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Underlying genetic factors of the VATER/VACTERL association with special emphasis on the “Renal” phenotype

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Abstract

The acronym VATER/VACTERL association (OMIM #192350) refers to the rare non-random co-occurrence of the following component features (CFs): vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). According to epidemiological studies, the majority of patients with VATER/VACTERL association present with a “Renal” phenotype comprising a large spectrum of congenital renal anomalies. Indeed, all of the identified human disease genes for the VATER/VACTERL association, namely *FGF8*, *FOXF1*, *HOXD13*, *LPP*, *TRAPI*, *ZIC3* have been associated with renal malformations. This review resumes these genotype-phenotype correlations and suggests that the elucidation of the genetic causes of the VATER/VACTERL association will ultimately provide insights into the genetic causes of the complete spectrum of congenital renal anomalies per se.

Keywords

VATER; VACTERL; association; renal; genetics

Introduction

The VATER/VACTERL association was first described as VATER association in 1972 by Quan and Smith as an acronym which identifies a non-random co-occurrence of vertebral anomalies (V), anal atresia (A), tracheoesophageal fistula and/or esophageal atresia (TE), radial dysplasia (R) [1]. In 1973, only one year later the same authors published an extended definition of the acronym now linking the letter R not only to radial dysplasia but also to renal anomalies [2]. In 1974, again one year later, Temtamy and Miller [3] added cardiac (C) and limb defects (L), and the acronym was revised to VACTERL association.

In the following years, several extensions of the clinical definition were proposed, including vascular anomalies “V”, auricular anomalies “A”, and rib anomalies “R” [4,5]. Furthermore, recent studies showed that besides the classical and the extended component features (CFs), many patients present with less frequent congenital anomalies, which still seem to be inherent to the association [6,7]. In addition to these clinical diagnostic criteria, several studies reported clinically-defined clusters depending on the type and spatial location of CFs (e.g. “upper vs. lower” VATER/VACTERL phenotypes) [8]. However, in his review on the “VACTERL/VATER association” Solomon [8] pointed out that these clusters may very well reflect variable diagnostic criteria instead of true distinct phenotypes. Irrespective of these extended CFs and the rare but inherent congenital anomalies, it is now generally accepted in the field of Medical Genetics, that the clinical diagnosis of the VATER/VACTERL association (OMIM %192350) requires the presence of at least three of the core component features (CFs): vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L) [2, 9,10].

The involvement of genetic factors in the development of this rare association is suggested by reports of familial occurrence, the increased prevalence of component features among first-degree relatives of affected individuals, high concordance rates among monozygotic twins, chromosomal (micro-) aberrations or single gene mutations in individuals with the VATER/VACTERL phenotype, as well as murine knock-out models. The present review aims to summarize the current knowledge on the underlying genetic factors of the VATER/VACTERL association with special emphasis on the “Renal” phenotype.

Frequency of “Renal” anomalies in patient cohorts with VATER/VACTERL association

Population based epidemiological studies in Europe and the United States reported birth prevalences [11–13] ranging from 1 in 10.000 to 1 in 40.000 [14]. Because of this low birth prevalence Botto and colleagues [13] studied infants with the clinical diagnosis of the VATER/VACTERL association in a combined registry of infants with multiple congenital anomalies from 17 birth defects registries worldwide that are part of the International Clearinghouse for Birth Defects Monitoring Systems. Here, they analysed the frequency of core CFs among patients with a clear clinical diagnosis of VATER/VACTERL association. Overall they looked at data from approximately 10 million infants born between 1983 and 1991. 286 of these infants presented with the clinical diagnosis of VATER/VACTERL association, defined by the presence of at least three core CFs. Interestingly, 231 of these 286 patients (80.8%) presented with renal anomalies (R) making this CF the second most common CF in this survey only preceded by anorectal malformations (A) (236 out of 286; 82.5%). In 2002, Cuschieri and the EUROCAT Working Group [15] carried out an extended survey and re-analyzed the dataset from 1980 through 1994. Probably due to better ultrasound screening they now found renal anomalies to be by far the most common CF among VATER/VACTERL patients. This epidemiological observation suggests that disease-causing genes involved in the etiology of the VATER/VACTERL association might also be involved in the development of renal anomalies per se.

