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# Ganglioside metabolism in a transgenic mouse model of Alzheimer's disease: expression of Chol-1α antigens in the brain

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## ABSTRACT

The accumulation of Aβ (amyloid β-protein) is one of the major pathological hallmarks in AD (Alzheimer's disease). Gangliosides, sialic acid-containing glycosphingolipids enriched in the nervous system and frequently used as biomarkers associated with the biochemical pathology of neurological disorders, have been suggested to be involved in the initial aggregation of Aβ. In the present study, we have examined ganglioside metabolism in the brain of a double-Tg (transgenic) mouse model of AD that co-expresses mouse/human chimaeric APP (amyloid precursor protein) with the Swedish mutation and human presenilin-1 with a deletion of exon 9. Although accumulation of Aβ was confirmed in the double-Tg mouse brains and sera, no statistically significant change was detected in the concentration and composition of major ganglio-N-tetraosyl-series gangliosides in the double-Tg brain. Most interestingly, Chol-1α antigens (cholinergic neuron-specific gangliosides), such as GT1aα and GQ1bα, which are minor species in the brain, were found to be increased in the double-Tg mouse brain. We interpret that the occurrence of these gangliosides may represent evidence for generation of cholinergic neurons in the AD brain, as a result of compensatory neurogenesis activated by the presence of Aβ.

Key words: Alzheimer's disease, amyloid β-peptide, Chol-1α antigen, cholinergic neuron, ganglioside, transgenic mouse.

## INTRODUCTION

AD (Alzheimer's disease) is a progressive degenerative disease of the brain. It is the most common form of dementia and is characterized clinically by progressive loss of cognitive functions such as memory and learning and eventual death. Neuronal loss and brain atrophy are major hallmarks of AD, particularly in the basal forebrain cholinergic nuclei and their targets in the cortex and hippocampus. Neuropathologically, AD is characterized by the occurrence of amyloid deposits or 'senile plaques' in the brain, which consist mainly of an aggregated variant of Aβ (amyloid β-protein) (Ariga et al., 2008). Aβ undergoes a conformational transition from random coil to ordered structure rich in β-sheets and fibrils (Selkoe, 2002). Thus, although earlier views were that the appearance of Aβ is a marker of disease progression, accumulating evidence has indicated that they actually play a role in the neurodegenerative process (Yankner and Lu, 2009). Among the many possible mechanisms, there have been reports indicating that Aβ may become 'seeds' for the formation of fibrils after the addition of lipid vesicles containing $G_{M1}$ (Yanagisawa, 2007).

Gangliosides are sialic acid-containing glycosphingolipids expressed primarily in the outer leaflet of the plasma membrane of all vertebrate cells and are particularly abundant in neurons of the nervous system (Yu, 1994). Several earlier studies showed changes of ganglioside expression in AD brain. For example, ganglioside levels decreased in most of

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**Abbreviations:** Aβ, amyloid β-peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; HPTLC, high-performance TLC; PSEN, presenilin; PSEN1dE9, PSEN-1 with a deletion of exon 9; Tg, transgenic; WT, wild-type.
The nomenclature for gangliosides is based on the system of Svennerholm (Svennerholm, 1963).

the brain regions, including the cerebral cortex, hippocampus, and basal telencephalon, and especially in the frontal cortex and white matter (Crino et al., 1989; Svennerholm and Gottfries, 1994). Svennerholm and Gottfries (1994) reported that the concentration of gangliosides was reduced to 58–70% of the control level in all four grey matter areas and to 81% in frontal white matter of AD type I (early-onset or familial) cases, whereas it was only significantly reduced in temporal cortex, hippocampus, and frontal white matter in AD type II (late-onset or sporadic) cases. Clearly, the reduced ganglioside levels are correlated with the severe loss of neurons in the affected brain. The major brain ganglio-N-tetraosyl-series ganglioside species (GT1b, GD1b, GD1a and $G_{M1}$) are significantly decreased in the frontal and temporal cortices and basal telencephalon of the brains of patients with AD compared with the respective areas in control brains (Kracun et al., 1990; Kracun et al., 1992). Brooksbank and McGovern (1989) and Crino et al. (1989) also reported changes of ganglioside composition in AD brains in which b-series gangliosides, such as GT1b and GD1b, showed a significant decrease, in contrast with a slight increase in GT1a, GD3, $G_{M1}$ and $G_{M2}$. Since gangliosides have a strong affinity for A$\beta$ (Ariga et al., 2001), they could possibly participate in conformational changes of A$\beta$ (Choo-Smith et al., 1997). For this reason, ganglioside metabolism has been considered to be closely associated with the pathogenesis of AD (Mutoh et al., 2006; Ariga et al., 2008).

