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CLINICAL RESEARCH ARTICLE

Spectrum of congenital anomalies among VACTERL cases: a EUROCAT population-based study

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BACKGROUND: The VACTERL (Vertebral anomalies, Anal atresia, Cardiac malformations, Tracheo-Esophageal fistula, Renal anomalies, Limb abnormalities) association is the non-random occurrence of at least three of these congenital anomalies: vertebral, anal, cardiac, tracheo-esophageal, renal, and limb anomalies. Diagnosing VACTERL patients is difficult, as many disorders have multiple features in common with VACTERL. The aims of this study were to clearly outline component features, describe the phenotypic spectrum among the largest group of VACTERL patients thus far reported, and to identify phenotypically similar subtypes.

METHODS: A case-only study was performed assessing data on 501 cases recorded with VACTERL in the JRC-EUROCAT (Joint Research Centre-European Surveillance of Congenital Anomalies) central database (birth years: 1980–2015). We differentiated between major and minor VACTERL features and anomalies outside the VACTERL spectrum to create a clear definition of VACTERL.

RESULTS: In total, 397 cases (79%) fulfilled our VACTERL diagnostic criteria. The most commonly observed major VACTERL features were anorectal malformations and esophageal atresia/tracheo-esophageal fistula (both occurring in 62% of VACTERL cases), followed by cardiac (57%), renal (51%), vertebral (33%), and limb anomalies (25%), in every possible combination. Three VACTERL subtypes were defined: STRICT-VACTERL, VACTERL-LIKE, and VACTERL-PLUS, based on severity and presence of additional congenital anomalies.

CONCLUSION: The clearly defined VACTERL component features and the VACTERL subtypes introduced will improve both clinical practice and etiologic research.

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INTRODUCTION

In 1973, Quan and Smith[1] described the non-random occurrence of congenital anomalies, including vertebral anomalies, anorectal malformations (ARM), esophageal atresia with or without tracheo-esophageal fistula (EA/TEF), radial anomalies, and renal dysplasia as the VATER association. A few years later, cardiac and other limb anomalies were added to the spectrum, and the VACTERL (Vertebral anomalies, Anal atresia, Cardiac malformations, Tracheo-Esophageal fistula, Renal anomalies, Limb abnormalities) association (VACTERL) was clinically confirmed.[2,3] The prevalence of VACTERL was 1 in 20,000 births in 2012–2016 according to the EUROCAT (European Surveillance of Congenital Anomalies) central database.[4] VACTERL usually occurs sporadically, but familial inheritance and increased prevalence of component features in first-degree relatives are also observed.[5–8] As the VACTERL etiology is still unknown, patients are generally clinically diagnosed when they have three or more VACTERL component features, without phenotypic or genetic evidence of an alternative diagnosis.[9–11] However, diagnosing these patients can be challenging. First of all, the phenotypic heterogeneity among VACTERL cases is large, because any combination of three VACTERL component features qualifies for a diagnosis. In addition, other disorders have multiple features in common with VACTERL, for example, MURCS association, Fanconi anemia, Townes–Brocks syndrome, caudal regression syndrome, and CHARGE syndrome.[9] As a result, diagnosing VACTERL becomes even more complex.

In order to obtain clarity about the phenotypic description of VACTERL, several studies have examined the frequency of the different component features and combinations of component features present among VACTERL cases.[7,12–18] Both the studies of Botto et al.[12] and Solomon et al.[17] showed that all VATER/VACTERL component features, except for pre-axial limb anomalies, were observed in more than 50% of cases. Several other studies have also focused on component features in VACTERL cases, but because of small study populations, the external validity of these study results can be questioned.[7,13–16,18] A detailed description of the classification of congenital anomalies that make up the VACTERL components is often missing in the literature, which limits the comparison of component feature frequencies among studies.[19] However, a detailed description of the classification is

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important, especially since the diagnostic criteria for VACTERL have been debated ever since VACTERL was described.[1] Clear guidelines for establishing a VACTERL diagnosis may contribute to earlier diagnosis and better management of affected children, and may help identify potential subtypes of VACTERL patients in which a different etiology is likely.[12]

The aim of our study was to describe clear diagnostic criteria for VACTERL and to investigate the frequency of the VACTERL component features in the largest group of VACTERL cases described to date. We describe the phenotypic heterogeneity in cases diagnosed with VACTERL obtained from population-based registries and identify phenotypically similar subtypes that may be helpful in etiologic research.

METHODS

EUROCAT data collection

The Joint Research Centre (JRC)-EUROCAT central database, operated by the European Commission's JRC in Ispra, Italy, contains standardized individual data on congenital anomalies that are collected by the member registries of the EUROCAT Network.[20] In this study, we used data from 29 full member EUROCAT registries in 16 European countries for the birth years 1980–2015 (Table 1). The data were ascertained through different data sources, such as hospital records, birth and death certificates, maternal questionnaires, and post-mortem examinations. All congenital anomalies, syndromes, and chromosomal abnormalities were coded according to the International Classification of Diseases (ICD) version 9 or 10 with the British Paediatric Association (BPA) one digit extension. Some registries also registered McKusick codes (also known as OMIM numbers). The data for this study were extracted by the JRC-EUROCAT Central Registry in February 2018. All 29 registries gave approval for their data to be included. As this study used anonymous data obtained from registries, it did not require ethical approval of the committee.

