WILEY AMERICAN JOURNAL OF medical genetics [PART A]

ORIGINAL ARTICLE

Phenotypic diversity of patients diagnosed with VACTERL association

Majid Husain[1] | Marina Dutra-Clarke[1] | Bryan Lemieux[1] | Marie Wencel[1] |
Benjamin D. Solomon[2] 🔘 | Virginia Kimonis[1] 🔘

[1]Division of Genetics and Genomic Medicine, Department of Pediatrics, University of California-Irvine, School of Medicine, Irvine, California, USA

[2]GeneDx, Gaithersburg, Maryland, USA

Correspondence
Virginia Kimonis, Division of Genetics and Genomic Medicine, Department of Pediatrics, University of California-Irvine, School of Medicine, 101 The City Drive S., ZOT 4482, Orange, CA 92868.
Email: vkimonis@uci.edu

The combination of vertebral, anal, cardiac, tracheo-esophageal, renal and limb anomalies termed VACTERL association, also referred to as VATER, has been used as a clinical descriptor and more recently, a diagnosis of exclusion, for a specific group of phenotypic manifestations that have been observed to co-occur non-randomly. Though the causes remain elusive and poorly understood in most patients, VACTERL association is thought to be due to defects in early embryogenesis and is likely genetically heterogeneous. We present data on 36 patients diagnosed with VACTERL association in addition to describing the phenotypic diversity of each component feature. Unique cases in our cohort include a patient with a 498.59 kb microdeletion in the 16p11.2 region and another with a 215 kb duplication in the 3p25.2 region. Our findings expand upon the current understanding of VACTERL association and guide future research aimed at determining its etiology.

KEYWORDS
16p11.2; 3p25.2, VACTERL, VACTERL association, VATER, VATER association

1 | INTRODUCTION

Quan and Smith (1972) coined the term 'VATER association' to describe a spectrum of co-occurring anomalies including Vertebral defects, Anal atresia, Tracheo-Esophageal fistula with or without esophageal Atresia and Renal anomalies/Radial dysplasia (Quan & Smith, 1972; Quan & Smith,1973). Subsequently, its description and acronym were expanded to include Cardiac anomalies and Limb defects – effectively decoupling renal/radial dysplasia in order to include anomalies of the extremities not limited exclusively to the radius (Temtamy & Miller, 1974). VACTERL association (OMIM 192350) is now the widely accepted contraction and descriptor of these co-occurring component features (CFs).

Importantly, this association is considered a diagnosis of exclusion, with the presence of three or more congenital malformations necessary for a VACTERL association diagnosis (Jenetzky et al., 2011; Quan & Smith, 1973; Solomon et al., 2012). Variation in utilization of these diagnostic criteria accounts for the differences and discrepancies between studies. For instance, a paired association between the CFs was previously sufficient to earn the 'VATER' designation and "R" in VACTERL denoted either renal anomalies or radial anomalies, or both, depending on the study. Therefore, the literature represents a

wide degree of interpretation. Not surprisingly, depending upon the diagnostic criteria used, VACTERL association's prevalence may be as high as 1 in 5000 live births (de Jong et al., 2008). It is thus crucial to be critical of the criteria used when evaluating relevant studies.

Furthermore, there must be no clinical or laboratory-based evidence of an alternate or overlapping diagnosis that share similar features with VACTERL association, including: Baller–Gerold syndrome, CHARGE syndrome, Currarino syndrome, deletion 22q11.2 syndrome, Fanconi anemia, Feingold syndrome, Fryns syndrome, MURCS association, oculo-auriculo-vertebral syndrome, Opitz G/BBB syndrome, Pallister-Hall syndrome, and Townes-Brocks syndrome, among others. Unlike many of these syndromes, which have a clearly defined etiology, VACTERL association is causally heterogeneous, and may include non-genetic or multifactorial etiologies. Since its initial description, a significant number of studies have focused on unraveling the causes and defining the clinical features despite the above-mentioned challenges.

For the purpose of our study, the presence of at least three CFs was required to assign the VACTERL designation; the "R" denotes only renal anomalies, with the "L" denoting limb anomalies. We report the phenotypic diversity of each CF in 36 patients diagnosed with

VACTERL association as well as case highlights with interesting genetic findings that will add to the current literature.

