CLINICAL RESEARCH ARTICLE

Cervical ribs and other abnormalities of the vertebral pattern in children with esophageal atresia and anorectal malformations

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BACKGROUND: In mammals the vertebral column has a constant pattern, particularly in the cervical region, which is likely the result of stabilizing selection. The prevalence of cervical ribs and extended vertebral pattern abnormalities is increased in deceased fetuses and neonates and associated with structural and chromosomal anomalies. The hypothesized close interaction between vertebral patterning and organogenesis in early embryogenesis could be studied in children with esophageal atresia and anorectal malformations by evaluating the radiographs of the vertebral column and the type and number of associated anomalies

METHODS: The vertebral pattern of 367 children diagnosed with an esophageal atresia ($n = 135$), anorectal malformation ($n = 215$), or both ($n = 17$) was assessed on radiographs.

RESULTS: The vertebral pattern was abnormal in 170/202 (84.2%) children in whom this could be assessed, and cervical ribs were present in 147/335 (43.9%). Extended vertebral pattern abnormalities became more frequent with an increasing number of associated structural abnormalities. Cervical ribs were particularly common in children with chromosomal and genetic abnormalities (18/20, 90.0%).

CONCLUSIONS: Cervical ribs are frequent and abnormal vertebral patterns are more extended in the presence of associated anomalies. These findings support the hypothesized selection against vertebral patterning variations and emphasize the relevance of assessment of the vertebral pattern.

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INTRODUCTION

The number of ribs and vertebrae in mammals has been remarkably stable during the course of evolution. The regular human vertebral pattern consists of 7 cervical vertebrae, 12 thoracic rib-bearing vertebrae, and 5 lumbar vertebrae. Variations in vertebral and/or rib number, especially at the cervical region, are rarely present in the general population.¹ This seems to be the result of a selection against changes in cervical vertebral number.² This selection is thought to be the result of indirect, rather than direct effects.³ It has been suggested that variations in the cervical vertebral pattern, such as the presence of a (rudimentary) cervical rib or multiple cervical ribs, are associated with fetal death or death at a young age.³ The higher prevalence of (rudimentary) cervical ribs in deceased fetuses and neonates and pediatric cancer patients compared to the low prevalence in the general population supports this hypothesis.^{1,2,4} Altered Homeobox (*Hox*) gene expression could play a role, because *Hox* genes are involved in both vertebral patterning and tumorigenesis,⁵ and cervical ribs have been observed in mice with *Hox4–6* mutations.^{6–12} Abnormalities involving the ribs and vertebrae have been described in patients with esophageal atresia (EA) and anorectal malformations (ARMs), particularly within the context of the

VACTER-L (Vertebral, Anal, Cardiac, TracheoEsophageal fistula, Renal and Limb anomalies) association.^{13–15} Despite the association with vertebral abnormalities, studies focusing on the pattern of the vertebral column and the presence of cervical ribs in patients with EA and/or ARM are lacking. The standard of care for children with EA and ARM involves screening for VACTER-L association, including imaging of the vertebral column. The availability of these radiographs and the high frequency of associated abnormalities in this population enable evaluation of the possible relationship between the type and the number of affected organ systems and the extent of disruption of the vertebral pattern. A higher prevalence of abnormalities involving the vertebral pattern in children with EA and ARM and more extended abnormalities of the vertebral pattern in children with associated abnormalities would support the hypothesized close interaction between vertebral patterning and organogenesis in early embryogenesis. It would also strengthen the postulated selection against vertebral variations and emphasize the value of a careful evaluation of the vertebral pattern. This study thereby provides the opportunity to investigate the principle of selection against variations of the vertebral pattern, regardless of the fact that EA and ARM are distinct anomalies. Contrary to previous

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Fig. 1 Schematic representation of different patterns of the vertebral column, with increasing extent of abnormalities from left to right.² R, regular pattern; CT, shift at the cervicothoracic boundary; CT_TL, shift at the cervicothoracic and thoracolumbar boundary; CT_TL_LS, shift at cervicothoracic, thoracolumbar, and lumbosacral boundary

studies in deceased fetuses and neonates,^{2,16,17} we aimed to determine the vertebral pattern in children with EA and/or ARM, of which the majority remained alive. The extent of the abnormalities of the vertebral pattern was related to the number of associated structural abnormalities and the presence of chromosomal or other genetic abnormalities.