Questionnaire

All participating registries received a questionnaire (see Supplemental Information) to obtain more detailed information on how VACTERL cases are registered in the specific registry.

Case definitions

A VATER/VACTERL diagnosis was based on ICD9 codes 759895 and 75989, ICD10 code Q8726, and OMIM/McKusick codes 192350, 314390, and 267950. Cases with an ICD9 code 75989 were only selected when VATER/VACTERL was specified in the text description, as this code is not specific for VATER/VACTERL. We included live births, fetal deaths/stillbirths (≥20 weeks of gestation), and terminations of pregnancy for fetal anomaly following prenatal diagnosis (termination of pregnancy for fetal anomaly (TOPFA)). Fetuses or infants with a diagnosis of VACTERL with hydrocephalus (VACTERL-H) were excluded, as VACTERL-H is a distinct condition with a suggested autosomal recessive or X-linked inheritance (OMIM %276950). In addition, we excluded cases with a syndrome that explains their phenotype.

VACTERL limits

We predefined a detailed classification of congenital anomalies that were considered to be part of VACTERL, in which we made a distinction between major and minor VACTERL features (Table 2). Major VACTERL features are congenital anomalies that are part of the classical VACTERL features. Minor VACTERL features are congenital anomalies not typically seen in VACTERL cases, but occurring in one of the organ systems that are commonly affected among VACTERL cases. The complete rationale behind the inclusion or exclusion of congenital anomalies as major or minor VACTERL features is provided in the Supplemental Information. In short, we included vertebral anomalies in the non-sacral spine as major features and sacral spine anomalies as minor features. All ARM types were included as major features, except for cloacal malformations. Most cardiac malformations affecting the cardiac chambers and connections or the aorta were included as major features, but anomalies affecting the cardiac valves, pulmonary artery, and great veins were considered minor features. All EA/TEF types were included as major features. Renal agenesis and other reduction defects of the kidneys were included as major features, whereas secondary effects, such as hydronephrosis or vesicoureteral reflux were included as minor features. Only the radial-ray anomalies, including thumb hypoplasia, were considered major features, while other upper limb anomalies were considered minor features.

In addition, we categorized congenital anomalies that were not considered to be part of VACTERL (Supplemental Information Table S1), again divided into major and minor congenital anomalies. As we provided a complete overview, not all congenital anomalies were necessarily observed within our study population. Minor congenital anomalies were defined based on the EUROCAT minor anomalies for exclusion (see Chapter 3.2 of EUROCAT Guide 1.4.[21]) All other congenital anomalies were considered major congenital anomalies. We made five exceptions for major congenital anomalies outside the VACTERL spectrum that frequently occur simultaneously with major VACTERL features: (1) Tethered cord is often observed in combination with ARM, and therefore not included when it occurred in combination. (2) Respiratory system anomalies can often be explained by cardiac anomalies, EA/TEF, or renal anomalies. Therefore, respiratory system anomalies are considered secondary anomalies and, consequently, they were not included. (3) As the embryology of the genitourinary (GU) system is partly shared with that of the anorectal and renal organ systems, GU anomalies were not considered when they occurred in combination with ARM or renal anomalies. (4) Congenital deformities of hip were not included either, as these are very common congenital anomalies. (5) Congenital anomalies affecting the lower limbs were not included either, as they are often included as limb component features for VACTERL in the clinic.

Predefined VACTERL subtypes

Individual congenital anomalies reported for the VACTERL cases in this study were judged by two independent researchers (M.G. and R.v.d.P) and a clinical geneticist (C.M.). When a case had both a major and a minor VACTERL component feature affecting the same organ system, only the major VACTERL feature was registered.

Based on the presence of major and minor VACTERL features, and possibly congenital anomalies outside the VACTERL spectrum, the VACTERL cases were subdivided into four predefined and mutually exclusive subtypes: (1) STRICT-VACTERL; (2) VACTERL-LIKE; (3) VACTERL-PLUS; and (4) NO-VACTERL. The STRICT-VACTERL subtype contains cases with ≥3 major VACTERL features and no major congenital anomalies outside the VACTERL spectrum, except for the restrictions mentioned above. In the VACTERL-LIKE subtype, we included cases with <3 major VACTERL features, but with additional minor VACTERL features adding up to ≥3 major and minor VACTERL features combined. Cases with only the major VACTERL components ARM and EA/TEF were also included in this subtype. Major congenital anomalies outside the VACTERL spectrum were not present in these cases either. In the VACTERL-PLUS subtype, we included all cases that fulfilled either the STRICT-VACTERL or the VACTERL-LIKE subtype criteria, but had additional major congenital anomalies outside the VACTERL spectrum. Finally, cases with <3 VACTERL component features not included in the VACTERL-LIKE subtype were included in the NO-VACTERL subtype and were excluded from all further analyses. In all subtypes, the cases may have additional minor congenital anomalies.

