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Neuronal Plasticity in the Developing and Aging Cerebral Cortices of Patients with Down Syndrome

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Abstract

Background: The aim of the study was to clarify the developmental and aging changes of Protein gene product 9.5 (PGP9.5) expression in granular and pyramidal neurons of the cerebral cortex comparing patients with Down syndrome (DS) and subjects without it.

Subjects and Methods: Fifty-four human brains were obtained from post-mortem samples of 24 subjects with DS (19 weeks gestation (GW) to 63 years of age) and 30 subjects without it (20 GW to 75 years of age). PGP9.5 expression in the frontal cerebri was qualitatively assessed in specific cortical layers and compared among 4 age groups (fetus, infant, child, and adult), and between the brains with and without DS.

Results: In subjects without DS, the expression of PGP9.5 in the cerebral cortex was highest from 30 to 39 GW, and then decreased with increasing age. In patients with DS, cortical PGP9.5 expression did not decrease with aging. Compared with samples from subjects without DS, those from patients with DS showed weaker PGP9.5 expression in layers 3 and 5 (pyramidal cell layers) of the infant group, but stronger expression in layer 4 (granule cell layer) of the child group, and in layers 2, 3, 4, and 6 of the adult group.

Conclusion: An increase of PGP9.5 expression in cortical granule neurons from children to adults with DS was found and suggests the existence of an important therapeutic window during which compensatory and plastic processes may influence the progression of cognitive impairment in individuals with DS.

Keywords: Down syndrome; Immunohistochemistry; Plasticity; Intellectual disabilities; Dementia

Introduction

Cognitive impairment is one of the most prominent features of Down syndrome (DS). Recent improvements medical care for patients with DS have led to a significantly enhanced quality of life [1,2]. However, the extended lifespan of patients with DS may lead to the development of cognitive problems such as dementia of Alzheimer's type (DAT) in elderly patients with DS [3]. Even in the absence of a clinical diagnosis of dementia, neuropathological findings associated with DAT (e.g., the accumulation of senile plaques (amyloid-β protein) and neurofibrillary tangles (hyperphosphorylated tau protein) [4,5] are commonly observed in the cerebral samples of adults with DS aged more than 30 years. Senile plaques contain the amyloid-β peptide that is derived from a longer amyloid precursorprotein (APP), the gene for which is located on chromosome 21. DS, otherwise known as trisomy 21, may be responsible for the over expression of APP, which is thought to be a causative factor in DAT pathogenesis. However, compensatory responses of APP have also been observed in DS brains prior to or in parallel with the development of DAT pathology [6], suggesting that further investigation of DS pathology associated with cognitive dysfunctions at different stages of development and aging may be important.

Protein gene product 9.5 (PGP9.5) has been widely used as a marker for the differentiating nervous system [7-9]. PGP9.5 is ubiquitin carboxyl terminal hydrolase, which was initially identified as a human neuron-specific protein, and then was observed to be expressed prominently in differentiating neurons in the brains of various species.

The present neuropathological study was conducted to investigate the temporal and spatial changes of PGP9.5 expression in the cerebral cortical layers of patients with DS and subjects without it.

Subjects and Methods

Human tissue specimen

Fifty-four human brains frontal cerebri were obtained from post-mortem samples of 24 subjects with DS (19 weeks gestation (GW) to 63 years of age) and 30 subjects without it (20 GW to 75 years of age) with written informed consent for scientific use of the samples by the patients or their family representatives. The subjects without DS (controls) were chosen from the samples without abnormal neuropathological findings at the primary post-mortem assessment. The causes of death in controls were either spontaneous abortion, cardiomyopathy, congenital heart disease, acute lymphocytic leukemia, squamous cell carcinoma, or liver cirrhosis. Because we intended to use these subjects without DS brain samples to represent normal brains, we confirmed in advance that there were no abnormal neuropathological findings in these samples.

The subjects with DS and controls were further assigned into 4 age groups of fetus (age <40 GW), infant (40 GW ≤ age <12 months of age), child (12 months old ≤ age <16 years old), and adult (16 years old

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≤ age) groups. The numbers of control and DS brains were 10 and 4 in fetus group, 10 and 8 in infant group, 7 and 4 in child group, and 3 and 8 in adult group respectively. Fetus group was further divided into two subgroups, and the numbers of control and DS brains <30 GW were 5 and 2, and those ≥ 30GW were 5 and 2 respectively.

