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Astroglia disturbances during development of the central nervous system in fetuses with Down's syndrome

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Abstract

Down's syndrome (DS), caused by aneuploidy of chromosome 21, is the most common chromosomal disorder. The most significant symptom of this disorder is mental retardation. Neuropathological changes found in the DS central nervous system (CNS), such as reduced number of neurons, alteration of synapses and synaptic spines or delayed myelination have been widely described. But there are only a few studies of DS-related glia disturbances. A growing number of astroglia new functions have recently been described. In our study we compared the number of astrocytes and radial glial cells in the frontal lobe of DS fetuses at 18-20 weeks of gestation with that observed in age-matched controls. We found a substantially increased number of glial fibrillary acid protein (GFAP) positive cells in all age range samples of DS brains. We also noticed that in our study astrocytes in DS brains seem to be morphologically more mature than in controls of corresponding age. The same observation was made for radial glia. Taking into consideration the role played by astroglia during CNS development we believe that any change in their number, reduced or increased, can affect CNS development and lead to disturbances of both neurogenesis and synaptogenesis.

A possible correlation between the increased number of astroglia and disturbances in CNS development is discussed.

Key words: *Down's syndrome, fetus, astrocytes, radial glia, brain development.*

Introduction

Down syndrome (DS), caused by autosomal aneuploidy of human chromosome 21, is the most common chromosomal disorder [3]. DS individuals are affected by various abnormalities in many organs and systems, but mental retardation, which can range from mild to severe, is the major anomaly [5]. Neuropathological changes found in the DS central nervous system (CNS) have been widely described and they include reduced neuron number, alteration of synapses and synaptic spines, and delayed myelination [1,2,22,25], to mention only a few. Hypoplasia and cortical hypocellularity are also very often described in fetuses with DS [9]. Defective neurogenesis and reduced neuron number in the development of CNS are believed to be the major cause of mental retardation [5]. Neurogenesis involves the migration of neurons from the ventricular zone to the cortical plate. The cortical plate forms an inside-out gradient

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of maturation, which means that inner layer 6 is formed first, followed by formation of outer layers 5, 4, 3, and 2 as the last one [4]. This process is guided, among others, by radial glial cells, which control the migration. The migration itself has at least three forms: two types of radial migration, somal translocation and glia-guided locomotion and one non-radial (tangential) migration [13]. During neurogenesis in the telencephalon a transient structure is formed, called the ganglionic eminence, which disappears near the term of delivery. The ganglionic eminence contains cells which later become neurons of basal ganglia and interneurons migrating to the cerebral cortex. This is an example of tangential migration. It is also a transitional target for axons going from the thalamus to the cerebral cortex and vice versa [24]. Later during gliogenesis radial glial cells differentiate mainly into astrocytes and also neurons [17]. Astrocytes, the major cell population in the brain, play a pivotal role in CNS development, but the knowledge of mechanisms underlying the generation of astroglia is still fragmentary. Recent studies have revealed that astrocytes perform a number of functions. As astrocytes form the largest cell population in the CNS, one of their most obvious functions is to provide structural support and stabilization for the brain tissue. They also demarcate the grey matter regions into functional compartments by establishing non-overlapping regions, cortical and subcortical [20]. Their end-feet processes are tightly organised around vessels, which contribute to the maintenance of the blood-brain barrier. They also ensure that nutrients, such as glucose, enter astrocytes on their way to neurons. Astrocytes also regulate the concentrations of ions and neurotransmitters in brain fluid (extracellular space). They actively remove neurotransmitters such as glutamine, dopamine, serotonin, and gamma-aminobutyric acid (GABA) from the synaptic cleft [14]. Neurotransmitters in the synaptic cleft can stimulate astrocytes to release neuroactive substances. These substances are known as gliotransmitters and can stimulate postsynaptic neurons, as well as presynaptic terminals [21]. In addition to neurotransmitter receptors, astrocytes contain ion receptors. During synaptic activity the increased concentration of extracellular K^+ is alleviated by astrocytic uptake. From there K^+ is ejected into the capillary blood. The role of astrocytes in CNS development still needs to be determined. However, we already know that they promote myelinating activity of oligodendrocytes. ATP released by electrically active neurons induces astrocytes to secrete cytokine leukaemia inhibitory factor, which is a regulatory protein and promotes myelinating activity of oligodendrocytes [12]. They are also involved in regulation of CNS synaptogenesis by releasing factors which influence synapse development [23]. One such factor is cholesterol, important for cell communication and memory [18]. There are reports that glia-derived signals enhance synaptic maturation by increasing the size of postsynaptic currents [19]. The aim of this study was to investigate the relationships of radial glial cells and astrocytes in the CNS of DS fetuses and compare them with age-matched controls.

