

# Potency of testicular somatic environment to support spermatogenesis in XX/Sry transgenic male mice

Mayuko Ishii<sup>1,\*</sup>, Tsuyoshi Tachiwano<sup>1,\*</sup>, Anshin Hoshino<sup>1,\*</sup>, Naoki Tsunekawa<sup>1</sup>, Ryuji Hiramatsu<sup>1</sup>, Shogo Matoba<sup>1</sup>, Masami Kanai-Azuma<sup>2</sup>, Hayato Kawakami<sup>2</sup>, Masamichi Kurohmaru<sup>1</sup> and Yoshiakira Kanai<sup>1,†</sup>

The sex-determining region of Chr Y (*Sry*) gene is sufficient to induce testis formation and the subsequent male development of internal and external genitalia in chromosomally female mice and humans. In XX sex-reversed males, such as XX/*Sry*-transgenic (XX/*Sry*) mice, however, testicular germ cells always disappear soon after birth because of germ cell-autonomous defects. Therefore, it remains unclear whether or not *Sry* alone is sufficient to induce a fully functional testicular soma capable of supporting complete spermatogenesis in the XX body. Here, we demonstrate that the testicular somatic environment of XX/*Sry* males is defective in supporting the later phases of spermatogenesis. Spermatogonial transplantation analyses using XX/*Sry* male mice revealed that donor XY spermatogonia are capable of proliferating, of entering meiosis and of differentiating to the round-spermatid stage. XY-donor-derived round spermatids, however, were frequently detached from the XX/*Sry* seminiferous epithelia and underwent cell death, resulting in severe deficiency of elongated spermatid stages. By contrast, immature XY seminiferous tubule segments transplanted under XX/*Sry* testis capsules clearly displayed proper differentiation into elongated spermatids in the transplanted XY-donor tubules. Microarray analysis of seminiferous tubules isolated from XX/*Sry* testes confirmed the missing expression of several Y-linked genes and the alterations in the expression profile of genes associated with spermiogenesis. Therefore, our findings indicate dysfunction of the somatic tubule components, probably Sertoli cells, of XX/*Sry* testes, highlighting the idea that *Sry* alone is insufficient to induce a fully functional Sertoli cell in XX mice.

**KEY WORDS:** *Sry*, Sertoli cell, Transplantation, Spermatogenesis, Spermiogenesis, Mouse

## INTRODUCTION

In many non-mammalian vertebrate species with a genetic sex-determination system, it has been shown that experimental and spontaneous sex-reversed XX or ZW males show complete spermatogenesis and the production of functional sperm (Yamamoto, 1955; Elbrecht and Smith, 1992; Hayes, 1998; Geffen and Evans, 2000; Nanda et al., 2003). In mammals, *Sry* is essential in pre-Sertoli cells for initiating male sex differentiation (Sinclair et al., 1990; Koopman et al., 1991). *Sry* alone is sufficient to promote testis formation and the subsequent male development of internal and external genitalia in chromosomally female mice (Koopman et al., 1991). However, XX sex-reversed males such as XX/*Sry*-transgenic (XX/*Sry*), XX*Sxr* and XXY mice, are always infertile because of the loss of spermatogonial germ cells soon after birth (Cattanach et al., 1971; Lue et al., 2001). In both XXY and XX*Sxr*<sup>d</sup> testes, XY or X*Sxr*<sup>d</sup>O germ cells occasionally survive to take part in spermatogenesis because of the loss of the second X chromosome in a progenitor cell (Lyon et al., 1981; Mroz et al., 1999; Hall et al., 2006). Such defects arising from a double X dosage are also sufficient to explain the germ cell-autonomous demise of XX spermatogonia in XX<->XY chimeric testes (Palmer and Bugoyne, 1991). Because Y-linked genes in spermatogenic cells are essential for spermatogenesis (Levy and Burgoyne, 1986; Mazeyrat et al., 2001; Toure et al., 2004), the germ cell demise in XX males is due to germ cell-autonomous defects caused by both the extra X- and the

missing Y-chromosome. Therefore, it still remains unclear whether or not XX sex-reversed males such as XX/*Sry* mice have a fully functional testicular somatic environment capable of supporting complete spermatogenesis in mammals.

In this report, in order to elucidate the potency of the XX/*Sry* testicular somatic environment, we examined the differentiation ability of donor XY spermatogonia in recipient XX/*Sry* testes, compared to germ cell-deficient *W/W*<sup>v</sup> testes, which were used as the XY control.

## MATERIALS AND METHODS

### Animals

For XY-donor spermatogenic cells, we used wild-type C57BL/6 [B6] mice, ROSA26 mice (B6×129 genetic background, Jackson Laboratories), Green mice [B6-Tg(CAG-EGFP), SLC, Japan], and Steel/Steeldickie (*Sl/Sl*<sup>d</sup>) mice (WB×B6; SLC). For recipient testes, the sex-reversed transgenic mouse line (B6/*Hsp-Sry* lines carrying the autosomally-located *Sry* transgene driven by a basal weak *Hsp70.3* promoter) (Kidokoro et al., 2005) and the germ cell-deficient *W/W*<sup>v</sup>-mutant line (WB×B6; SLC) were used in this study. The *Hsp-Sry* line displays XX testes at embryonic stages because of transgenic *Sry* expression in embryonic gonads at the sex-determining periods. Because XY *Hsp-Sry* males display normal spermatogenesis and fertility even after 1 year of age, the integration position and transgene misexpression elicit no appreciable defect in spermatogenesis in these mice.

### Transplantation of XY cells prepared from the immature testes

For spermatogonial transplantation, cell suspensions (including spermatogonial cells) were prepared from 10-day old testes of ROSA, Green, wild-type B6 or *Sl/Sl*<sup>d</sup> males. They were then transplanted into the testes of 8-week-old recipient XX/*Sry* and XY *W/W*<sup>v</sup> mice as described previously (Brinster and Zimmermann, 1994; Ogawa et al., 2000). At 2.5-3 months after transplantation, the recipient testes were dissected and processed for histological and histochemical analyses. Some recipient XX/*Sry* testes injected with enhanced green fluorescent protein (EGFP)-positive spermatogonial-cell suspensions (from Green mice) were also

<sup>1</sup>Department of Veterinary Anatomy, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan. <sup>2</sup>Department of Anatomy, Kyorin University School of Medicine, Mitaka, Tokyo 181-8611, Japan.

\*These authors contributed equally to this work

†Author for correspondence (e-mail: aykanai@mail.ecc.u-tokyo.ac.jp)

dissected 2.5 months after transplantation. Seminiferous tubules with EGFP-positive spermatogenic colonies were collected from these mice under the epifluorescence stereomicroscope and were further used as donors for the second transplantation experiment of seminiferous tubules, as follows.

The seminiferous tubule transplantation was carried out as described previously (Tanemura et al., 1996). Seminiferous tubules of the immature B6 testes (2-3 weeks of age) or fluorescent-positive seminiferous tubules of the primary-recipient XX/Sry testes were cut into small segments (1 cm in length), washed in DMEM medium to remove interstitial tissue and then transplanted under the testicular capsules of the recipient males. The recipient testes were examined histologically at 4 weeks after transplantation.