Immunohistochemistry

Whole brain samples were fixed in 4% formalin, and then embedded in paraffin wax; 4 μm thick sections of the brain tissue were immunohistochemically stained using streptavidin-biotin method. The sections were deparaffinized in xylene and then rehydrated in ethanol. Microwave irradiation was performed to retrieve the antigen after endogenous peroxidase activity was blocked with 3% H_2O_2in methanol. After cooling to room temperature, methanol/H_2O_2 was applied to the section to depress endogenous peroxidases. After three washes in Tris-buffered saline (TBS), the sections were incubated with anti-PGP9.5 (Ultraclone limited, diluted to 1:500, overnight at 4°C). After three washes in TBS, the biotinylated second antibodies and peroxidase-conjugated streptoavidin (simplestain MAX-PO (MULTI), Nichirei, Tokyo) were incubated on the sections (for 2 hours at room temperature). After three washes in TBS, the immunoproducts were visualized using diaminobenzidine (Nichirei, Tokyo).The sections were then counterstained with hematoxylin [10].

Cell density analysis

PGP9.5 positive neurons in cortical layers 2-6 were defined using x400 magnification as follows: negative (no positive neurons), mild (positive neurons <5%), moderate (5% ≤ positive neurons <50%), strong (50% ≤ positive neurons <95%), and very strong (95% ≤ positive neurons).

Statistical analysis

The level of PGP9.5 expression was compared first between different age groups, and then between the patients with DS and controls using Mann-Whitney U-test; for the multiple comparisons between the age groups, P-values<0.05 were considered to be significant. Finally, to identify the exact timing when PGP9.5 expression becomes significant, the fetus group of controls was further divided into two subgroups of (i) <30 GW and (ii) ≥ 30 GW; the level of PGP9.5 expression was compared using Mann-Whitney U-test; this analysis was not performed for DS samples because of the limited number of subjects in the fetus group (n=4).

Results

PGP9.5 expression in the cerebral cortex of controls

PGP9.5 positive neurons were commonly observed in both granular and pyramidal neurons. When the samples in all age groups were classified, there was difference in the expression of PGP9.5 in layers. Modest PGP9.5 expression was also seen in the neuropil of the layer-1 and in the white matter. Compared with the infant group, the expression of PGP9.5 was weak for the fetus group both in the pyramidal cell layers (layers 3 and 5; P=0.002 and 0.001 respectively) and the multiform layer (layer 6; P=0.001), for the child group in the layer 5 (P=0.01), and for the adult group in all layers (all P=0.007). Thus, in layers 3 and 5 (pyramidal cell layers), the expression of PGP9.5 increased from fetus to infant and child groups. However, in layers 2 and 4 (granular cell layers), the expression was similar in groups of fetus, infant and child, but decreased in adult group (Figure 1). When the PGP9.5 expression was compared between subgroups within the fetus group, the subjects ≥ 30 GW showed significantly stronger expression compared with the subjects <30 GW for all the layers. In the subjects ≥ 30 GW, the expression of PGP9.5 in the cerebral cortex was transiently increased and showed the highest from 30 to 39 GW.

PGP9.5 expression in the cerebral cortex of patients with DS

Mild to strong PGP9.5 expression was observed throughout the layers and the age groups; compared with the infant group, there was a trend that the level of PGP9.5 expression in the layer 4was stronger for the child group (P=0.028). No other difference between the age groups was observed (Figure 2).

Comparison of PGP9.5 expression between the patients with DS and controls' samples

The PGP 9.5 expression was compared between the patients with DS and controls, and showed differences in several age groups. In the layer 3 and 5 (pyramidal cell layers) of patients with DS samples, the expression was lower in infant group (P<0.05 and <0.005 respectively), and higher in adult group than controls (Figure 2). In the layers 2 and 4 (granular cell layers), the expression was similar in infant group to that of controls, higher in the layer 4 of the child group (P<0.05), and significantly higher in all the layers (layers 2-6) of the adult group (all P<0.05) (Figure1).

Discussion

The expression of PGP9.5 in the cerebral cortex of controls transiently increased during the late fetal period, and gradually declined thereafter. In contrast, PGP 9.5 expression in the cerebral cortex of the patients with DS was increased throughout life. Our current observations showed low expression in cortical layers 3 and 5 of the infant DS group; however, expression was higher in most layers of the adult DS group compared to the controls group. In particular, PGP9.5 expression was increased inlayer 4 from the period of childhood to adulthood.

