

Brain and cerebellar hemidysplasia in a case with ipsilateral body dysplasia and suspicion of CHILD syndrome

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Abstract

CHILD syndrome is an acronym for Congenital Hemidysplasia with Ichthyosiform nevus and Limb Defects. This is an X-linked dominant disorder affecting females with early lethality in hemizygous males. The clinical features are congenital hemidysplasia with ichthyosiform erythroderma and ipsilateral hypoplasia of limbs and other parts of the skeleton as well as defects of the brain, heart, kidney and lung. CHILD syndrome is caused by mutations in the NSDHL (steroid dehydrogenase-like protein) gene at Xq28, which affects cholesterol biosynthesis. A female premature newborn with left side body hemidysplasia and ipsilateral defects of the skin, visceral organs and brain is reported. Analysis of child DNA isolated from skin fibroblasts showed missense mutation c.1046A>G;PpY349C in the NSDHL gene that could cause the phenotype.

Key words: premature newborn, body and organs hemihypoplasia, brain hemidysplasia, lissencephaly type II, cerebellar malformation, NSDHL gene mutations, neuropathology.

Introduction

Cholesterol is an essential component of mammalian cell membranes and a precursor molecule for steroid hormones synthesis and bile acid. Additionally, cholesterol plays an important role in development of the central nervous system and signal transduction. Several malformation syndromes connected with defects of cholesterol biosynthesis are described. One of them is CHILD syndrome (OMIM 308050) caused by mutations in the *NSDHL* gene, located at the Xq28 region [8,10].

CHILD syndrome is an acronym for Congenital Hemidysplasia with Ichthyosiform nevus and Limb Defects [1,6]. This is an X-linked dominant disorder affecting females with early lethality in hemizygous males. The syndrome is characterized by congenital hemidysplasia with ichthyosiform erythroderma and ipsilateral hypoplasia of limbs and other parts of the skeleton as well as defects of the heart, kidney, lung and brain. Involvement of the CNS in CHILD syndrome is not very frequent. Happle et al. [6] in two of their own and 20 reported in literature CHILD syndrome cases mentioned brain ipsilateral involvement in

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six cases. Among them unilateral hypoplasia of the brain, dilated lateral ventricle [14], hypoplasia of the cranial nerves and the spinal cord [15], minor EEG anomalies, mild intellectual impairment, and decreased sensation to touch and heat on the affected side were found. Hebert et al. reported one case of CHILD syndrome with lumbar meningocele and hydrocephalus [9]. Neurosensory hearing loss in two out of 23 CHILD cases was also described by Bornholdt et al. [2].

We report a neuropathological description of the brain in a further case suspected of CHILD syndrome diagnosed in a premature newborn with left side developmental abnormalities affecting the skin, visceral organs and brain.

Case report

The proband was a fetus from the second pregnancy of healthy and nonconsanguineous parents (36-year old mother, 42-year old father). Family pedigree showed no MCA/MR cases but one spontaneous abortion in the 5th week of gestation was noted in the proband's mother. Primarily, the second pregnancy was bigeminal but one embryo was aborted in the 6th gestational week. There was no exposure to teratogenic agents during this pregnancy. Prenatal ultrasonography and MRI showed multiple malformations in the second fetus and pregnancy was terminated at 29 weeks of gestation. The newborn birth weight was 1070 g (25th centile), length 40 cm (75th centile), head circumference 27 cm (50-75th centile.) Apgar score was 4-6 points at 1st and 5th minutes respectively. Clinical evaluation after birth revealed body hypotrophy with hypoplasia of the left body side, ipsilateral hypoplasia of the skull, the left eye, mandible, arm and leg. There were no palmar dermatoglyphics on the left hand. There were areas of alopecia over the left parietal region of the scalp and cutis marmorata of the left body side with sharp midline demarcation. Echocardiography showed left heart hypoplasia without mitral and aortal valve defects. Brain ultrasonography showed hypotrophy of the left brain hemisphere with a large cyst communicating with the lateral ventricle and agenesis of the corpus callosum. The cerebellum had abnormal structure. The child died after one day. Clinical diagnosis before autopsy was: hypoplasia of the left part of the body with multiple congenital defects. Diagnosis of CHILD syndrome was suggested.