### Histology and immunohistochemistry

The transplanted testes were fixed in Bouin's solution or 4% paraformaldehyde solution and were then routinely embedded in paraffin. Paraffin sections (4  $\mu$ m) were subjected to conventional histological and immunohistochemical staining. For quantitative analysis of the incidence ratio of each advanced spermatid stage, we used periodic acid-Schiff (PAS)-stained transverse sections (three sections per testis) of the testes which had a higher contribution of donor XY germ cells (XX/Sry: six testes; XY W/W<sup>n</sup>: four testes). All seminiferous tubules in the three sections were classified by direct microscopic observation into tubules lacking donor germ cells, tubules with spermatocytes, or tubules with round (steps 1~7) or early (step 8~10)/late (steps 11~16) elongated spermatids. The incidence ratio of each spermatid stage represented the mean percentage of the relative tubule number  $\pm$  standard error (s.e.m.; number of tubules with spermatocytes was set at 100%).

For immunohistochemical staining, two consecutive sections were separately incubated with anti-MVH [2 ng/ml (Toyooka et al., 2001) provided by Dr Toshiaki Noce], anti-EGFP (1/3000 dilution; Molecular probes, OR) or anti-HSC70T [1/1000 dilution (Tsunekawa et al., 1999) provided by Dr Hirokazu Fujimoto] antibody at 4°C for 12 hours. After washing in TBS, the reaction was visualized with biotin-labeled secondary antibody in combination with Elite ABC kit (Vector Laboratories, CA).

For transmission electron microscopy, the transplanted testes were fixed in 4% glutaraldehyde at 4°C for 12 hours. After post-fixation with 1% OsO<sub>4</sub>, the specimens were dehydrated and embedded in Araldite M. Ultrathin sections were observed under a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Japan).

For LacZ staining, the transplanted testes were fixed with 1% PFA-0.2% glutaraldehyde-0.02% NP40-PBS at 4°C for 4 hours and were then subjected to whole-mount X-gal staining (Kanai-Azuma et al., 2002). Paraffin sections of the stained testes were prepared for histological analysis.

### RNA extraction, microarray and RT-PCR analyses

Whole testes and seminiferous tubules of 8-week-old XX/Sry and W/W<sup>n</sup> males were used for microarray expression analysis using the Affymetrix GeneChip system (Affymetrix, CA). After total RNA was extracted using a RNeasy Mini kit (Qiagen, MD), double-stranded cDNA and biotin-labeled cRNA were synthesized using One-Cycle cDNA Synthesis and IVT Labeling kits (Affymetrix), respectively. Fragmented biotin-labeled cRNA (20  $\mu$ g) was hybridized to the Affymetrix Mouse Expression Array MOE 430A for 16 hours at 45°C. The chips were washed, stained, scanned and then analyzed using Microarray Suite version 5.0 (Affymetrix), in accordance with the manufacturer's standard protocols. Differential expression was defined as a difference of twofold or more in both whole-testis and seminiferous tubule samples between two recipient males. The microarray data have been deposited in the Gene Expression Omnibus of NCBI (accession number: GSE5319).

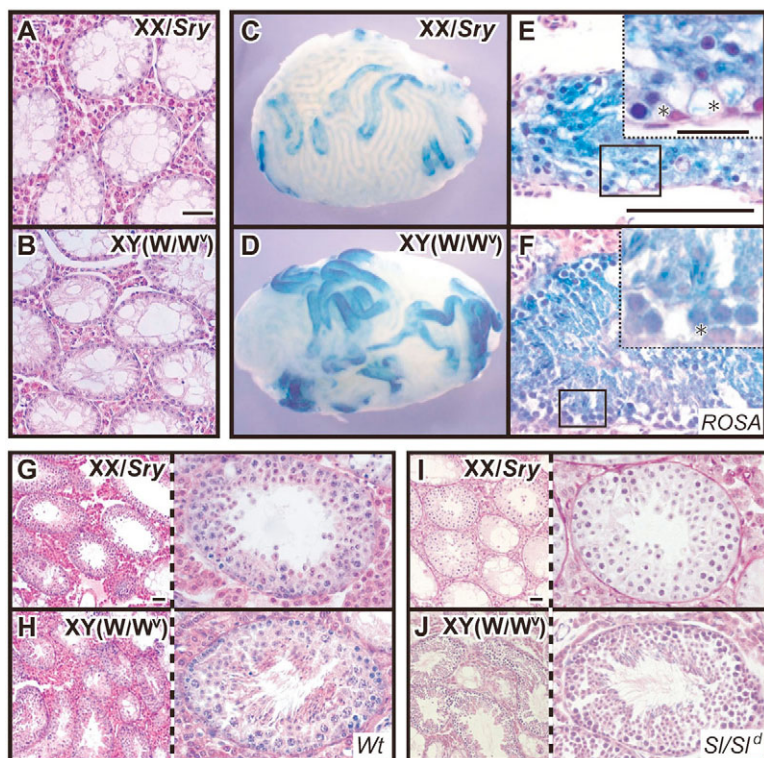
For reverse transcriptase (RT)-PCR analysis, the RNA of seminiferous tubules was treated with DNase I and was then reverse-transcribed using an oligo(dT) primer with a Superscript III cDNA synthesis kit (Invitrogen, CA). PCR was performed with 27-30 cycle amplifications at 94°C for 40 seconds, 55°C for 1 minute and 72°C for 1 minute by using the appropriate primer sets (see Table 1).

## RESULTS AND DISCUSSION

Before transplantation, recipient testes displayed no spermatogenic cells beyond the pre-leptotene spermatogonial stage in both XX/Sry and XY W/W<sup>n</sup>-mutant mice (Fig. 1A,B). First, we transplanted XY-donor testicular suspensions prepared from LacZ-positive ROSA26 pups into the seminiferous tubules of recipient testes and then examined their colonization patterns in the testes at 3 months after transplantation. LacZ staining revealed that XY spermatogonia were able to colonize the seminiferous tubules of recipient XX/Sry testes (Fig. 1C,D), in which only spermatogenic cells were positive (Fig. 1E,F). Histological analyses at 3 months after transplantation of

