Alzheimer's disease animal models and their use in AD drug-candidate screen

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Numerous transgenic mouse models for Alzheimer's disease (AD) have been generated to recapitulate the histological pathogenesis and behavioral phenotypes of AD brain. However, none of the existing models exhibits the full spectrum of AD symptoms, nor have all of the traits mimicked by the developed animal models been successfully represented within a single mouse line, indicating that the development of transgenic lines showing new features of the AD-like brain needs to be explored.

We created a transgenic mouse line Tg-βCTF99/B6 expressing the human βCTF99 in the brain of inbred C57BL/6 strain. Tg-βCTF99/B6 mouse brain at 12-16 months showed severely down-regulated calbindin, phospho-CREB, and Bcl-x_L expression, and up-regulated phospho-JNK, Bcl-2, Bad, and Bax expression. Neuronal cell density in the Tg-βCTF99/B6 cerebral cortex at 16-18 months was lower than that of the non-transgenic control, but not at 5 months. At 11-14 months, Tg-βCTF99/B6 mice displayed cognitive impairments and increased anxiety. Although increased anxiety is a symptom of AD, Tg2576 and APP23 mouse models, which are widely used in many laboratories partly because they show AD-like phenotypes such as Aβ-plaque deposition and cognitive deficits, do not show increased anxiety. Thus, not all of the desirable traits mimicked by developed animal models are successfully represented by a single mouse line.

Available animal models can be potentially used to address questions such as how prior occurrence of biochemical and histological changes specifically affects neuronal loss, plaque pathogenesis, and cognitive and psychiatric dysfunctions, which factors in the aging brain critically lead to such neuronal and behavioral impairments, and what strategy or drugs can be sued to delay or protect the pathophysiology of the AD-like brain. In conjunction with these issues, some concepts and tips that may be useful in AD drug-candidate screen will be discussed.

# Alzheimer's disease animal models and their utilization

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Anatomical view of Alzheimer's brain
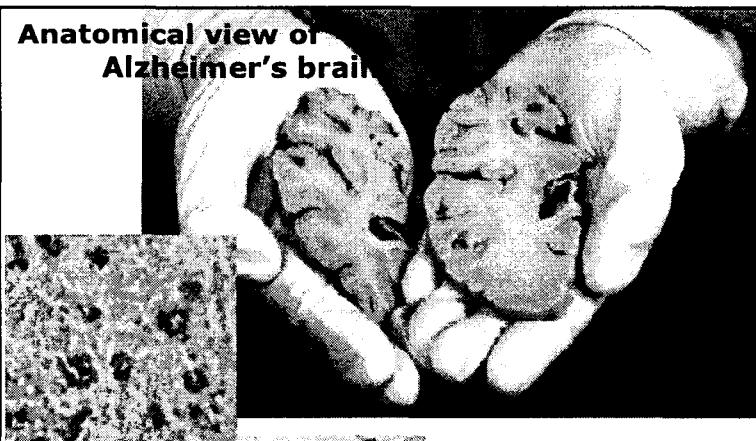
Histopathological changes
- amyloid plaque deposition
- neurofibrillary tangle
- neuronal cell loss
- gliosis

Neuronal loss and changes in dendritic morphology in AD
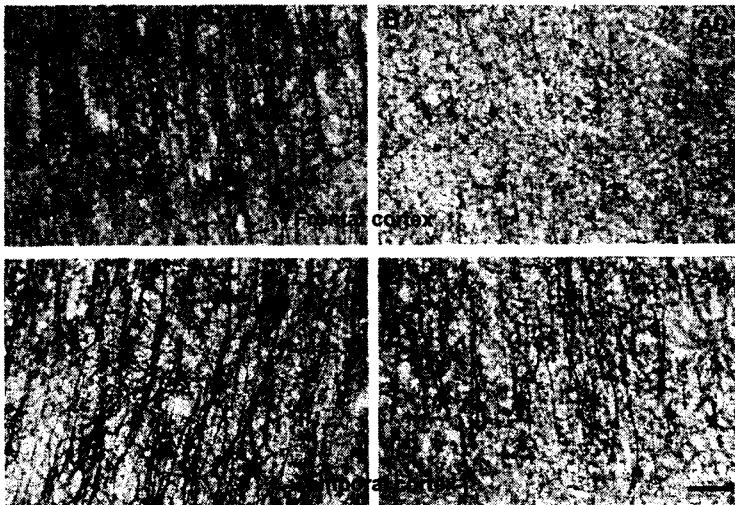
*Hof and Morrison (1999), Alzheimer Disease. 2nd. LWW.*

Familial Alzheimer's disease (FAD)

Gene	Chromosome	Age of onset	% of all cases	Penetrance	Known missense mutations
APP	21	45-66	<0.1	100 %	12
PS1	14q24.3	28-62	1-2	100 %	80
PS2	1q42.1	40-85	<0.1	100 %	5
ApoE4	19q12,13	>60	(risk factor)		
α2-M	12	>60	(risk factor)		

low-density lipoprotein receptor-related protein (LRP)
receptor for advanced glycation end products (RAGE)
insulin degrading enzyme (IDE)
neprilysin (NEP)
endothelin converting enzyme (ECE), angiotensin converting enzyme (ACE)
u-PA, tPA, plasmin, MMP2,9, furin, etc

