Chapter

Fetal Craniospinal Malformations: Aetiology and Diagnosis

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Abstract

The chapter discusses the aetiology and diagnostics of each fetal craniospinal disorder, particularly neural tube defects, ventriculomegaly, Dandy-Walker and Arnold-Chiari malformation, corpus callosum dysgenesis, iniencephaly, holoprosencephaly, microcephaly and kinked-brainstem. We aimed to highlight the usual ultrasound findings and genetic testing options.

Keywords: neural tube defects, anencephaly, exencephaly, spina bifida, ventriculomegaly, encephalocele, hydrocephalus, Dandy-Walker, Arnold-Chiari, corpus callosum, iniencephaly, kinked-brainstem, holoprosencephaly, microcephaly

1. Introduction

Our knowledge of the genetic background of the development of neurodevelopmental disorders is evolving. Today, ultrasound is a gold-standard diagnostic method for diagnosing developmental disorders. In addition to teratogenic causes, an increasing genetic background is being recognised for more and more fetal disorders. In addition to ultrasound diagnostics, the aim of this chapter is to investigate the genetic diagnostics of developmental disorders affecting the nervous system. In the case of malformations involving multiple organ systems, we investigate what chromosomal abnormalities or gene mutations may underlie each multiple disorder.

2. Fetal craniospinal malformations

2.1 Neural tube defects. Anencephaly/exencephaly. Spina bifida. Encephalocele

2.1.1 Epidemiology

Neural tube defects are the second most common structural-developmental malformations [1]. If the failure of neural tube closure is at the cranial end of the developing embryo, the disorder occurs in the form of anencephaly (initially exencephaly), if the failure is more caudal than cranial, it occurs in the form of spina bifida.

The prevalence of neural tube defects (NTDs) is 0.5–2/1000 live births, showing a heterogeneous geographical and ethnic distribution [2]. A genetic cause can be identified in 20% of cases [3].

2.1.2 Fetal morphology and prognosis

The brain of an anencephalic fetus is missing or missing in large parts. The form localising only to the cranium is called meroacrania, and extending to the foramen magnum is called holoacrania. If the spinal cord is also affected, the disorder is called craniorachischis.

The most severe neural tube closure disorder is caused by abnormal closure of the cranial section of the neural tube [4]. Anencephaly is a condition incompatible with life.

Spina bifida develops due to an abnormality in the closure of the neural tube caudally from the cranium. It basically affects the spinal region, with or without nerve tissue involvement. Its mildest form is spina bifida occulta, which is a defect of the vertebral arcs without affecting the underlying nerve tissue, most commonly in the sacral region, and usually causes no symptoms. In the case of spina bifida cystica, the lesion advances cystically. It can be closed (covered with skin or an opaque membrane) or open. If the cyst has meninx and cerebrospinal fluid but no nerve tissue in it, the lesion is called meningocele, if it also contains nerve tissue elements, the term is myelomeningocele [5]. The most severe form is myeloschisis (also known as rachischisis), which is an open lesion, so nothing covers the medullary (neural) plate [5]. After birth, spina bifida occulta usually does not cause symptoms, while spina bifida cystica can lead to paralysis of the lower extremities and urinary problems. Although myelomeningocele is a developmental disorder compatible with life, it is often associated with varying degrees of disability.

In encephalocele, the brain tissue with or without the meninges protrudes hernia-like through a pathological opening in the skull [5]. The most common site of its formation is os occipital [6]. Based on the grouping of Suwanwela and Suwanwela: cranial, frontoethmoidal, and basal encephalocele can be distinguished in addition to the occipital group [7].

2.1.3 Aetiology

To date, no clear genetic defect has been identified in the background of the development of NTDs, however, the role of a number of environmental and genetic predisposing factors is already known. It has been clinically demonstrated that folic acid supplementation significantly reduces the incidence of neural tube defects during the first trimester of pregnancy. Folic acid enrichment of the flour reduced the incidence of NTDs by 18% in 59 countries [8]. Folic acid, which is involved in purine and pyrimidine synthesis, is one of the cornerstones of DNA synthesis. Of course, not only isolated folic acid deficiency but any drug involved in folic acid synthesis may be associated with the development of anencephaly and other neural tube defects. These drugs such as antiepileptics (valproate or carbamazepine) and antimalarials (trimethoprim) are contraindicated during pregnancy without adequate folic acid supplementation, especially, in the first trimester.

In 1999, Shields et al. identified a heat-labile *MTHFR* C677T mutant gene that was present in the homozygous form of TT in 51 (18.8%) of 271 infants with neural tube defects (the homozygous form of CC did not increase the rate, whereas the heterozygous form of CT alone non-significantly increased the incidence of neural tube defects) [9].

Two members of the *SHROOM* gene family, the *SHROOM-2* and *SHROOM-3* genes have also been associated with the development of NTDs.

The role of the *SHROOM-3* gene in complex morphogenesis has long been demonstrated, and a mutation in a Loss of Function (LoF) (p.N594f) may be

associated with the development of neural tube defects. The Shroom-3 protein encoded by the gene is involved in the planar cell polarity (PCP) pathway, and in animal models, it has been found that its main function is to regulate the distribution of myosin II in cells [10].

A Chinese research team, Z. Chen et al. isolated 1.56 times as many rare variants in the *SHROOM-3* gene in live-born children with NTD than in the control group. In addition, the same research has also linked another member of the *SHROOM* family, the *SHROOM-2* gene mutation, to the development of neural tube defects. *SHROOM-2* is a gene localised on the short arm of the X chromosome (Xp22.2), which encodes a protein of the same name, Shroom-2. Shroom-2 is expressed by endothelial cells; its role in facilitating the development of the contractile network in endothelial cells. In infants with NTD cases, 4.5 times as many deleterious missense (D-Mis) variants were identified compared with the control group. In the same study, *SHROOM-2* variants were found in 42 of the 343 NTD cases, of which 15 mutations were identified. More than one *SHROOM-2* mutation was found in five of these samples [1].