**Table 1. PCR primers**

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Ddx3y (Dby)</i>	GGTCTGGAAAACTGCTGC	TTGGTGGCATTGTGCTCTGC
<i>Uty</i>	AAATGCAGCTCGGACCAAATC	CTGAATGATGTGAAGCTGTC
<i>Eif2s3y</i>	GGTGTCTGGAGCATTACC	AGAAACACTCTGCTGTGCTGTG
<i>Jarid1d (Smcy)</i>	CTCTCGTGGGATGAAGTCGATA	TCAACTCGTCGGCTTAGAGC
<i>Nxf2</i>	GCCTTCACGAGGATCTTCAT	AAGTATACTCCTGTGTAGCCTG
<i>Clca1</i>	ACCTAAAGAGGCTGGCTCAA	GAAAGGCCTCTCTATTCC
<i>Alcam</i>	CATTTCCCTGAGGAGAATG	AGTGTGACAGGCAGCCTTT
<i>Apod</i>	GAGTCTGATGGAACCATGA	TATCCAATCCGCTCCTCTCT
<i>Kctd14</i>	TCAACCTCAGAAATCTGC	GCCATGGTTGCTTGGTACTT
<i>Slc6a4</i>	ACATCTGGCGTTTTCCCTACAT	ATAAGGTGACCTCTCAGA
<i>119002H23Rik</i>	AGAGTGCAGACTCAGTGTAC	TTTTGACTCCTTCCAGATGCT
<i>Jam2</i>	ACATGTAACGGCTGCCAGCT	AGTGAGCTTCACTCTCCGAA
<i>Scara5</i>	ATGACTTGAAGCGCTGACT	CCGAATGCATGGAATGCGAA
<i>Ube1y1</i>	CTCTGAGTACATCCGTGG	CCTGCAGCTGGTAGAGTTC
<i>Xist</i>	CGGGGCTTGGTGGATGGAAAT	GCAATCCTGCTGAACTGC
<i>Klk16</i>	CTGACTTCAGCAATGACCTG	GCGTAACTGGCTCGAGAATA
<i>Asb12</i>	TATCTGGCTGCAGTCTATGG	TACACCAGGTATACCGCATG
<i>Sult1e1</i>	CAGAGCTTGTGGACAGAATC	TCTGCGGATAACTAAACGGG
<i>Klk6</i>	GCAGCATTACACCCGTCAAA	GTGCAATCCTTCATCTGCTG
<i>Col9a3</i>	TCAAGGTGTACTGGCATCA	TACCCGGCACATTGGGTTTA
<i>Rhbg</i>	CCACGAAGCTTATGGAGATG	CCAATAGATGTTCTGGGGC
<i>Klk27</i>	CAAGAGCAATGACCTGATGC	CTGGAACAGAGTAACACTGG
<i>Snrpn</i>	AGCAGGTGTACCTATTCCCC	ATTGGGTTTAGCGCATGGGA
<i>Klk24</i>	GCAACCTAAGGACAAAGAGCA	ATGGGTGGTCTCATACCAGG
<i>Klk9</i>	ACCGATTGGTCAGCAAAGC	CGCATTGGGTTTACCACATG
		GCATGACATCTGTCACTTC



**Fig. 1. Spermatogonial transplantation into testicular seminiferous tubules of XX/Sry and XY W/W<sup>v</sup> (control) males.** (A,B) HE staining of the intact XX/Sry and XY W/W<sup>v</sup> testes at 8 weeks of age, showing lack of germ cells in most tubules of both testes. (C–J) The recipient testes were injected with spermatogonial-cell suspension prepared from immature ROSA26 (C–F), wild-type (G,H) and *Sl/Sl<sup>d</sup>* (I,J) testes, and were then histologically examined at 3 months after transplantation. (C–F) LacZ staining of the transplanted testes, visualizing ROSA26-derived XY germ cells in XX/Sry (C,E) and XY W/W<sup>v</sup> (D,F) testes. Asterisks indicate LacZ-negative Sertoli cells located at the basal region of the seminiferous tubules. Insets show higher-magnification images of boxed area. (G–J) HE staining of XX/Sry (G,I) and XY W/W<sup>v</sup> (H,J) testes injected with the wild-type-derived (G,H) or *Sl/Sl<sup>d</sup>*-derived (I,J) testicular cell suspension. Higher-magnification images of recipient tubules with donor germ cells are shown on the right. Scale bars: 50  $\mu$ m in A,G,I and inset of E; 200  $\mu$ m in E.

wild-type (B6) testicular cells revealed no difference in the frequency of testes with settled donor germ cells between XX/Sry and XY W/W<sup>v</sup> males (number of testes with donor germ cells per total testes injected: 22/44 testes [50.0%] in XX/Sry males vs. 5/10 testes [50.0%] in XY W/W<sup>v</sup> males). In both XY W/W<sup>v</sup> and XX/Sry testes, spermatocytes and round spermatids were frequently observed inside the tubules (Fig. 1G,H). In order to exclude a possible contribution of donor-derived somatic cells in XX/Sry tubules, we also transplanted a cell suspension prepared from XY *Sl/Sl<sup>d</sup>* testes (normal spermatogonia, but defective Sertoli cells) (Zsebo et al., 1990; Ogawa et al., 2000), and obtained the same results as those observed in the transplantation of wild-type donor cells (Fig. 1I,J). Therefore, it was concluded that the XX sex-reversed male body is capable of supporting the settlement, proliferation and complete meiosis of XY germ cells even in the absence of other Y-linked genes except for *Sry*. Because several lines of XY-female mutant mice are able to produce litters (Lovell-Badge and Robertson, 1990; Capel et al., 1993), these findings indicate that, at least in mice, intrinsic differences between XX and XY somatic cells are not essential to promote haploid germ cell production in either sex.

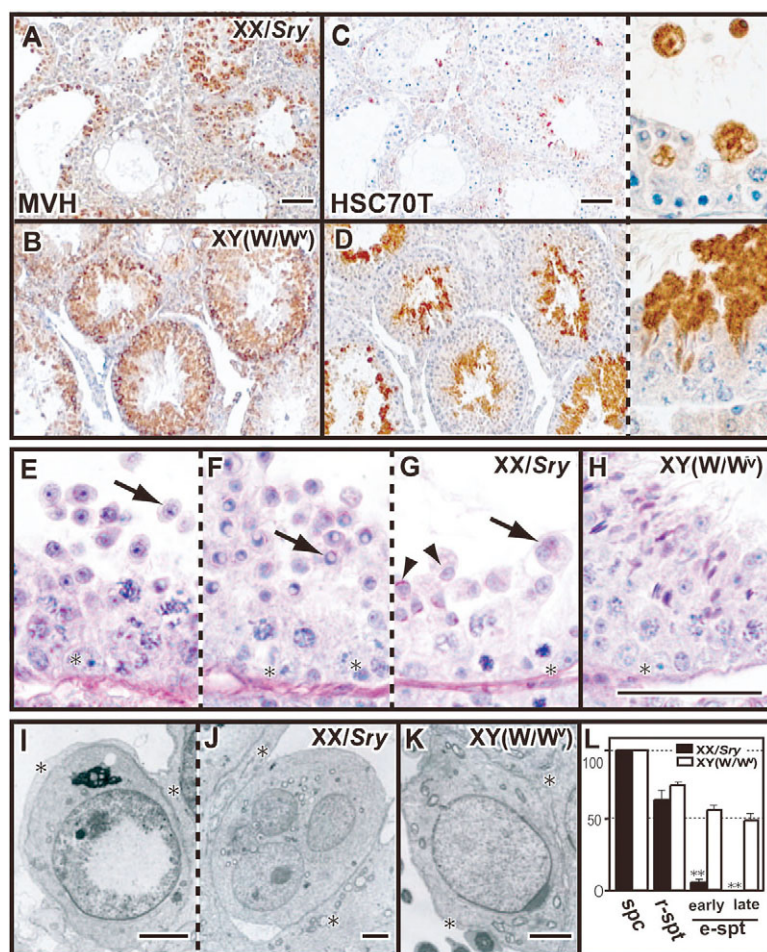
Interestingly, in contrast to the proper spermiogenesis observed in XY W/W<sup>v</sup> testes, no late elongated spermatids could be detected in any XX/Sry testes (Fig. 1G–J). Immunohistochemical analyses using anti-HSC70T (a marker specific for elongated spermatids) and anti-MVH (a marker specific for spermatocytes and round spermatids) antibodies confirmed defective formation of elongated spermatids from XY-donor cells in XX/Sry tubules (Fig. 2A–D). In the XY W/W<sup>v</sup> testes, HSC70T-positive cells with a sperm tail were frequently observed in most seminiferous tubules containing MVH-positive cells (Fig. 2B,D). By contrast, XX/Sry testes showed a drastic reduction of HSC70T-positive cells in MVH-positive seminiferous tubules (Fig. 2A,C).

