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Cerebellar cortical neurons misplaced in the white matter due to disturbed migration during development of human brain

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Abstract

The normal laminar organisation of the cerebellar cortex is the result of the precisely controlled migration, differentiation and maturation of the neurons. Occasionally the migrating neurons lose their proper way of migration and form nests of grey matter in the improper place. The aim of this study was to investigate the morphological features of the lost neurons in the cerebellar white matter during development, with particular emphasis on their localisation, arrangement and differentiation. We analyzed 31 fetal and infantile brains, aged from 28 gestational weeks to 18 postnatal months. We observed different morphological patterns of cerebellar heterotopias. Clusters of grey matter reflecting the cerebellar cortical pattern with well-defined molecular layer and altered granular and Purkinje cells were most frequently observed. The compact heterotopias were composed of bands or whirls of spindle and round granule cells situated closely together, while Purkinje neurons were completely disorganised. The ectopic cortex in the white matter with a normal layered structure containing all the components of the cerebellar cortex was localised by the large vessels. Aggregations of Purkinje cells scattered in the white matter without accompanying granule cells were observed. The evaluation of the biological features of the misplaced cerebellar cortical components showed high activity of neurons.

Key words: *cerebellum, heterotopy, misplaced neurons, human, development.*

Introduction

During prenatal and early postnatal development of the human cerebellum, neurons migrate from the germinal layers to their destined localisation in the cerebellar cortex [3,4]. The cerebellar neurons migrate radially and tangentially from two different germinal sources and stop at defined correct positions within the cerebellar cortex [1,3]. The normal laminar organisation of the cerebellar cortex is the result of the precisely controlled migration, differentiation and maturation of the neurons [41]. Occasionally the migrating neurons lose their proper way of migration and form nests of grey matter in the improper place. Heterotopia is a sign of altered migration with the abnormal distribution of neuronal cells within the white matter. Abnormal clusters of grey matter were described in trisomy 13

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and 21 [28,34,35], PAX6 mutation [39] and genetic metabolic disorders [29]. A high incidence of cerebellar heterotopias and dysplastic changes was detected in the brains of autistic subjects [42]. Small nests of misplaced neurons were also found as incidental lesions in normal brains [11,44].

The examination of the localisation, arrangement and maturation of the misplaced neurons at different stages of human cerebellar development was the aim of the study.

Material and methods

The analysed material comprised 910 infantile cases whose brains were examined in the Department of Developmental Neuropathology during 1984-2006. Based on post-mortem evaluation, 31 brains with abnormal clusters of neurons in the cerebellar white matter were chosen. The clinicopathological data of selected cases and the localisation of heterotopias are presented in Table I. Single dentate nucleus dysgenesias were excluded from the study; only those which were accompanied by other heterotopic foci were taken into consideration.

Neuropathological examination was performed on slides from the coronal sections of both cerebellar hemispheres and vermis at several levels. Formalin-fixed, paraffin-embedded tissue was serially sectioned at 6 µm and sections were routinely stained with cresyl violet and haematoxylin eosin. Representative sections mounted on silane-coated slides were processed for immunocytochemical reactions to synaptophysin (1 : 200, DAKO), calbindin (1 : 200, Sigma), and glial fibrillary acidic protein (1 : 1000, DAKO).

Results

Among cerebellar heterotopias, 14 were observed in the vermis, 13 in the flocculus, 15 in the interior white matter of hemispheres (*corpus medullare cerebelli*) and in 8 cases the dysgenesia of the dentate nucleus accompanied other improper nests. In 14 cases heterotopic neurons were settled in more than one localisation; among them, in five cases abnormal clusters of grey matter were observed in all three localisations (Fig. 1). In the infantile cases born under 40 gestational weeks, localisation in the white matter of the hemisphere dominated, while in the postnatal cases mostly flocculonodular dysplasia was observed.

Several types of cerebellar heterotopias can be distinguished relating to their morphological picture.

Clusters of grey matter reflecting the cerebellar cortical pattern with a well-defined molecular layer were most frequently observed in all three localisations (Fig. 2). A distinct, evident molecular layer was surrounded by a wide band of granular cells. The heterotopic groups of neurons were small, rounded or formed irregular large clusters. In the molecular layer, ectopic granule cells were observed. Spindle-shaped cells corresponding to the external granular

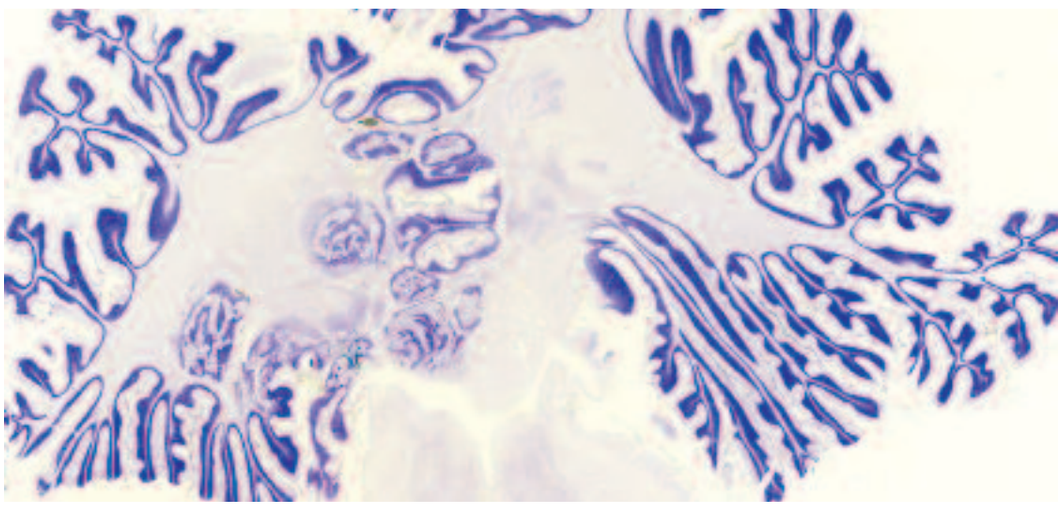

Fig. 1. Clusters of grey matter in the cerebellar white matter of hemispheres and vermis. Glass magnification.

