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Mutant Presenilin 2 Causes Abnormality in the Brain Lipid Profile in the Development of Alzheimer's Disease

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Mutation in the presenilin 2 (PS2mt) is known to be one of factors involved in the development of Alzheimer's disease (AD). It was recently revealed that an abnormality of lipid metabolism is a phenomenon occurring in AD. Therefore, the aim of this study was to investigate the potential relationship between the mutation of PS2 and alterations of the lipid profile within the brain. The results showed there increases in the levels of cholesterol, low density lipoprotein and triglyceride, but a decrease in the level of high density lipoprotein in brain tissues expressing mutant PS2. These findings indicated that PS2mt is involved in the abnormalities of the lipid profile, which could cause or result in the development of AD.

Key words: Transgenic mice, Presenilin 2, Alzheimer's disease, Cholesterol, Triglyceride, HDL cholesterol, LDL cholesterol

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive deterioration of cognition and memory. Recent studies have revealed abnormalities in lipid metabolism in brain ageing and the pathogenesis of late onset AD (Wellington *et al.*, 2004; Cutler *et al.*, 2004; Sawamura *et al.*, 2000). An elevated level of plasma cholesterol is supposed to be associated with an increased risk for the development of AD. The major apolipoprotein (ApoE) at levels of approximately 3-5 μg/mL in the cerebrospinal fluid has been shown to affect late onset AD (Mahley, 1998). The synthesis of ApoE is induced in response to central nervous system injury or disease, where it coordinates the mobilization and redistribution of cholesterol in the repair and maintenance of neuronal membranes (Ignatius *et al.*, 1986). Furthermore, intracellular cholesterol regulates the generation of Aβ peptides from amyloid precursor protein, which accumulate as amyloid plaques in the brains and cerebral blood vessels of AD patients. It has also been suggested that ApoE is a key mediator of Aβ metabolism (Shie *et al.*, 2002; Wahrle *et al.*, 2002; Burns *et al.*, 2003; Refolo *et al.*, 2001; Buxbaum *et al.*, 2001; Ehehalt *et al.*, 2003). Moreover, cholesterol-lowering drugs reduce the prevalence of AD (Refolo, 2001; Jick *et al.*, 2000; Fassbender *et al.*, 2001). Inhibition of cholesterol biosynthesis was found to reduce the amyloid burden in guinea pig and murine models of AD (Refolo *et al.*, 2001; Fassbender *et al.*, 2001). There are conflicting reports on whether the levels of plasma lipid or lipoprotein are altered in AD patients (Pappolla *et al.*, 2003; Tan *et al.*, 2003). However, several pieces of evidence indicate the possible roles of plasma cholesterol in the clearance of Aβ peptides, which are thought to bind to plasma lipoproteins after crossing the blood brain barrier in the pathogenesis of AD (Koudinov *et al.*, 2001; Koudinov *et al.*, 1998). These observations suggest that intracellular lipid metabolism in the peripheral or central nervous system may participate in the pathogenesis of AD.

Conversely, the majority of familial AD is supposed to be caused by mutations within the presenilin genes, although the involved mechanism remains to be fully understood (Mori *et al.*, 2002; Deng *et al.*, 1996; Janicki *et al.*, 1997; De Sarno *et al.*, 2001). Neuronal cells expressing

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N141I mutant PS2 were revealed to significantly increase apoptotic cell death compared to those expressing wild PS2 or untransfected control (Mori *et al.*, 2002; Deng *et al.*, 1996; Janicki *et al.*, 1997). The lack of memory in PS2 transgenic mice, particularly in cases of mutant PS2 transgenic mice, also suggests the involvement of the PS2 gene in the neuronal degeneration associated with AD (Hwang *et al.*, 2002; Lee *et al.*, 2006).

Therefore, the aim of this study was to investigate the possible relationship between the mutation of PS2 and the alteration of the brain lipid profile, which may be accompanied with neuronal cell death in the development of AD.