2 | METHODS

Patient data from the University of California, Irvine Medical Center, Miller Children's Hospital Long Beach and Children's Hospital of Orange County were collected through an IRB-approved protocol on VACTERL association, to expand our understanding of the clinical characteristics and to explore possible etiologies including potential genetic causes of VACTERL association. All patients were ascertained from inpatient and outpatient genetics consultations. All patients were evaluated by medical geneticists from the Department of Pediatrics, Division of Genetics and Genomic Medicine of the University of California, Irvine. Of the 36 patients, 33 were evaluated by a clinical geneticist in the NICU shortly after birth and 3 were evaluated at an outpatient genetics clinic. All patients in this cohort had a spinal x-ray and chromosome studies as the standard of care, 15 had microarray analysis and 21 had karyotype only. Nine patients had diepoxybutane (DEB) chromosome breakage studies to rule-out Fanconi anemia, all of which were normal. Only patients with a final diagnosis of VACTERL association after a complete physical exam and supporting imaging studies were included in the study. The diagnosis required at least three CFs as well as a lack of indications of an alternative diagnosis. The medical records of these patients were evaluated retrospectively

for the associated CFs. Additional data such as sex, birth weight/length, head circumference, gestational and postnatal age, prenatal care, known or reported teratogen exposure, and familial history of congenital anomalies or VACTERL association, was also ascertained. None of the patients in this cohort were related to one another. No patient had a family history of VACTERL association. Statistical studies included a descriptive analysis of the frequencies of the clinical features of the syndrome.

3 | RESULTS

Of the 36 patients included in our study, 18 (50%) were males and 18 (50%) were females based on chromosome studies. Of four patients with ambiguous genitalia, three were genotypically female.

The most commonly observed CF was cardiac anomalies (n = 30, 83%), followed by vertebral anomalies (n = 29, 80.6%), tracheoesophageal fistula/esophageal atresia (TEF/EA) (n = 25, 69.4%), renal anomalies (n = 23, 63.9%), anal atresia (n = 22, 61.1%), and limb anomalies (n = 19, 51.4%) (Table 1). The frequency of the six CFs in our cohort is compared to that of previously published studies in Table 2.

The most common cardiac anomaly was ventricular septal defect; vertebral anomaly was scoliosis; renal anomaly was renal agenesis; anorectal malformation was rectovestibular fistula; limb anomaly was hypoplasia or aplasia of the thumb. Notably, six patients with anal atresia also had duodenal atresia. All patients with TEF/EA were

TABLE 1 Comparison of the CF and its subtypes observed in our study

Component feature	N	Component feature	N	Component feature	N
Vertebral anomalies	29 (80.56%)	Anal anomalies	22 (61.1%)	Cardiac anomalies	30 (83.33%)
Other anomalies	16	Anal atresia	22	Ventricular septal defect	22
Scoliosis	12	Duodenal atresia	6	Valvular anomalies	15
Rib anomalies	10	Rectovestibular fistula	4	Other anomalies	**15**
Hemivertebrae	8	Rectovesicular fistula	1	Patent foramen ovale	14
Tethered cord	5	Other anomaly	5	Patent ductus arteriosus	13
Kyphosis	5			Cardiomyopathies	12
Transition vertebrae	4			Atrial septal defect	11
Fused vertebrae	4			Vascular anomalies	9
Sacral agenesis	4			Pulmonary anomalies	9
Butterfly vertebrae	3			Tetralogy of fallot	6
Wedge vertebrae	2			Aortic anomaly	2
Sacral dysgenesis	2			Double outlet right ventricle	2
Component feature	**N**	**Component feature**	**N**	**Component feature**	**N**
TEF/EA	25 (69.44%)	Renal anomalies	22 (61.1%)	Limb anomalies	18 (50%)
		Hydronephrosis	*15*	Thumb anomalies	10
		Renal agenesis	8	Lower limb anomalies	9
		Vesico-ureteral reflux	6	Radial anomalies	7
		Duplex	4	Other anomalies	8
		Cystic kidney disease	3	Thenar hypoplasia	3
		Horseshoe kidney	3	Clinodactyly	2
		Renal dysplasia	2	Syndactyly	2
		Ureteral anomalies	3	Limb reduction	2
		Bladder anomalies	2	Ulnar anomalies	2
				Brachydactyly	1