Material and methods

The study comprised 24 brains derived from human fetuses between 18 and 20 gestation weeks (GW), including 12 fetuses with genetically confirmed Down syndrome and 12 fetuses without obvious developmental or neuropathological abnormalities. All fetuses were derived from legal abortion. The brains were fixed in 4% buffered formalin, embedded in paraffin and cut into 5 μm coronal sections. The haematoxylin-eosin (H-E) method was used for routine staining. For immunohistochemical staining the following primary antibodies were used: glial fibrillary acid protein (GFAP) monoclonal antibody from Novocastra Laboratories (1 : 100 dilution, NCL-GFAP-GA5) and Vimentin monoclonal antibody from Novocastra Laboratories (1 : 100 dilution, NCL-VIM-V9). Immunoreaction was visualized using appropriate secondary antibody and streptavidin-biotin-peroxidase method. All sections were counterstained with haematoxylin and mounted for light microscope examination. The examination employed a Zeiss light microscope with Olympus digital camera. The quantitative analysis of astroglial cells was carried out in the frontal lobe, opposite the ganglionic eminence, along the lateral ventricle at 4 mm^2 (Fig. 1). The statistical significance was assessed using nonparametric Mann-Whitney U test. Probability of $p < 0.05$ was considered to indicate statistical significance.

Results

The quantitative analysis of GFAP positive cells was performed on the frontal lobe opposite to the

ganglionic eminence, along the lateral ventricle (Fig. 1). The number of GFAP positive cells in DS brains was compared with that in the age-matched controls. We observed a substantially increased number of GFAP positive cells in all age range samples of DS brains as compared with controls (Fig. 2). In the DS cases under study we found a slightly decreasing number of astrocytes during maturation between 19-20 GW, although without statistical significance (Fig. 2). At 18 GW we were able to find astrocytes with one process as well as more mature with developed processes (Figs. 3 and 4). In 19-20 GW astrocytes were generally more mature but less mature forms were still present (Figs. 5 and 6). We also assessed the number of radial glial cells. We observed a higher number of radial glial cells in DS brains than in age-matched controls with the exception of 20 GW. A slight increase in the number of radial glia was observed in DS brains between 18 and 19 GW while between 19 and 20 GW it substantially decreased (Fig. 7). We also noted that in our study astrocytes in DS brains seemed to be more mature than in controls of corresponding age. In DS there were more astrocytes with developed processes than in the age-matched control (Figs. 5 and 6). To the best of our knowledge there were no differences in vimentin staining in DS compared with controls (Figs. 8 and 9).

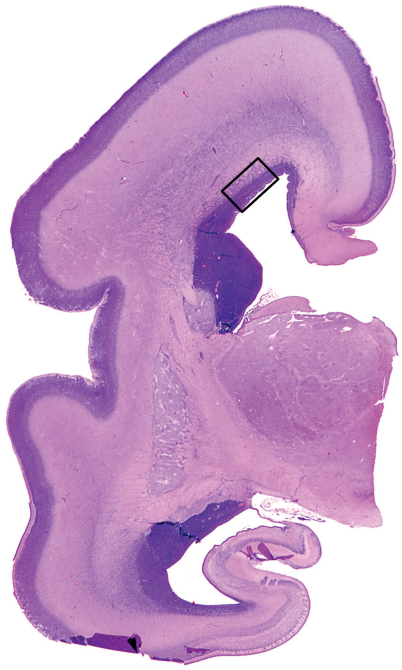

Fig. 1. Fetus brain coronal section 20 GW. HE staining. Square indicates the area of analysis.