Because the convergent extension is a critical point in neural tube closure, mutation of the gene encoding any other protein in the PCP system that regulates it leads to neural tube closure disorders. Mutation of PCP core genes such as *VANGL2* or *CELSR1* has been shown in mouse models to lead to the development of severe NTDs [11]. It is likely that genes encoding other proteins involved in the PCP signalling pathway may also be associated with neural tube closure disorders, however, human orthologs of the genes found in the experimental models have not yet been identified, so this may be part of further research.

Foetuses of untreated diabetic mothers are also more prone to neural tube defects, as elevated blood glucose levels lead to misfolded proteins, their accumulation and apoptosis of cells through non-enzymatic glycosylation.

This causes structural damage to organogenesis, especially the neuroepithelium. In animal models, high-dose folic acid supplementation has also been shown to reduce the incidence of neural tube defects associated with high blood glucose [12]. In terms of other environmental factors, hyperthermia and vitamin A deficiency may also lead to NTD, the former due to heat stress and the latter due to its role in the retinoic acid pathway resulting in the inadequate closure of the neural tube. Maternal obesity increases the chances of developing NTDs through hyperinsulinemia, and metabolic syndrome through its teratogenic effect due to oxidative stress [13].

2.1.4 Diagnostics

Anencephaly can be diagnosed in the first trimester (in this case, exencephaly is shown in the image), but can only be diagnosed with ultrasound in the second trimester with high certainty, median time to prenatal diagnosis is 20 weeks (16–24) [14]. Ultrasound signals that the upper part of the skull is missing and that no parenchymal tissue can be detected in the skull, however, the brainstem and occipital bone can be identified. In the coronal plane, a 'frog eye' or 'Mickey Mouse' symptom is seen, which is due to a lack of cranial bones and a protruding bulbus. In some cases, it is associated with polyhydramnios, which is the result of insufficient amniotic fluid ingestion by the fetus.

Spina bifida can be diagnosed in the second trimester, with a median time of 21 weeks (18–24). The time and accuracy of detection depend largely on the type of spina bifida and the position of the fetus, as certain positions make it very difficult to follow the spinal column. The direct signs are the openness of the vertebral arches and

herniation of the spinal cord, and the indirect signs are the lemon sign, the biconcave os frontale, and the banana sign, which is an abnormally bent, thin shape of the cerebellum. Ventriculomegaly due to cerebrospinal fluid flow disturbance is also common in foetuses with spina bifida, however, this ultrasound signal is not specific for diagnosis. Furthermore, the clivus-supraocciput angle is of diagnostic value; if it is less than 5 percentile, it raises the possibility of a form of neural tube defect associated with Chiari II malformation [15].

Thanks to modern technology, the availability of 3D and 4D ultrasound and MRI make it easier to diagnose neural tube defects so that in case of doubtful ultrasound findings, the diagnosis can be clarified by choosing another imaging modality. However, it should be noted that although these tests are much more accurate than conventional transabdominal and transvaginal ultrasound, their cost and limited availability make it essential to perform 2D ultrasound accurately and precisely, as it is still the most accessible, quickest and most economical method of diagnosis today.

In addition to imaging, laboratory tests can also support the diagnosis, as in 90% of cases, α-fetoprotein (AFP) levels are elevated in maternal blood and amniotic fluid, so this may be an additional tool to imaging. However, with the development of ultrasound, this test has been superseded.

In about one-tenth of cases, a chromosomal aberration or mutation has been identified as the cause of the neural tube defect, i.e., the majority of NTDs have a non-syndromic cause [16]. It can be seen that, although no clear environmental influence or genetic mutation has been identified as the cause of NTDs, it is likely that their development is multifactorial, i.e., genetic predisposing factors and environmental stresses contribute to their development.

2.1.5 Postnatal morphology and associated disorders

Postnatally, the anencephalic infant lacks a cranial bone (skull), the cerebellum is only a mass, shrunken. The ears are low set and deformed. Facial structures such as the eyes, nose and cheeks are large. The neck is short and spinal abnormalities may be present. The limbs are deformed, the thymus is abnormally large and pulmonary hypoplasia is often present. Spina bifida may be present with minimal external signs depending on the severity of the disease (in spina bifida occulta, only a darker patch or patch of hair-covered skin in the sacral region indicates a malformation). Depending on the region affected, a child with spina bifida lacks the structures covering the spinal column at the affected vertebrae and may have a herniated spinal cord; in meningocele and myelomeningocele, the protruding cyst is visible in the occipital region, with or without nerve tissue.

Neural tube defects are most commonly associated with renal abnormalities such as hydronephrosis, polycystic kidney disease, uni- or bilateral agenesis or unilateral hypoplasia. Cardiac malformations range from simple septal defects to complex cardiac malformations [17].

2.2 Congenital ventriculomegaly and hydrocephalus

2.2.1 Epidemiology

Ventriculomegaly is one of the most common pathological findings during antenatal ultrasound screening [18]. In severe cases, we are talking about hydrocephalus. The prevalence of hydrocephalus is 11/1000 live births [19].

2.2.2 Fetal morphology and prognosis

Hydrocephalus develops due to a progressive increase or impaired absorption of intraventricular cerebrospinal fluid (CSF) and its pathomechanism can be either obstructive or communicating [17, 20]. Increased pressure leads to the dilation of the ventricles, i.e., ventriculomegaly. If the brain volume thins due to the growth of the ventricles, we speak of hydrocephalus internus, if the volume of cerebrospinal fluid increases in the subarachnoid spaces, we speak of hydrocephalus externus. Macrocephaly can also develop with the growth of the bony skull [5], and the skull of such a fetus is larger than average.