To further examine whether this association is present in animal models of AD whose pathology is well defined, we investigated the ganglioside composition of the brain in a double-Tg (transgenic) mouse model of AD that co-expresses mouse/human chimaeric APP (amyloid precursor protein) with Swedish double-mutation (K595N/M596L) (APPswe) and human PSEN1dE9 [PSEN-1 (presenilin-1) with a deletion of exon 9], and compared it with that of age-matched WT (wild-type) controls. Since their introduction two decades ago (Hsiao et al., 1996; Hsiao, 1998), AD model mice overexpressing APP have been proven to be useful in testing hypotheses of AD pathogenesis and testing rational therapeutic strategies. On the other hand, these Tg models overexpressing human APP only develop a partial AD-like phenotype and only recapitulate certain features of human AD. Pathologically they show amyloid plaque deposits, elevated A$\beta$ levels, neuritic changes, phosphorylated tau protein epitopes, $\alpha$-synuclein-positive neuritis, gliotic reactions and inflammatory responses (for a review, see Turner, 2001). It is also interesting to note that cholinergic abnormalities exist in the immediate vicinity of amyloid plaques of these mutants (Sturchler-Pierrat et al., 1997; Wong et al., 1999). Given the salience of neuropathology in the cholinergic pathways mediating cognition in AD and the relative lack of information about ganglioside concentrations in these areas, we made additional efforts to examine gangliosides that may serve as biomarkers of cholinergic neurons in the present paper.

## MATERIALS AND METHODS

### Materials and animals

Authentic Chol-1$\alpha$ gangliosides, GT1a$\alpha$ and GQ1b$\alpha$, were isolated from bovine brains (Ando et al., 1992; Hirabayashi et al., 1992). The double-Tg mouse model of AD [B6.Cg-Tg (APPswe,PSEN1dE9)85Dbo/J] co-expressing APPswe and PSEN1dE9 (Jankowsky et al., 2001; Jankowsky et al., 2004) was purchased from JAX Mice and Services (Bar Harbor, ME, U.S.A.). Double-Tg and age-matched WT mice were maintained for 1 year according to the guidelines of the Institutional Animal Care and Use Committee of the Medical College of Georgia. Genotypes of mice were confirmed by PCR of genomic DNAs using specific primer sets for APPswe, PSEN1dE9 and mouse prion protein promoter. For serum preparation, blood samples were collected immediately by heart puncture after mice were anaesthetized. Mice were then killed by cervical dislocation, and the brains were quickly removed. The middle third of brains was sliced using a brain matrix (Braintree Scientific, Braintree, MA, U.S.A.) to prepare brain slices for immunohistochemical and biochemical analyses. For ganglioside analysis, the brain slices were stored at −80°C until use.

### Immunohistochemistry of A$\beta$s in brain sections

Coronal sections (12 μm thick) were prepared from brain slices using a cryostat and were mounted on frost-free glass slides. Each section was pretreated with 1% BSA in PBS for 15 min to block non-specific binding and then incubated with biotinylated monoclonal antibody against human A$\beta$ (clone 82E1; IBL-America, Minneapolis, MN, U.S.A.; 1:100 in 1% BSA in PBS) overnight at 4°C. The 82E1 antibody detects soluble and fibrillar $A\beta_{1-42}$ and $A\beta_{1-40}$. After washing with PBS, the section was incubated in 1% BSA in PBS containing rhodamine-conjugated anti-mouse IgG antibody for 2 h, followed by nuclear staining with Hoechst 33258 (Sigma–Aldrich, St. Louis, MO, U.S.A.) for 10 min. The slide was mounted and covered with a coverslip. The section was then examined using a fluorescence microscope.

### Quantification of $A\beta_{1-42}$ in sera

The concentrations of serum $A\beta_{1-42}$ in double-Tg and age-matched WT mice were determined using a Human $\beta$ Amyloid (1-42) ELISA kit, High-Sensitive (Wako Chemicals USA, Richmond, VA, U.S.A.). In brief, 100 μl of serum, prepared from blood samples by centrifugation (10000 rev./min for 10 min) and diluted to an appropriate titre in PBS, was placed into a well of a 96-well microtitre plate coated with anti-human $A\beta_{1-16}$ antibody and then incubated at 4°C overnight. After washing with a wash solution, the sample was incubated for 1 h in 100 μl of horseradish peroxide-conjugated anti-human $A\beta_{35-43}$ antibody (Fab′ fragment). The sandwiched $A\beta_{1-42}$ was visualized with a TMB (3,3′,5,5′-tetramethylbenzidine) solution and the absorbance was

measured at a wavelength of 450 nm using a microtitre plate reader after the addition of a stop solution.