MATERIALS AND METHODS

Mutant and wild PS2 transgenic mice

Transgenic mice expressing wild PS2 (PS2wt-Tg) and mutant PS2 (PS2mt-Tg), described elsewhere (Hwang *et al.*, 2002), were used in this study. Transgenic and age-matched control mice were handled in an accredited Korea FDA animal facility in accordance with the AAALAC International Animal Care policies. Mice were housed in cages under a strict light cycle (light on at 06:00 and off at 18:00), and given a standard irradiated chow diet (Purina Mills, St. Louis, MO) at libitum. The mice were maintained in a specified pathogen-free state.

Determination of triglyceride

The brains of the mice were removed immediately after exsanguinations, and frozen on dry ice. Chloroform-methanol (1:1) extracts of the brains were dried under nitrogen gas and re-extracted with chloroform–methanol (2:1). The lower phase was evaporated, and the residue extracted with isopropanol prior to analyses. Plasma was separated by centrifugation at 1,500 rpm for 20 min at 4°C. The levels of triglycerides were measured with an enzymatic colorimetric kit obtained from Roche for use with the Cobas Mira Chemstation (Boehringer Mannheim Corp., Germany). The triglycerides in the sample were hydrolyzed to glycerol and fatty acids using lipoprotein lipase. The glycerol was then phosphorylated to glycerol-3-phosphate using glycerol kinase and subsequently catalyzed with glycerol oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide was then reacted in the presence of peroxidase to form a chromogen, with the increases in the absorbance measured at 405 nm being proportional to the triglycerides concentration. The amount of triglyceride was express in units of mg/dl.

Determination of total cholesterol

The total cholesterol was measured using an enzymatic kit obtained from Roche for use with the Cobas Mira Chemstation (Boehringer Mannheim Corp., Germany). This method follows a two step approach. In the first step, cholesterol is desterified by the action of cholesterol esterase, and subsequently exposed to the action of cholesterol oxidase. This second step was coupled to a chromogen (color-forming compound), which can be measured using a spectrophotometer, with the increases in the absorbance due to the chromogen at 405 nm being proportional to the cholesterol concentration in the sample. The amount of total cholesterol was expressed in units of mg/dl.

Determination of high density lipoprotein

The high density lipoprotein (HDL) was measured using a HDL direct kit obtained from Roche for use with the Cobas Mira Chemstation (Boehringer Mannheim Corp., Germany). After elimination of the cholesterol in non-HDL lipoproteins, a second reaction mix was added and incubated for 10 min at 37°C, where the HDL cholesterol is selectively exposed to the action of cholesterol esterase and cholesterol oxidase in a color forming reaction, which can be monitored spectrophotometrically by measuring the absorbance at 405 nm. The results were expressed in units of mg/dl.

Determination of low density lipoprotein

Low density lipoprotein (LDL) was directly measured with a LDL cholesterol kit obtained from Roche for use with the Cobas Mira Chemstation (Boehringer Mannheim Corp., Germany). After the elimination of solubilized non-LDL lipoprotein particles, a second detergent specific for the solubilization of the LDL fraction was added in the presence of a chromogenic coupler for the detection of LDL cholesterol. The enzyme reaction in the presence of the coupler produces color proportional to the LDL cholesterol concentration. The results were expressed in units of mg/dl.

