnum-

Table 1 Backgrounds, vascular risk factors, and vascular complications in dementia with Lewy bodies (DLB) and Alzheimer's disease (AD)

Variable	DLB ($n = 25$)	AD ($n = 63$)
Age at death (years)	80.8 ±6.6	83.2 ±6.2
Age of onset (years)	75.1 ±6.9	75.4 ±7.4
Duration (months)	69.4 ±50.5	92.8 ±55.1
Hypertension	11 (44%)	27 (43%)
Hyperlipidemia	2 (8%)	2 (3%)
Heart disease	4 (16%)	11 (17%)
Diabetes mellitus	2 (8%)	10 (16%)
Tobacco use	10 (40%)	18 (29%)
Brain weight	1164 ±124	1048 ±118
Gross hemorrhage	5 (20%)	7 (11%)
Gross infarction	9 (36%)	25 (40%)
Microscopic hemorrhage	3 (12%)	4 (6%)
Microscopic infarction	10 (40%)	43 (68%)

Table 2 Logistic regression analysis of the differences between dementia with Lewy bodies and Alzheimer's disease

Factor	*P*-value	Odds ratio	95%CI
Basic factors			
Age at death (years)	0.11	0.33	0.85–1.27
Age of onset (years)	0.12	2.90	0.76–11.1
Duration (months)	0.17	1.08	0.97–1.21
Risk factors			
Hypertension	0.80	1.18	0.34–4.12
Hyperlipidemia	0.96	1.09	0.05–24.2
Heart disease	0.72	0.72	0.12–4.43
Diabetes mellitus	0.68	0.66	0.10–4.52
Tobacco use	0.62	1.36	0.40–4.65
Pathological factors			
Gross hemorrhage	0.03*	6.44	1.26–32.8
Gross infarction	0.39	1.96	0.42–9.13
Microscopic hemorrhage	0.81	0.76	0.78–7.35
Microscopic infarction	0.02*	0.15	0.03–0.70

*$P < 0.05$.

Table 3 Clinical pictures of dementia with Lewy bodies cases with and without vascular complications

Variable	Vascular complication ($n = 17$)	Vascular complication ($n = 8$)	*t*-value	χ^2	*P*-value
Age at death (years)	80.5 ±7.5	81.5 ±4.2	−0.34	–	0.74
Age at onset (years)	74.4 ±7.4	77.8 ±4.6	−1.17	–	0.26
Duration (months)	79.9 ±55.0	48.6 ±40.4	1.42	–	0.18
Memory disturbance as the initial symptom	5 (29%)	6 (75%)	–	4.59	0.03*
Visual hallucinations	13 (76%)	5 (63%)	–	0.53	0.47
Delusions	10 (76%)	4 (50%)	–	0.17	0.68
Wandering	6 (35%)	4 (50%)	–	0.49	0.48
Parkinsonism	7 (41%)	4 (50%)	–	0.17	0.68

*$P < 0.05$.

bered those with hemorrhages on microscopic inspection, as most of the hemorrhages identified grossly were subdural hemorrhages and were not observed on thin sections. There was a significant difference in the frequency of microscopic infarcts between DLB and AD (OR = 0.15, $P = 0.02$), but no significant difference was found in the frequency of microscopic hemorrhages (OR = 0.76, $P = 0.81$). In the DLB cases, no significant difference in vascular complications was found between the cases with mild neurofibrillary pathology of a Braak tangle stage of 2 or 3 and those with relatively severe pathology, with a Braak tangle stage of 4 ($\chi^2 = 0.414$, $P = 0.52$).

Clinical symptoms of dementia with Lewy bodies with and without vascular complications

On the hypothesis that the core symptoms of DLB might be affected by vascular complications, such as hemorrhage and infarction, differences in the frequency of the core symptoms were compared between the DLB cases with and without vascular complications (Table 3). In contrast to our assumption, there was no significant difference in the frequency of parkinsonism ($\chi^2 = 0.17$, $P = 0.68$), wandering ($\chi^2 = 0.49$, $P = 0.48$), delusions ($\chi^2 = 0.17$, $P = 0.68$) or visual hallucinations ($\chi^2 = 0.53$, $P = 0.47$). Likewise, there was no significant difference in the age of onset ($t = -1.17$, $P = 0.26$) or duration of the disease ($t = 1.42$, $P = 0.18$). However, the frequency of the cases whose initial symptom was memory disturbance was 29% in DLB cases with vascular complications and 75% in those without vascular complications; this difference was significant ($\chi^2 = 0.59$, $P = 0.03$). Parkinsonism was more common as the initial symptom in DLB cases with vascular complications.

DISCUSSION

In the present study, we investigated the frequency of concomitant vascular pathologies in DLB and showed that the frequency of microinfarcts in DLB is lower than in AD. This result is consistent with the findings reported by Jellinger, who found the frequency of cerebrovascular lesions in DLB to be lower than in PD and AD.[12,16] No significant differences in the major vascular risk factors, including hypertension, hyperlipidemia, diabetes mellitus, and tobacco use, were found between DLB and AD in this study, although some reports have argued that the differ-

ence in frequency of vascular complications between PD and AD might be related to differences in concomitant vascular risk factors.[5] Our results indicate that the different frequency of vascular risk factors is not the only explanation for the difference in vascular complications observed in DLB and AD, although the reason remains unclear. However, the frequency of grossly indentified hemorrhages in DLB was higher than in AD; most of the lesions were subdural hemorrhages, possibly related to head trauma. This seems to be explained by the fact that falls are common among DLB patients, together with the exacerbation of parkinsonism.

The precise mechanism of less frequent concurrent microinfarcts in DLB is not known, but an increased frequency of vascular pathologies in AD seems to explain this discrepancy, at least in part. Amyloid deposition within cerebral vessels, or cerebral amyloid angiopathy (CAA), is common in advanced age and even more common in AD.[17] Although sporadic CAA is usually clinically silent, it can be associated with a number of clinical manifestations, including cerebral hemorrhage and infarction.[18–20] Other possible explanations of the higher frequency of vascular lesions in AD include vascular nitric oxide (NO) release disorder and nitric oxide synthase (NOS) dysfunction.[21] Chronic cerebral hypoperfusion resulting from aging and vascular risk factors, such as hypertension, hyperglycemia or hyperlipidemia, can stimulate a rapid release of NO via activation of NOS on the endothelium, inducing further basement membrane thickening. This progressive change in the microvasculature can then promote a decrease in glucose and oxygen delivery to neuronal and glial cells, which results in neurodegeneration, including the accumulation of abnormal proteins and neuronal cell death. This mechanism via NO is possibly involved in the development of AD pathologies,[22] although it remains unclear if this also promotes the development of DLB pathologies.

Despite the lower incidence of microinfarcts in DLB, the difference in frequency of large or gross infarcts was not significant between DLB and AD. This seems to be related to the differing frequency of gross infarcts and microinfarcts in AD; gross infarcts were less frequently found in AD cases. Although the specific risk factors for lacunar strokes are broadly similar to those in large ischemic stroke patients, it is likely that hypertension is significantly more common among lacunar stroke patients than among those with other forms of ischemic stroke.[23] The difference in vascular risk factors, however, was not significant between AD cases with gross infarction and those with microinfarction (data not shown). The reason for this discrepancy remains to be elucidated.