The CSF is produced by the choroid plexus, circulates in the ventricles, then exits through the fourth ventricle into the subarachnoid space, where it is absorbed by the granulationes arachnoideae and finally drained through the venous sinuses into the systemic circulation. 1/3 of the CSF enters the lymphatic circulation, however, pathological alterations of this have not yet been demonstrated in human models [19].

The prognosis of hydrocephalus depends on its severity and the success of prenatal treatment. Of the 90 cases of hydrocephalus followed up by Yamasaki et al., 17% resulted in death, 21% were diagnosed with severe retardation, 13% with moderate retardation and 26% with mild retardation. A normal phenotype was described in 23% of cases [21].

The classification of ventriculomegaly depends on the degree of dilatation detected on ultrasound: mild ventriculomegaly between 10–12 mm, moderate ventriculomegaly between 13–15 mm and severe ventriculomegaly above 15 mm. The measurement is taken at the atrium of the lateral ventricle, the point where the temporal and posterior horns converge. This is a fixed value between 15 and 40 weeks of pregnancy [22].

If no abnormality is found in genetic testing and no other associated abnormality is present, mild ventriculomegaly is not considered pathological, and postnatally 90% of these cases present a normal phenotype, i.e., the wider ventricle is considered a normal variant [22].

2.2.3 Aetiology

Congenital hydrocephalus can be syndromic or non-syndromic, but in half of the cases, it is idiopathic [20]. The most common form of congenital hydrocephalus is the X-linked monogenic *L1CAM* mutation. The gene product of *L1CAM* is a protein that plays a key role in neuronal migration.

Hydrocephalus due to the *L1CAM* mutation is one of the most severe forms associated with stenosis of the aqueduct of Sylvius, known as HSAS (Hydrocephalus with Stenosis of the Aqueduct of Sylvius). It is often associated with corpus callosum agenesia or hypoplasia, adducted thumb and other structural cerebellar abnormalities [20]. Another form of X-linked congenital hydrocephalus is associated with *AP1S2* mutation [23].

Mutations in the *MPDZ* gene lead to primary ependymal malformations, including hydrocephalus [20]. *MPDZ* encodes a protein that regulates tight junction function and is also likely involved in the PCP pathway [24]. Mutations in this gene at 9p23 lead to autosomal recessive non-syndromic hydrocephalus [25]. Another form of autosomal recessive hydrocephalus is caused by mutations in *CCDC88C*. This gene encodes a protein called Daple, which interacts with Dishevelled protein to regulate cell migration. The Dishevelled protein is a member of the non-canonical Wnt pathway [26].

In addition to these well-studied genes, two others have recently been identified that are associated with the development of hydrocephalus. The *EML1* gene (14q32.2) encodes a microtubule system-related protein that is also involved in the PCP pathway. The gene mutation results in abnormal development of the posterior part of the skull, leading to severe hydrocephalus [23]. Also in this study, Shaheen et al. described another mutation, *WDR81* (17p13.3), which leads to severe hydrocephalus with cerebellar hypoplasia.

Neural tube defects are often associated with hydrocephalus. This may be due to common genetic factors and environmental aetiology, and pathological spinal development may itself be a physical barrier to CSF. Arachnoid cysts may also form a physical barrier in the pathway of cerebrospinal fluid. As arachnoid cysts occur in 15% of Phelan-McDermid syndromes, this syndrome is also often associated with hydrocephalus [19]. Other syndromes include mucopolysaccharidosis, Sotos syndrome and Rothmund-Thomson syndrome. Cytogenetic abnormalities have also been associated with the disorder, such as microdeletion of 9q22.3, partial trisomy of chromosome 1, but hydrocephalus is also common in Patau, Edwards and Down syndromes [19].

Ventriculomegaly/hydrocephalus may occur in isolated cases as a consequence of TORCH (Toxoplasma, Rubeola, Cytomegalovirus, Herpes simplex and other viruses) infection during pregnancy, or in rare cases due to congenital tumours such as choroid plexus papilloma [21].

2.2.4 Diagnostics

In terms of ultrasound diagnostics, it should be noted that in the first trimester, physiological ventricular dilatation is present, so hydrocephalus can only be diagnosed with certainty after the 14th week. The first characteristic ultrasound sign is asymmetry of the choroid plexus [27]. The top of the fourth ventricle may show an abnormal image and the absence of foramina Magendii and Luschka is common [17]. Due to the progressive nature of hydrocephalus, it may develop throughout pregnancy and even after birth without any previous ultrasound signal. Thus, the time to diagnosis also varies widely; Yamasaki et al., in their study of 156 cases of hydrocephalus, found the diagnosis to be made between 13 and 40 weeks (51% of cases were already diagnosed before 28 weeks). Breeze et al., also reported similar data, with a median time to diagnosis of 28 weeks (16–36) [28].

In addition to ultrasound, if ventriculomegaly or hydrocephalus is suspected, a Magnetic Resonance Imaging (MRI) scan may be useful, as it is a more accurate and reliable way of showing the development of brain structures and their possible malformations than ultrasound. However, it should be taken into account that, in addition to the general disadvantages of MRI (difficult availability, high costs), the fact that the fetal movement makes the findings more difficult or impossible to evaluate in antenatal diagnosis is a particular difficulty [29].

2.2.5 Postnatal morphology and associated disorders

The neonate with ventriculomegaly/hydrocephalus has macrocephaly, which may progress postnatally [30]. The disorder is often associated with neural tube defects due to common genetic predisposing factors.

2.3 Dandy-Walker malformation

2.3.1 Epidemiology

The incidence of this malformation is 0.33/1000 live births [31], i.e., it is a relatively rare disorder.

2.3.2 Fetal morphology and prognosis

The Dandy-Walker malformation includes dilatation of the fourth ventricle with hypoplasia or agenesis of the vermis of the cerebellum. A pseudocyst often develops at the base of the posterior fossa. Survival is low (about half of cases) [5].