Table I. Clinicopathological data

No	Age	Clinicopathological data	Localisation of heterotopias			
			Ver.	Fl.	H.	D.n.
1	26/28 gw	Perinatal hypoxia, twin I, periventricular haemorrhage	+		+	+
2	26/28 gw	Perinatal hypoxia, twin II, periventricular haemorrhage	+		+	
3	28 gw	Perinatal hypoxia, intraventricular haemorrhage		+		
4	30 gw	Down syndrome, congenital heart defect, gastrointestinal anomaly, leukomalacia periventricularis		+	+	+
5	32/34	Respiratory distress syndrome, anoxic cerebral lesions	+			
6	34/36	Septic shock, pneumonia, hepatomegaly, periventricular white matter lesion			+	
7	34/36 gw	Pregnancy anhydramnios, congenital disorders, hypoxic-ischaemic encephalopathy			+	+
8	34/36 gw	Pneumonia, disseminated vascular coagulation, congenital disorders, hypoxic encephalopathy		+	+	
9	36 gw	Spina bifida malformation, hydrocephalus, urinary tract anomalies, white matter lesions	+		+	
10	36 gw	No data			+	
11	37 gw	Respiratory distress syndrome, no cerebral lesions	+		+	
12	38 gw	Placental abruption, stillborn, disseminated intravascular coagulation, subarachnoid haemorrhage	+			
13	40 gw	Congenital heart defect, cerebral oedema			+	
14	40 gw	Down syndrome, pneumonia, diathesis haemorrhagica, hypoxic-ischaemic encephalopathy			+	
15	40 gw	Malformation syndrome, hydrocephalus	+			
16	40 gw	Sepsis, congenital heart defect, meningoencephalitis	+			
17	40 gw	Congenital heart failure	+			+
18	2 w.	Pneumonia, pneumothorax, periventricular leukomalacia		+		
19	3 w.	Malformation syndrome, hydrocephalus	+	+		
20	3 w.	Congenital heart failure, perinatal asphyxia, leukomalacia		+	+	+
21	1 mo.	Congenital anomaly gastroschisis, circulatory shock, cerebral haemorrhage		+		
22	1.5 mo.	Congenital heart defect, pneumonia, hyperaemia cerebri	+		+	
23	1.5 mo.	Toxic colitis, anoxic lesions	+		+	+
24	2 mo.	Sepsis, gastro-colitis acute, jaundice, hypoxic encephalopathy		+		
25	3 mo.	Congenital heart defect, hyperaemia cerebri		+		+
26	3 mo.	Turner's syndrome, hydrocephalus	+			
27	5 mo.	Down syndrome, heart defect, multiple foci of cerebral necroses		+		
28	5 mo.	No data		+		
29	6 mo.	Down syndrome, heart defect, hepatomegaly, pneumonia, hypoxic encephalopathy		+	+	+
30	8 mo.	Pneumonia, hypoxic encephalopathy	+			
31	1.5 y.	Down syndrome, heart defect, surgery, cerebral hyperaemia		+		

ver. – vermis, Fl. – flocculonodular lobe, H. – white matter of the hemisphere, "arbour vitae", D.n. – dentate nucleus, gw – gestational weeks, w. – weeks, mo. – months, y. – year

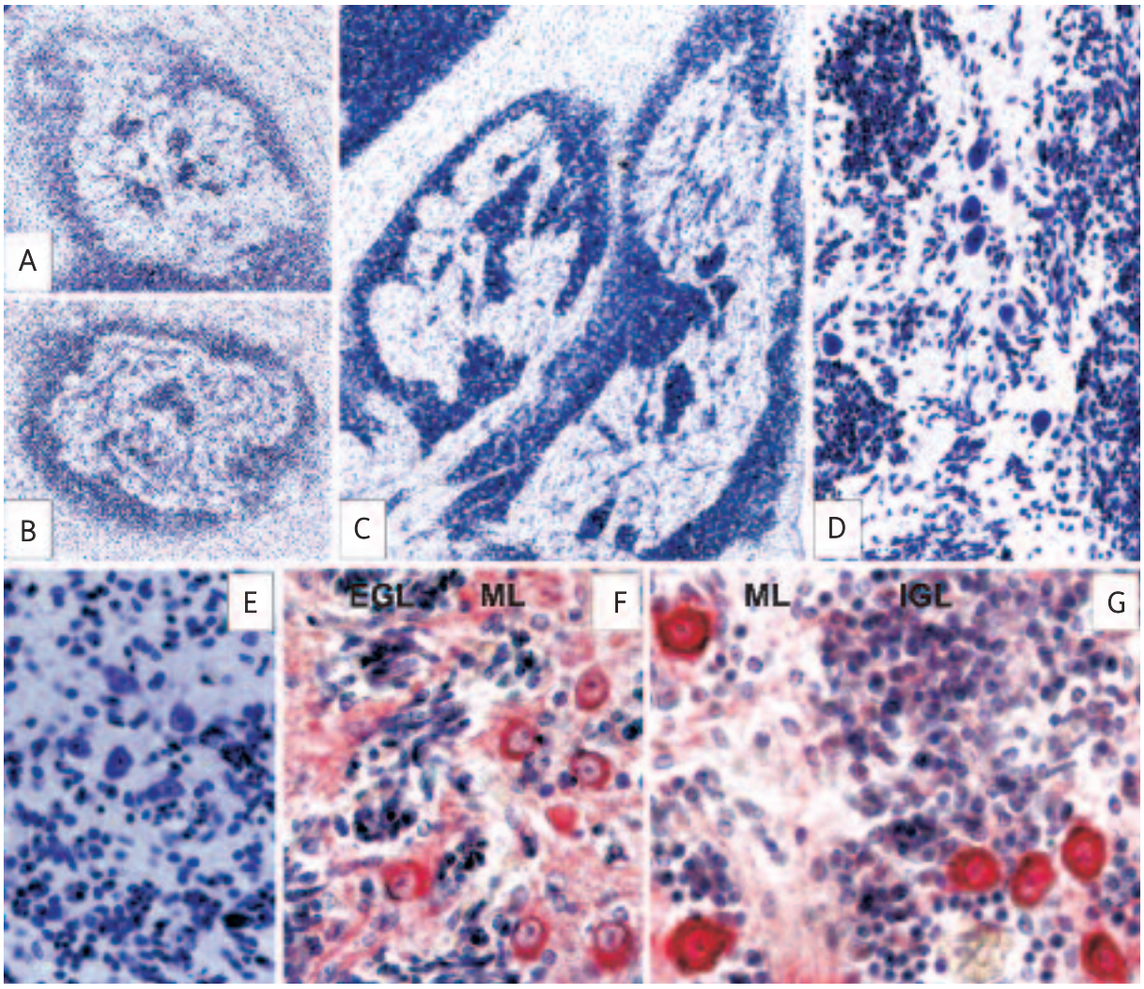

Fig. 2. Heterotopic group of disarranged neurons with distinct molecular layer. **A, B)** Small, rounded clusters; orig. magn. 40×. **C)** Large, irregular heterotopy; orig. magn. 40×. **D)** Purkinje neurons irregularly dispersed within molecular layer; orig. magn. 100×. **E)** Purkinje neurons intermingled with granule cells; orig. magn. 100×. **F)** Calbindin positive Purkinje neurons in the molecular layer; orig. magn. 200×. **G)** Calbindin positive Purkinje cells dispersed in the molecular layer and among granular cells; orig. magn. 200×.

