Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2011 March ; 91(3): 192–194. doi:10.1002/bdra.20768.

VACTERL Association and Mitochondrial Dysfunction

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Summary

BACKGROUND—VACTERL association includes the presence of malformations affecting the vertebrae, anus, heart, trachea and esophagus, kidneys, and limbs. The causes remain largely unknown, but rare patients with mitochondrial dysfunction have been reported. Although clinical signs and symptoms consistent with possible mitochondrial disease are not uncommon in patients with VACTERL association, the necessary testing to confirm mitochondrial dysfunction is infrequently performed.

METHODS—We describe a patient with relatively classic signs of VACTERL association who had an onset of clinical signs of mitochondrial dysfunction at 13 months of age. These signs included progressive muscle weakness, autonomic dysregulation, episodic hypoglycemia, and exocrine pancreatic dysfunction. The patient was later shown to have evidence of mitochondrial dysfunction (cytochrome c oxidase deficiency).

CONCLUSIONS—Abnormal mitochondrial function may be associated with VACTERL association, and clinicians who encounter patients with VACTERL association should have a low threshold for considering mitochondrial dysfunction.

Keywords

VACTERL; VACTERL association; VATER; VATER association; mitochondria

INTRODUCTION

VACTERL association, which consists of a combination of vertebral anomalies, anal atresia, cardiac malformations, tracheo-esophageal (TE) fistula, renal anomalies, and limb abnormalities, is relatively common, with a prevalence estimated at approximately 1 in 10,000 live births (Khoury et al., 1983). Diagnostic criteria are controversial, and the many overlapping conditions make diagnosis challenging (Rittler et al., 1996; Källén et al., 2001). Although there is evidence of inheritance in some families, genetic and other causes remain poorly understood, likely at least partially due to heterogeneity (Khoury et al., 1983; Solomon et al., 2010a; Solomon et al., 2010b). Purported causes in human patients include mutations affecting *HOXD13*, *ZIC3*, and *PTEN*, and cytogenetically-visible and submicroscopic copy number variations, whereas animal models provide evidence that the Sonic hedgehog and related signaling pathways may be involved (Aynaci et al., 1996; Cinti

et al., 2001; Kim et al., 2001; Reardon et al., 2001; Walsh et al., 2001; Garcia-Barceló et al., 2008; de Jong et al., 2010; Wessels et al., 2010).

To date, five patients have been reported with both VACTERL association and mitochondrial dysfunction (Table 1; Cormier-Daire et al., 1996a; Cormier-Daire et al., 1996b; Damian et al., 1996; Stone and Biesecker, 1997; von Kleist-Retzow et al., 2003; Thauvin-Robinet et al., 2006). Through our National Human Genome Research Institute Institutional Review Board-approved protocol on VACTERL association, we collected data on patients with VACTERL association. Including the central patient described here, 5 of 105 patients (4.8%) in our cohort of patients with VACTERL association were independently (i.e., by outside clinicians) felt to have clinical features highly suspicious of mitochondrial dysfunction. It is critical to note that invasive testing has not been performed in these latter patients to more definitively test for mitochondrial dysfunction (standard clinical biochemical analyses on blood and urine failed to yield evidence for other explanatory biochemical disorders), and thus, solid evidence for mitochondrial dysfunction has not been established. Clinical signs of possible mitochondrial dysfunction in these patients included: abnormal glycemic control, cyclical vomiting, developmental delay, dysautonomia, intestinal malabsorption, pancreatic insufficiency, positive response to “mitochondrial cocktail” treatment, ptosis, and episodic weakness. Of these five patients, one patient, who is described in detail here, underwent the necessary invasive testing to clearly demonstrate mitochondrial dysfunction.

CASE REPORT

The patient was a 3-year-old girl with the following features of VACTERL association: absent C1 vertebra, supernumerary lumbar vertebrae, hypoplastic sacrum/coccyx, fatty filum terminale with tethered spinal cord, and three fused ribs; an anorectal malformation including a cloaca consisting of a common urogenital sinus and a duplex vagina with midline septum; type C TE fistula; right renal agenesis with moderate left hydronephrosis and vesicoureteral reflux. She had no cardiac or limb malformations, and no other major organ anomalies. Both she and her father were additionally clinically diagnosed with hypermobile type Ehlers–Danlos syndrome, but family history was otherwise noncontributory. The diagnosis of hypermobile type Ehlers–Danlos was based on the patient’s hypermobility (9/9 on the Beighton hypermobility scale), very soft, fragile skin with unusual scarring, and poor skin healing. Skin biopsy was not performed, but testing on peripheral blood did not reveal mutations associated with other forms of Ehlers–Danlos syndrome.