Table 1. Overview of the EUROCAT registries and the number and prevalence of the VACTERL patients, which were included in the study

Country	Registry	Years covered by registry	Total of live and stillbirths in the population	VACTERL cases (*N*)	VACTERL prevalence[a]
Austria	Styria	1985–2012	327,302	7	0.21
Belgium	Antwerp	1989–2015	427,101	25	0.59
Croatia	Zagreb	1983–2015	206,837	3	0.15
France	Auvergne	2002–2014	149,305	12	0.80
	Brittany	2011–2015	179,180	15	0.84
	French West Indies	2009–2015	68,665	1	0.15
	Ile de la Reunion	2001–2015	218,584	4	0.18
	Paris	1981–2015	1,134,068	15	0.13
Germany	Mainz	1990–2014	85,315	10	1.17
	Saxony Anhalt	1980–2015	543,762	32	0.59
Ireland	Cork and Kerry	1996–2015	179,563	9	0.50
	South East Ireland	2005–2014	74,527	1	0.13
Italy	Emilia Romagna	1981–2015	1,022,236	10	0.10
	Tuscany	1980–2015	770,279	3	0.04
Malta	Malta	1986–2015	137,337	4	0.29
The Netherlands	Northern Netherlands	1981–2015	577,868	50	0.87
Norway	Norway	1999–2012	836,535	3	0.04
Poland	Wielkopolska	1999–2015	626,876	2	0.03
Portugal	South Portugal	1990–2015	408,832	3	0.07
Spain	Basque Country	1990–2014	458,334	12	0.26
	Valencia Region	2007–2015	446,903	13	0.29
Switzerland	Vaud	1989–2015	207,593	15	0.72
Ukraine	OMNI-Net	2005–2015	333,189	4	0.12
UK	East Midlands and South Yorkshire	1998–2012	998,655	13	0.13
	Northern England	2000–2015	512,608	30	0.59
	South West England	2005–2015	545,302	19	0.35
	Thames Valley	1991–2015	411,928	19	0.46
	Wales	1998–2015	602,776	40	0.66
	Wessex	1994–2015	615,000	23	0.37
Total	29 registries	1980–2015	13,106,460	397	0.30

[a]Rate of live births, stillbirths, and terminations of pregnancy for fetal anomaly following prenatal diagnosis present in this study, per 10,000 births

Statistical analysis

Statistical analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Infant and maternal characteristics assessed were: gender, birth year, birth type (live birth, fetal death/stillbirth, TOPFA), survival (>1 week postpartum), gestational age (in completed weeks), birth weight (in g), twin pregnancy (vs. singleton pregnancy), and maternal age at birth (in years).

Descriptive analyses were performed for the frequencies of the VACTERL component features and the different combinations of VACTERL component features in the total group of VACTERL cases and in the predefined subtypes. In addition, we stratified for gender to see whether the phenotypes were different between male and female cases. χ^2 tests were performed to test whether the differences between VACTERL subtypes and between male and female cases were statistically significant after Bonferroni correction for the number of comparisons ($p < 0.003$, corresponding to 16 tests).

In order to identify phenotypes that would cluster together, we performed exploratory factor analyses based on principal components for the major VACTERL features and the total of major and minor VACTERL features in the total group of VACTERL cases. Clusters containing three or more VACTERL features with a minimum factor loading of 0.5 were considered clinically relevant.

Comparison with the literature

We clearly defined all VACTERL component features, within and outside VACTERL spectrum, which enabled us to compare our results with the existing literature. For comparison, we selected the two largest studies that were published thus far, which included 286 and 60 VACTERL cases.[12,17] Additionally, we compared our study results with the most recently published study that included 36 VACTERL cases[13] and with one of the earliest studies including 50 VACTERL cases.[3]

RESULTS

Registries

In total, 29 EUROCAT registries participated in this study and completed the study questionnaire. Overall, there was broad consensus about the registration of VACTERL cases, as all registries recorded a VACTERL diagnosis if it was made in the clinic. In one registry, a VACTERL diagnosis was also recorded if a case had three or more congenital anomalies being part of the VACTERL

Table 2. Specification of the anomalies commonly associated with VACTERL (based on the ICD10 coding for congenital anomalies)

