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Case Report

# Elimination of amyloid precursor protein in senile plaques in the brain of a patient with Alzheimer-type dementia and Down syndrome

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**Abstract**

The average lifespan of individuals with Down syndrome has approximately doubled over the past three decades to 55–60 years. To reveal the pathogenic process of Alzheimer-type dementia in individuals with Down syndrome, we immunohistochemically examined senile plaque formation in the cerebral cortex in the autopsy brain and compared findings with our previous studies. We described a 52-year-old female with Down syndrome who developed progressively more frequent myoclonus following cognitive decline and died at the age of 59 years. Her karyotype [46XX, inv(9)(p12q13), i(21)(q10)] included triplication of the gene for amyloid precursor protein and the Down syndrome critical region. On microscopy, very few gamma-aminobutyric acid-ergic (GABAergic) neurons, in the form of small granular cells, in the cortex and Purkinje cells in the cerebellum were visible. In our previous study, amyloid precursor protein immunoreactivity was first noted in senile plaques at the age of 32 years. In this patient, even though amyloid β immunoreactivity was detected in the cores of senile plaques and diffuse plaques, amyloid precursor protein immunoreactivity was not noted in senile plaques in the frontal cortex. Amyloid precursor protein and its derivative amyloid-β play an important role in the formation of senile plaques and the time course of immunoreactive expression may be related to the pathogenic process of Alzheimer-type dementia.


*Keywords:* Down syndrome; Amyloid precursor protein; Amyloid beta; Senile plaque; Gene dosage effect; Alzheimer-type dementia; Myoclonic epilepsy

## 1. Introduction

The incidence of dementia in Down syndrome (DS) is estimated to be greater than 25% in individuals older than 35 years [1]. This number has been shown to increase with advancing age in relation to triplication and overexpression of amyloid precursor gene on chromosome 21 [1]. We report a patient with DS presenting with myoclonic epilepsy following cognitive decline.

To reveal the pathogenic process of Alzheimer-type dementia and late-onset myoclonic epilepsy, we examined the brain during autopsy using immunohistochemistry.

## 2. Case report

### 2.1. Clinical presentation

A 52-year-old woman with DS presented with a 6-month history of cognitive decline, consistent with

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dementia. She was born to non-consanguineous parents with a normal birth history. The patient had no congenital heart disease nor history of surgical operations. She had developed a progressive cognitive deterioration, characterized by indifference, uncooperativeness, apathy, depression, and socially deficient communication. Her behavior affected her activities of daily living, such as dressing and using public transportation. On examination, we found that the patient had small low-set ears, a saddle nose, and single palmar crease, consistent with typical findings in patients with DS. Laboratory examinations showed no abnormalities in thyroid function or Vitamin B12 and folic acid levels. Neurological examination revealed horizontal nystagmus and slurred speech. Brain computed tomography showed mild brainstem and cortical atrophy at initial presentation (Fig. 1). While treatment with donepezil, an acetylcholinesterase inhibitor, brought some initial benefit, the cognitive decline progressed. Nine months after the onset of cognitive symptoms, whole body myoclonus beginning from subtle tremors in a single extremity was noted. The electroencephalogram exhibited delta waves predominant in the frontal regions with generalized intermittent slowing. Photic stimulation did not elicit an abnormal response.

Phenobarbital treatment was initiated with no subsequent change in the frequency of myoclonus. Valproate sodium or piracetam was prescribed and had no effect on myoclonus. Levetiracetam was prescribed with moderate improvement of the myoclonus. The patient died of respiratory failure at 59 years of age.