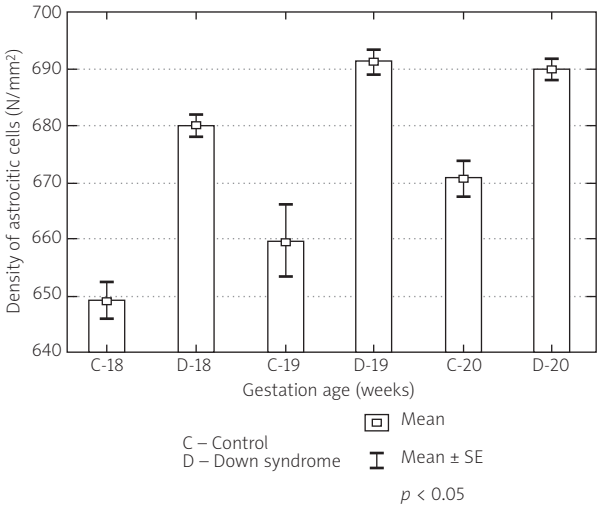

Fig. 2. Density of astrocytes in the frontal lobe of Down syndrome fetus and control.

Discussion

We examined the brains of fetuses between 18 and 20 weeks of gestation. Our results demonstrated a highly significant difference in the number of GFAP positive cells between controls and DS cases in the frontal lobe. The astrocytes were also morphologically more mature than those in controls. Moreover, in the frontal lobe of DS cases there was a significant difference in the number of radial glial cells as compared to the control group, except 20 GW, which may indicate earlier maturation. The results of our study were consistent with those obtained by Guidi *et al.* [10]. They examined the hippocampal region of fetuses between 17 and 21 GW and observed a higher percentage of cells with astrocytic phenotype in DS fetuses, accompanied by general hypocellularity of all examined structures. Our findings were also confirmed by studies on TS65Dn mice, the mouse model of Down's syndrome. Contestabile *et al.* [7] reported in their phenotypic analysis of hippocampal dentate gyrus and neocortical germinal matrix that the number of cells with astrocytic phenotype is higher than in controls [7]. Both studies were performed on the hippocampal region. The data presented in our study indicate that the same observation can be made in other regions of the brain. It is interesting to note that not only astroglia was increased in DS fetuses. It also appears

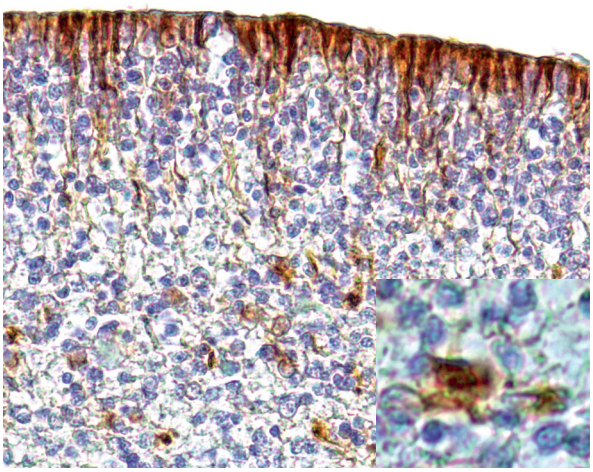

Fig. 3. Frontal lobe, control, 18 GW, GFAP. × 200. Insert: astroglia cells × 400.

