Fetal Pathology of Neural Tube Defects – An Overview of 68 Cases

Fetalpathologie der Neuralrohrdefekte – ein Überblick über 68 NTD-Fälle

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Key words
neural tube defects, spina bifida, encephalocele, Chiari II malformation, fetal pathology

Schlüsselwörter
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ABSTRACT

Introduction The prevalence of neural tube defects worldwide is 1–2 per 1000 neonates. Neural tube defects result from a disturbance of neurulation in the 3rd or 4th week of development and thus represent the earliest manifestation of organ malformation. Neural tube defects (NTD) are classified into cranial dysraphism leading to anencephaly or meningoencephalocele and spinal dysraphism with or without meningomyelocele. In isolated form they have multifactorial causes, and the empirical risk of recurrence in Central Europe is 2%. As associated malformations they tend to occur sporadically, and in monogenic syndromes they follow Mendelian inheritance patterns with a high risk of recurrence.

Patients Autopsies were performed on 68 fetuses following a prenatal diagnosis of NTD and induced abortion.

Results The incidence of NTDs in our autopsied fetuses was 8% and 11% in fetuses with malformations. The percentage of fetuses with anencephaly, encephalocele or spina bifida was 24, 18, and 60%*, respectively. Analysis of the sex distribution showed a female preponderance in cranial dysraphisms but the sex distribution of spina bifida cases was equal. The extent and localization of NTDs varied, with lumbar-sacral cases clearly predominating. The proportion of isolated, associated and syndromic neural tube defects was 56, 23.5 and 20.6% respectively. In the majority of syndromes, the neural tube defect represented a not previously observed syndromic feature.

Conclusion The high proportion of NTDs with monogenic background underlines the importance of a syndrome oriented fetal pathology. At the very least it requires a thorough photographic and radiographic documentation of the fetal phenotype to enable the genetic counsellor to identify a syndromic disorder. This is necessary to determine the risk of recurrence, arrange confirming mutation analyses and offer targeted prenatal diagnosis in subsequent pregnancies.

ZUSAMMENFASSUNG

Einleitung Neuralrohrdefekte zeigen weltweit eine Prävalenz von 1–2 : 1000 unter Neugeborenen. Sie beruhen auf einer gestörten Neurulation in der 3.–4. Entwicklungswoche und stellen damit die früheste Manifestation einer Organfehlbildung
Introduction

Neural tube defects (NTD) are failures of parts of the neural tube to close. They originate during the 3rd and 4th week of development. As dorsal dysraphisms they represent the earliest organ malformation in humans [1–3]. Neural tube closure starts in the mid-section and continues outward in cranial and caudal directions with midline fusion of the neural fold, and ends with closure of the rostral and caudal neuropore on the 25th and 27th developmental day. While open NTDs result from failure of neural tube closure during neurulation, skin-covered NTDs are considered “post-neurulation defects” resulting from non-separation of the neural tube from the overlying ectoderm after the 4th week of development [2, 4]. The type and localization of an NTD will therefore indicate its determination period. The caudal third develops into the spinal cord, while the rostral two thirds develop into the brain. Depending on the localization, extent and resulting complications, NTDs can be lethal perinatally or may be associated with functional defects post partum such as incontinence, paresis and sensory deficits below the level of the lesion [3].

NTDs develop from primary genetic, secondary teratogenic or multifactorial disorders of organogenesis [4–6]. They present as exencephaly or anencephaly (AC), iniencephaly, meningoencephalocele (MEC) or spina bifida (SB) with or without meningomyelocele (MMC) in association with craniolisis, rachischisis or combined craniolischisis. Because of the exposed position of the defect NTDs are often detected early on prenatal ultrasound [7, 8]. Maternal folic acid supply pre- and post-conception has been found to be effective in primary prevention [9]. Fetal surgery to close spina bifida aperta (SBA) and prevent complications has also been carried out successfully. Infants operated in utero show improved motor and sensory functions in the lower limbs and a lower incidence of type II Chiari malformations (CM-II); surgery reduces the necessity of ventriculoperitoneal shunt to treat hydrocephalus by half and improves psychomotor development compared to infants with SBA who were operated postnatally [10–12].

Chiari malformation is the most significant condition associated with spina bifida. It occurs as one of four types and refers to a downward displacement and herniation of parts of the cerebellum, the brain stem and the medulla oblongata through an extended occipital foramen magnum into the spinal canal [13]. It may be followed by obstruction of cerebrospinal fluid outflow and thus causes non-communicating hydrocephalus [3]. In contrast to CM-I, CM-III and IV, CM-II is associated with caudal spina bifida and is present in around 75% of lumbosacral NTDs, of which 85.4% have associated hydrocephalus [8]. The increased cerebral pressure with compression of the cerebellum, brainstem and cervical spinal cord can result in nerve palsy, dyspnea and dysphagia [14]. In fetuses, the so-called “lemon and/or banana sign” is a sonographic indication of CM-II. The pathogenesis of CM-II is still not clear [13]. One of the oldest theories is the traction theory. It postulates that normal ascension of the caudal spinal cord is prevented by fixation at the level of the lumbosacral spina bifida. Another theory proposes that hydrocephalus is the primary event and that herniation and even spina bifida are secondary to pressure-related rupture of the neural tube. A third theory has focused on the hindrances to normal distension of the embryonic ventricular system because of the early loss of cerebrospinal fluid following failure of neural tube closure. This limits normal basicranial growth, leading to hypoplasia of the posterior fossa, and herniation in fetuses with normal brain growth. The lack of space prevents expansion of the medulla oblongata and pons and is responsible for its elongation and the characteristic Z-shaped kink. But none of the theories can provide a comprehensive explanation of the complex relationships at the cranio-cervical junction [15, 16].