Microarray analysis initially showed a maternally-inherited deletion at 4q35.1 detected with one clone and confirmed by FISH (RP11-173M11). Subsequent performance of an 180K custom oligonucleotide microarray (Baylor College of Medicine Medical Genetics Laboratories) refined the deleted region deletion to a maximum size of 1.37 Mb, with karyotype revised to 46,XX,arr cgh 4q35.1q35.2 (187,321-768-188,694-589) 3 1. Genes in the deleted region, which contains a number of much smaller copy number variations (CNVs) identified in normal controls, include *CYP4V2*, *KLKB2*, *F11*, *MTRNR1A*, and *FAT1*, none of which can be clearly related to her phenotype, especially as her mother, from whom the deletion was inherited, is unaffected.

Evidence for mitochondrial dysfunction began at 13 months of age with progressive muscle weakness, autonomic dysregulation, hypoglycemic episodes, exocrine pancreatic dysfunction, and decline of gastrointestinal function, eventually leading to total parenteral nutrition dependency due to visceral hyperalgesia, dysmotility, and malabsorption. Muscle biopsy showed a mild increase in fiber size variability but no other findings consistent with

mitochondrial disease, including via electron microscopy. However, analysis of electron transport chain (ETC) activity showed normal activity of complexes I, II, and III, but complex IV, or cytochrome c oxidase, activity was 45.4 $\mu\text{mol}/\text{min}/\text{g}$ weight, well below the control range (148.9 $\pm$ 67.2 $\mu\text{mol}/\text{min}/\text{g}$ weight). Repeat ETC analysis in isolated mitochondria confirmed a profound and reproducible complex IV deficiency. Citrate synthase activity and content in both ETC analyses were normal, confirming good sample quality and normal mitochondrial content. Whole mitochondrial genome sequencing, and sequencing of DNA *Polymerase Gamma 1* and 2 (*POLG1* and *POLG2*) and Thymidine Phosphorylase did not reveal abnormalities.

DISCUSSION

The causes of VACTERL association are largely unknown, but in a subset of patients the condition seems to be associated with mitochondrial dysfunction, suggesting that it is important for clinicians encountering patients with VACTERL association to screen for clinical findings suggestive of mitochondrial dysfunction.

Demonstrating a causal link between VACTERL association and mitochondrial dysfunction is more difficult. Mitochondrial dysfunction is well known to affect organ systems that require high energy for proper function. Reports such as this one support the idea that congenital malformations may be related to mitochondrial deficiency. The mechanism by which this might occur is not well understood, although efforts have been made toward understanding possible effects of mitochondrial dysfunction in early embryogenesis. For example, an antisense morpholino zebrafish model for the *surfl*, *coxaa*, and *coxab* genes encoding the cytochrome c oxidase enzyme subunits, have a consistent phenotype that includes structural malformations. In this model, apoptosis is dramatically higher in tissues in which malformations occur, whereas the effects on other tissues (such as the heart) seem to be more related to energy deficiency (Baden et al., 2007). Other human diseases involving congenital malformations, most famously Glutaric Aciduria type II, also result from mitochondrial dysfunction (Goodman et al., 1983; Cormier–Daire et al., 1996a; Cormier–Daire et al., 1996b). Ultimately, appropriate energy availability may be required in a temporospatially sensitive manner to allow normal tissue development and organization (Stone and Biesecker, 1997; von Kleist–Retzow et al., 2003). However, it is possible that mitochondrial dysfunction and congenital malformations in the patient described here are both secondary to an as yet unidentified process.

Acknowledgments

The authors would like to express their gratitude to the participating patients and families, and also thank Dr. Maximilian Muenke for his support and mentorship.

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Table 1
Features of patients with VACTERL association and proven mitochondrial dysfunction

Patient	V	A	C	TE	R	L	Other anomalies	Evidence for mitochondrial dysfunction	Reference
1	+	-	-	+	-	+	?	Complex IV deficiency (unclear which tissue tested)	Cormier-Daire et al., 1996; Stone and Biesecker, 1997
2	+	+	?	-	+	+	-	np 3243 A>G point mutation in mitochondrial DNA (100% in affected kidney)	Damian et al., 1996
3	+	+	-	+	-	-	-	Complex IV deficiency by liver biopsy	von Kleist-Retzow et al., 2003
4 ^b	?	?	?	?	?	?	?	Unclear	von Kleist-Retzow et al., 2003
5	+	-	-	+	-	+	Minor facial dysmorphism, pyloric stenosis, hip dislocation	Complex IV deficiency by liver biopsy	Thauvin-Robinet et al., 2006
6	+	+	-	+	+	-	Genitourinary anomalies; hypomobile Ehlers-Danlos	Complex IV deficiency by muscle biopsy	This report

^aEsophageal atresia only reported.

^b Although this patient was reported as having VACTERL association as well as mitochondrial dysfunction, details are unclear; the authors of this paper were contacted and confirmed the presence of both conditions, though further details are not available.

V, Vertebral anomalies; A, anal atresia; C, cardiac malformations; T, trachea; E, esophageal fistula; R, renal anomalies; L, limb.