Changes in PGP9.5 expression during development and aging

According to previous studies that used the Golgi staining technique, the dendritic spines of cerebral cortical neurons increase with age in both children with DS and controls; however, the increase of the spines is slower in patients with DS than in controls. After 20 years of age, spine density decreases slowly in the brains of controls, but decreases more rapidly in the brains of adults with DS [11,12]. In our current study, the expression of PGP9.5 in the cerebral cortex of controls was at its peak from 30 to 39 GW, when gestational ages of the fetus group were reassessed in greater detail, and then gradually decreased. The older subgroup showed the significantly highest level of PGP9.5 expression, thus, the activation of PGP9.5 occurs during the last weeks of pregnancy. Such a transient increase of PGP9.5 expression may be associated with dendritic expansion or synapse formation, as these phenomena occur most prominently at the same stage of development.

Persistent expression and elevations of PGP9.5 in patients with DS

In current study on the infant group, PGP9.5-positive neurons in the pyramidal cell layers were less prominent in patients with DS than in controls, whereas those in the granule cell layers were not different between patients with DS and controls. Conversely, in the adult groups, patients with DS showed more prominent PGP9.5 expression than controls in both the granule and pyramidal cell layers, consistent with the increased expression of PGP9.5 in the brains of patients with

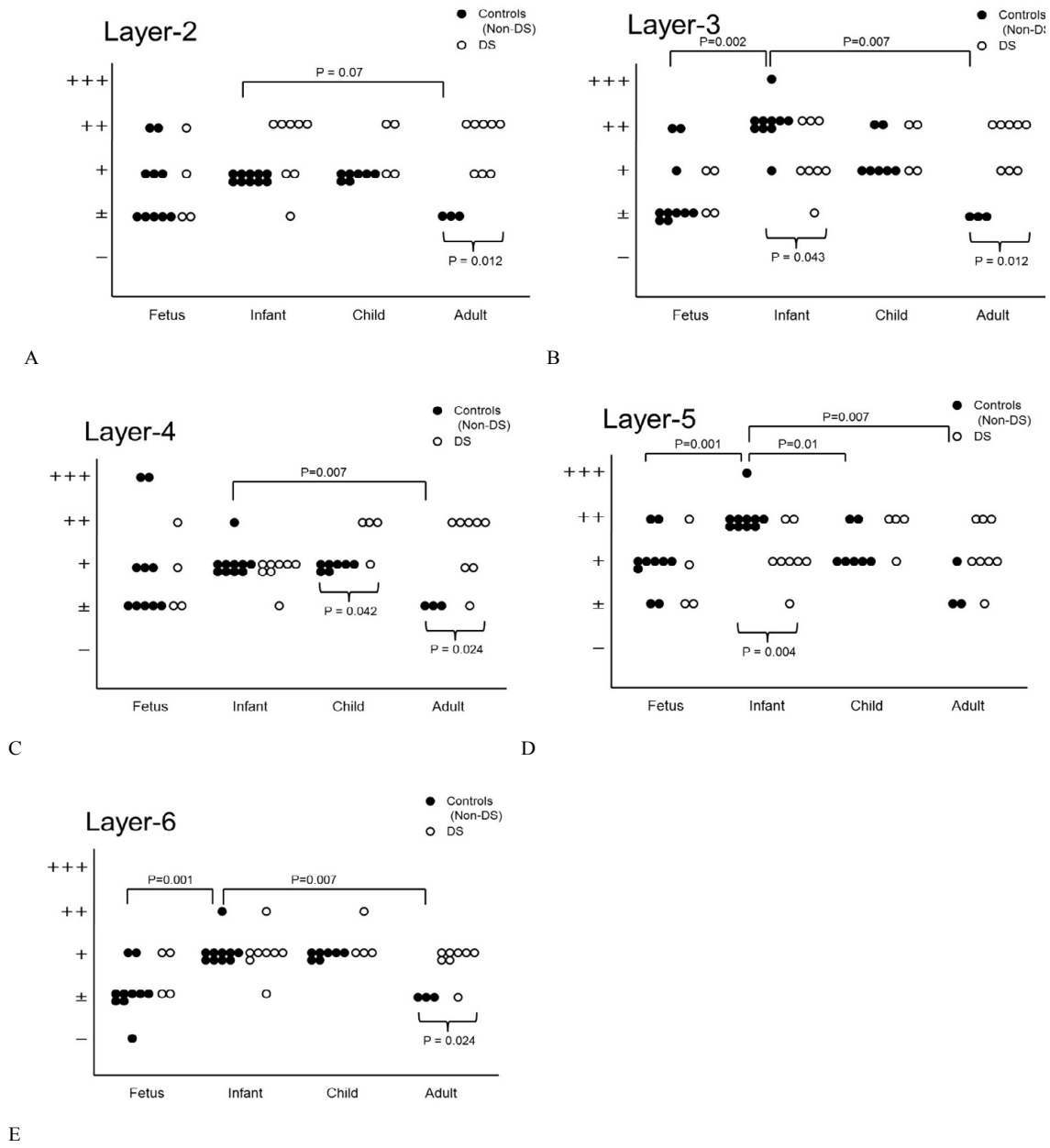

Figure1: PGP9.5 expression in the frontal cortex in the patients with DS and controls.
(A) the layer 2 (B) the layer 3 (C) the layer 4
