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Astroglia and microglia in cerebellar neuronal migration disturbances

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Abstract

Between the neuronal and glial cells there is a close relationship conditioning a tight morphological correlation and proper functional interplay. Disturbed interaction between glial and neuronal components leads to inappropriate neural circuits. The reflection of the failure of neural circuit organisation is the picture of morphological changes of neurons and glia. The appearance of microglia and astroglia was analysed in a defectively formed cellular network due to cerebellar neuronal migration disturbances. Focal disruption of neuron migration leads to their differentiation in an abnormal position manifested as heterotopias and cortical anomalies. Neurons that had lost their proper migratory way and heterotopically settled in the white matter were encircled by GFAP-positive astrocytes, with morphology appropriate for surrounding white matter. The microglial cells infiltrated the parenchyma within the heterotopic neurons playing a role in their elimination. In the cerebellar cortical malformations astrocytes were grouped near the Purkinje cells. In the minimal cortical dysplasia the increased number of astrocytes supported the neurons. Impaired morphological components of the glial-pial barrier were observed. In the massive cortical malformations a few degenerated astrocytes followed the disarranged Purkinje cells, while microglia and Bergmann glia fibres were not present. Absence of cells supporting and organizing the cerebellar cortex had an effect on loss of Purkinje cell shape, their disorientation and abnormal position. The appearance and localisation of the astroglia and microglia in the abnormal cerebellar circuitry due to migration disturbances is dependent on the pathomechanism of the anomalies.

Key words: *cerebellum, development, migration failure, cortical malformation, glia*

Introduction

At every stage of central nervous system development neurons have a close association with, and functional dependence on, astroglial cells. The developmental function of astroglia is the migrational guidance of neurons by radial glia. After guiding neuronal migration, radial glia differentiate into astrocytes and spread in the white matter, granular layer, and in the Purkinje cell layer as a Bergmann glia. Trophic support of neurons, synaptogenesis and synaptic remodelling, and homeostasis of neuronal microenvironment are the normal functions of astrocytes. Signalling between astrocytes and neurons contri-

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butes to the coordination of numerous physiological processes: synaptogenesis, synchronisation of network activity, control of microcirculation, modulation of the strength of synaptic transmission [2].

During development microglia invade central nervous tissue at very early stages, before or concomitantly to neurogenesis [16], and are able to secrete a variety of cytokines that have been implicated in all aspects of neuronal functions [8]. Microglial cells participate in many of the complex morphogenic and histogenic processes which take place during the development of the CNS in order to establish the complex network of connections. Microglia are involved in synaptogenesis and apoptosis, influencing the fate of neurons in the developing brain [3,16]. The localization of microglial cells and their morphological forms correlate precisely with the appropriate stages of brain development [14].

The cerebellum develops over an extremely long period of time extending from the early embryonic phase until the first postnatal years [1,11,18]. Agents that interrupt normal growth may have different effects depending on the stage of development. Focal disruption of neuron migration early in development leads to localized cortical dysplasia and heterotopias [4]. In general, neurons differentiate in an abnormal position. The cerebellar heterotopias exist in the white matter of the hemispheres and consist of nests of large cells at times resembling Purkinje cells, which may or may not have an admixture of granular neurons. When an agent acts after the Purkinje cells have matured but the granular cells are still dividing and migrating, diffuse lesions affecting the entire granular layer may appear [10]. Granule cells are mixed with Purkinje cells instead of forming respective layers.

A close relationship between neurons and glia exists in the proper development of the cerebellum. The examination of neuron-glia interactions in abnormal cerebellar circuitry is the aim of the study. The morphological appearance of astroglia and microglia is analysed in the defectively formed cellular cerebellar network as a result of migration disturbances.

Material and Methods

The study was performed on the brains of 12 infants selected according to the diagnosis of migration anomalies found in routine post-mortem examination. Two groups of neuronal migration disturbances were taken into consideration. The first constituted abnormal groups of neurons arrested in the white matter of hemispheres during their way to the cortex. The second represented disorganized cortical layering and gyrus formation. The disorganized cortical layering and gyrus formation foci varied in size from small foci of marked disarrangement of cortical neurons to a blurred layered pattern of cerebellar cortex. Six cerebella with different types of heterotopias (age 36-40 gestational weeks) and 6 cerebella with cortical malformations (age 36-40 gestational weeks) were studied. The clinicopathological data of these cases are shown in Table I. Age-matched brains diagnosed as nonpathological were taken as controls.

