The localization of cells with XX and XY in gonadal tissues associated with ovotesticular disorder of sexual development with a 46, XX/46, XY karyotype

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Background

Individuals with a mixed 46, XX and XY karyotype have gonads with either an ovary in one side and a testis in the other side or an ovotestis in which both ovarian and testicular tissues exist in the same gonadal tissue. Such conditions are categorized as the ovotesticular disorder of sexual development (ODSD). The question arises as to how cells with 46, XY and 46, XX distribute in ovotestes and separated bisexual gonads in 46, XX and 46, XY ODSD [1)].

Objective

The aim of this study was to investigate the distribution of sex chromosomes (XX and XY) in testicular and ovarian tissues in gonads associated with individuals with 46, XX and 46, XY.

Methods

Six gonadal tissues from three patients with a 46, XX/46, XY karyotype were available for immunofluorescence in situ hybridization (FISH), immunohistochemistry (IHC) and immunofluorescence (IF).

1) We reviewed histopathological features of the gonads and performed FISH for X and Y chromosomes to examine the distribution of sex chromosomes in the testicular and ovarian structures.
2) The localization of SOX9 (testicular marker, BAF3075, R&D SYSTEMS, goat polyclonal) in Sertoli cells and FOXL2 (ovarian marker, IMG-328, IMEGENEX, goat polyclonal) in ovarian follicular epithelia[2)] was ascertained by IHC and IF.

Results

1. The clinical, pathological, and cytogenetic data of three patients are summarized in Table 1.

2. Histopathology of the gonads (Fig. 1)
Case 1 had an ovotestis in the left gonad (A, B) and an ovary in the right gonad (C).
Case 2 had a testis in the left (D) and an ovary in the other gonad (E).
Case 3 had a dysgenetic testis in the left gonad (F) and a normal appearing testis in the right gonad (G).
No ovarian tissue was evident because the histological examination was performed on biopsy specimens which occasionally contain too little gonadal parenchyme to reliably have any potential ovarian tissues.

3. FISH analysis for X and Y chromosomes in the gonads (Fig. 2)
Sertoli cells with XX signals were scattered in seminiferous tubules of the ovotestis (Case 1 : Fig. 2C), testes (Case 2, 3 : Fig. 2D, F) where most Sertoli cells showed XY signals. Conversely, a small amount of ovarian epithelial cells with XY signals were unequivocally present in ovarian follicles in the ovotestis (Case 1 : Fig. 2A, B) and ovary (Case 2 : Fig. 2E) where the majority of cells had XX signals (Fig. 2A, B, E).

4. IHC and IF for SOX9 and FOXL2 in the ovotestis (Fig. 3)
Since XX cells were observed in the seminiferous tubules and XY cells were seen in the ovarian follicles, we examined whether or not a testicular lineage marker (SOX9) is expressed in the ovarian tissue and an ovarian marker (FOXL2) is expressed in the testicular tissue. SOX9 was exclusively expressed in the nucleus of Sertoli cells and FOXL2 was also exclusively expressed in the nucleus of ovarian follicular epithelial cells in Case 1 (Fig. 3A-D).
Case 2 and 3 showed the same results as Case 1.

Discussion

- It is indicated that precursor sex cord cells with an XX karyotype incorporated in testicular tissues might be able to alter its lineage and differentiate into Sertoli cells because all of the epithelial cells in the seminiferous tubules solely expressed SOX9. Similarly, precursor sex cord cells with an XY karyotype distributed in ovarian tissues might be able to differentiate into ovarian follicular epithelia because all of ovarian follicular epithelia solely expressed FOXL2 (Table 2).
- It is suggested that the destiny of individual gonadal epithelial cells is influenced by local environmental factors rather than by the sex chromosome type.