(D) the layer 5 (E) the layer 6
PGP9.5 positive neurons in cortical layers 2-6 were defined using 400x magnification as follows: negative (-; no positive neurons), mild (± ; positive neurons <5%), moderate (+; 5% ≤ positive neurons <50%), strong (++; 50% ≤ positive neurons <95%), and very strong (+++; 95% ≤ positive neurons).

DS, although gradually declining expression was observed in the brains of controls.

Increased PGP9.5 immunoreactivity has also been observed in the brains with periventricular leukomalacia (PVL) by Nakabayashi et al. [10] indeed, marked PGP9.5 expression was observed in white matter and granule cell layers (layers 2 and 4) adjacent to the necrotic lesions of PVL, but not in the pyramidal cell layers [12]. The characteristic increase in the numbers of axons in the white matter and granule neurons closeto PVL lesions most likely reflects compensatory mechanisms in response to axonal damage and transneuronal degeneration of pyramidal neurons.

Kanaumi et al. [13] conducted a study using brain samples from patients with DS and an immunohistochemical antibody to cystathionine β-synthase (CBS). CBS is encoded by a gene localized in the brain region critical to DS, and plays a role in the production of hydrogen sulfide (H_2S) in the brain, which has an important cellular function [13]. In their human controls, CBS reactivity appeared in the cortical layers of the temporal lobe from the age of 14 GWs, followed by a marked increase during the late fetal period, and a slight reduction after infancy; in contrast, only weak CBS expression was observed in adults of controls. CBS-positive cells are mainly astrocytes, but CBS positive neurons were transiently found around 35 GWs. These