In the 1970s, the prevalence of NTDs in Europe was still ~ 2 per 1000 births [17]. Following improvements in prenatal diagnosis, the prevalence of NTDs at birth dropped to < 1% [18, 19]. However, the prevalence of NTDs varies across different geographical regions and populations, as has been demonstrated impressively by a ninefold difference found in the frequency of NTDs between 8% in Ireland and 0.9% in Japan [3, 18, 20, 21]. Nasofrontal encephaloceles are rare in Europe with a prevalence at birth of 1:40–50 000 but are 10 times more common in Southeast Asia. In the
population of North Thailand the reported incidence even reaches 1/3500 [22]. A higher rate of NTDs is expected in the early stages of pregnancy [17, 23]. A Japanese study found a 4 times higher incidence of NTD (3.4–6.7 %) in first-trimester embryos who were aborted for socio-medical reasons [24]. NTDs were found in 3.6–8.8 % of cases of early spontaneous miscarriage. The majority of them had chromosomal anomalies [18, 25, 26].

We report here on 68 fetal neural tube defects out of a total of 815 autopsied fetuses. The main focus is put on phenotypical distribution patterns, the syndromic classification and the consequences for recurrence risks and prenatal diagnosability.

Material and Method

Fetopathological studies were carried out in 68 fetuses diagnosed with neural tube defects on prenatal ultrasound. The catchment area for the fetuses included prenatal centers in the “old” federal states of Germany; however, after 2010 most of the fetuses were obtained from the department of prenatal medicine of the University Hospital of Gießen and Marburg (UKGM).

Fetopathological examination included body measurement, careful inspection of the external appearance, in particular of the facial aspect, opening of the body cavity with removal of the organs, magnifier-assisted macroscopic preparation of the organs (LUXO WAVE PLUS magnifier lamp 3.5 dpt, Glamox Luxo Lighting GmbH, Hildesheim, Germany), microscopic examination and separate neuropathological examination of the central nervous system. The separate stages of the examination were documented photographically using a repro stand with a motorized height-adjustable camera mount (Kaiser Fototechnik GmbH & Co. KG, Buchen, Germany). All photographs were taken with a digital camera (Canon Power Shot G5). The camera was linked to a PC with a monitor showing an enlarged display to monitor the quality of the images and for further image processing (Adobe Photoshop); data were stored on a central server.

Radiological examinations were carried out using a cabinet X-ray system (Faxitron X-ray LX-60, Rohde & Schwarz GmbH & Co. KG, Cologne, Germany). The fetuses were fixed to the X-ray plates with adhesive tape. The exposure time for images of the entire body (posterior-anterior or anterior-posterior view and from the side) was 3–4.5 s at 20–35 kV depending on the size of the fetus.

Genetic investigations required the parents’ informed consent. This was obtained together with their consent to the fetopathological examination. Chromosome analysis was carried out after short and long-term culturing. Analysis was done using chorionic villi or amnion cells prenatally or using umbilical cord or fetal tissue, preferably obtained from the fetal Achilles tendon, post abortion. Umbilical cord, fetal Achilles tendon and muscle tissue was frozen and stored for use in molecular genetic analysis. Genomic DNA was extracted using standard procedures [28]. DNA was sent to a molecular genetic laboratory for mutation analysis. Sequencing of coding exons and adjacent intronic regions of the analyzed genes was done after amplification with PCR (polymerase chain reaction) by Sanger sequencing. If a syndrome was diagnosed clinically based on mutations in different genes, analysis was carried out using an NGS mutation panel [29].

Results

A total of 618 out of 815 cases autopsied for fetopathological examination in Marburg between 2004 and 5/2016 had malformations; 68 of these fetuses had a neural tube defect. In the majority of cases (64/68), the pregnancy was terminated by induced abortion. Fetal ages ranged from the 13th to the 36th week of gestation. Distribution of maternal age for the age groups < 20/21–25/26–30/31–35/36–40/41–45 years was 3/14/17/25/5/4. The ratio of “isolated” to “associated” to “syndromic” forms was 38 : 16 : 14. The NTD rate in our autopsied fetuses was 8 %. It was 11 % for the cases with malformations (Tables 1, 2 and 35).