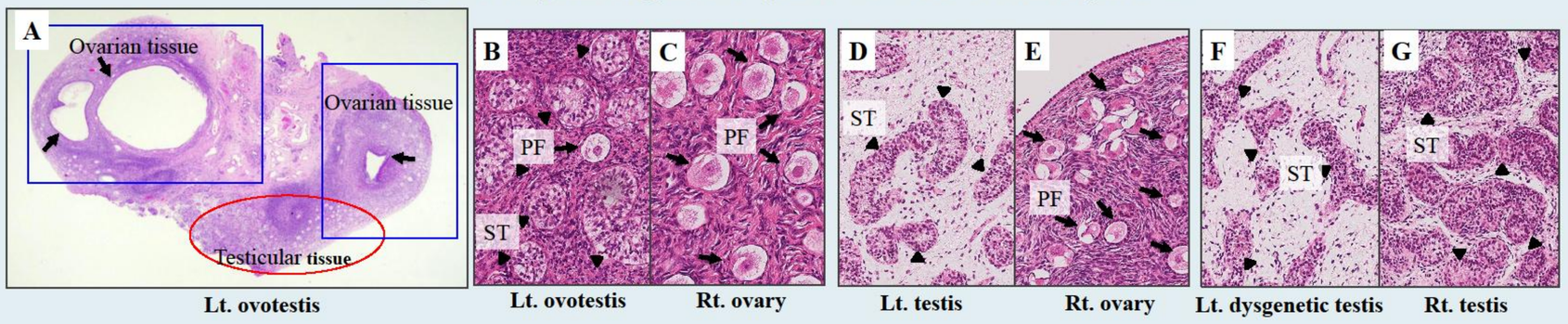
Table 2 Relationship between sex chromosomes and sex lineage makers

Cell type		SOX9	FOXL2
Sertoli cells	XY	+	-
	XX	+	-
Ovarian follicular epithelial cells	XX	-	+
	XY	-	+

Table 1 Clinical, pathological, and cytogenetic data

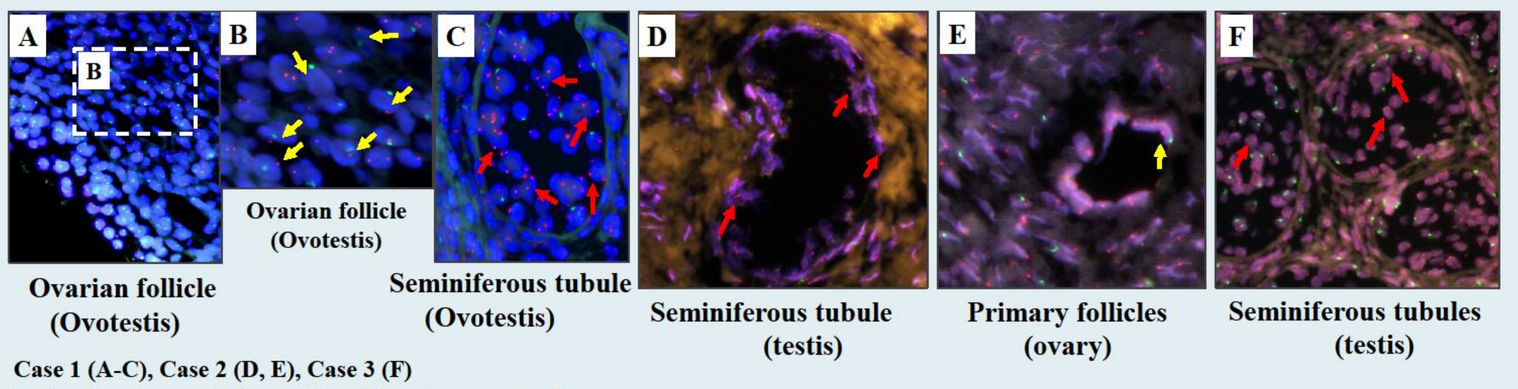
Case	Gender of rearing	Age-at-gonadectomy or biopsy	External genitalia	Karyotype (Peripheral blood)	Gonads Left/ Right
1	Male	3 years old (lt. gonadectomy;rt. biopsy)	micropenis, lt. cryptorchism, hypospadias	46, XX [81] / 46, XY [19]	Ovotestis /Ovary (Fig.1A, B /Fig.1C)
2	Male	10 months old (lt. gonadectomy;rt. biopsy)	micropenis, lt. cryptorchism, hypospadias	46, XX [13] / 46, XY [7]	Hypoplastic testis / Ovary (Fig.1D/Fig.1E)
3	Male	13 months old (lt. and rt. biopsy)	micropenis, hypospadias	46, XX [25] / 46, XY [5]	Dysgenetic testis / Testis (Fig.1F/Fig.1G)

