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

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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*^{a} testes, XY or X*Sxr*^{a}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*^{v} 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*^{d}) 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*^{v}-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*^{d} males. They were then transplanted into the testes of 8-week-old recipient XX/*Sry* and XY *W/W*^{v} 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

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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 μm) 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*^{v}: 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 +/– 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_4, 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*^{v} 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 μg) 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*^{v}-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′)
Ddx3y (*Dby*)	GGTCTGGAAAAACTGCTGC	TTGGTGGCATTGTGTCCTGC
Uty	AAATGCAGCTCGGACCAAATC	CTGAATGATGTGAAGCTGTC
		AGAAACACTCTGCTGTGCTGTG
Eif2s3y	GGTGCTGTTGGAGCATTACC	TCAACTCGTCGGCTTAGAGC
Jarid1d (*Smcy*)	CTCTCGTGGGGATGAAGTCGATA	AAGTATACTCCTGTGTAGCCTG
Nxf2	GCCTTCACGAGGATCTTCAT	GAAAGGCCTCTCTCTATTCC
Clca1	ACCTAAAGAGGCTGGCTCAA	AGTGTTGACAGGCAGCCTTT
Alcam	CATTTCCCCTGAGGAGAATG	TATCCAATCCGCTCCTCTCT
Apod	GAGTCCTGATGGAACCATGA	GCCATGGTTGCTTGGTACTT
Kctd14	TCAACCTCAGAAATCTGC	ATAAGGTGACCTCTCAGA
Slc6a4	ACATCTGGCGTTTTCCCTACAT	TTTTGACTCCTTTCCAGATGCT
1190002H23Rik	AGAGTGCAGACTCAGTGTAC	AGTGAGCTTCACTCTCCGAA
Jam2	ACATGTAACGGCTGCCAGCT	CCGAATGCATGGAATGCGAA
Scara5	ATGACTTGAAGGCGCTGACT	CCTGCAGCTGGTAGAGTTCC
Ube1y1	CTCTGAGTACATCCGTGG	GCAATCCTGCTGAACTGC
Xist	CGGGGCTTGGTGGATGGAAAT	GCGTAACTGGCTCGAGAATA
Klk16	CTGACTTCAGCAATGACCTG	TACACCAGGTATACCGCATG
Asb12	TATCTGGCTGCAGTCTATGG	TCTGCGGATAACTAAACGGG
Sult1e1	CAGAGCTTGTGGACAGAATC	GTGCAATCCTTCATCTGCTG
Klk6	GCAGCATTACACCCGTCAAA	TACCCGGCACATTGGGTTTA
Col9a3	TCAAGGTGTACCTGGCATCA	CCAATAGATGTTCTTGGGGC
Rhbg	CCACGAAGCTTATGGAGATG	CTGGAACAGAGTAACACTGG
Klk27	CAAGAGCAATGACCTGATGC	ATTGGGTTTAGCGCATGGGA
Snrpn	AGCAGGTGTACCTATTCCCC	ATGGGTGGTCTCATACCAGG
Klk24	GCAACCTAAGGACAAGAGCA	CGCATTGGGTTTACCACATG
Klk9	ACCGATTGGTCAGCAAAAGC	GCATGACATCTGTCACCTTC