TABLE 2 Comparison of the frequency of CFs in our cohort with previously published studies

	This study 2016 N = 36 (%)	Oral et al. (2012) N = 28 (%)	Solomon et al. (2010) N = 60 (%)	Källén et al. (2001) N = 51 (%)	Botto et al. (1997) N = 286 (%)	Rittler et al. (1996) N = 21 (%)	Weaver et al. (1986) N = 46 (%)
V	29 (80.5)	26 (96.4)	47 (78)	42 (82)	231 (81)	13 (62)	27 (59)
A	22 (61.1)	19 (67.8)	33 (55)	46 (90)	236 (83)	15 (71)	26 (57)
C	30 (83.3)	14 (45.0)	48 (80)	–	–	8 (38)	36 (78)
TE	25 (69.4)	17 (60.7)	31 (52)	42 (82)	168 (59)	12 (57)	31 (67)
R	22 (61.1)	15 (53.5)	43 (72)	–	231 (81)	11 (52)	29 (61)
L	18 (50)	15 (53.5)	28 (47)	25 (49)	111 (39)	11 (52)	-

classified as type C with a proximal esophageal atresia and a distal fistula. Unique among those patients with TEF/EA included one with a porcine bronchus, and another with a subglottic laryngeal atresia (type II) with an associated accessory trachea. The frequency of the various subtypes of CFs in our cohort is presented in Table 1.

Genitourinary anomalies occurred in 11 of the 36 (30.6%) patients in our cohort, representing 5 of 18 female patients (27.7%) and 6 of 18 male patients (33.3%). The most common genitourinary (GU) anomaly was cryptorchidism in males (n = 5/7; 71.4%) followed by ambiguous genitalia in females (n = 3/5; 60.0%) (Table 3). While 10 of the 11 patients (91%) had GU anomalies in conjunction with an imperforate anus and/or renal anomalies, only one presented without a co-occurring anal or renal anomaly. Of note, a 2-vessel cord, specifically, a single umbilical artery was detected in 8 of our 36 patients (22.2%) and four patients were noted to have dysmorphic facies. Supporting Information Table 1 lists all 36 patients with their reported CFs.

The frequencies of the different combinations of CFs of VACTERL association are depicted in Supporting Information Table 2. A triad of any of the CFs was most common (n = 14; 38.9%), followed by a tetrad (n = 13; 36.1%), hexad (n = 5; 13.9%), and lastly a pentad (n = 4; 11.1%). The most frequent shared combination of CFs was 'VACTERL' (n = 5; 13.9%), followed by 'VACTE' (n = 3; 8.3%) and 'ACTE' (n = 3; 8.3%).

TABLE 3 Description of GU anomalies

Patient	A	R	Sex	GU anomalies (n = 11; 30.6%)
6	x	x	F	Posterior labial fusion, single urogenital orifice
7	x		F	Absent labia minora
15	x	x	F	Ambiguous genitalia (bifid vagina), Persistent cloaca with hydrocolpos
25	x	x	F	Ambiguous genitalia with clitoromegaly, non-descript uterine/ovarian/fallopian anomaly
9	x	x	F	Ambiguous genitalia/Microphallus, Absent GU orifice/agenesis of vaginal vestibule
8	x		M	Microphallus
10	x	x	M	Cryptorchidism
13		x	M	Cryptorchidism
22	x	x	M	Ambiguous genitalia, hypospadias, cryptorchidism, absent scrotal raphe
31			M	Meatal stenosis
33		x	M	Cryptorchidism

A = anorectal malformation; R = renal anomaly.

With respect to genetic findings, 15 patients in this cohort had a microarray analysis and 21 patients had karyotype only. There was one male with a 498.59 kb microdeletion in the 16p11.2 region (chr16: 29,500,284-29,998,874; hg18) and one female with a 215 kb duplication in the 3p25.2 region (chr3: 12,823,965-13,038,645; hg19), the latter with no known clinical phenotype described in the literature when searched in PubMed or in the Unique database.