METHODS

Subjects

All patients born between January 2006 and July 2017, and diagnosed with an EA and/or ARM at the Erasmus University Medical Center Sophia Children's Hospital Rotterdam, were included.

Protocol

All neonates born with a congenital anatomical malformation are enclosed in a protocolized program tailored to the specific malformation. Neonates with an EA and/or ARM are included in the VACTER-L program. Part of this program is a radiographic examination of the vertebral column. These radiographs were made both in an anterior–posterior and a lateral view (5.6–12.6 mAs, 40–46 kV, Philips Optimus ZBM3/NZR91).

Measurements

If a rib on the most cranial or most caudal thoracic vertebra had a length of less than half of the rib of the adjacent thoracic vertebra, it was considered rudimentary.² If the length of the transverse processes of the seventh cervical vertebra was more than the transverse process of the first thoracic vertebra, it was considered a rudimentary cervical rib. If the rib was longer than half of the first thoracic rib, it was considered a (complete) cervical rib.² If the uppermost cervical vertebrae were not visualized, the CT boundary was determined based on the characteristics of the transverse processes. The transverse process of the first thoracic vertebra extends in a superior direction, while the transverse process of the seventh cervical vertebra is directed inferiorly.¹⁸ In the presence of supernumerary non-cervical ribs, the ribs were considered thoracic if 5 or more caudal vertebrae without ribs were present, and considered lumbar if 4 or fewer caudal vertebrae were present. If the total number of vertebrae and ribs could be assessed, the pattern was classified according to ten Broek et al.² (see Fig. 1). Changes occurring at the cervical region are considered more serious than those at thoracic or lumbar

regions. The radiographs of a subset of 30 patients were reviewed twice by the same reviewer and once by a second reviewer to determine the intraobserver and interobserver agreement. Data concerning pregnancy and outcome, type of (associated) abnormalities, surgery, and chromosomal or genetic analyses were retrieved from medical electronic patient files. The presence of associated abnormalities was classified according to EUROCAT (European Registration of Congenital Anomalies and Twins)¹⁹ and based on autopsy, postnatal inspection, surgical reports, and/or postnatal radiographic examinations. VACTER-L association was defined as the presence of ≥ 3 of the following: vertebral abnormalities, anal atresia, cardiac defects, tracheal anomalies, including tracheoesophageal fistula, EA, renal and radial abnormalities, and other limb abnormalities and the absence of a different syndrome with similar features.²⁰

Data analysis

SPSS (IBM Corp released 2013, IBM SPSS statistics for windows, version 24.0; IBM Corp, Armonk, NY) was used for statistical analysis. Descriptive analyses were used to evaluate the prevalence of different vertebral patterns and cervical ribs. χ^2 and Fisher's exact test were used to compare categorical data between groups. Independent *t* tests or one-way analysis of variance was used to compare continuous normally distributed data between two or more than two groups. Non-parametric tests were used for analysis of continuous non-normally distributed data. A *p* value < 0.05 was considered significant. For determination of the level of intraobserver reliability, Cohen's κ measure of agreement test was used. κ Values between 0.41 and 0.60 were considered to be moderate, between 0.61 and 0.80 to be substantial, and between 0.81 and 1.00 were considered almost perfect.²¹ If the vertebral pattern or the presence of cervical ribs could not be determined, cases were excluded from the respective analyses. This study was approved by the ethics committee of the Erasmus University Medical Center Rotterdam (MEC-2017-1080).