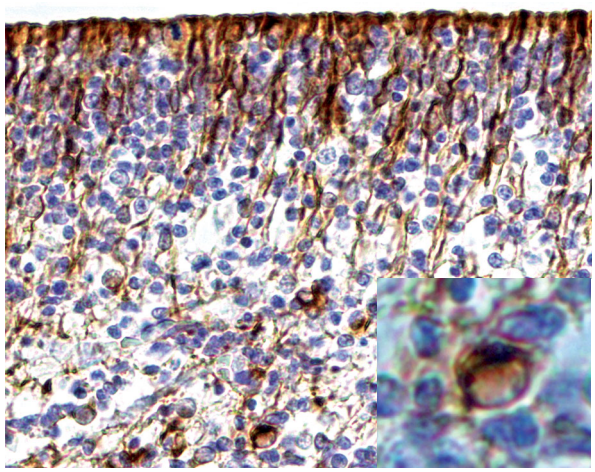

Fig. 4. Frontal lobe, Down syndrome fetus, 18 GW, GFAP. × 200. Insert: astroglia cells × 400.

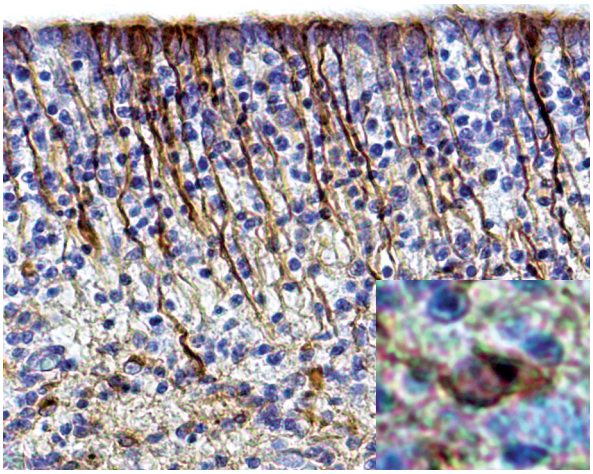

Fig. 5. Frontal lobe, control, 20 GW, GFAP. × 200. Insert: astroglia cells. × 400.

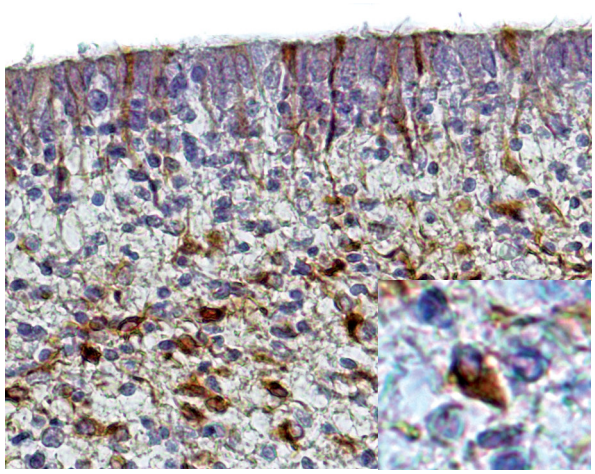

Fig. 6. Frontal lobe, Down syndrome fetus, 20 GW, GFAP. × 200. Insert: astroglia cells. × 400.

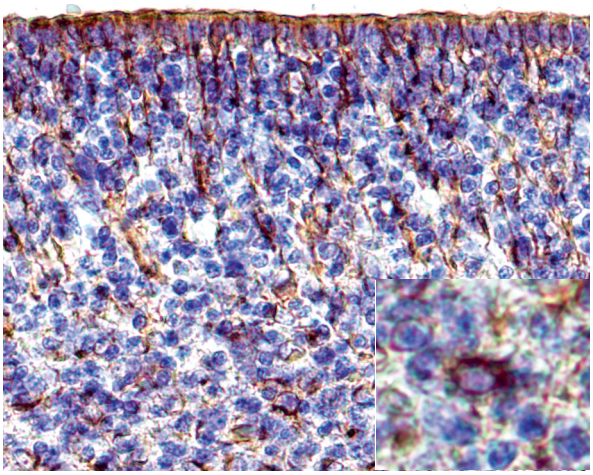

Fig. 8. Frontal lobe, control, 20 GW. Vimentin × 200. Insert: × 400.

