

# P2-259 Differences of sex development with chromosomal mosaicism: histological characterization and immunohistochemistry markers in gonads during childhood

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## INTRODUCTION

Differences/disorders of sex development (DSD) comprise a group of congenital conditions, affecting human sex determination and/or differentiation. Patients with DSD are classified in: sex chromosome DSD, 46,XY DSD and 46,XX DSD. Sex chromosome disorders, including sex chromosome mosaicism, result in a large clinical spectrum. There is scarce information about the histological pattern of gonads from these patients.

## OBJECTIVE

The aim of this study was to characterize the histology and cell markers pattern in gonads of patients with chromosomal mosaicism.

## CLINICAL MATERIAL AND METHODS

- Gonadal biopsies from 13 prepubertal patients with chromosomal mosaicism, including chromosome Y were studied.
- Six were rearing as male (M) and 7 as female (F).
- Patients were divided in two subgroups (G), according to external genitalia phenotype:
  - G1, with atypical genitalia:** n=7, chronological age (CA) at biopsy, was 1.75, 0.25-12 years (y) expressed as median and range. Five patients were rearing as M and two as F.
  - G2, Turner syndrome:** n=6, CA at biopsy was 13.8, 3.5-18.8 y, all patients were reared as females.
- H&E sections from gonadal biopsies were observed by two specialists.
- Immunohistochemical (IHC) analysis:**
  - Sertoli cells (anti-**SOX9** goat polyclonal 1:500, AF3075, R&D Systems).
  - Ovarian follicular cells (anti-**FOXL2** goat polyclonal 1:250, ab5096, Abcam).
  - Pluripotent germ cells (anti-**OCT3/4** mouse monoclonal 1:50, sc-5279, Santa Cruz Biotechnology).

## RESULTS

**Table 1.** Histological characteristics of gonadal biopsies from prepubertal patients.

	Testis	Ovary	Müllerian remnants	Epididymis	Streak	Testis & Müllerian remnants
<b>G1</b>	6/7 (86%)	0/7 (0%)	3/7 (43%)	2/7 (29%)	3/7 (43%)	2/7 (29%)
<b>G2</b>	2/6 (33%)	1/6 (17%)	5/6 (83%)	1/6 (17%)	3/6 (50%)	1/6 (17%)
<b>Total</b>	8/13 (62%)	1/13 (8%)	8/13 (62%)	3/13 (23%)	6/13 (46%)	3/13 (23%)

In 4 samples of 2 patients of G2 gonadoblastoma, embryonic carcinoma and dysgerminoma were found.

Of the samples that presented testicular parenchyma, 43% had structures compatible with Müllerian remnants.

**Table 2.** Immunohistochemical characterization of gonadal biopsies from prepubertal patients.

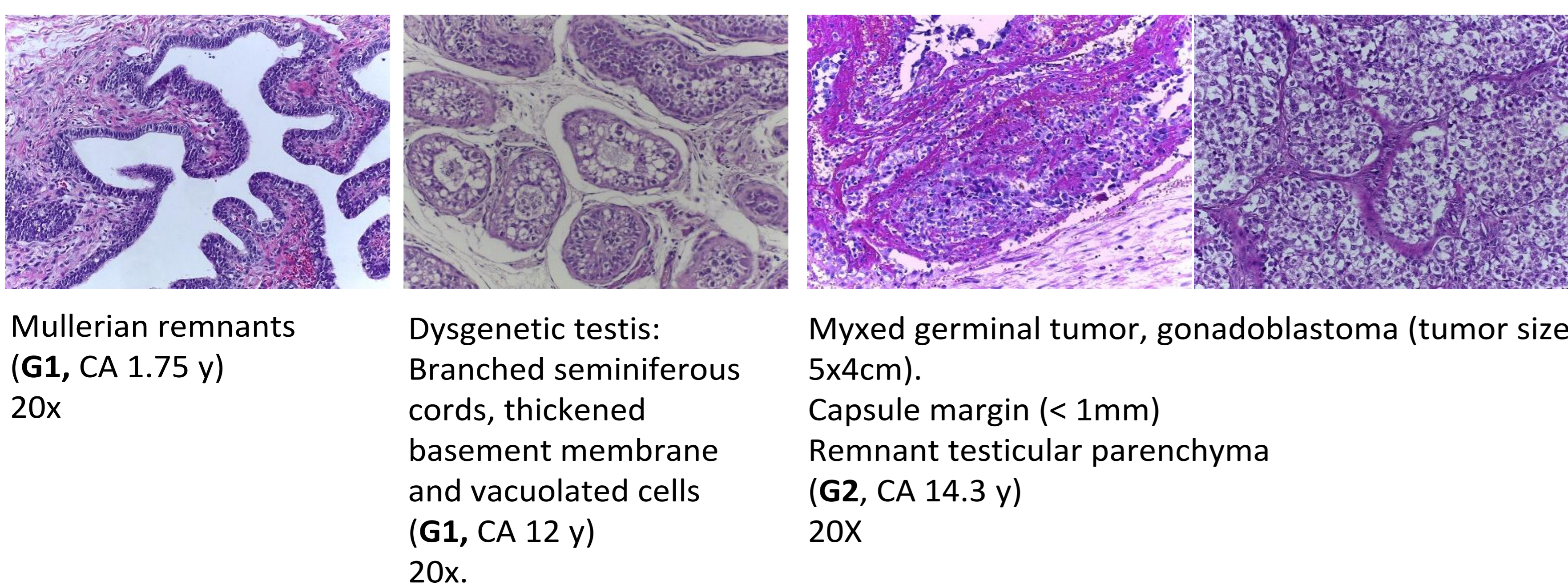
	OCT 3/4 +	SOX9 +	FOXL2 +
<b>G1</b>	57% <sup>a</sup>	75% <sup>b</sup>	33%
<b>G2</b>	67%	50% <sup>c</sup>	100%
<b>Total</b>	60%	67%	60%

<sup>a</sup> OCT 3/4 expression in G1 corresponded to testicular parenchyma in all cases.