All brains were fixed in formalin, and blocks of cerebellum were embedded in paraffin. Histopathological and immunohistochemical examination were conducted on coronal cerebellar sections.

The sections of specimens of cerebellum were stained with haematoxylin-eosin and cresyl violet. The representative slides were incubated in primary

Table I. Clinicopathological data

Nr	Age g.w.	Survival	Clinico-pathological data
group of heterotopies			
1	36	1 h	Down's syndrome, congenital heart failure
2	36	0	anhydramnion, foetal malformations
3	36	3 h	bilateral bronchopneumonia, low birth weight
4	38	1 d	malformations of the digestive system
5	40	2 h	congenital heart failure, low birth weight
6	40	1 d	malformations of the digestive system, postoperative shock
group of cortical malformations			
7	36	2 h	multiple malformations of the urinary tract, digestive and skeletal systems
8	37	2 d	pneumonia
9	40	3 h	multicystic dysplasia of kidneys
10	40	4 h	shock, sudden cardiac arrest, low birth weight
11	40	0	congenital heart failure
12	40	1 h	Down's syndrome, congenital heart malformation

g.w. – gestational weeks; h – hours; d – days

antibodies generated against calbindin D-28k (Sigma, dilution 1:200) for Purkinje cells detection, glial fibrillary acidic protein (GFAP) (DAKO 1:1000) for astrocytes, and ferritin (Sigma, 1:1000) for microglia. Immunoreaction was visualized using appropriate secondary antibody and an alkaline phosphatase--avidin-biotin conjugate or ABC complex conjugated with peroxidase. Finally all sections were dehydrated, cleared and mounted for light microscope examination. Some sections were counterstained lightly with cresyl violet. To clarify the relationship between the Bergmann glia and Purkinje cells, double immunolabelling of GFAP and calbindin was performed.

Results

In the cerebellar gyri at age 36-40 gestational weeks the GFAP-positive astroglial cells are dispersed in the cortex and in the white matter (Fig. 1A). Small cells with long thin processes are seen in the internal granule layer (Fig. 1B). The cells with clearly visible cytoplasm and few short processes are present mainly in the white matter (Fig. 1C). GFAP-positive "palisade-like" formation of the Bergmann glia fibres is observed in the molecular layer of the cerebellar cortex (Fig. 1D). The microglia cells infiltrate the cortex and white matter. Fully ramified microglia are located in all the cortical layers: external and internal granular, molecular, Purkinje cell layer (Fig. 1E).

In the cases with displaced neurons the clusters of cerebellar cortical neurons were abnormally located in the hemispheric white matter. The heterotopias consisted mostly of Purkinje cells. Purkinje cells did not form a monolayer, but were irregularly dispersed in the white matter or scattered among whirls or stripes of granules and spindle cells (Fig. 2 A, B). The Purkinje cells were well differentiated, with a dendritic branching tree. The expression of calbindin closely resembled normotopic neurons.

The groups of neurons were surrounded by star--shaped astroglial GFAP-positive cells. Among the arrested neurons GFAP-positive cells were not present (Fig. 3). The ramified microglial cells penetrated within the neurons and were located near the Purkinje cells. Microglial cells were ramified, with small bodies and short processes (Fig. 4 A, B).

The second group of cases presented the displaced neurons in the cerebellar cortex. In four cases the discrete malformative cortical lesions were observed like fusion of external granular layers or nests of Purkinje neurons dislocated to the internal granular layer (Fig. 5). The fused external cortical layers were underlined by GFAP-immunopositive astrocytes located in the molecular, Purkinje cells and external granule layers. The cells increased in