Sporadic Alzheimer's disease

- More than 80-90% of all AD cases
- caused by <u>non-genetic or environmental risk factors</u>
- **Aging:** is the most prevalent risk factor
    → applicable for all AD cases
- Clinical and epidemiological studies show that
    - acid-forming food
        (ex, food high in dietary fat or total energy)
    - dioxins, aluminum, lead
    - viral infections
    could act as environmental risk factors for AD
        (Grant et al. J Alzheimers Dis. 4 (2002):179-189)

→ It is disputable if these factors can be counted as salient non-genetic risk factors that affect most of the population in developed countries
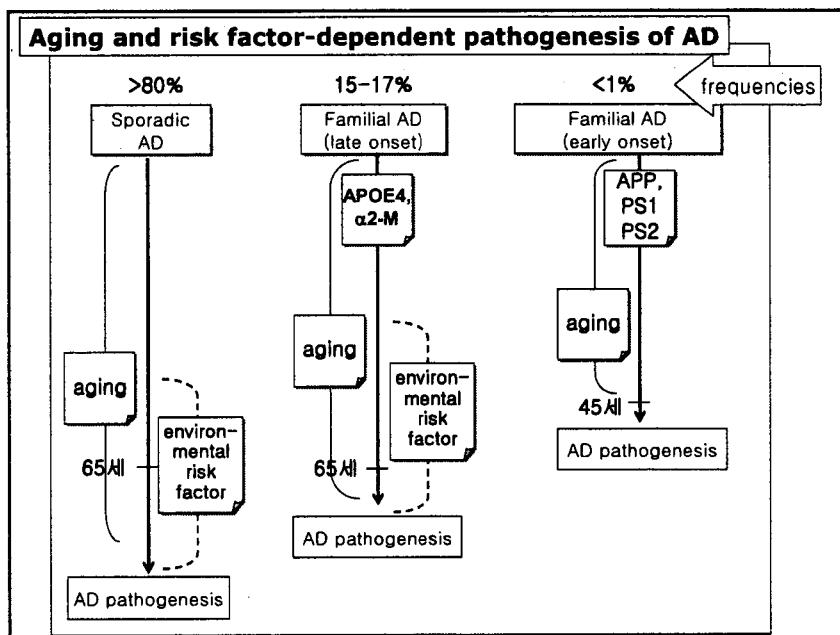
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Aging and risk factor-dependent pathogenesis of AD

>80%	15-17%	<1%	frequencies
Sporadic AD	Familial AD (late onset)	Familial AD (early onset)	

APOE4, α2-M

APP, PS1 PS2

aging

aging

aging

65세

environmental risk factor

65세

environmental risk factor

45세

AD pathogenesis

AD pathogenesis

AD pathogenesis

## Pathological hallmarks of Alzheimer's Disease

1) **Histological phenotypes**

  a. <u>amyloid plaque, neurofibrillary tangle (NFT)</u>

 b. <u>neuronal cell loss,</u> gliosis
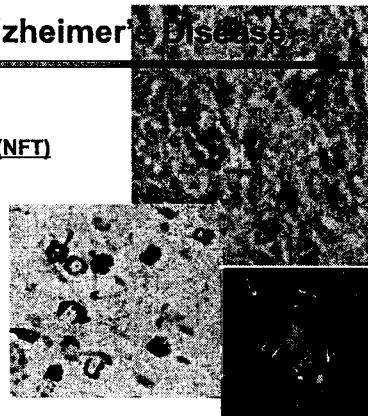
2) **Behavioral phenotypes**

  a. **Cognitive symptoms**

    - <u>learning and memory impairment</u>

  b. **Psychological symptoms**

    - **mood disorders** : <u>anxiety,</u> slowed thinking, tension, irritability

                  sadness, social withdrawal, depression

    - **other behavioral changes** : calculation defect, Language defect,

                     violence , crying, wandering


Structure of APP and FAD associated mutations

Sac II (11) Bgl I (165) Kpn I (186) BspH I (1351) Xho I (1250) Sac I (1942) Bgl II (2119) Spe I (2708) Xmn I (2722) Nde I (2810) Hind III (3080) Sph I (3473)

1   125   2437   3556

Met → app-1f   exon 7 exon 8 app-2f →   Aβ42 Stop ← app-1r   3'-UTR

← app-2r

ORF (125-2437)
exon 7 (990-1157)
exon 8 (1158-1215)
Aβ42 (2138-2263)

APP770
APP751 = APP770 - exon 8
APP695 = APP770 - exon 8 -exon 7

β-secretase   α-secretase   γ secretase

KM DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA TVIV

NL   (Hendriks mutation) G   I,G,F

(Swedish double mutation)   ("717" mutation)

Q (Dutch mutation)

Generation of Tg-βCTF99/B6 mice in inbred C57BL/6

(A)

PDGF-βCTF99-pA

[PDGF-β] — [SP] [■] [CT] — [SV40pA]

"V717F" mutation

PDGF-intron-βCTF99-pA

[PDGF-β] — [intron] — [SP] [■] [CT] — [SV40pA]

"V717F" mutation

(B)

