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CASE REPORT

DUPLICATION OF THE *SOX3* GENE IN AN SRY-NEGATIVE 46,XX MALE WITH ASSOCIATED CONGENITAL ANOMALIES OF KIDNEYS AND THE URINARY TRACT: CASE REPORT AND REVIEW OF THE LITERATURE

Tasic V[1], Mitrotti A[2], Riepe FG[3], Kulle AE[3], Laban N[1], Polenakovic M[4], Plaseska-Karanfilska D[4], Sanna-Cherchi S[2], Kostovski M[1], Gucev Z[1,*]

***Corresponding Author:** Professor Dr. Zoran Gucev, University Children's Hospital, Medical Faculty Skopje, ul. Majka Tereza 17, 1000 Skopje, Republic of Macedonia. Mobile: +389-70-279-742. E-mail: gucevz@ gmail.com

ABSTRACT

Disorders of sex development (DSD) are a group of rare conditions characterized by discrepancy between chromosomal sex, gonads and external genitalia. Congenital abnormalities of the kidney and urinary tract are often associated with DSD, mostly in multiple malformation syndromes. We describe the case of an 11-year-old Caucasian boy, with right kidney hypoplasia and hypospadias. Genome-wide copy number variation (CNV) analysis revealed a unique duplication of about 550 kb on chromosome Xq27, and a 46,XX karyotype, consistent with a sex reversal phenotype. This region includes multiple genes, and, among these, *SOX3* emerged as the main phenotypic driver. This is the fifth case reporting a genomic imbalance involving the *SOX3* gene in a 46,XX SRY-negative male, and the first with associated renal malformations. Our data provide plausible links between *SOX3* gene dosage and kidney malformations. It is noteworthy that the current and reported *SOX3* gene duplications are below the detection threshold of standard karyotypes and were found only by analyzing CNVs using DNA microarrays. Therefore, all 46,XX SRY-negative males should be screened for *SOX3* gene duplications with DNA microarrays.

Keywords: Congenital anomalies of kidneys and the urinary tract (CAKUT); Copy number variations (CNVs); Disorders of sex development (DSD).

[1] Medical Faculty Skopje, Skopje, Republic of Macedonia

[2] Division of Nephrology, Columbia University, New York, NY, USA

[3] Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University Hospital Schleswig-Holstein, Christian-Albrechts University Kiel, Kiel, Germany

[4] Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov," Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

INTRODUCTION

Sex in humans is genetically determined and is defined by the sex chromosomes (XY for males and XX for females) and by the development of gender specific anatomy, physiology and behavior. A complete or partial mismatch between genetic sex and phenotypic sex results in disorders of sexual development (DSD). Disorders of sexual development in humans have a frequency of at least one in 100 live births [1], while the frequency of "corrective" genital surgery is estimated to be between one and two per 1000 live births. There is a wide spectrum of DSD ranging from hypospadias (incidences variable from one in 500 to six in 250 births) [2] to ambiguous genitalia (incidence one in 4500 births) [3] and complete sex reversal (46,XY females and 46,XX males; one in 20,000 births) [4]. Congenital malformations of the kidney and DSD are often described in association, in the broad spectrum of multiple malformation syndromes, as it happens in Smith-Lemli-Opitz Syndrome (OMIM: 270400), a complex syndrome characterized by congenital kidney and ureteric abnormalities associated with genital anomalies and inadequate sexual hormone production [5,6]. Mutations of Wilms tumor 1 (*WT1*) gene may lead to Denys-Drash syndrome (OMIM: 19408), Frasier syndrome (OMIM: 13668) or Wilms aniridia genitourinar renal (WAGR) syndrome (OMIM: 194072), which are characterized by kidney and genitourinary diseases in association with internal and external genitalia defects [7-10] and mostly 46,XY female phenotypes. The sex-determining region Y (SRY) is considered to be the main regulator of male sex determination in mammals [1,11]. The main function of SRY in sex determination is to upregulate its direct target gene *SOX9*, thus initiating Sertoli cell differentiation [12,13,14]. The SRY-related HMG box-containing gene 3 (*SOX3*) is a member of a family of 20 *SOX* genes, structurally similar to

SRY [15]. This gene is located on the X chromosome and it consists of a single exon [16]. The *SOX3* gene encodes for a transcription factor expressed in the central nervous system (CNS) of vertebrate embryos, which is essential for pituitary, craniofacial and neuronal development [15-22].