Periodic acid-Schiff (PAS) staining also demonstrated that round spermatids were sometimes detached from the seminiferous epithelia and were located in the lumen of XX/Sry testes (Fig. 2E). They frequently exhibited apoptotic-like cell death, with typical crescent-like condensation of the chromatin (Fig. 2F,I). Some large, multi-nucleated giant cells of spermatids could also be observed in XX/Sry testes (Fig. 2G,J). By contrast, no anomalies were detectable in the XY W/W<sup>v</sup> testes, in which round and elongated spermatids displayed normal morphology similar to that seen in intact XY testes (Fig. 2H,K).

The incidence ratio of each advanced spermatid stage was estimated as the number of seminiferous tubules with round or early/late elongated spermatids relative to the total number of tubules with spermatocytes (Fig. 2L). In XY W/W<sup>v</sup> testes, the incidence ratio of round and elongated spermatids was approximately 75% and 50%, respectively. In XX/Sry testes, the incidence of round spermatids was approximately 65%, which did not significantly differ from that of W/W<sup>v</sup> testes. In XX/Sry testes, however, the incidence of early-elongated spermatids was significantly ( $P < 0.01$ ) reduced (only 5.9%), with no elongated spermatids detected after step 11. These data suggest that the XX/Sry testicular somatic environment is defective in the maintenance of round spermatids and their differentiation into spermatozoa.

Next, in order to evaluate the potency of the interstitial environment of XX/Sry testes, we prepared seminiferous tubule segments from immature wild-type testes at 2–3 weeks of age. At this stage, the testes consist mainly of spermatogonia and spermatocytes. These tubule segments were transplanted under the testis capsules of XX/Sry and XY W/W<sup>v</sup> males, and then histologically examined at 4 weeks after transplantation. It was shown that proper differentiation of donor XY germ cells into elongated spermatids was detected in four independent grafts that





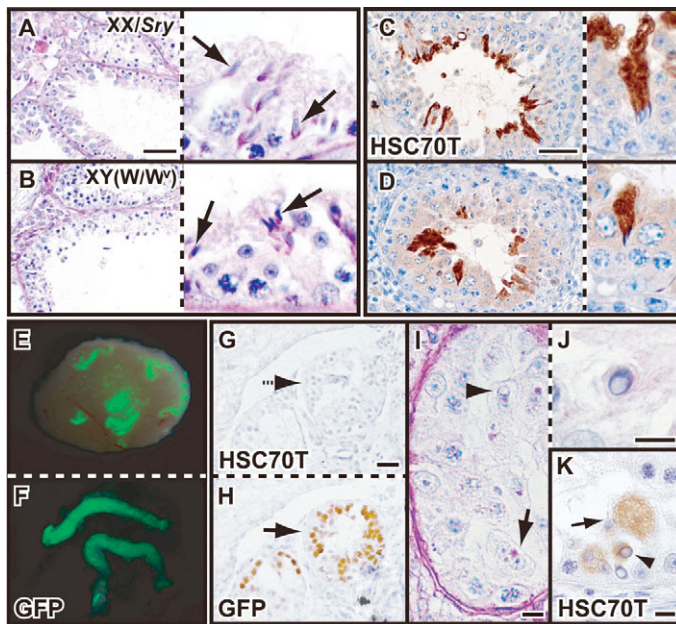
**Fig. 2. Defective spermiogenesis of donor XY germ cells in recipient seminiferous tubules of XX/Sry males.** (A-D) Anti-MVH (A,B) and anti-HSC70T (C,D) stainings of two serial sections of XX/Sry and XY W/W<sup>v</sup> testes (brown staining). (C,D) Insets show higher-magnification images of HSC70T-positive spermatids: notice the abnormal morphology of all HSC70T-positive cells in XX/Sry tubules. (E-K) PAS staining (red, E-H) and transmission electron microscopic (I-K) analyses of transplanted XX/Sry and XY W/W<sup>v</sup> testes, showing three main types of degenerating round spermatids (arrows) in recipient XX/Sry tubules: detached (E), piknotic (F,I) and large multi-nucleated giant cells (G,J). Arrowhead in G shows early elongated spermatids at steps 8-10. Asterisks indicate Sertoli cells in E-H; Sertoli cell process in I-K. (L) Quantitative analysis of transplanted XX/Sry and XY W/W<sup>v</sup> testes showing the number of seminiferous tubules with round (steps 1-7; r-spt) or early (steps 8-10)/late (steps 11-16) elongated spermatids (e-spt) relative to the total number of tubules with spermatocytes (spc). The bar represents mean  $\pm$  standard error; asterisks indicate a statistically significant difference between the XX/Sry and XY W/W<sup>v</sup> testes (\*\* $P < 0.01$ ). Scale bars: 50  $\mu$ m in A-H; 2  $\mu$ m in I-K.

survived inside the recipient XX/Sry testes (Fig. 3A-D). These data indicate that the interstitial environment of XX/Sry testes is capable of supporting normal spermiogenesis, which in turn suggests the defective environment inside the seminiferous tubules of the XX/Sry testis. Moreover, we prepared the seminiferous tubules composed of XX/Sry soma and EGFP-positive XY spermatogenic colonies from the recipient XX/Sry testes 2.5 months after spermatogonial transplantation (Fig. 3E,F). The EGFP-positive tubule segments were further transplanted under the testis capsules of the secondary recipient XY W/W<sup>v</sup> males. At 4 weeks after tubule transplantation, it was shown that no restoration of spermiogenesis in EGFP-positive XY spermatogenic colonies was detected in all XX/Sry tubule segments that survived in the testicular interstitium of the secondary XY W/W<sup>v</sup> recipients ( $n=4$ ; Fig. 3G-K). These findings clearly indicate dysfunction of the somatic tubule component, probably Sertoli cells, in XX/Sry testes.

