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Clinical and neuropathological picture of familial encephalopathy with bifunctional protein deficiency

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Abstract

Peroxisomal diseases are a heterogeneous group of genetic metabolic disorders which are caused by incorrect biogenesis of peroxisomes or a defect in activity of particular enzymes located in those organelles.
D-bifunctional protein (D-BP) deficiency belongs to the second group of peroxisomal diseases characterised by dysfunction of a single peroxisomal enzyme. Bifunctional protein is a catalyst in the second and third stage of the β-oxidation of fatty acids. Gene locus of bifunctional protein deficiency comprises chromosomes 5q2 and 3p23-p22.
The authors present two siblings with progressing family encephalopathy. In the younger brother the diagnosis of a bifunctional protein deficiency was made. The girl died before a diagnosis was made; however, due to the presence of a very similar clinical condition a suspicion arises that the girl had a peroxisomal disease. In the siblings were ascertained characteristic dysmorphic features, delayed psychomotor development, polymorphic epileptic seizures and generalized muscular hypotonia with areflexia.
The neuropathological findings were consistent in general with MRI findings showing features of hypomyelination. Also neuron heterotopias that were found in autopsy are a form of pathology typical for D-BP.

Key words: *bifunctional protein deficiency, peroxisomal disorders, children*

Introduction

Peroxisomal diseases form a heterogeneous group of genetically conditioned metabolic diseases that occur as a result of abnormal biogenesis of peroxisomes or defects in the activity of enzymes localized in these organelles. Except for X-linked adrenoleukodystrophy (ALD) all peroxisomal diseases are inherited in an autosomal recessive way. Their prevalence varies within the range 1:25000 to 1:50000 [4-6]. Peroxisomal enzymes are involved in oxidation of very long-chain fatty acids (VLCFA), in bile acid metabolism, in pipecolic and phytanic acid degradation, plasmalogen synthesis and in intracellular neutralization of peroxides.

Recently peroxisomal diseases can be classified into three groups [6]:

- the first group caused by disorders in peroxisome biogenesis (absence or deficiency) accompanied by numerous enzymatic defects (Zellweger spec-

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trum (ZS), neonatal adrenoleukodystrophy (NALD), infantile Refsum's disease (IRD));
- in the second group the structure of peroxides is normal, only one peroxisomal enzyme is deficient (bifunctional protein deficiency, X-linked adrenoleukodystrophy, acatalasemia, hyperoxaluria type 1, pseudo-Zellweger syndrome, Acyl-Coa deficiency);
- the third group includes diseases with changes in peroxisome structure and enzymatic defects (pseudo-Zellweger syndrome, rhizomelic chondrodysplasia punctata).

Patient presentation

Patient NM

11-month girl born to healthy, unrelated parents. The child from a normal first pregnancy, born after 39 weeks of gestation by Caesarean section (indication: breech presentation of the fetus), with birth weight of 3500 g, Apgar score – 8 points. On admission physical examination revealed dysmorphic features, systolic cardiac murmur. Neurological examination: head circumference (HC) – 46.5 cm (90-97 pc), horizontal nystagmus, opsoclonia, hypotonia with areflexia, poor spontaneous motor activity. Psychomotor development of the child estimated at 1 month.

From the first day of her life the patient suffered from respiratory distress and polymorphic epileptic seizures. For epilepsy treatment phenobarbital, valproic acid and carbamazepine were administered. At the age of 2 months MRI of the head demonstrated changes suggesting demyelinization of white matter of CNS, widening of the cerebral ventricular system and basal cisterns. All in all the results of MRI examination were consistent with or at least strongly suggestive of leukodystrophy. The findings of the remaining additional tests are presented in Table I. The girl died at 14 months of age.

In the autopsy examination, the white matter showed gently and not uniformly lowered consistency. The ventricular system was broadened. There were no macroscopic unequivocally evident morphological changes. For the histopathological investigations samples taken from the lobes of the brain, basal ganglia, thalamus, cerebellum and brain stem were typically processed to the paraffin blocks. The slides were stained with haematoxylin-eosin (HE), Kluver-Barrer (KB) method, and immunohistochemically with antibody against glial fibrillary acidic protein – GFAP (1:100). Antibodies for GFAP and other reagents for immunohistochemistry were purchased in DAKO.