Anencephalies (AC)

There were 16 cases (23.5 %) with anencephaly and cranio(rachio)schisis among the 68 cases with NTDs, two of which were delivered by cesarean section because of discordant monochorionic diamniotic and dichorionic triamniotic multiple pregnancy. The female to male ratio was f : m = 10 : 6. Findings for the 12 cytogenetically investigated fetuses were unremarkable. The 16 fetuses with anencephaly were grouped into 8 cases with meroencephaly, 3 cases with holocrania and 5 cases with craniorachischisis. On the fetogram the holocraunia presented as partial defects of the parietal and temporal bones and of the frontal bone; holocraunia additionally presented with partial defects of the occipital bone and a split foramen magnum (Figs. 1a to d). Craniorachischisis was accompanied by defects of adjacent vertebral arches and an open vertebral canal. In 2 cases, the opening extended to L1, in 1 case to L2 and in a further 2 cases to L5 (Fig. 1e). Craniorachischisis was accompanied by kyphoscoliosis or lordosis. Facial dysmorphisms included long nose, macrognathia and a proptosis in cases with defects of the orbital roof (Fig. 1c). Overlength of the limbs was another characteristic feature. Histological examination of the cerebrovascular membrane overlying the skull base showed islands of neural tissue and strongly vascularized arachnoid membranes. Eyes and optic nerves distal to the chiasm were unremarkable. Secondary renal gland hypoplasia can be explained by hypoplasia or necrosis of the pituitary gland.

Classification into clinical entities

There were 10 cases with “isolated” anencephaly. Schisis association was diagnosed in 4 cases with additional omphalocoele, diaphragmatic defect, and cleft lip and palate. One of these cases additionally had amniotic rupture sequence. One case had associated pseudo-median cleft lip and palate and cebophageo-holoprosencephaly, probably of genetic origin. One case corresponded to a TRAP sequence. One isolated case was a recurrence.

Meningoencephalocele (MEC)

Meningoencephalocele was found in 12 of the 68 NTD cases (17.7 %) with a female to male ratio of f : m = 8 : 4. Occipital MEC was present in 10 cases. One case presented with isolated nasofrontal MEC next to a defect of the frontal and ethmoid bone, visi-
### Table 1  Syndromic and associated neural tube defects.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gestational week</th>
<th>Sex</th>
<th>Type</th>
<th>Diagnosis</th>
<th>Malformations consequent to NTD</th>
<th>Cause</th>
<th>Syndrome-specific and other concomitant malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC 5</td>
<td>14 m</td>
<td>M-AC</td>
<td>Midline development defect? HPE syndrome?</td>
<td>∅</td>
<td>monogenic?</td>
<td>Celocephaly, pseudo-median cleft lip and palate (holopros-encephaly complex)</td>
<td></td>
</tr>
<tr>
<td>AC 6</td>
<td>15 + 4 f</td>
<td>M-AC</td>
<td>Schisis association</td>
<td>∅</td>
<td>sporadic</td>
<td>Bilateral cleft lip and palate</td>
<td></td>
</tr>
<tr>
<td>AC 7</td>
<td>15 + 5 f</td>
<td>M-AC</td>
<td>Schisis association/ amn. rupture seq.</td>
<td>∅</td>
<td>sporadic</td>
<td>Congenital limb defects, omphalocele</td>
<td></td>
</tr>
<tr>
<td>AC 8</td>
<td>16 + 2 m</td>
<td>M-AC</td>
<td>Schisis association</td>
<td>∅</td>
<td>sporadic</td>
<td>Bilateral CDH</td>
<td></td>
</tr>
<tr>
<td>AC 15</td>
<td>23 f</td>
<td>CRS</td>
<td>Schisis association</td>
<td>∅</td>
<td>sporadic</td>
<td>Bilateral cleft lip and palate, rib and vertebral defects, scoliosis of the cervical spine</td>
<td></td>
</tr>
<tr>
<td>AC 16</td>
<td>19 + 4 m</td>
<td>CRS</td>
<td>TRAP sequence discord. Twins</td>
<td>∅</td>
<td>sporadic</td>
<td>Acardia, defects of the liver, spleen, kidneys, pancreas, facial and abdominal cleft, limb defects, kyphoscoliosis</td>
<td></td>
</tr>
</tbody>
</table>

n = 6 f:m = 3:3 1 × genetic? 4 × schisis association 1/4 amn. rupture seq. 1 × TRAP seq. 1 × monogenic? 5 × sporadic

**MEC syndrome**

| MEC 8    | 21 + 5 f         | High occ. large | 13q syndrome microcephaly | chromosomal sporadic Ring 13 | Defects of the 1st finger and toe ray, cardiac and renal malformations et al. |
| MEC 9    | 18 + 5 m         | Double MEC high + low occ. covered | Meckel-Gruber syndrome | DWC | AR monogenic MKS3 mutation | MEC + poly cystic kidneys et al. (MKS without polydactyly) |
| MEC 10   | 18 + 4 m         | High occ., small | Peters-plus syndrome | DWC + H | AR monogenic B3GALT1 mutation Rec. | Peters anomaly of the anterior chamber of the eye, cleft lip and palate, PL SVC |
| MEC 11 + SB 41 | 16 f | Double high occ. covered + SB occulta L5–S1 | Noonan syndrome SB occ. | AD monogenic PTPN11 mutation c.226G>C | Hydrops, cystic hygroma, cardiac defect (HLH) et al. |
| MEC 12   | 18 m             | Low occ. small, covered | OFD 6 | DWC + H | AR monogenic Rec. | Brachymelia, meso axial hexa-dactyly, HLH, incomplete midline lower cleft lip, hypothy laric hamartoma et al. |