2.3.3 Aetiology

Genetically heterogeneous in origin, several mutations have been described in recent years. However, one of the main "suspects" are the *ZIC1* and *ZIC4* genes, located at 3q24. In terms of inheritance, autosomal dominant, recessive and X-linked inheritance patterns have been reported, so it may be present as part of trisomy 9 (AR) or 6p (AD), but it may also be associated with Aicardi syndrome.

It is common in Edwards syndrome. However, in addition to genetic causes, a number of environmental factors may contribute to its development, such as maternal alcoholism or severe diabetes mellitus, as well as TORCH infection in the first trimester [17].

2.3.4 Diagnostics

Dandy-Walker malformation can be diagnosed by ultrasound at the earliest at week 11, but it should be noted that isolated dilatation of the fourth ventricle may be physiological during early development. In addition, the cerebellar vermis is not fully developed until the second trimester. In conclusion, an accurate diagnosis is only possible during the second trimester [27].

2.3.5 Postnatal morphology and associated disorders

Since the disorder mainly affects the cerebellum, in case of survival, postnatally the disorder may be marked by muscle movement disorders, learning difficulties and mental retardation. Hydrocephalus and consequent macrocephaly often develop due to inhibition of cerebrospinal fluid drainage [17]. It is often associated with hyperdactyly, syndactyly, renal, hepatic and pancreatic alloplasia and abnormal retina [32].

2.4 Arnold-Chiari malformation

2.4.1 Epidemiology

There are four types of Arnold-Chiari syndrome. Its prevalence is 0.9/1000 live births [33].

2.4.2 Fetal morphology and prognosis

In type I, the cerebellar tonsils are ectopic, with a part of the tonsils pressing into the foramen magnum, often in isolation. In type II, there is cerebellar hypoplasia with myelomeningocele, part of the tonsils and the elongated distal part of the brainstem protruding into the foramen magnum. In type III, the cerebellum is herniated due to the absence of occipital bone and spina bifida. In type IV, the most severe type, the cerebellum itself is hypoplastic [17]. Structural deformities lead to hydrocephalus. A lesion with a poor prognosis.

2.4.3 Aetiology

In the majority of cases, Arnold-Chiari malformation is multifactorial, and external environmental factors may also play a role in the development of the disease.

A precise genetic mutation has not yet been identified. It is assumed to be the result of mutations in proteins involved in the Sonic hedgehog and Wnt pathways, suggesting that there is an overlap at the gene level between mutations causing neural tube defects and Arnold-Chiari malformation, but this requires further research. Its aetiology is probably multifactorial and it cannot be ruled out that various environmental factors also contribute to the development of the phenotype.

Syringomyelia is often described in this pathology. In syringomyelia, the cavity formation observed in the nervous system may be due to residual formations from embryonic age, but may also occur as a result of haemorrhage or inflammation.

2.4.4 Diagnostics

An ultrasound scan in the second trimester of pregnancy can raise suspicion of the lesion, and if necessary, an MRI scan can confirm the diagnosis.

2.4.5 Postnatal morphology and associated disorders

Type I occurs in 3–5% of patients with Klippel-Feil syndrome, suggesting that abnormal *GDF6* and *GDF3* function may also be associated with the development of the syndrome [33].

Syringomyelia, which is often associated with type II, is rarely hereditary and may be associated with the following additional pathological conditions and gene mutations: hydrocephalus (*NF1, NES, GFAP, FGFR2, AQP4*), spina bifida (*GDF6*, *GDF3*) and other spinal deformities (*VDR, POC5, NF1, GH1, GFAP, GDF3*), various neurological tumours: astrocytoma (*NRAS, NF1, NES, GFAP, COL1A1*), neurilemmoma (*NF1, NES, GFAP*), ependymoma (*NES, GFAP*).

In addition, syringomyelia may also be caused by tissue weakness, such as in Ehlers-Danlos syndrome (mutations in *COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, TNXB, ADAMTS2, PLOD1, B4GALT7, DSE, D4ST1/CHST14* genes) or Marfan syndrome (mutation in *FBN1* gene).

2.5 Corpus callosum agenesia/dysgenesis

2.5.1 Epidemiology

The prevalence of malformations of the corpus callosum is 0.25/1000 live births. In terms of aetiology, 30–45% of cases are due to genetic causes, 10% to chromosomal

abnormalities and 20–35% are associated with a genetic syndrome. In some cases, environmental factors (e.g., maternal alcohol consumption) also lead to corpus callosum dys- or agenesis [34].

2.5.2 Fetal morphology and prognosis

The corpus callosum is one of the five major cerebral commissures and is one of the largest white matter-containing tract in the brain. Its role is to connect the right and left hemispheres of the brain, and it is thought that 2–3% of the cortical fibres are passing through it. Its main function is to coordinate the hemispheres of the brain and to integrate sensory and motor functions [35].

Isolated corpus callosum dys- or agenesis is a disorder compatible with life, however, approximately 25% of foetuses with isolated corpus callosum agenesis/dysgenesis diagnosed antenatally will later have an intellectual disability. In addition, mild social or learning deficits may occur even with normal intelligence [35]. If the developmental disorder is part of a syndrome, the outcome of the disease depends on the particular syndrome.

2.5.3 Aetiology

Known chromosomal abnormalities affect chromosomes 1, 4, 6, 8 and 17. The most common type is a deletion, including the 1q42-q44 deletion causing corpus callosum dys- or agenesis of variable severity [35]. As most of the proteins encoded by these regions regulate or are involved in a key moment in nervous system development, corpus callosum dys- or agenesis as an isolated developmental disorder does not occur in any of the chromosomal disorders, but is often associated with microcephaly, hydrocephalus or craniofacial abnormalities.

Inheritance patterns include autosomal dominant, recessive and X-linked hereditary syndromes.