The following neuropathological changes were found. In the white matter of the brain and cere-

Table I. Gestation and delivery period

	Patient NM (11 mo)	Patient NW (6 mo)
pregnancy	normal	normal
delivery	caesarean section	caesarean section
birth weight	3500 g	3250 g
birth head circumference	37 cm	35 cm
Apgar score	8 points	10 points

Table II. Clinical picture of the patients

	Patient NM (11 mo)	Patient NW (6 mo)
respiratory distress	since 1st day of life	since 1st day of life
epileptic seizures	polymorphic (Pb, VPA, CBZ)	polymorphic (Pb, VPA, CBZ, NZP)
psychomotor delay	at the level of 1st month of life	at the level of the 1st day of life

Table III. Results of additional tests

	Patient NM (11 mo)	Patient NW (6 mo)
cerebrospinal fluid	normal	normal
EEG	generalized paroxysmal changes (with right parietal area predominance)	generalized paroxysmal changes
ENG, EMG	myopathic pattern	decreased conduction velocity 26.4 m/s
ophthalmological examination	normal	except for horizontal nystagmus – normal
cardiologic evaluation	pulmonary hypertension	normal

bellum there were features of hypomyelination manifesting as poorly bordered areas of "paleness" in slides stained with KB (Fig. 1). The level of severity of hypomyelination was not uniform. Noticeably in subcortical zones the white matter was almost normally myelinated. In the whole white matter more or less intensive gliosis was noted (Fig. 2). Gliosis in the white matter is formed by astrocytes only mildly hypertrophic with few and short processes (Fig. 2). This is in contrast with gliosis observed in the grey matter in the brain cortex and basal ganglia where astrocytes are strongly hypertrophic (large, with ample perinuclear cytoplasm), rich in long processes, and especially numerous around vessels, sometimes as if "encrusting" them (Fig. 3). Gliosis was also noted in the cerebellum, especially in the (internal) granular layer of the cortex (Fig. 4). In turn, in the molecular layer of the cerebellar cortex strongly GFAP-immunopositive cell processes formed a "palisade-like" structure (Fig. 4). They supposedly represent embryonic radial glia and their presence (as well as the

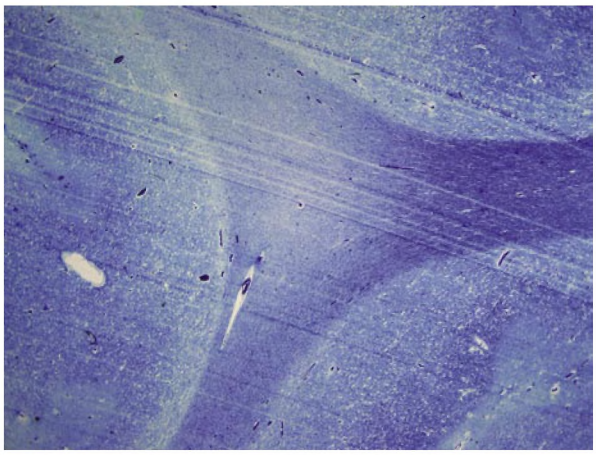

Fig. 1. Cerebral white matter. The zone of imperfect myelination is seen (poorly bordered "paleness"). KB method. Macroscopic photograph

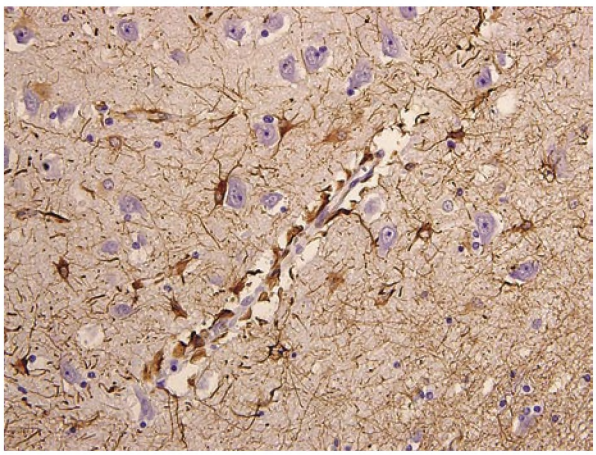

Fig. 3. Grey matter of cerebral cortex – gliosis with strongly hypertrophic astrocytes (large sized with ample perinuclear cytoplasm and with numerous long processes) especially densely surrounding blood vessels. GFAP IHCh. Magn. × 200