VACTERL component feature	Major VACTERL features (including ICD10 codes)	Minor VACTERL features (including ICD10 codes)
Vertebral	• Congenital scoliosis due to congenital bony malformation (Q763), including hemivertebrae, fusion, or failure of segmentation with scoliosis • Other congenital malformations of spine, not associated with scoliosis (Q7640 Q7642), including congenital absence or fusion of spine, hemivertebrae, malformation of spine, and supernumerary vertebrae (specified in the non-sacral region) • Combination of unspecified vertebral anomalies with rib anomalies (Q765 or Q766)	• Klippel Feil (Q761) • Scoliosis without specification: "due to bony malformation" (Q675) • Other congenital malformations of spine, not associated with scoliosis (Q7641), including congenital absence or fusion of spine, hemivertebrae, malformation of spine, and supernumerary vertebrae (unspecified or specified in the sacral spine) • Other congenital malformations of spine, not associated with scoliosis (Q764), including kyphosis, lordosis, malformation of lumbosacral (joint) region, platyspondyly • Cervical rib and other congenital anomalies of ribs (Q765, Q766), including accessory rib, congenital absence, cervical rib, fusion, or malformation of ribs
Anorectal	• Congenital absence, atresia, and stenosis of rectum with or without fistula (Q420, Q421) • Congenital absence, atresia, and stenosis of anus with or without fistula (Q422, Q423), including congenital fistula of rectum and anus (Q436), congenital rectovaginal fistula (Q522), and congenital urethra-rectal fistula (Q6474) • Ectopic anus (Q435)	• Persistent cloaca (Q437) • Cloacal exstrophy (Q641)
Cardiac	• Congenital malformations of the cardiac chambers and connections (Q20), excluding isomerism of atrial appendages (Q206) • Congenital malformations of cardiac septa (Q21) • Congenital malformations of great arteries, including patent ductus arteriosus (only registered if GA ≥37 weeks) and anomalies affecting the aorta (Q250–Q254)	• Isomerism of atrial appendages (Q206) • Congenital malformations of valves (Q22, Q23) • Other congenital malformations of the heart (Q24) • Congenital malformations of the great arteries affecting the pulmonary artery, other and unspecified great arteries (Q255–Q259) • Congenital malformations of great veins (Q26)
Tracheo-esophageal	• Esophageal atresia without fistula (Q390) • Esophageal atresia with tracheo-esophageal fistula (Q391), including broncho-esophageal fistula • Congenital tracheo-esophageal fistula without atresia (Q392)	
Renal	• Renal agenesis and other reduction defects of kidney (Q60) • Renal dysplasia (Q614) • Lobulated, fused, and horseshoe kidney (Q631)	• Polycystic kidneys (Q611, Q612, Q613), medullary cystic kidney, other cystic kidney diseases, and unspecified cystic kidney diseases (Q615, Q618, Q619) • Congenital obstructive defects of renal pelvis and congenital malformations of ureter (Q62) • Other congenital malformations of kidney (Q63) excluding lobulated, fused, and horseshoe kidney (Q631)
(Upper) Limb	• Accessory thumb(s) (Q691) • Congenital absence of hand and finger(s), thumb affected (Q7131), or Q713 with describing thumb defects in text • Longitudinal reduction defect of radius (Q714), including clubhand • Other reduction defects of upper limb(s) (Q718) with text information describing thumb defects • Other congenital malformations of upper limb(s) (Q74) with text information describing thumb defects	• Congenital deformity of finger(s) and hand (Q681) • Accessory finger(s) (Q690) • Fused fingers (Q700), webbed fingers (Q701), and polysyndactyly (Q704) • Congenital absence of (parts of) upper limbs (Q710–712), longitudinal reduction defects of ulna (Q715), lobster-claw hand (Q716), other and unspecified reduction defects of upper limbs (Q718–Q719), or Q713 without describing thumb defects in text • Other congenital malformations of upper limb(s) (Q740)

The rationale for the division into major and minor VACTERL features is provided in the Supplemental Information
GA gestational age

spectrum. In most registries, the person who makes the clinical diagnosis is either pediatrician ($N = 24$) or clinical geneticist ($N = 26$). All registries also code the individual congenital anomalies present in the VATER/VACTERL cases. In 16 registries, genetic testing is performed to rule out other diagnoses with overlapping features.

Case characteristics

In total, 545 cases had a VACTERL diagnosis recorded in the central JRC-EUROCAT database. After exclusion of 10 cases without information on individual anomalies present, 21 cases with VACTERL-H, 13 cases with selected syndromes in addition to VACTERL, and 104 cases that we classified as "NO-VACTERL," 397 cases remained for analyses. The excluded syndromes were Fanconi anemia, caudal regression syndrome, Goldenhar syndrome, sirenomelia, and the 22q11 deletion syndrome. Of the cases included, 213 cases (54%) were categorized as STRICT-VACTERL, 82 (20%) as VACTERL-LIKE, and 102 (26%) as VACTERL-PLUS.

The characteristics of our study population are listed in Table 3. In the total VACTERL group, the majority of cases were male (65%) and more than 75% of the cases were born alive between 2000 and 2015. Of these live born cases, 87% survived beyond the first week postpartum, 44% were delivered preterm, and 51% had a

Table 3. Characteristics of VACTERL patients among the total group of patients and in the VACTERL subtypes

	Total group of VACTERL patients ($N = 397$, 100%)[a], *N* (%)	STRICT-VACTERL ($N = 213$, 54%)[a], *N* (%)	VACTERL-LIKE ($N = 82$, 20%)[a], *N* (%)	VACTERL-PLUS ($N = 102$, 26%)[a], *N* (%)
Gender				
Male	256 (65.1)	133 (62.7)	53 (65.4)	70 (70.0)
Female	137 (34.9)	79 (37.3)	28 (34.6)	30 (30.0)
Year of birth or TOPFA				
1980–1989	22 (5.5)	11 (5.2)	4 (4.9)	7 (6.9)
1990–1999	72 (18.1)	38 (17.8)	16 (19.5)	18 (17.6)
2000–2009	166 (41.8)	85 (39.9)	39 (47.6)	42 (41.2)
2010–2015	137 (34.5)	79 (37.1)	23 (28.0)	35 (34.5)
Type of birth				
Live birth	301 (75.8)	156 (73.2)	75 (91.5)	70 (68.6)
Stillbirth	13 (3.3)	7 (3.3)	1 (1.2)	5 (4.9)
TOPFA	83 (20.9)	50 (23.5)	6 (7.3)	27 (26.5)
Survival (>1 week postpartum)[b]	256 (87.4)	135 (88.2)	69 (95.8)	52 (76.5)
Preterm birth (<37 weeks)[b]	128 (44.0)	62 (41.3)	30 (41.7)	36 (52.2)
Low birth weight (<2500 g)[b]	151 (51.2)	72 (47.4)	40 (54.1)	39 (56.5)
Twin pregnancy	22 (5.6)	13 (6.1)	5 (6.1)	4 (4.0)
Maternal age at birth (≥35 years)	68 (17.9)	39 (19.0)	8 (10.1)	21 (21.9)