layer neurons were loosely scattered or grouped in separate small clumps within the molecular band, mainly in cases up to 34 gestational weeks old. In older cases, clusters of ectopic granular cells, variable in size, were observed within the deformed molecular layer forming the labyrinth. The dysplastic molecular layer mixed with ectopic neurons was surrounded by a continuous band of round cells corresponding to internal granule layer neurons. The Purkinje cells did not form a monolayer. Small groups of Purkinje neurons were irregularly dispersed within the distorted molecular layer or intermingled with granule cells. Purkinje cells displayed intense calbindin immunoreactivity. Their cell bodies were strongly stained, but their processes were pale. The calbindin immunoreactivity imparted an intense coloration to background tissue near the Purkinje neurons where their dendrites arborize. The intensity of staining increased with the developmental maturation of cell soma. The groups of neurons were surrounded by astroglial GFAP-positive cells (Fig. 3). Among the arrested granular neurons, few astroglial cells showed GFAP immunoreactivity. The GFAP cells were small with thin, tiny processes. Their shape and

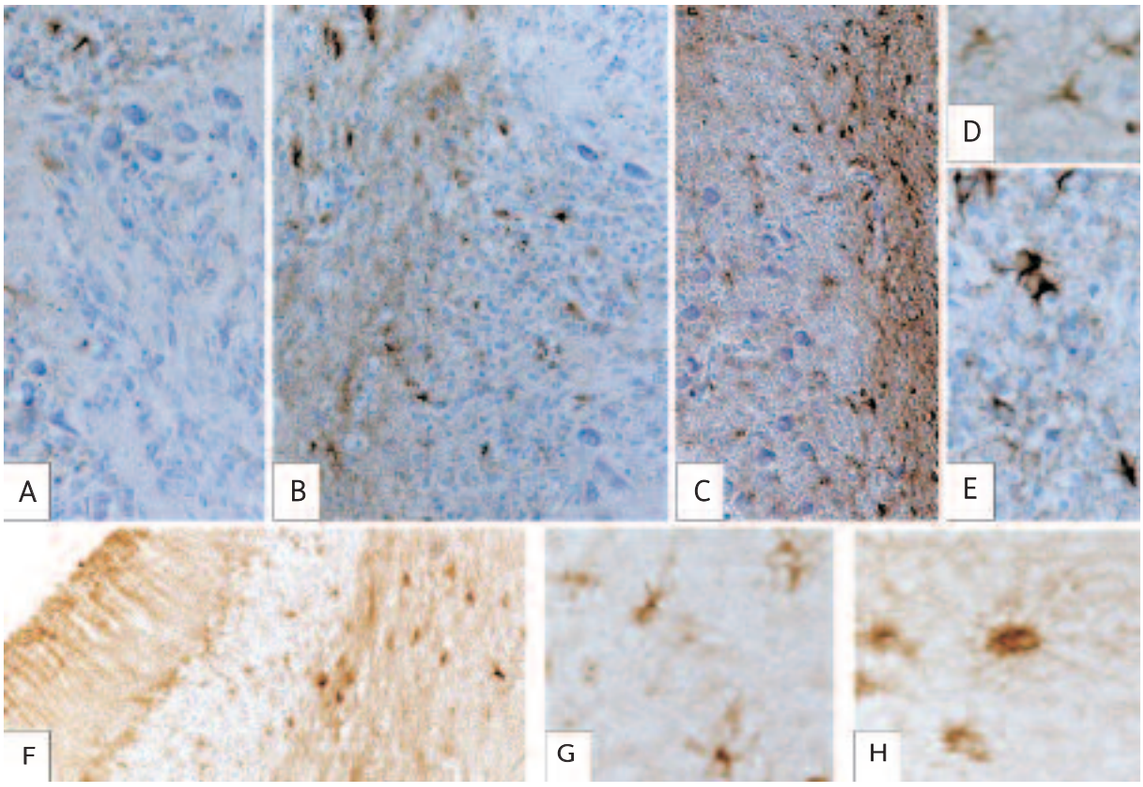

Fig. 3. GFAP-positive astroglial cells surrounding the nests of neurons. **A, B)** Heterotopy composed of all cerebellar neurons; orig. magn 100×. **C)** Heterotopy composed of Purkinje cells; orig. magn. 100×. **D)** GFAP-positive fine cells among the arrested granule cells; orig. magn. 400×. **E)** GFAP positive cells at the border of heterotopy with white matter; orig. magn. 400×. **F)** GFAP positive cells in normal cortex, newborn; orig. magn. 400×. **G)** GFAP immunoreactive cells in the internal granule layer; orig. magn. 40×. **H)** GFAP immunoreactive cells in the white matter; orig. magn. 400×.

appearance corresponded to the astroglial cells observed in the normal internal granular layer. There was no GFAP reaction in the molecular layer, or near the Purkinje cells.

In the heterotopias arranged in solid pattern the cytoarchitecture of the molecular and granule layers and the Purkinje cells was completely disorganised (Fig. 4). Densely packed cells without a well distinguishable molecular layer were observed mainly in the flocculonodular lobe, near the ventricle. The compact heterotopias were composed of bands of spindle and granule cells situated closely together. In some of the heterotopic clusters, irregular minimal islands of the molecular layer were observed. The spindle cells were evident in all cases. In the younger cases the spindle neurons predominated in the morphological picture, while in older ones they formed strips or bands mixed with the round granule cells. The closely arranged granule neurons frequently forming whirls were interspersed with Purkinje cells. Purkinje cells were arranged in groups of a few of them in the immature cases. In postnatal cases, single Purkinje neurons were scattered haphazardly and disarranged, in an improper place within the granule cells. All of them expressed calbindin immunoreactivity.

Synaptophysin reactivity in the heterotopias was observed in the neurons corresponding to the internal granular layer and in Purkinje cells (Fig. 5). The spindle cells corresponding to the external granular layer did not show any synaptophysin reactivity. The Purkinje cells intermingled with granule cells exhibited evident coarse-grained immunoreactivity only on their soma, and faint on the processes and