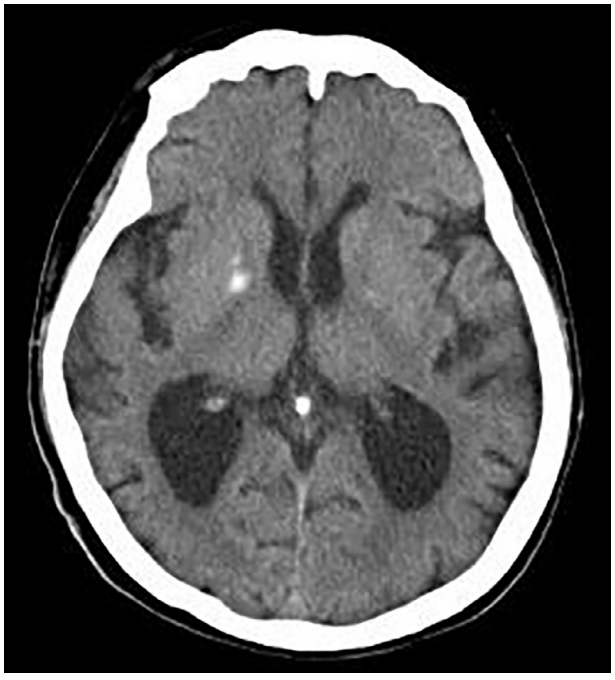

Fig. 1. Brain axial computed tomography shows mild cortical atrophy, enlargement of bilateral ventricles, and calcification in basal ganglia at 52 years of age.

The present study was conducted with approval from our center's Board of Ethics, which includes third-party members. Informed consent was obtained from adult sibling.

### 2.2. Assessment of genetic background

Genetic assessment found a karyotype of 46XX, inv (9)(p12q13), i(21)(q10). Inversion of chromosome 9 is a major chromosomal polymorphism [2]. Down syndrome resulted from a partial trisomy 21q(21q10 → qter) including both the genes for amyloid precursor protein mapping to 21q21.2 [3] and the Down syndrome critical region (DSCR) due to a *de novo* unbalanced translocation (Fig. 2).

### 2.3. Material and methods

Autopsy was performed within 24 h of death. Brain tissues were fixed in 10% formalin for two weeks, embedded in paraffin, and then cut into 7-μm-thick sections. The slides were deparaffinized and then immersed in 0.3% hydrogen peroxidase in methanol for 20 min to inactive endogenous peroxidase activity. The specimens were heated in a microwave oven for 9 min at 90 °C for antigen retrieval, and then incubated with 10% normal goat serum for β-amyloid (Aβ) or amyloid precursor protein (APP) to block non-specific binding. They were incubated overnight at 4 °C with anti-human Aβ(1–42) (IBL, Japan: Rabbit polyclonal) (1:100) [3] or APP recognizing an epitope located between amino acids 60 and 100 in the N-terminal part of APP (clone22C11; Millpore, USA) (prediluted) [3]. To visualize immunoreactivity, the slides were incubated at room temperature for 60 min with a biotinylated rabbit anti-mouse IgG (Nichirei; prediluted) for Aβ or a biotinylated goat anti-rabbit IgG (Nichirei; prediluted) for APP, and then with peroxidase-conjugated streptavidin (Nichirei; prediluted) for 30 min. Between each step, the sections were thoroughly washed with phosphate-buffered saline (PBS; pH 7.4). The immune products were visualized using 0.02% diamino-benzidine (Dojin) in 50 mM Tris-HCL (pH 7.6) containing 0.0006% hydrogen peroxidase.

### 2.4. Neuropathological findings

The autopsy brain was abnormally rounded and weighed 729 g before formalin fixation. The cerebellum and brainstem were small, and the occipital contour was flatter than normal. The frontal lobe was reduced in its anteroposterior diameter and the superior temporal gyrus was poorly developed.