TOPFA termination of pregnancy for fetal anomaly
[a]Numbers do not add up to total number due to missing values: gender 1%, birth year 0%, birth type 0%, survival 3%, preterm birth 3%, low birth weight 2%, twin pregnancy 0.3%, and maternal age at birth 4%
[b]Only calculated for live births

low birth weight. Among all VACTERL cases, 6% were from twin pregnancies, and 18% had a mother aged ≥35 years. When we stratified for gender, we observed 4% stillbirths and 22% TOPFAs for male cases compared to 2% stillbirths and 17% TOPFAs for female cases ($p = 0.13$). Among male compared to female live births, we found lower percentages of preterm deliveries (41% vs. 48%, $p = 0.26$) and low birth weight (46% vs. 60%, $p = 0.02$).

Among the different VACTERL subtypes, we observed differences in case characteristics, for example, in the percentage of live births, stillbirths, and TOPFAs ($p = 0.005$). The most striking differences were seen between VACTERL-LIKE and VACTERL-PLUS for live births (92% vs. 69%) and TOPFA (7% vs. 27%). For survival beyond the first week postpartum, similar differences were observed among the three subgroups ($p = 0.002$), with survival being highest in the VACTERL-LIKE subtype (96%) and lowest in the VACTERL-PLUS subtype (77%). The latter subtype also included a slightly higher percentage of preterm infants (52%) than the other subtypes (41% and 42%; $p = 0.29$). The percentages of cases with low birth weight varied between 47% for the STRICT-VACTERL subtype and 57% for the VACTERL-PLUS subtype ($p = 0.38$). For twin pregnancies no major differences were observed. Maternal age appeared to be lower in the VACTERL-LIKE subtype, with only 10% of mothers being ≥35 years old compared to 19% and 22% in the other subtypes, although this difference was not statistically significant ($p = 0.11$).

Frequencies of congenital anomalies

VACTERL features. In the total VACTERL group, the most commonly observed major VACTERL features were ARM and EA/TEF (both 62%), followed by cardiac defects (57%), renal defects (51%), vertebral defects (33%), and limb defects (25%) (Table 4). When we also included the minor VACTERL features, all VACTERL component features were observed in minimally 62% of the cases, except for limb anomalies (32%). In the STRICT-VACTERL subtype, the percentages of major VACTERL features were much higher than those of minor VACTERL features, whereas this was reversed for vertebral anomalies in the VACTERL-LIKE subtype. In addition, the percentages of major and minor renal and limb anomalies were almost equal in the latter subtype. In the VACTERL-PLUS subtype, EA/TEF was less frequently observed in comparison with the two other VACTERL subtypes. Major renal anomalies and minor cardiac anomalies were observed more frequently in comparison to the STRICT-VACTERL and VACTERL-LIKE subtypes. The percentages of the other VACTERL component features lie between that of the STRICT-VACTERL and VACTERL-LIKE subtype.

In Table 3, we showed that our VACTERL study population contained more male than female cases. To study whether the VACTERL phenotypes differed between male and female cases, the analyses were stratified by gender (Supplemental Information Table S2). Major ARM features were observed less frequently in female (56%) compared to male (65%) cases in the total group ($p = 0.06$). This difference became more apparent in the VACTERL-LIKE (36% vs. 57%, $p = 0.07$) and VACTERL-PLUS subtypes (43% vs. 61%, $p = 0.10$), although the smaller numbers hamper interpretation. In contrast, major cardiac features were observed more frequently in female (65%) compared to male (54%) cases in the total group ($p = 0.03$) and in the VACTERL-LIKE subtype (50% vs. 28%, $p = 0.05$). Concerning EA/TEF, the percentages seemed to be higher for female cases in the VACTERL-PLUS subtype (63% vs. 43%, $p = 0.06$) and lower for female cases in the VACTERL-LIKE subtype (57% vs. 70%; $p = 0.25$). Renal anomalies were more frequently observed among male compared to female cases in the STRICT-VACTERL subtype (62% vs. 49%, $p = 0.06$), as were limb defects in the VACTERL-PLUS subtype (31% vs. 13%, $p = 0.06$).

The component features ARM and EA/TEF are considered core features of VACTERL, as these are more specific congenital anomalies with a more clear delineation compared to the other

Table 4. Absolute numbers and percentages (%) of VACTERL component features and additional congenital anomalies among the total group of VACTERL patients and in the VACTERL subtypes