Non-tg | Tg-βCTF99 (-intron) F9 F13 | Tg-βCTF99 (+intron) F7 F17

28s
18s
→ endogenous APP
→ transgene transcript

28s
18s
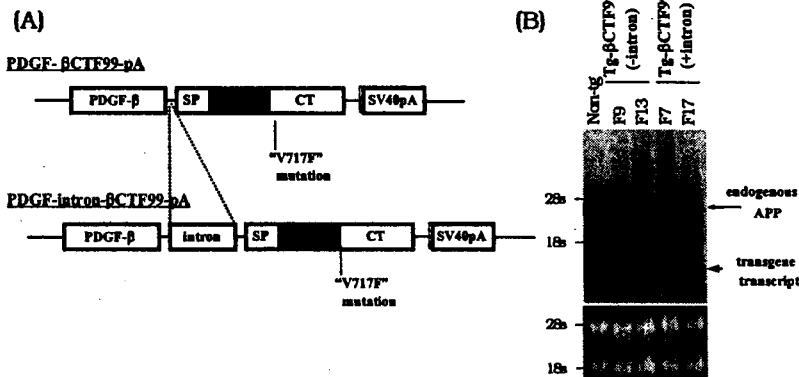
Analyses of the transgene expression in Tg-βCTF99/B6 mice

(A)

[kDa] Non-tg   Tg-βCTF99   Tg-βCTF99

17 —   → β CTF
        → α CTF

anti-CTF (Sigma)

7 —

Relative level
3.0
2.5
2.0
1.5
1.0
0.5
0

□ β CTF
■ α CTF

Non-tg   Tg-βCTF99
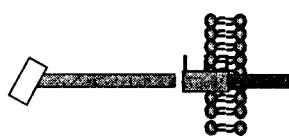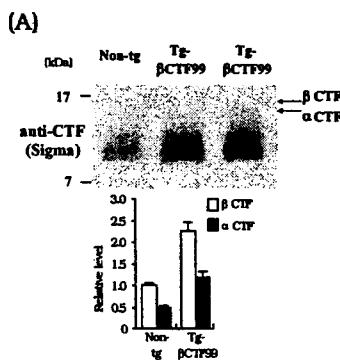
Expression of calcium-binding proteins in the brain of Tg-βCTF99/B6
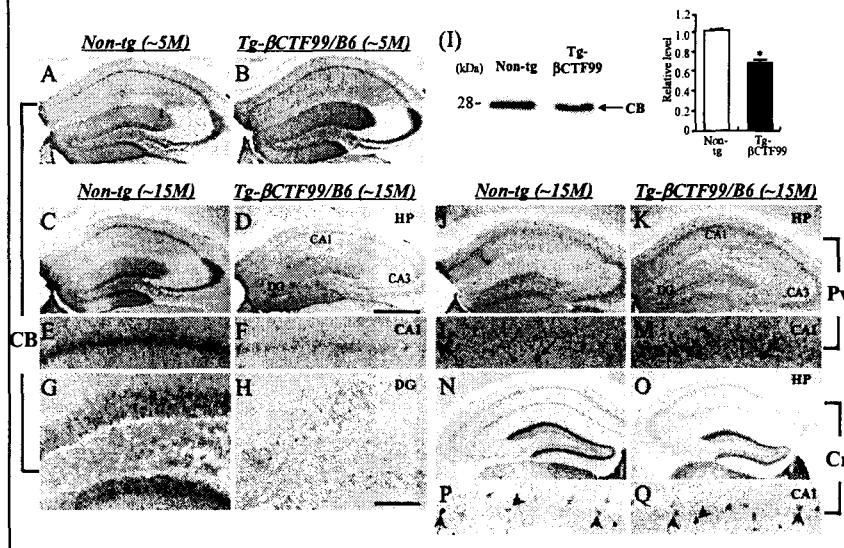
## Activated JNK pathway in the brain of Tg-βCTF99/B6
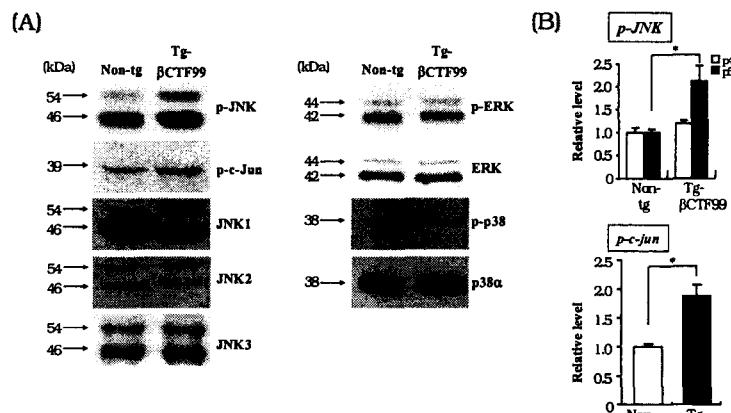
Reduced expression of phospho-CREB in the brain of Tg-βCTF99/B6
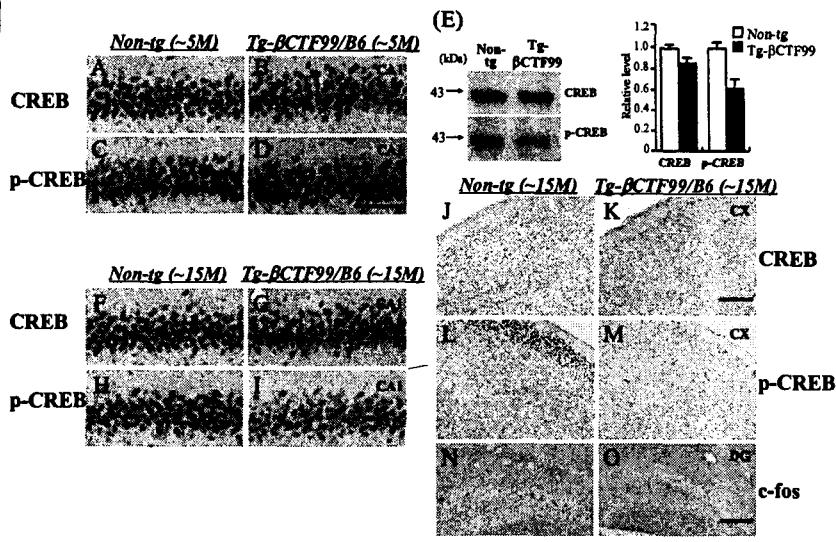
Tg-βCTF99/B6 shows the reduced neuronal cell numbers and simpler neuritic morphology in the brain
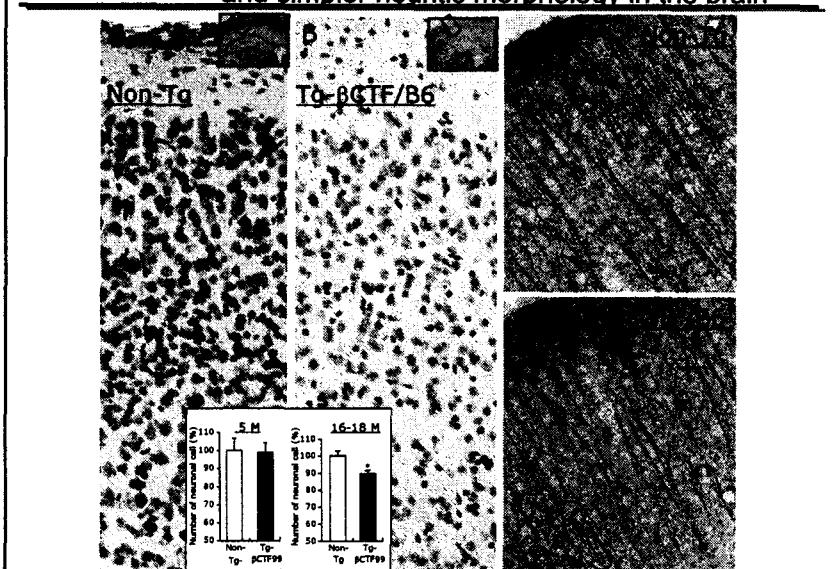
**Altered spatial learning and memory retention of Tg-βCTF99/B6**