Prior human genetics studies implicated *SOX3* in brain development and gender determination. Laumonnier *et al.* [17] described a pericentric inversion of the X chromosome involving the IL1RAPL at Xp21.3 and the polyalanine repeat of *SOX3* at Xq26.3, in a 10-year-old girl affected a by mild memory deficiency, strabism, speech impairment and hypotonia; because previous studies showed that female carriers of microdeletions involving IL1RAPL do not show intellectual disability [23,24], the phenotype was likely attributable to another gene in the duplicated region. Analysis of an independent family segregating X-linked intellectual disability demonstrated an in-frame duplication of 33 bp involving a polyalanine repeat of *SOX3*, thus pointing to *SOX3* mutations as the cause of neurodevelopmental delay.

A sub-microscopic duplication of 685.6 kb at Xq27.1 involving *SOX3*, has been reported in two siblings affected by hypopituitarism and abnormalities of corpus callosum, while a duplication involving the *SOX3* polyalanine repeat was identified in three male siblings from another family, segregating panhypopituarism and abnormalities of the pituitary gland: all of these patients had absent infundibulum and did not present an intellectual disability [18]. A duplication of 3.9 Mb involving the Xq27 region containing *SOX3*, has been reported in males affected by X-linked hypopituitarism [25]. In a study of 16 SRY-negative 46,XX male patients, DNA microarray analysis showed genomic rearrangements of the *SOX3* regulatory region in three patients, two duplications and one deletion: the CNVs involved genomic regions in close proximity of *SOX3* in all three patients [26].

Interestingly, a recent report described a SRY-negative, 46,XX boy affected by ovotesticular DSD, with hypo-spadias and cryptorchidism with a *de novo* duplication of a 502 kb fragment of the long arm of chromosome X, involving *SOX3*, as well as *RPS17P17*, *CDR* and *MIR 320D2*. The role of the *RPS17P17*, *CDR1* and *MIR320D2* genes has not been investigated [27]. In summary, SOX3 genetic variants have been associated with X-linked intellectual disability with isolated growth hormone deficiency as well as X-linked panhypopituitarism and 46,XX sex reversal in males. Until now, no other developmental phenotypes have been associated to *SOX3* gene dosage.

CASE PRESENTATION

We were consulted on a 11-year-old white Caucasian male for the findings of hypoplasia of the right kidney and coronal moderate hypospadias, after surgical correction of the urethra anomaly. He was the first child of a non consanguineous couple. His parents and younger sister were healthy. His intelligence was normal (IQ 92) and he had no other anomalies. The behavior, growth and development were all normal. His testes volume was >4 mL and the penis length was 5 cm. Abdominal ultrasound and magnetic resonance imaging (MRI) did not show internal female genitalia, and confirmed right kidney hypoplasia (Figure 1, Table 1). The left kidney size was 80 × 32 mm, while the right kidney size was 57 × 23 mm.