This study corroborated some clinical features of DLB. The mean duration of DLB tends to be shorter than AD. Studies comparing DLB and AD suggest that the mean duration of illness is shorter in DLB patients than in AD patients, although this is still controversial.[24] However, the mean brain weight was significantly greater in DLB than in AD. This is consistent with the results of neuroimaging studies showing the preservation of the hippocampus and medial temporal lobe volume in DLB.[25]

In DLB cases, vascular complications are associated with the initial symptoms, although no apparent association was found in this study between the clinical symptoms and vascular pathologies over the whole clinical course. Memory disturbance was common as the initial symptom in DLB cases without vascular complication, while parkinsonism was more common in those with vascular lesions. The basal ganglia are vulnerable to vascular changes in general and might be responsible for parkinsonism in DLB cases with vascular complications.

In conclusion, grossly identified hemorrhages, mainly related to trauma, were more common in DLB, while microinfarcts were less common in DLB than AD, although the reason remains unclear. These vascular complications might affect the clinical manifestations of DLB, in particular the initial symptoms: memory disturbance is seen in cases without vascular complications and parkinsonism in those with complications.

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Alzheimer's Disease – An Interactive Perspective

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Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disorder that is characterized by a progressive loss in memory and deterioration of the higher cognitive functions. The brain of an individual with AD exhibits extracellular senile plaques of aggregated amyloid-beta-peptide (Aβ), intracellular neurofibrillary tangles (NFTs) that consist of hyperphosphorylated tau protein (P-tau) and a profound loss of basal forebrain cholinergic neurons that innervate the hippocampus and the neocortex. Recent data obtained via genomics, proteomics and molecular genetics, have gleaned new information with regard to the physiological and pathophysiological functions of the amyloid precursor protein (APP) and its cleavage product Aβ. This review glances over several aspects that may play a major role in the pathogenesis of AD providing an insight into APP's and Aβ's interplay with other cellular systems.

Keywords: Alzheimer's disease, apoptosis, cell death, neurodegeneration.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by impaired memory and cognitive functions. Though several genetic defects have been identified in patients with a family history of this disease, the majority of AD cases is late-onset (over the age of 65) and involves individuals with no known genetic predisposition [1-2]. However, the discovery that the ε4 allele of apolipoprotein E (ApoE, with its gene localized to chromosome (Chr) 19) predisposes to AD, provides one major genetic risk factor for late-onset AD. Studies have correlated the inheritance of one or two ε4 alleles with the increased likelihood of developing AD, making its mean age of onset earlier as compared to subjects harboring ε2 and/or ε3 alleles [3-6].

AD is characterized predominantly by the presence of neuritic amyloid plaques, cerebrovascular amyloidosis and neurofibrillary tangles. Proteolytic processing of the amyloid precursor protein (APP, Chr21) generates the amyloid-beta peptide (Aβ) and has been implicated in the pathogenesis of AD. Evidence shows that mutations in the APP encoding gene and also in Presenilin-1 (PS-1, Chr14) and Presenilin-2 (PS-2, Chr1), which lead to early-onset AD, are associated with excess Aβ deposition in the brains of AD patients. However, the mechanism leading to the selective neurodegeneration of nerve cells and synaptic connections preceding the emergence of dementia in patients, particularly in sporadic AD, still remains elusive [1, 2, 7].

APP's proteolytic fragments, including Aβ and APP-C-terminal fragments (CTFs), have been reported to cause apoptosis suggesting that the deposition of Aβ is a central disease-causing event. However, the physiological function of APP and whether this function is related to the proteolytic processing of APP remain unclear. Interestingly, the metabolism of APP resembles that of Notch, a cell surface receptor essential for commitment during cell differentiation [8-10]. Infact, many type-I membrane proteins, including CD44, ErbB4, neuregulin-1, alcadein (Alc) and $p75^{NTR}$, have recently been found to be cleaved first at their extracellular juxtamembrane region and subsequently at an intramembrane region by a γ-secretase and cleavage of these type-I membrane proteins generates and releases their cytoplasmic domain, which is hypothesized, in synergy with other transcriptional regulatory factors, to play an important role in gene transactivation [10-13].

APP has insofar been localized to various membranous structures in the cell such as the endoplasmic reticulum and Golgi compartments, as well as to the cell membrane. After arriving at the cell membrane, APP is eventually re-internalized and subsequently subjected to lysosomal proteolytic events, although enzyme cleavage of APP also occurs in the Golgi and on the cell surface. Of paramount interest is that APP has been detected on the membranes of synaptic preparations and has been further localized to postsynaptic densities, axons and dendrites. In addition, APP is enriched at adhesion patches in close proximity to other proteins such as integrins, and a portion of APP is localized to unique cholesterol and GM1 ganglioside-rich membrane microdomains that are thought to be involved in signal transduction and proteolytic processing events [2, 7].

In the neuron as well, intracellular processing of APP, which leads to the formation and release of Aβ-peptide, occurs at all cellular sorting stations such as the endosomal system, the trans-Golgi network and the endoplasmic reticulum (ER) as well as at the cell surface. Aβ is also produced in axonal membrane compartments lacking any ER, Golgi or endosomal marker proteins. The concomitant release of APP's intracellular domain (AICD) followed by

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subsequent retrograde transport of AICD to the cell body could be a vital defense mechanism, transmitting either an injury signal that initiates neuroprotective gene transcription or to start a pro-apoptotic signal transduction pathway to mediate cell death in response to pathological accumulation of Aβ intracellularly [14]. Moreover, it is also possible that the proteolytic cleavage of APP might be a simple physiological action tantamount to aiding in detachment of a transporting vesicle from a motor protein (kinesin) upon arrival at the nerve terminal. Thus, the precise cleavage-process of APP and the potential physiological role of Aβ and AICD have yet to be unravelled [1, 2, 7, 15].

The amyloid deposits in the brains of AD patients are principally composed of the 39–43 amino acid residue Aβ, which is derived from the large APP (Fig. 1). Most of the Aβ species contain 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acids. Three proteolytic activities have been identified that bear on the production of Aβ from APP [1]. The predominant cleavage event by α-secretase (releasing CTF83) occurs in the extracellular domain of APP within the Aβ sequence, thus precluding the formation of the highly fibrillogenic Aβ petides. Three related metalloproteases of the ADAM family (a disintegrin and metalloprotease; ADAM-9, ADAM-10 and ADAM-17) can exert this α-secretase activity [16, 17]. This process has been shown to be catalyzed by various mechanisms and molecules such as protein kinase-C action, metabotropic glutamate receptor agonists, reactive oxygen species (ROS) or nitric oxide that acts as a retrograde messenger in synaptic transmission [18-21]. Alternatively, the extracellular domain of APP can be cleaved at the amino-terminus of Aβ by the β-secretase BACE (releasing CTF99) [22, 23] and a soluble enzyme, carboxipeptidase-B which is localized in the cytosol of neurons and some microglial cells [24]. BACE1 knock-out mice are phenotypically normal suggesting that perturbing the β-secretase pathway, by inhibiting cleavage at the β-site to favor the α-site, is not toxic and may be a suitable route for the development of therapeutics [25].