### MEC associated monogenic?

| MEC 5 + SB 42 | 24 + 5 f | Double low occ. + MMC, Th2–5 | Dandy-Walker malformation + occ. cephalocele? (# 6092222) | DWC + H + SB thor | monogenic (AD)? | Wedge-shaped thoracic vertebrae and thoracic hemivertebrae |
| MEC 6      | 21 m     | High occ. small, covered | Congenital malformation complex? | DWC + H | monogenic? Rec. | Agenesis of corpus callosum |
| MEC 7      | 19 f     | Low occ. small, covered | # 6092222? | DWC + H | monogenic (AD)? | Shortened 1st metacarpal bones, cervical ribs |
Table 1  Syndromic and associated neural tube defects.  (Continued)

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gestational week</th>
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<th>Cause</th>
<th>Syndrome-specific and other concomitant malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEC association</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEC 3</td>
<td>15 + 5</td>
<td>f</td>
<td>nasopharyngeal</td>
<td>schisis association (pentalogy of Cantrell)</td>
<td>H?</td>
<td>unknown sporadic?</td>
<td>Thoracogastroschisis, partial defects of the sternum, diaphragm and pericardium, cardiac defect, ectopy of the lung + abdominal organs, cleft lip and palate, unilateral dysmelia</td>
</tr>
<tr>
<td>MEC 4</td>
<td>14 + 5</td>
<td>f</td>
<td>occipito-cervical, large</td>
<td>schisis association</td>
<td>? unclear</td>
<td>Sporadic</td>
<td>Iniencephaly, omphalocele, VSD, CoA</td>
</tr>
<tr>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td>5 × syndrome 3 × ass. monogenic? 2 × schisis association</td>
<td>1 × chromosomal 4 × monogenic 3 × mono-genic? 2 × sporadic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB 32</td>
<td>18</td>
<td>m</td>
<td>LS-MMC L2–S2</td>
<td>trisomy 18 (Ts 18)</td>
<td>CM-II + H?</td>
<td>chromosomal 47,XY,+18</td>
<td>Left thumb ray defect, cleft palate, facial dysmorphisms, VSD, 2-Ao valve et al.</td>
</tr>
<tr>
<td>SB 33</td>
<td>20 + 4</td>
<td>f</td>
<td>CTL-SB C1–S3</td>
<td>Ts 18</td>
<td>CM-II + H</td>
<td>chromosomal 47,XX,+18</td>
<td>Lumbar gibbus, right-sided CDH, omphalocele, VSD, 2-pulm valve et al.</td>
</tr>
<tr>
<td>SB 34</td>
<td>25</td>
<td>m</td>
<td>LS-SB L1–S2</td>
<td>Ts 18</td>
<td>CM-II + H?</td>
<td>chromosomal 47,XY,+18</td>
<td>Kyphosis of the lumbar spine, bilateral radial aplasia, right-sided cleft lip and palate, VSD, 2-pulm + 2-Ao valve et al.</td>
</tr>
<tr>
<td>SB 35</td>
<td>21 + 4</td>
<td>f</td>
<td>LS-SB L2–S2</td>
<td>Triploidy</td>
<td>CM-II HPE</td>
<td>chromosomal 69,XXX</td>
<td>Holoprosencephaly and cebocephaly, syndactyly of 3rd–4th fingers and 2nd–3rd toes, left cystic kidney et al.</td>
</tr>
<tr>
<td>SB 36</td>
<td>21</td>
<td>m</td>
<td>LS-SB L2–S1</td>
<td>Bardet-Biedl syndrome (BBS)</td>
<td>CM-II, micro-ceph., H Ø</td>
<td>AR monogenic BBS4 mutation Rec.</td>
<td>Meckel syndrome: postaxial polydactyly of the right hand and feet (bilateral), medullary cystic kidneys</td>
</tr>
<tr>
<td>SB 37</td>
<td>19</td>
<td>f</td>
<td>LS-MMC L2–S1 + CT-SB occulta</td>
<td>CMNS</td>
<td>CM-II H Ø</td>
<td>AR monogenic</td>
<td>SCDO, thoracic kyphoscoliosis, omphalocele, urogenital malformation et al.</td>
</tr>
<tr>
<td>SB 38</td>
<td>21 + 5</td>
<td>m</td>
<td>L−MMC L2–L5</td>
<td>CMNS</td>
<td>CM-II Ø H Ø</td>
<td>AR monogenic</td>
<td>SCDO, urogenital malformation, bilateral talipes et al.</td>
</tr>
<tr>
<td>SB 39</td>
<td>20</td>
<td>m</td>
<td>LS-MMC L2–S4</td>
<td>Fryns syndrome (FS)</td>
<td>CM-II Ø H Ø</td>
<td>AR monogenic gene unknown</td>
<td>Fryns syndrome-like appearance, hydrops, bilateral CDH, omphalocele, complex cardiac, GI and urogenital malformations</td>
</tr>
<tr>
<td>SB 40</td>
<td>20</td>
<td>m</td>
<td>presacral/ anterior MMC L5–S5</td>
<td>Robinow syndrome (RS)</td>
<td>CM-II Ø H Ø</td>
<td>AD/AR monogenic</td>
<td>Cystic hygroma, cleft palate, preauricular appendages, complex cardiac defect, hypospadias, anal atresia et al.</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
<td></td>
<td></td>
<td>3 × Ts 18 1 × triploidy 1 × BBS/1 × FS 2 × CMNS/1 × RS</td>
<td>4 × chromosomal 5 × monogenic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued next page
ble as an uncovered cord-like prolapse of a ruptured encephal- 
cele sac. One case presented as nasopharyngeal MEC with schisis 
association (pentalogy of Cantrell) over a transsphenoidal defect.