Morris Water Maze Test

(A) 7M
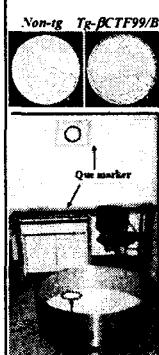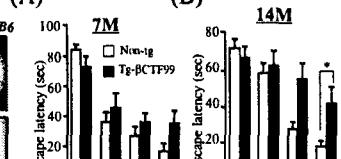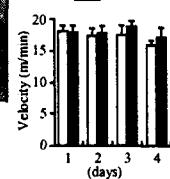
(B) 14M
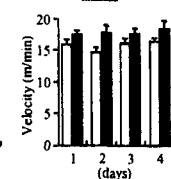
*Passive Avoidance Test*

(C) 7M

(D) 14M
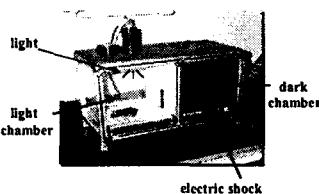
(E) 7M
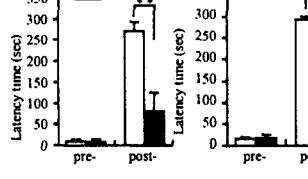
(F) 14M
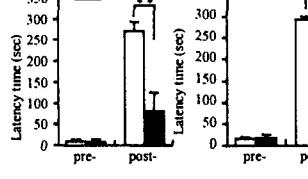
Anxiety-like behaviors of Tg-βCTF99/B6 in the elevated plus maze test

11M
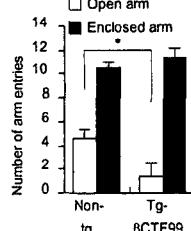
➡ **Increased anxiety**

Normal locomotor activity and motor coordination of Tg-βCTF99/B6

Open Field Test
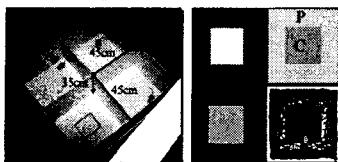
Rota-rod Test
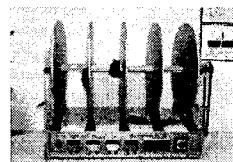
(A) 7M
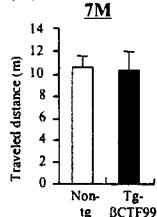
(B) 14M
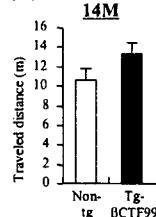
(C) 5.5 M
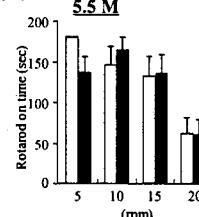
(D) 11 M
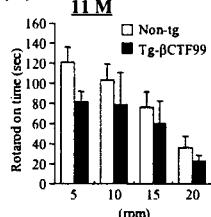
□ Non-tg
■ Tg-βCTF99