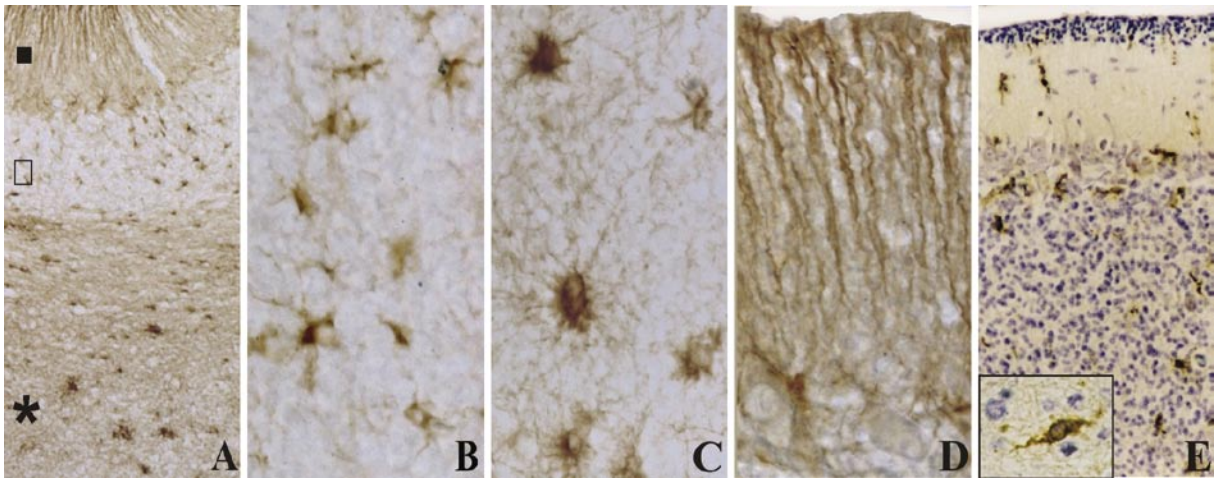

Fig. 1. Cerebellum. Newborn 36 gestational weeks old. (A) GFAP-positive astroglial cells in the cerebellar gyrus. ■ molecular layer, □ internal granule layer, * white matter. Original magnification × 4; (B) GFAP-positive astroglial cells in the internal granule layer. GFAP, orig. magn. × 40; (C) GFAP-positive astroglial cells in the white matter. GFAP, orig. magn. × 40; (D) GFAP-positive palisade-like formation of the Bergmann glia fibres in the molecular layer. GFAP, orig. magn. × 60; (E) Microglia in the cerebellar cortex. Ferritin, orig. magn. × 10. Insert: ramified microglia cell × 60

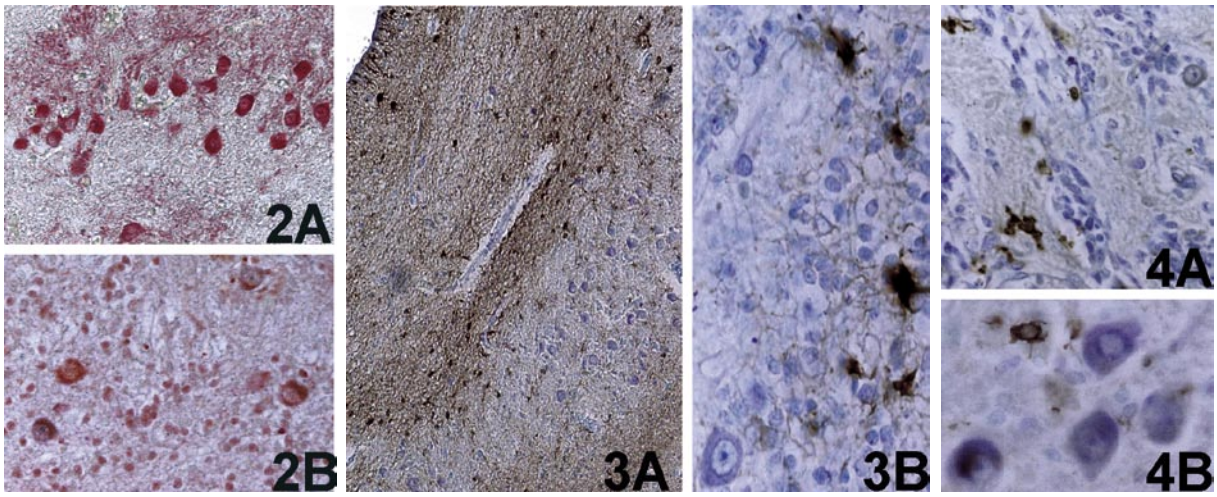

Fig. 2. (A) Nest of Purkinje cells located in the white matter; (B) Purkinje cells intermingled with granule cells – heterotopy in the white matter. Calbindin, orig. magn. × 20

Fig. 3. GFAP-positive astroglial cells in the white matter surrounding the heterotopy. GFAP. (A) Orig. magn. × 4; (B) Orig. magn. × 40