### Ganglioside isolation and HPTLC (high-performance TLC)

Gangliosides were isolated from brain slices containing hippocampal/cortical tissue (150–210 mg wet weight) of double-Tg and age-matched WT mice as previously described (Ariga et al., 1988). In brief, total lipids were extracted sequentially with 5 ml each of chloroform/methanol (1:2, 1:1 and 2:1, by volume) and chloroform/methanol/water (30:60:8, by volume; solvent A). The combined lipid extracts were evaporated and redissolved in 5 ml of solvent A and applied to a diethylaminoethyl-Sephadex A-25 column (acetate form, 2 ml bed volume), followed by elution with 20 ml of solvent A to remove neutral lipids. The acidic lipids, including gangliosides, were then eluted with 20 ml of chloroform/methanol/0.8 M sodium acetate in water (30:60:8, by volume), followed by desalting using Sep-Pak Cartridge column chromatography (Waters, Milford, MA, U.S.A.; Kubo and Hoshi, 1985). Lipid-bound sialic acid contents in the acidic lipid fraction were determined by the resorcinol/hydrochloric acid reagent (Svennerholm, 1957). A portion of the acidic lipid fraction, containing a ganglioside sample with 5 μg of sialic acid, was applied to an HPTLC plate and developed with chloroform/methanol/0.2% calcium chloride in water (50:45:10, by volume). After development, gangliosides on HPTLC plates were visualized by spraying the orcinol/sulfuric acid reagent and heating at 100°C and then quantified by densitometric analysis. Statistical comparison of the data was performed by the Student's *t* test.

### HPTLC immunostaining

HPTLC immunostaining was performed as previously described (Ngamukote et al., 2007). After developing the HPTLC plate as described above, the plate was coated with an n-hexane solution containing 0.4% polyisobutylmethacrylate (polymer; Sigma–Aldrich) for 1 min. After drying, the plate was incubated for 2 h at room temperature with an anti-Chol-1α monoclonal antibody (GGR-41; IgG; Kusunoki et al., 1993) diluted with 1% BSA in PBS. The plate was then incubated for 1 h with horseradish peroxide-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.) diluted with 1% BSA in PBS. Bands recognized by the antibody were detected using the Western Lightening Western Blot Chemiluminescence reagent (PerkinElmer Life and Analytical Sciences, Boston, MA, U.S.A.). Chol-1α antigens were quantified by densitometric analysis. After the plate was dipped in chloroform to remove excess polymer, ganglioside bands were visualized by spraying orcinol/sulfuric acid reagent and heating at 100°C.

## RESULTS

### Immunohistochemical detection of Aβ in double-Tg mouse brains

First, we confirmed whether our double-Tg mice co-expressing APPswe and PSEN1dE9 represent a valid model of the plaque formation in AD. Figure 1 shows immunohistochemical localization of Aβ in the cortex of AD double-Tg mice. It has been reported that these double-Tg mice develop substantial Aβ deposits in their brains by 6 months of age (Jankowsky et al., 2004). Consistently, the appearance of Aβ staining (shown in red) was massive and mostly in the cortical region of the brains of 1-year-old double-Tg mice, and was confined to the extracellular areas, except in the cerebral vessels where it lined the vessel wall (Figure 1). There were no gender-related differences in the immunostaining (results not shown), consistent with the notion that Aβ deposits in the brain are a general pathological hallmark in AD (Selkoe, 2002). As expected, the WT mice did not show any evidence of Aβ staining (results not shown).

### Concentration of $A\beta_{1-42}$ in double-Tg mouse sera

Figure 2 shows the concentrations of serum $A\beta_{1-42}$ in double-Tg and age-matched WT mice determined by ELISA using anti-$A\beta_{1-16}$ antibody and anti-$A\beta_{1-42}$ antibody. The $A\beta_{1-42}$ level

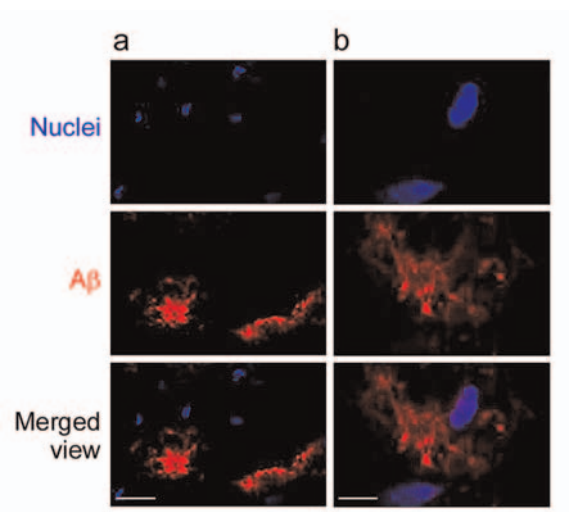


**Figure 1 Immunohistochemical localization of Aβ in the cortex of double-Tg mice co-expressing APPswe and PSEN1dE9**

The coronal brain sections are 12 μm thick. Nuclei (blue) and Aβ (red) were stained with Hoechst 33258 and anti-human Aβ antibody respectively. (**a**) Low-magnification view; (**b**) high-magnification view. Scale bar: (**a**) 20 μm and (**b**) 5 μm.