Pathological hallmarks of Alzheimer's Disease

1) Histological phenotypes

  a. <u>amyloid plaque, neurofibrillary tangle (NFT)</u>

  b. <u>neuronal cell loss</u>, gliosis
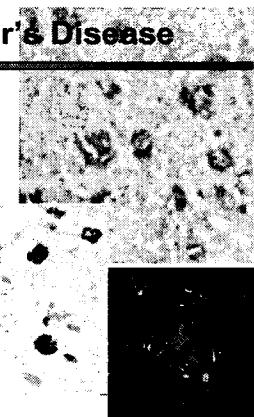
2) Behavioral phenotypes

  a. **Cognitive symptoms**

    - <u>learning and memory impairment</u>

  b. **Psychological symptoms**

    - **mood disorders** : <u>anxiety,</u> slowed thinking, tension, irritability

                         sadness, social withdrawal, depression

    - **other behavioral changes** : calculation defect, Language defect,

                                violence , crying, wandering

Table 1 Summary of the primary APP-based transgenic models of Alzheimer's disease

|	Transgene (mutation)	Promoter	Background	Phenotype						Initial reference
				DP	AP	Glia	NFT	ND	Cog	
NSEAPP	APP₇₅₁	NSE	JU	■	∗	∗	~	~	■	Quon et al. (1991)
PDAPP	APP_V717F	PDGF	B6 × D2 × SW	■	■	■	~	~	■	Games et al. (1995)
Tg2576	APP₆₉₅ (K670N,M671L)	PrP	B6 × SJL	■	■	■	~	-	■	Hsiao et al. (1996)
APP23	APP₇₅₁ (K670N,M671L)	Thy 1	B6 × D2 (B6 bX)	■	■	■	~	■	■	Sturchler-Pierrat et al. (1997)
PSAPP	APP₆₉₅ (K670N,M671L) + PS1_M146L	PrP + PDGF	B6 × D2 × SW	■	■	■	~	~	■	Holcomb et al. (1998)
CRND8	APP₆₉₅ (K670N,M671L + V717F)	PrP	C3H × B6	~	■	■	~	?	■	Chishti et al. (2001)
JNPL3	TAU_P301L	PrP	B6 × D2 × SW	~	-	■	■	?	?∗∗	Lewis et al. (2000)
TAPP	TAU_P301L + APP₆₉₅ (K670N,M671L)	PrP + PrP	B6 × D2 × SW	■	■	■	■	?	?∗∗	Lewis et al. (2001)

Promoters: NSE, neuron-specific enolase; PDGF, platelet-derived growth factor; PrP, prion protein. Mouse strains: B6, C57BL/6J; D2, DBA2; SW, Swiss Webster; bX, backcross. Transgene: APP₆₉₅, amyloid precursor protein (isoform); PS1, presenilin 1; Mutations: APP (K670N,M671L), Swedish mutation; APP V717F, London mutation. Phenotype: DP, diffuse (preamyloid) plaques; AP, amyloid plaques; Glia, gliosis (astrogliosis and microgliosis); NFT, neurofibrillary tangles; ND, neurodegeneration; Cog, cognitive impairment. For phenotype: ■, positive; -, negative; ?, unknown.
∗Amyloid plaques and gliosis seen in rare cases (about 5%);
∗∗Cognitive impairment may be difficult to demonstrate given the early neurological phenotype seen in these mice.

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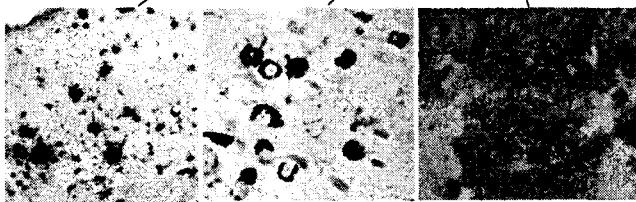
0 months   12 months   24 months

PDAPP
Tg2576
APP23
PSAPP
CRND8
JNPL3
TAPP

Legend:
- Amyloid plaques
- Neuronal cell loss
- Neurofibrillary tangles
- Gliosis
- Cognitive impairment
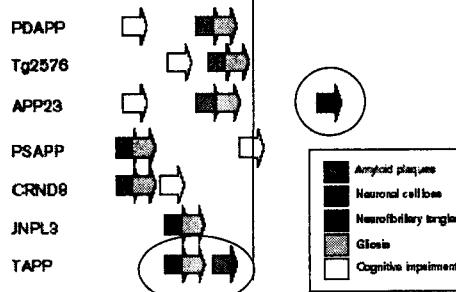
An outline of the sequence of pathology and apparent relationship to cognitive change in the PDAPP, Tg2576, APP23, PSAPP, CRND8, JNPL3 and TAPP mouse models of Alzheimer's disease (AD). Note that only the TAPP mouse exhibits both amyloid plaque and tangle pathology, and neurodegeneration has only been unequivocally reported in the APP23 mouse. Of further note is the apparently variable relationship between pathology and cognitive impairment across the various mouse models. In some mouse lines, e.g. PDAPP, robust cognitive impairment seems evident before plaques can be identified, yet in other lines, e.g. PSAPP, robust cognitive impairment only seems evident after amyloid plaque deposition. See Section 3 for a fuller description (and references) for each mouse line.