During the amyloidogenic pathway, APP is first cleaved at the N-terminus of Aβ sequence by BACE, to produce a soluble ectodomain, sAPPβ, and a membrane-anchored C-terminal fragment, CTFβ (CTF99). CTFβ is then subse-

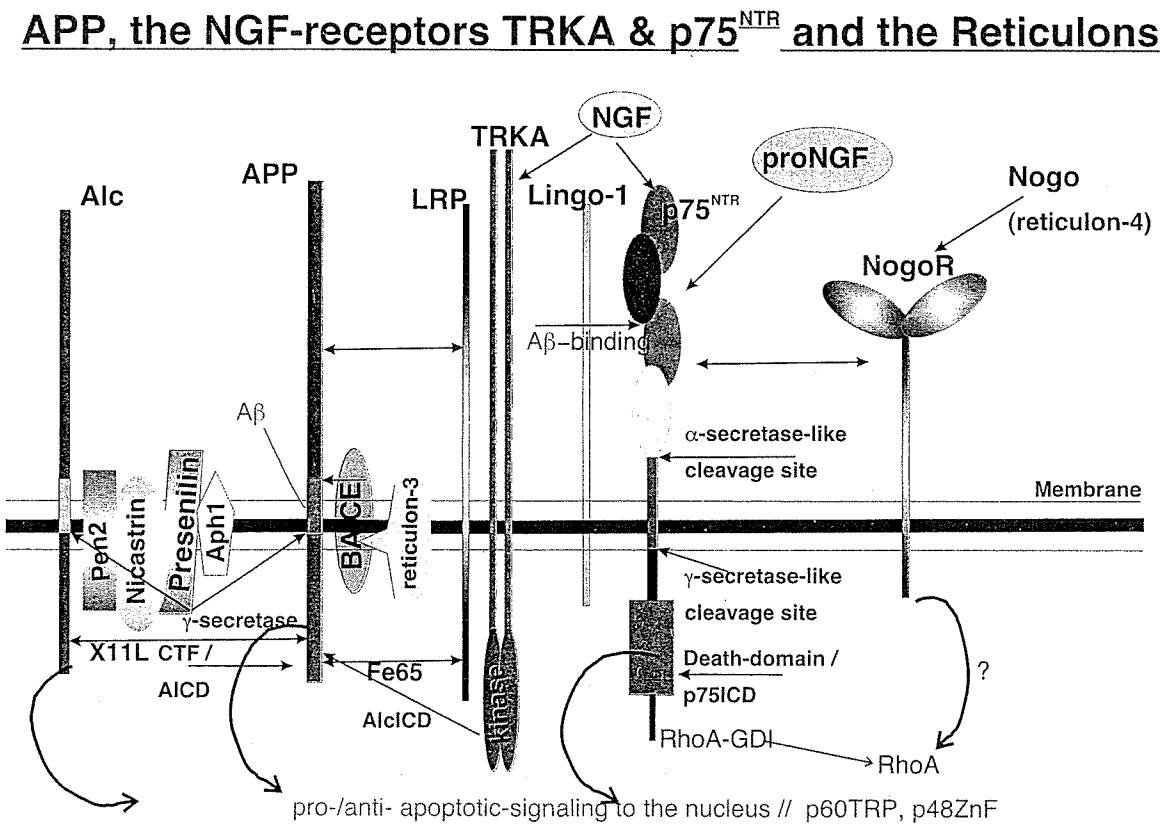

Fig. (1). Schematic representation of the Amyloid Precursor Protein APP and its various cleavage sites. AICD = APP's intracellular signaling domain; CHO = glycolisation sites; CT = C-terminus; CTF = C-terminal fragment; KPI = Kunitz-type-Protease-Inhibitory domain; sAPP = soluble APP; SP = signal peptide; TM = transmembrane domain; α, β, γ, ε, ζ = cleavage sites of the different specific secretases. Blue letters indicate currently known missense mutations that give rise to increased cleavage of APP. Upon cleavage at the different sites, various CTFs can be released. Whether CTF59, CTF57 or CTF50 is finally the (patho-) physiological AICD remains to be shown. After cleavage of APP at the α-site or β-site, sAPPα or sAPPβ are released into the extra-cellular space. A comparison of APP's and $p75^{NTR}$'s γ-cleavage sites shows to some extent homologous sequences.

quently cleaved within the transmembrane domain by a γ-secretase to produce the full-length Aβ (for instance, $A\beta_{42}$) and the intracellular domain AICD. The γ-secretase has been identified as a type I membrane-associated aspartyl protease. Observations of knockout studies of the PS-1 and PS-2 genes show the abolishment of γ-secretase mediated cleavage of APP. Furthermore, that two aspartate residues in two transmembrane domains of Presenilin have been identified as critical for the γ-secretase activity suggest that Presenilin may be the γ-secretase [26]. However, $A\beta_{42}$ peptide fragments are still produced in PS-1 and PS-2 double-knock-out mice, thus pointing to additional enzymes with γ-secretase activity [27, 28]. Recently, several other molecules, viz nicastrin, Aph1, and Pen2, have been identified as essential components of the γ-secretase protein complex of which Presenilin may function as the catalytic subunit [29]. The carboxyl terminal of APP then undergoes intramembrane proteolysis by this Presenilin-dependent γ-secretase activity, releasing Aβ, as well as the small cytoplasmic fragments of APP (AICDs such as CTF59 and CTF57, (CTF53 and CTF50) reflecting the predominant cleavage sites of γ-secretase in APP). These cleavage events probably contribute to AD-related neurodegeneration in two ways: release of Aβ and liberation of bioactive C-terminal domains from membrane bound APP [1, 30]. The predominant cytoplasmic fragment generated first by γ-secretase cleavage of APP is predicted to be CTF59. It has been hypothesized that this culminates finally in nuclear signaling via CTF50 and the activation of genes required for APP-mediated cellular processes. A consequence of Presenilin mutations, for instance, that cause familial AD, may be an increase in the alternative pro-apoptotic CTF57 which accompanies the (disturbed CTF50-processing and) production of a more toxic 42 amino-acid peptide $A\beta_{42}$. Notably recent sequence analysis indicates the N-terminus of AICD as starting at residue 50 of the Aβ sequence, which is 7–9 amino acids away from the C-termini of $A\beta_{40}$ and $A\beta_{42}$. This led subsequently to the discovery of the ε-cleavage site between $A\beta_{49}$ and $A\beta_{50}$. This recent identification of an ε-cleavage site within APP has shed new light on the possible physiological signaling of Aβ and AICD [31].

However, neither the intermediate Aβ peptide, which ends at the ε-cleavage site, nor the C-terminal fragment, which starts with an N-terminus generated by γ-cleavage, has ever been detected leading to a potential analytical and experimental paradox. One possibility is that γ- and ε-cleavages occur simultaneously. The other possibility may be that additional cleavages between the γ- and ε-cleavages exist. Indeed, a new cleavage site identified at $A\beta_{46}$ has been designated as ζ-cleavage site and interestingly later on identified as the APP717 mutation site. This ζ-cleavage is within the transmembrane domain and is a Presenilin-dependent event. Furthermore, it has been shown that the new ζ-cleavage is inhibited by γ-secretase inhibitors known as transition state analogs but less affected by inhibitors known as non-transition state γ-secretase inhibitors [32].