A very severe form of X-linked dominant (XLD) inheritance is Aicardi syndrome, which is incompatible with life in male foetuses but also has high premature mortality in girls. In addition to corpus callosum agenesis, it is associated with infantile seizures (infantile spasm) and the development of chorioretinal lacunes [36].

The autosomal dominant form is frontonasal dysplasia, Goldenhar syndrome; autosomal recessive form is Andermann syndrome, craniotelencephalic dysplasia, Da Silva or Leigh syndrome. Isolated corpus callosum dys- or agenesis can be inherited in an autosomal recessive, X-linked recessive (XLR) or autosomal recessive (AR) manner [17].

2.5.4 Diagnostics

Developmental abnormalities of the corpus callosum are difficult to detect before 18 weeks [35]. Ultrasonography shows colpocephaly, a high-lying enlarged third ventricle with absent or abnormal morphology of the corpus callosum [34]. These may confirm the suspicion, as in ultrasound diagnostics there is always the question of whether the absence of a formula is not only due to the position of the fetus, or possibly to a technician error. In their 2012 study, Santo et al. found that the number of false-positive ultrasound findings can be as high as 20%. An MRI scan after 22 weeks can confirm or refute the ultrasound findings with high certainty [37].

2.5.5 Post-natal morphology and associated abnormalities

Corpus callosum dys- or agenesis is often associated with microcephaly, hydrocephalus or craniofacial abnormalities [17]. Therefore, both the postnatal picture and the associated abnormalities are influenced by the gene mutation that results in the disorder.

2.6 Holoprosencephaly

2.6.1 Epidemiology

Holoprosencephaly is a midline malformation of the cranium and face. Its prevalence is estimated to be between 0.2 and 0.06 per 1000 live births [17, 38].

2.6.2 Fetal morphology and prognosis

The three main types of holoprosencephaly are lobar, semi-lobar and alobar. The most severe form is alobar, where midline separation is completely absent and the blister of the telencephalon does not separate. Typically, the corpus callosum and the third ventricle are absent, and cyclopia and proboscis are present. In the semilobar form, the frontal and parietal lobes are usually not separated bilaterally, but all septations, especially in the posterior region, are observed. Microphtalmia or anophtalmia, nasal malformations may also be associated. In the least severe form, the lobar form, the two hemispheres are essentially retained, with varying degrees of fusion between the two halves. The nose may be depressed with eyes sitting close, but the facial phenotype may be completely normal [39].

The prognosis depends on the severity of the holoprosencephaly. Mortality is high in alobar cases.

2.6.3 Aetiology

As with all neural tube defects, the development of holoprosencephaly is multifactorial, with both genetic and environmental influences contributing to its occurrence [40].

It is often associated with chromosomal abnormalities, most commonly with trisomy (Patau syndrome) or deletion of chromosome 13, but may also be associated with trisomy and deletion of chromosomes 18 and 21. Monogenic syndromes have also been associated with foetuses with holoprosencephaly, such as ARH (autosomal recessive holoprosencephaly), ADH (autosomal dominant holoprosencephaly), Váradi-Papp syndrome (AR), Grote syndrome (AR), Steinfield syndrome (AD) or holoprosencephaly-fetal akinesia syndrome (XL) (Wainwright, 2005). Environmental influences have also been implicated in the development of holoprosencephaly, such as maternal alcohol consumption during pregnancy and insulin-dependent diabetes mellitus.

A clear genetic mutation has been identified in the background of 15–20% of holoprosencephalic disorders [41]. Since the Sonic hedgehog signalling pathway is responsible for the regulation of the ventral phase of nervous system development and for the separation of the brain vesicles, it is understandable that genes affecting this mutation and their dysfunctional protein products would also be involved in this pathway. Mutations in the *SHH* gene itself have been demonstrated to underlie

holoprosencephalic retardation since 1996 [42]. Later, mutations in several members of the signalling pathway were identified, including mutations in *PTCH1* and *GLI2*. The Six-3 protein has not yet been linked to any of the signalling pathways, but mutations in *SIX3* are responsible for about 1.3% of holoprosencephaly cases. Six-3 is thought to play a role in the Wnt pathway [43].

There are also correlations between the development of neural tube defects and holoprosencephaly due to their common predisposing factors. K. Shiota found 14 cases of exencephaly or myeloschisis in 150 embryos with holoprosencephaly, but no correlation was found between holoprosencephaly and the severity of the neural tube defect. Diabetic mothers have a higher risk of developing both holoprosencephaly and neural tube defects [41].

2.6.4 Diagnostics

It can be diagnosed prenatally by transabdominal or transvaginal ultrasound, however, some of the milder lobar forms are difficult to diagnose by ultrasound. The median time to diagnosis of holoprosencephaly is 12 weeks (10–14) [27]. In the alobar type, morphological abnormalities of the face (cyclopia, ethmocephaly, cebocephaly) and absence of the choroid plexus in the lateral ventricles are well diagnosed by ultrasound. Dorsal cysts, ventriculomegaly and absence of the cavum septum pellucidum may also be associated findings [44]. An ultrasound sign of semilobar holoprosencephaly is incomplete separation of the hemispheric nuclei and fused thalamus. Both types are often associated with polydactyly, renal dysplasia, omphalocele and hydrops [44].

2.6.5 Postnatal morphology and associated disorders

Kaliaperumal et al. found a 95% mortality rate in alobar holoprosencephaly after antenatal diagnosis. Even mild cases are associated with severe postnatal complications, often requiring neurosurgery and intensive care [45].

2.7 Microcephaly

2.7.1 Epidemiology

Microcephaly is a deviation of at least three standard deviations of head circumference from the mean for given sex and age at fetal maturity [46]. A microcephaly finding is a clinical finding in itself, not a diagnosis [47]. Primary microcephaly is defined as a diagnosis made before 36 weeks of gestation, secondary microcephaly develops after birth [47]. The incidence of primary microcephaly is 0.16–0.025/1000 live births [17].

