Y chromosome aberration in a patient with cloacal-bladder exstrophy-epispadias complex: an unusual finding

Aberração cromossômica do Y em uma paciente com extrofia de bexiga e de cloaca e epispadia: um achado raro

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SUMMARY

Chromosome aberrations or genetic syndromes associated with cloacal-bladder exstrophy complex have rarely been reported. The aim of this report is to describe a 14 year-old female Brazilian patient with a complex urogenital malformation, short stature, lack of secondary sexual characteristics and Y chromosome aberration. A girl with cloacal bladder exstrophy complex was referred for evaluation of short stature and absence of secondary sexual characteristics. Pre-pubertal levels of gonadotropins and sex steroids were observed at the beginning of monitoring, but follow-up showed a progressive increase in testosterone levels. The patient underwent gonadectomy and testicular tissue was identified without dysgenetic characteristics. She had a 46,X,inv(Y)(p11.1q11.2) karyotype, normal *SRY* sequence, and no Y deletions. The pericentric inversion of Y chromosome apparently did not contribute to the development of the complex urogenital malformation in this patient. Currently, no teratogenic agent, environmental factor, or defective genes have been recognized as etiologic factors for this type of urogenital malformation. Arq Bras Endocrinol Metab. 2013;57(2):148-52

SUMÁRIO

Aberrações cromossômicas ou síndromes genéticas associadas ao complexo extrofia de bexiga e de cloaca e epispadia são raramente relatadas. O objetivo é descrever uma paciente brasileira com 14 anos que apresenta uma malformação urogenital complexa, baixa estatura, ausência de características sexuais secundárias e alteração do cromossomo Y. Uma menina com extrofia de bexiga e de cloaca e epispadia foi encaminhada para avaliação de baixa estatura e ausência de desenvolvimento de características sexuais secundárias. Níveis pré-puberais de gonadotrofinas e esteroides sexuais foram observados no início da avaliação, mas durante o seguimento notou-se um aumento progressivo dos níveis de testosterona. Ela foi submetida à gonadectomia e identificou-se a presença de testículos sem características disgenéticas. O cariótipo era 46,X,inv(Y)(p11.1q11.2), com sequência normal do *SRY* e ausência de deleções do Y. A inversão pericêntrica do cromossomo Y, aparentemente, não contribuiu para o desenvolvimento da malformação urogenital complexa nessa paciente. Atualmente, nenhum agente teratogênico, fator ambiental ou mutações gênicas foram reconhecidos como fatores etiológicos para essa malformação urogenital. Arq Bras Endocrinol Metab. 2013;57(2):148-52

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INTRODUCTION

The cloacal-bladder exstrophy-epispadias complex (BEEC) is an anterior midline defect with variable expression comprising anomalies involving the abdominal wall, pelvis, urinary tract, genitalia and, occasionally, the spine and anus (1). This rare congenital anomaly is characterized by a clinical spectrum ranging from isolated epispadias to classic bladder exstrophy (CBE), to its most severe form, cloacal exstrophy (CE) or "OEIS" complex (omphalocele, exstrophy of the bladder, imperforate anus, and spinal defects). The incidence of BEEC varies with regard to ethnic background, sex, and phenotypic expression, but it is twice as common among males (2).

The great majority of BEEC cases are classified as non-syndromic, and the etiology of this disorder is still unknown. No single teratogenic agent or environmental factor that could play a role in the expression of BEEC, has been identified. However, Reutter and cols., studying a large cohort of patients with BEEC and their families, concluded that smoking and medical radiation during the first trimester of pregnancy might be associated with a more severe BEEC phenotype (2). Chromosomal aberrations or genetic syndromes associated with BEEC have only rarely been reported.

CASE REPORT

The present study was approved by the Ethics Committee of the Hospital das Clinicas, University of Sao Paulo Medical School. Written consent was obtained from the patient's mother.