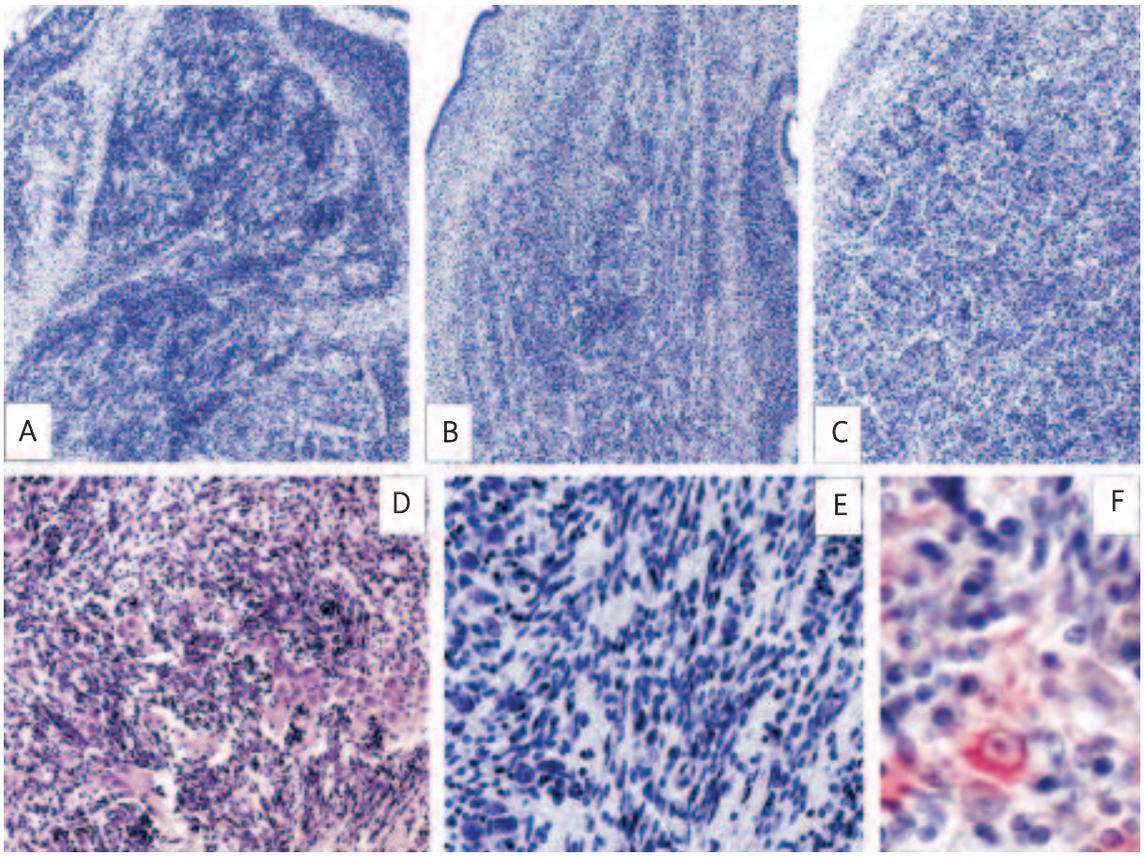

Fig. 4. The compact pattern of heterotopy. **A-C)** The densely packed spindle and granular neurons; orig. magn. 40×. **D)** Small group of Purkinje neurons situated among whirls of elongated cells; orig. magn. 100×. **E)** A few Purkinje neurons scattered among the bands of spindle and round granular cells; orig. magn. 100×. **F)** Calbindin positive Purkinje cell among round granule neurons; orig. magn. 400×.

in the surrounding background. In the grey matter nests with predominance of Purkinje cells and a small admixture of spindle neurons, intense grained deposits were seen on the Purkinje neurons' soma and scattered within the surrounding tissue. Within the round granule cells, indistinct synaptophysin reactivity was demonstrated. The molecular layer displayed a very weak reaction in comparison to the reactivity in the normal laminated cerebellar cortical layer.

Nests of Purkinje cells scattered in the white matter without accompanying granule cells were observed in some cases (Fig. 6). Size of the foci varied from large aggregations of Purkinje cells to a few neurons. The Purkinje cells dispersed haphazardly in white matter were morphologically well developed and strongly immunostained with calbindin antibody. The dendrites of Purkinje cells were well visible and exhibited high calbindin immunoreactivity. Heterotopic neurons within the white matter displayed strong granular synaptophysin reactivity at the cell body and their processes. Around some neurons the synaptophysin droplets were arranged linearly along their long projections and within surrounding white matter. The nests of Purkinje cells were surrounded by GFAP-positive astroglial cells (Fig. 3). The astroglial cells with clearly visible cytoplasm and a few short processes were similar to those observed in the white matter. Among the arrested Purkinje neurons GFAP-positive cells were not present.

The ectopic cortex in the white matter with a normal layered structure containing all the components of the cerebellar cortex was localised by the large vessels (Fig. 7). In the white matter centre, sin-

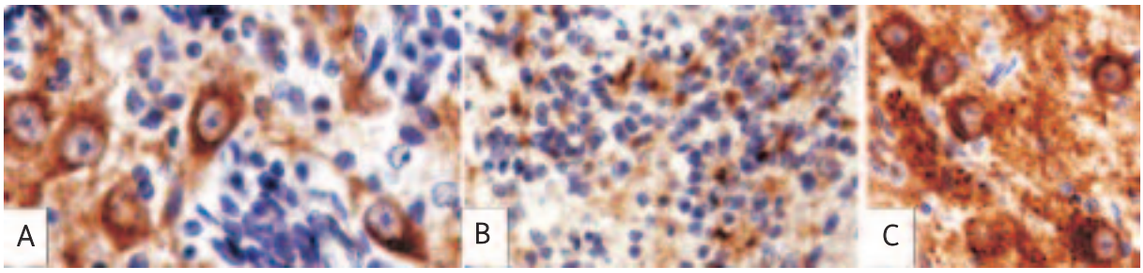

Fig. 5. Synaptophysin reactivity in the heterotopias. **A)** Synaptophysin reactivity at the surface membranes of Purkinje cells; orig. magn. 400×. **B)** Scattered foci of synaptophysin activity in the internal granular layer; orig. magn. 400×. **C)** Grained deposits on the Purkinje cell soma and scattered within the surrounding tissue; orig. magn. 400×.

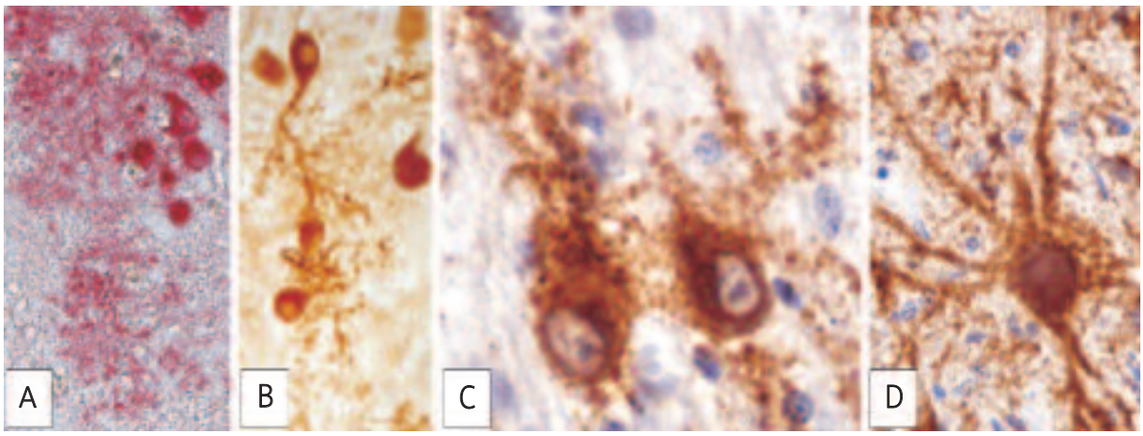

Fig. 6. Nests of Purkinje neurons without accompanying granule cells. **A, B)** Calbindin positive Purkinje cells haphazardly dispersed in the white matter with well visible dendritic tree; orig. magn. 400×. **C)** Strong granular synaptophysin reactivity at the cell body of Purkinje cells and their processes; orig. magn. 600×. **D)** Synaptophysin droplets arranged linearly at the cell body and along its projections; orig. magn. 600×.