Uses of AD animal models

1. searching for in vitro data vs. in vivo data
2. studies on the mechanism underlying the <u>plaque pathogenesis</u>, <u>tangle formation</u>, and <u>other biochemical, histological changes</u>
3. <u>genetic studies</u> by breeding AD mice with other genetically engineered mice
4. studies on the progressive alterations of the <u>cognitive, and psychological function</u>
5. in efficacy tests of AD drug-candidates
6. searching for environmental risk factors that cause sporadic AD

Tg2576 (by Dr. Karen Hsiao)

1. Transgenic mice expressing the mutant form of hAPP (APPswe)
2. Hsiao et al. (1996). Science 274:99-102
3. produce β-amyloidosis and gliosis starting from 9-12 months
3. 공급처: Taconic, 샘타코 (국내)
4. Tg2576 is established in the C57BL6/J and SJL hybrid
   - C57BL6/J x SJL → C57BL6/J & SJL hybrid
   - B6/SJL hybrid x Tg2576 (C57BL6/J & SJL)
   → non-Tg, Tg2576 (B6/SJL)

C57BL6/J
SJL
C57BL6/J & SJL hybrid (C57BL6/J & SJL)
Tg2576
their non-Tg control
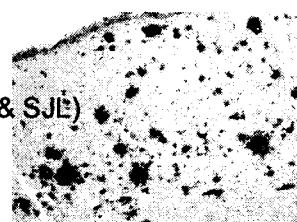
Tg-APPswe/PS1dE9 (by Dr. Borchelt DR)

1. Transgenic mice expressing the mutant form of human   APP and PS1 (APPswe/PS1dE9) in the brain

   - Jankowsky et al., Biomol Eng. 17 (2001):157-165

   - Jankowsky et al., J Neuropathol Exp Neurol. 62 (2003):1220-1227

2. produce β-amyloidosis and gliosis starting from 6-7 months

3. 공급처: JAX

4. 국내 공급업체: ㈜오리엔트

C57BL6/J
C3H
C57BL6/J/C3
Tg-APP/PS1
their non-Tg

Tg2576 mice

C57BL6/J
SJL

C57BL/6J/SJL hybrid

C57BL6/J x SJL

C57BL/6J/SJL hybrid x Tg2576

Non-Tg   Tg2576

Non-Tg	Drug 투여 대조군	Tg2576 Drug 미투여 대조군
Non-Tg	Drug 미투여 대조군	Tg2576 Drug 투여군 < Dose1 / Dose2
		Tg2576 Drug 투여 대조군 (positive control)

6-8개월간 투여

9-10 M   16-18M

Histological exam
Behavioral test
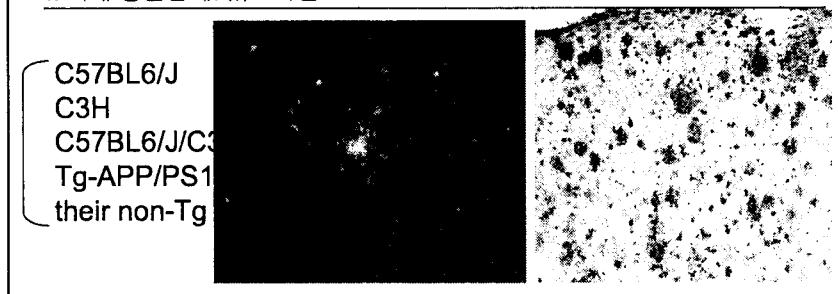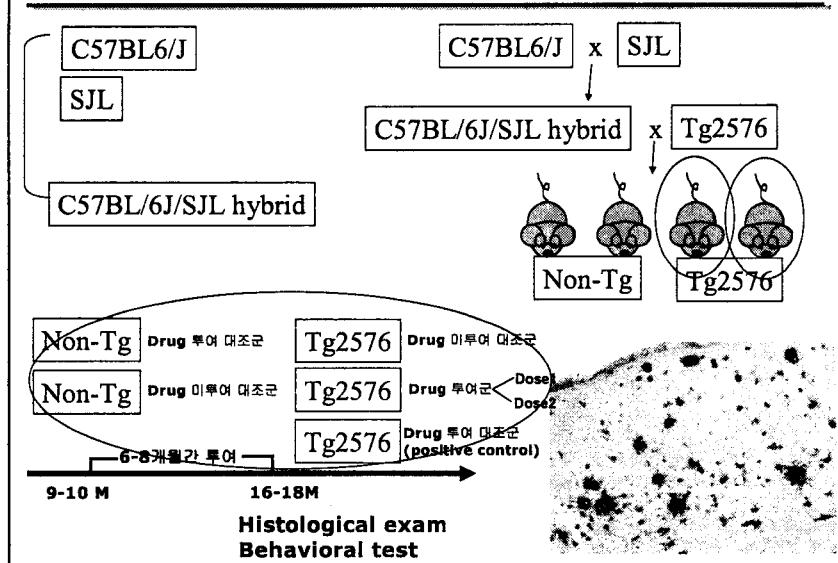
Immunological analyses of plaque pathogenesis
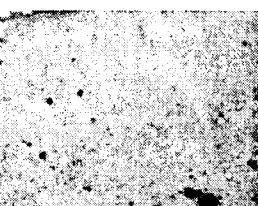
Tg (9 month)   Tg (15 month)   Tg (15 month) treated with cpd X
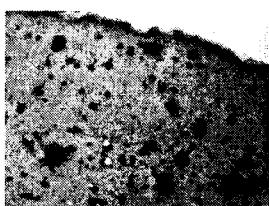
Modified pathogenesis