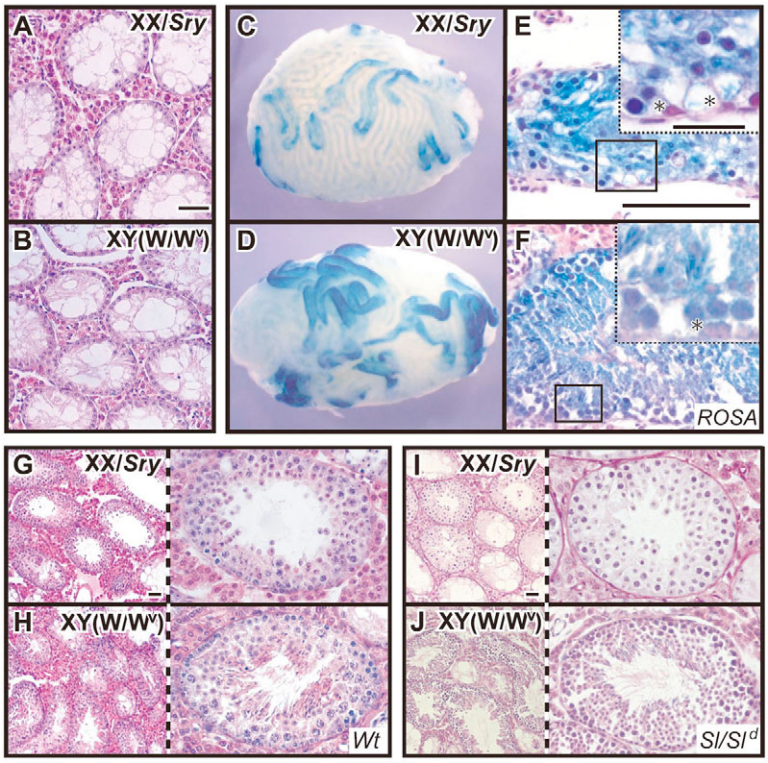

Fig. 1. Spermatogonial transplantation into testicular seminiferous tubules of XX/Sry and XY W/W^v (control) males. (**A**,**B**) HE staining of the intact XX/*Sry* and XY W/W^v testes at 8 weeks of age, showing lack of germ cells in most tubules of both testes. (**C-J**) The recipient testes were injected with spermatogonial-cell suspension prepared from immature *ROSA26* (C-F), wild-type (G,H) and *Sl/Sl^d* (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^v (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^v (H,J) testes injected with the wild-type- derived (G,H) or *Sl/Sl^d*-derived (I,J) testicular cell suspension. Higher-magnification images of recipient tubules with donor germ cells are shown on the right. Scale bars: 50 μm in A,G,I and inset of E; 200 μm 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^v* 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^v* males). In both XY *W/W^v* 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^d* 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^v* 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^v* 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^v* 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^v* 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^v* 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^v* 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

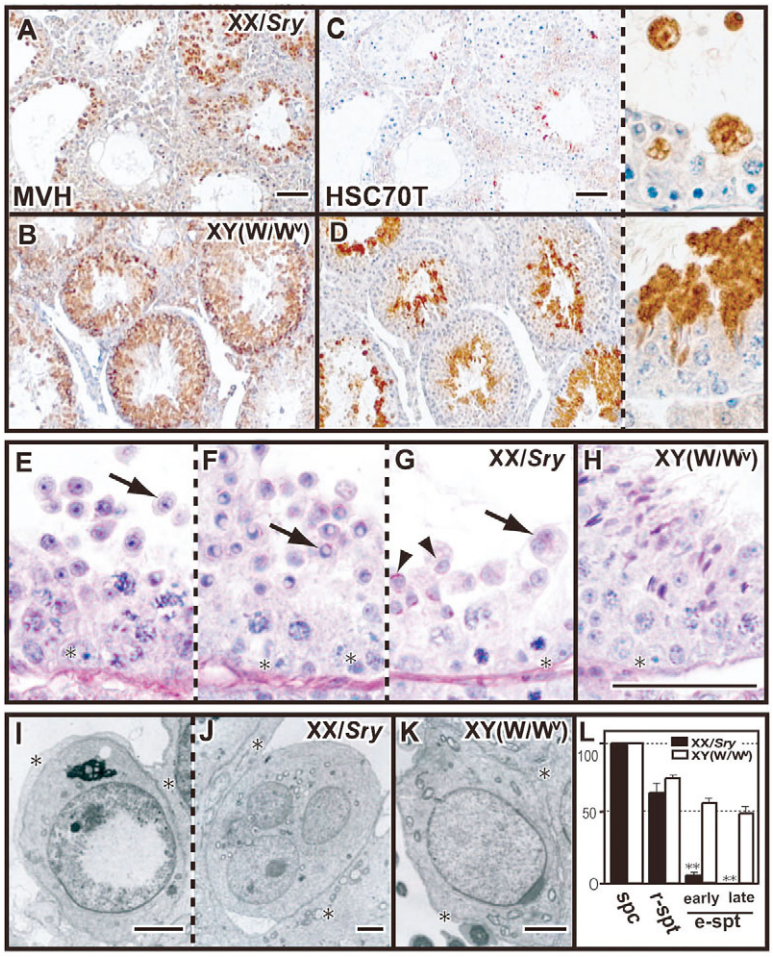

Fig. 2. Defective spermiogenesis of donor XY germ cells in recipient seminiferous tubules of XX/Sry males. (**A-D**) Anti-MVH (A,B) and anti-HSC70T (C,D) stainings of two serial sections of XX/*Sry* and XY W/W^v testes (brown staining). (C,D) Insets show higher-magnification images of HSC70T-positive spermatids: notice the abnormal morphology of all HSP70T-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^v 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^v 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 +/– standard error; asterisks indicate a statistically significant difference between the XX/*Sry* and XY W/W^v testes (**P<0.01). Scale bars: 50 μm in A-H; 2 μm 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*^{v} 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*^{v} 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*^{v} 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*^{v} 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*^{b}-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