An infant was born from a nonconsanguineous couple by normal vaginal delivery. Multiple anomalies were present at birth, including omphalocele, cloacal exstrophy, imperforate anus, ambiguous genitalia with prominent rugated labioscrotal folds, and no apparent genital tubercle. Abdominal sonography showed left-kidney agenesia. The family history was unremarkable, with three healthy siblings (one female and two males), and no other similarly affected individuals. The mother had short stature without sexual dysfunction, was 26 years of age and the father 29 years old at the time of the child's birth. At the age of three days, the patient was submitted to corrective surgery. A colostomy was created, and the cloacal exstrophy and abdominal wall were closed. At four years of age, she underwent surgery to close the colostomy. In the same year, the (exstrophic) bladder was closed and enlarged with the ileum and colon bowel. The bladder neck was closed and the construction of a urinary continent stoma was performed with the cecal appendices (Mitrofanoff principle). External female genitalia were created. Latter, other surgeries were performed to correct an enterocutaneous fistula and to remove bladder lithiasis.

At 14 years of age she was referred to an endocrinologist to evaluate her short stature and absence of development of secondary sexual characteristics. Physical examination revealed (Table 1): height 135 cm, (SDS -4.3 female; SDS -3.7 male), height age of 11.5 years and bone age of 12 years, and low weight 38.8 kg (SDS -2.9 female; SDS -2.3 male), low-set hairline, shield-like chest with widely separated nipples, acne in the dorsal area, hair in the nasolabial region, and deep voice. Neuropsychomotor and intellectual development were normal for the patient's age.

Genital examination identified labia major with excess of skin and posterior fusion, excess of skin in the clitoris region without palpable tissue, a single perineal opening, and Tanner B1P1.

Initial hormonal data showed prepubertal levels of gonadotropins and sex steroids, but in the follow-up, testosterone levels gradually increased (Table 1).

Table 1. Clinical and laboratory follow-up of the patient with BEEC and 46,X,inv(Y)(p11.1q11.2) karyotype

Chronological age (yrs)	14.8	15.1	15.4	16.0*	16.5**	17.5
Bone age (yrs)	10.0	11.0	12.0	13.0	13.5	NP
Weight (kg)	28.5	32.1	35.8	38.8	40.4	46.6
W (SDS) female	-2.9	-2.7	-2.3	-2.1	-1.9	-1.2
W (SDS) male	-2.3	-2.2	-2.0	-2.1	-1.9	-1.8
Height (cm)	135.0	137.0	142.0	146.0	148.0	152.9
H (SDS) female	-4.3	-4.1	-3.3	-2.7	-2.4	-1.6
H (SDS) male	-3.7	-4.0	-3.5	-3.7	-3.8	-3.2
Puberty (Tanner stage)	B1/P1	B1/P1	B1/P2	B1/P4	B2/P4	B2/P4
LH (UI/L)	< 0.1	3.2	6.0	12.4	16.1	63.4
FSH (UI/L)	< 0.1	3.9	6.3	12.1	12.6	78.2
Testosterone (ng/dL)	< 11	21	113	190	191	< 11
Estradiol (pg/mL)	< 13	< 13	16	NP	NP	14

B: breast; F: female; H: height; M: male; NP: not performed; P: pubic hair; SDS: standard deviation score; W: weight.

* Data obtained before the first surgery; ** Data obtained before the second surgery.

Normal hormone levels: female *pre-pubertal range* – LH: ≤ 0.6 IU/L; FSH: ≤ 3.2 IU/L; testosterone: < 14 ng/dL; estradiol: < 21 pg/mL; *pubertal range* – LH: 1.1-6.3 IU/L; FSH: 1.4 – 5.7 IU/L; testosterone: ≤ 98 ng/dL; estradiol: 22 – 232 pg/mL; male *pre-pubertal range-testosterone*: < 19 ng/dL; *pubertal range* – testosterone: ≤ 669 ng/dL; estradiol: ≤ 35 pg/mL.

An abdominal CT scan was not able to identify gonadal tissue. She was submitted to a laparotomy to remove the gonads, but only the right testis was identified and excised. Some fibrous tissue was removed on the supposition that it was the left gonad. Histological examination showed normal testicular tissue on the right side, and Mullerian structures were not identified. After the surgery, testosterone levels remained elevated (198 ng/dL), confirming the presence of the other testis. The patient underwent surgery in which the testis was identified near the left kidney and removed. Histological analysis showed normal testicular tissue. Psychological evaluation was performed to elucidate her gender orientation, which turned out to be female.