The extra X- and/or missing Y-chromosome in XX/Sry testes could be the primary cause of their defective spermiogenesis. In XXY mice, spermatogonia are eliminated within the first few days of postnatal life (Mroz et al., 1999; Lue et al., 2001). However, rare breakthrough patches of spermatogenesis, composed of XY germ cells, are observed in the testes of adult mice (Hall et al., 2006). Some men with non-mosaic Klinefelter syndrome are also able to produce functional sperm from 46,XY spermatogonial cells, because live-births involving 47,XXY fathers are almost always chromosomally normal, with as many 46,XY as 46,XX children (Lanfranco et al., 2004). These reports, therefore, indicate that the

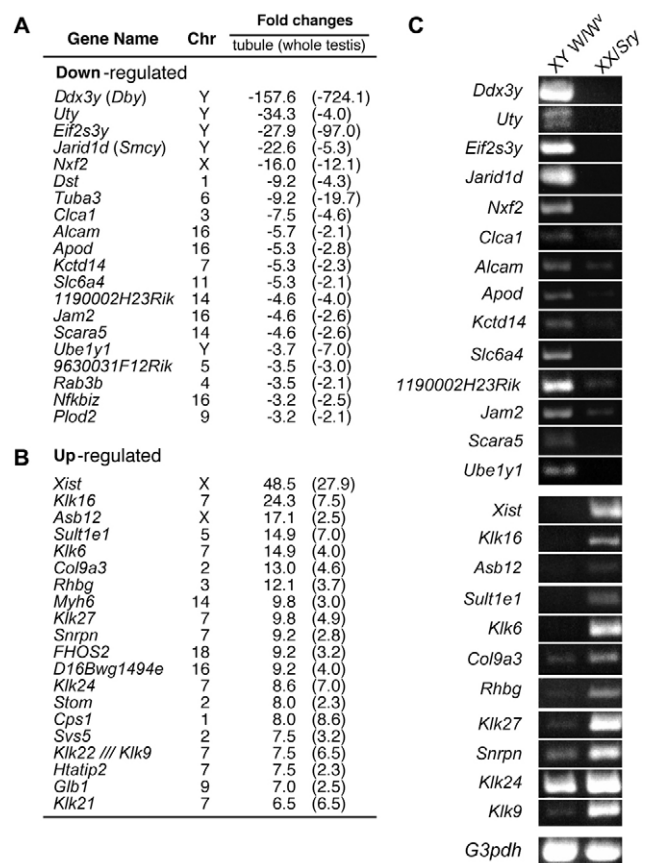
XXY testicular somatic environment is capable of supporting complete spermatogenesis of XY spermatogonia. This, in turn, suggests that the primary cause of the defective XX/Sry somatic environment is likely to be attributable to the absence of other Y-linked genes rather than to the presence of the extra X chromosome. In order to understand the molecular basis of defective XX/Sry testes, we performed microarray analyses of isolated seminiferous tubules from XX/Sry and W/W<sup>v</sup> testes. Despite their histological similarity, the present microarray screens identified 48 downregulated and 93 up-regulated genes in both seminiferous tubule fractions and whole testis of the XX/Sry testes compared with those of W/W<sup>v</sup> testes (Fig. 4, and see Tables 1, 2 in the supplementary material). Among the downregulated genes, the expression of five Y-linked genes – *Ddx3y* (previously known as *Dby*), *Uty*, *Eif2s3y*, *Jarid1d* (previously known as *Smcy*) and *Ube1y1* – was missing in the XX/Sry tubules. Because these genes are all mapped in the *Sxr*<sup>b</sup>-deletion-interval region (the essential region for germ cell development after the early postnatal period) of the mouse Y chromosome (Burgoyne, 1998; Mazeyrat et al., 2001), they may be the Y-linked candidate genes for spermiogenic failure in XX/Sry Sertoli cells.

Interestingly, we also found several autosomal genes whose expression was reduced in the seminiferous tubules of XX/Sry testes (Fig. 4), including some genes involved in ion channel and/or transport molecules (*Clca1*, *Kctd14*, *Slc6a4*) and cytoskeletal and/or cell-junction components (*Dst*, *Tuba3*, *Alcam*, *Jam2*). Ion transport regulations in Sertoli cells are important for the secretion



**Fig. 3. Transplantation of seminiferous tubule segments under testis capsules of XX/Sry and XY W/W<sup>v</sup> males.** (A-K) Segments of immature XY seminiferous tubules (2–3 weeks old, wild type) were transplanted under the testis capsules of recipient XX/Sry (A,C) and XY W/W<sup>v</sup> (B,D) males. Tubule segments of the primary-recipient XX/Sry testes with EGFP-positive XY spermatogenic colonies (2.5 months after spermatogonial transplantation using Green mice as donors; E,F) were further transplanted under the testis capsules of the secondary XY W/W<sup>v</sup> recipients (G-K). All grafts were then histologically examined at 4 weeks after transplantation. (A,B) PAS staining showing elongated spermatids inside transplanted XY seminiferous tubules (arrows) of both recipient testes. (C,D) Immunohistochemical staining with anti-HSC70T antibody visualizing normal elongated spermatids of donor seminiferous tubules transplanted in XX/Sry testes. Insets show higher-magnification images of the donor seminiferous tubules. (E,F) Fluorescent micrographs showing a primary XX/Sry recipient testis (E) and two isolated tubule segments (F) with EGFP-positive XY spermatogenic colonies. (G-K) Anti-HSC70T and anti-EGFP immunohistochemical staining (two serial sections in G,H; K) and PAS staining (I,J) showing the absence of normal elongated spermatids (broken arrow in G; H), and the presence of pyknotic (arrowhead in I,K; J) and abnormally large (arrow in I,K) spermatids of XY spermatogenic colonies inside the donor XX/Sry seminiferous tubules transplanted in the secondary XY W/W<sup>v</sup> recipients testes. Scale bars: 50  $\mu$ m in A-H; 10  $\mu$ m in I-K.

of a potassium- and chloride-rich fluid in spermiogenesis (Hinton and Setchell, 1993; Pace et al., 2000). JAM2, an immunoglobulin-superfamily protein mediating homophilic and heterophilic interactions, is expressed on the Sertoli cell surface facing round and elongated spermatids (Gliki et al., 2004). JAM3, a partner molecule for trans-interactions with JAM2, is essential for the differentiation of round spermatids into spermatozoa (Gliki et al., 2004), suggesting a possible function of JAM2 in spermiogenesis. By contrast, of the 93 genes up-regulated in XX/Sry tubules, six members of the kallikrein gene family [*Klk1b16* (previously known as *Klk16*), *Klk6*, *Klk1b27* (previously known as *Klk27*), *Klk1b24* (previously known as *Klk24*), *Klk1b22* (previously known as *Klk22*), *Klk9* and *Klk1b21* (previously known as *Klk22*)] were found within the top 20 up-regulated genes in XX/Sry tubules. The kallikrein genes encode the tissue-specific protease required to



**Fig. 4. Microarray and RT-PCR analyses of gene expression in isolated seminiferous tubules from XX/Sry and XY W/W<sup>v</sup> testes.**

(A,B) Microarray expression analysis showing the top 20 genes that were (A) downregulated and (B) up-regulated in XX/Sry testes. The fold change represents the difference in the expression level in seminiferous tubules between XX/Sry and XY W/W<sup>v</sup> testes; fold change in parentheses showing the difference in expression level in whole testis. (C) RT-PCR analysis of genes selected from the top-20 genes that were up- and down-regulated in XX/Sry seminiferous tubules.

liberate kinins, small peptide hormones involved in multiple physiological processes. Kinin (bradykinin) receptors are highly expressed in spermatocytes and round spermatids, indicating a possible function of the kallikrein-kinin system in the local regulation of later spermatogenesis [see Monsees et al. (Monsees et al., 2002) and references therein]. Therefore, it is likely that such misregulation of several spermiogenesis-regulatory genes in XX/Sry tubules reflects the inability of these tubules to support the maintenance of round spermatids and their differentiation into spermatozoa. Further spermatogonial transplantation and microarray analyses using XXY, XXSx<sup>r</sup>, XO/Sry and XSx<sup>r</sup>O testes would be required to resolve whether these transcriptional changes in XX/Sry tubules are a consequence of the Y-gene deficiency or of the double X dosage.