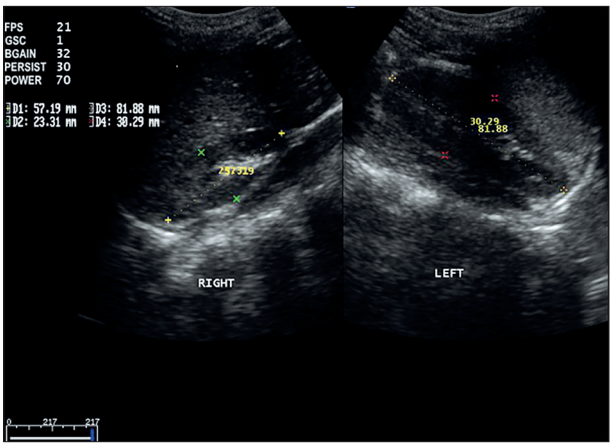

Figure 1. Ultrasound images showing hypoplasia of the right kidney measuring 57 × 23 mm compared to a normal size left kidney 80 × 32 mm.

The patient was investigated as part of a study approved by the institutional review board at our International Centre for genetic Engineering and Biotechnology in Skopje (Republic of Macedonia) and at the Department of Nephrology, Columbia University, New York, NY, USA. This patient was already reported as part of our prior study on copy number variations (CNVs) in kidney malformations [28].

An additional 23 patients were selected to perform targeted Sanger resequencing of *SOX3*. We selected 23 males affected by urinary tract developmental defects (10 renal hypodysplasia; three vescicoureteral reflux; two posterior urethral valve; four obstructive uropathy; one bladder anomaly, one ectopic, one accessory kidney and one horseshoe kidney) and associated DSD (11 hypospadias, nine cryptorchidism, one epispadia and one congenital hidrocele).

Endocrine Analysis. Plasma concentrations of steroid hormones, comprising mineralocorticoids, glucocorticoids and androgens, were determined using UPLC Quattro Premier/Xe system (Waters, Milford, MA, USA) as previously described [29-31].

In brief, aliquots of plasma samples, calibrator and controls with a volume of 0.1 mL were combined with an internal standard mixture to monitor recovery. All samples were extracted using Oasis MAX SPE system Plates (Waters).

Table 1. Comparison of our patient characteristics with cases reported in the literature.

References	**[26]**	**[26]**	**[26]**	**[27]**	**This Study**
Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	M-30	M-19; M-26 (histology)	M-19 months	M-30 months	M-11
Height	165 cm	167.5 cm	75 cm	87.8 cm (11.8 kg)	148 cm (42 kg)
Penis size		10.2 cm long; 2.6 cm wide	3.4 cm long	32 mm long; 13 mm wide	5 cm
Testicular size	~ 5 mL	~6 mL	right testicle appear smaller than left testicle		4 mL
Genitals and testes		scrotal hypoplasia; retractile testes; histology: atrophic changes with loss of normal hypoplastic scrotum; spermatogenesis; thickening and hyalinization of the tubular basal lamina and diminished number of interstitial cells; normal spermatic cords		cryptochidism; hypospadias	moderate coronal hypospadias
Secondary sexual characteristics	normal	Tanner stage 5 pubic hair and penile development with small testes; onset age 13 years	NA	NA	
Developmental issues		gender dysphoria from 6 years; referred to behavioral therapist	microcephaly; developmental delay; growth retardation	none	crossdressing
CAKUT	–	–	–	–	hypospadias; kidney hypodysplasia
Genetic alterations	two microduplications of ~123 and 85 kb, the former of which spanned the entire *SOX3* gene	microdeletion; a single 343 kb immediately upstream of *SOX3*, suggesting that altered regulation of *SOX3* is the cause of XX male sex reversal	a large ~6 Mb duplication that encompasses *SOX3* and at least 18 additional distally located genes	*de novo* duplication (0.5 Mb) at Xq27.1 comprising *SOX3*, *CDR1* and *MIR320D2*	a unique 550 kb duplication involving *SOX3*, the non coding RNA *LINC00632*, *AK054921*, *CDR1* and the miRNA *MIR320D2*

NA: not available; CAKUT: congenital anomalies of the kidneys and urinary tract.

Genetic Analyses. After receiving informed consent, collected according to the Ethics Board of the Macedonian Academy of Sciences and Arts (Skopje, Republic of Macedonia), genomic DNA was obtained from peripheral blood samples using standard methods. Genome wide genotyping was conducted on patient MCD_13 using Illumina 610-Quad chip (Illumina Inc., San Diego, CA, USA) [32].