MEC is a liquor-filled sac like herniation of meningeal and/or 
brain tissue through a circumscribed cranial defect. Occipital MECs 
are located in the upper or lower squamous occipital bone with in-
volve ment of the posterior fontanelle or of the foramen magnum.
They can lead to microcephaly depending on their size (Figs. 2b and  
d). In 5 cases, the MEC was covered by skin. One case with 
Meckel-Gruber syndrome (MKS) presented with a “double MEC” 
at the level of the upper and lower occipital bone (Figs. 2a and  
c). Double NTDs presented as MEC + MMC (meningoencephalocele) 
in a case with Noonan syndrome and in one case with associated

Dandy-Walker cyst (Figs. 3a to d). A case with iniencephaly 
showed craniorachischisis with progression of the MEC into MMC.

Classification into clinical entities

Two cases corresponded to “isolated” MEC, with one case 
associated with maternal pre-gestational diabetes (PGD) and cardiac 
malformation in a sibling. Associated cleft lip and palate and ab-
dominal clefts or malformations and dysmorphisms allowed 
2 cases to be classified as schisis association or pentalogy of 
Cantrell and 5 cases as syndromal disorders. The diagnosed syn-
dromes included del(13q) syndrome (Fig. 2d), MKS, Peters-plus 
syndrome (PPS) [30], Noonan syndrome (NS) and orofaciiodigital 
syndrome (OFD6), with the latter diagnosed after molecular anal-
alysis had excluded Pallister-Hall syndrome (Tables 1 and 2). The

### Table 1 Syndromic and associated neural tube defects. (Continued)

<table>
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<th>Case no.</th>
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<tr>
<td>SB 27</td>
<td>15 + 5</td>
<td>m</td>
<td>LS-MMCM</td>
<td>OEIS</td>
<td>CM-II Ø HC Ø</td>
<td>sporadic/multifactorial</td>
<td>Omphalence, cloacal exstrophy, imperforate anus, SB, scoliosis</td>
</tr>
</tbody>
</table>
| SB 28    | 16              | f   | LS-MMCM | OEIS     | CM-II H Ø   | sporadic/multifactorial | Omphalence, cloacal exstrophy, imperforate anus, SB, kypho-
scoliosis |
| SB 29    | 20 + 5          | f   | S-MMCM | OEIS     | CM-II H Ø   | sporadic/multifactorial | Omphalence, cloacal exstrophy, imperforate anus, SB |
| SB 30    | 19              | m   | TLS-MMCM | CDFD     | CM-II + H   | sporadic | Right-sided renal agenesis |
| SB 31    | 19              | f   | LS-MMCM | CDFD     | CM-II + H   | sporadic | Bilateral renal agenesis, ano-
rectal atresia, Mullerian duct anomalies, vertebral anomalies |

n = 5

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rectal atresia, Mullerian duct anomalies, vertebral anomalies |

n = 5

### Table 2 Numerical distribution of NTD groups.

<table>
<thead>
<tr>
<th></th>
<th>w:m</th>
<th>Syndrome</th>
<th>Ass. monogenic?</th>
<th>Associations</th>
<th>Ø-isolated</th>
<th>isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC n = 16</td>
<td>23.5</td>
<td>10:6</td>
<td>0/16 = 0%</td>
<td>1/16 = 6.25%</td>
<td>5/16 = 31.25%</td>
<td>6/16 = 37.5%</td>
</tr>
<tr>
<td>MEC n = 12</td>
<td>17.7</td>
<td>8:4</td>
<td>5/12 = 41.6%</td>
<td>3/12 = 25%</td>
<td>2/12 = 16.66%</td>
<td>10/12 = 83.3%</td>
</tr>
<tr>
<td>SB n = 40</td>
<td>58.8</td>
<td>20:20</td>
<td>9/40 = 22.5%</td>
<td>0/40 = 0%</td>
<td>5/40 = 12.5%</td>
<td>14/40 = 35%</td>
</tr>
<tr>
<td>NTD n = 68</td>
<td>100%</td>
<td>38:30</td>
<td>14/68 = 20.6%</td>
<td>4/68 = 5.9%</td>
<td>12/68 = 17.64%</td>
<td>30/68 = 44%</td>
</tr>
</tbody>
</table>

AC = anencephaly; ass. monogenic = associated monogenic; MEC = meningoencephalocele; AC = anencephaly; MMC = meningomyelocele; MDU = pericardial defect; NTD = neural tube defect; SB = spina bifida
diagnoses were confirmed by cytogenetic identification of ring chromosome 13 or by molecular genetic identification of mutations in the MKS3, B3GALTL and PTPN11 gene in 3 cases. In 3 syndromic (MKS, PPS, OFD6) and 3 non-syndromic cases, occipital MEC was associated with Dandy-Walker cyst (DWC) and hydrocephalus. Four cases (3 of them syndromic) were recurrences (▶ Tables 1 and 2).