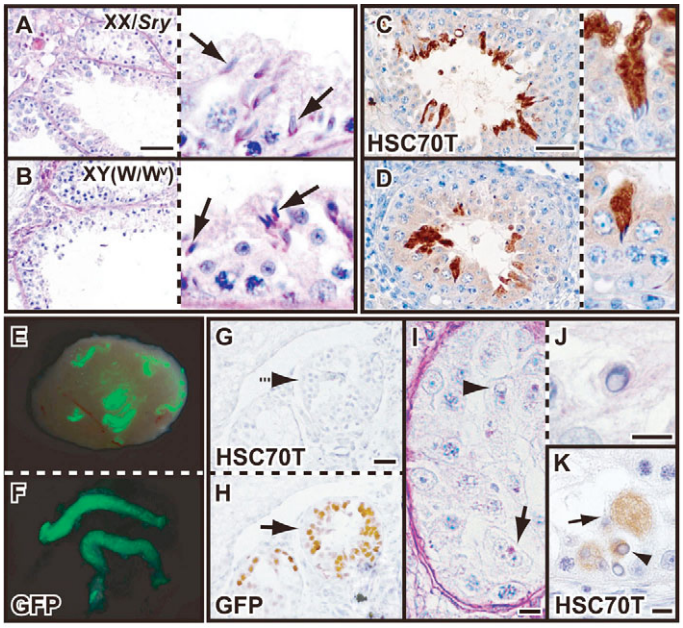

Fig. 3. Transplantation of seminiferous tubule segments under testis capsules of XX/Sry and XY W/W^v males. (**A-K**) Segments of immature XY seminiferous tubules (2-3 weeks old, wild type) were transplanted under the testis capsules of recipient XX/Sry (A,C) and XY W/W^v (B,D) males. Tubule segments 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^v 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^v recipients testes. Scale bars: 50 μm in A-H; 10 μm in I-K.

A

Gene Name	Chr	Fold changes tubule	(whole testis)
Down-regulated			
Ddx3y (Dby)	Y	-157.6	(-724.1)
Uty	Y	-34.3	(-4.0)
Eif2s3y	Y	-27.9	(-97.0)
Jarid1d (Smcy)	Y	-22.6	(-5.3)
Nxf2	X	-16.0	(-12.1)
Dst	1	-9.2	(-4.3)
Tuba3	6	-9.2	(-19.7)
Clca1	3	-7.5	(-4.6)
Alcam	16	-5.7	(-2.1)
Apod	16	-5.3	(-2.8)
Kctd14	7	-5.3	(-2.3)
Slc6a4	11	-5.3	(-2.1)
1190002H23Rik	14	-4.6	(-4.0)
Jam2	16	-4.6	(-2.6)
Scara5	14	-4.6	(-2.6)
Ube1y1	Y	-3.7	(-7.0)
9630031F12Rik	5	-3.5	(-3.0)
Rab3b	4	-3.5	(-2.1)
Nfkbiz	16	-3.2	(-2.5)
Plod2	9	-3.2	(-2.1)

B

Up-regulated			
Xist	X	48.5	(27.9)
Klk16	7	24.3	(7.5)
Asb12	X	17.1	(2.5)
Sult1e1	5	14.9	(7.0)
Klk6	7	14.9	(4.0)
Col9a3	2	13.0	(4.6)
Rhbg	3	12.1	(3.7)
Myh6	14	9.8	(3.0)
Klk27	7	9.8	(4.9)
Snrpn	7	9.2	(2.8)
FHOS2	18	9.2	(3.2)
D16Bwg1494e	16	9.2	(4.0)
Klk24	7	8.6	(7.0)
Stom	2	8.0	(2.3)
Cps1	1	8.0	(8.6)
Svs5	2	7.5	(3.2)
Klk22 /// Klk9	7	7.5	(6.5)
Htatip2	7	7.5	(2.3)
Glb1	9	7.0	(2.5)
Klk21	7	6.5	(6.5)