APP'S PHYSIOLOGICAL ROLE

A general observation regarding APP's physiological function is that it acts at the cellular level in diverse processes such as axonal transport, cell adhesion, cholesterol metabolism and gene transcription. Surprisingly Aβ is also produced during normal metabolism, suggesting that it may play important roles during normal cell function, and thus its normal physiological function might differ either quantitatively or qualitatively when steady-state endogenous levels are elevated during disease conditions. It would also be prudent to consider if Aβ exerts different effects when present intracellularly versus extracellularly. Hence it is imperative to know and understand the basic cell biological functions of Aβ in its various states and locations [7, 34].

There is no doubt that Aβ is toxic to various types of cultured cells and potentiates long-term damaging effects when injected directly into the brain [33]. However, Aβ is found in a variety of forms and association states and it is tedious to quantify these individual forms *in vivo*. It is rather apparent that the aggregated states of the peptide must be taken into account when measuring the effects of Aβ. While the effects of aggregated (fibrillar) and toxic Aβ had been intensively investigated previously (e.g. [34]), the acute effects of soluble monomeric, dimeric and oligomeric forms of amyloid still need detailed analysis, as these can produce sub-toxic alterations on neuronal function.

Several studies have found that Aβ dimers are produced at the membrane as well as intracellularly and can thereafter be secreted. Several proteins that can bind secreted Aβ have been discovered. One of these proteins is the apolipoprotein-E (ApoE), which binds to many proteolytic fragments of APP [35, 36]. Aβ competes with lipid molecules for ApoE, indicating that Aβ may alter cholesterol uptake and homeostasis.

Another receptor that binds Aβ and seemingly mediates its removal and degradation is the receptor for advanced glycosylation endproducts (RAGE) which is expressed on microglia and hippocampal neurons [37].

Due to these subtle but undeniable interactions, scientists resumed investigations into the possible physiological role of APP and, in particular, of the Aβ peptide that might function to compensate in impaired cholesterol dynamics and the associated breaks in neuronal network integrity, neurotransmission, synaptic plasticity, learning and memory. In recent years, we have witnessed the accumulation of convincing evidence that links AD and Aβ generation with lipid homeostasis, and it has been suggested that cellular lipid metabolism controls APP processing.

APP AND CHOLESTEROL

Aβ modulates the membrane's biophysical properties particularly with regards to cell membrane fluidity, which is essential for the functioning of a number of receptors critically dependent on finely-tuned homeostasis of cholesterol and lipid rafts. In this respect it is worth to mention that amyloidogenic processing of APP depends on lipid rafts [38].

It has been highlighted in several studies that Aβ production is sensitive to cholesterol levels and lipid trafficking. Enhanced cholesterol levels leads to increased Aβ production. Furthermore, cholesterol depletion reduces γ-secretase activity and results in reduced Aβ generation. Aβ production and deposition are tightly associated with changes in cho-

lesterol levels [38, 39]. Lipid levels vary in response to changes in diet, and physical or neuronal activity. Similarly, Aβ levels change in response to several conditions, including cholesterol and other factors. Brain cholesterol levels increase during the progression of AD and hypercholesterolaemia is an early risk factor in the development of AD. As a consequence, cholesterol-lowering drugs are being considered as a potential treatment for AD. Hydroxymethylglutaryl-CoA reductase (HMGR) and sphingomyelinases (SMases) are the main enzymes that regulate cholesterol biosynthesis and sphingomyelin (SM) levels, respectively. Both cholesterol and SM metabolism involves APP processing. Furthermore, $A\beta_{42}$ directly activates neutral SMase and downregulates SM levels, whereas $A\beta_{40}$ reduces cholesterol *de novo* synthesis by inhibition of HMGR activity. This process is strictly dependent on γ-secretase activity. In line with altered $A\beta_{40}$ and $A\beta_{42}$ generation, pathological Presenilin mutations result in increased cholesterol and decreased SM levels [38-41]. These findings also corroborate the proposed hypothesis that changes of Aβ biochemistry in AD is – inter alia – a functional compensatory phenomenon aimed at counterbalancing impaired cholesterol dynamics and associated synaptic plasticity.

APP AND SYNAPTIC TRANSMISSION

The various APP fragments, including Aβ, exhibit the capacity to exert powerful regulatory control over key neural functions including cell excitability, synaptic transmission and long-term potentiation (LTP), both acutely and over the long-term for neural plasticity [42].

Data from mice with APP-null mutations show a multitude of alterations with respect to neural structure and function, including gliosis, decreased neocortical and hippocampal levels of synaptophysin, reduced dendritic lengths in hippocampal neurons, reduced survival of cultured neurons and impaired LTP. Basal excitatory synaptic transmission and paired-pulse facilitation are not affected although basal GABAergic (gamma-aminobutyric acid) inhibitory synaptic transmission is somewhat reduced. Despite the reduced level of inhibition, which would be expected to promote synaptic plasticity, LTP appears significantly impaired. The inhibition of LTP may be due in part to the reduction in frequency-dependent suppression of inhibitory currents observed in these mice, although this has not been definitively demonstrated. However, while it still remains to be demonstrated whether full-length APP or one of its fragments appears to be really involved and necessary for normal excitatory transmission in the hippocampus, one or more of these polypeptides may be crucial for the induction or maintenance of LTP and synaptic plasticity [43-45].

The release of sAPPα and sAPPβ occurs constitutively, but stimulation of various neurotransmitter receptors, for instance metabotropic glutamate (mGlu1) and muscarinic acetylcholine (mACh, m1+m3, but not m2 or m4) receptors, in hippocampal, striatal, cortical and cerebellar neurons, leads to an increase of sAPP via a PKC-dependent mechanism [18, 46-48]. In the hippocampal perforant pathway, *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP is involved in this mechanism [49]. sAPP can exert proliferative and neurotrophic effects partially via excitoprotective and Calcium-dependent activity. Thus, endogenous sAPP might be an important regulator of synaptic plasticity and neurotoxicity, affecting memory and learning through its effects on cell excitability and NMDA receptor-mediated processes, particularly when secretion is stimulated by neural activity and the activation of modulatory neurotransmitter receptors [48-51]. Binding partners insofar identified for sAPP include serum proteins, cell surface proteins and extracellular matrix proteins. ApoE (has three alleles (E2, E3, E4) with the E4 allele being cited as a potential risk factor for AD) for instance, can bind sAPP in serum and may regulate its clearance [35]. ApoE and its complexes are produced by glial cells and internalized by neurons following binding to surface receptors such as low-density lipoprotein (LDL) receptors (e.g. LRP) or scavenger receptors. LRP can bind directly to sAPP at the cell surface and indirectly to Aβ via ApoE to mediate their internalization. Full-length APP also interacts with LRP via the intracellular adaptor protein Fe65, potentially regulating its internalization and trafficking [52].

Aβ's involvement in neurotransmission and synaptic plasticity is additionally supported by several studies showing an increase in synaptic APP with learning capacity in rats or by neuronal-activity-dependent secretion of natural Aβ [38-40, 50, 53].