Fig. 4. (A) Microglial cells dispersed within the heterotopy; (B) Microglial cell by the Purkinje cell. (A, B) Ferritin, × 40

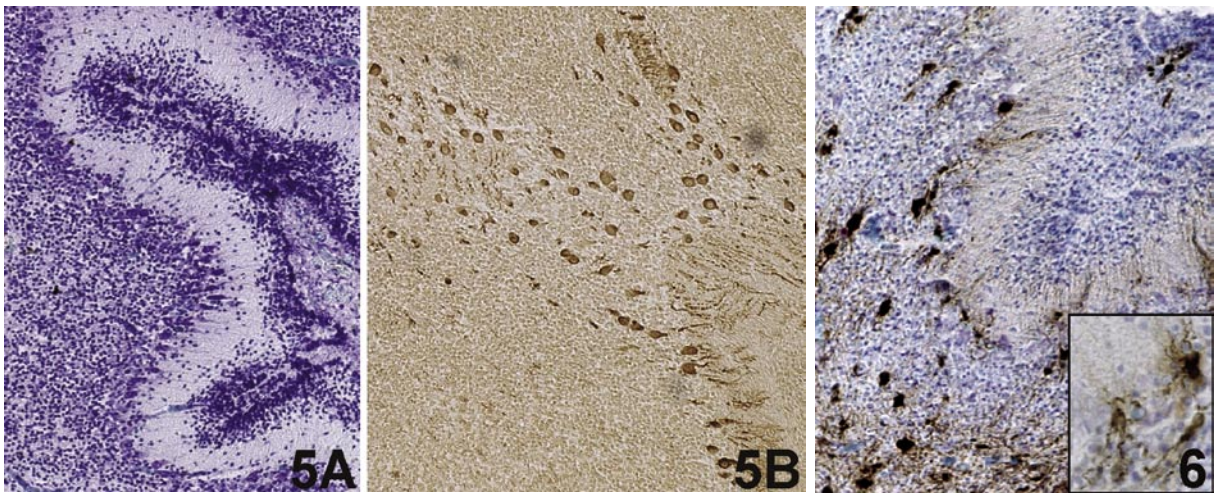

Fig. 5. (A) The fusion of the external granule layers of the neighbouring convolutions. Cresyl violet × 4; (B) Purkinje cells transgressing the internal granular layer. Calbindin, × 4

Fig. 6. GFAP-positive astrocytes in the cerebellar cortex around the adhesion of external granule layers. Insert: astrocytes in the Purkinje cell layer. GFAP, × 10; insert × 40

number, size and GFAP staining intensity. The GFAP-positive astrocytes were located closely to neurons, mainly the Purkinje cells (Fig. 6). The Bergmann glia fibres in the molecular layer were less evident. GFAP staining was weak. The microglia colonized the upper part of the cerebellar cortex, infiltrating mostly the Purkinje cell and molecular layers like in the age-matched controls.

In two cases the normal layered pattern of the cerebellar cortex was completely disorganized. The external granule cells did not form a proper external granule layer, but moved deeper, forming irregular

aggregates that invaded cerebellar tissue. Laminated structure of the cortex was disturbed. The narrow molecular layer was occasionally visible, but mostly it had disappeared. In the preserved fragments of the molecular layer, GFAP-positive Bergmann glia fibres were absent. The Purkinje cells presented short fragments of monolayer located among the granule cell mass (Fig. 7). They also formed improperly arranged clusters. Purkinje neurons were abnormal, atrophic, with disorganised dendrites. In large parts of cerebellar tissue Purkinje cells were not observed; they did not migrate to the place of residence at all. GFAP-positive astrocytes (Fig. 8) followed the Purkinje cells embedded in the granule neuron mass. In the areas devoid of Purkinje cells, astrocytes were also lacking. The astrocytes were degenerated, with vacuoles in the cytoplasm, without processes. In the vicinity of the malformed cortex normal astroglial GFAP-positive cells were seen.

The microglial reaction was very weak. Cells produced faint labelling. Only a few microglia cell shadows were observed (Fig. 9).