	Total group of VACTERL patients ($N = 397$)	STRICT-VACTERL ($N = 213$)	VACTERL-LIKE ($N = 82$)	VACTERL-PLUS ($N = 102$)
VACTERL component feature				
Vertebral				
Major	131 (33.0)	95 (44.6)	6 (7.3)	30 (29.4)
Minor	130 (32.7)	42 (19.7)	47 (57.3)	41 (40.2)
Total	261 (65.7)	137 (64.3)	53 (64.6)	71 (69.6)
Anorectal				
Major	247 (62.2)	148 (69.5)	41 (50.0)	58 (56.9)
Minor	12 (3.0)	2 (0.9)	6 (7.3)	4 (3.9)
Total	259 (65.2)	150 (70.4)	47 (57.3)	62 (60.8)
Cardiac				
Major	227 (57.2)	144 (67.6)	29 (35.4)	54 (52.9)
Minor	26 (6.5)	7 (3.3)	8 (9.8)	11 (10.8)
Total	253 (63.7)	151 (70.9)	37 (45.1)	65 (63.7)
Tracheo-esophageal				
Major	247 (62.2)	144 (67.6)	53 (64.6)	50 (49.0)
Renal				
Major	202 (50.9)	122 (57.3)	21 (25.6)	59 (57.8)
Minor	65 (16.4)	28 (13.1)	23 (28.0)	14 (13.7)
Total	267 (67.3)	150 (70.4)	44 (53.7)	73 (71.6)
Limb				
Major	98 (24.7)	63 (29.6)	9 (11.0)	26 (25.5)
Minor	27 (6.8)	9 (4.2)	12 (14.6)	6 (5.9)
Total	125 (31.5)	72 (33.8)	21 (25.6)	32 (31.4)
Additional congenital anomalies[a]				
Congenital anomalies outside the VACTERL spectrum	102 (25.7)	–	–	102 (100)
Tethered cord	9 (2.3)	4 (1.9)	3 (3.7)	2 (2.0)
Respiratory system anomalies	45 (11.3)	19 (8.9)	6 (7.3)	20 (19.6)
Genitourinary anomalies	63 (15.9)	38 (17.8)	9 (11.0)	16 (15.7)
Hip anomalies	6 (1.5)	3 (1.4)	1 (1.2)	2 (2.0)
Lower limb anomalies	50 (12.6)	26 (12.2)	8 (9.8)	16 (15.7)

[a]All additional congenital anomalies were listed as major congenital anomalies outside the VACTERL spectrum, with five exceptions: tethered cord, respiratory system anomalies, genitourinary anomalies, hip anomalies, and lower limb anomalies

VACTERL component features.[11,19] In this study, 358 cases (90%) had either the major component features ARM or EA/TEF or both. We observed ARM in 111 cases (28%) and EA/TEF in 111 cases (28%), while 136 cases (34%) had both ARM and EA/TEF in the total VACTERL group.

Congenital anomalies not part of VACTERL. Congenital anomalies outside the VACTERL spectrum occurred frequently in our study population, as 26% had one or more additional major congenital anomalies (Table 4). By definition, these cases were included in the VACTERL-PLUS subtype. Congenital anomalies outside the VACTERL spectrum were observed in all organ systems, but the digestive system was most often affected, including, for example, duodenal atresia or malrotation of the colon. When we stratified for gender, we did not observe substantial differences in the percentages of congenital anomalies outside the VACTERL spectrum (Supplemental Information Table S2).

Tethered cord was observed in nine cases, all in combination with ARM. GU anomalies were observed in 63 cases (16%) and in only four of them, the GU anomaly was not present in combination with ARM or renal defects. Respiratory anomalies were observed in 11% and lower limb anomalies in 13% of the total VACTERL group. If we were to include lower limb anomalies within the VACTERL spectrum, 41% of the total population would have had a limb component feature for VACTERL, instead of the 32% of cases with major or minor upper limb anomalies reported.

Combinations of VACTERL component features

In the total group, 69% had three or more major VACTERL features. A triad of major VACTERL component features was observed in 49%, a tetrad in 15%, and a pentad in 4%, while the full VACTERL spectrum was observed in only one case. In the STRICT-VACTERL subtype, we observed a triad of VACTERL component features in 71% of the cases, a tetrad in 22%, a pentad in 7%, and the full spectrum in one case. Due to the inclusion criteria for the VACTERL-LIKE subtype, it did not contain cases with three or more major VACTERL features, but in the VACTERL-PLUS subtype, 43% of the cases had a triad, 15% a tetrad, and 2% a pentad of major VACTERL features.

Many different combinations of component features were observed in the total group of VACTERL cases, illustrating the large heterogeneity (Supplemental Information Table S3). Among all combinations, eight combinations of congenital anomalies were observed at or above the arbitrary threshold of 10 cases: ACTE, ATER, CTER, ACR, VCTE, VAR, ACTER, and VAC, with V the abbreviation for vertebral anomalies, A for ARM, C for cardiac anomalies, TE for EA/TEF, R for renal anomalies, and L for limb anomalies. When minor VACTERL features were included (Supplemental Information Table S4), new combinations of congenital anomalies were observed in 10 or more cases: VCR, VATE, VTER, VACR, VATER, VCTER, VACTER, and VACRL. Vertebral defects were present among all of these new combinations, as a direct result of the vertebral component feature having the highest percentage of minor VACTERL features. Logically, ARM or EA/TEF were part of almost every combination of congenital anomalies, as these were observed most frequently.

In summary, no clear subtypes of phenotypically similar VACTERL cases were observed because of the large heterogeneity of combinations of major VACTERL component features. These findings were supported by the exploratory factor analyses in which we did not identify clusters of three or more major or major and minor VACTERL features.