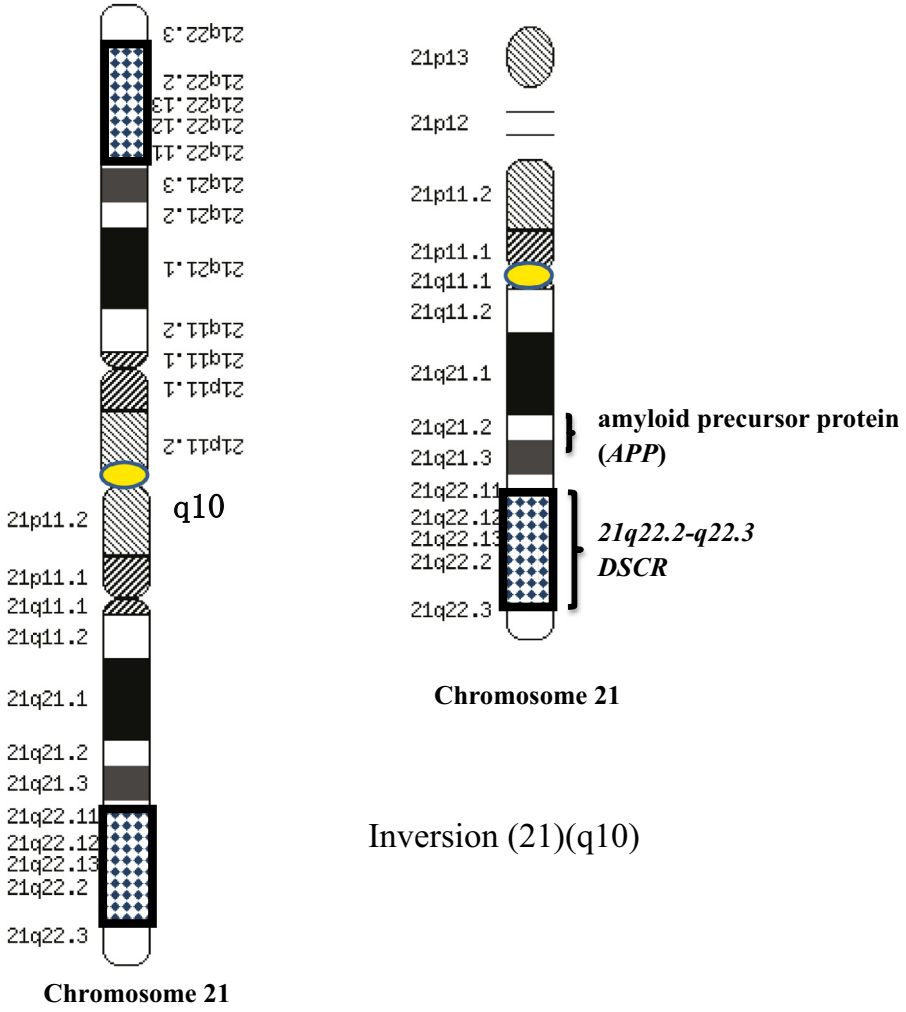


Fig. 2. A de novo unbalanced translocation 21q(21q10 → qter) including the genes for both the amyloid precursor protein mapping in 21q21.2 and Down syndrome critical region.

### 2.5. Microscopic findings

Microscopic examination revealed atrophy of the cerebellar cortex with a severe loss of Purkinje and granular cells. The frontal and temporal cortex showed a reduced number of pyramidal neurons and small granular cells and decreased neuronal densities. Abnormal neuronal morphology in terms of neurofibrillary tangles and neuronal distribution were most marked in cortical layer IV. Neither hypoxic-ischemic changes to neurons nor infiltration of inflammatory cells were seen. Aβ-positive immunoreactivity was detected in the core of senile plaques and diffuse plaques, and detected diffusely in the frontal cortex, hippocampus, and entorhinal cortex (Figs. 3a and 3b). On the contrary, APP immunostaining was not detected in either mature plaques or in diffuse plaques (Fig. 3c).