Discussion

The migration of neurons requires the arrangement of multiple molecular events, including the selection of a pathway, the formation of adhesive interactions with cellular and extracellular substrates and the cooperation of cytoskeletal components [1]. The consecutive steps of cerebellum development lead to formation of cerebellar circuitry and further differentiation of neurons and glia. Interplay between astroglia, microglia, neurons and the other non-neuronal cells is necessary to form the proper cerebellar circuitry. During normal development of cerebellum the glial and neuronal cells migrate from the place of origin to the place of residence in the correct laminar position within the cerebellar cortex. Distinct genetic mutations and environmental toxins can affect neuronal migration, influence interactions between glial and neuronal components and result in a spectrum of morphological changes which are the consequence of aberrant migration.

The Purkinje cells migrate along radial glia through the future white matter toward the cortical plate [12]. Most, if not all, glial precursors in the cerebellum take the form of radial glial cells. Radial fibres arising near the ventricular zone do not reach the pial surface but terminate in the vascular wall in the intermediate zone [19]. The majority of them transform into astroglial cells of the white matter. Arrested or excessive migration leads cerebellar neurons to differentiate in a heterotopic position. Heterotopic neurons form

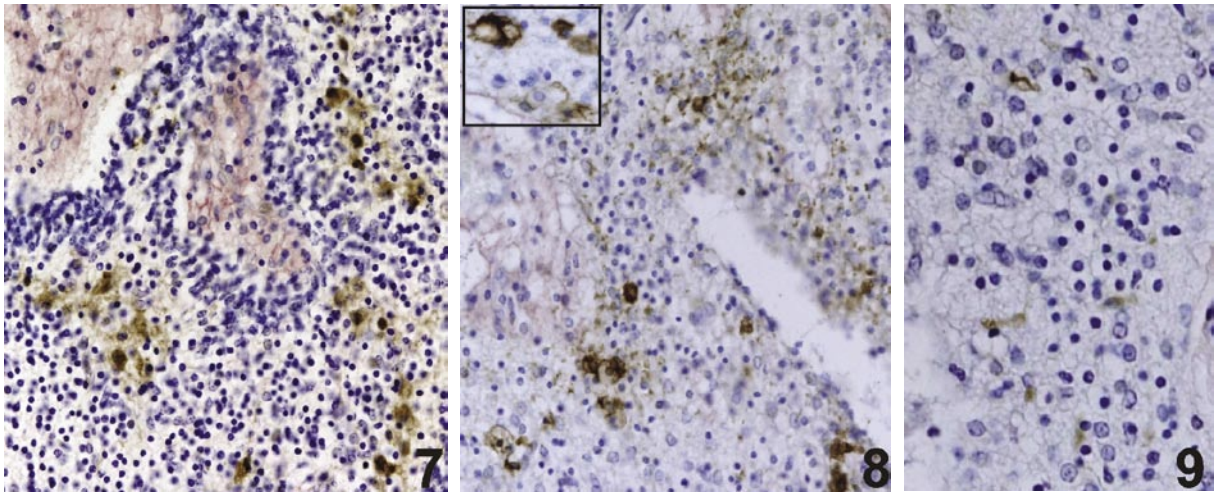

Fig. 7. Disturbed laminated structure of the cerebellar cortex. Abnormal Purkinje cell positioning and disarranged molecular layer. Calbindin, × 20

Fig. 8. GFAP-positive astrocytes following the Purkinje cells. GFAP, × 20. Insert: the degenerated astrocyte. GFAP, × 60

Fig. 9. Microglia shadows among the disorganized unlayered cerebellar cortex. Ferritin, × 60

essentially normal afferent and efferent connections. Recent data show that heterotopic neurons can be contacted by environmental, that is local, fibres that normally never innervate the neocortex. This dual connectivity leads heterotopias to form bridges between their environmental and the original network. The intraheterotopic network may be organised very differently from the normotopic neocortical network. The ramification pattern of the dendritic tree of heterotopic neurons is different from normotopic neurons [4]. In the nests of heterotopic neurons the astroglia were grouped in the border of surrounding white matter. Their appearance corresponded with the picture of normal astrocytes existing in this structure. Ramified microglia occurred by the heterotopic neurons, as cells regulating and controlling their apoptosis and synaptic properties.