All the patients were older than 3 months.

<sup>b</sup> SOX9 was present in the nucleus of Sertoli cells inside the seminiferous cords in G1.

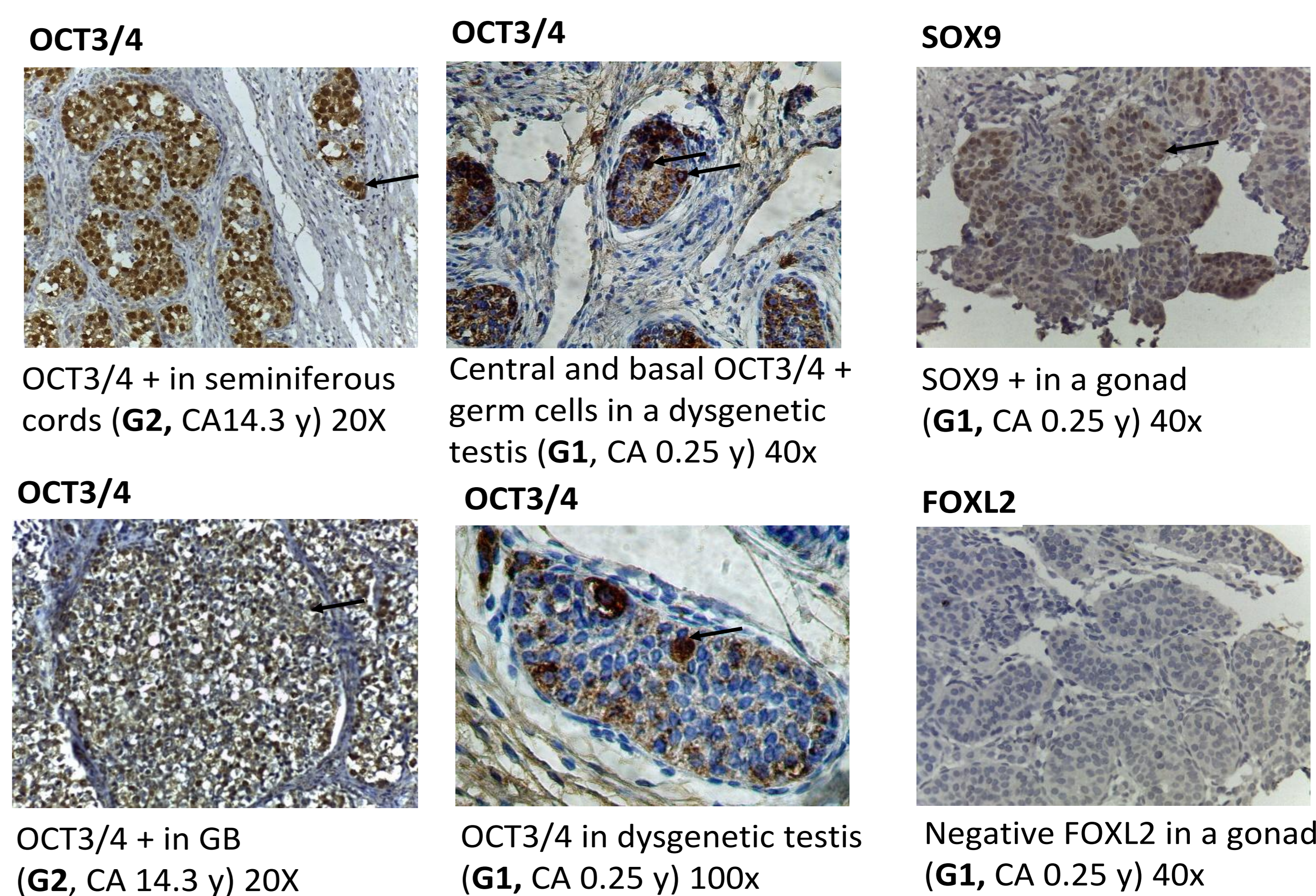
<sup>c</sup> Positive expression of SOX9 was found in isolated nuclei of tissues of G2 without seminiferous cords.



Müllerian remnants (G1, CA 1.75 y) 20x

Dysgenetic testis: Branched seminiferous cords, thickened basement membrane and vacuolated cells (G1, CA 12 y) 20x.

Myxed germinal tumor, gonadoblastoma (tumor size 5x4cm). Capsule margin (< 1mm) Remnant testicular parenchyma (G2, CA 14.3 y) 20X



OCT3/4 + in seminiferous cords (G2, CA14.3 y) 20X

Central and basal OCT3/4 + germ cells in a dysgenetic testis (G1, CA 0.25 y) 40x

SOX9 + in a gonad (G1, CA 0.25 y) 40x

OCT3/4 + in GB (G2, CA 14.3 y) 20X

OCT3/4 in dysgenetic testis (G1, CA 0.25 y) 100x

Negative FOXL2 in a gonad (G1, CA 0.25 y) 40x

## CONCLUSIONS

- The complexity of the tissues corresponding to patients with chromosomal mosaicism requires a deep histological and immunohistochemical analysis that allow the characterization of cell types and cell cancer risk.
- Samples of G1 (2/7) showed testicular parenchyma and Müllerian remnants, which might indicate an early alteration in the function of the Sertoli cell.
- The expression of Sertoli cell marker SOX9 in streak tissues of G2, suggests an increased risk of gonadoblastoma development.