One possible mechanism of Aβ-dependent neurotransmission regulation is through facilitation of membrane depolarization and calcium influx via L-type voltage-dependent Calcium channels. Increased calcium influx could promote synaptic plasticity and on the other hand, if calcium levels rise too dramatically this could down-regulate plasticity mechanisms as calcium channels and NMDA receptors are subjected to feed-back inhibition by calcium, and in the extreme scenario induce toxicity. While soluble Aβ fragments appear to exert no direct acute effects on glutamatergic synaptic transmission as mediated by AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, their effects on NMDA receptor function have been somewhat more controversial. While some reports suggest an enhancement in NMDA receptor-mediated currents (for instance in granule cells), others have shown rather a reversible inhibition of NMDA receptor-mediated field potentials. However, evidences point rather to a depressive action of Aβ on NMDA receptor function, although the extent to which these effects occur depends on cell type and amyloid concentration [50, 54-56].

While till now Aβ peptides may not have major direct effects on glutamatergic synaptic transmission, there is now convincing evidence that Aβ regulates signaling through nicotinic acetylcholine (nACh) receptors. For example, Aβ binding causes a significant inhibition of nicotinic currents in hippocampal pyramidal cells [57]. A wide range of Aβ peptides block ACh release from nerve terminals in the cortex and hippocampus [58], an effect that could be explained by inhibition of nACh receptors (nAChR) located on the cholinergic terminals. Several lines of evidence reveal that Aβ regulates cholinergic neurotransmission through its effects on both presynaptic activity and the postsynaptic activation of nAChRs. Of particular relevance to neuronal signaling is the high-affinity binding of $A\beta_{42}$ to nAChRs, including both

alpha7-containing nAChRs and alpha4-beta2-nAChRs [59, 60]. In isolated membranes, $A\beta_{42}$ shows a much greater affinity for the Calcium channel forming alpha7-nAChRs [58], but functional studies suggest that *in vivo*, the effect of Aβ is slightly greater on the non-alpha7 containing receptors. Since the link between AChR dysfunction and AD is well established, the direct binding of Aβ to such receptors could provide the beginnings of a mechanistic link between Aβ and cognitive dysfunction and offers a potential way for the treatment of AD [61, 62].

Aβ can affect both induction and consolidation processes of LTP [55]. Inhibition of LTP in the hippocampus is observed during the administration of both synthesized peptides and naturally secreted oligomers of Aβ. LTP inhibition is dependent on oligomer formation, since Aβ monomers appear to be inactive against LTP [63]. Transgenic mouse models of AD have also been used to demonstrate that overexpression of human APP (for instance with the Swedish mutations (K670N, M671L, APP_{695}SWE) or using the PDAPP mice (V717F) (a transgenic mouse model expressing APP under the control of the platelet-derived growth factor promotor)) leads to impaired LTP presumably attributed to the direct effects of (pre-aggregated) Aβ [64-66]. The mechanisms by which Aβ peptides reduce glutamatergic transmission, inhibit synaptic plasticity and inhibit LTP still remain elusive though. Although a direct effect on the NMDA-receptor or $GABA_A$-receptor has not been observed [56], a recent study reports that application of Aβ promotes endocytosis of NMDA receptors in cortical neurons. Correspondingly, neurons from a genetic mouse model of AD expressed reduced amounts of surface NMDA receptors. Reducing Aβ levels by treating neurons with a γ-secretase inhibitor restored surface expression of NMDA receptors. Consistent with these data, Aβ application produced a rapid and persistent depression of NMDA-evoked currents in cortical neurons. Aβ-dependent endocytosis of NMDA receptors required the alpha-7 nAChR, protein phosphatase PP2B and the striatal enriched tyrosine phosphatase STEP. Dephosphorylation of the NMDA receptor subunit NR2B furthermore correlated with receptor endocytosis. These data indicate a signaling relationship between the nACh- and the NMDA receptor systems and it reveals a new mechanism by which Aβ can cause synaptic dysfunction that may contribute to AD pathology [67].

It has been proposed that changes in the activity of the different fragments as a result of altered APP processing during the course of AD may also contribute to cognitive dysfunction. With these new developments researchers are now focusing more on elucidating the physiological actions of soluble Aβ, and the normal functions performed by APP both in the brain and other organs, in order to fully comprehend the disease process. Despite advances in our understanding of the basic biological roles of APP, the normal physiological function(s) of APP in learning and memory remains still unclear. Appropriate expression of APP appears to be necessary for the neuroplastic events that accompany learning and memory. Several studies have demonstrated APP to be necessary for learning and memory but none have related a functional significance to the neuroplastic events that accompany memory consolidation [50].

Notably APP expression becomes transiently decreased in the rat hippocampal dentate gyrus during memory consolidation. Neuronal APP_{695} expression becomes transiently reduced through association with endosomal adaptin proteins and enhanced internalization. In contrast, internalization of glial-associated APP containing the Kunitz protease inhibitor-like domain (APP-KPI) appears dependent on the low-density lipoprotein receptor-related protein LRP. LRP modulates APP-KPI expression and hence may operate during the early stages of memory consolidation. ApoE is also required for the regulation of cholesterol content in synaptic plasma membranes and its uptake, via lipoprotein receptors, is increased during hippocampal synaptic remodeling. Synaptic restructuring is positively regulated by glial cells and is, in part, dependent on their release of ApoE-conjugated cholesterol [38-40, 50].

Internalization of APP isoforms is observed only in dentate gyrus, a region that receives extensive sensory input from the perforant path, and probably relates to the learning-associated restructuring of the perforant path terminals. Memory-associated APP processing in both neuronal and glial compartments points to a potential role for glial mediated unsheathing of synaptic connections, an occurrence during synaptic restructuring that accompanies memory consolidation. These observations may have a direct relevance to understanding the pathophysiology of AD as Aβ is formed following internalization of cell surface APP into the endosomal compartment. Internalization and degradation of APP_{695} restricted to the dentate gyrus suggests that the most intensive processing occurs in the terminals of the perforant path and in a period just prior to the extensive synaptic remodelling that accompanies acquisition of spatial learning and avoidance conditioning paradigms [68].

APP AND SIGNALING

Neurotrophic factors have attracted much attention for their potential as a remedy for neurological disorders. In this aspect, nerve growth factor (NGF) has generated a great interest as a potential target for the treatment of AD. This interest is based on the observation that cholinergic neurons of the basal forebrain (CBF), which provide the major source of cholinergic innervation to the cerebral cortex and hippocampus, undergo selective and severe degeneration in advanced AD and the survival of these CBF neurons depends upon the availability of NGF and its receptors, namely TRKA and $p75^{NTR}$.