Fig. 1 Histopathology of the gonads of 46, XX/46, XY patients



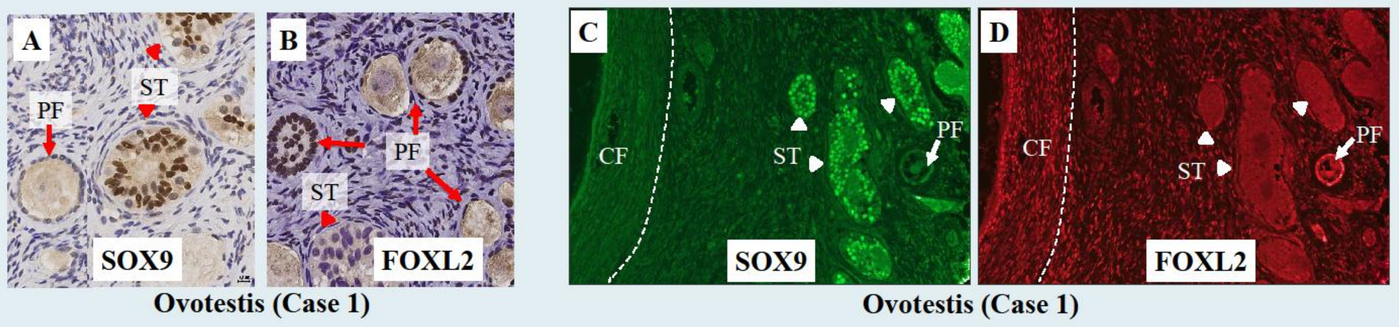
Case 1 (A-C), Case 2 (D, E), Case 3 (F, G)
An ovotestis consisting of areas containing ovarian tissue (surrounded by blue rectangles) and an area involving testicular tissue (surrounded by a red circle) (A). The left gonad showing an ovotestis composed of a mixture of primary ovarian follicles (PF, indicated by arrows) and seminiferous tubules (ST, indicated by arrow heads) (B) and the right gonad is composed of ovarian tissue only (C). The left gonad consists only of testicular tissue with hypoplastic seminiferous tubules (D) and the right gonad is comprised only of ovarian tissue (E). The left gonad had seminiferous tubules only; however they are abnormally shaped and branched in an edematous stroma, i.e., dysgenetic testis (F) and the right gonad consisted only of seminiferous tubules (G).

Fig. 2 FISH analysis for X and Y chromosomes in the gonads



Case 1 (A-C), Case 2 (D, E), Case 3 (F)
Red and green signals denote X and Y probe, respectively.
XY signals are included in follicular epithelial cells in the ovotestis (A). An area surrounded by broken lines highlighting the presence of XY signals (yellow arrow) in the follicular epithelial cells in the ovotestis (B). XX signals (red arrows) are involved in Sertoli cells in the seminiferous tubules in the ovotestis (C). Similar to Case 1, XX and XY signals are observed in seminiferous tubules (D) and ovarian follicles (E) respectively (Case 2). Seminiferous tubules containing XX signals (F) (Case 3).

Fig. 3 IHC (A, B,) and IF (C, D) for SOX9 and FOXL2 in the ovotestis



SOX9 is exclusively expressed in the nucleus of Sertoli cells in the seminiferous tubules (ST, indicated by arrow heads) (A, C) and FOXL2 is also exclusively expressed in the nucleus of ovarian follicular epithelial cells. Primary follicles (PF) are indicated by arrows (B, D). Consecutive sections using IF confirmed that the expression of FOXL2 is absent in the seminiferous tubules where nuclear SOX9 (green signal) is expressed (C) and the lack of SOX9 expression in the epithelia of the primary follicles and cystic follicles (CF) where nuclear FOXL2 (red signal) is expressed (D).

Reference:
1) Vilain E. Adv Exp Med Biol.707:105-6, 2011 2) Nishina N, et al. Medicine (Baltimore).Apr;94(14):e720. 2015

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