The radial fibres arising in the intermediate zone of the cerebellum reach the pial surface. In the cerebellar cortex they convert into the Bergmann glia. Bergmann glial cells are the essential organizers of the cerebellar cortex, especially through normal anatomical arrangement of Purkinje cells and their dendrites and by ensuring the survival of granule cells [19]. Bergmann fibres of the developing cerebellum constitute a palisade-like glial framework in the molecular layer. During development, fibres are known to associate with migrating granule cells [1]. The granule cells migrate inwards from the proliferative zone in the external granular layer, just below the pial surface membrane, along the Bergmann glial fibres. The granule cells attach the glial fibres, pass through molecular layer, by Purkinje cells and beneath the Purkinje cell layer form the mature internal granular layer. The Bergmann glia is also defined as a Purkinje neuron-associated astrocyte influencing the dendritogenesis and synaptogenesis of these cells. During the cerebellar development the palisade-like radial organisation is replaced with a reticular glial meshwork. The rod-like domain of Bergmann fibres provides structural substrates for growing dendrites to promote directional growth and arborisation. Cerebellar cortex is characterised by dynamic expansions of the molecular layer as a result of active dendritogenesis and synaptogenesis of Purkinje cells and also of the internal granular layer as a result of massive granule cell production and migration. Bergmann glial cells contribute to the supply of the glial framework and substrates to these newly expanded regions [19]. In the massive cerebellar cortical malformations the framework of Bergmann glia was not visible. The narrow molecular layer was only occasionally noticed; mostly it had disappeared. The absence of cells supporting and organizing the cerebellar cortex had an effect on the loss of Purkinje cells' shape, their disorientation and abnormal position. The normal layered pattern of the cerebellar cortex was disorganized. The affecting role of the altered framework of Bergmann glia on the cortical laminar disorganisation was suggested by Yue and co-workers. Premature differentiation of Bergmann glia fibres led to extensive layering defects. Severe granule neuron migration defects and abnormal laminar formation were observed in impaired Bergmann glia [20].

An observation worth noting in the cerebellar cortical malformation with blurred layered pattern is the paucity of microglia. Microglial cells within the developing central nervous system (CNS) originate from mesodermic precursors of haematopoietic lineage that enter the nervous parenchyma from the meninges, ventricular space and blood stream [5,17]. Microglial precursors colonize the CNS by two mechanisms: migration and proliferation. In the cortical layers and white matter intermediate microglial cells migrate from the ventricular zone to the deep cortical plate along radial and tangential pathways. Bergmann glia in the cerebellar cortex might guide radial microglial migration. Proliferative activity of amoeboid microglia occurs during normal CNS development. Due to proliferation microglia increase in number and widely disperse through the CNS during normal development. After reaching their final location microglial cells progressively acquire a ramified morphology, first becoming intermediate, scarcely ramified microglial cells and finally mature fully ramified microglia [14]. Astrocytes appear to play an important role in determining the mature microglial phenotype by releasing products influencing the differentiation of microglial cells. In our cases with massive cortical anomalies the functional interaction between microglia and astroglia was disturbed. The degenerated astrocytes following a few residual Purkinje cells affected the microglial cells. Marin-Teva et al. [13] described that alteration of developing microglia impacts synaptic properties. Their data provide evidence that the loss of function of a microglial protein expressed during perinatal stages results in alterations in synaptic function and plasticity. In our cases with disarranged cortical lamination and abnormal neuronal positioning the

interaction between microglia and neurons was evidently altered.

In the discrete cerebellar cortical malformations the morphological appearance of astroglia and microglia was different. Developmental events in the superficial part of the cerebellum appear to be critical for the formation of gyri [7] and important for the generation of discrete cortical disturbances. Meningeal cells constitute part of the superficial glia limitans and produce components of both the interstitial matrix and the basement membrane. The other part of the cerebellar glia limitans consists of the end-feet of Bergmann glial fibres. In the cases with discrete malformations GFAP-positive glia limitans disappeared around the fusion of adjacent folia; the Bergmann fibres staining was weak. The glial-pial barrier was incompletely formed. Impaired formation of the glia limitans may result in the disruption of laminar structures of the cerebellar cortex. A similar picture was observed in anomalous formation of cortical convolutions and in discrete glioneuronal malformative lesions of the cerebral cortex [6,9].

Mercier and Hatton [15] describe a meningeoglial cellular network that courses through all layers of meninges bordering or joining the vasculature and extends into the periventricular subependymal layer. They suggested that all the cell types of this cooperative network may communicate and control cellular proliferation, growth and differentiation. The alteration of the glia limitans and the meningeal component may affect the neuro- and gliogenesis and this disturbance could be important in anomalous formation of the cerebellar cortex. In the discrete malformative lesions the intensively stained GFAP-positive astrocytes located in the molecular, Purkinje cell and external granule layers underlined misshapen convolutions. The cells increased in number and size were located close to neurons and supported mainly the Purkinje cells.