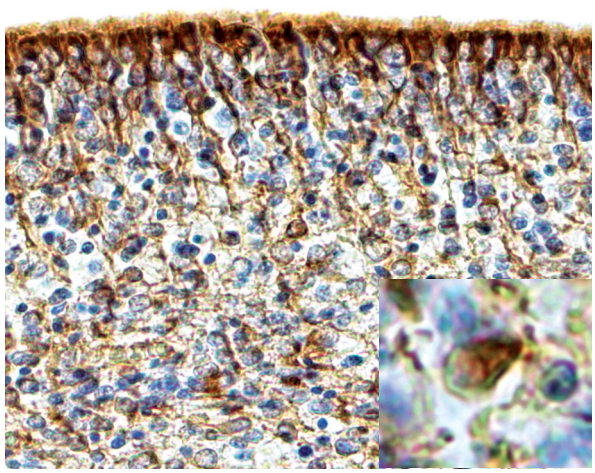

Fig. 9. Frontal lobe, Down syndrome fetus, 20 GW. Vimentin × 200. Insert: × 400.

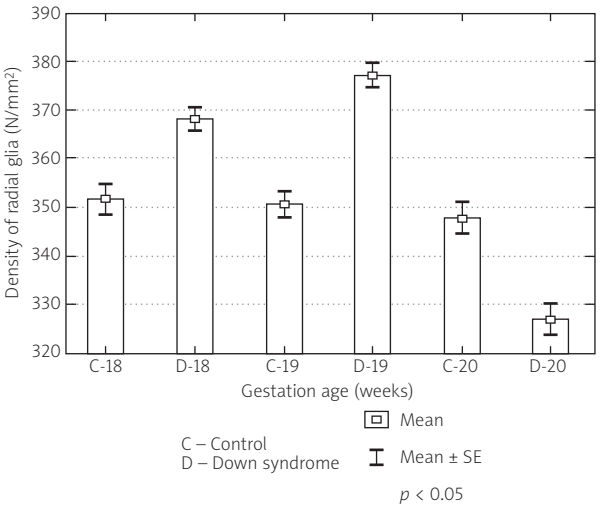

Fig. 7. Density of radial glia in frontal lobe of Down syndrome fetus and control.

that the number of ramified microglial cells was significantly higher than in normal fetuses [26]. As recently demonstrated by Herrera *et al*. [11] it may be essential to understand the role of altered astrogliogenesis in DS pathology. A study performed on Ts65Dn mice showed a possible relationship between the enhanced level of synaptojanin-1 expression and the increased number of astrocytes. The synaptojanin-1 gene is located on chromosome 21 and the product of that gene is involved in the development of astrocytes [11]. The above-mentioned authors also pointed out that an imbalance between neuron cells (decreased number) and astroglia cells (increased number) can lead to cognitive impairments. Despite the well-known fact that the dysfunction of astrocytes plays an essential role in the pathogenesis of CNS disorders, it is believed that altered astrogliogenesis is one of the major causes of mental retardation in Down's syndrome. This is a relatively new approach and only a few papers addressing this problem have been published to date [8]. One such paper suggests that astroglial alterations progress with age, being involved in the development of Alzheimer's type of dementia in adult life of DS individuals [6]. This type of dementia is typical for adults with Down's syndrome. The disorder of neuron-glia interactions in migration disturbances does not occur only in DS [15,16,27]. Taking into consideration the role of astroglia during development we believe that any change in its quantity, reduced or increased, can affect CNS development and lead to disturbances of both neurogenesis and synaptogenesis. We believe that this relevance need to be further explored as altered astrogliogenesis has a great impact on prenatal, as well as postnatal CNS development, which in consequence can lead to mental retardation as part of the Down's syndrome phenotype.

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