Prenatal and maternal history was essentially non-contributory; four mothers (11.1%) reported gestational diabetes, two reported pre-eclampsia, and one reported polyhydramnios and "double bubble" sign was identified on ultrasound. Significantly, one mother reported a history of polysubstance abuse (marijuana, amphetamines & cocaine) and another was diagnosed with stage IV metastatic rhabdomyosarcoma. Other prenatal and birth history include one case of severe intrauterine growth restriction, one born preterm at 32 weeks gestational age, and one was a twin conceived through IVF (twin B was reportedly unaffected). There was one report of consanguinity. One patient in this cohort has a sibling with bilateral anotia, bifid thumb, tracheaesophageal fistula, self-resolved atrial septal defect, scoliosis, cord syrinx, and hearing loss. Both this patient and the sibling have had a normal single nucleotide polymorphism (SNP) microarray, but it is unknown whether or not they have had DEB studies to rule out Fanconi anemia. Additionally, one patient reportedly had a sibling with a cystic kidney and short neck. Family history for the rest of the patients in this cohort was otherwise non-contributory.

4 | DISCUSSION

4.1 | Novel etiologies for VACTERL association

Adding to the ever-increasing reports of abnormal microarrays or chromosomal abnormalities associated with VATER/VACTERL association (Cinti et al., 2001; Prieto, Garcia, Elder, Zinn, & Baker, 2007; van der Veken et al., 2010; Winberg et al., 2014), two of our patients were identified with abnormal microarray studies. Since many features of VACTERL association and chromosomal disorders overlap, it can be difficult to differentiate them in the prenatal/perinatal period. Thus, it is paramount that chromosomal disorders be excluded by microarray (Hernando, Plaja, Rigola, & Fuster, 2002; Santos, Nogueira, Pinto, Cerveira, & Pereira, 2013; Solomon et al., 2012).

One patient in our cohort had a 498.59 kb microdeletion in the 16p11.2 region (chr16: 29,500,284-29,998,874; hg18). Loss of material from 16p11.2 is increasingly recognized as one of the most common structural chromosome disorders, and has been identified in

roughly 1 in 100 individuals with autism spectrum disorder (Miller et al., 2015). Deletion of 16p11.2 has been widely associated with developmental delay and intellectual disability, though frequency of birth defects of all types is only slightly increased (vertebral anomalies being the most frequent). Furthermore, healthy individuals have been found to have 16p11.2 microdeletions, with no reported medical problems or birth defects. Its co-occurrence with VACTERL association may be coincidental and likely suggestive of a dual-diagnosis rather than causative because it has been so well characterized. However, we cannot rule out the possibility of a mutation in one of the other alleles in that region of 16p11.2, or other genetic variants in combination with the 16p11.2 microdeletion, which together could be causative of the VACTERL phenotype. Vertebral (scoliosis and multiple mid-lower thoracic segmentation anomalies), cardiac (VSD, aortic arch hypoplasia), and limb anomalies (clinodactyly) have been reported in other cases of 16p11.2 microdeletion. A previously reported dysmorphic baby with a de novo interstitial deletion of band 16p11.2 showed four of the six VACTERL association core components: vertebral anomalies (hemivertebra at level L1), cardiac defects (tetralogy of Fallot with pulmonary atresia), renal anomalies (unilateral renal agenesis and unilateral multiple renal cysts), limb anomalies (cubital deviation of the hands, talipes varus and articular limitation), and cryptorchidism (Hernando et al., 2002). A 593-kb microdeletion of 16p11.2 (chr16: 29,528,190-30,104,842; hg18) was found in a 3-year-old boy with vertebral anomalies (multiple hemivertebrae and missing ribs) as well as genitourinary anomalies (hydrocele testis) (Shimojima, Inoue, Fujii, Ohno, & Yamamoto, 2009). Another patient with limb anomalies (small hands with distally tapered fingers, short toes) and a genitourinary anomaly (microphallus) was reported to have a similar microdeletion (Fernandez et al., 2010). Two patients – one with a limb anomaly (polydactyly) and the other with both a limb anomaly (hypoplastic, non-articulating thumbs) and a vertebral anomaly (extra-thoracic and transition vertebrae) were found to have de novo interstitial deletions of 16p11.2 (Wat et al., 2011). Interestingly, two other patients with heterozygous deletions of 16p11.2 were found to have long cervico-thoracic syringomyelias (chr16: 29,528,190-30,107,184) (Schaaf et al., 2011).