RESULTS

The cohort consisted of 215 patients with an ARM (58.6%), 135 with an EA (36.8%), and 17 patients with both an EA and ARM (4.6%). The patient characteristics are shown in Table 1. An EA and/or ARM was diagnosed after birth in most patients; 80 cases (21.8%) had been referred for a prenatal advanced anomaly scan, because of polyhydramnios or suspicion of structural

Table 1. Patient characteristics in the cohort of patients with EA, ARM, or EA and ARM

	EA (n = 135, 36.8%)	ARM (n = 215, 58.6%)	EA and ARM (n = 17, 4.6%)	Total (n = 367) (%)	P value
Gender					0.11
Male	83 (61.5)	109 (50.7)	11 (64.7)	203 (55.3)	
Female	52 (38.5)	106 (49.3)	6 (35.3)	164 (44.7)	
Twins	10 (7.4)	12 (5.6)	0	22 (6.0)	0.63
Advanced anomaly scan	27 (20.0)	46 (21.4)	7 (41.2) ^a	80 (21.8)	0.02
Abnormal findings ^b	17 (63.0)	31 (67.4)	7 (100)	55 (68.8)	0.15
Polyhydramnios	20 (74.1)	2 (4.3) ^a	6 (85.7)	28 (35.0)	0.00
GA at delivery (weeks)	38 (25–42) ^a	38 (26–42) ^a	36 (28–40) ^a	38 (25–42)	0.00
GA (weeks)					
<32	6 (4.4)	6 (2.8)	2 (11.8)	14 (3.8)	
32–37	40 (29.6)	26 (12.1)	8 (47.1)	74 (20.2)	
≥37	85 (63.0)	146 (67.9)	7 (41.2)	238 (64.9)	
Unknown	4 (3.0)	37 (17.2)	0	41 (11.2)	
Birth weight (g)	2810 (725–4300) ^a	3103 (1010–5010) ^a	2280 (1080–3440) ^a	2948 (725–5010)	0.00
Birth weight category					
<p10	41 (30.4)	35 (16.3)	8 (47.1)	84 (22.9)	
p10–p90	86 (63.7)	131 (60.9)	9 (52.9)	226 (61.6)	
>p90	1 (0.7)	8 (3.7)	0	9 (2.5)	
Unknown	7 (5.2)	41 (19.1)	0	48 (13.1)	
Associated structural anomalies					0.03
No	42 (31.1)	78 (36.3)	1 (5.9) ^a	120 (32.6)	
Yes	93 (68.9)	133 (61.9)	16 (94.1) ^a	244 (66.3)	
Unknown	0	4 (1.9)	0	4 (1.1)	
VACTER-L association	16 (11.9)	40 (18.6)	14 (82.4) ^a	70 (19.1)	0.00
(Neonatal) death	6 (4.4)	8 (3.7)	1 (5.9)	15 (4.1)	0.64
<1 week after birth	2 (33.3)	2 (25.0)	1 (100)	4 (26.7)	
<1 year after birth	3 (50.0)	6 (75.0)		10 (66.7)	
>1 year after birth	1 (16.7)	0		1 (6.7)	
Age at last control (years) ^c	6 (0–16)	7 (0–12)	8 (1–14)	7 (0–16)	0.61
Surgery					0.00
Yes	132 (97.8)	174 (80.9) ^a	17 (100)	323 (88.0)	
No	3 (2.2)	41 (19.1) ^a	0	44 (12.0)	

Data are presented as numbers and percentages or median and range. GA gestational age, VACTER-L individuals with ≥3 of the following abnormalities: cerebral abnormalities, anal atresia, cardiac defects, and tracheal anomalies, including tracheoesophageal fistula, esophageal atresia, renal and radial abnormalities, and other limb abnormalities. *P*-values <0.05 are marked in bold

^aSignificant differences between the anomaly group with and both other anomaly groups

^bThis included any ultrasound abnormality, except for polyhydramnios and single umbilical artery

^cNeonatal deaths excluded from analysis

abnormalities. Polyhydramnios was present in most of the prenatally referred cases with an EA or EA and ARM (20/27, 74.1% and 6/7, 85.7%, respectively). A structural abnormality was detected in approximately two-thirds (55/80, 68.8%) of the fetal ultrasound scans. This included any abnormality, excluding polyhydramnios or a single umbilical artery, but not necessarily a suspected EA or ARM. Patients with an EA or EA and ARM were born at a significantly earlier gestational age ($p < 0.001$) and had a significantly lower birth weight compared to patients with an ARM ($p < 0.001$). The criteria for VACTER-L association were met in 70 patients (19.1%). The prevalence was highest in children with an EA and ARM (14/17, 82.4%), which is consistent given the fact that two out of three associated features requested for VACTER-L association were already present in these children. The number of thoracic ribs, the presence of (rudimentary) cervical ribs, and the presence of lumbar ribs could be determined in 339 (92.4%), 335 (91.3%), and 353 (96.2%) children, respectively. In 165 (45.0%) cases, the vertebral pattern could not be classified, mainly because