Familial VATER/VACTERL association with the “Renal” phenotype

In 2012 we reviewed the familial occurrence of the VATER/VACTERL association by performing a systematic literature search accessing the electronic databases NCBI (www.ncbi.nlm.nih.gov/pubmed) and DIMDI (www.dimdi.de/static/de/index.html), and congress reports concerning the following “Medical Subject Headings” (MeSH): VATER, VACTERL, association, esophageal atresia, anorectal malformation, congenital heart defect, limb anomalies, renal anomalies, and vertebral anomalies [16]. We identified in total 12 families, in which at least one member exhibited three or more core CFs of the VATER/VACTERL association, and at least one additional member had a minimum of one CF [16]. In some of these families, the association appeared to follow a Mendelian mode of inheritance [16–24]. In almost all of the families, at least one of the affected members presented with renal anomalies [16, 18, 20, 22–24] providing additional support, that among families with a possible involvement of genetic factors in disease development, these genetic factors might also be involved in the development of the renal anomalies.

Chromosomal (micro-)aberrations in patients with VATER/VACTERL and VATER/VACTERL-like phenotypes

To date, cytogenetic and molecular analyses have revealed chromosomal (micro-) aberrations in at least 13 patients with the full clinical picture of the VATER/VACTERL association (see Table 1). These include: (i) deletions of 5q11.2 [25], 6q [26], 7q35-qter [27], distal 13q [28,29], 19p13.3 [30], and 20q13.33 [31]; (ii) duplications on 1q41, 2q37.3, and 8q24.3 [32], 9q [33] and at 22q11.21 [34]; (iii) deletion at Yq with duplication at Yp [35], and deletion of 9p24.3-p24.1 with duplication of 18q12.3-q23 [36]; (iv) supernumerary der(22) syndrome [37]; (v) mosaicism for supernumerary ring chromosome 12 [38] or 18 [39]; and (vi) partial monosomy 16p13.3pter/partial trisomy 16q22qter [40]. The smallest of these microaberrations was identified in the study by Hilger et al. [32], describing a *de novo* microduplication at 2q37.3 with an estimated size of 25kb. The duplication comprised exons 3–4 of one splice variant of Calpain-10 (*CAPN10*) and exons 3–6 of *GPR35*, encoding the G-protein-coupled receptor 35. The duplication carrying index case was an aborted female fetus. The pregnancy was terminated at 30+1 weeks of gestation because of sonographically proven severe bilateral renal dysplasia. Macroscopic and histological findings obtained at autopsy, revealed a cloacal malformation with anal atresia, bilateral renal dysplasia, urethral agenesis, a secondary bell-shaped thorax, and Potter-sequence facies due to anhydramnion. *Gpr35* expression studies in mice at embryonic day (E) 14.5 showed detectable transcripts in, amongst other tissues, genital tubercle and rectum. However, mutation analysis of *GPR35* in 192 VATER/VACTERL and VATER/VACTERL-like patients did not identify any sequence variant of likely pathological significance.

Genetically engineered VATER/VACTERL murine mutant models with the “Renal” phenotype

Shh, Gli2, Gli3

Lately mutant murine models provided some clues and several candidate genes to the etiology of the VATER/VACTERL association. The vast majority of these genetically engineered mice present with congenital renal anomalies (see Table 2). Here genetically engineered mice with mutations in genes of the Sonic hedgehog (SHH) signaling cascade namely *Shh*, *Gli2*, and *Gli3* show the complete spectrum of VATER/VACTERL phenotype, including variable renal anomalies like unilateral renal agenesis, horseshoe or ectopic kidney [41]. In human, various allelic phenotypes have been associated with heterozygous *SHH* mutations (MIM #142945, Holoprosencephaly 3; MIM #611638, Microphthalmia with coloboma 5), *GLI2* (MIM #610829, Holoprosencephaly 9; MIM #615849, Culler-Jones syndrome), and *GLI3* (MIM #175700, Greig cephalopolysyndactyly syndrome; MIM #146510, Pallister-Hall syndrome). However, none of these syndromic phenotypes in human completely resembles the classic human VATER/VACTERL association.