Comparison with the literature. When we compared the results of our study with a selection from the literature (Table 5), we noticed that: (a) most studies did not report the exact congenital anomalies that were included among the different component features clearly; (b) the frequencies of the component features ARM and EA/TEF in our study were quite consistent with that reported in the literature, except for the study by Khoury et al.[3] that reported much lower numbers; and (c) the number of the remaining component features became more comparable with the existing literature when we also included the minor VACTERL features.

Table 5. Distribution of the different congenital anomalies according to the current study and the literature

Component feature	Major features (total group of VACTERL patients, $N = 397$), *N* (%)	Major features (STRICT-VACTERL, $N = 213$), *N* (%)	Major and minor features (total group of VACTERL patients, $N = 397$), *N* (%)	Major and minor features (STRICT-VACTERL, $N = 213$), *N* (%)	Husain et al.[13] ($N = 36$), *N* (%)	Solomon et al.[8] ($N = 60$), *N* (%)	Botto et al.[12] ($N = 286$), *N* (%)	Khoury et al.[3] ($N = 50$), *N* (%)
V	131 (33%)	95 (45%)	261 (66%)	137 (64%)	29 (81%)	47 (78%)	190 (66%)	18 (36%)
A	247 (62%)	148 (70%)	259 (65%)	150 (70%)	22 (61%)	33 (55%)	236 (83%)	20 (40%)
C	227 (57%)	144 (68%)	253 (64%)	151 (71%)	30 (83%)	48 (80%)	136 (48%)	40 (80%)
TE	247 (62%)	144 (68%)	247 (62%)	144 (68%)	25 (69%)	31 (52%)	168 (59%)	12 (24%)
R	202 (51%)	122 (57%)	267 (67%)	150 (70%)	22 (61%)	43 (72%)	231 (81%)	41 (82%)
L	98 (25%)	63 (30%)	125 (32%)	72 (34%)	18 (50%)	28 (47%)	111 (39%)[a]	34 (68%)

V vertebral anomalies, *A* anorectal anomalies, *C* cardiac anomalies, *TE* tracheo-esophageal anomalies, *R* renal anomalies, *L* limb anomalies, *NS* not shown
[a]$N = 100$ (35%) of the limb anomalies were pre-axial

DISCUSSION

We aimed to aid clinical practice and etiologic research concerning VACTERL by presenting clear guidelines for the diagnosis of VACTERL. Strict diagnostic guidelines for VACTERL have been debated ever since VACTERL was described. A European-wide consensus is reached amongst participating EUROCAT registries regarding these diagnostic guidelines. We showed that a large amount of heterogeneity exists among VACTERL phenotypes, as almost every combination of component features is observed. Because of this heterogeneity, no phenotypically similar VACTERL subtypes were identified. The VACTERL subtypes STRICT-VACTERL, VACTERL-LIKE, and VACTERL-PLUS that were introduced in this study were predefined, based on the severity of the congenital anomalies and the presence or absence of additional congenital anomalies outside the VACTERL spectrum. These subtypes can be used as subgroups in etiologic studies to aid the identification of genetic and environmental risk factors.

A major strength of this study is its size, as we have included the largest population-based cohort of European cases with a clinical diagnosis of VACTERL thus far reported. We did not assign the VACTERL diagnosis ourselves, but used the clinical diagnosis, registered in EUROCAT, to select the cases. The possibility of sampling bias in studies that select cases based on the presence of certain core component features, such as ARM or EA/TEF[12] or the severity of the phenotype,[17] was limited in our study as data from population-based congenital anomaly registries were used.

A limitation of population-based studies is the possibility of incomplete ascertainment of VACTERL cases among the participating registries. Cases are registered for monitoring purposes and in the event of fetal death or infant death autopsies are not always performed. The possibility remains that only the most obvious major congenital anomalies were diagnosed and that cases were not screened for additional congenital anomalies that are not immediately evident. Therefore, asymptomatic cardiac or renal congenital anomalies, for example, may have been missed. This may have resulted in an underrepresentation of certain combinations of VACTERL component features and even in cases not being diagnosed with VACTERL at all. However, incomplete ascertainment is mitigated in EUROCAT because the registries need to adhere to the EUROCAT guidelines and multiple sources are used for ascertainment. In addition, data quality indicators (DQIs) are used to assess key elements of the data, including completeness of case ascertainment and accuracy of the diagnosis.[22] The DQIs allow registries to evaluate their performance in relation to others, provide direction for improvement of the data collection and case ascertainment, and allow annual assessments to monitor improvement.[22]

We excluded cases with syndromes that could explain their phenotype. However, only 16 of the 29 registries indicated that they use genetic testing to rule out other diagnoses with overlapping features, such as CHARGE or Townes–Brocks syndrome. In almost half of the cases in this study, genetic testing was performed, but this mainly comprised karyotyping or the use of chromosomal microarrays. Detailed genetic testing such as whole-exome sequencing (WES) or whole-genome sequencing (WGS) is the only test to confirm the presence of a syndrome that has overlap with VACTERL, especially when not all clinical features of the syndrome are expressed. WES or WGS was generally not performed at the time the cases were registered in EUROCAT, as these genetic tests were not available in the earlier years of the registry. Therefore, we cannot rule out that some VACTERL cases in this study have in reality genetic evidence of a syndrome that has multiple features in common with VACTERL. However, when genetic testing was performed after initial inclusion of the patient in EUROCAT and this resulted in an alternative diagnosis, the diagnoses was usually updated and overwrote the VACTERL diagnosis within EUROCAT registries.