Control(wt) Tg-AD Tg-AD with cpd X
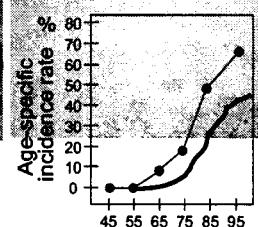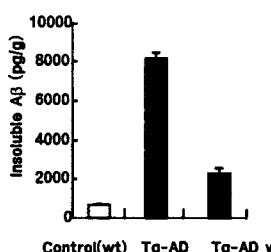
Tg-βCTF99/B6
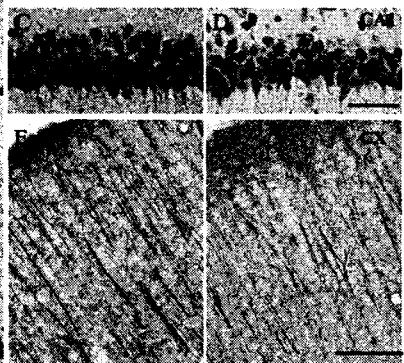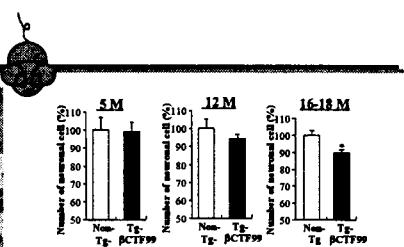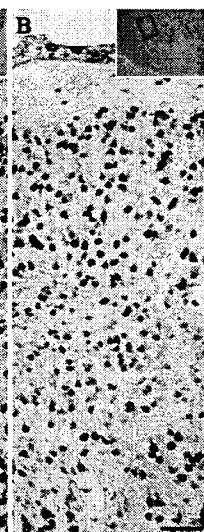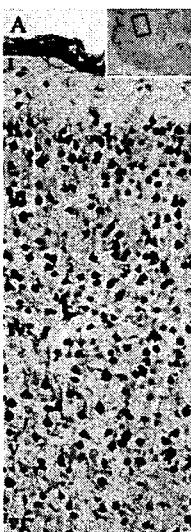
Table 2 Summary of studies conducted to examine novel therapies in transgenic models of Alzheimer's disease

| Approach	Treatment	Transgenic model	Result	Reference
Aβ antibody	(1) 11 × Aβ42 immunity (11 months duration)	PDAPP	(1) 13 months age, HC Aβ load – controls, 2.3%; treated, 0%	Schenk et al. (1999)
	(2) post plaque Aβ42 immunity (4–7 months)		(2) 18 months age, HC Aβ load – controls, 4.9%; treated, 0%	
	9 × Aβ42 immunizations (8 months duration)	Tg2576	16 months age, Cx Aβ load – controls, 4%; treated, 1%	Morgan et al. (2000)
	5 × immunizations (14 weeks duration)	CRND8	25 weeks age, HC ~ 50% reduction in Aβ plaque area	Janus et al. (2000)
	Aβ antibody (m266; passive) (5 months duration)	PDAPP	9 months age, reduction in plaque load, defined as number of animals with no plaque	DeMattos et al. (2001)
	6 × Aβ42 immunizations (3–4 months duration)	Tg2576	7–15 months age; evidence for primary effect versus new plaque (7–8 months) accumulation compared to clearance of pre-existing plaques (18 months)	Das et al. (2001)
	8 × Aβ homologous peptide (7 months duration)	Tg2576	18–20 months age, 80–90% reduction in HC and Cx Aβ load, 574 redn. Aβ (soluble); reduced inflammatory markers	Sigurdsson et al. (2001)
	4 × Aβ antibody (BAM-10; passive) (12 days' duration)	Tg2576	9–11 months age, CNS Aβ (insoluble) soluble Aβ amounts unch[anged]	Kotilinek et al. (2002)
γ-secretase inhibition	DAPT (10–100 mg/kg p.o.) 3 h pt	PDAPP	15–50% reduction Aβ in Cx	Dovey et al. (2001)
	DAPT (10–100 mg/kg s.c.) 3 h pt	Tg2576	6 months age, 80–40% reduction Aβ in Cx and plasma; 17 months age, NSE on Aβ Cx	Lanz et al. (2003)
	LY411575 (1–3 mg/kg p.o. × 90 days)	PDAPP	12 months age, 80–90% reduction in Aβ in Cx May et al. (2001) and plaque load	May et al. (2001)
Anti-inflammatory	Ibuprofen (6 months duration in diet)	Tg2576	16 months age 50% reduction in CNS Aβ load, reduction IL-1β and GFAP	Lim et al. (2000)
	NCX 2216 (NSAID) (6 months duration in diet)	PSAPP	12 months age, 45% redn. Aβ load in Cx/HC, microglia activation	Jantzen et al. (2002)
	Curcumin (6 months duration in diet)	Tg2576	16 months age 40–50% reduction CNS Aβ load, reduction IL-1β and oxidized proteins	Lim et al. (2001)
Cu/Zn chelator	Clioquinol (30 mg/kg per day × 9 weeks)	Tg2576	21 months age, 40–50% reduction CNS Aβ	Cherny et al. (2001)
Cholesterol lowering	BM15.766 (250 mg/kg per day × 8 weeks)	PSAPP	13 weeks age, ~50% reduction CNS Aβ (threshold reduction in plasma [cholesterol])	Refolo et al. (2001)
Oestrogen lowering	Ovariectomy ± oestradiol supplement (surgery at 13 weeks)	Tg2576	7 months age, ovariectomy increased CNS Aβ (soluble); oestradiol produced dose-related decrease in Aβ	Zheng et al. (2002)
Plasma Aβ sequestration	Gelsolin (0.6 mg/kg per 2 day i.p. × 3 weeks)	PSAPP	13 weeks age, ~50% reduction in CNS Aβ and HC/Cx plaque (diffuse?) load	Matsuoka et al. (2003)
	GM1 (15 mg/kg per 2 day i.p. 2 weeks)		13 weeks age, ~ 40–50% reduction in CNS Aβ	
Nicotine	Nicotine (200 µg/ml drinking fluid × 5.5 months duration)	Tg2576	14.5 months age, 50–80% reduction in insoluble CX Aβ. NSE versus soluble Aβ. Significant reduction in plaque burden	Nordberg et al. (2002)