General autopsy showed apart from hypoplasia of the left side of the face and body, hypoplasia of the left atrium and ventricles of the heart without other malformations, unilobar left lung with narrowing of the ipsilateral pulmonary artery, hypoplasia of the left kidney and adrenal gland, hyperpigmented skin nevus located over the right side of the scalp and only two umbilical vessels. Microscopic structure of the inner organs was normal.

Material and Methods

The brain was fixed in formalin. Then, specimens from the cerebral hemispheres, brain stem and cerebellum were taken and embedded in paraffin. The sections were stained with haematoxylin-eosin (H-E) and Klüver-Barrera methods, and GFAP and DC 34 immunoreactions were done.

Karyotype in peripheral blood lymphocytes was performed using routine methods of cytogenetic analysis.

DNA for molecular investigations was isolated from cultured fibroblasts of the skin. Sequence analysis of the coding exons of *NSDHL* and *EBP* genes was performed.

All investigations were performed in the Institut für Allgemeine Humangenetik der Universität Marburg in Germany.

Results of cytogenetic and molecular investigations

Cytogenetic analysis in peripheral blood lymphocytes showed normal female karyotype – 46, XX.

In the *EBP* gene no mutation was found. In the *NSDHL* gene, missense c.1046A>G; p.Y349C mutation was detected in the proband. Presence of this mutation was excluded in the proband's mother.

Results of neuropathology examination

Gross neuropathological evaluation showed asymmetry of the brain, cerebellum and brain stem with normal for age development and size of the right side structures but with hypoplasia and dysplasia of the left one. The left brain hemisphere showed lissencephalic appearance with focal cobblestone surface (Fig. 1). On the frontal brain sections the left hemisphere had a balloon-like appearance, and there was a lack of corpus callosum (Fig. 2). The only

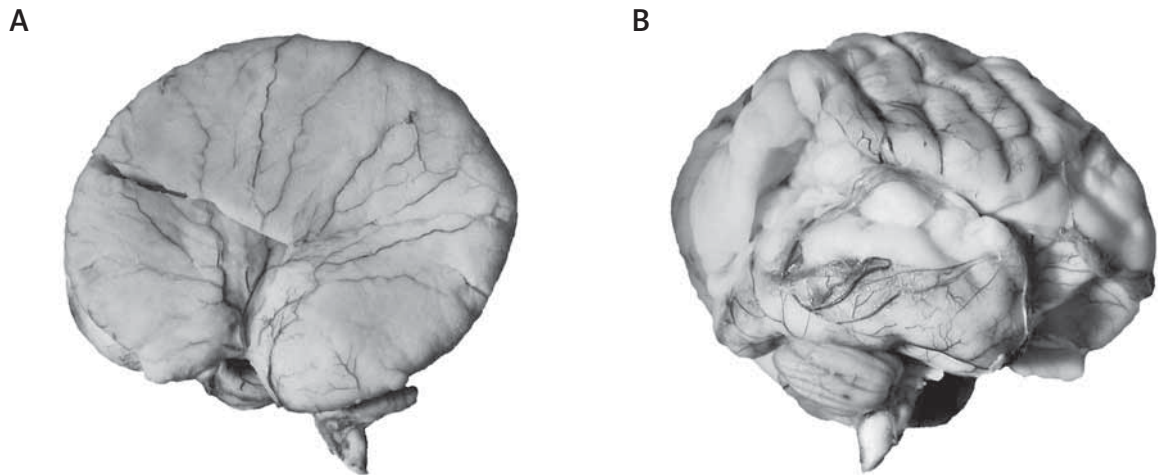


Fig. 1. Lateral view of the brain hemispheres: A. Left hemisphere showing lack of convolutions (lissencephaly) with cobblestone appearance of the surface; B. right hemisphere with normal for age convolutions

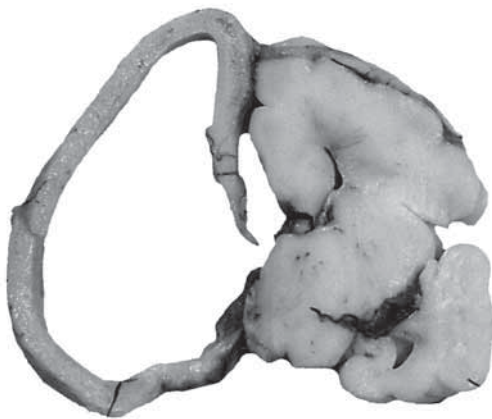


Fig. 2. Normal for age appearance of the right hemisphere and lissencephalic, balloon-like left hemisphere. Lack of corpus callosum and left side thalamus. Frontal section.