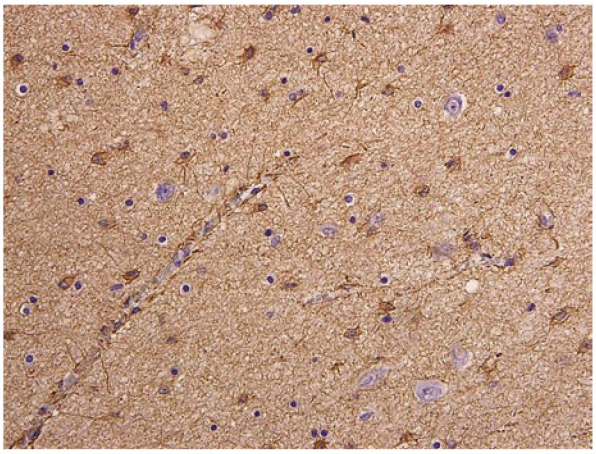

Fig. 2. Gliosis in cerebral white matter with relatively few and only mildly hypertrophic astrocytes with scanty processes. GFAP IHCh. Magn. × 200

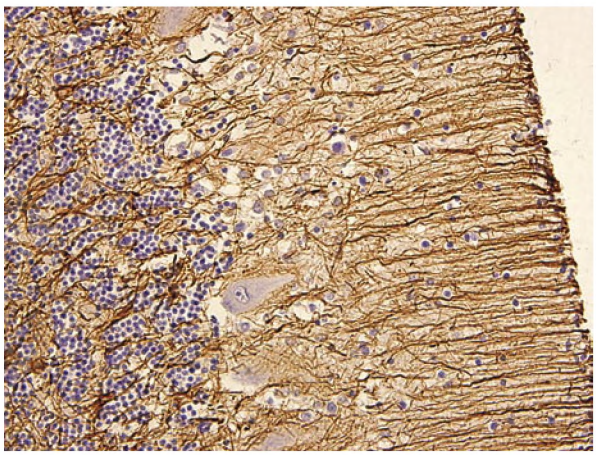

Fig. 4. GFAP-positive "palisade-like" formation in molecular layer of cerebellar cortex supposedly representing embryonic radial glia seems to be a feature of delayed maturation of cerebellum. In the internal granular layer of the cerebellar cortex there are numerous hypertrophic reactive astrocytes. GFAP IHCh. Magn. × 200

remnants of external granular layer – see Fig. 10) reflects improper (delayed) maturation. Neurons in the brain cortex showed marked but not specific degenerative changes with prominent but probably to some degree artifactual perineuronal vacuolisation (Fig. 5). Focally in the brain cortex some cells with "empty" nuclei were noted (Fig. 6). These cells may be degenerated neurones but at least some of them may represent so-called Alzheimer type II cells. Purkinje cells of cerebellar cortex showed marked features of degeneration of various morphology (Fig. 7). It seems that the most striking and interesting were the multiple foci of neuronal heterotopias that were found first of all in the cerebellum but sporadically also in the cerebrum. In the cerebellum the heterotopias had the form either of large clusters of cells morphologically equivalent to Purkinje cells or even singular cells misplaced into white matter (Figs. 8, 9). In the molecular layer of the cerebellar cortex the number of cells was focally increased and there were numerable remnants of the external granular layer (Fig. 10). It seems that these findings could be interpreted as the manifestation of improper or "incomplete" migration of neuroblasts from the fetal external granular layer. Moreover, here and there some small cerebellar "pseudofolia" suggesting

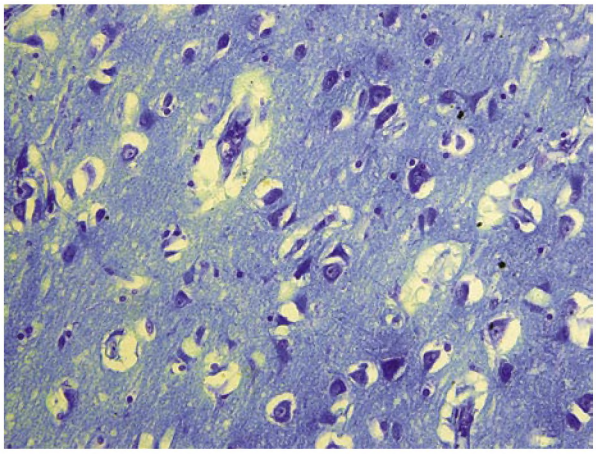

Fig. 5. Non-specific degeneration of neurons in neocortex. There is marked vacuolisation in neuropil especially around neurons (probably partially artifactual). KB method. Magn. × 200