Copy number variation analysis was performed as previously described and data were compared to 21,575 multiethnic controls [28,33-35]. Briefly, genotype calls and quality-control analyses were conducted using GenomeStudio v.2010.3 (Illumina Inc.) and PLINK software [36]. Standardized genotyping methods implemented by the PennCNV program [37] were used for genome-wide CNV calls. The human reference genome hg18 (NCBI build 36.1, March 2006) was the reference assembly used to map the CNVs. The annotation of the CNVs was then performed using the UCSC RefGene and RefExon (CNVision program) [38].

Specific primers were designed to direct polymerase chain reaction (PCR) at the exon and exon-intron boundaries of *SOX3*, and bidirectional Sanger sequencing was performed by BigDye® terminator (Nimagen BV, Nijmegen, The Netherlands) reaction followed by a run on an automatic capillary DNA sequencer. Sequence and alignment was conducted using Sequencer 5.4 software (Gene Codes Corp., Ann Arbor, MI, USA).

An adreno corticotropic hormone (ACTH) test showed normal basal and stimulated 17OH-progesterone excluding a form of 46,XX DSD due to 21-hydroxylase deficiency. The 11-deoxycorticosterone (DOC) and 11-deoxycortisol were normal at both baseline and after ACTH stimulation, excluding 11-hydroxylase deficiency. Cortisol levels were in the mid-normal range at baseline and responded to stimulation, excluding primary adrenal insufficiency.

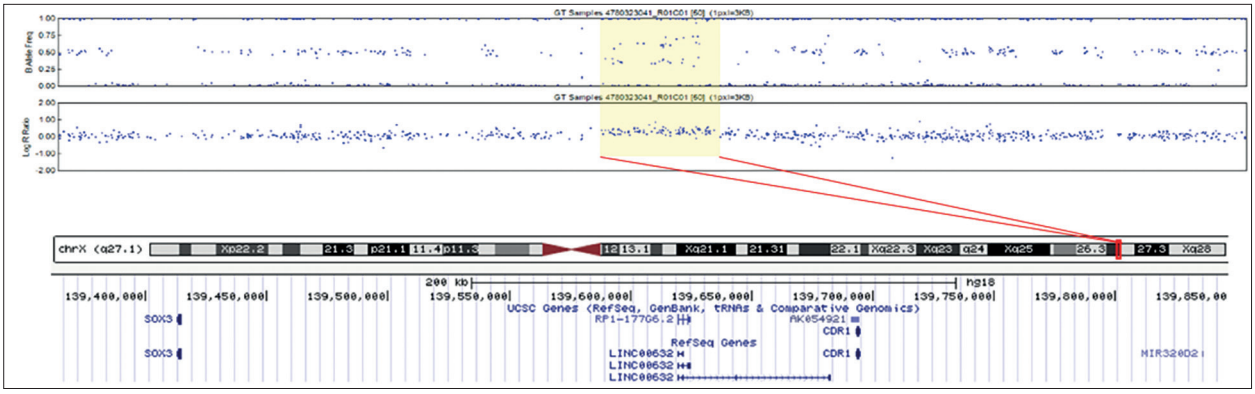

Figure 2. The 550 kb duplication at Xq27 (ChrX: 139,360,520-139,908,320), involving *SOX3*, the non coding RNA *LINC00632*, *AK054921*, *CDR1* and the miRNA *MIR320D2*.

The hCG (human chorionic gonadotrpin) test found testosterone in the low-normal range for male sex and age at baseline. After stimulation, it raised up to 146.0 ng/mL indicating the presence of functional Leydig cells targeted by hCG. The stimulated ratio A:T was below 1, not supporting 17-β-hydroxysteroid dehydrogenase type 3 deficiency. The stimulated ratio T:DHT was 5.6, not supporting 5 α-reductase insufficiency. Microarray-based copy number analysis was previously performed in this patient as part of a larger study on congenital kidney defects [28].

