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Yq Microdeletion in a Patient with VACTERL Association and Shawl Scrotum with Bifid Scrotum: A Real Pathogenetic Association or a Coincidence?

Stefano Tumini[a] Melissa Alfonsi[b] Silvia Carinci[a] Elisena Morizio[b] Ivana Antonucci[c] Valentina Gatta[c] Gabriele Lisi[d] Pierluigi Lelli Chiesa[d] Giuseppe Calabrese[b] Liborio Stuppia[c] Chiara Palka[b]

[a]Department of Pediatrics, "G. d'Annunzio" University, Chieti, Italy; [b]Genetic Unit, Department of Oral Sciences, Nano and Biotechnologies, "G. d'Annunzio" University, Chieti, Italy; [c]Department of Psychological, Humanities and Territory Sciences, School of Medicine and Health Sciences, "G. d'Annunzio" University, Chieti, Italy; [d]Department of Pediatric Surgery, "G. d'Annunzio" University, Pescara, Italy

Established Facts

- VACTERL association is defined by the occurrence of congenital malformations: vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, and limb defects.
- No genetic alterations have been discovered except for some sporadic chromosomal rearrangements and gene mutations.
- The AZFc region is frequently deleted in infertile men with severe oligozoospermia or azoospermia.
- *BPY2* (previously *VCY2*) interacts with a ubiquitin-protein ligase, involved in the SHH pathway which is known to be implicated in the genesis of VACTERL association.

Novel Insights

- This is the first report of a Yq11.223q11.23 microdeletion associated with VACTERL association and shawl scrotum with bifid scrotum.

Keywords
Shawl scrotum with bifid scrotum · Sonic hedgehog pathway · Ubiquitin-protein ligase E3A · VACTERL · *BPY2*

Abstract
VACTERL association is defined by the occurrence of congenital malformations: vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, and limb defects. No genetic alterations have been discovered except for some sporadic

E-Mail karger@karger.com
www.karger.com/cgr

Chiara Palka
Genetic Unit, Department of Oral Sciences, Nano and Biotechnologies
"G. d'Annunzio" University
Via dei Vestini 5, IT–66100 Chieti (Italy)
E-Mail chiarapalka@libero.it

chromosomal rearrangements and gene mutations. We report a boy with VACTERL association and shawl scrotum with bifid scrotum who presented with a de novo Yq11.223q11.23 microdeletion identified by array CGH. The deletion spans 3.1 Mb and encompasses several genes in the AZFc region, frequently deleted in infertile men with severe oligozoospermia or azoospermia. Herein, we discuss the possible explanation for this unusual genotype-phenotype correlation. We suggest that the deletion of the *BPY2* (previously *VCY2*) gene, located in the AZFc region and involved in spermatogenesis, contributed to the genesis of the phenotype. In fact, *BPY2* interacts with a ubiquitin-protein ligase, involved in the SHH pathway which is known to be implicated in the genesis of VACTERL association.

VACTERL association is characterized by the occurrence of at least 3 of these cardinal congenital malformations: vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, and limb defects [Botto et al., 1997; Solomon, 2011, 2018]. A defect in blastogenesis has been suggested as a possible etiology for this malformation spectrum [Martínez-Frías et al., 1998]. Although the majority of cases are sporadic, there is evidence for inheritance [Solomon et al., 2010]. Furthermore, chromosomal abnormalities have been described in some patients, such as 13q deletion, ring 12 and 16q chromosomes, 9q duplication as well as mitochondrial mutations, and *PTEN*, *HOXD13*, and *ZIC3* mutations [Aynaci et al., 1996; Damian et al., 1996; McNeal et al., 1977; Cinti et al., 2001; Reardon et al., 2001; Walsh et al., 2001; Garcia-Barceló et al., 2008; Wessels et al., 2010; Zhang et al., 2017; Solomon, 2018].

Recently, Aguinaga et al. [2010] studied the *SHH* gene in a group of 10 patients who fulfilled 3 or more of the criteria for VACTERL association, based on the observation that *Shh* mutant mice exhibited a spectrum of anomalies similar to those observed in VACTERL patients [Kim et al., 2001; Aguinaga et al., 2010]. The study did not demonstrate any evidence of sequence changes in the patients.