Spina bifida (SB)

Spina bifida with and without MMC was the biggest group of neural tube defects with an incidence of 61.8% (n = 40 + 2*). In 2 of these cases* SB was associated with MEC (see above). The sex ratio was almost even at f: m = 20 + 2*: 20. One syndromic case was a recurrence. There was a familial predisposition in 3 isolated cases. The extent of non-closure ranged from 2 vertebrae (L5–S1) in SB occulta to a maximum of 10 vertebrae (L1–S5) in spina bifida aperta (SBA). Affected locations were 1× cervico-thoracolumbo-sacral, 1× thoracic*, 1× thoracolumbar (2,38% each), 4× thoracolumbosacral (9,52%), 2× lumbar (5%), 30 + 1*× lumbosacral (77,5%), 1× sacral and 1× presacral in 1 syndromic case (RS see below). In 29 of 42 cases, SB occurred in combination with MMC, i.e. the cystocele was elevated above skin level. X-ray images of the concomitant rachischisis showed separation of the vertebral arches (▶ Fig. 5b). The MMC was covered by a delicate membrane over an oval skin defect with raised edges (▶ Figs. 4a and 5c). In 7 cases with pronounced forms of spina bifida it was accompanied by partially gibbus-like kyphoscoliosis (▶ Figs. 5a and c). Vertebral anomalies were present in 7 cases; 1 case presented with diastematomyelia. Type II Chiari malformation (CM- II) was found on autopsy in all 26 cases with isolated SB, 24 of whom had hydrocephalus. CM-II was also found in 6 of the 9 syndromic cases – 3 × with hydrocephalus – and in 3 of the 5 association cases, 2 of which had hydrocephalus. Hypoplasia of the posterior cranial fossa and elongation and kinking of the medulla oblongata caused by inhibition of pontine expansion were already visible in the NTD fetus (▶ Fig. 6). Histological examination showed prolapsing meninges on SB cross-section and parts of the neural plate with an open central canal and an open subarachnoid space (▶ Figs. 4b and c). There were no signs of inflammation.

* Two NTD cases with double NTD (MEC + MMC) were listed in both the MEC and the SB groups, increasing the number of SB cases to 40 + 2.
Classification into clinical entities

Isolated SB was found in 26 of the 42 cases with spina bifida. Five SB cases were present as associated forms occurring concomitantly with OEIS complex (omphalocele, cloacal extrophy, imperforate anus, spinal anomalies) (3 ×) or caudal developmental field defect (2 ×). 9 + 1 * cases with SB could be attributed to a chromosomal (3 × Ts 18, 1 × triploidy) or monogenic syndrome. The latter included the autosomal recessive Bardet-Biedl syndrome (BBS) [29], Fryns syndrome (FS) and the autosomal dominant Robinow syndrome (RS) and Noonan syndrome (NS) (see above under MEC*). Two cases presented with severe spondylocostal dysostosis and concomitant urogenital malformations as part of the autosomal recessive Casamassima-Morton-Nance syndrome (CMNS). In the cases with BBS and NS, syndrome diagnosis was confirmed by the detection of mutations of the BBS4 and PTPN11 genes.

Prenatal findings on ultrasound

Exencephaly and anencephaly were already detectable on sonography in the first trimester of pregnancy. Characteristic features found in the longitudinal view included reduced crown-rump length, absent cranial vault and, in some cases, exposed neural tissue with cystic/solid parts floating in the amniotic fluid. Because of the absence of the bony skullcap, in the frontal view the fetal face is limited cranially by the orbits ("frog-eye sign"). Measurement of the biparietal diameter is not possible.

In cases with MEC, ultrasound showed the bony defect through which brain tissue (MEC) or meninges (MC) herniated, and the connection between the contents of the herniated sac and the intracranial space. Diagnosis of SBA was based on imaging of the fetal spine in all three planes. In the transverse and longitudinal planes, MMC or MC presented as a protrusion over the ossification centers with elevation of the neural placode above the level of the skin. In ruptured MMCs this was located at skin level with interruption of the skin contour. A neurological deficit was documented as pes equinovarus with reduced leg motor activity. In open spinal NTDs, typical intracranial signs were found in the horizontal plane in the second trimester of pregnancy; they included CM-II, obliterated cisterna magna and caudally displaced, usually hypoplastic cerebellum. The typical "banana sign" was visible in almost all cases.
Liquor circulation disorders resulted in a concavity of the parietal bones (“lemon sign”). Microcephaly was also often present. The severity of the concomitant hydrocephalus ranged from “borderline” to pronounced internal hydrocephalus. Closed spinal dysraphism, which occurs with diastematomyelia, was more difficult to detect as there are no secondary cranial changes. One open NTD was already detectable in the first trimester of pregnancy, based on the measurement of “intracranial translucency” (IT, 4th ventricle) and further parameters of the posterior fossa, e.g. the cisterna magna.