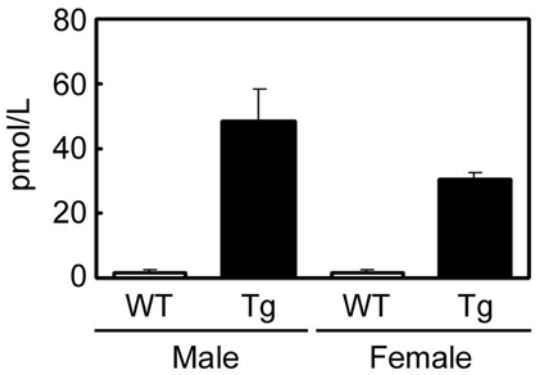


**Figure 2 Serum A$\beta_{1-42}$ concentration in double-Tg and age-matched WT mice**
Serum A$\beta_{1-42}$ concentrations were quantified using a Human $\beta$ Amyloid (1–42) ELISA kit, High-Sensitive (Wako Chemicals USA); $n$=3–4.

was significantly high in sera of double-Tg mice, but not in sera of WT mice. In human AD, elevated levels of serum A$\beta$ were reported to be associated with vascular risk factors of AD (Sundelof et al., 2008; Abdullah et al., 2009). Longitudinal evaluations of serum A$\beta$ should provide a better understanding of the significance of this association in AD aetiology (Abdullah et al., 2009). Using these double-Tg mice as a model of AD, we then evaluated the metabolism of brain gangliosides.

## Composition of major gangliosides in double-Tg mouse brains

In human AD, several earlier studies documented abnormal ganglioside metabolism in brain regions of demented patients, in which the ganglioside levels were significantly reduced (Crino et al., 1989; Svennerholm and Gottfries, 1994). In addition, analysis of the ratios of a- and b-series gangliosides revealed that b-series gangliosides were preferentially decreased in brain regions of demented AD patients (Crino et al., 1989). The decreased levels of gangliosides are consistent with the degradation of cortical neurons, especially in the frontal cortex (Kalanj et al., 1991), and a reduced density of nerve endings in the brain regions of demented AD patients (Svennerholm and Gottfries, 1994). Hence, these findings suggest that abnormal ganglioside metabolism coincides with the affected cortical areas of neurodegeneration that afflicts AD.

In contrast with these human studies, we found no significant differences in the lipid-bound sialic acid content in the brain slices containing hippocampal/cortical tissue prepared from double-Tg and age-matched WT mice (Table 1). In addition, there was no significant difference in the expression levels of major ganglio-N-tetraosyl gangliosides ($G_{M1}$, GD1a, GD1b and GT1b) in the brains between double-Tg and age-matched WT mice (Figure 3a and Table 1). This is consistent with the report by Sawamura et al. (2000) who also did not detect notable changes in major ganglioside patterns in the brains of mutant PSEN-2 double-Tg mice despite the remarkable increase in the level of A$\beta_{1-42}$ and significantly lower levels of glycerophospholipids and sphingomyelin. In addition, Bernardo et al. (2009) did not find significant differences in a- or b-series gangliosides between WT and double-Tg mice expressing APPswe and PSEN1dE9. These studies as well as our present results indicate no significant changes in major brain ganglioside metabolism in AD model mice, despite the presence of massive accumulation of A$\beta$ deposits in the brains of these animals.

## Composition of minor gangliosides in double-Tg mouse brains

Several investigators have documented alteration of minor gangliosides in human AD. Kracun et al. (1990) and Kalanj et al. (1991) reported that the levels of simple gangliosides, such as $G_{M2}$ and $G_{M3}$, were elevated in the frontal and parietal cortices in AD brains; the elevation of $G_{M2}$ and $G_{M3}$ in these areas may correlate with an accelerated lysosomal degradation of gangliosides occurring during neuronal death and astrogliosis respectively. Barrier et al. (2007) also reported an increase in $G_{M2}$ and $G_{M3}$ within the cortices of Tg mice expressing human APP751 with Swedish and London mutations and human PSEN-1 (M146L). In the present study, $G_{M3}$ was slightly increased in double-Tg mouse brains compared with WT mouse brains (Table 1); the increase was statistically significant for $G_{M3}$ in female double-Tg mouse brains. We attribute the small and significantly elevated $G_{M3}$ to the gliotic reactions or invading macrophages that occur more prominently in female Tg mice than in the males. We did not find any significant difference in the content of $G_{M2}$ between double-Tg and WT mouse brains (Table 1).