gle or multiple annular cortical circuits were observed. The cytoarchitecture of the molecular and granule layers and the Purkinje cells was preserved corresponding to the developmental age. In younger cases, the nests of ectopic cortex included both external and internal granule layers; in 6-month-old case the external granule cells presented a discontinuous single layer, mostly disappeared. The monolayer of Purkinje cells displayed intense calbindin immunoreactivity; cell bodies were strongly stained. Staining intensity of calbindin in the ectopic Purkinje neurons was higher than that of neurons in the normal cortex. The synaptophysin immunoreactivity was evident in the molecular and internal granule layers and in the Purkinje cells. The reaction was stronger than in the corresponding neurons of the cerebellar hemispheric cortex in the same case. The morphological appearance, calbindin immunoreactivity of the Purkinje neurons and more intense synaptophysin immunoreactivity in the molecular and granular layer neurons showed a greater maturity of misplaced ectopic neurons than the relevant cortical cells observed in the same case.

Discussion

All the processes of cell proliferation and maturation, cell migration, neuronal differentiation, and Purkinje cell dendrite growth are differently involved in the patterning of the cerebellar architecture.

The cerebellum contains two main progenitor compartments: the cerebellar ventricular zone and the

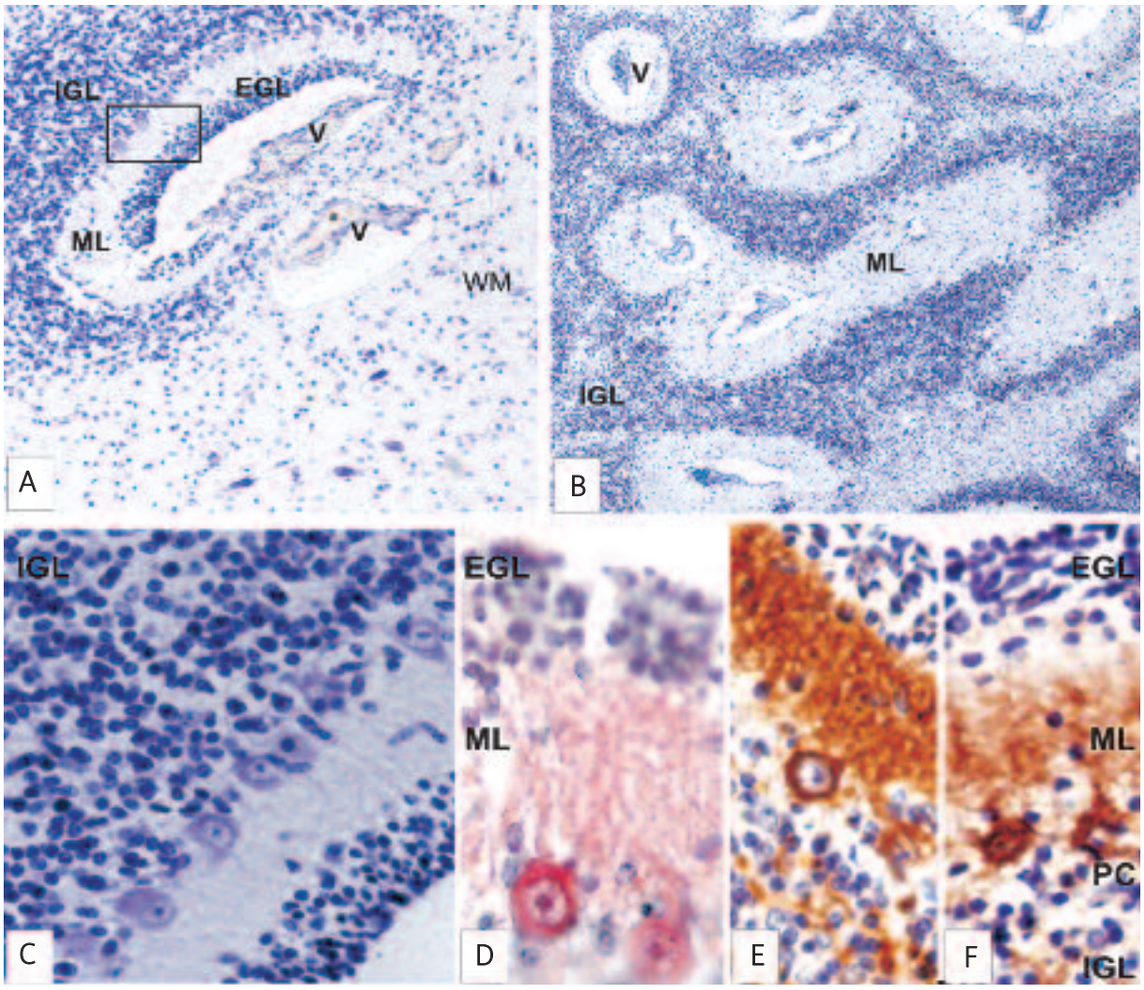

Fig. 7. The ectopic cortex in the white matter localized by the large vessels. **A)** Single annular fragment of the cerebellar cortex; orig. magn. 40×. **B)** Multiple annular circuits in the white matter by the vessels; orig. magn. 40×. **C)** Fragment of the layered cortex, insert from Fig. 7A; orig. magn. 400×. **D)** Calbindin reactivity in Purkinje cell; orig. magn. 400×. **E)** Synaptophysin reactivity evident in layers of heterotopia; orig. magn. 400×. **F)** Synaptophysin immunoreactivity of the cerebellar hemispheric cortex in the same case; orig. magn. 400×.

rhombic lip [12,41]. The ventricular zone is a source of all GABAergic neurons including Purkinje cells and inhibitory interneurons, while the rhombic lip is the source of glutamatergic granule cells [6-8]. Newly generated neurons must move for long distances along specific pathways to reach their final destination.