Between the neuronal and glial cells there exists a close relationship conditioning a tight morphological correlation and proper functional interplay. The disturbed interdependence between glial and neuronal components leads to inappropriate neural circuits. The reflection of the failure of neural circuit organization in the cerebellum is the picture of morphological changes of neurons and glia. The appearance and localization of the astroglia and microglia in abnormal cerebellar circuitry due to migration disturbances is dependent on the pathomechanism of the anomalies.

It is interesting that among our cases the majority have other than migrational, congenital malformations of internal organs. The lesions originating from different ontogenic periods indicate remitted or prolonged influence of teratogenic factors. Teratogens may alter gene expression and hinder differentiation of tissue not only in the cerebellum, but also in other systems.

References

1. Altman J, Bayer SA. Development of the cerebellar system in relation to its evolution, structure and functions. CRC Press, Boca Raton, Florida 1997.
2. Bellamy TC. Interactions between Purkinje neurones and Bergmann glia. Cerebellum 2006; 5: 116-126.
3. Bessis A, Bechade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. Glia 2007; 55: 233-238.
4. Chevassus-au-Louis N, Represa A. The right neuron at the wrong place: biology of heterotopic neurons in cortical neuronal migration disorders, with special reference to associated pathologies. Cell Mol Life Sci 1999; 55: 1206-1215.
5. Cuadros MA, Navascues J. The origin and differentiation of microglial cells during development. Prog Neurobiol 1998; 56: 173--189.
6. Dąmbska M, Laure-Kamionowska M. The role of Glial-Pial barrier lesions and impaired vascularization in anomalous formation of cortical convolutions. Brain Dev 2001; 23: 223-227.
7. Friede RL. Developmental neuropathology. 2nd ed. Berlin Heidelberg New York: Springer-Verlag; 1989: 361-371.
8. Hanisch UK. Microglia as a source and target of cytokines. Glia 2002; 40: 140-155.
9. Laure-Kamionowska M, Maslinska D, Raczkowska B. Discrete glioneuronal malformative lesions in the foetal and infantile cerebral cortex. Folia Neuropathol 2002; 40: 183-191.
10. Laure-Kamionowska M, Taraszewska A, Maślińska D, Raczkowska B. Faulty position of cerebellar cortical neurons as a sequel of disturbed neuronal migration. Folia Neuropathol 2006; 44: 327-332.
11. Lavezzi AM, Ottaviani G, Terni L, Matturri L. Histological and biological developmental characterization of the human cerebellar cortex. Int J Dev Neurosci 2006; 24: 365-371.
12. Mai JK, Ashwell KWS. Fetal development of the Central Nervous System. Cerebellum and precerebellar nuclei. In: Paxinos G, Mai JK (eds.) The human nervous system. Elsevier, San Diego 2004; pp. 78-80.
13. Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. Microglia promote the death of developing Purkinje cells. Neuron 2004; 41: 535-547.
14. Maslinska D, Laure-Kamionowska M, Kaliszek A. Morphological forms and localization of microglial cells in the developing human cerebellum. Folia Neuropathol 1998; 36: 145-151.
15. Mercier F, Hatton G. Immunocytochemical basis for a meningeo--glial network. J Comp Neurol 2000; 420: 445-465.
16. Monier A, Adle-Biassette H, Delezoide AL, Evrard P, Gressens P, Verney C. Entry and distribution of microglial cells in human

embryonic and fetal cerebral cortex. J Neuropathol Exp Neurol 2007; 66: 372-382.
17. Navascués J, Calvente R, Marín-Teva JL, Cuadros MA. Entry, dispersion and differentiation of microglia in the developing central nervous system. An Acad Bras Cienc 2000; 72: 91-102.
18. Wang VY, Zoghbi HY. Genetic regulation of cerebellar development. Nat Rev Neurosci 2001; 2: 484-491.
19. Yamada K, Watanabe M. Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. Anat Sci Int 2002; 77: 94-108.
20. Yue Q, Groszer M, Gil JS, Berk AJ, Messing A, Wu H, Liu X. PTEN deletion in Bergmann glia leads to premature differentiation and affects laminar organization. Development 2005; 132: 3281--3291.