the cervical vertebral column was not entirely visualized on the available radiographic examinations, which were made according to the protocol. The number of vertebrae and ribs in children with an EA and/or ARM and in the total population is provided in Table S1. As is shown in Fig. 2, a regular vertebral pattern was seen in 32/202 (15.8%) children in whom this could be assessed. A cervical patterning abnormality (including cervicothoracic (CT), CT and lumbosacral (CT_LS), cervicothoracic and thoracolumbar (CT_TL), and all three vertebral regions (CT_TL_LS) was seen in 137/202 (67.8%). The vertebral pattern with a variation at the CT boundary exclusively was the most common pattern ($n = 64$, 32.2%). Cervical ribs were present in 147/335 (43.9%), and most of them were rudimentary (138/147, 93.9%). A statistically significant difference between the prevalence of cervical ribs was not found between patients with an EA, ARM, or both ($p = 0.12$). Twelve thoracic rib pairs were present in 229/339 (67.6%) of the total study population in whom this could be assessed, while 110 (32.4%) had a deviating number. Of the 353 children (96.2%) in

Fig. 2 The vertebral pattern in the total group and subgroups categorized according to type of abnormality (left) and in patients with only gastrointestinal abnormalities, including EA and/or ARM (1), or associated abnormalities in an increasing number of organ systems (right middle), or in patients with associated anomalies, categorized according to affected organ system (right). The total number of cases in which the vertebral pattern could be assessed is depicted above the bars. R, regular pattern; CT, shift at the cervicothoracic boundary; TL, shift at the thoracic lumbar boundary; LS, shift at the lumbar sacral boundary; CT_TL, shift at the cervicothoracic and thoracolumbar boundary; CT_TL_LS, shift at cervicothoracic, thoracolumbar, and lumbosacral boundary; EA, esophageal atresia; ARM, anorectal malformation

Fig. 3 Radiograph (left) and three-dimensional reconstruction of computed tomography image (right) showing bilateral rudimentary cervical ribs (arrows) in an infant born with an esophageal atresia

whom the presence of lumbar ribs could be assessed, 34 (9.6%) had lumbar ribs. The frequency of widespread abnormalities of the vertebral pattern increased in children with an increasing number of affected organ systems, other than the gastrointestinal system (Fig. 3). The prevalence of the most abnormal vertebral pattern, involving all vertebral boundaries (CT-TL-LS), increased from 3.4% if only the gastrointestinal system was affected, to 25.0% if abnormalities were present in ≥ 5 different organ systems. An abnormal vertebral pattern involving the CT region showed an increase from 59.3 to 91.6% in children with an abnormality involving only the gastrointestinal system and children with abnormalities in ≥ 5 different organ systems. Abnormalities involving the cardiovascular and urogenital system were the most common associated anomalies. Within the subgroups with associated skeletal anomalies and limb defects, CT boundary pattern variations were most frequent, but the distribution of the vertebral patterns between the subgroups with associated abnormalities in different organ systems did not show evident differences (Fig. S1). Neonatal death occurred in 15/367 (4.1%). These occurred in neonates with trisomy 18 ($n=2$), severe structural anomalies for which non-intervention and palliative care was provided ($n=4$), sepsis ($n=4$), sinus thrombus ($n=1$), left ventricular thrombus complicating extracorporeal membrane oxygenation ($n=1$), or systemic hypoperfusion after receiving a relatively large cardiac shunt ($n=1$). The definitive cause of death