RESULTS

Effect of the PS2 mutation upon the total cholesterol in PS2 transgenic mice brains

To study whether the mutation of presenilin 2 is involved in the abnormality of lipid metabolism in AD, PS2 transgenic mice were generated as an *in vivo* AD model. The results showed that the cholesterol level in all the brains of the PS2mt-Tg mice was higher than in the brains of non-Tg and PS2wt-Tg mice at all the ages investigated (Fig. 1). No change in the total cholesterol in the brain tissues between 2 and 6 months of age (young mice) was observed. The increases in the cholesterol levels in PS2mt-Tg and PS2wt-Tg were clearer in the brains of older. As shown in Fig. 1, the total cholesterol levels in

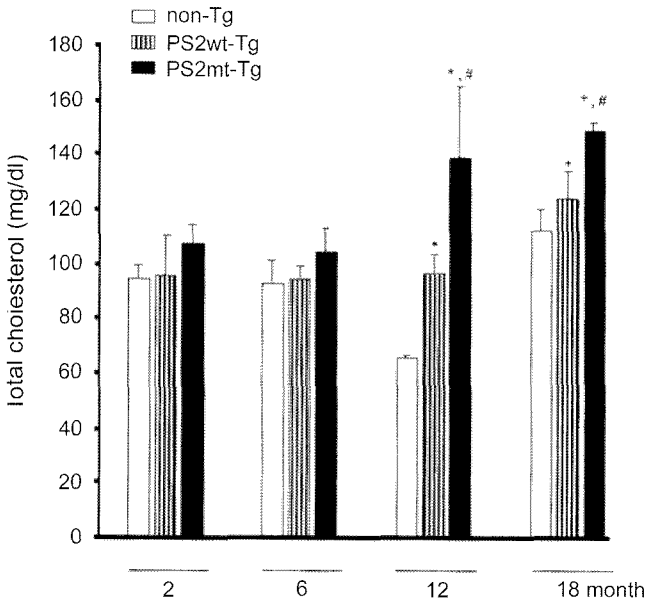

Fig. 1. Total cholesterol levels in the brain tissues of mice: non-Tg, PS2wt-Tg and PS2mt-Tg at all ages; 2, 6, 12 and 18 months. The data represent the mean ± SEM (bars) values determined from three independent experiments (n=6). *significant difference from non-Tg mice, #significant difference from 2 month old mice.

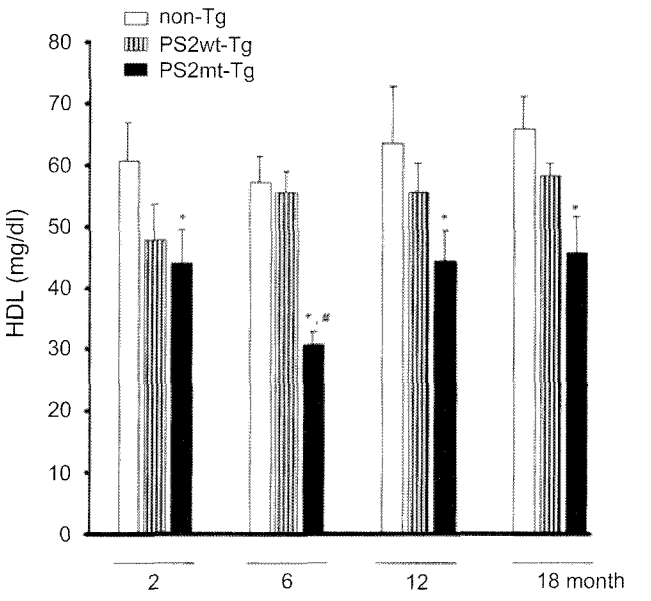

Fig. 2. High density lipoprotein (HDL) cholesterol levels in mice brain tissues: non-Tg, PS2wt-Tg and PS2mt-Tg at all ages; 2, 6, 12 and 18 months. The data represent the mean ± SEM (bars) values determined from three independent experiments (n=6). *significant difference from non-Tg mice, #significant difference from 2 month old mice.