Another patient in our cohort found to have a 215 kb duplication in the 3p25.2 region (chr3: 12,823,965-13,038,64; hg19) exhibited four core CFs (VACR), including: vertebral (fused ribs with six on right and levoscoliosis of her thoracic spine), anal (imperforate anus with a rectovestibular fistula), cardiac (VSD with a PDA and PFO and deviated axis) and renal (cross-fused renal ectopia with duplex collecting system and associated hydronephrosis). A 215 kb micro-duplication at 3p25.2 encompassing *RAF1* was reported in a case associated with syndromic congenital heart disease suggestive of Noonan syndrome (chr3: 12,526,807-12,781,123; hg19) (Luo et al., 2012). Interestingly, in addition to the cardiac defects in the patient (tetralogy of Fallot, secundum ASD, and persistent left superior vena cave), dysmorphic facies, short stature and cognitive delay, he also exhibited limb anomalies (short right forearm, complete radial aplasia with absence of thumbs/radial carpals and syndactyly between the index and long fingers). Future studies of VACTERL association-like phenotypes associated with microdeletions and microduplications such as this novel

3p25.2 region may provide insights to elucidate the etiology of this complex heterogeneous disorder.

Given that the VACTERL association phenotype describes a combination of congenital malformations, it is logical that abnormalities in the genes involved in regulating development as well as those moderating the complex network of signaling pathways––such as Sonic hedgehog (SHH), may be the culprits (Kim, Kim, & Hui, 2001). Research in determining its genetic etiology has focused on previously identified candidate genes, namely, *FGF8*, *FOXF1*, *HOXD13*, *LPP*, *TRAP1* and *ZIC3* (Reutter, Hilger, Hildebrandt, & Ludwig, 2016). Additionally in an effort to identify the genetic cause of VACTERL association, Shi et al. (2017) performed genomic sequencing in families and identified pathogenic variants in gene encoding enzymes of the kynurenine pathway, namely 3-hydroxyanthranilic acid 3, 4-dioxygenase (HAAO) and kynureninase (KYNU), resulting in disruption of de novo synthesis and deficiency of nicotinamide adenine dinucleotide (NAD) – a vital co-factor for embryogenesis. Defects similar to patients developed in null mice for these two genes owing to NAD deficiency and niacin supplementation during gestation prevented the malformation in mice. This study, and perhaps additional analysis of variant genes encoding other aspects of NAD processing, such pathways involved in its uptake, transport, and salvage, will pave the way to increasing our understanding of the genetic and environmental causes of congenital malformations and associations such as VACTERL.

Cytogenetic and molecular testing thus continue to provide potentially explanatory genomic causes in patients with VATER/VACTERL association, though it may be likely that multiple interacting genetic and environmental factors contribute to the VACTERL association phenotype (Aynaci, Celep, Karagüzel, Baki, & Yildiran, 1996; de Jong et al., 2010; Dworschak et al., 2013; Hilger et al., 2013; McNeal, Skoglund, & Francke, 1977; Peddibhotla et al., 2013; Schramm et al., 2011; Solomon et al., 2011b; Walsh, Vance, & Weaver, 2001; Yamada et al., 2009; Zen et al., 2010). Although a possible causal relationship has been proposed between malformations observed in VACTERL association and maternal diabetes (Becerra, Khoury, Cordero, & Erickson, 1990; Castori, Rinaldi, Capocaccia, Roggini, & Grammatico, 2008; Janssen, Rothman, & Schwartz, 1996; Mills, 2010; Pedersen, Tygstrup, & Pedersen, 1964), our study cannot substantiate this hypothesis. Regarding the mother with Stage IV metastatic rhabdomyosarcoma, she was initially diagnosed 32 months prior to her delivery. She completed chemotherapy treatment 3 months prior to her first prenatal ultrasound at 9 weeks gestational age. These chemotherapy agents are not recognized to be embryotoxic. Interestingly, although rhabdomyosarcoma is associated with genetic cancer syndromes such as Li-Fraumeni, Beckwith-Wiedemann, Costello, and Neurofibromatosis type 1, most cases are sporadic. Therefore, no further investigation was made into its unlikely and distant connection to VACTERL association. While no concrete relation between teratogenic exposure *in utero*, beyond its already known effects, and the development of VACTERL association has been established, several studies have shown evidence for the role of inheritance in VACTERL association – implying a role for genetic loci (Auchterlonie & White, 1982; Becker, Hernandez, Dipietro, & Coran, 2005; Finer, Bowen, & Dunbar, 1978; Fuhrmann, Rieger, & Vogel, 1958; Hilger et al., 2012; Kim et al., 2001; Khoury, Cordero, Greenberg, James, & Erickson,