In conclusion, we show here that XX/Sry testicular soma is not capable of supporting the differentiation and morphogenesis of haploid germ cells into spermatozoa. This is probably due to the dysfunction of the XX/Sry Sertoli cells, highlighting the idea that Sry alone is insufficient to induce a fully functional Sertoli cell in XX mice.



The authors wish to thank Drs A. Greenfield and J. Bowles for their critical reading of and comments on the manuscript; Drs H. Fujimoto, T. Noce, T. Tabata, M. Fujisawa, T. Kidokoro and M. Ishii for their support in this study; and M. Fukuda, T. W. Tay, T. Yasugi and I. Yagihashi for their technical and secretarial assistance. This work was supported by financial grants from the Ministry of Education, Science, Sports and Culture of Japan.

#### Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/134/3/449/DC1>

#### References

- Brinster, R. L. and Zimmermann, J. W.** (1994). Spermatogenesis following male germ-cell transplantation. *Proc. Natl. Acad. Sci. USA* **91**, 11298-11302.
- Burgoyne, P. S.** (1998). The role of Y-encoded genes in mammalian spermatogenesis. *Semin. Cell Dev. Biol.* **9**, 423-432.
- Capel, B., Rasberry, C., Dyson, J., Bishop, C. E., Simpson, E., Vivian, N., Lovell-Badge, R., Rastan, S. and Cattanach, B. M.** (1993). Deletion of Y chromosome sequences located outside the testis determining region can cause XY female sex reversal. *Nat. Genet.* **5**, 301-307.
- Cattanach, B. M., Pollard, C. E. and Hawker, S. G.** (1971). Sex-reversed mice: XX and XO males. *Cytogenetics* **10**, 318-337.
- Elbrecht, A. and Smith, R. G.** (1992). Aromatase enzyme activity and sex determination in chickens. *Science* **255**, 467-470.
- Geffen, A. J. and Evans, J. P.** (2000). Sperm traits and fertilization success of male and sex-reversed female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **182**, 61-72.
- Gliki, G., Ebnert, K., Aurrand-Lions, M., Imhof, B. A. and Adams, R. H.** (2004). Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C. *Nature* **431**, 320-324.
- Hall, H., Hunt, P. and Hassold, T.** (2006). Meiosis and sex chromosome aneuploidy: how meiotic errors cause aneuploidy; how aneuploidy causes meiotic errors. *Curr. Opin. Genet. Dev.* **16**, 323-329.
- Hayes, T. B.** (1998). Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. *J. Exp. Zool.* **281**, 373-399.
- Hinton, B. T. and Setchell, B. P.** (1993). Fluid secretion and movement. In *The Sertoli Cell* (ed. L. D. Russell and M. D. Griswold), pp. 249-267. Clearwater, FL: Cache River Press.
- Kanai-Azuma, M., Kanai, Y., Gad, J. M., Tajima, Y., Taya, C., Kurohmaru, M., Sanai, Y., Yonekawa, H., Yazaki, K., Tam, P. P. et al.** (2002). Depletion of definitive gut endoderm in Sox17-null mutant mice. *Development* **129**, 2367-2379.
- Kidokoro, T., Matoba, S., Hiramatsu, R., Fujisawa, M., Kanai-Azuma, M., Taya, C., Kurohmaru, M., Kawakami, H., Hayashi, Y., Kanai, Y. et al.** (2005). Influence on spatiotemporal patterns of a male-specific Sox9 activation by ectopic Sry expression during early phases of testis differentiation in mice. *Dev. Biol.* **278**, 511-525.
- Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. and Lovell-Badge, R.** (1991). Male development of chromosomally female mice transgenic for Sry. *Nature* **351**, 117-121.
- Lanfranco, F., Kamischke, A., Zitzmann, M. and Nieschlag, E.** (2004). Klinefelter's syndrome. *Lancet* **364**, 273-283.
- Levy, E. R. and Burgoyne, P. S.** (1986). The fate of XO germ cells in the testes of XO/XY and XO/XY/XY mouse mosaics: evidence for a spermatogenesis gene on the mouse Y chromosome. *Cytogenet. Cell Genet.* **42**, 208-213.
- Lovell-Badge, R. and Robertson, E.** (1990). XY female mice resulting from a heritable mutation in the primary testis-determining gene, Tdy. *Development* **109**, 635-646.
- Lue, Y., Rao, P. N., Sinha Hikim, A. P., Im, M., Salameh, W. A., Yen, P. H., Wang, C. and Swerdloff, R. S.** (2001). XXY male mice: an experimental model for Klinefelter syndrome. *Endocrinology* **142**, 1461-1470.
- Lyon, M. F., Cattanach, B. M. and Charlton, H. M.** (1981). Genes affecting sex differentiation in mammals. In *Mechanisms of Sex Differentiation in Animals and Man* (ed. C. R. Austin and R. G. Edwards), pp. 329-386. New York: Academic Press.
- Mazeyrat, S., Saut, N., Grigoriev, V., Mahadevaiah, S. K., Ojarikre, O. A., Rattigan, A., Bishop, C., Eicher, E. M., Mitchell, M. J. and Burgoyne, P. S.** (2001). A Y-encoded subunit of the translation initiation factor Eif2 is essential for mouse spermatogenesis. *Nat. Genet.* **29**, 49-53.
- Monsees, T. K., Blocher, S., Heidorn, F., Winkler, A., Siems, W. E., Muller-Esterl, W., Hayatpour, J., Miska, W. and Schill, W. B.** (2002). Expression and location of the bradykinin B2 receptor in rat testis. *Biol. Reprod.* **67**, 1832-1839.
- Mroz, K., Carrel, L. and Hunt, P. A.** (1999). Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. *Dev. Biol.* **207**, 229-238.
- Nanda, I., Hornung, U., Kondo, M., Schmid, M. and Scharl, M.** (2003). Common spontaneous sex-reversed XX males of the medaka *Oryzias latipes*. *Genetics* **163**, 245-251.
- Ogawa, T., Dobrinski, I., Avarbock, M. R. and Brinster, R. L.** (2000). Transplantation of male germ line stem cells restores fertility in infertile mice. *Nat. Med.* **6**, 29-34.
- Pace, A. J., Lee, E., Athirakul, K., Coffman, T. M., O'Brien, D. A. and Koller, B. H.** (2000). Failure of spermatogenesis in mouse lines deficient in the Na(+)-K(+)-2Cl(-) cotransporter. *J. Clin. Invest.* **105**, 441-450.
- Palmer, S. J. and Burgoyne, P. S.** (1991). In situ analysis of fetal, prepubertal and adult XX—XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development* **112**, 265-268.
- Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., Foster, J. W., Frischauf, A. M., Lovell-Badge, R. and Goodfellow, P. N.** (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* **346**, 240-244.
- Tanemura, K., Kanai, Y., Kanai-Azuma, M., Kurohmaru, M., Kuramoto, K., Yazaki, K. and Hayashi, Y.** (1996). Reinitiation of spermatogonial mitotic differentiation in inactive old BDF1 mouse seminiferous tubules transplanted to W/Wv mouse testis. *Biol. Reprod.* **55**, 1237-1242.
- Toue, A., Szot, M., Mahadevaiah, S. K., Rattigan, A., Ojarikre, O. A. and Burgoyne, P. S.** (2004). A new deletion of the mouse Y chromosome long arm associated with the loss of Ssty expression, abnormal sperm development and sterility. *Genetics* **166**, 901-912.
- Toyooka, Y., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M. and Noce, T.** (2000). Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mech. Dev.* **93**, 139-149.
- Tsunekawa, N., Matsumoto, M., Tone, S., Nishida, T. and Fujimoto, H.** (1999). The Hsp70 homolog gene, Hsc70t, is expressed under translational control during mouse spermiogenesis. *Mol. Reprod. Dev.* **52**, 383-391.
- Yamamoto, T.** (1955). Progeny of artificially induced sex-reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male. *Genetics* **40**, 406-419.
- Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R. Y., Birkett, N. C., Okino, K. H., Murdock, D. C. et al.** (1990). Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* **63**, 213-224.