In our 11-year-old male patient affected by renal dystrophy (RHD) and DSD (MCD_13), the microarray analysis showed an unique duplication of about 550 kb of the chromosome region Xq27, involving multiple genes and transcripts: *SOX3*, *RP1-177G6* and *CDR1*, the non coding RNA *LINC00632*, and the miRNA *MIR320D2* [28] (Figure 2). None of the genes within the duplication locus has previously been reported to be in association with kidney and urinary tract phenotypes [39,40]. The chromosomal microarray analysis confirmed the 46,XX female karyotype. Parental DNA material was not available to test segregation; therefore, we could not verify if the Xq27 duplication was a *de novo* or inherited genomic imbalance. No causal mutations were detected in the 23 male patients selected for targeted resequencing indicating that *SOX3* coding variants might be a very rare cause of urinary tract malformations associated with DSDs.

DISCUSSION

There are four cases reported with *SOX3* duplications in 46,XX SRY-negative males in the literature [26,27,41] (Table 1). Two of the 46,XX male patients, 30 and 26 years old, respectively, reported by Sutton *et al.* [26], had normal intelligence and growth; the third one had developmental delay, growth retardation and microcephaly. The patient described by Grinspon *et al.* [27] had normal growth and intelligence, but was affected by hypospadias and cryptorchidism, with ovotestis and hypoplastic testis. Histology analysis showed atrophic changes and loss of normal spermatogenesis. Our patient's clinical phenotype was characterized by normal development and intelligence, DSD characterized by hypospadias and males genitalia with 46,XX karyotype, and, unique compared to all other reported patients in the literature, hypoplasia of the left kidney. Interestingly, our patient, as well as the patient described by by Grinspon *et al.* [27], both with karyotype 46,XX SRY-negative, were characterized by duplications involving the Xq27, encompassing the same genes: *SOX3*, the non coding RNA *LINC00632*, *AK054921*, *CDR1* and the miRNA *MIR320D2*.

The question is whether the kidney defect observed in our patient is biologically related to the duplication of *SOX3* or the other genes in the CNV, or if it represents a coincidental finding. Analysis of publicly available expression data (www.gudmap.org) indicates high expression of *Sox3* in the mouse developing bladder neck at embryonic day E13.5, thus suggesting a possible link to lower urinary tract malformations. The *SOX3* gene is known to be regulated by *PBX1* through direct interaction with its transcription binding site [42]. Interestingly, another patient with renal hypodysplasia from our cohort, was found to carry a *de novo* 0.51 kb deletion affecting *PBX1* [28]. Inactivation of Pbx1 in the mouse results in urinary malformations including renal agenesis and hypodysplasia [43]. Finally, a recent report implicates haploinsufficiency of PBX1 in the pathogenesis of syndromic forms of congenital anomalies of the kidney and urinary tract [44].

These data provide plausible links between *SOX3* gene dosage and kidney malformations. Formal proof of a causal link will require additional genetic and functional data. It is noteworthy that the current and reported *SOX3* duplications are below the detection threshold of standard karyotype and were found only by analyzing CNVs using

DNA microarrays. Therefore, it is important to convey that all 46,XX SRY-negative males should be screened for *SOX3* duplications with DNA microarrays.

We report a case of an 11-year-old male with a duplication of chromosome Xq27, involving *SOX3*, and leading to a male sex reversal and, possibly, kidney hypoplasia. This is the second case of 46,XX SRY-negative affected by DSD and characterized by CNV involving the SOX3 locus, described so far. We speculate that the genomic duplication involving *SOX3* could be responsible not only for pituitary hormone deficiencies in humans and male sex reversal, but also for CAKUT. All 46,XX SRY-negative patients, should be screened for duplications affecting *SOX3*.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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