In addition to several adapter proteins, one of the more interesting aspects of APP is its function as a kinesin-I membrane receptor and this suggests that APP and kinesin-I are crucial carrier components mediating the axonal transport of cargo proteins such as BACE, PS-1, GAP43, synapsin and the high affinity NGF receptor TRKA. Interestingly, the carboxy-terminus mediates binding of APP to the motor protein (kinesin light chain (KLC) of kinesin-I), whereas the Aβ region might be an essential part of the sorting signal for axonally transported vesicles [15, 69-72]. This raises the possibility that axonal damage might induce APP proteolysis to release the AICD, which might then deliver an injury signal to the cell body. Evidently after cleavage, AICD is released into the cytoplasm and tends to relocalize to the nu-

cleus [73-75]. This process is enhanced when the adaptor protein Fe65 is bound to APP, which probably confers increased stability. The APP-binding proteins MENA (the mammalian homolog of the Drosophila Enabled gene) and Fe65 provide also a mechanism for coupling APP to the cell cytoskeleton. Over-expression of both APP and Fe65 can increase cell motility [76], thereby signifying their potential importance in morphological remodeling which may be necessary for long-term synaptic plasticity. Additionally, Fe65 can bind at least two transcriptionally relevant proteins, Tip60 (a histone acetyltransferase) and CP2 (alpha-globin transcription factor, also called LSF or LBP-1) [77]. Another report demonstrates that 14-3-3γ facilitates Fe65-dependent gene transactivation by forming a complex containing AICD and Fe65, and phosphorylation of AICD down-regulates Fe65-dependent gene transactivation through the dissociation of 14-3-3γ and/or Fe65 from AICD. These findings offer profound implications that multiple interactions of AICD with Fe65 and 14-3-3γ modulate Fe65-dependent gene transactivation [78].

A further remarkable discovery is that the neuronal Alcadein (Alc), which is a type-I transmembrane protein, interacts with the phosphotyrosine interaction domain of X11-Like (X11L, also called APP-binding-family-A-member-2 (APBA2)) and can initiate the formation of a tripartite complex comprised of Alc, X11L, and APP. This complex stabilizes intracellular APP metabolism and significantly suppresses Aβ production by slowing APP maturation. Alc and X11L can also form a tripartite complex with CTF99/CTFβ and this complex inhibits the interaction of CTF99 with PS-1 which suppresses the γ-cleavage of CTF99 thereby inhibiting Aβ formation. In AD brains, APP was found to colocalize with Alc in the dystrophic neurites of senile plaques. These observations indicate that APP exists in protein complexes composed of X11L and Alc that regulate APP metabolism, including Aβ production in neurons, and that this regulatory mechanism may be perturbed in AD. APP-X11L-Alc tripartite complex not only suppresses the metabolic cleavages of APP but the metabolism of Alc markedly resembles that of APP. Alc is subjected to a primary cleavage event that releases its extracellular domain. It then undergoes a secondary Presenilin-dependent γ-cleavage that leads to the secretion of the Aβ-like peptide and the liberation of an intracellular domain fragment (AlcICD). However, when Alc is in the tripartite complex, it escapes from these cleavages, as does APP. AlcICD also suppresses the Fe65-dependent gene transactivation activity of AICD, probably because AlcICD competes with AICD for binding to Fe65. Neuronal Alc and APP are coordinately metabolized and their cleaved cytoplasmic fragments are reciprocally involved in the regulation of Fe65-dependent gene transactivation. Any imbalance in the metabolism of Alc and APP may influence the Fe65-dependent gene transactivation, which together with increased secretion of Aβ may contribute to neural disorders [11, 12]. However, a direct involvement of Tip60 or Fe65 in AD is currently unknown.

APP, NGF AND THE SUNDAY DRIVER SYD

APP, TRKA, PS-1 and GAP43 have been observed to be located in the same axonal transport vesicle [69-72]. Rather coincidentally APP may also interact with the 'Sunday-driver' (SYD), a protein candidate strongly implicated in apoptosis and more importantly, in vesicle transportation [79]. It might be interesting to mention at this point an anti-NGF-antibody animal model showing typical features of AD [80-82]. The aged anti-NGF transgenic mice acquire an age-dependent neurodegenerative pathology including deposition of amyloid plaques, insoluble and hyperphosphorylated tau, and neurofibrillary tangles in cortical and hippocampal neurons. Aged anti-NGF mice also display extensive neuronal loss throughout the cortex, cholinergic deficit in the basal forebrain, and behavioral deficits. These results demonstrate that a deficit in the signaling and/or transport of NGF leads to neurodegeneration. The overall picture is strikingly reminiscent of human AD. Similarly, it has been suggested that genetic reduction of KLC should enhance AD-like pathology in, for instance, an APP-FAD (APP mutants associated with familial AD) over-expressing mouse [15]. Thus, as APP plays a major role in kinesin-I-dependent axonal transport, deregulation of intracellular transport mechanisms – caused either by APP-FAD or by a deteriorated TRKA-NGF signaling – might be one crucial aspect for the initiation of neurodegeneration in AD.

The protein encoded by the 'Sunday-driver' (SYD) gene is required for the functional interaction of kinesin-I with axonal cargo [83, 84]. The SYD protein, with its cytoplasmic C- and N-terminal domains and a central transmembrane domain, is associated via its N-terminus with KLC. Taking into account that APP can interact with SYD and JIP1b/2 [14, 85] it is, thereby, of interest that SYD (also called JSAP1 or JIP3) also acts as a scaffolding protein for the JNK/MAPK (c-Jun N-terminal kinase, mitogen-activated kinase) cascade [86-88], particularly, because the SYD-JNK3 pathway is required for signaling upon nerve injury and stress-induced neuronal apoptosis - and APP seems to be directly involved in regulating those apoptotic signaling pathways [89-93]. This finding hints at a potential link between intracellular APP-mediated apoptotic signaling and microtubule-dependent transport, because mutations in APP may connect APP-signaling with the JNK pathway [92, 94].

Another exciting discovery is that the NGF receptor TRKA modulates APP phosphorylation to couple APP via the Shc/Grb2 adaptor proteins – similar to the TRKA-signaling – to cellular pathways generally associated with proliferation and survival, including the Ras/MAPK pathway and the PI3K/PKB (phosphatidylinositol 3-kinase, protein kinasese-B) signal transduction cascade [95-98]. Considering the fact that in AD the NGF/NGF-receptor system is functionally disturbed [99-102], an impaired NGF-TRKA-signaling cascade may affect APP- and SYD-transport and signaling, thus mediating apoptosis via: (i) a retrograde transport of AICD, (ii) the SYD-activated JNK/p38MAPK pathway, or (iii) an inhibited ras/MAPK-PI3K-signaling. However, further experiments are needed to determine the extent of crosstalk between APP, SYD, TRKA, kinesin-I and JNK proteins and recent results concerning the anterograde and retrograde transport of neurotrophins may probably help to intensify studies about APP's and SYD's transport and JNK-mediated signaling mechanisms [103-105].

APP AND P75^{NTR}

A further reciprocal action between the NGF and the APP systems that can be contemplated upon is the interplay between Aβ and its binding to the low affinity neurotrophin receptor p75^{NTR}. Whilest p75^{NTR} is able to mediate an anti- or pro-apoptotic signal, the Aβ-p75^{NTR} signal transduction pathway and its impact on AD is still an unsolved problem [106-111]. At this point, it is of special interest to note that p75^{NTR} is probably processed by a γ-secretase in a similar way as APP, releasing p75ICD transmitting a signal to the nucleus [112] (Fig. 2). Another interesting similarity is p75^{NTR}'s involvement in triggering small GTP-binding protein signaling pathways [111] and the activation of G-proteins by APP as revealed by recent findings. Although the downstream effects of Go by APP are completely unknown [113-115], the significance of G-proteins in directing neural activity and synaptic plasticity is well established and there are numerous possible ways in which APP-mediated G-protein regulation could influence higher cognitive functions.