Pcsk5

Szumaska and colleagues [42] identified an ethylnitrosourea (ENU)-induced recessive mouse mutation (Vcc) resulting from a p.Cys470Arg exchange in the proprotein convertase subtilisin/kexin type 5 (PCSK5), resembling all CF of the human VATER/VACTERL association including bilateral renal agenesis. Nevertheless, to date, all candidate gene sequencing studies in human VATER/VACTERL patients revealed only heterozygous missense variants and one heterozygous frameshift variant [36,42,43]. To determine the origin of the variants, the authors of these studies sequenced the family samples that were available and found all of the respective variants to be transmitted by an unaffected parent. Assuming a pathogenic effect of these *PCSK5* variants, an autosomal recessive model of inheritance seems to be most consistent with these findings, as a model of reduced penetrance in the parents would imply, that at least some of the parents are mildly affected. Hence, further exploration of the non-coding regulatory regions of *PCSK5* in order to identify the second hit is warranted.

Ptf1a

Recently, the molecular basis of *Danforth's short tail* (*Sd*) mouse [44] was elucidated by three groups who reported the insertion of a retrotransposon in the 5' regulatory domain of the murine *Ptf1a* gene, encoding pancreas specific transcription factor 1A [45–47]. As a consequence and contrary to wildtype littermates, *Sd* mice showed ectopic *Ptf1a* expression in the notochord and hindgut at E8.5 to E9.5, which extended to the cloaca and mesonephros at E10.5 and to the pancreatic bud at E10.5 and E11.5 [46]. The resultant phenotype of this *Sd* mutation not only causes reduction or absence of kidneys, but mirrors the complete phenotype of the human VATER/VACTERL association. While *PTF1A* had been previously confirmed as an autosomal-recessive disease-causing gene for human “Pancreatic and cerebellar agenesis” (MIM #609069), *PTF1A* sequence analysis of 103 VATER/VACTERL and VATER/VACTERL-like patients however, could not identify any pathogenic alterations

affecting the coding region and 1.5 kb of its 5' flanking sequence [48]. These findings suggest that mutations in *PTF1A* do not play a significant role in the development of human VATER/VACTERL association.

Ift172

Friedland-Little and colleagues [49] identified a recessive ENU-induced mutation, *avc1* (atrioventricular canal 1), causing VACTERL-H phenotype including vertebral anomalies, anal atresia, cardiac defects, tracheoesophageal anomalies, renal dysplasia, limb anomalies and hydrocephalus. They identified an *avc1*-specific polymorphism in *Ift172*, an intraflagellar transport gene. They showed that *avc1* is a hypomorphic mutation of intraflagellar transport protein 172 (Ift172), required for ciliogenesis and Shh signaling. The authors described renal dysplasia with hypoplastic glomeruli in four of nine *avc1* mutant embryos, compared with the normal phenotype in six wild-type mice [49]. In human, homozygous and compound heterozygous mutations in *IFT172* have been proven to cause the phenotypes of retinitis pigmentosa 71 (MIM #616394) or short-rib thoracic dysplasia 10 with or without polydactyly (MIM #615630).

While no human phenotype has been associated with mutations in *PCSK5*, various allelic human phenotypes have been detected with heterozygous mutations in *SHH*, *GLI2*, and *GLI3*, as well as with homozygous or compound heterozygous mutations in *PTF1A* and *IFT172* (see above). Of these, only heterozygous mutations in *GLI3* and homozygous or compound heterozygous mutations in *IFT172* are associated with micro- and macroscopic renal anomalies in human.