non-Tg and PS2wt-Tg brains were about 94 mg/dl at 6 months of age, increase to approximately 96 mg/dl at 12 months and to 112±8 mg/dl (non-Tg) and 124±10 mg/dl (PS2wt-Tg) at 18 months. Particularly, the minimum cholesterol level (65.3±1.2 mg/dl) in non-Tg brains was detected at 12 months (Fig. 1). Similar results were detected in the brains of PS2mt-Tg mice. At 2 and 6 months of age, the total cholesterol in PS2mt-Tg samples was about 107 mg/dl, but increase to 139±26 and 148±3.2 mg/dl at 12 and 18 months, respectively (Fig. 1). The total cholesterol contents were always higher in PS2mt-Tg brains compared to PS2wt-Tg (1.1 fold increase at 2 and 6 months, 1.4 fold increase at 12 months and 1.2 fold increase at 18 months), and especially higher than in non-Tg brains (1.1 fold increase at 2 and 6 months, 2.1 fold increase at 12 months and 1.3 fold increase at 18 months) (Fig. 1).

Decrease in HDL level in mutant PS2 transgenic mice brains

The results showed that the level of HDL decreased in the brains of PS2wt-Tg and PS2mt-Tg mice, particularly in the PS2mt-Tg compared to non-Tg mice. The changes of the levels of HDL in PS2wt-Tg, PS2mt-Tg or non-Tg brains seemed to be independent of age. The levels of HDL in non-Tg brains (about 60±6 mg/dl) did not significantly increase from 2 to 18 month of age (Fig. 2). Similar results were observed in the brains of PS2wt-Tg mice (45±5 mg/dl) as well as PS2mt-Tg mice, but an unusual exception was shown in the brains of 6 month old PS2mt-Tg mice [showing a minimum HDL value (30.6±2 mg/dl) (Fig. 2)].

Increase in LDL levels in mutant PS2 transgenic mice brains

The LDL levels were increased in all the brains of PS2mt-Tg mice compared to non-Tg and PS2wt-Tg mice (Fig. 3). In young mice, increases in the level of LDL occurred not only in PS2mt-Tg, but also in PS2wt-Tg and non-Tg mice brains. The highest level was shown in the brains of 6 month old mice. However, in older mice, the LDL levels were only slightly higher in the brains of PS2mt-Tg mice compared to non-Tg mice, while the LDL levels in the brains of PS2wt-Tg mice were equal to those of non-Tg mice. The LDL level in non-Tg brains was increased from 15.3±3 mg/dl at 2 months to 23.3±8 mg/dl at 6 months, but decreased to 11.6±1.8 and 14.7±1.7 mg/dl at 12 and 18 months of age, respectively (Fig. 3). Similar results were obtained in the brains of PS2wt-Tg mice. There were increases in the levels of LDL in the brains of PS2wt-Tg mice from 26.6±8.5 mg/dl at 2 months to 36.3±7.4 mg/dl at 6 months, but decreases to 11.6±2 and 16±1.5 mg/dl at 12 and 18 months of age, respectively (Fig. 3). The LDL levels in the brains of PS2mt-Tg mice were always higher than in those of non-Tg and PS2wt-Tg mice. The levels of LDL in PS2mt-Tg brains were increased from 28.6±8 mg/dl at 2 months to 54±9.8 mg/dl at 6 months, but then decreased to a consistent level of 27±8 mg/dl at 12 and 18 months of age (Fig. 3).