The VACTERL cases were collected in the period 1980–2015, in which diagnostics changed dramatically. Access to ultrasound increased the prenatal detection of congenital anomalies and greatly facilitated diagnosing VACTERL. The focus of the EUROCAT registries mainly lies on major congenital anomalies, as minor congenital anomalies are only registered in EUROCAT when major congenital anomalies are present. Therefore, the accuracy of reporting minor congenital anomalies is probably not as complete as for major congenital anomalies. On the other hand, the majority of cases (>75%) were born or detected between 2000 and 2015, so this will not have influenced our study results to a large extent. In addition, we minimized the possible impact of the changes in diagnostics by differentiating between major and minor VACTERL features and additional anomalies, as minor congenital anomalies are more easily missed.

Regarding the study population characteristics, we observed differences compared to the literature. We observed a male predominance, with 65% of the cases being male. Male predominance was described before,[3,7,15] but was never as striking as in this study. However, no large differences were observed in the percentages of major and minor component features illustrating that VACTERL is not phenotypically different between males and females. In this study, 44% of the live born infants were delivered preterm, whereas other studies showed percentages between 20 and 33%.[3,7,15] This discrepancy may partly be explained by different selection criteria for the cases in previous studies. In our study, but also in that of Oral et al.,[15] cases were selected based on a final VACTERL diagnosis, whereas other studies included cases based on the presence of a certain number of component features.[3,7] Khoury et al.[3] found that 54% had a low birth weight, which was comparable to our findings.

We did not detect phenotypically similar subtypes of VACTERL cases based on the combination of congenital anomalies present, apart from the clear distinction between the predefined VACTERL subtypes. Some differences regarding case characteristics exist among these three subtypes, reflecting the complexity and severity of the congenital anomalies. The VACTERL-LIKE cases had the highest percentages of live births (92%) and survival beyond the first week postpartum (96%) compared to the other VACTERL subtypes, suggesting that these cases were less severely affected. The percentages of live births and survival beyond the first week postpartum were lowest in the VACTERL-PLUS subtype (67% and 77%). The additional anomalies may have resulted in more severely affected cases, and additionally, may point towards another possible diagnosis. It can be argued that cases with additional major congenital anomalies outside the VACTERL spectrum are not typical VACTERL cases, but actually have an undiagnosed syndrome. Therefore, genetic testing to rule out syndromes that have overlap with VACTERL is most important in the VACTERL-PLUS subtype.

In the comparison of the frequencies of component features between our study and three other studies,[12,13,17] the percentages of ARM and EA/TEF were similar, most likely because these component features are clearly defined congenital anomalies. This is also reflected in the low percentages of minor VACTERL features for the ARM and EA/TEF components. Vertebral, cardiac, renal, and limb anomalies are less clearly defined, as these comprise a broad spectrum of congenital anomalies that differ in severity and in timing of appearance.[19] In addition, it is hard to compare frequencies among studies, as the specific congenital anomalies included as component features were often not specified. We observed more similarity between our study and the literature when we included both major and minor VACTERL features, although the latter are not typically seen in VACTERL cases. The comparison with the literature, however, seems to indicate that minor VACTERL features are often considered part of VACTERL, which increases the heterogeneity of the VACTERL case group unnecessarily. This illustrates why clear definitions for the diagnosis of VACTERL are important.

GU anomalies were neither considered part of the VACTERL spectrum nor included in VACTERL-PLUS anomalies when occurring in combination with a renal or anorectal anomaly, as their embryology is partly shared.[23] Several studies reported relatively high frequencies of VACTERL cases with GU anomalies.[12,13,23] Husain et al.[13] even proposed to consider another revision of the acronym to include GU anomalies as a component feature of VACTERL. As major GU anomalies are less frequently observed than all other VACTERL component features, we do not support this extension.

In conclusion, we present clear guidelines for the congenital anomalies that should be considered part of VACTERL, for which consensus was reached. A clear definition of VACTERL may help the timely diagnosis of VACTERL cases, improve immediate survival and long-term prognosis of children with this condition, and avoid incorrect diagnosis of VACTERL, which is harmful not only for the patient but also for parents when considering the risks in another pregnancy.

We were not able to identify VACTERL subtypes that show phenotypic similarity, but we did present predefined subtypes of VACTERL cases based on the inclusion of major and minor VACTERL features and additional congenital anomalies. These more homogeneous subtypes may aid the future identification of environmental and genetic risk factors involved in the etiology of VACTERL. The VACTERL patient group is very heterogeneous, and it is questionable whether all subtypes should be included when studying risk factors. It is important to exclude all NO-VACTERL cases, as these cases do not fulfill the diagnostic criteria for VACTERL. Furthermore, the STRICT-VACTERL subtype may be the only group of true VACTERL patients, and the inclusion of the VACTERL-LIKE and VACTERL-PLUS subtype may dilute the magnitude of the risk estimates that are identified in etiologic studies. Therefore, we would encourage researchers and physicians to consider using the guidelines and subtypes that we provide here, both in the clinic and in future etiologic studies.

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All authors meet the *Pediatric Research* authorship requirements. They have contributed to the conception and design, acquisition of the data, or analysis and interpretation of the data; they have drafted the article or revised it critically for important intellectual content; and all authors gave their final approval of the version to be published.

ADDITIONAL INFORMATION

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