A rarely noted finding in VACTERL association is penoscrotal transposition [Walsh et al., 2001]. It is a rare congenital anomaly in which the scrotum is located superior and anterior in relation to the penis. Penoscrotal transposition can present with a wide spectrum of anomalies which go from simple shawl scrotum to very extreme transposition [Gershoni-Baruch and Zekaria, 1996; Pinke et al., 2001]. The latter malformation also has been reported previously in children with distal 13q deletions, suggesting that the critical region maps between 13q33.1 and 13qtel [MacKenzie et al., 1994; Parida et al., 1995; Bartsch et al., 1996; Boduroglu et al., 1998; Kirchhoff et al., 2009]. The *EFNB2* gene identified in this chromosome segment has been suggested to control urorectal development, and it is a candidate gene for genital malformations in males [Walczak-Sztulpa et al., 2008]. Furthermore, a partial deletion of the long arm of the normal Y chromosome has been reported in a boy with hypospadia and incomplete penoscrotal transposition, inherited from his normal father. It seems likely that the genital abnormalities of this patient did not result from the deleted Y chromosome, but from some prenatal disturbance [Fujita et al., 1983].

Here, we describe the first case of a male child with VACTERL association and shawl scrotum with bifid scrotum, carrying a 3.1-Mb microdeletion at Yq11.223q11.23, identified by array CGH, encompassing several genes in the AZFc region.

Case Report

The proband is a 1-year-old Italian male child born to unrelated parents. Family history was unremarkable. During pregnancy, an amniocentesis was performed because of an abnormal triple-test at 15 weeks of gestation. The fetal karyotype resulted 46,XY. Delivery was performed at 37 gestational weeks by cesarean section, after a pregnancy with intrauterine growth restriction identified by fetal ultrasound at 8 months. His birth length was 50 cm (50th centile), weight 2,470 kg (10th centile), and head circumference 35 cm (25–50th centile). At birth, an anorectal malformation was noticed characterized by an invertography as an anal atresia and a rectocutaneous fistula associated with a posterior hypospadia and a shawl scrotum with bifid scrotum (Fig. 1A, B). The anomaly was surgically corrected with a posterior sagittal anorectoplasty and correction of the fistula before the first month of life. Furthermore, he presented a preaxial polydactyly of the right hand. An abdominal ultrasound showed renal anomalies, (third grade vescico-ureteral reflux with an incomplete duplex ureters on the left side) while a spine radiography identified vertebral anomalies (C6–C7 schisis, T12 emivertebra, and a left convex scoliosis). A heart ultrasound resulted normal.

Methods and Results

Metaphase chromosomes were harvested from phytohemagglutinin-stimulated peripheral lymphocytes and G-banded using standard techniques. The karyotype was defined according to the International System for Human Cytogenetic Nomenclature [iSCN 2009] and resulted normal 46,XY. Array CGH was carried out using the 575-kb resolution BAC array (Cytochip BlueGnome), according to the recommendations of the manufacturer. It detected a Yq chromosome deletion bridging from clone RP11-70G12, at position 25.49 Mb, to clone RP11-557B9, at position 28.59 Mb. Clone RP11-263A15, at position 25412514 Mb, showed normal copy number resulting as adjacent to the Yq breakpoint,