Human VATER/VACTERL disease genes and their contribution to the “Renal” phenotype

TRAP1

Saisawat et al. [50] most recently identified *TRAP1* as the first autosomal-recessive disease causing gene for the full clinical picture of the VATER/VACTERL association, as well as a cause for isolated congenital abnormalities of the kidneys and the urinary tract (CAKUT). In this study, recessive mutations in the gene encoding TNF receptor-associated protein 1 (TRAP1) were found in two families with severe vesicoureteral reflux and in three families with VATER/VACTERL association (including right duplex kidney or right renal agenesis in two of these three cases). TRAP1 is a heat-shock protein 90-related mitochondrial chaperone, possibly involved in antiapoptotic and endoplasmic reticulum stress signaling. *Trap1* is expressed in renal epithelia of the developing mouse kidney at E13.5 and in the kidney of adult rats, most prominently in proximal tubules and in thick medullary ascending limbs of Henle's loop [50]. Furthermore, Yan et al investigating several heat shock proteins and their embryonic function in mice, found *Trap1* to be involved in forelimb development, another organ system affected by the VATER/VACTERL association [51].

ZIC3

Besides the classic VATER/VACTERL association, an associated condition presents with hydrocephalus. In view of its X-linked transmission, it is termed X-linked VATER/

VACTERL-hydrocephalus (or VACTERL-H), which can be caused by mutations in the *ZIC3* gene. According to the studies presented in the following the hydrocephalus is an optional feature that does not obligatory occur in *ZIC3* caused VATER/VACTERL association. In 2010, Wessels and colleagues [52] described a male newborn with VATER/VACTERL association including a unilateral multicystic kidney. As the clinical picture of this patient overlapped with that of X-linked heterotaxy caused by *ZIC3* mutations, the *ZIC3* coding region was sequenced revealing a 6-nucleotide insertion in a GCC repeat of the *ZIC3* gene. The resultant polyalanine expansion was not found in 192 ethnically matched controls, nor was it present in the mother, suggesting it occurred *de novo* in the patient. Recently mutation analysis of *ZIC3* identified a recurrent disease-causing mutation (c.49G>T, p.Gly17Cys) in four patients with VATER/VACTERL and VATER/VACTERL-like phenotypes [53]. The first male patient presented with three CFs, namely, recto-vesical fistula, atrial septal defect, and right renal agenesis with grade IV–V vesicoureteral reflux on the left side. Additional features were cryptorchidism and penoscrotal transposition. He inherited the *ZIC3* p.Gly17Cys mutation from his unaffected mother. Furthermore, the p.Gly17Cys mutation was detected in a sister and her brother. The girl presented with vestibular fistula, high-grade vesicoureteral reflux, and 13 ribs on both sides. Her brother presented with recto-prostatic fistula and atrial septal defect. Testing for skewed X-chromosome inactivation in the family showed, that the mutant allele was predominant (only 9% inactivated), whereas the paternal allele was nearly 91% inactivated. The same active allele in the sister was shared by her brother, which explains why both siblings were similarly affected. The fourth p.Gly17Cys patient presented with recto-urethral fistula and right-sided ectopic kidney with grade I vesicoureteral reflux. In addition, he displayed penoscrotal transposition and glandular hypospadias [53]. While *ZIC3* is known to regulate left-right body asymmetry during embryonic development it remains unclear how *ZIC3* is involved in early renal development [54]. Since several of the VATER/VACTERL patients with *ZIC3* mutations show unilateral renal anomalies this might be attributable to a disturbed regulation of body asymmetry during embryonic development and point towards *ZIC3* regulation of renal development.

Human disease genes in patients with VATER/VACTERL-like phenotypes and renal anomalies

In a few cases, distinct gene mutations have been reported, with mostly VATER/VACTERL-like phenotypes. Mutations in these genes were almost always found in single patients rendering their contribution to the development of the association uncertain (see Table 2).

HOXD13

In a female with tetralogy of Fallot, bilateral hydronephrosis and hydroureters, anorectal malformation, and inter-phalangeal joints of her 4th and 5th toes, resembling a VATER/VACTERL ('ACR') phenotype, Garcia-Barceló et al. [55] identified a heterozygous 21 base-pair *de novo* deletion in the *HOXD13* gene. According to the "Mouse Genome Database (MGD)" none of the *HOXD13* cre mice displays renal anomalies [56]. Accordingly *Hoxd13* is not expressed in the mouse renal progenitor structures (expression data from E11.5 – E17) [57]. Hence, *HOXD13* does not seem to play a major role in the development of renal anomalies in the context of the VATER/VACTERL association.