1983; McMullen, Karnes, Moir, & Michels, 1996; Nezarati & McLeod, 1999; Say, Balci, Pirnar, & Hi Csönmez, 1971; Solomon et al., 2010b).

4.2 | Discussion of our results and comparison to other studies

Although the CFs observed in patients with VACTERL association may arise as isolated anomalies, they tend to co-occur more frequently than expected by chance. For example, the relative incidence of TEF/EA in newborns is approximately 1 in 3500; however, TEF/EA occurs in isolation only about 50% of the time, the remainder of the cases present most frequently with VACTERL association (Shaw-Smith, 2006). Furthermore, VACTERL association itself is phenotypically more diverse than previously thought; for this reason, it is imperative to recognize co-occurring anomalies and know the range of congenital malformations that comprise the six CFs in order to truly understand this association and diagnose it accurately.

A wide variety of vertebral anomalies were observed in our cohort. With respect to segmentation defects and their location, hemivertebrae were more frequently found in the lumbar region, followed by the thoracic region; "butterfly vertebrae" were isolated to the thoracic region while "wedge vertebrae" were in the lumbar region only. Fused vertebrae were found throughout the thoraco-lumbar spine. While the exact location of the vertebral defects may not be immediately relevant, the abnormal spine curvature caused by these costovertebral anomalies often provides the clinician with the first indication of an underlying vertebral abnormality warranting further investigation to prevent long term sequelae (Chen et al., 2016; Raam, Pineda-Alvarez, Hadley, & Solomon, 2011). Not surprisingly, our patients with scoliosis or kyphosis revealed accompanying vertebral anomalies post investigative imaging. 31% of our patients with vertebral anomalies had accompanying rib anomalies, is consistent with several other studies (Wattanasirichaigoon, Prasad, Schneider, Evans, & Korf, 2003; Weaver, Mapstone, & Yu, 1986).

Other findings associated with vertebral anomalies in our cohort included distal cord lipomas, syringohydromyelia, lack of intervertebral disks, absence of vertebral pedicles, and spina-bifida occulta, although the latter is recognized as a normal variant. Notably, although the inclusion of dysplastic sacral vertebrae (typically associated with anorectal malformations) as vertebral malformations for the diagnosis of VACTERL association is controversial (Ladd & Gross, 1934; Lawhon, MacEwen, & Bunnell, 1986; Rittler, Paz, & Castilla, 1996), this study did not exclude those anomalies.

With respect to anorectal malformations, imperforate anus/anal atresia have been reported in 55–90% of patients with VACTERL association (Botto et al., 1997; Lawhon, MacEwen, & Bunnell, 1986; Solomon et al., 2010a). Our study corroborates these findings and highlights the need for a high index of suspicion for other associated GI and GU malformations, namely rectovestibular/rectovesicular fistulas and duodenal atresia that may not be as apparent during a postnatal physical examination as the clinically obvious imperforate anus (Solomon et al., 2011a). Awareness and prompt diagnosis can aid in immediate treatment as opposed to waiting for the patient to present with signs and symptoms of obstruction or associated morbidities (Rittler et al., 1996).