In human cerebellar ontogenesis, Purkinje cells are formed in the ventricular zone early in embryonal life and between 9 and 13 gestational weeks start to migrate radially directly to their definitive cortical sites. They mature much later, residing in the cortex in an undifferentiated form until the advanced development of the cortical architecture occurs [11]. The period over the 28th week of gestation is of crucial importance for maturation of Purkinje cells and for development of their structural and functional associations with arriving climbing fibres.

Neurons of the granular layer of the cerebellar cortex migrate from the rhombic lip. Granule cell precursors migrate tangentially along the cerebellar surface to form a transient external granular layer.

The external granular layer forms at 10/11 gestational weeks. The cells of the external granule layer actively proliferate and express factors Zic1 and RU49, important for regulating the rate of cell division [7]. Granule neurons secrete fibroblast growth factor 9 to control formation of the Bergmann glia fibre scaffold [24]. Finally granule cells migrate inward through the molecular layer between clusters of Purkinje cells with the aid of Bergmann radial glia fibres, forming the internal granule layer [16,17,45]. From the second month of postnatal life the external granule layer progressively decreases and by 12 months totally disappears [22].

In some cases the route of neuronal migration to the appropriate position in the cerebellar cortex is disturbed and the cortical neurons settle improperly in the white matter. The groups of lost cortical neurons exhibit different forms of cytoarchitecture abnormalities.

The most frequently observed group of disarranged neurons displayed a wide, distinct molecular-like layer with nests of ectopic granule cells, Purkinje neurons dispersed irregularly within molecular or granule cell layers, and few spindle neurons, only in the youngest cases. The morphological picture corresponded to the image of dysplastic lesions described by Friede [11] or malformations of cerebellar cortex [21]. The abnormal appearance of molecular and granular layers predominated in the cytoarchitectural features of this heterotopy.

It is difficult to define affected developmental pathways and to establish molecular targets that can be involved in the altered arrangement of cells. There are many molecules regulating the correct migration of neurons and involved in the appropriate positioning of cells [31,36].

The study of Qin and co-workers demonstrated that bone morphogenetic protein BMP signalling plays a crucial role in the embryonic proper formation of the cerebellum. Close regulation of BMP signalling is required for specification of granule cells and Purkinje neurons maintenance during cerebellar development [32]. Altered BMP signalling results in breakdown of granule cell differentiation. The ectopic groups of granular neurons are formed in the cerebellar white matter, and also Purkinje cells are disorganized and ectopically located [27,32].

Disturbance in the functioning of another factor, netrin-1, can play a role in the formation of improper neural circuits. Netrins are a family of extracellular proteins that direct cell and axon migration during embryogenesis. The proteins are responsible for directing neuronal migration, regulation of cell adhesion, maturation of cell morphology, and cell survival [33]. Netrin-1 functions as a guide for cerebellar granule neurons and regulates the tangential migration of granule cell precursors [2].

The tangential migration of granule cell precursors is also controlled by molecules such as stromal cell-derived factor or chemokine receptor 4, which orient and keep them at the cerebellar surface. The deficiency of these molecules causes that granule cell precursors abnormally invade the cerebellar parenchyma [9].

The reelin-disabled-1 signalling pathway, through the components reelin, adapter protein Dab1, reelin receptors VLDLR and ApoER2, is critically involved in the positioning of migrating cells. Human cerebellar cortex is particularly dependent on the integrity of the reelin-Dab1 pathway [30].

In the further regulation of cell fate, the Shh molecular and the Notch signalling pathways play a crucial role [41]. The Shh expressed by Purkinje cells influences the proliferation of granule cell precursors and is important for Bergmann glia development. The Jagged1 ligand for members of the Notch family is required for the formation and maintenance of Bergmann glial cells in the cerebellum. Along Bergmann glia fibres, granule cells migrate to form the internal granule layer. Loss of Jagged1 results in aberrant granule cell migration and their ectopic differentiation in the molecular layer [43]. Displaced granule cells in the molecular layer of the cerebellar cortex were also observed in mice treated with methylazoxymethanol [47]. The granule cells were mixed with Purkinje neurons instead of forming layers.

In the dysplastic heterotopy with abnormally arranged molecular and granular layers the main neuronal subtypes differentiated but formed clusters instead of organizing into typical cerebellar layers. The impaired migration of neuronal precursors led to accumulation of these cells in the improper place and to generation of aberrant interactions between neuronal and glial components. Attention must be paid to the absence of GFAP-positive glial cells among the heterotopias, especially in the molecular and Purkinje cell layers. The framework of Bergmann glia fibres in the molecular layer was not visible. The failure of Bergmann glia influenced the neuronal disorganisation, particularly granular neu-

rons. Ectopias of external granule layer precursors resulting from absence of the Bergmann glia scaffold were described in the protein O-mannose of UDP-N-acetylglucosaminyl transferase 1 knockout mice [23]. However, Hoser and co-workers suggest existence of an alternative migration mechanism for granule cell migration in the absence of Bergmann glial fibres [14].

Among our cases, a relatively large group of misplaced cortical neurons in the white matter constitutes compact heterotopias composed of bands of spindle and round granule cells situated closely together. These lost neurons are localized in the flocculonodular lobe near the ventricle. The tightly adjacent bands of small round and elongated granular cells closely correspond to the morphological pattern of the external granular layer. In human cerebella after 30 gestational weeks the external granular layer is formed by 6-8 rows of densely packed small round cells, and at term this layer presents a superficial zone of small round cells and a deeper stripe of bipolar, elongated neurons [22]. The excessive accumulation of external granule layer cells in the form of waves predominated in the appearance of compact heterotopy. In this type of heterotopias the few Purkinje neurons were scattered haphazardly and disarranged in the improper place within the granule cells. There is a close interaction between external granular neurons and Purkinje cells. In the normal cortical development, signalling from external granular layer appears to be crucial to terminate migration of Purkinje cells and induce the monolayer arrangement. Settling of Purkinje neurons in the cerebellar cortex is regulated by molecular signals issued by granule cells [8]. In the cortical regions where granule cells are disrupted, Purkinje cells fail to align and form clusters [15]. Paucity of Purkinje cells and their grouping in clusters in the solid heterotopias may be a symptom of a fault that exists in granular cells.

In some heterotopias only Purkinje cells were collected in the white matter as a result of defective dispersion. Purkinje cells migrate along radial glia through the future white matter toward the cortical plate [26]. Arrested migration causes that neurons fail to reach their proper destination and instead aggregate in clusters. The nests of Purkinje cells were devoid of GFAP positive astroglial cells. The supporting and organizing influence of glia on the Purkinje cells in cerebellar anomalies was described previously [19].