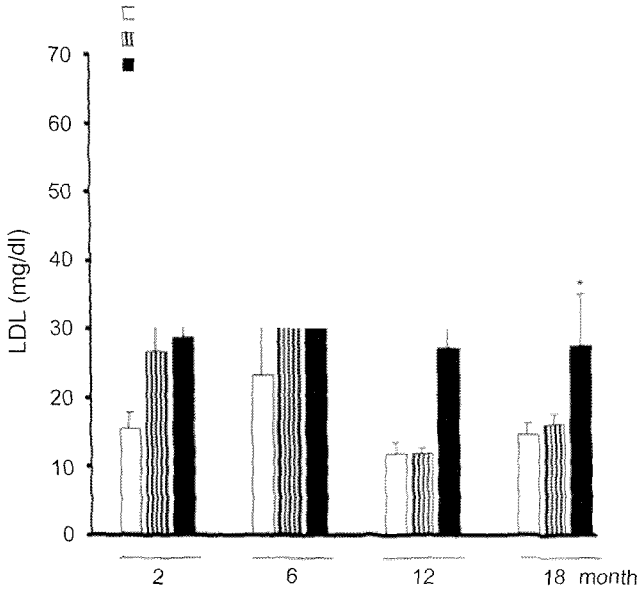

Fig. 3. Low density lipoprotein (LDL) cholesterol levels of mice brain tissues: non-Tg, PS2wt-Tg and PS2mt-Tg at all ages; 2, 6, 12 and 18 months. The data represent the mean ± SEM (bars) values determined from three independent experiments (n=6). *significant difference from non-Tg mice, #significant difference from 2 month old mice.

Alteration of triglyceride levels in mutant PS2 transgenic mice brains

The triglyceride levels in the brains of Tg mice were increased compared to those in non-Tg mice at all ages, particularly in PS2mt-Tg mice (Fig. 4). In the brains of non-Tg mice, there were increases in the levels of triglyceride from 80±14.7 mg/dl at 2 months to 100±2.3 mg/dl at 6 months, and to 118±5.6 mg/dl at 18 months of age (Fig. 4). Similarly, the triglyceride levels in the brains of PS2wt-Tg mice were also increased from 92.5±3.7 mg/dl at 2 months to 121.6±7.5 and 135.6±8 mg/dl at 12 and 18 months, respectively (Fig. 4). At 2 months of age, the triglyceride level in the brains of PS2mt-Tg mice was 89.7±7.8 mg/dl, but at 6 months the level was 138.3±8.8 mg/dl, which increased to 168±19.3 mg/dl at 18 months (Fig. 4). The triglyceride levels were higher in old mice, with the increase appearing to be age-dependent in the brains of PS2wt-Tg and PS2mt-Tg mice (Fig. 4).

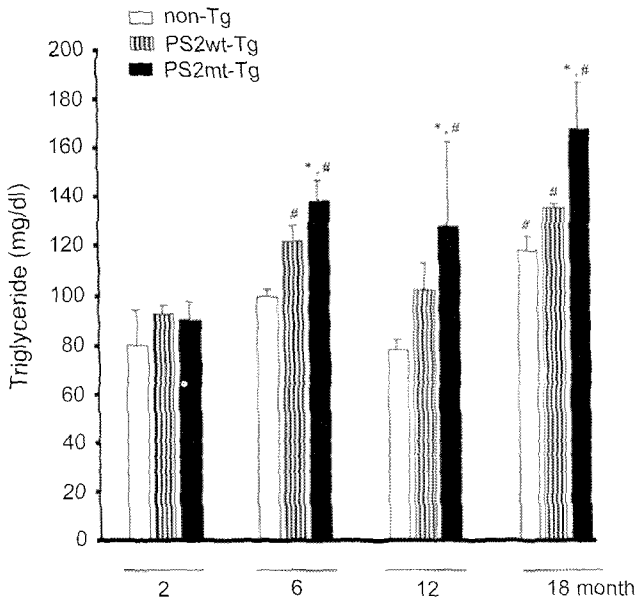

Fig. 4. Triglyceride levels of mice brain tissues: non-Tg, PS2wt-Tg and PS2mt-Tg at all ages; 2, 6, 12 and 18 months. The data represent the mean ± SEM (bars) values determined from three independent experiments (n=6). *significant difference from non-Tg mice, #significant difference from 2 month old mice.