Cardiac malformations are reportedly not more common in patients with VACTERL association than in other multiple malformation syndromes. Our study, like others, found cardiac anomalies to be the most common followed by vertebral anomalies (Chen et al., 2016; Rittler et al., 1996). Cardiac anomalies in our cohort occurred at similar rates as prior studies with frequencies ranging from 40% to 80% (Botto et al., 1997; Källén, Mastroiacovo, Castilla, Robert, & Källén, 2001; Lawhon, MacEwen, & Bunnell, 1986). The variable rates of cardiac malformation in VACTERL association may be explained by ascertainment bias of studies utilizing malformation registries versus studies based on patients seen in outpatient genetic clinics, because severe cardiac malformations result in a high rate of mortality (Solomon, 2011). Not surprisingly, because our cohort comes from specialty children's hospitals affiliated with obstetric divisions managing high-risk pregnancies, we accordingly report a high rate of cardiac anomalies. Additionally, while the presence of normal, age-based findings such as an isolated patent ductus arteriosus (PDA) and/or patent foramen ovale (PFO) may be considered a cardiac malformation to loosely satisfy the diagnostic criteria of VACTERL association, nearly all except one of the PDAs/PFOs we report were accompanied by a range of structural heart anomalies. These include valvular abnormalities (pulmonary atresia, bicuspid aortic valve, and stenosis) and vascular abnormalities (malposition of great arteries and hypoplastic aortic arch). Consequently, our patients exhibited signs and symptoms of these serious cardiac malformations such as mild-severe valvular regurgitation with resulting dilated/hypertrophic cardiomyopathies as well as conduction defects and arrhythmias ranging from bundle-branch blocks to ectopic atrial tachycardia.

Compared to our 69.4%, TEF/EAs occur in 50–80% of patients with VACTERL association (Botto et al., 1997; Lawhon, MacEwen, & Bunnell, 1986; Solomon et al., 2010a). The TEF/EAs represent all subtypes, A –E (or H-type) with or without EA, with type C being the most common (Spitz, 2007). While many patients are diagnosed prenatally (maternal polyhydramnios or absent gastric bubble on ultrasound) or immediately postnatally (failure to pass nasogastric tube) (Evans, Greenberg, & Erdile, 1999) and undergo surgical repair within the first few days of life, numerous complications must be managed thereafter (Chen et al., 2016). For example, 44% of our patients suffered from gastroesophageal reflux, 16% reported (and we conjecture that more suffered) chronic respiratory infections and 8% developed tracheomalacia. Other complications include reactive airway disease and co-occurring pulmonary anomalies (Avcu et al., 2009; Solomon, Raam, Pineda-Alvarez, 2011). In one case, our patient with a porcine bronchus developed a gastrocutaneous fistula with an associated hiatal hernia in addition to circumferential superficial glottis ulceration. In patients with TEF/EA, recognizing the complications and anticipating course of management and treatment is vital in preventing significant morbidity (Scott, 1993).

Similar to the subtypes of TEFs, there are a variety of renal anomalies reported in 50–80% of patients with VACTERL association (Ahn, Mendoza, Kaplan, & Reznik, 2009; Botto et al., 1997; Källén et al., 2001; Lawhon, MacEwen, & Bunnell, 1986; Solomon et al., 2010a). 61.1% of our patients had anomalies ranging from renal dysgenesis (unilateral agenesis), congenital cystic renal disease (multicystic dysplastic kidneys – Potter type II), renal morphological anomalies

(horseshoe kidney, cross-fused renal ectopia), congenital renal positional anomalies (pelvic kidney), to ureteral and bladder anomalies (duplicated collecting systems, congenital megaureter, ureteroceles and bladder diverticuli). Many patients in our cohort were reported to have complications secondary to these malformations namely hydronephrosis due to vesicoureteral reflux or ureterovesical junction obstruction and frequent urinary tract infections contributing to renal failure. Therefore, having a high index of suspicion and performing a careful physical examination and abdominal ultrasound is paramount when detecting these renal anomalies that would otherwise go undetected and consequently result in significant morbidly (Evans, Vitez, & Czeizel, 1992).