Thus, for our understanding of neurodegenerative processes in AD we need an enlightenment of the physiological role of APP and its processed products AICD and Aβ. Moreover, the cross-talk among APP's and NGF-receptor's signaling cascades will be an interesting issue for further explorations.

APP AND NEUROFIBRILLARY PATHOLOGY: TAU AND MAPS

AD has also been assigned to the heterogeneous group of tauopathies. The intraneuronal lesions can be differentiated from lesions associated with Pick's disease, corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease, or other tauopathies. Only a few types of nerve cells in specific regions of the brain become diseased in AD and the pathological process begins at predisposed induction sites and advances in a topographically predictable sequence. Whereas some neuronal types, cortical areas, and subcortical nuclei remain untouched, others sustain severe damage. By pinpointing the locations and severity of the pathology, stages in the evolution of neurofibrillary lesions and amy-

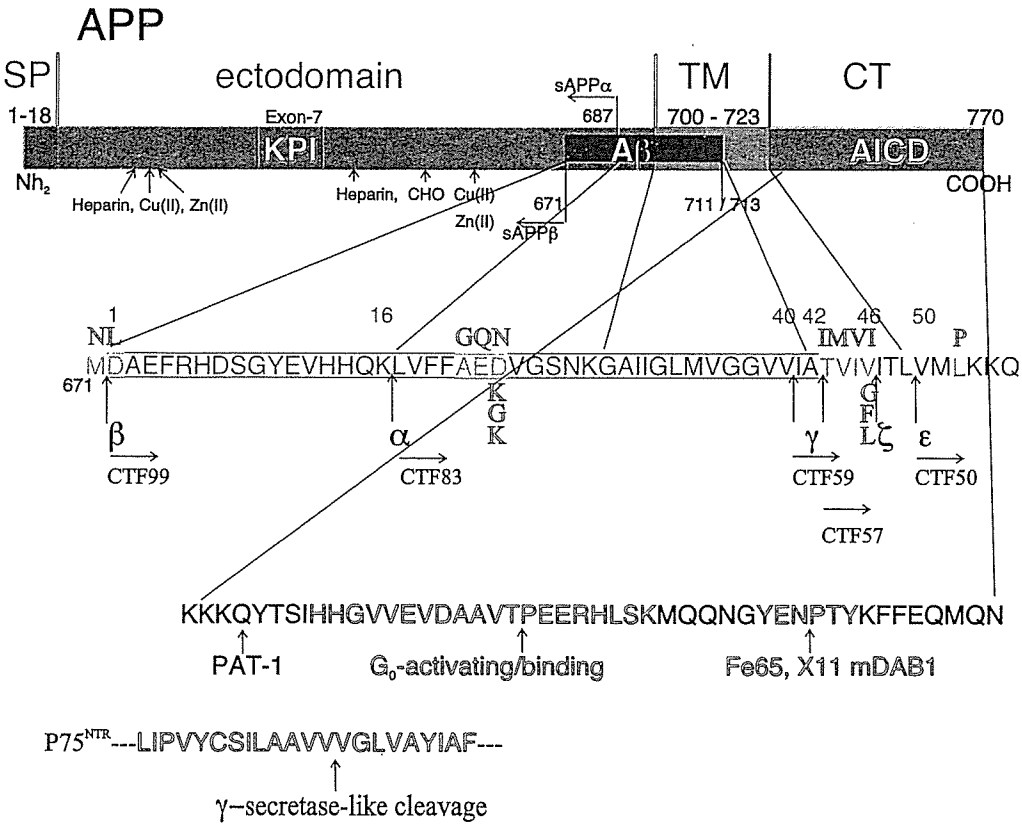

Fig. (2). Potential interactions among different receptors involved in AD including the NGF receptors TRKA and p75^{NTR}, APP and the reticulons. The protein complex, consisting of Presenilin, Nicastrin, Pen2 and Aph1, controls the γ-cleavage site of APP. The reticulons control the β-cleavage-site of APP indirectly via interacting with BACE. An increase in the reticulon protein reduces the production of Aβ. Alcadein (Alc) forms a protein complex with APP and X11L thereby suppressing Aβ production. The metabolism of Alc resembles that of APP including the Presenilin-dependent γ-cleavage mechanism. NGF can modulate intracellular APP signaling by direct phosphorylation of APP via the NGF receptor kinase TRKA. In contrast, Aβ can mediate a pro- or anti-apoptotic signal by stimulating the p75^{NTR} receptor. Upon cleavage, APP's intracellular domain (AICD) and p75ICD are released to translocate into the nucleus where they can activate gene transcription. P60TRP and p48ZnF are probably involved in these signaling cascades.

loid-deposition can be differentiated [116-118]. The occipital lobe cortex remains functionally almost normal during the progression of AD, even in patients at the terminal stage. By contrast, the temporal lobe cortex, being one of the most fragile parts of the brain, is extremely vulnerable to neuronal death and pathological changes and thus, affected during the early stages in AD.

According to the 'amyloid cascade hypothesis', Aβ is neurotoxic and causes neuronal degeneration, including neurofibrillary pathology and, consequently, dementia. Thus for the amyloid hypothesis to be correct, there should be a link between elevations in Aβ and the hyperphosphorylation of tau. This has been verified to some extent by the application of double (mutant APP and mutant tau) transgenic mice that develop significantly increased levels of neurofibrillary tangles and by primary neuronal culture clearly showing that Aβ induces phosphorylation of tau [119, 120].

However, this hypothesis is inconsistent with (i) the presence of Aβ burden in normal aged brain similar to that in AD, (ii) the occurrence of neurofibrillary degeneration and dementia in the absence of β-amyloidosis in several tauopathies, (iii) the co-segregation of the disease with certain mutations in tau in inherited FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17), and (iv) the failure to produce significant neurofibrillary pathology and neurodegeneration in APP, PS-1 and PS-2 transgenic mice, which develop considerable Aβ plaque pathology (though this might be due to species differences), [2, 118]. Recent findings, however, suggest that neurofibrillary pathology and Aβ deposition could be independent processes that might interact secondarily [121].

In AD brains a several-fold increase in tau protein levels has been observed and this increase is in the form of abnormally hyperphosphorylated tau (P-tau) which results from an imbalance between the activities of tau kinases and tau phosphatases (e.g. PP2A) [122]. This P-tau can be detected in two subcellular pools: (i) as polymers of neurofibrillary tangles of paired helical filaments (PHF) mixed with straight filaments (SF); and (ii) as a non-fibrillized form in the cytosol [123]. The tau polymerized into neurofibrillary tangles is apparently inert and the cytosolic P-tau does not interact with tubulin/microtubules. P-tau sequesters normal tau and the other two neuronal microtubule-associated proteins, MAP1 and MAP2, leading to inhibition and disassembly of microtubules, thus, causing a breakdown of the neuronal microtubule network. Besides tau, MAP1B is aberrantly hyperphosphorylated by the proline-dependent protein kinase GSK3β [124].