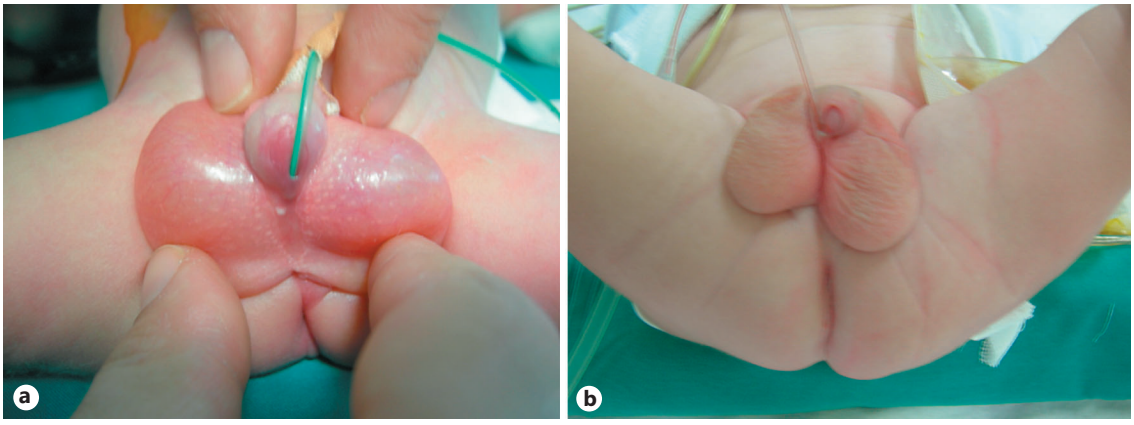

Fig. 1. Posterior hypospadias (**a**) and penoscrotal transposition with bifid scrotum (**b**).

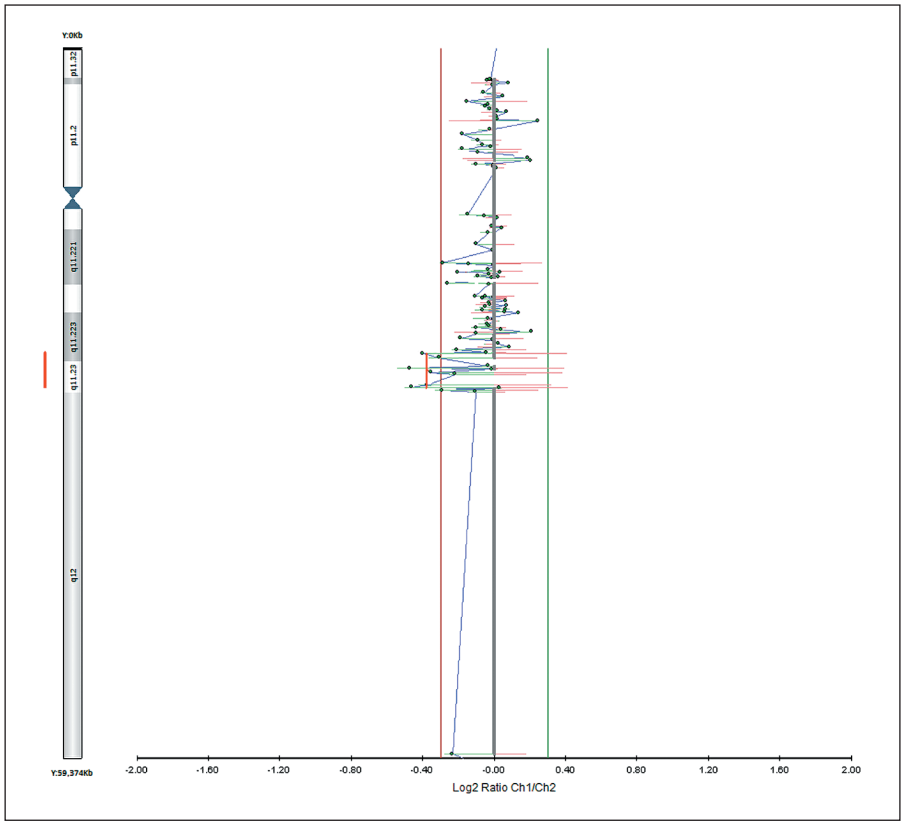

Fig. 2. Yq11.223q11.23 microdeletion.

thus occurring between AZFa and AZFb (Fig. 2). These results allowed one to establish that proximal Yq region including AZFa and AZFb loci was preserved, while distal Yq including the AZFc locus was deleted. The Yq deletion detected by array-CGH analysis was confirmed by the microdeletion Y Kit (AMPLI-Y Chromosome UE, BIRD, Italy). The final karyotype was interpreted as 46,XY,del(Y)(Yq11.223;q11.23) de novo. The parents' karyotype and array-CGH analysis were normal.