Lastly, limb anomalies have been reported in approximately 40–50% of patients with VACTERL association (Botto et al., 1997; Källén et al., 2001; Solomon et al., 2010a; Solomon, 2011; Stoll, Alembik, Dott, & Roth, 2007). Traditionally, only radial anomalies and thumb aplasia/hypoplasia comprised the types of limb anomalies in VACTERL association; other anomalies, including those of the lower limb, were thought to be erroneously ascribed to VACTERL association (Solomon, 2011). However, just as with the other core CFs, there exists a diverse variety and degree of severity of limb anomalies that characterizes this CF, and we argue that these should be included if there is no other syndrome in the differential diagnosis of VACTERL association that would otherwise explain these malformations. The limb anomalies reported in our study included ulnar, digit anomalies (clinodactyly, syndactyly and brachydactyly, and rarely radial and thumb anomalies) and various co-occurring lower limb anomalies.

4.3 | Discussion of GU findings

Additionally, albeit not included in the description of VACTERL association, it is equally important to be knowledgeable of other anomalies that tend to co-occur with VACTERL association–specifically with anal and renal anomalies. For instance, our study shows a high prevalence of GU anomalies in patients diagnosed with VACTERL association. Previous studies corroborate our findings and report the occurrence of GU anomalies in up to 25% of patients with VACTERL association (Ahn et al., 2009; Lawhon, McEwen, & Bunnell, 1986; Paidas, Morreale, Holoski, Lund, & Hutchins, 1999; Rittler et al. 1996). This is not surprising given that the embryology and developmental biology of the GU tract is deeply intertwined with that of the renal systems and hindgut; therefore, malformations affecting these systems are expected to occur together (Kluth, Hillen, & Lambrecht, 1995; Lau & Caty, 2006; Nievelstein, van der Werff, Verbeek, Valk, & Vermeij-Keers, 1998; Rich, Brock, & Peña, 1988). Of note, not all patients with GU anomalies have associated anal or renal anomalies (Ahn et al., 2009). Nevertheless, statistically-based studies advocating for ambiguous genitalia to be included as a core CF have also suggested that GU anomalies may in fact be a "secondary" feature of VACTERL association (Botto et al., 1997; Solomon et al., 2010a). It is time to consider another expansion or revision of the acronym to include GU anomalies as a core CF of VACTERL association– not only for the sake of completion, but also for an accurate all-encompassing diagnosis facilitating timely identification and treatment to prevent associated

morbidity and mortality (Tongsong, Wanapirak, Piyamongkol, & Sudasana, 1999).

Lastly, studies have also implicated the antenatal finding of a 2-vessel cord, namely a single umbilical artery occurring in 20–50% of the patients and reported at 22.2% in our cohort as a crucial, and often the first clue in the diagnosis in patients with VACTERL association (Botto et al., 1997; de Jong et al., 2008).

5 | SUMMARY

In summary, the data from our cohort of patients with VACTERL association supports that of previous studies with respect to the overall prevalence of each of the six CFs as well as the relatively high prevalence of GU anomalies found in these patients. Due to the essentially unknown etiologies of VACTERL association, there exists a large inconsistency in the way clinical geneticists approach patients presenting with features consistent with VACTERL association, from diagnostic criteria to the genetic testing approach (Solomon et al., 2012). We anticipate that by detailing the range of clinical characteristics of patients with VACTERL association as well as its associated GU anomalies, clinicians will practice with a higher index of suspicion and employ a more streamlined approach to identifying, characterizing, cataloging, and treating these patients.

Limitations to this study include the inherent problems associated with retrospective studies because of the variable methods employed by the diagnosing geneticists in ascertaining, investigating co-morbidities and reporting pertinent information in our cohort. As the study aims to provide a description of the phenotypic diversity of VACTERL association, the relatively small sample size is a limitation. Also, given this study's scope and limited genetic findings coupled with the overall lack of current knowledge regarding VACTERL association's etiology, the conclusions, comparisons and discussions of the study are exploratory at this time.

CONFLICT OF INTEREST

None.

ORCID

Benjamin D. Solomon http://orcid.org/0000-0002-8826-1023
Virginia Kimonis http://orcid.org/0000-0003-1567-4449

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