2.7.2 Fetal morphology and prognosis

Primary microcephaly is a static condition [48]. Phenotypic microcephaly is associated with varying degrees of cognitive deficits depending on the mutation, in addition, to head circumferential abnormalities, but weight, height and other external variations are not common. Imaging studies show normal brain morphology [48]. The prognosis depends on the type of mutation and is generally good, but in the majority of cases, severe deterioration in the quality of life is to be expected.

2.7.3 Aetiology

In terms of aetiology, microcephaly may be caused by a reduction or absence of neurogenesis (due to Cytomegalovirus (CMV) infection, chromosomal abnormality or primary autosomal recessive microcephaly), a prenatal destructive process (e.g., hypoxia, ischaemia) or a rare genetic syndrome.

TORCH infection suffered antenatally, especially in the first trimester, also increases the chance of developing microcephaly [47]. In addition to TORCH pathogens, the Zika virus has received particularly high press coverage in recent years due to the increased number of cases in the US. The association between Zika virus infection during pregnancy and primary microcephaly was quickly shown to be significant. 41,473 pregnant women infected with Zika virus were studied in Brazil between 2015 and 2016. Of these, 1950 cases of microcephaly associated with infection were recorded [49]. The almost 5% case rate is a huge increase compared to the average of 0.02%, and therefore more attention should be paid to mapping the teratogenic effects of Zika virus and preventing infection.

In particular, exposure to harmful substances such as maternal alcohol consumption in the first trimester increases the risk of developing microcephaly in addition to neural tube defects and hydrocephalus. Since fetal hypoxia during pregnancy can also lead to the development of microcephaly, special attention should be paid to pregnant women with placental insufficiency [47].

Isolated microcephaly is an autosomal recessively inherited disorder. Its pathomechanism is a disorder of neurogenic tissue mitotic activity, with normal cell migration and apoptosis [48]. Currently, 18 genes have been identified in the pathogenesis of primary microcephaly. All are members of the MCPH (autosomal recessive primary microcephaly) gene family.

In addition to the autosomal recessive form, mutations in other gene families are known to lead to primary microcephaly. These include *KIF2A, KIF5C* and *KIF11* from the kinesin family, and *TUBG1*, *TUBB2B* and *TUBA1A* from the tubulin family. The proteins encoded by these genes also contribute to the physiological function of the microtubule system.

Among chromosomal abnormalities, microcephaly is often associated with Patau, Edwards and Down syndromes [17].

2.7.4 Diagnostics

Ultrasound diagnosis is made by calculating the head circumference, calculated from the biparietal diameter and the occipitofrontal diameter. This derived value is compared with the mean value for the developmental stage and sex, and if it is at least two [50], in other sources three [46], standard deviations lower than the mean, it raises the possibility of microcephaly.

However, it is worth noting that individual variations may occur without organic deviation, and therefore further examinations to exclude false positivity is always important in the diagnosis of microcephaly. Other imaging, MRI, 3D or 4D ultrasound may be helpful. If a genetic abnormality is suspected, depending on the gestational age, amniocentesis and detailed genetic testing should be considered, especially if microcephaly is associated with other suspected signs (e.g. Intrauterine Growth Restriction, IUGR).

2.7.5 Postnatal morphology and associated disorders

Microcephaly may be associated with certain syndromes. One of these is the autosomal recessive Meier-Gorlin syndrome, which is caused by mutations in the *ORC1, ORC4, and ORC6, CDT1* or *CDC6* gene. The syndrome is one of the primordial dwarfisms and is characterised by intrauterine growth retardation, absence of patella, small ears and microcephaly. Pulmonary emphysema is common [51]. Other notable conditions include Nijmegen-Breakage syndrome, Ligase IV syndrome, Warsaw-Breakage syndrome, severe combined immunodeficiency (SCID) or Bloom syndrome [52].

2.8 Sacrococcygeal teratoma

2.8.1 Epidemiology

Sacrococcygeal teratoma is the most common neonatal tumour with a prevalence of 0.027/1000 live births. Its origin is pluripotent cell proliferation with tissue from all three germinal discs. The origin of the cells is remnant cells of the primitive streak or primordial germ cells [53]. It is more common in female foetuses, with a 4:1 ratio [54].

2.8.2 Fetal morphology and prognosis

The typical site of the tumour is the sacral region, hence its name, and it can often grow very large. In terms of pathology, it can be benign (mature) or malignant (immature). The majority of tumours (90%) are benign [55]. The tumour may be cystic or solid, as well as mixed in appearance. Often it may degenerate secondarily, calcify, or may contain haemorrhagic or necrotic regions [55].

The Altman classification was established based on the anatomical location of the tumour. Altman I is largely located externally, II has an associated intrapelvic tumour, III is largely located in the abdominal cavity, and IV is predominantly located presacrally, often without an externally visible tumour [56].

At prenatal diagnosis, the prognosis is poor, with frequent intrauterine death, mainly due to cardiac failure. In contrast, the prognosis is excellent after surgical intervention for postnatally diagnosed sacrococcygeal teratomas [54].

2.8.3 Aetiology

Sacrococcygeal teratoma is rarely associated with chromosomal abnormalities. There is literature evidence that sacrococcygeal teratoma can be associated with partial 13q22 trisomy [57]. Mutations associated with the 12p region are common in adult germ cell tumours, but this mutation has not been detected in "pure" sacrococcygeal teratomas [58]. However, the 12p mutation is common in sacrococcygeal teratomas where a yolk sac component is also present in the tumour. Based on this, Emans et al. suggest that sacrococcygeal teratomas should be classified into two groups depending on the absence or presence of the 12p isochromosome [59].