Cytogenetic analysis of lymphocyte and gonadal tissue showed a 46,X,inv(Y)(p11.1q11.2) karyotype (Figure 1). FISH analysis confirmed the presence of the *SRY* gene (Yp11.31), DYZ3 (centromere) and DYZ1 (Yq12) regions of the Y chromosome, and clarified the mechanism that generated the Y chromosome inversion (Figure 1). Eight *loci* of the Y chromosome: PAR1, *SRY*, *TSPY*, *AMELY (*Yp*)*, DYZ3 (centromere), DYS280, DYS1, and DYZ1 (Yq) were amplified by PCR using genomic DNA extracted from blood, indicating that these regions were preserved. The *SRY* gene sequence was normal. The mother's karyotype was 45,X[10]/46,XX[113].

DISCUSSION

Advanced parental age (3), increased parity even after adjusting for age (4) and *in vitro* fertilization (5) have been reported as risk factors to BEEC development. Spontaneous errors of development such as somatic mutation or complex gene-environment interactions may be responsible for BEEC.

A role of genetic factors in the pathogenesis of BEEC has also been suggested. This hypothesis was based on observations of rare familial cases, high but incomplete concordance in monozygotic twins, and a single report of increased recurrence risk for BEEC in the offspring of an affected parent (6-8).

Cytogenetic and molecular analyses have revealed chromosomal anomalies in a few patients with BEEC. Numerical chromosomal aberrations (47,XXX; 47,XXY; 47,XYY; 45,X/46,XX) were observed. In some of these cases Down syndrome was observed (9). Aneuploidy of sex chromosomes in some of these cases might point to *loci* involved in the formation of BEEC (1). The observation of different sexes in two subsequent spontaneous