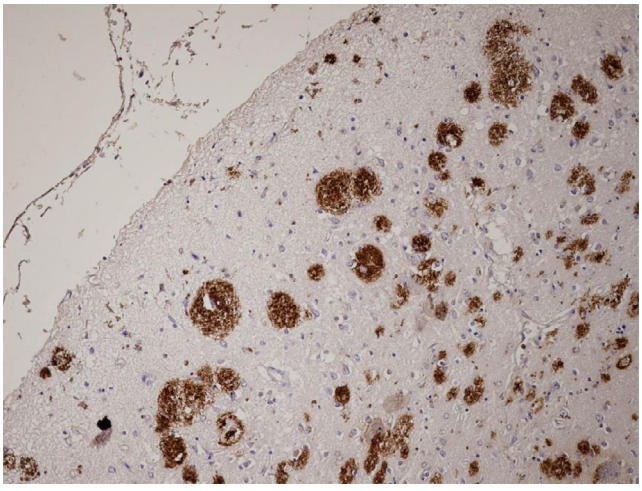

Fig. 3a. β-Amyloid (1–42) immunoreactivity is noted in diffuse or mature plaques in the frontal cortex. (×100 magnification).

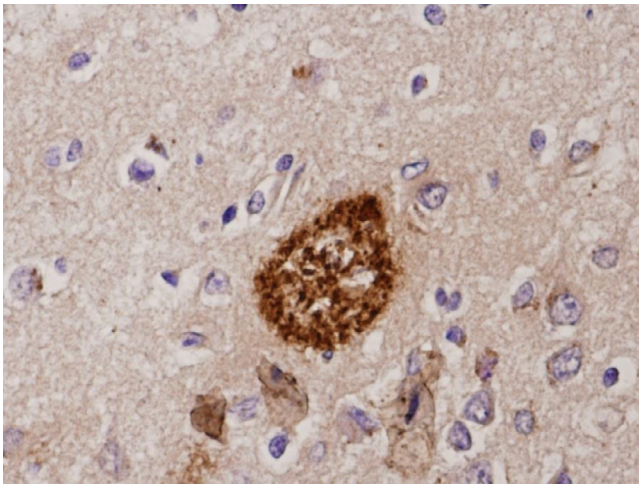

Fig. 3b. β-Amyloid (1–42) immunoreactivity is noted in the core of a senile plaque in the frontal cortex (×800 magnification).

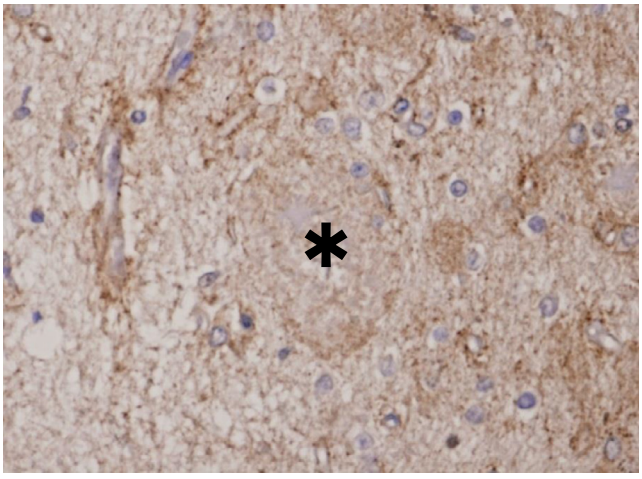


Fig. 3c. APP immunoreactivity is not detected in a senile plaque (*) on the serial section of Fig. 3b (×800 magnification).

## 3. Discussion

### 3.1. Early Alzheimer-type dementia in DS

In the clinical course of dementia in DS, it is difficult to distinguish between cognitive deterioration and the degree of pre-existing intellectual disability.

The early symptoms of Alzheimer's dementia seem to be different in adults with DS from those in typically developed adults. In the general population, episodic memory and orientation disorders are usually the first signs of developing Alzheimer's dementia [4]. On the contrary, frontal lobe symptoms such as indifference, uncooperativeness, apathy, depression, and socially deficient communication, are common in DS [4]. Early Aβ senile plaque deposition in the frontal lobes rather than hippocampal atrophy was reported to cause predominantly frontal signs in patients with DS with dementia instead of impaired episodic memory in patients without DS [5]. In this patient, Aβ positive senile plaques were detected not only in the frontal cortex, but also in the hippocampus and entorhinal cortex.