DISCUSSION

The lack of memory in PS2 transgenic mice, particularly in mutant PS2 transgenic mice, is correlated with the increased expression of PS2 wild (PS2wt) and PS2 mutant (PS2mt) types in the cortex and hippocampus, as described in a previous study (Hwang *et al.*, 2002). The involvement of the PS2 mutation in the abnormality of lipid metabolism in AD is unclear, although a clue that intracellular cholesterol transport alters the PS localization in neuronal cells suggests the possibility of a cholesterol-dependent trafficking PS (Runz *et al.*, 2002). In this study, how mutant PS2 influences the lipid profile in an AD model of the PS2 transgenic mice brains was investigated. An increase in the amount of total cholesterol accompanying the increase in the levels of triglyceride occurred in the brains of PS2mt-Tg compared to normal mice indicates that the mutant PS2 may be involved in these abnormalities of the lipid profile. Moreover, the age-dependent increases in the levels of triglyceride and cholesterol were clearer in PS2mt-Tg than in non-Tg mice, which address the abnormal effect of mutant PS2 in the lipid metabolism of the cells.

Cholesterol and triglyceride are known to be required for the formation and maintenance of cell membrane permeability and fluidity, as well as for cellular signaling and other forms of cellular biosynthesis (Simons *et al.*, 2000; Galbete *et al.*, 2000). Therefore, alterations to the cholesterol and triglyceride contained within cells may reflect an abnormality in the cell membrane properties influencing the other forms of metabolism concerned, including Aβ generation and the signaling transmission of cells. In support of this notion, another study has reported that the cholesterol levels were increased in a vulnerable brain region, but not in a non-vulnerable brain region in AD patients (Cutler *et al.*, 2004). It has been suggested that psychoactive drugs increase the triglyceride change in the membrane fluidity and receptor function (Diebold *et al.*, 1998). There is accumulating data supporting the

hypothesis that alterations in cholesterol levels influence the development of AD by affecting the formation and distribution Aβ within cholesterol rich membranes (Runz *et al.*, 2002; Subasinghe *et al.*, 2003; Refolo *et al.*, 2000). Furthermore, the decrease in the levels of HDL as opposed to the increase in levels of LDL, which are supposed to be 'good and bad cholesterol' for cells, respectively, occurred in the brains of PS2mt-Tg mice, demonstrating that mutant PS2 may cause cellular degeneration via alteration of the lipid transport mechanism in cells. The HDL level is consistent at all ages, suggesting HDL may be a stable component in the cell structure and not an age dependent agent. Thus, alternations in the level of HDL could influence the function of brain or cause damage to neuronal cells. Mutant PS2 may influence the maintenance of the levels of HDL, which may also be involved in the risk of developing AD (Michikawa, 2003). In contrast, LDL may play a role in age related cell activities during the life of cells, which alter the levels of LDL in the brains of non-Tg and PS2wt-Tg mice at all ages investigated. Our results have also demonstrated the existence of a potential LDL level regulation mechanism during each term of cell life. In the case of cells expressing mutant PS2, the LDL levels were always higher, suggesting mutant PS2 may also affect the membrane fluidity leading to disturbance of the membrane function, including APP processing, which is involved in the development of AD (Irizarry *et al.*, 2004). Overall, the level of LDL was higher in the brains of PS2mt-Tg mice, but the increase in the level of LDL was higher in relatively young (6 months old) than older mice. The reason for this is not clear, and it may not be significant, since there was much variation among the animals, and the corresponding HDL level was proportionally decreased at the same time. Remarkably, the levels of total cholesterol, triglyceride and LDL, which are supposed to be risk factors for AD (Tan *et al.*, 2003; Refolo *et al.*, 2001), in the brain tissues of mice expressing PS2mt were always higher than in normal brains, and also age dependent, indicating the noteworthy contribution of PS2mt to neurodegeneration during ageing.

In conclusion, this study elucidated the involvement of PS2 in the lipid profile of the brain. The mutation of PS2 alters the lipid metabolism, which may disturb the regulation of lipid mechanism in neuronal cells, and in turn influence the neuronal function, rendering the lack of memory in PS2mt transgenic mice. These findings, therefore, could be useful in the development of an appropriate therapeutic intervention for targeting mutant PS2-induced AD cases.

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