Presence of nests of ectopic cortex in the white matter is explained by some authors as arrest of normal migration because of impediment of the migrating pathway by a blood vessel [10,13]. The localization of lost cortical neurons adjacent to blood vessels may be related to the effects of vascular endothelial growth factor. Vascular endothelial growth factor plays a role in angiogenesis, is critical for vessel patterning and also regulates granule cell migration during cerebellar development [37].

When the neurons reach their terminal position they begin to expand their dendritic tree to establish synaptic connections with growing axons [41].

The synaptophysin reactivity around disoriented Purkinje cells and within clusters of disorganized granule cells should be referred to the proper development of synapses in the normal cerebellar cortex. As was described by Sarnat, during normal development the cerebellar cortex shows increasing immunoreactivity of the molecular layer at 25-34 weeks, and by term there is evident synaptophysin activity within the molecular zone. The Purkinje cells in late gestation, 35-40 weeks, show strong coarsely granular immunoreactivity outlining their somatic membranes, but this does not extend to the basal dendrite or its branches within the molecular zone. The external granular layer showed no synaptophysin immunoreactivity at any age. The internal granular layer showed only scattered small foci of activity at 25-35 weeks and more widely distributed and stronger reactivity by term [38].

A similar picture of the distribution of synaptophysin reactivity occurred in the nests of normal layered ectopic cortex by the large vessels.

In our cases of compact heterotopias, synaptophysin reactivity was seen only on the soma of Purkinje neurons and within the round granule cells. The molecular layer displayed a very faint reaction; the synapse formation between terminal axons of granule cells and dendrites of Purkinje neurons was disturbed.

Heterotopic Purkinje cells dispersed haphazardly in the white matter presented strong granular synaptophysin reactivity not only at the cell body but also at their processes, suggesting intensive formation of synapses. There was finely beaded linear synaptophysin reactivity scattered within surrounding white matter and arranged linearly along their long projections, probably representing synaptic vesicles forming in unmyelinated axons.

The calbindin-D28k immunoreactivity in cerebellar cortical neurons lost in the white matter showed an intense reaction consistent with the previously described results [20]. The Purkinje cells located in the cerebellar heterotopias were immunostained with calbindin antibody. The intrauterine harmful agents that disturb migration of the cerebellar neurons did not affect content of calcium binding protein in misoriented neurons located in the wrong place. Since calretinin, calbindin and parvalbumin are thought to be cytosolic calcium buffers that modify the spatiotemporal aspects of calcium transients in cells [40], they may stabilize homeostasis and facilitate development and maturation of Purkinje cells located in heterotopias. The calbindin protein may function differently at different developmental stages [18]. During gestation the calbindin immunopositive Purkinje cells increase in size and exhibit pronounced dendritic arborisation associated with dendritic synapse formation and stabilisation. In the groups consisting of all cortical neurons arranged in a random manner, only the cell bodies of Purkinje neurons were calbindin positive, while their processes were negative. Limited intrinsic information between these cells is not sufficient for the proper development of their dendrites. In the nests composed only of Purkinje neurons, the dendrites of Purkinje cells were well visible and exhibited high calbindin immunoreactivity; however, they did not branch properly. In the environment devoid of granule cells, Purkinje neurons were badly aligned, with disoriented and less branched dendrites. The ramification pattern of the dendritic tree of heterotopic neurons is different from normotopic cells [10].

Radial glia processes influence the shape of the dendritic tree of Purkinje cells [25]. The radial fibres of Bergmann glia form synapses with Purkinje cell dendrites [5,46]. Glia play a role in directing Purkinje cell morphology; more dendrites grow while in contact with glia.

The formation of the cerebellar cortex is a long, complex process that requires integration of interactions between multiple neuronal populations and migratory patterns established by neuron glia interactions. Cell-to-cell communication plays a role to specify cell fate and regulate proper development. Many factors are important for cerebellar development and are involved in survival and differentiation of cerebellar neurons and circuitry. Wiring of neuronal circuits relies on precise spatial positioning of neurons and axons [37]. Genetically or environmentally disturbed cell-cell interactions may result in migration problems and may lead to failed placing and arrangement of neurons in the improper place. The misplaced neuronal cells in the cerebellar white matter are not a homogeneous group and differ in the patterning and morphogenesis. The various pathomechanism of injury is reflected in the different morphological features of altered cell arrangement. The neurons fail to form normal layers, are not appropriately sorted and intermingled. The arrangement of lost neuronal groups varied, but heterotopic neurons in all groups closely resemble normotopic cerebellar neurons. The form of neuronal components is consistent with normal maturing cerebellar neurons. The intraheterotopic network may be organised differently from the normotopic cortical circuitry, but the neurons are probably normal, functional cells with abnormal connections.

References

1. Abraham H, Tornoczky T, Kosztolanyi G, Seress L. Cell formation in the cortical layers of the developing human cerebellum. Int Dev Neurosci 2001; 19: 53-62.
2. Alcantara S, Ruiz M, DeCastro F, Soriano E, Sotelo C. Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system. Development 2000; 127: 1359-1372.
3. Altman J, Bayer SA. Development of the cerebellar system in relation to its evolution, structure and functions. CRC Press, New York 1997.
4. Armstrong CL, Hawkes R. Pattern formation in the cerebellar cortex. Biochem Cell Biol 2000; 78: 551-562.
5. Bellamy TC. Interactions between Purkinje neurones and Bergmann glia. Cerebellum 2006; 5: 116-126.
6. Cameron DB, Kasai K, Jiang Y, Hu T, Saeki Y, Komuro H. Four distinct phases of basket/stellate cell migration after entering their final destination (the molecular layer) in the developing cerebellum. Dev Biol 2009; 332: 309-324.
7. Carletti B, Rossi F. Neurogenesis in the cerebellum. Neuroscientist 2008; 14: 91-100.
8. Carletti B, Wiliams IM, Leto K, Nakajima K, Magrassi L, Rossi F. Time constrains and positional cues in the developing cerebellum regulate Purkinje cell placement in the cortical architecture. Dev Biol 2008; 317: 147-160.
9. Chedotal A. Should I stay or should I go? Becoming a granule cell. Trends Neurosci 2010; 33: 163-172.
10. 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.