## Increase of Chol-1$\alpha$ antigens (GT1a$\alpha$ andGQ1b$\alpha$) in double-Tg mouse brains

The most consistent and interesting finding of the present study, however, is the increased expression of Chol-1$\alpha$ antigens, GT1a$\alpha$ and GQ1b$\alpha$, in the brain of double-Tg mice (Figures 3b and 4). These gangliosides are normally minor species in the brain and serve as markers of cholinergic neurons (Ando et al., 1992; Hirabayashi et al., 1992). The expression of Chol-1$\alpha$ antigens in rat brain regions such as the hippocampus is developmentally regulated, and their concentrations increase with aging (Derrington and Borroni, 1990). Chol-1$\alpha$ antigens are first detected between days 10 and 20 of postnatal age, reaching adult levels on day 50 in normal rat brains (Derrington et al., 1990). In the present study, we found that the expression levels of Chol-1$\alpha$ antigens, especially GQ1b$\alpha$, were elevated in double-Tg mouse brains as compared with those in WT mouse brains (Figures 3b and 4). The increase was especially significant in female double-Tg mouse brains. No significant differences were found in the expression of GT1a$\alpha$ and GQ1b$\alpha$ between male and female WT mouse brains. These observations may reflect a change in specific ganglioside metabolism associated with the pathological processes underlying AD.