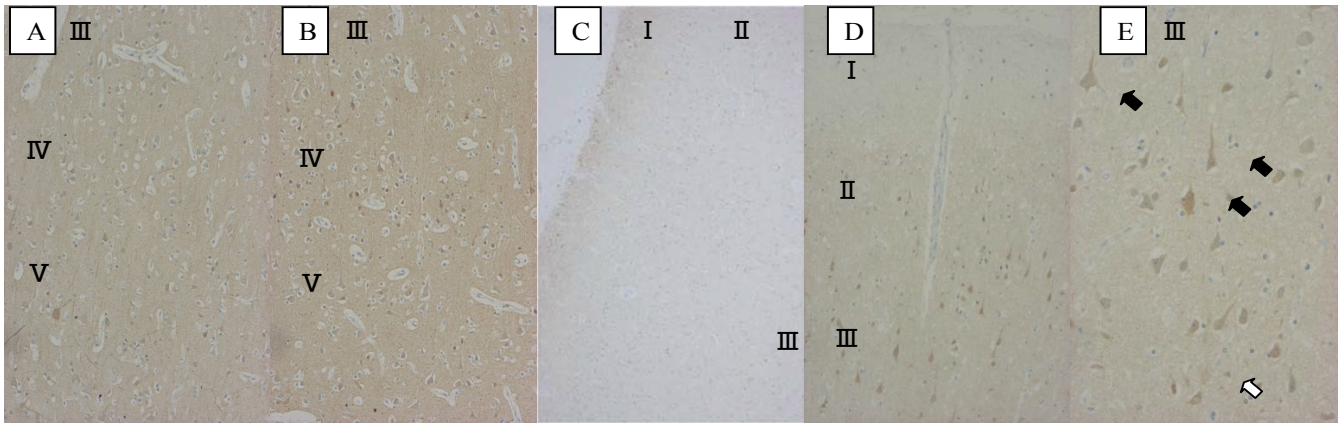

Figure 2: Immunohistological pictures of the cerebral cortex of the neonates (38 GW) of control (A) and Down syndrome (B). Immunohistological picture of the cerebral cortex an adult of control (C) and Down syndrome (D,E). (Arrows show immunoreactive neurons.)

developmental changes of CBS are similar to those of PGP9.5, and the increase of CBS may be related to the activation of neurons. In the brains of patients with DS, a different pattern of expression was evident. CBS-positive cells were observed in the entire cerebral cortex, predominantly in the granule cell layers. In the brains of patients with DS and DAT, CBS expression was especially prominent around senile plaques [13].

Such an increase of CBS expression in the granule cell layer appears to be consistent with our current findings of GPS9.5. CBS expression is seen mainly in astrocytes whereas PGP9.5 is found in developing neurons in the cerebral cortex. However, both CBS and PGP9.5 expression increases predominantly in the granule cell layers in the cerebral cortex of patients with DS. Given the absence of adult controls with marked PGP9.5 expression in the cortical layers, increased PGP9.5 expression in patients with DS might be associated with compensatory mechanisms of neural transmission in cortical inter neurons, or the plasticity that leads to synaptic reduction and dendritic atrophy [14] rather than being directly associated with nervous system degeneration. Although there are some reports that aberrant inhibitory neurons may be linked to cognitive impairments in DS model mice [15], our results of persistently increased PGP9.5 expression in granule and pyramidal cell layers of human DS subjects suggest a role for neuronal plasticity and compensation.

Characteristics of the brain in elderly patients with DS

In the cerebral cortices of adult patients with DS, pathological changes such as senile plaques and neurofibrillary changes appear earlier in life and increase with aging, as compared to similar changes in controls. Neurofibrillary pathology appears slightly after senile plaques become evident, and is more widespread throughout the brain than are the senile plaques. Neurofibrillary pathology is especially prominent in the entorhinal cortex, and corresponding drop-out of nerve cells is often observed in patients with DS and DAT. In our study of elderly patients with DS and DAT, neurons that were strongly positive and neurons that were negative for PGP9.5 were found in mixed populations in the cerebral cortex. In a study that used positron-emission tomography, adults with DS showed higher glucose metabolic rates (GMR) in the cerebral cortex than age-matched controls [16]. If increased GMR corresponds to early compensatory neural responses that occur during the development of dementia, this function may be observed even before the neuropathology of DAT becomes overt. Our current findings that PGP9.5 remained high (or tended to increase in cortical layer4) in older children and young adults with DS and continued to persist thereafter support early and prolonged commitment of compensatory mechanisms in the cellular network of patients with DS.

In an experimental setting, an increase in brain growth factor molecules has been observed, supporting the existence of compensatory mechanisms that may be important in patients with DS. This goes with the fact that physical exertion and altered environmental factors may promote the maintenance of cerebral functioning in patients with DS via the well-recognized effects of exercise and environment on growth factor expression [17,18]. Increased neurogenesis and synaptogenesis, followed by reduced amyloid-β deposition, have been observed after wheel running in mouse models of Alzheimer disease [19,20], further emphasizing the potential of continuous rehabilitation in the face of cognitive impairment.

Limitations

Although we were able to obtain a moderate number of brain samples for this study, the subject number was still too small to allow statistical comparisons within each specific age group. Further studies with larger sample populations are needed to confirm and extend these data.

Conclusion

In the brains of controls, PGP9.5 expression was found to be increased towards the end of fetal life, and gradually declined after childhood. Compared with brains of controls, persistent or increased expression of PGP9.5 was found in the 4th layer of the cerebral cortex of DS children and adults, which may be associated with the characteristic neuronal plasticity and compensation before or during DAT. We need further investigations on the potential compensatory mechanism of DS brains to maintain the cerebral function and to improve the prolonged rehabilitation with cortical stimulation for the patients with DS from childhood.

Acknowledgement

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