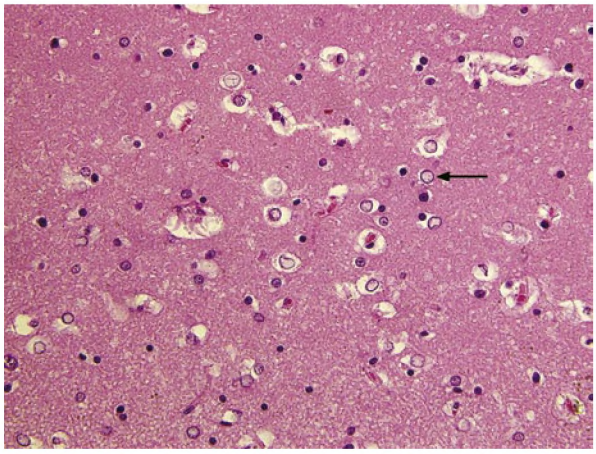

Fig. 6. Cells in cerebral cortex (molecular layer) with "empty" nuclei (one of them marked with an arrow) supposedly consistent with "Alzheimer type II cells". HE. Magn. × 200

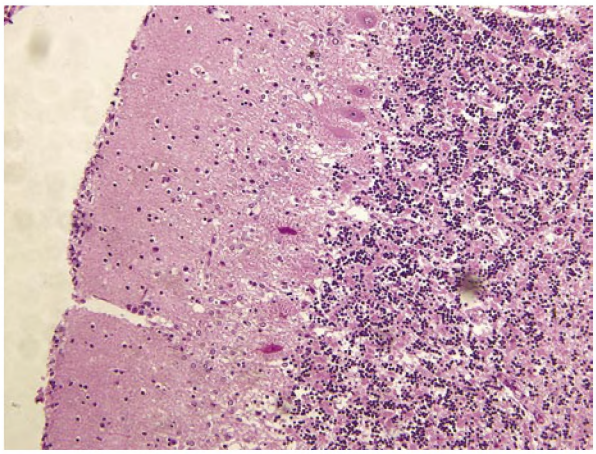

Fig. 7. Degeneration of Purkinje cells in cerebellar cortex. HE. Magn. × 100

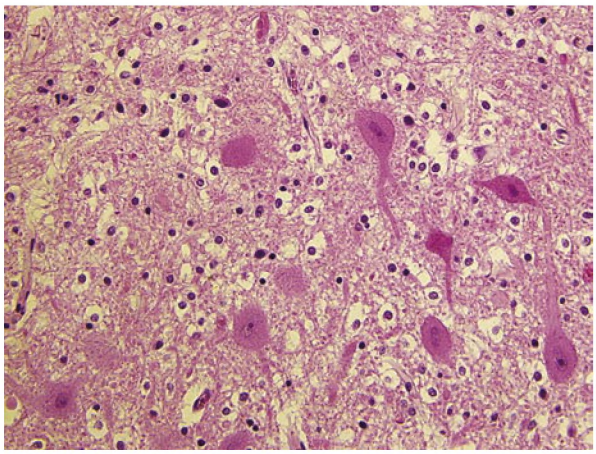

Fig. 8. Cluster of heterotopic Purkinje cells in white matter of cerebellum. HE. Magn. × 200

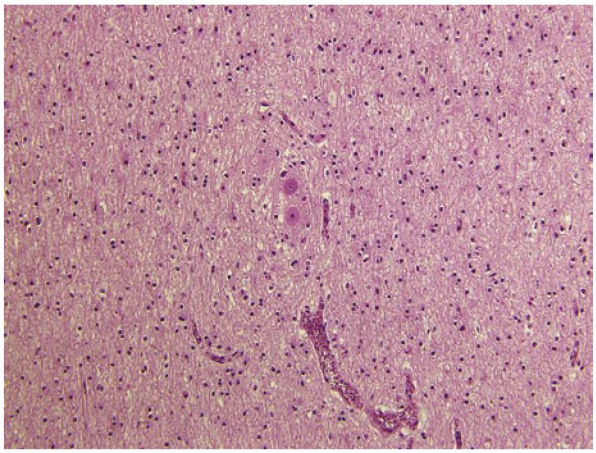

Fig. 9. Two heterotopic Purkinje cells in white matter of cerebellum. HE Magn. × 100