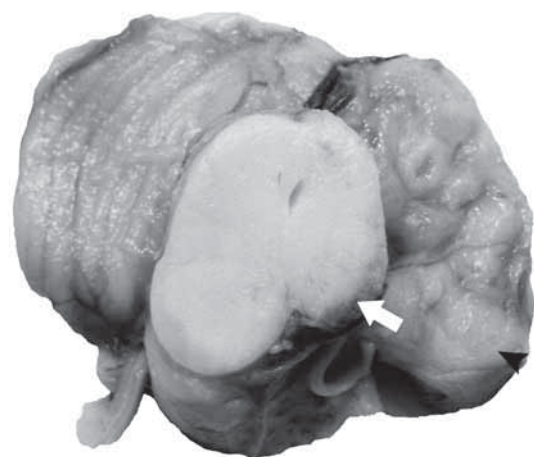


Fig. 3. Cerebellum and mesencephalon: the left half of mesencephalon smaller than right one with hypoplasia of left crus cerebri (arrow); poorly convoluted left cerebellum hemisphere (head arrow)

connection between both cerebral hemispheres was in the region of the hypothalamus. The left cerebellar hemisphere had wide, irregular structure of folia. The brain stem disclosed left side hypoplasia (Fig. 3). Microscopic evaluation showed appropriate to gestational age development of the right brain and cerebellar hemispheres as well as right side of the brain stem. The left brain hemisphere showed diffuse dysplastic changes with multiple nests of grey matter with small clefts between the nervous tissue without any laminar structures and differentiation on the cortex and white matter. There were also submeningeal glioneuronal ectopias (Fig. 4). There were a

few structures resembling brain gyri, surrounded by clefts with wide, thin-walled vessels (Fig. 5).

In the left brain hemisphere there was a small amount of germinal matrix according to fetal age as well as compared with the amount of germinal matrix in the right brain hemisphere. In the basal part of the left brain hemisphere there were foci of neurons resembling neurons of basal ganglia, but without organization normal for those structures. There was also a small part of cortex with micropolygyric appearance. In that part of the brain there were a lot of calcifications in the subventricular area and some of

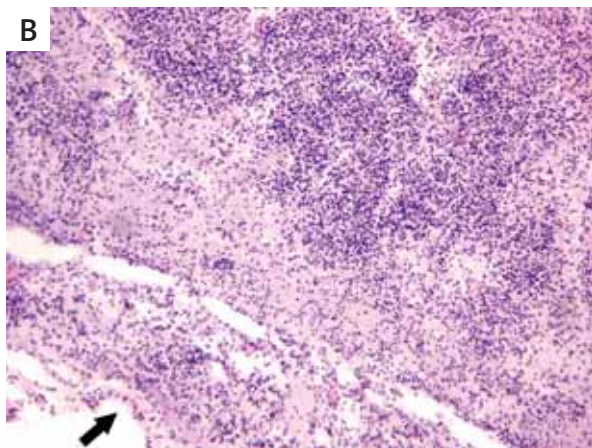


Fig. 4. A. Appearance of the left brain hemisphere with bizarre dysplasia: there are multiple nests of grey matter without any laminar structures (arrows) with narrow clefts (arrow heads). Lupe, Kluver-Barrera method; B. Haphazard cellular organization with glioneural ectopia into the cleft (arrow). Kluver-Barrera method. Magn. $\times 200$

the vessels had calcification of the wall. The inner part of the left hemisphere was devoid of ependyma and was lined by glial fibres. The left cerebellar hemisphere showed diffuse dysplastic changes: folia were small, and some of them were coupled together, giving a smooth external surface. There were also seen