FGF8

The *FGF8* gene encodes a transcription factor involved in multiple embryonic signaling cascades and also in regulation of *SHH* expression. A recent *FGF8* analysis by Zeidler and colleagues [58] in 78 patients with VATER/VACTERL association or VATER/VACTERL-like phenotype revealed two different mutations. While the p.Gly29_Arg34dup mutation in a patient with five CFs ('ACTERL') including horseshoe kidney has not been reported yet, the p.Pro26Leu substitution in a patient with two CFs ('AR'), comprising right-sided renal dysplasia, had earlier been found in a patient with Kallmann syndrome (KS) who did not show any CFs of the VATER/VACTERL association [59]. Interestingly, both patients had bilateral cryptorchidism, a phenotypic feature in *FGF8* associated KS. Each mutation was paternally inherited. However, besides delayed puberty in both and unilateral cryptorchidism in one of the fathers, they were otherwise healthy. This suggests a variable expressivity for mutations in *FGF8* in which patients with VATER/VACTERL association may constitute the severe end [58]. According to MGD *Fgf8*^{tm1.3Mrt}/*Fgf8*^{tm1.4Mrt} mice display abnormal kidney development and absent renal glomerulus [56]. Furthermore, *Fgf8* is expressed from E11.5 – E15 in the metanephros of the mouse suggesting that *FGF8* might play a role in the formation of renal anomalies within the VATER/VACTERL association spectrum [57].

LPP

Arrington et al. [60] detected haploinsufficiency of the *LPP* gene in a patient with VATER/VACTERL association including a "Renal" phenotype. This patient presented with tetralogy of Fallot, rib anomalies, hypospadias, small kidneys and esophageal atresia, yielding the phenotype 'CTER'. *LPP* codes for the LIM domain containing preferred translocation partner in lipoma, which has been shown to bind PEA3 (polyomavirus enhancer activator 3 homolog), an ETS domain transcription factor involved in the SHH signaling pathway [61]. However, *LPP* gene analysis detected no deleterious sequence changes in 70 additional patients with VATER/VACTERL association [62]. None of the *Lpp* associated mouse phenotypes in MGD display renal anomalies [56]. Furthermore, *Lpp* shows absent or only ambiguous expression in the mouse metanephros substructures at E15 [57]. Hence, *LPP* does not seem to play a major role in the development of renal anomalies in the context of the VATER/VACTERL association.

FOXF1

The transcription factor *FOXF1*, a downstream target of the SHH pathway plays an important role in epithelium-mesenchyme signaling [63]. Most recently, a heterozygous *de novo* *FOXF1* missense mutation was found in a patient with a VATER/VACTERL-like phenotype ('AR'), who presented with an anorectal malformation, left-sided renal agenesis and glandular hypospadias [53]. In 2009, Stankiewicz et al. [64] had reported several patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV) displaying different heterozygous genomic deletions and point mutations in *FOXF1*. All these patients also showed additional malformations in VATER/VACTERL organ systems, and in all of these systems, except for the kidney, strong *Foxf1* expression was noted in mice at E12.5 and E13.5 [53]. Moreover, *Foxf1* heterozygote mutant mice displayed tracheo-esophageal fistulas [65], one hallmark of the VATER/VACTERL

association. Why *Foxf1* did not show significant staining in mouse kidney and why the patient reported by Hilger et al. [53] did not develop ACDMPV remains to be elucidated, but the resultant phenotype might be dependent on residual function and amount of FOXF1 protein [64].