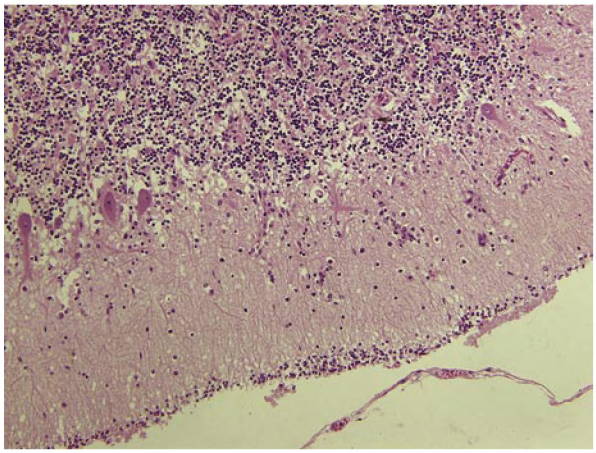

Fig. 10. Increased number of cells in molecular layer of cerebellar cortex. Remnants of the external granular layer are also seen. It seems that these findings suggest improper ("unfinished") migratory process of the cells from the external granular layer. HE. Magn. × 100

cerebellar microgyria were noted. Also the cerebral cortex focally showed disordered architecture.

Patient NW

6-month boy (brother of the NM patient) from a normal second pregnancy, born in 40th week, with body weight of 3250 g by Caesarean section, Apgar score – 10 points. In the boy, similarly to his sister, from the first day of his life respiratory distress and generalized muscular hypotonia accompanied with areflexia were observed.

The first epileptic seizures appeared in the neonatal period as left-sided focal seizures, myoclonus and tonic seizures. Valproic acid and phenobarbital were administered in the first stage of the treatment and temporary control of seizures was obtained. Due to the reintensification of polymorphic epileptic seizures and the appearance of cluster seizures (requiring hospitalization at an intensive care unit), epilepsy treatment was modified (including Clonazepam) with a satisfactory effect. In the MRI of the head performed in the 3rd month of life dysmyelination in the area of the occipital lobes was demonstrated.

On admission physical examination revealed: hypotrepsia, dysmorphic features, systolic cardiac murmur. The neurological evaluation showed: head circumference HC-45 cm (90-97 pc) the presence of posture reflexes, generalized muscular hypotonia, lack of tendon reflexes, marked atrophy of distal muscle groups and very poor spontaneous motor activity. Psychomotor development was estimated at one month of age.

In the control MRI examination of the head at 6 months of age no progression of myelinization process was revealed. The result of the cerebrospinal fluid examination was normal.

Based on metabolic work-up we excluded aminoacidopathies, organic acidurias, lactic acidosis, lysosomal storage disorders, mitochondrial diseases and urea cycle disorders.

Suspecting NALD serum VLCFA levels were determined, revealing abnormal results. In order to establish a final diagnosis the levels of VLCFA, plasmogens and pipecolic acid were determined in the cultures of skin fibroblasts and also immunocytochemical analysis of peroxisomes was performed. The results of the above-mentioned tests suggested the lack of a single enzyme – bifunctional protein. The C (carbon) concentration 26:0; C 26:1 and the proportion C24/C22 and C26/C22 were elevated, which indicated the defect of peroxisomal oxidation of fatty acids (VLCFA in serum: *C26:0* 3.150 ug/ml, normal values: 0.24±14; *C26:1* 2.93 ug/ml, normal values: 0.11±0.04; *C24:C22* 1.576 normal values: 0.78±0.1; *C26:C22* 0.21, normal values: 0.01±0.003; VLCFA in fibroblasts: *C26:0* 0.469 ug/mg protein, normal values: 0.07±0.04, *C26:1* 0.506 ug/mg protein, normal values: 0.09±0.07; *C26:0/C22:0* 0.848, normal values:

0.08±0.03). Phytanic acid oxidation was diminished: 4.175 pmol/h/mg protein (normal values: 13.2). On the basis of immunocytochemical analysis of peroxisomes the presence of ALDP and protein catalase was established.

Eventually, D-bifunctional protein deficiency was diagnosed with features of encephalopathy. The child died at 18 months of age. Due to the lack of consent of his parents postmortem examination was not performed.