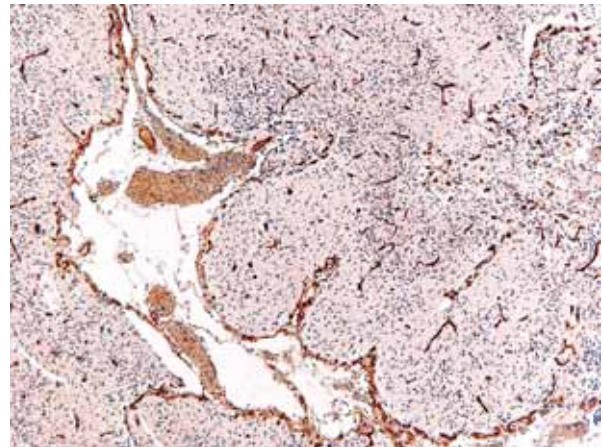


Fig. 5. Small convolutions without normal cellular structure, surrounded by the cleft with thin-walled, wide vessels. CD34, $\times 100$

various sized cavities with meningeal tissue and thin-walled vessels inside. These cavities were surrounded by normal cerebellar cortex (Fig. 6) or only by granular cells. In the deep white matter of the left cerebellar hemisphere there was a large heterotopic focus formed by cavities similar to cavities described above and by foci with some laminar structures resembling cerebellar cortex (Fig. 7).

Discussion

CHILD syndrome is characterized by hemidysplasia with ichthyosiform erythroderma with clear-cut demarcation in the middle line, ipsilateral limb and organ defects. More often the right side of the body is affected. The skin changes are either present at birth or develop during the first weeks of life [3,6]. The presented case was a premature newborn aged 29 weeks of gestation with left body side dysplastic changes and internal organs. There were not skin changes typical for CHILD syndrome with ichthyosiform erythroderma; however, areas of alopecia described as typical for CHILD syndrome were present from birth. Because of prematurity and early death there was no possibility to observe evaluation of this kind of skin abnormalities.

Left side of the body and ipsilateral organs (eye, left part of the heart, lung, kidney and adrenal gland) were smaller than the right one without other abnormalities. The most severe dysplastic changes were noted in the brain and cerebellar hemispheres ipsi-

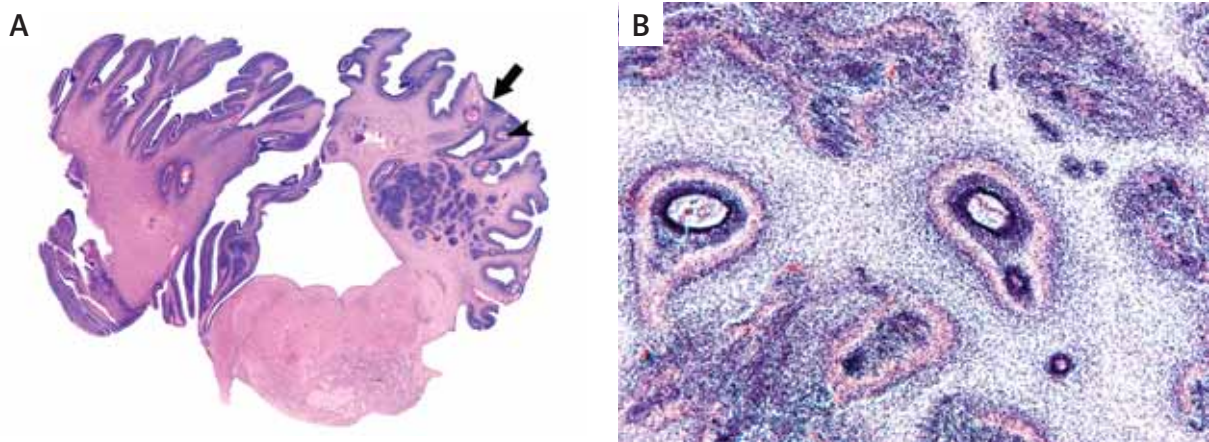


Fig. 6. Cerebellum and pons: A. Left cerebellar hemisphere with diffuse dysplastic changes: small folia, some of them coupled together giving a smooth surface (arrow), varying in size cavities (arrow head) and heterotopia in white matter. The left side of the pons smaller than the right one with lack of the left pyramidal tract. Lupe. HE; B. cavities surrounded by normal cerebellar cortex with meningeal tissue and thin-walled vessels inside. HE. Origin. magn. $\times 200$