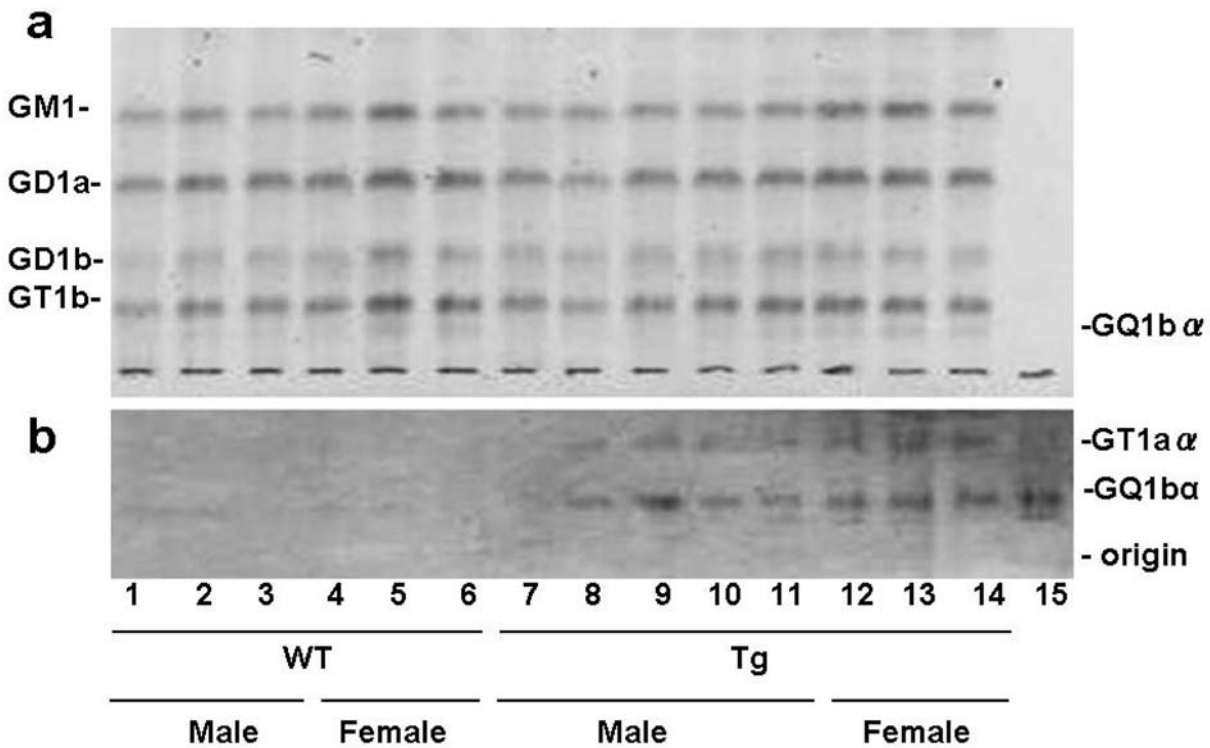


**Figure 3** **Expression of gangliosides in double-Tg and age-matched WT mouse brains**
Lanes 1–3, male WT mouse brain gangliosides; lanes 4–6, female WT mouse brain gangliosides; lanes 7–11, male double-Tg mouse brain gangliosides; lanes 12–14, female double-Tg mouse brain gangliosides; lane 15, authentic GT1aα (4 ng) and GQ1bα (20 ng). Gangliosides were separated by HPTLC with a solvent system of chloroform/methanol/0.2% $CaCl_2$ (55:45:10, by volume) and visualized by (**a**) orcinol/sulfuric acid staining or (**b**) immunostaining with an anti-Chol-1α monoclonal antibody (GGR-41).

## DISCUSSION

In this study, we examined ganglioside metabolism in the brains of double-Tg mice expressing APPswe and PSEN1dE9, a model of amyloid aggregation and associated neuropathology of AD. No statistically significant change in major brain gangliosides was detected, as reported previously (Sawamura et al., 2000). Among minor brain gangliosides, however, the expression of $G_{M3}$ exhibited a slight increase in the female double-Tg mouse brains. Most importantly, an increase in the cholinergic neuron-specific gangliosides, GT1aα and GQ1bα, also known as Chol-1α antigens, was found in the double-Tg mouse brains. In AD patient sera, the presence of antibodies

**Table 1** **Lipid-bound NeuAc contents in gangliosides in double-Tg and age-matched WT mouse brains (mean ± S.D.; $n=3–7$)**

| | WT | | Tg | |
|---|---|---|---|---|
| **Ganglioside** | **Male** | **Female** | **Male** | **Female** |
| Expressed in terms of a percentage | | | | |
| $G_{M3}$ | 1.3 ± 0.1 | 1.0 ± 0.4 | 1.6 ± 0.3 | 3.1 ± 0.3* |
| $G_{M2}$ | 0.6 ± 0.1 | 0.6 ± 0.3 | 0.9 ± 0.3 | 1.0 ± 0.4 |
| $G_{M1}$ | 19.8 ± 0.5 | 18.3 ± 1.1 | 18.0 ± 2.0 | 20.3 ± 0.9 |
| GD3 | 1.3 ± 0.2 | 0.9 ± 0.4 | 0.7 ± 0.3 | 0.8 ± 0.4 |
| GD1a | 28.3 ± 0.4 | 32.2 ± 1.4 | 26.5 ± 1.6 | 30.4 ± 0.6 |
| GD2 | 2.7 ± 0.4 | 2.6 ± 0.8 | 1.9 ± 0.4 | 1.7 ± 0.5 |
| GT1a | 1.7 ± 0.1 | 1.6 ± 0.4 | 1.5 ± 0.2 | 1.8 ± 0.5 |
| GD1b | 17.0 ± 0.1 | 15.4 ± 1.4 | 18.0 ± 0.7 | 14.6 ± 0.5 |
| GT1b | 24.0 ± 0.4 | 23.3 ± 0.8 | 26.2 ± 1.1 | 23.0 ± 1.3 |
| GQ1b | 3.2 ± 0.2 | 3.8 ± 0.9 | 4.0 ± 0.8 | 3.3 ± 0.8 |
| Expressed in terms of μg/g of wet weight | | | | |
| NeuAc | 341.9 ± 35.4 | 347.3 ± 60.7 | 340.7 ± 33.5 | 344.2 ± 11.0 |

* $P<0.05$.

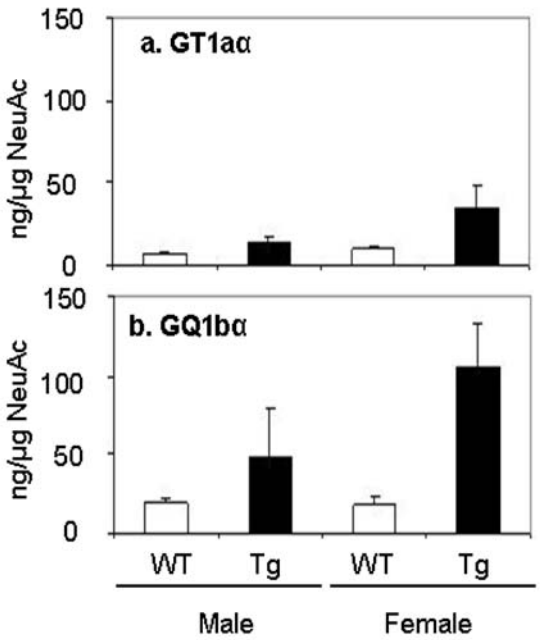


**Figure 4 The content of Chol-1$\alpha$ antigens, GT1a$\alpha$ (a) and GQ1b$\alpha$ (b), in double-Tg and age-matched WT mouse brains**