Discussion

We present the first case of a de novo Yq11.223q11.23 microdeletion in a male with VACTERL association and shawl scrotum with bifid scrotum. The microdeletion includes 10 genes in the AZFc region and, in particular, the *BPY2* gene. We question whether the proband's pheno-

type is related to the Yq11.223q11.23 microdeletion and, especially, to the loss of *BPY2*.

Recent research has shown that mice with a deletion of the *Shh* gene develop anomalies similar to those observed in VACTERL association [Arsić et al., 2002]. In fact, the expression of *Shh* begins shortly after gastrulation and is of crucial importance for the normal embryonal development of many organs and structures [Arsić et al., 2007]. Nevertheless, no mutations in humans have yet been reported [Aguinaga et al., 2010]. To date in humans, mutations in *SHH* have been associated only with holoprosencephaly [Roessler et al., 1996].

The *BPY2* gene is a testis-specific protein which encodes a 13.9-kDa protein with 106 amino acids. The association between *BPY2* and male infertility is known [Stuppia et al., 2001]. For the first time, Tiepolo and Zuffardi [1976] observed the presence of genes controlling spermatogenesis on the long arm of the Y chromosome. Different reports have demonstrated that about 10–15% of the infertile patients with normal karyotype are carriers of Yq microdeletions [Stuppia et al.,1996a; Wong et al., 2002]. These microdeletions are clustered within 3 nonoverlapping hot spot regions, defined as AZFa, AZFb, and AZFc [Vogt et al., 1996]. So far, at least 12 genes have been isolated from these regions [Wong et al., 2002], including *BPY2* which is located in the AZFc region in chromosome Yq. It has been reported to be deleted in infertile men with severe oligozoospermia or azoospermia [Stuppia et al., 1996b, 1997; Repping et al., 2004]. However, to date no single nucleotide variants of *BPY2* have been identified in patients with infertility.

BPY2 interacts directly with a ubiquitin-protein ligase E3A (UBE3A) [Wong et al., 2002]. Mutations of *UBE3A* have been reported in patients affected by Angelman syndrome and autism spectrum disorders [Greer et al., 2010]. *UBE3A* is involved in the SHH pathway which is known to be implicated in the genesis of VACTERL association [Wong et al., 2002; Solomon, 2011; Robbins et al., 2012]. In fact, previous experimental studies show that the BPY2 protein interacts with the HECT domain of UBE3A whose ubiquitination may be required for BPY2 function [Wong et al., 2002].

In conclusion, this is the first case of a Yq11.2 deletion, involving the *BPY2* gene, and VACTERL association with shawl scrotum with bifid scrotum. To date, no reports of such phenotypic consequences of a Yq11.2 deletion or of a haploinsufficiency of *BPY2* have been published. As a result, it is not possible to speculate that the deletion of this gene contributed to the genesis of the phenotype, as well as excluding it as a coincidence. Penoscrotal transposition has rarely been reported in VACTERL association [Walsh et al., 2001]. So this association could be coincidental. Furthermore, we have to consider that VACTERL association, and even more the shawl scrotum with bifid scrotum, are rare diseases in comparison to AZFc microdeletions. On the other hand, to date several chromosomal microdeletions/microduplications are reported in literature in affected and unaffected people. As a consequence, they are considered to be of uncertain significance because of some genetic model explanations such as reduced penetrance, variable expressivity, or the different sex expression. Therefore, the present aberration can represent modifiers or contributors to a multifactorial mode of inheritance. Finally, we have to consider that the shawl scrotum with bifid scrotum has rarely been reported in VACTERL association, and these associations could be coincidental.

Further cases will be needed to confirm the mechanisms suggested in this report and whether array CGH is warranted in patients with certain phenotypic effects.

Statement of Ethics

All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, with the 1964 Helsinki declaration, and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

S. Tumini and S. Carinci were responsible for the clinical data and participated in study design and coordination. M. Alfonsi and E. Morizio performed the karyotype and the array-CGH analysis. I. Antonucci and V. Gatta performed the Y study. G. Lisi and P. Lelli Chiesa were responsible for clinical and chirurgical diagnoses. G. Calabrese helped to draft the paper. L. Stuppia gave the final approval of the version to be published. C. Palka was responsible for clinical issue, participated in the paper's design and coordination, and drafted the manuscript.

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