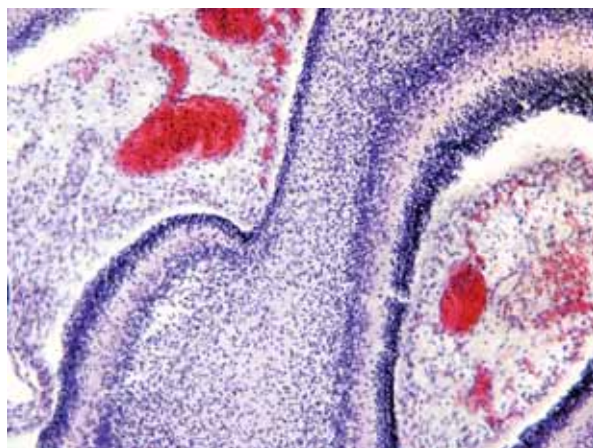


Fig. 7. Heterotopic focus in the deep white matter with cavital and non-cavital dysplastic changes. HE. Origin. magn. $\times 100$

lateral to the body and organ dysplasia. It seems as if the two halves of the brain developed separately, according to different programs: the right half of the brain and cerebellum developed appropriately to the gestational age and had normal structure without any signs of malformations. In the left brain and cerebellar hemispheres there were morphological changes which were a consequence of disturbances in proliferation and migration. The left part of the brain was hypoplastic with a small, according to gestational age, amount of germinal cells. The structure of the left brain hemisphere showed lissence-

phaly type II. There was also evidence of disturbed connections between the neural and meningeal structures. The left cerebellar hemisphere showed severe structural disturbances similar in character to the malformations in the brain hemisphere, also with abnormal connections between neural and meningeal structures. Similar cerebellar malformations were reported by Laure-Kamionowska et al. [11] in two cases of stillborn premature newborns with other congenital malformations. The disturbance of structure in the brain stem with lack of cortical tracts in the left part of the brain stem should be considered as the consequence of malformation of the left brain hemisphere with lack of structures which are the source of cortical tracts.

It is known that heterozygous nonsense and heterozygous missense mutations in the *NSDHL* gene are responsible for most cases of CHILD syndrome [10]. However, mutations in the *EBP* gene have also been found in some patients with CHILD syndrome phenotype [4]. Genotype–phenotype correlations have yet to be elucidated, but patients with *NSDHL* mutations tend to have more severe phenotype and persistent skin involvement than those with *EBP* mutations. Happle has proposed that midline early embryonic “organizer” cells expressing the mutant *NSDHL* gene could affect the process of X-inactivation itself in CHILD syndrome and alter the patterning of a large developmental field on one side of the body [6]. Typical cutaneous manifesta-

tions in CHILD syndrome are predominantly right sided, although *NSDHL* mutation was also detected in a CHILD female with left-sided involvement likely in the presented proband [12].

“It is known that cholesterol contributes fundamentally to the development and function of CNS and bones”. Over the past decade, the identification of multiple congenital anomaly/mental retardation syndromes due to inborn errors of cholesterol synthesis has underscored the importance of cholesterol synthesis in normal development. According to König et al. (2000) CHILD syndrome can be added to the list of developmental disorders associated with mutations affecting cholesterol synthesis [10,12].

The *NSDHL* gene has been localized to Xq28 and encodes for 3-beta-hydroxysteroid dehydrogenase involved in the oxidative decarboxylation of sterol C-4 methyl groups in the cholesterol biosynthetic pathway [5].

Molecular analysis revealed a missense mutation in the *NSDHL* gene in our proband. This mutation was not observed among 100 investigated control persons from Europe but the same mutation was detected, previously, in a patient from Japan. The same amino acid was also mutated in a patient from France into histidine (personal communication of Professor Grzeschik). Obviously, this codon represents a mutation hotspot. The change affects an amino acid which is highly conserved from yeast to man. Therefore, it seems that it could cause the proband's phenotype.

Unfortunately, because of the early death of our proband, evaluation of cholesterol and other metabolites as well as chromatographic determination of sterol concentration in the affected skin were not available. Comparison of clinical manifestation of CHILD syndrome presented by our proband and cases reported in the literature showed that this diagnosis should be considered. Molecular studies showed a “novel”, previously unknown mutation in Europe.

It is very important for genetic counselling that the mother of the proband is not a carrier of this mutation. The risk of recurrence of CHILD syndrome for further pregnancies is near population risk, but germinal mosaicism cannot be excluded.

The presented case documented detailed neuropathological changes and clinical aspects of a very rare malformation syndrome. Practical benefits of molecular studies for the family and their correct interpretation are important for genetic counselling.

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