Discussion

Malformations were found in 618 of 815 autopsied fetuses. 68 of the 618 fetuses with malformations (11%) had neural tube defects (NTD). 23.5% of NTDs were anencephalies (AC), 17.7% were meningoencephaloceles (MEC) and 61.8%* had spina bifida (SB) with or without meningomyelocele (MMC). A comparable study from Copenhagen reported a rate of 14% NTDs in 693 fetuses with malformations. The ratio of AC:MEC:SB in their study was 43.3:17.5:39.2%, similar to the ratio of 44:7:49% reported in a Canadian study [31, 32]. The discrepancies in the distribution reported in their studies and our autopsied cohort could be explained by the high prenatal detection rate of smaller NTDs. When related to the overall figure of 20,363 pregnancies monitored by ultrasound by the Prenatal Center Gießen/Marburg between 2004–5/2016, the figure of 38 fetuses with NTD in this cohort gives an NTD rate of 0.19%. This corresponds to the overall prevalence rates reported above.

While the sex ratio for SB was evenly distributed (f:m = 22*:20), the rate of cranial NTDs was almost twice as high in female fetuses compared to males (f:m = 19:9). This was confirmed by a retrospective analysis of 85 cranial NTDs from the years 1976–1983 with a sex ratio of f:m = 56:29 [personal communication H.R.]. One explanation could be that many of the affected male embryos with severe cranial defects are miscarried in early pregnancy and are therefore not documented. This assumption is supported by the balanced gender ratio reported for early NTD embryos [27].
NTDs in female fetuses has been explained by the hypothesis that female embryos have a slower growth during neurulation, thus exposing them to harmful environmental effects over a longer period of time [33–36].

Three fetuses had double NTDs with MEC + MMC in one case each of Noonan syndrome (NS) and of associated Dandy-Walker cyst (DWC). Characteristic double upper and lower occipital MEC was observed in a case of Meckel syndrome (MKS) [37]. The extent of the neural tube defect in spina bifida ranged from 2 (spina bifida occulta) to 10 vertebrae and in total craniorachischisis included the entire spine. As reported in the literature, the largest group of NTDs concerned lumbosacral NTDs in 77.5% of the cases [31, 32]. Spina bifida occulta is usually asymptomatic and skin covered. It may represent an incidental finding on X-ray – as in our Noonan case – or it may become apparent postnatally by a circumscribed hypertrichosis or pigmented nevus. An anterior sacral meningocele may initially be suggestive of the Currarino triad (ASP association = anorectal and sacral malformations and mass in the presacral space with meningocele or teratoma). In our case, however, the fetus had Robinow syndrome (RS). In almost all cases, the NTD was diagnosed prenatally on ultrasound, in some cases already in the 1st trimester of pregnancy, and was subsequently confirmed at autopsy. In only 2 cases the MEC was not detected on ultrasound. In the fetus with Peters-plus syndrome (PPS) the MEC and SB occulta were too small, and in the fetus with Noonan syndrome (NS) the MEC was hidden in the nuchal hygroma.

The ratio of isolated to associated and syndromic NTDs was 55.9: 23.5 : 20.6%. One case was classified as embryopathy associated with maternal pre-gestational diabetes. While “association” is defined as a “non-random co-occurrence of certain anomalies of unknown cause”, the term “syndrome” refers to a “relatively constant and characteristic malformation pattern of genetic origin” – as long as “teratogenic syndrome like phenotypes” are correctly designated as “embryopathies”. Associations should also be differentiated from “sequential changes” such as CM-II and hydrocephalus. For non-syndromic NTDs we have to refer to an empirical risk of recurrence (RR), which is reported to be 2% for isolated forms, i.e. 10 times the geographical prevalence if only one child is affected and 20 times the geographical prevalence with two affected children [3, 36, 38]. Associations and non-hereditary structural chromosomal anomalies, such as ring chromosome 13, are sporadic. Numerical chromosome anomalies resulting from meiotic failure are largely determined by maternal age. Triploidies in diandry and digyny have a RR of 1–1.5%, independent of maternal age. In contrast, monogenic syndromes follow the pattern of Mendelian inheritance with a high RR. For autosomal recessive disorders such as MKS it is 25%. Autosomal dominant disorders like Noonan syndrome (NS) usually result from new mutations, and their RR in sibs is therefore not increased. However, if one parent is affected, the RR is 50%; if germ cell mosaicism is present in one of the parents the RR is ~ 1%. Diagnosing the syndrome makes it possible to carry out goal directed prenatal ultrasound investigations in subsequent pregnancies which go beyond the search for a single feature such as NTD. Targeted gene analyses can be carried out prenatally to detect or exclude known gene mutations. This underlines the importance or even necessity for syndrome-based diagnostic investigations at fetal autopsies and

**Fig. 4** “In-plane” view of lumbosacral spina bifida (L2–S1) in a female fetus at 19 + 2 weeks of gestation showing an oval soft tissue skin defect with strongly raised edges, a wide vertebral arch (Wb) fissure, exposed spinal canal with exposed non-closed neural plate (a) and cross-sectional view of the spina bifida (c). Cross-sectional histological image showing a flat neural plate with an open neural groove and open subarachnoid space (HE × 1.25) (b).
the targeted search for mutations, or at least for a photographic and radiographic documentation of anomalies which will allow retrospective syndrome assignment.