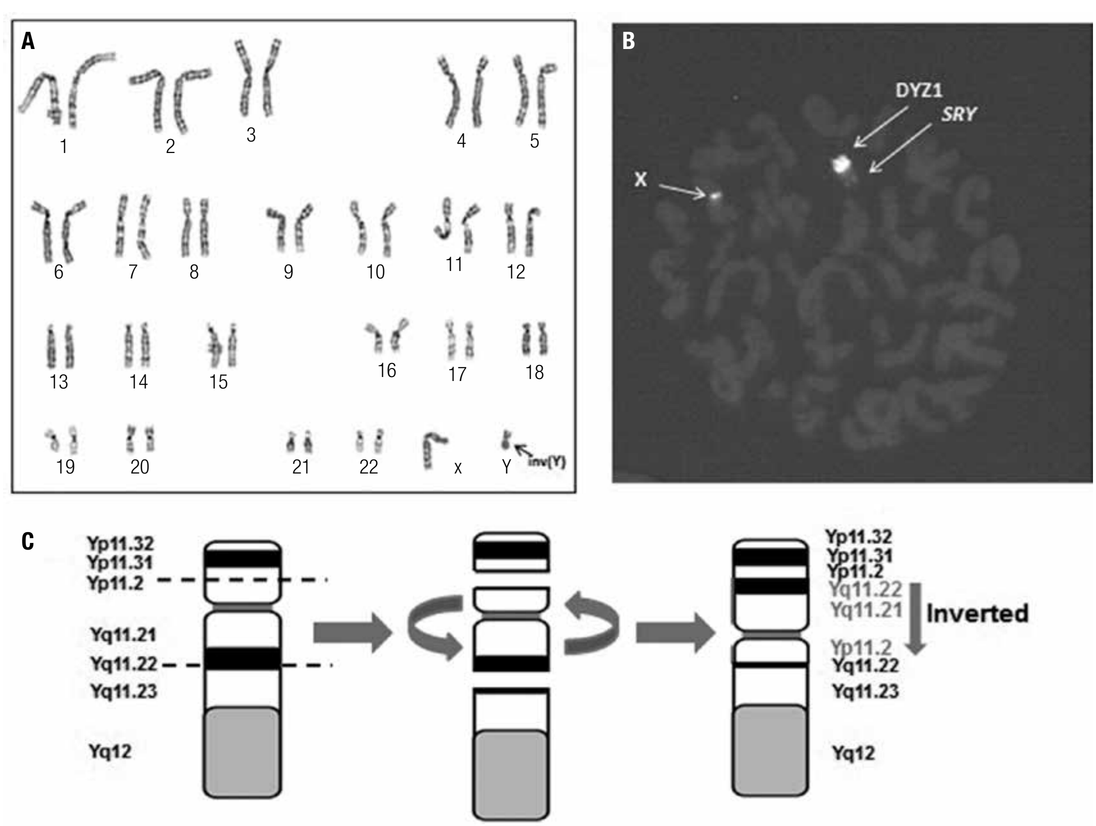

Figure 1. (**A**) Karyotype BTGW: 46,X,inv(Y)(p11.1q11.2); (**B**) Metaphase chromosome spread submitted by FISH technique, indicating the X chromosome, and the locations of the *SRY* gene and of the DXZ1 *locus* on the Y chromosome (arrows); (**C**) Schematic representation of the mechanism that generates pericentric inversion of the Y chromosome.

abortions, however, did not support this hypothesis (8). Apparently, none of these chromosomal abnormalities could be confirmed as the cause of this disorder.

Structural aberrations involving the chromosome 9 at region q32-ter have been identified in six BEEC cases (1). The *SF-1* (Steroidogenic Factor 1; 9q33.3) and *SET* genes (Suppressor of variegation, Enhancer of zeste and Thrithorax; 9q34.11) have been investigated in patients with BEEC (10). However, no mutations were detected in these studies. Nonetheless, other genes located in this region might be involved in the etiology of BEEC.

Our patient had 46,X,inv(Y)(p11.1q11.2) karyotype, and apparently neither large deletions in Y chromosome nor *SRY* gene mutations were identified. Possibly, the inverted Y chromosome was inherited from the father, but the father's blood sample was not available.

Pericentric inversion of the Y chromosome occurs in approximately 1:1,000 males in the general population, and it is considered a chromosome heteromorphism that does not influence male phenotype (11). However, in the literature, there are some cases of pericentric inversion of the Y chromosome leading to XY female with gonadal dysgenesis and no *SRY* mutations. Gimelli and cols. reported a young woman with gonadal dysgenesis, a gonadoblastoma and a dysgerminoma in both gonads, normal external genitalia and a 46,X,inv(Y)(p11.31q12) karyotype (12). This inversion led to a silencing of *SRY* due to the position-effect variegation, which caused the gonadal dysgenesis (12). Mitsuhashi and cols. also described a XY female with normal external genitalia, 46,X,inv(Y)(p11.2q11.2) karyotype, and gonadal dysgenesis. This inversion did not lead to position-effect variegation, but the streak gonads presented abnormally prolonged *SRY* expression. Thus, the authors believed that the regulation of the *SRY* gene was impaired, causing gonadal dysgenesis (13). Different from the previously described patients, in the patient described here, histological study identified a testis without characteristics of a dysgenetic gonad, and no abnormalities in the encoding region of *SRY* or 5'-UTR were detected.

Interestingly, our patient's mother had a 45,X lineage in the karyotype consistent with Turner syndrome. The age of the patient's mother was considered in the cytogenetic analysis, because of the loss of sex chromosomes with increasing age (14). She had some Turner stigmata, including webbed neck, short stature, recurrent otitis with hearing loss, and facial dysmorphia, but she had four spontaneous pregnancies. Despite the fact that women with Turner syndrome present a high risk of having malformed offspring (15), the other three siblings were phenotypically normal. Also, there are no cases in the literature describing women with Turner syndrome who had been pregnant with offspring with BEEC. At the moment, no defective gene has been recognized as the cause of BEEC development, neither has any teratogenic agent or environmental factor. The Y chromosome aberration identified in this patient with a complex urogenital malformation is an unusual finding, and apparently did not contribute to the development of the disease. The mother's chromosomal condition was not related to BEEC, either.

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