P2-P345

Histopathologic characterization of patients with 46,XX testicular and ovotesticular disorders of sex development

MS Touzon, ML Galluzzo Mutti, P Ramirez, N Perez Garrido, R Marino, M Bailez, M Costanzo, G Guercio, E Vaiani, M Ciaccio, MA Rivarola, A Belgorosky, E Berensztein

Hospital de Pediatría Garrahan, Argentina

Authors have nothing to disclose

Introduction

Sex development is a process that directs both the bi-potential gonads to become either a testis or an ovary, and the consequent differentiation of internal ducts and external genitalia.

Disorders of sex development (DSD) are those congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. These individuals can be classified according to their karyotype in: sex chromosome DSD, 46,XY DSD and 46,XX DSD.

Objective

The aim of this study was to characterize the histology of 46,XX DSD Testicular (T) / ovotesticular (OT) prepubertal gonads.

Clinical material and methods

14 patients with 46,XX T/OT DSD

25 Studied gonads

- Age of **biopsy/gonadectomy**: 1.17(0.08-4.17) years (median and range)
- ***SRY*** detection by PCR and/or MLPA in blood samples of all patients and in DNA from gonads from 8 patients.
- **Immunohistochemical (IHC) analysis:**
  - Sertoli cells (anti-**SOX9** goat polyclonal 1:500, AF3075).
  - Ovarian follicular cells (anti-**FOXL2** goat polyclonal 1:250, ab5096).
  - Pluripotent germ cells (anti-**OCT3/4** mouse monoclonal 1:50, sc-5279).
- As **negative controls**, normal serum of each species respectively was used instead of the primary antibody. No specific immunoreactivity was detected in these sections. Immunostudies were carried out twice, and no difference between duplicates was detected in the staining pattern.

Results

Table 1. Clinical material and characteristics of the gonads

Cases	Total 14	Sex of rearing (M/F)	CA (months)	OCT ¾ +	GB/UGT	OCT 3/4 + & GB/ UGT
OT	12	M:8 F:4	1.16 - 50 (M= 14)	3	6 (3/3)	3 (2/1)
T	2	M:2 F:0	5-26 (M= 15.5)	1	0	0

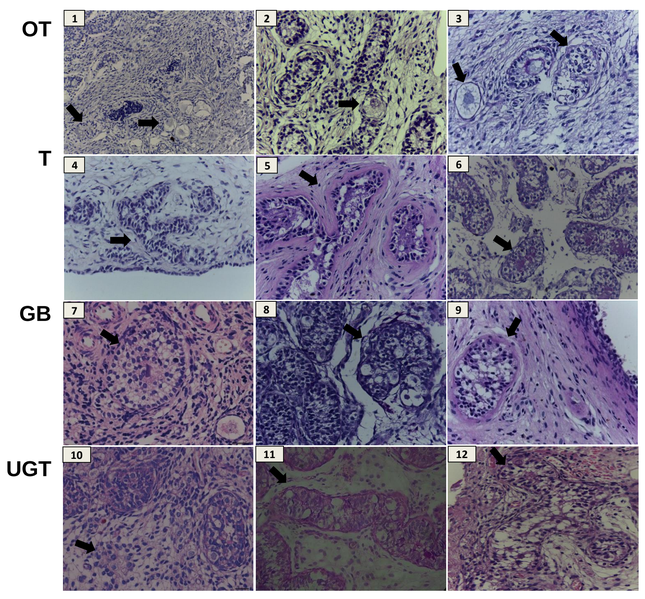
Twenty one gonads (corresponding to 12 patients) showed ovotesticular characteristics and 4 (2 patients) showed only testicular parenchyma. OT: ovotestis, T: testis, M: male, F: female, CA: chronological age, GB: gonadoblastoma, UGT: undifferentiated gonadal tissue

Table 2. IHC staining pattern

Cases	IHC		
	SOX9 +	FOXL2 +	OCT ¾ +
3 GB	2	1	1
3 UGT	2*	2*	2

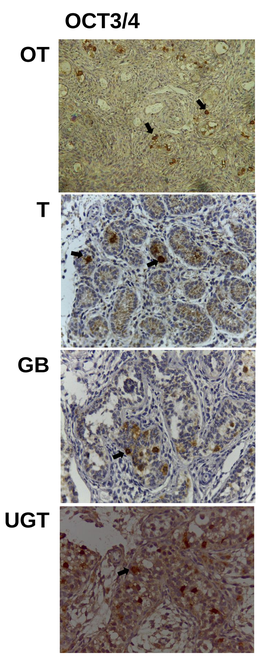
OCT3/4 was positive in 6 gonads (3 patients): 4 with UGT features (2 patients) and 2 with the presence of GB (1 patient).
* One sample was not available for IHC