11. Friede RL. Developmental Neuropathology. 2nd ed. Springer Verlag, Berlin 1989; p. 363.
12. Goldowitz D, Hamre K. The cells and molecules that make a cerebellum. Trends Neurosci 1998; 21: 375-382.
13. Hori A. Causes of neuronal heterotopia other than migration disturbances. Neuropathology 2006; 26: 540-543.
14. Hoser M, Baader SL, Bösl MR, Ihmer A, Wegner M, Sock E. Prolonged glial expression of Sox4 in the CNS leads to architectural cerebellar defects and ataxia. J Neurosci 2007; 27: 5495-5505.
15. Jensen P, Smeyne R, Goldowitz D. Analysis of cerebellar development in math1 null embryos and chimeras. J Neurosci 2004; 24: 2202-2211.
16. Komuro H, Yacubova E, Yacubova E, Rakic P. Mode and tempo of tangential cell migration in the cerebellar external granular layer. J Neurosci 2001; 21: 527-540.
17. Komuro H, Yacubova E. Recent advances in cerebellar granule cell migration. Cell Mol Life Sci 2003; 60: 1084-1098.
18. Kwong WH, Chan WY, Lee KKH, Fan M, Yew DT. Neurotransmitters, neuropeptides, and calcium binding proteins in the developing human cerebellum: a review. Histochem J 2000; 32: 521-534.
19. Laure-Kamionowska M, Maslinska D. Astroglia and microglia in cerebellar neuronal migration disturbances. Folia Neuropathol 2007; 45: 205-212.
20. Laure-Kamionowska M, Maślińska D. Calbindin positive Purkinje cells in the pathology of human cerebellum occurring at the time of its development. Folia Neuropathol 2009; 47: 300-305.
21. Laure-Kamionowska M, Taraszewska A, Maslinska D, Raczkowska B. Faulty position of cerebellar cortical neurons as a sequel of disturbed neuronal migration Folia Neuropathol 2006; 44: 327-332.
22. Lavezzi AM, Ottaviani G, Terni L, Matturi L. histological and biological developmental characterization of the human cerebellar cortex. Int J Dev Neurosci 2006; 24: 365-371.
23. Li X, Zhang P, Yang Y, Xiong Y, Qi Y, Hu H. Differentiation and developmental origin of cerebellar granule neuron ectopia in protein O-mannose UDP-N-acetylglucosaminyl transferase 1 knockout mice. Neurosci 2008; 152: 391-406.
24. Lin Y, Chen L, Lin Ch, Luo Y, Tsai RYl, Wang F. Neuron-derived FGF9 is essential for scaffold formation of Bergmann radial fibers and migration of granule neurons in the cerebellum. Dev Biol 2009; 329: 44-54.
25. Lordkipanidze T, Dunaevsky A. Purkinje cell dendrites grow in alignment with Bergmann glia. Glia 2005; 51: 229-234.
26. 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.
27. Ming JE, Elkan M, Tang K, Golden JA. Type I bone morphogenetic protein receptors are expressed on cerebellar granular neurons and a constitutively active form of the type IA receptor induces cerebellar abnormalities. Neurosci 2002; 114: 849-857.
28. Norman RM. Neuropathological findings in trisomies 13-15 and 17-18 with special reference to the cerebellum. Dev Med Child Neurol 1966; 8: 170-177.
29. Paprocka J, Jamroz E, Adamek D, Stradomska T, Głuszkiewicz E, Grzybowska-Chlebowczyk U, Marszał E. Clinical and neuropathological picture of familial encephalopathy with bifunctional protein deficiency. Folia Neuropathol 2007; 45: 213-219.
30. Perez-Garcia CG, Tissir F, Goffinet AM, Meyer G. Reelin receptors in developing laminated brain structures of mouse and human. Eur J Neurosci 2004; 20: 2827-2832.
31. Porcionatto MA.The extracellular matrix provides directional cues for neuronal migration during cerebellar development. Braz J Med Biol Res 2006; 39: 313-320.
32. Qin L, Wine-Lee L, Ahn KJ, Crenshaw EB 3rd.Genetic analyses demonstrate that bone morphogenetic protein signaling is required for embryonic cerebellar development. J Neurosci 2006; 26: 1896-1905.
33. Rajasekharan S, Kennedy TE. The Netrin protein family. Genome Biol 2009; 10: Article Number: 239: DOI: 10.1186/gb-2009-10-9-239; http://genomebiology.com/2009/10/9/239.
34. Rorke LB, Fogelson MH, Riggs HE. Cerebellar heterotopias in infancy. Dev Med Child Neurol 1968; 10: 644-650.
35. Rorke LB. A perspective: the role of disordered genetic control of neurogenesis in the pathogenesis of migration disorders. J Neuropathol Exp Neurol 1994; 53: 105-117.
36. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol 2010; 341: 126-140.
37. Ruiz de Almodovar C, Coulon C, Salin PA, Knevels E, Chounlamountri N, Poesen K, Hermans K, Lambrechts D, Van Geyte K, Dhondt J, Dresselaers T, Renaud J, Aragones J, Zacchigna S, Geudens I, Gall D, Stroobants S, Mutin M, Dassonville K, Storkebaum E, Jordan BF, Eriksson U, Moons L, D'Hooge R, Haigh JJ, Belin MF, Schiffmann S, Van Hecke P, Gallez B, Vinckier S, Chédotal A, Honnorat J, Thomasset N, Carmeliet P, Meissirel C. Matrix-binding vascular endothelial growth factor (VEGF) isoforms guide granule cell migration in the cerebellum via VEGF receptor Flk1. J Neurosci 2010; 30: 15052-15066.
38. Sarnat HB, Born DE. Synaptophysin immunocytochemistry with thermal intensification: a marker of terminal axonal maturation in the human fetal nervous system. Brain Dev 1999; 21: 41-50.
39. Schmidt-Sidor B, Szymańska K, Williamson K, Heyningen V, Roszkowski T, Wierzba-Bobrowicz T, Zaremba J. Malformations of the brain in two fetuses with a compound heterozygosity for two PAX6 mutations. Folia Neuropathol 2009; 47: 371-382.
40. Schwaller B, Meyer M, Schiffmann S. "New" functions for "old" proteins: The role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin in cerebellar physiology. Studies with knockout mice. Cerebellum 2002; 1: 241-258.
41. Sotello C. Cellular and genetic regulation of the development of the cerebellar system. Prog Neurobiol 2004; 72: 295-339.
42. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba-Bobrowicz T, de Leon M, Louis LAS, Cohen IL, London E, Brown WT, Wisniewski T.

The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 2010; 119: 755-770.
43. Weller M, Krautler N, Mantei N, Suter U, Taylor V. Jagged1 ablation results in cerebellar granule cell migration defects and depletion of Bergmann glia. Dev Neurosci 2006; 28: 70-80.
44. Yachnis AT, Rorke LB, Trojanowski JQ. Cerebellar dysplasias in humans: development and possible relationship to glial and primitive neuroectodermal tumors of the cerebellar vermis. J Neuropathol Exp Neurol 1994; 63: 61-71.
45. Yacubova E, Komuro H. Intrinsic program for migration of cerebellar granule cells *in vitro*. J Neurosci 2002; 22: 5966-5981.
46. Yamada K, Watanabe M. Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. Anat Sci Int 2002; 77: 94-108.
47. Yamanaka H, Obata K. Displaced granule cells in the molecular layer of the cerebellar cortex in mice treated with methylazoxymethanol. Neurosci Lett 2004; 358: 132-136.