could not be extracted from the medical file in two cases. In 9 of the 15 deceased neonates the vertebral pattern could be assessed. An abnormal vertebral pattern that involved the CT boundary pattern variation was found in 8/9 (88.9%), which is higher compared to the children who were alive (129/193, 66.8%), but this was not a statistically significant difference ($p=0.28$). Most patients had a normal GTG-banded karyotype ($n=186$). Similarly, most molecular karyotypes were normal ($n=136$) or included a copy number variation with uncertain significance ($n=25$). However, GTG-banded karyotyping ($n=151$), molecular karyotyping ($n=104$), or both ($n=86$) resulted in the identification of 12 aneuploidies. Moreover, seven pathogenic structural anomalies were identified. Suspicion of a specific syndrome based on their phenotypical spectrum of anomalies and subsequent sequencing of the gene(s) of interest (either with whole-exome sequencing or Sanger sequencing, $n=54$) had a higher diagnostic yield ($n=13$). Table 2 shows the characteristics of the vertebral pattern in the cases with abnormal chromosomal or DNA results. Cervical ribs were frequent in aneuploidies (8/12, 66.7%). In children with chromosomal and genetic abnormalities in whom the entire vertebral pattern could be assessed, abnormalities including the CT region were very common (18/20, 90.0%). The intraobserver agreement for the presence of cervical ribs and the number of thoracic ribs was almost perfect ($\kappa=0.86$ and 0.84 , respectively) and substantial ($\kappa=0.79$) for the presence of lumbar ribs. The intraobserver agreement for determination of the vertebral pattern was moderate ($\kappa=0.49$). The interobserver agreement for the presence of cervical ribs, lumbar ribs and the number of thoracic ribs was almost perfect ($\kappa=0.86$, 1.00 , and 0.81 , respectively). The interobserver agreement for the vertebral pattern was moderate ($\kappa=0.53$). The lower agreement in determination of the vertebral pattern was mainly due to differences between scoring the number of cervical vertebrae as “non-assessable” or “normal” and different scoring of the number of lumbar vertebrae.

DISCUSSION

The prevalence of cervical ribs and other abnormalities of the vertebral pattern was remarkably high in this cohort of live-born

Table 2. The vertebral pattern in cases with abnormal chromosomal, SNP array, or DNA results

Syndrome	Cervical rib present (n/ total n)	Vertebral pattern (n)	Structural abnormality (n)
Trisomy 21	5/7	R CT (2) CT_TL CT_TL_LS Unknown (2)	ARM EA (1), ARM (1) EA ARM EA (1), ARM (1)
Monosomy X	1/1	CT_TL	ARM
Trisomy 18	1/2	CT_TL_LS Unknown	EA ARM
Triple X syndrome	1/2	CT_TL Unknown	EA and ARM EA
Cat eye syndrome	2/2	CT (2)	ARM (2)
4p16.3 deletion	1/1	CT_TL	EA
1q21.1 duplication	0/1	R	ARM
3q22–q24 deletion	1/1	CT	ARM
22q11 deletion	0/1	Unknown	ARM
CHARGE syndrome	4/4	CT (1) CT_TL (2) CT_TL_LS (1)	EA EA (2) EA
Feingold syndrome	1/1	CT_TL_LS	EA
Holt Oram syndrome	0/1	Unknown	ARM
De Grouchy syndrome	0/1	Unknown	ARM
Mandibulofacial dysostosis	1/1	Unknown	EA
Townes Brocks	0/1	Unknown	ARM
Mosaic mutation <i>USP9X</i> gene	1/1	CT_TL	ARM
STAR syndrome	0/1	Unknown	ARM
Ulnar mammary syndrome	0/1	Unknown	ARM
Smith Magenis syndrome	0/1	Unknown	ARM
Kabuki syndrome	1/1	CT_TL	ARM
Fanconi anemia	0/1	Unknown	EA and ARM
Total	20/33	R (2) CT (6) CT_TL (8) CT_TL_LS (4) Unknown (13)	EA: 12 ARM: 19 EA and ARM: 2