[a]PSAPP mice also studied, however NSE of treatment on cortex Aβ load.
[b]Attenuation of cognitive impairment also reported following treatment
[c]Similar result demonstrated in PSAPP line
For each treatment the duration is given in parentheses. The result is presented as the age of animal at time of analysis, and a primary endpoint presented. Usually other endpoints have also been measured (see specific reference for further details).
NSE, no significant effect; HC, hippocampus; Cx, cortex

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Drug	Maker	Mechanism of action	Approved	Sales	Side effects
Donepezil (Aricept)	Eisai Inc.	Cholinesterase inhibitor, prevents the breakdown of acetylcholine in the brain	1997	$1.1 billion	Nausea, diarrhea, insomnia, vomiting, muscle cramps, fatigue, anorexia
Memantine (Namenda)	Forest Pharmaceuticals	NMDA receptor antagonist, blocks toxic effects associated with excess glutamate and regulates glutamate activation	2003	$498 million	Dizziness, headache, confusion, constipation
Rivastigmine (Exelon)	Novartis Pharmaceuticals Corporation	Cholinesterase inhibitor, prevents the breakdown of acetylcholine and butyrylcholine in the brain	2000	$226 million	Nausea, vomiting, loss of appetite, indigestion, weakness/lack of energy, dizziness, diarrhea, headache, stomach pain
Galantamine (Razadyne)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor, prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2001	$223 million	Nausea, vomiting, diarrhea, anorexia, weight loss
Galantamine (Razadyne ER)	Ortho-McNeil Neurologics Inc.	Cholinesterase inhibitor, prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine	2005	$24 million	Nausea, vomiting, diarrhea, anorexia, weight loss

	Drug	Manufacturer	Medicine's action	Status	Side effects
New drugs	3APS (Alzhemed)	Neurochem, Inc.	Inhibits amyloid-beta aggregates, binds and reduces soluble amyloid-beta	Phase 3	Nausea, vomiting
	MPC-7869 (Flurizan)	Myriad Pharmaceuticals	NSAID derivative; inhibits amyloid-beta aggregates and reduces their levels of amyloid-beta with little or no anti-inflammatory effect	Phase 3	None disclosed
	AAB-001	Elan Pharmaceuticals	monoclonal antibody binds to and clears amyloid-beta, is designed to directly deliver antibodies to amyloid-beta	Phase 2	None disclosed
	Neramexane	Forest Laboratories	NMDA receptor antagonist; blocks the effects of excessive glutamate at the receptor	Phase 3	None disclosed
Drugs for other conditions	Simvastatin (Zocor)	Merck	Statin; reduces cholesterol-carrying protein that promotes amyloid-beta aggregation	Phase 3	None disclosed for this trial, but Zocor has been known to cause nausea, diarrhea, abdominal pain and muscle cramps
	VP4896	Voyager Pharmaceutical	Hormone drug leuprolide acetate; decreases amount of luteinizing hormone in body, might prevent brain cell death	Phase 3	None disclosed
	Valproate	Manufacturer not disclosed	Anticonvulsant drug; neuroprotective properties may delay clinical progression of Alzheimer disease	Phase 3	None disclosed
Dietary supplements	Ginkgo biloba		Antioxidants neutralize free radicals and may reduce or prevent the damage they cause in brain cells	Phase 3	Headache, upset stomach, allergic reactions
	Vitamin E Selenium		Antioxidants neutralize free-radicals and may reduce or prevent the damage they cause in brain cells	Phase 3	None disclosed

Target practice: Most candidates being tested for Alzheimer disease are based on the amyloid hypothesis.

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알츠하임머성 치매의 약물학적 제어의 의미 및 목표

- AD 발병 예측선
- AD 증상 임계선
- AD 증상
- (C)
- (B')
- (B)
- (A)
- Modified pathogenesis
- 연령

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