LETTER TO THE EDITOR

46,XX ovotesticular DSD associated with a *SOX3* gene duplication in a *SRY*-negative boy

Dear Editor,

Ovotesticular disorders of sex development (DSD) are characterized by the coexistence of ovarian and testicular tissue. Although some individuals carry a Y chromosome, explaining testicular development, 46,XX is the commonest karyotype, ranging from 65% to 90% of patients with ovotesticular DSD.[1] In about one-third of 46,XX ovotesticular DSD cases, the *SRY* gene is present, owing to an abnormal translocation to the X chromosome or to an autosome. In the remaining cases, *SRY* is absent; however, the mechanism responsible for the development of testicular tissue is poorly understood.

An effort to accurately establish the aetiological diagnosis in patients with DSD is important for the provision of proper genetic counselling and long-term management. Using a genome-wide strategy, we identified the first case of *SRY*-negative 46,XX ovotesticular DSD associated with a *SOX3* duplication in a boy presenting with hypospadias and bilateral cryptorchidism.

A boy aged 2 years 6 months was referred to us for hypospadias and bilateral cryptorchidism. Parents were nonconsanguineous, and there was no remarkable family history. At the moment of referral, his weight (11·8 kg, −0·82 SDS) and height (87·8 cm, −1·36 SDS) were within the normal ranges. He had a trophic phallus 32 mm long and 13 mm wide, with coronal hypospadias and nonpalpable gonads. No other dysmorphic features were observed.

Because hypospadias and cryptorchidism are signs of foetal undervirilization, a DSD was suspected. Hormonal laboratory exploration (Fig. 1a) showed low serum testosterone levels after a 3 × 1500 IU hCG stimulation test, and low basal AMH, suggesting the presence of scarce functional testicular tissue. Serum LH and FSH were normal for age. The patient was subjected to surgical procedures for the correction of hypospadias and the exploration of the reproductive tract. At exploratory laparotomy, the right gonad was biopsied, followed by orchiopexy. The left gonad was macroscopically judged very atypical; therefore, gonadectomy was performed. The histologic evaluation revealed the presence of bilateral ovotestes (Fig. 1b and c). Testicular tissue of both gonads had features of testicular dysgenesis: dichotomic bifurcation of testicular cords containing enlarged germ cells with hyperchromatic nuclei, penetration of thin albuginea by seminiferous cords and increased interstitial fibrous tissue. The ovarian tissue had primordial and primary follicles. On the left side, two ducts were identified: the medial one with typical features of an epididymis and a lateral one, rudimentary, with Müllerian epithelium (Fig. 1d and e).

Peripheral blood karyotype, using high-resolution GTG banding, was 46,XX, with polymorphisms 15 ps+,21 ps+ in 100 metaphases. The karyotypes of the parents were normal. No *SRY* or other Y chromosome sequences were found by PCR and by chromosomal microarray. Therefore, we sought to determine a genetic aetiology that could explain the diagnosis of 46,XX ovotesticular DSD in the absence of *SRY*.

A genomewide scan of 850 000 tag SNPs was conducted, using the Illumina CytoSNP-850k BeadChip according to the manufacturer's specifications (Illumina). GenCall scores <0·15 at any locus were considered 'no calls'. Image data were analysed using the Chromosome Viewer tool contained in Genome Studio (Illumina). The metric used was the log R ratio, which is the log (base 2) ratio of the observed normalized R value for a SNP divided by the expected normalized R value (under manufacturer's specifications). In addition, an allele frequency analysis was applied for all SNPs. All genomic positions were based upon NCBI Build 37 (dbSNP version 130). The whole-genome SNP array showed a *de novo* gain at Xq27·1. The duplicated region was around 0·5 Mb and encompassed the *SOX3* gene (arr[hg19] Xq27·1(139,541,737-140,043,863)x3). Other genes included were *RPS17P17, CDR1* and *MIR320D2* (Fig. 1f).

Metaphase FISH analysis, using BAC probe RP4595A18 (BlueGnome, Illumina, San Diego CA, USA), excluded *SOX3* signal probe in any autosomal chromosome and suggested a tandem duplication of *SOX3* on the X chromosome (Fig. 1g). FISH analysis in both parents ruled out any abnormality (not shown), indicating that the rearrangement in the proband was *de novo*.