Figure 1. Histological analysis of the patients' gonads

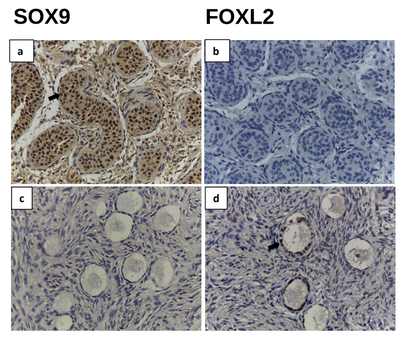


OT, ovotestis, 1 (34m) dysgenetic testis with seminiferous cords and ovarian follicles, 20x; 2 (14m) Meandering and dysgenetic seminiferous cords, one of them with an oocyte-like cell inside,40x; 3 (1.5m) Left Gonad with primordial and primary follicles, and seminiferous cords, 40x.
T, testis, 4 (24m) Branched seminiferous cords in tunica albugínea, and scarce germ cells ,20x; 5 and 6 (3.7m) Rigth gonad, seminiferous tubules, with a significantly thick basal membrane, vacuolated cells, Sertoli cells with pycnotic nucleus and eosinophilic cytoplasm, cell detritus in the lumen,40x.
GB, gonadoblastoma, 7 (3m) Left gonad, oval shape structure with germ cells, sex cord cells, and basement membrane deposits, 40x, 8 (1.5m) Testicular area of an ovotesticular gonad, nests ressembling GB, 40x, 9 (22m) Oval structure with two types of cells and a thick membrane, 40x;
UGT, undifferentiated gonadal tissue, 10 (1.16m) Right Gonad with an incipient GB and clusters of germs cells and somatic cells isolated in fibrous tissue, 40x; 11 (14m) Right gonad with UGT like structures, 40x; 12 (3.7m) Right gonad with UGT, 40x.

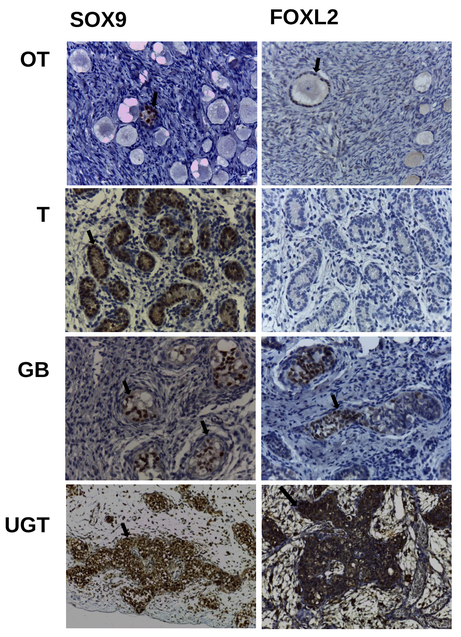
Figure 2. SOX9, FOXL2 and OCT3/4 immunoexpression



Expression of OCT3/4 in OT (3m) 20x, T (5m) 40x, GB (14m) 40x, and UGT (3m) 40x.



Positive SOX9 expression in Sertoli cell nuclei in seminiferous cords (a) and **negative FOXL2** (b) in the first biopsy of a patient (3.7m) (40x) and **Negative SOX9** and **positive FOXL2** (d) in granulosa cell nuclei in the second biopsy of the same patient 13 years later (13.3y) (40x).



OT: (50m) **Positive SOX9** expression in cell nuclei in small cords and **positive FOXL2** nuclei of granulosa cells. 40x.

T (5m) **Positive SOX9** and negative FOXL2 in testicular cords , 40x.

GB (14m) **Positive** nuclear expression of **SOX9** and **FOXL2** , 40x.

UGT (3m) **Positive SOX9** and **FOXL2** expression in cells of branched cords, 20x and 40x.

Conclusions

- **A careful histological analysis is crucial for the diagnosis. Nevertheless, the addition of several IHC markers is important to achieve a thorough characterization of the gonads.**
- **In all testicular parenchyma signs of dysgenesis were found.**
- **A second biopsy in 2 former testicular cases revealed the presence of ovarian parenchyma.**
- **Considering the histopathological findings in early childhood, a close clinical follow up of patients with a specialized DSD team is suggested.**

Discussion

- **How representative is a biopsy?**
- **How important are SOX9 and FOXL2 in histopathological assessment of DSD gonads ?**

References

- Hersmus R et al. The biology of germ cell tumors in Disorders of Sex Development 2016.
- Faure A et al. Testicular biopsy in prepubertal boys: a worthwhile minor surgical procedure? 2016.
- Idrees M T et al. WHO 2016 Classification of Testicular Non-Germ Cell Tumours: A Review and Update from the International Society of Urological Pathology Testis Consultation Panel 2016.
- Spoor J et al. Histological Assessment of Gonads in DSD: Relevance for Clinical Management 2017.
- Ulbright T M Gonadoblastoma and selected other aspects of gonadal pathology in young patients with disorders of sex development 2014.