Discussion

Structural analysis of bifunctional protein revealed the presence of three domains: the N-terminal domain (1-323 amino acids) which bears the 3-hydroxyacyl-CoA dehydrogenase activity, the central domain (324-595 amino acids) which shows 2-enoyl--CoA hydratase activity, and the C-terminal domain (597-737 amino acids) which presents a great degree of homology to the sterole transfer protein and catalyzes *in vitro* the transit of 7-dehydrocholesterol and phosphatydylcholine through cellular membranes [4]. Depending on the localization of a molecular defect patients with D-bifunctional protein deficiency (D-BP, D-bifunctional protein deficiency, OMIM#261515) can be divided into three groups: as patients with the dehydrogenase and hydratase complex defect or with isolated defects of the enzymes mentioned. D-bifunctional protein deficiency may be caused by a mutation in the *HSD187D4* gene. The gene locus includes 5q2 [6]. The bifunctional protein exists in two isomers: L and D. A significant difference lies in the specificity towards the substrate. Only the D-bifunctional protein catalyzes the creation process of ketoacyl-CoA derivatives from straight chain as well as branched chain fatty acids. Similarly, dicarboxylic fatty acids undergo β-oxidation not within mitochondria, as it had previously been believed, but among others, due to the presence of D-bifunctional protein [2].

Table IV. Biochemical markers in peroxisomal disorders

Analysis	Results
routine clinical chemistry	↓ cholesterol, ↓ glucose, ↑ transaminases, ↑ serum iron, ↑ transferrin (in plasma or serum)
very long chain fatty acids	↑↑↑ (in plasma, serum or cultured fibroblasts)
ratio of C26/C22	↑↑↑
plasmalogens	↓↓ (in whole blood or erythrocyte membranes)
phytanic and pristanic acids	↑ (in plasma or serum, levels dependent on dietary intake)
bile acids	↑ individual metabolites (differentiation in plasma or urine)
pipecolic acid	↑ (in plasma, urine or bile by GC/MS)
organic acids	dicarboxylic aciduria, often characteristic ↑ of 2-hydroxysebacic acid

The patient presented in this paper is a rare case of an isolated defect of peroxisomal fatty acid oxidation. A very similar clinical picture in his sister suggests the presence of a peroxisomal disease in the girl. The basic diagnostic difficulty lies in the differentiation between a great number of chronic diseases accompanied by hypotonia, epileptic seizures and dysmorphic features. Very often patients with peroxisomal diseases do not present with the full spectrum of the disease. According to Poll-The and Saudubray peroxisomal disorders may be clinically diagnosed based on the presence of dysmorphy, neurological symptoms and renal and hepatic dysfunction [6].

In peroxisomal diseases, among other metabolic diseases, epileptic seizures appear relatively often. Focal seizures, for example, occur in 80% of Zellweger syndrome patients, whereas generalized seizures and myoclonia are observed as early as in the neonatal period. Similarly, in NALD tonic seizures and myoclonia are accompanied by hypsarrhythmia in an EEG tracing [1,2,6].

Among neurological symptoms encephalopathy, seizures, hypotonia and neuropathy are of primary importance, whereas during the ophthalmological examination quite often retinopathy, optic dysplasia and cataract can be observed. The first symptoms are usually not accompanied by metabolic decompensation features [6,7,8]. Peroxisomal diseases most frequently demonstrate themselves as chronic encephalopathy with its onset in infancy or alternatively in early childhood or as progressive neurological disorders at school age. According to recent studies the clinical course of the disease is more severe in patients with D-bifunctional protein deficiency in comparison to the remaining entities from the same group. Neuroimaging studies of BPD demonstrate disturbed white matter myelinization (mainly in occipital lobes and the cerebellum), neuronal migration disorder (most frequently polymicrogyria), focal

heterotopias, dysplasia of inferior olives, and periventricular cysts. The MR image resembles lesions found in NALD and ALD. The neuropathological findings in child NM were consistent in general with MRI findings showing features of hypomyelination. Also neuron heterotopias that were found in autopsy are a form of pathology typical for D-BP [6]. As was shown especially in Fig. 4 and Fig. 10 the neuropathological findings in the cerebellum suggest the impairment of its development probably with the defect of migration and maturation of neurons derived from the external granular layer. Another interesting finding seems to be the apparent occurrence of two types of gliosis: in white matter formed by rather "slim" astrocytes, and in grey matter composed of large, markedly hypertrophic ones. The biochemical marker for bifunctional protein deficiency is the increase of serum VCLFA levels and fibroblast VCLFA levels as well as the increase in concentrations of acid bile metabolites (Table IV).

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