When testicular tissue develops in an *SRY*-negative XX foetus, two different mechanisms can be foreseen:[2] the increased expression of pro-testicular genes, like *SOX9*, or the insufficient expression of pro-ovarian/antitesticular genes, like *WNT4* or *RSPO1*, whose expression is pivotal in early embryonic gonadogenesis to stabilize β-catenin and counteract SOX family genes, thus preventing the formation of the coelomic vessels,[3] an essential step that engages the hitherto undifferentiated gonadal ridge into the testicular differentiation pathway. Here, we report the first case of an *SRY*-negative patient with a 46,XX ovotesticular DSD associated with a duplication of a 502-kb fragment of the long arm of the X chromosome encompassing *SOX3* and its regulatory sequences. Although we cannot rule out a pathogenic effect of a potential overdosage of *RPS17P17, CDR1 and MIR320D2*, also included in the duplicated region of the present case, our results are in line with the evidence in mice indicating that, in the absence of *SRY,* gain of function of *SOX3* is responsible for partial testicular differentiation in the foetal XX gonad.[4] *SOX3* is a single exon gene located at Xq27·1, which encodes a protein that is most similar to SRY and that is required for normal brain, pituitary and craniofacial development in mice and humans. Although SOX3 does not seem to be required for normal testicular differentiation, its overexpression in the

(a)

Laboratory determination	Result in proband	Normal values for age*
Testosterone (ng l^{-1})		
Basal	<10	<10–10
Post hCG	30	>150
AMH (pmol l^{-1})	216	807–1460
LH (IU l^{-1})	<0·10	<0·10–0·43
FSH (IU l^{-1})	0·73	0·26–1·37

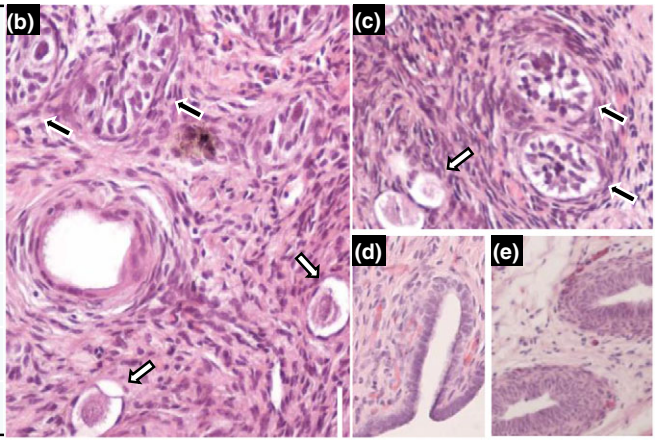

Fig. 1 (a) Laboratory findings in the proband (*Normal values for age from ref.[6]), (b) and (c) histology of the gonads showing left (b) and right (c) ovotestes, with the coexistence of seminiferous tubules (black arrows) and primordial follicles (white arrows) in both gonads; H&E 20x. (d) Polypoid projections with fibrous core and columnar epithelium with cilia, corresponding to the rudimentary Fallopian tube; H&E 20x. (e) Tubules with pseudostratified columnar epithelium and external layer of smooth muscle, corresponding to an epididymis; H&E 20x. (f) Illumina 850K SNP array performed in proband's DNA showed a duplication encompassing *SOX3 gene* (in the box). (g) Proband's inverted DAPI FISH results with probe RP4595A18 (SOX3, red) showed one of the two signals significantly increased (in the circle) on the metaphase chromosomes.

developing XX mouse gonad provokes testicular development, by synergizing with SF1 to upregulate SOX9 expression.[4] It should, however, be noticed that a *SOX3* duplication may not be sufficient to induce testicular differentiation when the regulatory sequences are lacking in the duplicated DNA fragment.[5]

This is the first case of *SRY*-negative 46,XX patient with ambiguous genitalia associated with a *SOX3* duplication presenting with bilateral ovotestes, thus phenocopying a transgenic mice overexpressing SOX3 generated using a 37-kb murine *Sox3* genomic fragment[4]. Our report provides evidence in humans for a pathogenic role of a *SOX3* duplication in ovotesticular DSD in 46,XX *SRY*-negative individuals and enlarges the spectrum of molecular diagnoses for such condition which can also be associated with overexpression of *SOX9*, a clear testis-determining gene, or to insufficient expression of *WNT4* or *RSPO1*.

Acknowledgements

This work was partially financed by grants PICT-2012-0441 of the Fondo Nacional de Promoción Científica, Argentina, PIP-11220120100279 of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, and Project S2010/BMD-2396 (ENDOSCREEN) of Programme Biomedicina 2010 (Comunidad de Madrid), Spain.

Declaration of interest

The authors declare no conflict of interest.

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doi: 10.1111/cen.13126

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