Rarely, sacrococcygeal teratoma may be part of the Currarino triad. It is an autosomal dominant inherited disorder with mutations in the *HLXB9* gene in the 7q36 region and is associated with anorectal malformations and presacral tissue proliferation and tumours

in addition to teratoma. Presacral tumours associated with the Currarino triad have a much lower chance of malignancy than non-syndromic forms [60].

In other rare cases, it may be associated with 3q trisomy, resulting in a Cornelia de Lange syndrome-like phenotype (short stature, bone developmental malformations, mental retardation, facial developmental abnormalities) [59].

2.8.4 Diagnostics

One of the most important suspicious signs is a larger uterus compared to the gestational age. This is caused by the size of the tumour or by the associated polyhydramnios. The visible tumour mass required to confirm the suspicion may be seen on ultrasound from as early as week 13, but its differential diagnosis is difficult, as the visible mass in the sacral region may also be pseudocyst, obstructive uropathy or meconium. In such cases, as is often the case in neurodevelopmental disorders, it is worthwhile to have an additional MRI scan.

An enlarged placenta and/or fetal hydrops may cause the mother to develop a condition similar to eclampsia, maternal mirror syndrome.

2.8.5 Postnatal morphology and associated disorders

The American Academy of Paediatrics Surgical Section (AAPSS) has developed a classification system that allows inferring the chance of malignancy and future complications depending on the presence of presacral and external tumours, the diagnosis, the success of the resection. In the I to IV scheme, grade I is the mildest with the least tendency to malignancy, while grade IV is the most severe and most likely to malign [55].

Associated abnormalities are usually consequential, so obstruction of the urinary tract, hydronephrosis, rectal atresia, bony malformation of the sacral region as well as fetal hydrops may occur [55]. Hip dysplasia and hydronephrosis may also be associated with sacrococcygeal teratoma in an unconsequential manner, so screening for these is essential both ante- and postnatally [56].

2.9 Kinked brainstem

2.9.1 Epidemiology

Kinked brainstem is an extremely rare condition. It is often only recognised postnatally. Precise figures on its incidence are not yet available.

2.9.2 Fetal morphology and prognosis

A kinked brainstem (twisted brainstem, fractured brainstem, also known as a Z-shaped brainstem) is a rare lesion, a sign of severe neurodysgenesis [61] on pre- or postnatal brain MRI scans. It usually occurs in association with other neurodevelopmental disorders and has a poor prognosis [62].

The posterior fossa is formed early during gestation. Brainstem folding occurs between the third and eighth week, with cerebellum development complete by the 16th week of gestation. Between the third and fifth week, the forebrain folds in accordance with the developing brainstem structures, creating the flexura cephalica, flexura pontis and flexura cervicalis. In the kinked brainstem, the angle of the

flexures is increased, normal brainstem and cerebellar development are inhibited, and cerebellar hypoplasia is, therefore, an associated abnormality in almost all cases [61].

2.9.3 Aetiology

So far, three syndromes have been identified in which kinked brainstem is present as an associated disorder: alpha-dystroglycanopathies (e.g., Walker-Warburg syndrome), tubulinopathies and X-linked hydrocephalus.

Alpha-dystroglycanopathies are heterogeneous congenital muscular dystrophies with brain, muscle and eye involvement [63]. At the more severe end of the spectrum is autosomal recessive Walker-Warburg syndrome, a defect in O-mannosyltransferase. It is often associated with ocular abnormalities (e.g. microphthalmia, retinal detachment), but these can often only be diagnosed after birth. Other alpha-dystroglycanopathies include muscle-eye-brain disease, Fukuyama muscular and cerebral dystrophy and muscle-eye-brain disease with bilateral multicystic leukodystrophy.

Alpha-dystroglycanopathy may be suspected if cobblestone lissencephaly is present. The trunks may be enlarged. Encephalocele is not a diagnostic criterion but may confirm suspicion [61, 62].

The genes identified so far that cause alpha-dystroglycanopathy are *FKRP, FKTN, POMT1, POMT2, POMGnT1, LARGE, ISPD, GTDC2, DAG1, TMEM5, B3GALNT2, B3GNT1, GMPPB, SGK196, DPM1, DPM2, DPM3, DOLK* [64].

There are two types of tubolinopathy, a more severe and a milder form. The more severe form is associated with microlissencephaly and 'kinked brainstem', the milder form is associated with more non-specific nervous system abnormalities. Three genes have been identified so far in its background: *TUBA1A* (chromosome 12), *TUBB2B* (chromosome 6) and *TUBB3* (chromosome 16) [65].

X-linked hydrocephalus is caused by the *L1CAM* (X-chromosome) mutation. It is suspected if the fetus is a boy and the cerebral aqueduct is not detectable on MRI even with a high T2 signal (however, this is difficult to diagnose if the fetus is small or moves around a lot during the scan). Spasticity and adduction of the thumbs may be associated (mainly seen on dynamic ultrasound, but not a diagnostic criterion). Usually, the hemispheres and trunks are not affected by the lesion [62].

2.9.4 Diagnostics

The abnormality is usually diagnosed prenatally during an MRI scan for suspected ventriculomegaly or other intracranial lesions. If the anomaly has not been diagnosed prenatally, a newborn with a kinked brainstem will require intensive care and will be in poor condition. Regardless of the associated abnormalities, the newborn presents with a variety of neurological symptoms, hypotonia and seizures [62].

2.9.5 Postnatal morphology and associated disorders

The kinked brainstem refers to the increase in the angle of the pontomesencephalic transition, exact figures have not been described so far. The brainstem may dislocate posteriorly or anteriorly at the midbrain bridge level. It is often associated with other intracranial abnormalities. Cerebellar hypoplasia is almost always present.

Other associated abnormalities may include ventriculomegaly, dys- or agenesis of the corpus callosum, delayed cortical development, neuron migration

disorders (e.g., lissencephaly), Dandy-Walker malformation, vertex encephalocele, abnormal cerebral hemispheres or abnormal head size (micro- or macrocephaly) [61].