Our 14 syndromic NTDs included 5 chromosomal in part prenatally diagnosed cases [Ts 18, triploidy, del (13q)]. The rate of 7.4% for chromosomal NTDs equals the rates of 6.5–7% reported in the literature. The percentage of spina bifida with a chromosomal anomaly among our NTDs was 12.5% compared to the reported rates of 9–10% [31,32,39]. In the 9 monogenic syndromes, most NTDs and associated malformations had been detected on prenatal ultrasound. However, assignment to specific syndromal entities was made only after abortion during assessment of the autopsy findings.

The only syndrome in our cohort where the NTD was a syndrome-specific feature was MKS [37]. NTD is fairly common in Ts 18 and was found in 12% of all of our Ts 18 cases. NTD occasionally...

**Fig. 5** Thoraco-lumbo-sacral spina bifida in a male fetus of 21 + 6 weeks of gestation. The fetus shows gibbus-like kyphosis (↘), wide median fissure at the spinal arches Th8–S2, wedged vertebrae and hemivertebrae in the thoraco-lumbo-sacral region on lateral and posterior-anterior X-ray (a and b). Dorsal view of the open spinal canal with exposed necrotic neural tissue (c).

**Fig. 6** Chiari II malformation (CM-II) in the female fetus (20th week of gestation) shown in **Fig. 4**. The skull and spinal canal have been opened dorsally; there is herniation of parts of the cerebellum and medulla oblongata through the distended occipital foramen magnum (* edge) into the spinal canal (a). Brainstem and upper spinal cord show distinct elongation and Z-shaped kinking and lateral ventricles are dilated due to hydrocephalus as shown by a longitudinal section through the right cerebral hemisphere (b).
is observed with triploidy, del(13q) and spondylocostal dysostosis (SCDO) or Casamassima-Morton-Nance syndrome (CMNS). A case of Fryns syndrome (FS) with associated craniorachischisis was recently described for the first time [4]. NTDs had not previously been reported for any of the other syndromic cases in our cohort. According to the “London Dysmorphology Database” around 88 syndromes include MEC among their characteristics and 62 include MMC [41]. However, this list does not enclose the syndromes PPS, NS, orofaciodigital syndrome (OFD6), Bardet-Biedl syndrome (BBS), FS and RS which we observed in our cohort and which could therefore be added to the list. It is worth noting that fetuses with syndromic disease can be severely affected and present with unusual features, since syndromes are defined by their postnatal appearance and fetuses with severe manifestation of the disorder often die prenatally. In 4 cases (MKS, PPS, NS, BBS) the clinical diagnosis of the syndrome was confirmed by molecular genetic analysis. No molecular analysis was carried out in the remaining cases.

Schisis association, OEIS and caudal developmental field defect as observed in our cohort, represent well-known sporadic disorders. The association of MEC and DWC and of AC with cebocephaly, which we found in non-syndromic form in 3 cases and 1 case respectively, points to a genetic or monogenic background with a higher RR and would increase the percentage of genetic NTDs to 26.5%. In fact, one of these 4 cases was a recurrence. The malformation risk of maternal PGD is 2–9 times higher compared to non-diabetic women. It involves cardiac malformations in the first instance, followed by malformations of the CNS, such as NTD. It is noteworthy that there appeared to be a connection between PGD and anencephaly or encephalocoele but not between PGD and SB [6, 42]. Our case of isolated occipital encephalocoele coupled with maternal PGD and a sibling with cardiac malformation emphasizes this concomitance.

Conclusion

In this study of the pathologies of 68 fetuses with neural tube defects (NTD), the NTD rate calculated for a total of 618 fetuses with malformations was 11% which is comparable to the rates reported in the literature. When the study investigated the frequency distribution of anencephaly (AC), meningoencephalocoele (MEC) or spina bifida (SB), the number of fetuses with SB or MEC found in our cohort was significantly higher. This could be partly explained by the higher rate of detection of smaller defects. The evenly balanced ratio of females to males among the cases with caudal NTD and the clear female predominance in the cases with severe cranial NTDs would be consistent with a higher death rate of severely affected male fetuses in early pregnancy. The most significant finding in our study was the high percentage of NTDs of genetic origin (26.5%). Monogenic syndromes predominated, while only 7.4% of cases with NTD were caused by pre- or postnatally detectable chromosome anomalies. The high risk of recurrence for monogenic disorders and the finding that NTD was often not included in the spectrum of features reported for a specific syndrome and that therefore ultrasound investigations in subsequent pregnancies, focusing on NTDs only, will not be useful, emphasize the importance and necessity of syndrome-based investigation in fetal pathology, a targeted search for syndrome-related mutations, and careful photographic and radiographic documentation of fetal malformations and dysmorphisms. It would be very much in the interest of pregnant women and their family planning if a fetal pathologist with experience in syndromology would be more closely involved into prenatal medicine.

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Conflict of Interest

The authors declare that during compilation of the article no conflicts of interest were present as defined in the recommendations of the International Committee of Medical Journal Editors.

References