### 3.2. Late-onset myoclonic epilepsy (LOMEDS)

Epilepsy is a well-recognized complication of DS. Forty percent of those that develop an epilepsy syndrome present in infancy with West syndrome. Another 40% will present after the third decade with the onset of dementia as late-onset myoclonic epilepsy (LOMEDS) [6].

LOMEDS in DS is characterized by seizure onset after progressive dementia, and myoclonus or tonic-clonic seizures.

In this case, the decreased number of neurons, especially small granular cells in the cortex and Purkinje cells in the cerebellum, that play a role in GABAergic inhibition may have contributed to the pathogenesis of LOMEDS. Regarding treatment, the number of epileptic seizures can be reduced in many patients by using new antiepileptic drugs, such as levetiracetam. This is the only drug demonstrated to decrease seizures frequency in mouse models with overexpression of human APP [7] and the clinical course of this patient supported this proposal.

### 3.3. Senile plaques formation

DS with trisomy of chromosome 21 (included trisomy of DSCR) inevitably develop Alzheimer-type neuropathology with increasing age. The overproduction of APP localized at 21q21.2, near the critical region for DS, leads to early onset Aβ plaques [3]. Sequential cleavage of APP by the proteolytic enzyme γ-secretase and β-secretase results in the production and accumulation of Aβ in senile plaques [8]. Genetic assessment of this patient revealed the triplication of APP in the cause of a *de novo* unbalanced translocation.

In a previous study, the senile plaques containing N-terminal of APP is found more frequently in dementia in DS rather than Alzheimer's disease or early onset Alzheimer's disease with presenilin-1 mutations [9].

As presented in our previous neuropathological study that used frontal lobe specimens, N-terminal APP immunoreactivity was first noted in senile plaques at 32 years of age [10]. In this 59-year-old patient, even though Aβ immunoreactivity was detected in the cores of senile and diffuse plaques, N-terminal APP immunoreactivity was not noted in senile plaques (Table 1). These results suggest that the process of Aβ peptide molecules cleaved from APP may be completed by 59 years of age.

In the general population, the presence of the ε4 allelic variant of apolipoprotein E (apo-E) gene has been

Table 1
Our previous neuropathological studies.

| Expression in senile plaques in the frontal cortices with DS | Coding on | 25yo | 32yo | 59yo |
|---|---|---|---|---|
| Apolipoprotein-E | Chromosome 19 | +** | +** | Not examined |
| APP | Chromosome 21 | −** | +** | − |
| β amyloid | Chromosome 21 | −* | +* | ++ |

−: negative immunoreactivity, +: positive immunoreactivity, ++: positive immunoreactivity in a more widespread distribution
* Ref. [3].
** Ref. [10].

linked to earlier onset of Alzheimer's disease [11]. Apo-E is produced by a single gene on 19q13.2 and the ε4 allele, which is inherited from each parent, acts as a risk factor for the age-specific manifestation of Alzheimer-type dementia with DS [11]. Using computerized image analysis techniques, Aβ deposition in the entorhinal cortex and hippocampus has been seen to be increased in apo-E gene ε4 carriers with DS [12]. In our previous immunohistochemical study using frontal cortex from a patient with DS, apo-E immuno-positive senile plaques were detected at 25 years of age, appearing earlier than APP and Aβ expression [10] (Table 1). Focusing on senile plaque formation, apo-E expression was detected at 25 years of age, APP expression at 32 years of age, and subsequent elimination by 59 years of age. By age 59 years, the process of Aβ peptide molecules cleaved from APP may have been completed and the time course of immunoreactive expression may be related to the pathogenic process of Alzheimer-type dementia in DS.

In conclusion, we described a 59-year-old patient with myoclonic epilepsy following dementia with DS. In neuropathological examinations, Aβ peptides in senile plaques were found to be already cleaved from APP and the immunoreactivity was not detected. The time course of immunoreactivity may be related to the pathogenic process in Alzheimer-type dementia in patients with DS. These findings have increasing importance given the increasing life expectancy in adult patients with DS.

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