Polymicrogyria and cobblestone lissencephaly are strongly suggestive of alpha-dystroglycanopathy, but the assessment of neuronal migration is difficult prenatally because the fetal brain is brainstem-rich until about 16 weeks gestation. Cerebellar cysts may also be present, however, these do not develop until the second week after birth [63].

The complicating factor is the secondary damage to cortical structures due to preexisting ventriculomegaly and hydrocephalus.

In a review of seven cases, Stroustrup et al. found that in two cases, the "kinked brainstem" was misidentified as a cerebellum on ultrasound [61]. Since the posterior fossa and the brainstem area are difficult to examine by ultrasound, it is advisable to request an MRI scan in case of a suspicious finding.

Theoretically, the abnormality can be diagnosed from week 7, but in practice, it is usually detected during the second-trimester screening.

2.10 Iniencephaly

2.10.1 Epidemiology

Iniencephaly is a complex malformation characterised by the absence of a neck, pronounced cervicothoracic lordosis and spina bifida.

Its prevalence is very rare and varies between 1 and 0.02/1000 live births, however, the actual prevalence may be higher, as iniencephaly is not always described as part of complex disorders [66].

2.10.2 Fetal morphology and prognosis

It is characterised by the complete or partial absence of the os occipital scales and of the cervical and thoracic vertebrae, an irregular fusion of the existing vertebrae and absence of the neck due to abnormalities in the closure of the vertebral arch. The foramen magnum is wider, while the posterior fossa is usually smaller. Due to the high degree of lordosis of the cervicothoracic spinal segment, the head is strongly tilted backwards, the face is upward (so-called "stargazing") and the trunk is shortened. It is often associated with other neural tube defects, open spina bifida or anencephaly. The disease has a very poor prognosis. If born alive, the newborn dies within a few hours (although one case has been described where the affected individual survived to adulthood and retained his or her intellect) [67].

We distinguish between two types of iniencephaly, iniencephaly apertus (with encephalocele) and iniencephaly clausus (without encephalocele) [68].

2.10.3 Aetiology

Its occurrence is predominantly sporadic. It is more common in female foetuses. Environmental effects have been described in association with maternal syphilis and drug intoxication. Chen et al. found chromosomal abnormalities in 5 of 16 cases studied (two cases of trisomy 18, two cases of trisomy of chromosome 13 mosaic and one case of monosomy of chromosome X mosaic) [66].

Since this is a type of neural tube defect, adequate folate supplementation can reduce the chances of its development, and all the factors described above as leading to the development of neural tube defects also contribute to the development of iniencephaly.

2.10.4 Diagnostics

Cuillier et al. diagnosed iniencephaly by transvaginal ultrasound at the 9th gestational week as the earliest on the basis of acrania, encephalocele and shortened spine [69]. Iniencephaly can be diagnosed with certainty from gestational week 13. Ultrasound signs include extreme dorsiflexion of the head, abnormally short and deformed spine and occipital meningocele. Polyhydramnios is always present.

In terms of differential diagnosis, it should be distinguished from cervical hyperextension, prenatal teratoma, lymphangioma, cervical myelomeningocele, Klipper-Feil and Jarcho-Levin syndromes.

Klipper-Feil syndrome is caused by a failure of segmentation of the cervical vertebrae early in gestation. There is no spina bifida in this case. There are usually associated neurological symptoms, often associated with deafness. Most cases are sporadic, but autosomal dominant and recessive forms have been described [66]. Klipper-Feil syndrome can be subsequently treated surgically [68].

2.10.5 Postnatal morphology and associated disorders

In 84% of cases, other anomalies are also associated: anencephaly, encephalocele, hydrocephalus, cyclopia, mandibular defect, cleft lip and palate, cardiovascular anomalies, diaphragmatic hernia, omphalocele, gastroschisis, situs inversus, ren polycysticum, arthrogriposis. It may also be associated with an undescribed frequency of Dandy-Walker malformation, hydronephrosis, atresia of the gastrointestinal system and umbilical artery singularis [66].

2.11 The epigenetics of fetal craniospinal malformations

As the cause of craniospinal malformations is usually multifactorial, it is understandable that epigenetic pathways should play an important role in neurulation, although it is yet a poorly researched area. The relation between neural tube defects and epigenetic pathways is the most studied part.

Multiple ways have been identified which affect the formation of neural tubes epigenetically. The most reviewed topic is DNA methylation, although only animal studies are available. In DNA methyltransferases *DNMT3A* and *DNMT3B* knocked-out mice neural tube defects are more common. It is also possible that *DNMT3L* plays a role in the process too, although it is less researched [71]. Mouse models have also been made to examine histone acetyltransferases, and it has been identified that histone acetyltransferases *GCN5* and *CBP* are both play a significant role in neurulation. It is supposed that it has an impact on human neural tube development too. Nucleosome positioning, another example of epigenetic regulation, is also studied in association with NTDs, as a subtype of ATP-dependent chromatin remodelling complex, the SWI/SNF-related nucleosome remodelling BAF complex has been proved to play an important role in the relation of neural tube closing in mice. Also, micro RNAs, like CECR2 have been proven to cause exencephaly in animal models [70].

It is clear that the epigenetic pathways do not function on their own, but rather as a part of a complex system. It would be important to examine and understand more about that as a part of future research.

3. Conclusion

In summary, by the second trimester, developmental disorders affecting the nervous system can be diagnosed with a high degree of certainty by ultrasound, but in case of doubtful findings, additional imaging tests should be performed. The development of these disorders is multifactorial; both environmental and genetic factors play a role. In the case of an abnormal ultrasound finding, genetic testing should be performed to confirm the finding and to rule out inherited mutations in subsequent pregnancies.

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