R regular pattern, CT shift at the cervicothoracic boundary, CT_TL shift at the cervicothoracic and thoracolumbar boundary, CT_TL_LS shift at cervicothoracic, thoracolumbar, and lumbosacral boundary, EA esophageal atresia, ARM anorectal malformation

neonates with an EA and/or ARM, compared to the reported frequencies in the general population (0.2–8.0%). It was comparable to the frequencies described in deceased fetuses and neonates with and without structural abnormalities.^{1,2,16} The prevalence of cervical ribs and abnormal vertebral patterns in children with chromosomal anomalies was high and similar to previous studies.^{22,23} As expected, the frequency of cervical ribs and the extent of abnormalities in the vertebral pattern increased when multiple organ systems were affected. This finding is in line

with the study of ten Broek et al.,² who included deceased fetuses and neonates, and strengthens the hypothesis that cervical ribs and other vertebral patterning abnormalities are markers of disrupted embryonic development.¹⁶ The co-occurrence of abnormalities of the vertebral pattern and structural abnormalities in other organ systems can be explained by the “developmental field theory,” entailing that during blastogenesis, the embryo consists of a relatively undifferentiated primary field, from which the organ primordia will arise at a later stage. A disruption of blastogenic processes could therefore have effects on multiple organ systems.^{24,25} The specific timing and duration of the disruption would explain the variability in the type and number of affected organ systems and seems to be of more importance than the disrupting factor itself.²⁶ This implies that an interruption earlier in blastogenesis would affect more progenitor fields, which leads to associated defects in multiple organ systems.²⁷ Segmentation of the vertebral column takes place during blastogenesis.²⁴ Because the vertebral column develops in a craniocaudal direction,²⁸ abnormalities involving the cervical region would commonly co-occur with abnormalities in multiple organ systems, leading to a stronger selection against cervical patterning abnormalities than against abnormalities involving caudal vertebral regions.

This study supports the hypothesized indirect selection against variations in (cervical) vertebral patterning, which is likely due to the concomitant occurrence of abnormalities of the vertebral pattern and the development of other organ systems. The proportion of patients with cervical ribs and abnormalities involving multiple vertebral regions clearly increased in patients with a higher number of associated anomalies. A significant proportion of these patients, as well as all patients with an EA, would have died postnatally without receiving adequate treatment, thereby preventing this trait from becoming more prevalent. Because of the association of more extended abnormalities in vertebral patterning with multiple abnormalities and chromosomal anomalies, information regarding the vertebral pattern in patients with an EA and/or ARM appears to be relevant for the assessment of the neonatal condition. The presence of (rudimentary) cervical ribs is not always mentioned in radiology reports and seems to be underreported.^{29,30} Our findings stress the importance of a critical evaluation of the vertebral pattern and the cervical region in particular. In children with cervical ribs or extensive abnormalities of the vertebral pattern, clinicians should be particularly alert to the presence of associated anomalies. In addition to a physical examination, screening for urogenital and cardiovascular abnormalities is recommended. Assessment of the vertebral pattern may not only be of value for neonates with an EA and/or ARM, as was demonstrated by this study, but could also apply to neonates with abnormalities in other organ systems. A limitation of this study is the insufficient quality of some of the radiographic images for the assessment of the (cervical) vertebral pattern and cervical ribs, because radiographs were made in the context of clinical care. This might have led to an underestimation of the prevalence of rudimentary cervical ribs. Second, a control group consisting of healthy children was lacking, due to ethical considerations. Several studies reporting on the prevalence of vertebral patterning abnormalities in the general population were available for comparison.

Strengths of this study are the inclusion of a relatively large group with both isolated and associated EA and/or ARM and the strong intraobserver and interobserver agreement for the presence of cervical ribs and assessment of the thoracic vertebral region, which were most relevant considering our hypothesis. In conclusion, the high frequency of cervical ribs, particularly in children with multiple associated abnormalities, supports the hypothesis of a selection against variations in cervical vertebral patterning. Disruptions of early embryonic developmental processes seem to play a role in the occurrence of abnormalities in both the vertebral pattern and other organ systems.

Determination of the number of vertebrae and ribs is relevant in children with an EA and/or ARM, because the extent of abnormalities of the vertebral pattern could be an indication of the degree of disturbed embryonic development.

AUTHOR CONTRIBUTIONS

P.C.S., A.J.E., and T.E.C.-O. contributed to the design of the study. M.B., P.C.S., and E.B. acquired and analyzed data. All authors interpreted data. P.C.S. and M.B. drafted the manuscript and all authors critically revised it for content. The final version of the manuscript was approved by all authors.

ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41390-019-0631-1>) contains supplementary material, which is available to authorized users.

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