**Table S1. Downregulated genes**

Gene name	Gene description	Chr. location	Fold changes	
			Tubule	Whole testis
<i>Ddx3y</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Y	-157.6	-724.1
<i>Uty</i>	ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	Y	-34.3	-4.0
<i>Eif2s3y</i>	eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked	Y	-27.9	-97.0
<i>Jarid1d</i>	jumonji, AT rich interactive domain 1D (Rbp2 like)	Y	-22.6	-5.3
<i>Nxf2</i>	nuclear RNA export factor 2	X	-16.0	-12.1
<i>Tuba3</i>	tubulin, alpha 3	6	-9.2	-19.7
<i>Dst</i>	dystonin	1	-9.2	-4.3
<i>Clca1</i>	chloride channel calcium activated 1	3	-7.5	-4.6
<i>Alcam</i>	activated leukocyte cell adhesion molecule	16	-5.7	-2.1
<i>Apod</i>	apolipoprotein D	16	-5.3	-2.8
<i>Kctd14</i>	potassium channel tetramerisation domain containing 14	7	-5.3	-2.3
<i>Slc6a4</i>	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	11	-5.3	-2.1
<i>1190002H23Rik</i>	RIKEN cDNA 1190002H23 gene	14	-4.6	-4.0
<i>Jam2</i>	junction adhesion molecule 2	16	-4.6	-2.6
<i>Scara5</i>	scavenger receptor class A, member 5 (putative)	14	-4.6	-2.6
<i>Ube1y1</i>	ubiquitin-activating enzyme E1, Chr Y 1	Y	-3.7	-7.0
<i>9630031F12Rik</i>	RIKEN cDNA 9630031F12 gene	5	-3.5	-3.0
<i>Rab3b</i>	RAB3B, member RAS oncogene family	4	-3.5	-2.1
<i>Nfkbiz</i>	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	16	-3.2	-2.5
<i>Plod2</i>	procollagen lysine, 2-oxoglutarate 5-dioxygenase 2	9	-3.2	-2.1
<i>Morc1</i>	microorchidia 1	16	-3.0	-7.0
<i>BC022960</i>	cDNA sequence BC022960	X	-3.0	-3.0
<i>Trim7</i>	tripartite motif protein 7	11	-3.0	-2.1
<i>Cxcr4</i>	chemokine (C-X-C motif) receptor 4	1	-2.8	-2.8
<i>Dusp15</i>	dual specificity phosphatase-like 15	2	-2.8	-2.0
<i>Apoa2</i>	apolipoprotein A-II	1	-2.6	-4.6
<i>Id4</i>	inhibitor of DNA binding 4	13	-2.6	-3.2
<i>Cd83</i>	CD83 antigen	13	-2.6	-2.5
<i>Ehd1</i>	EH-domain containing 1	19	-2.6	-2.3
<i>3230401M21Rik</i>	RIKEN cDNA 3230401M21 gene	18	-2.5	-2.3
<i>MGI:1916782</i>	homeobox only domain	5	-2.5	-2.1
<i>Bcl7c</i>	B-cell CLL/lymphoma 7C	7	-2.5	-2.0
<i>Dtymk</i>	deoxythymidylate kinase	1	-2.3	-2.8
<i>Cp</i>	ceruloplasmin	3	-2.3	-2.5
<i>Foxq1</i>	forkhead box Q1	13	-2.3	-2.1
<i>Tyro3</i>	TYRO3 protein tyrosine kinase 3	2	-2.3	-2.0
<i>Hells</i>	helicase, lymphoid specific	19	-2.1	-7.0
<i>Ris2</i>	retroviral integration site 2	8	-2.1	-2.6
<i>Gadd45b</i>	growth arrest and DNA-damage-inducible 45 beta	10	-2.1	-2.5
<i>Edn1</i>	endothelin 1	13	-2.1	-2.3
<i>Stk4</i>	serine/threonine kinase 4	2	-2.1	-2.3
<i>Galnt7</i>	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7	8	-2.1	-2.0
<i>Rarres2</i>	retinoic acid receptor responder (tazarotene induced) 2	6	-2.1	-2.0
<i>Zcchc3</i>	zinc finger, CCHC domain containing 3	2	-2.0	-3.2
<i>Ctnnal1</i>	catenin (cadherin associated protein), alpha-like 1	4	-2.0	-2.3
<i>Plagl1</i>	pleiomorphic adenoma gene-like 1	10	-2.0	-2.3
<i>1300007B12Rik</i>	RIKEN cDNA 1300007B12 gene	1	-2.0	-2.1
<i>4930588M11Rik</i>	RIKEN cDNA 4930588M11 gene	3	-2.0	-2.1