GT1a$\alpha$ and GQ1b$\alpha$ were quantified by densitometric analysis of HPTLC immunostaining; $n$=3–8. Statistical analyses were performed by the Student's $t$ test. For GT1a$\alpha$, $P<0.01$ (Tg male compared with WT male); $P<0.04$ (Tg female compared with WT female); $P<0.05$ (Tg male compared with Tg female) respectively. For GQ1b$\alpha$, $P<0.03$ (Tg male compared with WT male); $P<0.02$ (Tg female compared with WT female); $P<0.02$ (Tg male compared with Tg female) respectively.

that bind specifically to cholinergic neurons has been reported (Chapman et al., 1988). The increase in these antibodies in the patients may be attributed to the increase in Chol-1$\alpha$ antigens in AD brains found in the study. As discussed below, it is possible that these Chol-1$\alpha$ antigens are involved in the aetiology of AD by preferentially affecting cholinergic neurons.

Chol-1$\alpha$ antigens are marker glycolipids of mature cholinergic terminals (Richardson et al., 1982; Derrington and Borroni, 1990). These antigens are expressed relatively late during the maturation process of the cholinergic synapse in rat brains (Derrington and Borroni, 1990). *In vitro*, their expression levels were found to be significantly higher in the rat hippocampus and septum slices treated with nerve growth factor, which stimulates the maintenance of cholinergic neurons (Derrington et al., 1990; Gahwiler et al., 1990). Consistent with these reports, Chol-1 antigens were found to co-localize with choline acetyltransferase in the human central nervous system (Whittaker et al., 1992). In addition, Chol-1$\alpha$ antigens have been suggested to play functional roles in cholinergic neurons. For instance, it has been reported that treatment with anti-Chol-1$\alpha$ monoclonal antibody inhibited the release of acetylcholine from synaptosomes prepared from rat brains (Ando et al., 2004). Also, administration of the anti-Chol-1$\alpha$ antibody to rats markably suppressed the memory and learning abilities. On the other hand, treatment with Chol-1$\alpha$ antigens induced choline uptake by synaptosomes; as a result of increased choline uptake, acetylcholine synthesis was enhanced. These findings indicate that Chol-1$\alpha$ antigens can ameliorate decreased functions of synapses in aged brains, suggesting that Chol-1$\alpha$ antigens play a pivotal role in cholinergic synaptic transmission and participate in cognitive functions (Ando et al., 1998). Presynaptic degenerative changes in basal forebrain cholinergic nuclei have been observed in AD brain. Similarly, A$\beta_{1-42}$ has been suggested to induce the death of basal forebrain cholinergic neurons in mice via p75 neurotrophin receptor (Sotthibundhu et al., 2008). It was expected that up-regulation of Chol-1$\alpha$ antigens in cholinergic neurons represents evidence of a compensatory mechanism for the decline of cholinergic function in the AD model mouse brains. This interpretation is particularly relevant in light of the fact that there is little if any neurodegeneration in the double-Tg mouse.

On the other hand, there also is a possibility that Chol-1$\alpha$ antigens are involved in neurogenesis in AD brains. Although the occurrence of neurogenesis in brains in human AD patients is still controversial (Waldau and Shetty, 2008), a growing body of evidence has accumulated indicating an increase of adult neurogenesis not only in APP-overexpressing mouse model brains, but also in AD patient brains (Jin et al., 2004b, 2004a; Lopez-Toledano and Shelanski, 2004). It has been consistently reported that A$\beta$ is capable of stimulating the proliferation of neural stem cells and adult neurogenesis (Lopez-Toledano and Shelanski, 2004; Sotthibundhu et al., 2009). In contrast, other studies have suggested that A$\beta$ has cytotoxic effects on neural stem cells and disrupts neurogenesis (Haughey et al., 2002a, 2002b; Millet et al., 2005). It has also been reported that A$\beta_{1-40}$ and $G_{M1}$ co-operatively induce cell death in mouse neural stem cells (Yanagisawa et al., 2010). These reports clearly suggest neurogenic or neurotoxic effects of A$\beta$ in neurogenesis in AD brains, which needs to be resolved. In light of our finding that there is an increase in the level of Chol-1$\alpha$ antigens in the Tg-model animals, it is tempting to suggest that the increase is associated with neurogenesis. Although we have no direct evidence of this association, Chol-1$\alpha$ antigens, GT1a$\alpha$ and GQ1b$\alpha$, have been reported to be expressed in mouse neural stem cells (Ngamukote et al., 2007). This report can be taken as indirect evidence that Chol-1$\alpha$ antigens are involved in neurogenesis. Further, neurogenesis may also be involved in the compensatory mechanism for the decline of cholinergic function in AD brains as discussed above. There is the intriguing possibility that degeneration of the cholinergic pathways in early stages of the disease could result in a rebound of neurogenesis at later stages of the disease, which is expected to be reflected by the appearance of Chol-1$\alpha$ antigens in the brain. If this possibility were true, it would open a window of opportunity for the treatment of the disease by promoting neurogenesis. We fully realize that increased neurogenesis alone is not sufficient to produce new, functional neurons. It would be important, therefore, to