While the loss of functional tau can be partially compensated by the other two neuronal microtubule-associated proteins, MAP1A/MAP1B and MAP2, hyperphosphorylation of tau can also result in gain-of-function toxicity. The toxic property of the P-tau appears to be solely attributed to its hyperphosphorylation and the affected neurons antagonize this toxic P-tau both by continually synthesizing new normal tau and by packaging P-tau into inert PHF/SF making it resistant to proteolysis by the Calcium-activated neutral protease resulting in an increase of tau protein levels in AD [117]. This slow but progressive retrograde neurodegeneration results in synaptic loss when it reaches a certain threshold point and manifests then in the clinical expression of the disease which is characteristic of AD and other tauopathies [118].

Recent detailed AD brain site-specific gene expression pattern analyses provide insight into potential mechanisms responsible for the induction of AD-specific pathogenetic processes. The authors demonstrate that MAP1B and MAP2 show different expression patterns in AD and control subjects [125, 126]. These findings point to the fact that the two genes of the same family have different functional roles during the progression of AD. The observations that a) the expression of MAP1B was lower in AD, b) the expression of MAP2 was higher in AD and that c) the loss of MAP1B function resulted in inhibition of neurite outgrowth and cell death point to the fact that MAP1B function cannot be fully compensated by other MAPs [125, 127-129]. But the potential compensatory role played by MAP2 for MAP1B at early stages of AD is shown by the occipital lobe analysis, suggesting that this is one possible factor that may help to protect the occipital lobe against cell death in AD [128]. The down-regulation of proteins such as dystonin-1eB, neuronal ankyrin-2 and HSP90 in the temporal lobe of AD patients further strengthens the hypothesis that changes in the expression of cytoskeletal proteins promote tangle development temporally before plaque formation in AD.

APP AND THE RETICULONS

Another interesting aspect of AD is the disregulation of the reticulon gene expression [125]. The reticulon family of proteins has four members, RTN1, RTN2, RTN3 and RTN4 (also known as Nogo), the last of which is well known for its role in inhibiting neuritic outgrowth after injury. The reticulon family members are binding partners of BACE1. Reticulon proteins block access of BACE1 to APP and as a result reduce the cleavage of this protein. In the brain, BACE1 mainly colocalizes with RTN3 in neurons, whereas RTN4 is more enriched in oligodendrocytes. Inhibiting the activity of BACE1 or reducing levels of BACE1 *in vivo* decreases the production of Aβ. An increase in the expression of any reticulon protein substantially reduces the production of Aβ. Conversely, lowering the expression of RTN3 increases the secretion of Aβ, suggesting that reticulon proteins are negative modulators of BACE1. Thus, changes in the reticulon protein expressions in AD brain – down-regulation of neuronal RTN3 [125] - are likely to affect cellular Aβ and the formation of amyloid plaques in AD [130, 131].

AD, THE UBIQUITIN-PROTEASOME SYSTEM AND APOPTOSIS

Increasingly, newfound evidence indicates that the accumulation of aberrant or misfolded proteins, protofibril formation, failure of axonal and dendritic transport and ubiquitin-proteasome system dysfunction altogether represent unifying events in slowly progressive neurodegenerative disorders such as AD. Ubiquitin-positive deposits are histopathologically found in patients with AD. Unfortunately, it is not understood why ubiquitin accumulates in intra- and extra-cellular deposits or how it is involved in AD pathogenesis. Recent evidence has elucidated the molecular mecha-

nism involved during the ubiquitin-proteasome system (UPS) malfunction in AD. The neurotoxicity and proteasome inhibition by Aβ are mediated by increased E2-25K (ubiquitin-conjugating enzyme, also called Hip-2) in the brains of patients with AD. Evidently, E2-25K/Hip-2 is required for the neurotoxicity that is mediated by an ubiquitin-B mutant (UBB+1), which is a potent inhibitor of proteasomes that is detected in patients with AD [132]. Another report shows that the E3-ubiquitin ligase CHIP (carboxyl terminus of the Hsc70-interacting protein) ubiquitinizes tau, thus preventing cell death [133]. Newly acquired data also reveal an increased expression of TRIM37 and TRIM32 ('RING-finger'-motif-containing proteins) in occipital lobes of AD brains, an area with nearly no pathological changes during early stages of AD and from which the neuroprotective factor Humanin has been isolated recently [125, 134]. It is also appropriate to note that TRIM32/37 code for tripartite motif family proteins, which have anti-apoptotic and E3-ubiquitin ligase properties [135, 136] and reduced expression of those proteins leads to a decrease in neuronal survival. However, further research is required to identify the components of the UPS that are involved in the pathogenesis of AD [137].

Several other genes have been identified with a potential link to AD and which may interfere with Aβ-mediated toxicity, such as: p33MONOX, p18SRP, p60TRP, p48ZnF, COX-VIIb or POU2F1. While p48ZnF seems to control apoptosis via its interaction with other crucial transcription factors (SOX4, PCBP1, ZnF313), p60TRP may modulate tau phosphorylation via the interference of PP2A's activity [125, 138-142]. However, a detailed analysis is necessary to reveal their potential role during the progression of AD.

CONCLUSION

Since the advent of the new millennium, various strategies have been developed for the treatment and prevention of AD. Neurotransmitter-based therapies with ACh-esterase-inhibitors and NMDA receptor antagonists are in current use; anti-inflammatory and anti-oxidative approaches as well as compounds to block Aβ aggregation are being tested in the clinic; and β/γ-secretase inhibitors designed to reduce generation of Aβ peptides are under development. A more challenging step is the vaccination-based therapeutic trial against Aβ. Several research groups have reported cognitive benefits of an Aβ vaccine in transgenic mouse models of AD overexpressing Aβ, developing senile plaques and exhibiting associated memory performance deficits [143, 144]. The promise of cognitive benefits following Aβ vaccination in animal models has generated enormous interest in vaccination as a therapeutic strategy for AD patients. However, a first clinical trial has been abandoned due to the development of meningoencephalitis in some cases. The treatment of AD with Aβ vaccines remains yet a controversy [145-148]. Another promising approach is the application of NGF as a potential remedy for the treatment of AD. However, delivering NGF to the brain in a safe manner is still challenging because NGF does not cross the blood-brain barrier when administered peripherally. Yet when infused into the brain ventricular system, NGF causes intolerable side effects from its broad distribution, including pain and Schwann cell migration into the spinal cord and medulla. Nonetheless, recent experiments show that intranasal administration of NGF rescues recognition memory deficits in an anti-NGF transgenic mouse model which shows typical features of AD [149]. In addition, a brain site-specific gene delivery method provides sufficient quantities of NGF to support neuronal survival at restricted sites to avoid adverse site effects. A recent phase-I clinical trial study of NGF gene therapy for AD provides promising data [150, 151].

In conclusion, the data summarized above sheds new insights on several proteins that appear to be involved in the regulation of neuronal survival and death in AD. However, further studies on the possible compensatory roles played by certain genes in AD [134] is a fundamental prerequisite for possible therapeutic applications of such genes in the CNS and should provide more information about a potential genetic-based AD-therapy. Thus, the connection between these proteins and AD will be an interesting topic for additional investigations and further experiments are necessary to clarify the fundamental neurobiology of AD.

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