Table S2. Up-regulated genes

Gene name	Gene description	Chr. location	Fold changes	
			Tubule	Whole testis
<i>Xist</i>	inactive X specific transcripts	X	48.5	27.9
<i>Klik16</i>	kallikrein 16	7	24.3	7.5
<i>Asb12</i>	ankyrin repeat and SOCS box-containing protein 12	X	17.1	2.5
<i>Sult1e1</i>	sulfotransferase family 1E, member 1	5	14.9	7.0
<i>Klik6</i>	kallikrein 6	7	14.9	4.0
<i>Col9a3</i>	procollagen, type IX, alpha 3	2	13.0	4.6
<i>Rhbg</i>	Rhesus blood group-associated B glycoprotein	3	12.1	3.7
<i>Klik27</i>	kallikrein 27	7	9.8	4.9
<i>Myh6</i>	myosin, heavy polypeptide 6, cardiac muscle, alpha	14	9.8	3.0
<i>D16Bwg1494e</i>	DNA segment, Chr 16, Brigham & Women's Genetics 1494 expressed	16	9.2	4.0
<i>FHOS2</i>	formin-family protein FHOS2	18	9.2	3.2
<i>Snrpn</i>	small nuclear ribonucleoprotein N	7	9.2	2.8
<i>Klik24</i>	kallikrein 24	7	8.6	7.0
<i>Cps1</i>	carbamoyl-phosphate synthetase 1	1	8.0	8.6
<i>Stom</i>	stomatin	2	8.0	2.3
<i>Klik22 /// Klik9</i>	kallikrein 22 /// kallikrein 9	7	7.5	6.5
<i>Svs5</i>	seminal vesicle secretion 5	2	7.5	3.2
<i>Htatip2</i>	HIV-1 tat interactive protein 2, homolog (human)	7	7.5	2.3
<i>Glb1</i>	galactosidase, beta 1	9	7.0	2.5
<i>Klik21</i>	kallikrein 21	7	6.5	6.5
<i>Thrsp</i>	thyroid hormone responsive SPOT14 homolog (Rattus)	7	6.5	4.9
<i>Abcb1a</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	5	6.5	2.8
<i>RIKEN cDNA 1100001H23</i>	1100001H23Rik	6	6.5	6.5
<i>Slc39a8</i>	solute carrier family 39 (metal ion transporter), member 8	3	6.1	4.3
<i>Akr1c12</i>	aldo-keto reductase family 1, member C12	13	6.1	2.0
<i>Spp1</i>	secreted phosphoprotein 1	5	5.7	5.3
<i>Gpx7</i>	glutathione peroxidase 7	4	5.7	2.3
<i>Anxa3</i>	annexin A3	5	5.7	2.0
<i>Asb9</i>	ankyrin repeat and SOCS box-containing protein 9	-	5.3	2.5
<i>Masp1</i>	mannan-binding lectin serine peptidase 1	16	4.9	2.6
<i>Klik1</i>	kallikrein 1	7	4.6	5.7
<i>A930025J12Rik</i>	RIKEN cDNA A930025J12 gene	5	4.6	4.0
<i>Hsd17b3</i>	hydroxysteroid (17-beta) dehydrogenase 3	-	4.0	2.1
<i>Ephx1</i>	epoxide hydrolase 1, microsomal	1	3.7	2.3
<i>Rcn1</i>	reticulocalbin 1	2	3.7	2.1
<i>LOC544986</i>	similar to hypothetical protein LOC67055	14	3.5	3.2
<i>Kit</i>	kit oncogene	5	3.5	2.0
<i>Vnn1</i>	vanin 1	10	3.2	4.0
<i>Mbp</i>	myelin basic protein	18	3.2	2.8
<i>Hsd3b1</i>	hydroxysteroid dehydrogenase-1, delta<5>-3-beta	3	3.2	2.5
<i>Akr1c13</i>	aldo-keto reductase family 1, member C13	13	3.2	2.3
<i>Itih2</i>	inter-alpha trypsin inhibitor, heavy chain 2	2	3.2	2.3
<i>Pcolce</i>	procollagen C-endopeptidase enhancer protein	5	3.2	2.0
<i>Rdh11</i>	retinol dehydrogenase 11	12	3.0	3.7
<i>Wnt5a</i>	wingless-related MMTV integration site 5A	14	3.0	2.8
<i>4933407N01Rik</i>	RIKEN cDNA 4933407N01 gene	11	3.0	2.3
<i>Plxnd1</i>	Plexin D1 (Plxnd1), mRNA	6	3.0	2.3
<i>Txk</i>	TXK tyrosine kinase	5	3.0	2.3
<i>Zfp185</i>	zinc finger protein 185	X	3.0	2.0
<i>Tnfrsf12a</i>	tumor necrosis factor receptor superfamily, member 12a	17	2.8	2.8
<i>Tcn2</i>	transcobalamin 2	11	2.8	2.6
<i>Plp1</i>	proteolipid protein (myelin) 1	X	2.8	2.5
<i>MGI:1889205</i>	plasma glutamate carboxypeptidase	15	2.8	2.1
<i>Cd36</i>	CD36 antigen	5	2.8	2.0
<i>Slc39a8</i>	solute carrier family 39 (metal ion transporter), member 8	3	2.6	5.7
<i>Synpo</i>	synaptopodin	18	2.6	3.5
<i>Bscl2</i>	Bernardinelli-Seip congenital lipodystrophy 2 homolog (human)	19	2.6	3.0
<i>Frzb</i>	frizzled-related protein	2	2.6	2.5
<i>Car4</i>	carbonic anhydrase 4	11	2.6	2.1
<i>Cyp2d22</i>	cytochrome P450, family 2, subfamily d, polypeptide 22	-	2.6	2.1
<i>Pcolce</i>	procollagen C-endopeptidase enhancer protein	5	2.6	2.1
<i>Txndc5</i>	thioredoxin domain containing 5	13	2.6	2.1
<i>2310016C16Rik</i>	RIKEN cDNA 2310016C16 gene	13	2.6	2.0
<i>Myadm</i>	myeloid-associated differentiation marker	7	2.6	2.0
<i>Pld3</i>	phospholipase D family, member 3	7	2.6	2.0
<i>Olig1</i>	oligodendrocyte transcription factor 1	16	2.5	2.6
<i>Hfe</i>	hemochromatosis	13	2.5	2.5
<i>Tceal3</i>	transcription elongation factor A (SII)-like 3	X	2.5	2.5
<i>4632428N05Rik</i>	RIKEN cDNA 4632428N05 gene	10	2.5	2.3
<i>Ctps2</i>	cytidine 5'-triphosphate synthase 2	X	2.5	2.3
<i>Trp53inp1</i>	transformation related protein 53 inducible nuclear protein 1	4	2.5	2.3
<i>Sesn3</i>	sestrin 3	9	2.5	2.1



Ndrp4	N-myc downstream regulated gene 4	8	2.5	2.0
septin 6	37504	X	2.5	2.5
Dnaja4	DnaJ (Hsp40) homolog, subfamily A, member 4	9	2.3	4.0
Slc25a29	solute carrier family 25, member 29	12	2.3	2.8
Bbox1	butyrobetaine (gamma), 2-oxoglutarate dioxygenase 1	2	2.1	5.3
Atp6v0e2	ATPase, H+ transporting, lysosomal, V0 subunit E isoform 2	6	2.1	2.3
Dspg3	dermatan sulphate proteoglycan 3	10	2.1	2.3
Mdfic	MyoD family inhibitor domain containing	6	2.1	2.0
9530028C05	hypothetical protein 9530028C05	6	2.0	5.3
A130022J15Rik	RIKEN cDNA A130022J15 gene	6	2.0	2.8
Acacb	acetyl-Coenzyme A carboxylase beta	5	2.0	2.8
Dap	death-associated protein	15	2.0	2.8
Utx	ubiquitously transcribed tetratricopeptide repeat gene, X chromosome	X	2.0	2.8
Vcam1	vascular cell adhesion molecule 1	3	2.0	2.8
Gpx3	glutathione peroxidase 3	11	2.0	2.6
Sc5d	sterol-C5-desaturase homolog	9	2.0	2.5
Tmem71	ransmembrane protein 71	15	2.0	2.5
Fkbp9	FK506 binding protein 9	6	2.0	2.1
Acsl6	acyl-CoA synthetase long-chain family member 6	11	2.0	2.0
Actn3	actinin alpha 3	19	2.0	2.0
Lmo2	LIM domain only 2	2	2.0	2.0