Monogenic syndromes resembling the clinical picture of the “Renal” VATER/VACTERL phenotype

Several known monogenic syndromes resemble the full clinical picture of the “Renal” VATER/VACTERL phenotype, e.g. Baller Gerold syndrome (MIM #218600; autosomal-recessive; *RECQL4*), Townes Brocks syndrome (MIM #107480; autosomal-dominant; *SALL1*), and Fanconi anemia (FA) complementation group (i.e. FANCB, FANCF or FANCL). Here, Faivre et al. [66] found in over 200 FA patients, that approximately 5% were judged to have a VATER/VACTERL or VATER/VACTERL-like phenotype. In a recent review, Lubinsky [67] concludes that the frequency and number of CFs of the VATER/VACTERL association with FA correlate with the severity and onset of hematopoietic and malignancy issues. Here, radial anomalies are the most common CF, interestingly followed by renal anomalies.

Summary

Although the identified genetic causes underlying the VATER/VACTERL association are heterogeneous and gene-gene interactions have yet to be elucidated, all of the identified human disease genes are associated with “Renal” phenotypes. This is in accordance with the observation, that the majority of patients with VATER/VACTERL association presents with a “Renal” phenotype, suggesting that the elucidation of the genetic causes of the VATER/VACTERL association might very well provide insights into the genetic causes of isolated renal anomalies per se.

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Table 1
Chromosomal (micro-)aberrations in patients with VATER/VACTERL and VATER/VACTERL-like phenotypes

Chromosomal region	Size	Gain/Loss/Other	Study
5q11.2	~ 51.32 – 55.00 Mb	Loss	25
6q	-	Loss	26
46,XX,arr 7q35q36(147,314,780-158,781,397)x1	11.5 Mb	Loss	27
13q31.1-qter	~ 31.1 Mb	Loss	28
13q31.2-qter	28.5 Mb	Loss	29
13q33.2-qter	9.5 Mb	Loss	29
19p13.3	810 kb	Loss	30
20q13.33	0.7 Mb	Loss	31
1q41	135 kb	Gain	32
2q37.3	25 kb	Gain	32
8q24.3	120 kb	Gain	32
9q	-	Gain	33
22q11.21	2.51 – 2.54 Mb	Gain	34
(Yp) [46,X,i(Yp):isochromosome Yp]	-	Ring chromosome	35
del(9)(p24.3p24.1) dup(18)(q12.3q23).	7.34 Mb 34.3 Mb	Terminal deletion of 9p Terminal duplication 18q	36
del(X)(p22.2p22.2)	0.01 – 0.04 Mb	Hemizygous deletion	36
47,XX,+der(22)t(11;22)(q23;q11.2)mat	13.2 Mb 2.7 Mb	Gain	37
46,XY/47,XY+r(12)(p12.1q12)	~ 23 Mb	Ring chromosome	38
47,XXY,+r(18)(q10q11.2)[4].nuc ish 18q11.2 (RP11-79F3x8) [30/28]/46,XY[26]dn	In 11–13% of peripheral blood lymphocytes the additional ring chromosome lead to an octasomy of ~5 Mb of the pericentromeric region of chromosome 18	Ring chromosome	39
partial monosomy 16p13.3-pter partial trisomy 16q22-qter	~ 5.3 Mb ~ 1.5 Mb	Loss Gain	40

Table 2
Murine and human VATER/VACTERL candidate genes displaying “Renal” phenotypes

Candidate gene	Human chromosomal region	Murine derived candidate genes +/-	Human derived candidate genes +/-	Study
<i>IFT172</i>	2p23.3	+	+	49
<i>GLI2</i>	2q14	+	-	41
<i>HOXD13</i>	2q31.1	-	+	53
<i>LPP</i>	3q28	-	+	56-58
<i>GLI3</i>	7p13	+	+	41
<i>SHH</i>	7q36	+	-	41
<i>PCSK5</i>	9q21.23	+	-	36,42,43
<i>PTF1A</i>	10p12.2	+	-	44-48
<i>FGF8</i>	10q24.32	-	+	54,55
<i>TRAP1</i>	16p13.3	+	+	50
<i>FOXFI</i>	16q24.1	+	+	52, 59-61
<i>ZIC3</i>	Xq26.3	+	+	51,52