determine whether the new neurons migrate and incorporate properly into the hippocampal formation. In this regard, the discovery of adult neurogenesis raises the possibility that the nervous system has an intrinsic capacity for repair. Perhaps more controversially, it raises the question as to whether impaired or failed neurogenesis may contribute to the decline in neurodegenerative diseases (Abdipranoto et al., 2008). Future studies are clearly needed to examine whether it is possible to promote neurogenesis to achieve functional recovery in AD.

In the present study, the increase in Chol-1α was significantly higher in female double-Tg mice. It has been generally recognized that the prevalence of AD is also higher in women. However, the aetiology of high risk of AD in women is unknown. A role for oestrogen, by influencing brain development (pre- or post-menarche) or senescence (post-menopause), is a leading hypothesis. Some studies suggest a protective effect of oestrogen replacement therapy in the risk of developing AD (Turner, 2001). In addition to the prevalence of AD, virtually all cholinergic characteristics have been reported to be affected by sex differences. Berger-Sweeney (2003) has reported that sexual dimorphism in the development of the cholinergic basal forebrain system could have significant consequences for cognitive performance in sex differences during development and aging. In normal animals, basal acetylcholine concentrations, high-affinity choline uptake and choline acetyltransferase activity are different between females and males (Rhodes and Rubin, 1999). Cholinergic areas, such as amygdala and hippocampus, are larger in male than those in female (Madeira and Lieberman, 1995). Oestrogen, which may be involved in the high prevalence of AD in women, can modulate neurogenesis, differentiation, growth and survival of neurons in development and throughout the life of animals (Tanapat et al., 1999; Schaevitz and Berger-Sweeney, 2005). In fact, it has been reported that female mice have more proliferative cells in dentate gyrus than male mice in an oestrogen-level-dependent manner (Tanapat et al., 1999). It is expected that the increase in Chol-1α gangliosides in female double-Tg mice compared with male double-Tg mice is attributable to neurogenesis and the increase in cholinergic neurons in the high levels of oestrogen. The intriguing possibility that a higher neurogenesis occurs in female Tg mice certainly merits further experimentation.

Finally, AD model animals with disrupted ganglioside biosynthesis have also been used to examine the relationship between ganglioside metabolism and aspects of AD. For example, Bernardo et al. (2009) analysed the AD model of Tg mice expressing APPswe and PSEN1dE9 crossbred with mice deficient in GD3-synthase, which catalyses the synthesis of b-series gangliosides. In the triple mutant mice, b-series gangliosides, including GD3, were completely absent, but $G_{M1}$ and GD1a were significantly increased by 63.8% and 50.8% respectively. On the other hand, Oikawa et al. (2009) crossbred Tg mice expressing human APP having Swedish and London mutations with $G_{M2}$-synthase-knockout mice in which $G_{M1}$, GD1a, GD1b and GT1b were completely missing but $G_{M3}$ and GD3 were abundantly expressed (Takamiya et al., 1996). The mutant mice expressing no $G_{M1}$ showed a significant increase of Aβ accumulation in vascular tissues and formation of dysphonic-form amyloid angiopathy in the brain. These results suggest that b-series gangliosides derived from GD3-synthase could contribute to Aβ accumulation in the brain of these mutants. Indeed, Ariga et al. (2001) showed a strong correlation between the number of sialic acid residues on a ganglioside and its affinity for Aβ. In this model system the more complex b-series gangliosides had higher affinity for Aβ than the a-series gangliosides. Thus inhibition of GD3-synthase can be a novel therapeutic target to combat the cognitive deficits, amyloid plaque formation and neurodegeneration seen in AD. Targeting GD3-synthase would also have the effect of reducing levels of GQ1bα and GT1aα. It would be interesting to examine how this pattern of changes affects neurogenesis, AD-related neuropathology and cognitive functions.

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