C

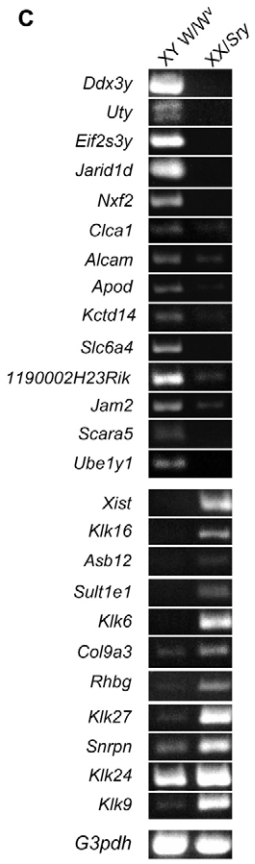

Fig. 4. Microarray and RT-PCR analyses of gene expression in isolated seminiferous tubules from XX/*Sry* and XY W/W^v testes. (**A**,**B**) Microarray expression analysis showing the top 20 genes that were (A) downregulated and (B) up-regulated in XX/Sry testes. The fold change represents the difference in the expression level in seminiferous tubules between XX/Sry and XY W/W^v 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.

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 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, XX*Sxr^b*, XO/*Sry* and X*Sxr^b*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.

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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., Ebnet, 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/XYY 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 Schartl, 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, prepuberal 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.

Toure, 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
Ddx3y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Y	-157.6	-724.1
Uty	ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	Y	-34.3	-4.0
Eif2s3y	eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked	Y	-27.9	-97.0
Jarid1d	jumonji, AT rich interactive domain 1D (Rbp2 like)	Y	-22.6	-5.3
Nxf2	nuclear RNA export factor 2	X	-16.0	-12.1
Tuba3	tubulin, alpha 3	6	-9.2	-19.7
Dst	dystonin	1	-9.2	-4.3
Clca1	chloride channel calcium activated 1	3	-7.5	-4.6
Alcam	activated leukocyte cell adhesion molecule	16	-5.7	-2.1
Apod	apolipoprotein D	16	-5.3	-2.8
Kctd14	potassium channel tetramerisation domain containing 14	7	-5.3	-2.3
Slc6a4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	11	-5.3	-2.1
1190002H23Rik	RIKEN cDNA 1190002H23 gene	14	-4.6	-4.0
Jam2	junction adhesion molecule 2	16	-4.6	-2.6
Scara5	scavenger receptor class A, member 5 (putative)	14	-4.6	-2.6
Ube1y1	ubiquitin-activating enzyme E1, Chr Y 1	Y	-3.7	-7.0
9630031F12Rik	RIKEN cDNA 9630031F12 gene	5	-3.5	-3.0
Rab3b	RAB3B, member RAS oncogene family	4	-3.5	-2.1
Nfkbiz	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	16	-3.2	-2.5
Plod2	procollagen lysine, 2-oxoglutarate 5-dioxygenase 2	9	-3.2	-2.1
Morc1	microrchidia 1	16	-3.0	-7.0
BC022960	cDNA sequence BC022960	X	-3.0	-3.0
Trim7	tripartite motif protein 7	11	-3.0	-2.1
Cxcr4	chemokine (C-X-C motif) receptor 4	1	-2.8	-2.8
Dusp15	dual specificity phosphatase-like 15	2	-2.8	-2.0
Apoa2	apolipoprotein A-II	1	-2.6	-4.6
Id4	inhibitor of DNA binding 4	13	-2.6	-3.2
Cd83	CD83 antigen	13	-2.6	-2.5
Ehd1	EH-domain containing 1	19	-2.6	-2.3
3230401M21Rik	RIKEN cDNA 3230401M21 gene	18	-2.5	-2.3
MGI:1916782	homeobox only domain	5	-2.5	-2.1
Bcl7c	B-cell CLL/lymphoma 7C	7	-2.5	-2.0
Dtymk	deoxythymidylate kinase	1	-2.3	-2.8
Cp	ceruloplasmin	3	-2.3	-2.5
Foxq1	forkhead box Q1	13	-2.3	-2.1
Tyro3	TYRO3 protein tyrosine kinase 3	2	-2.3	-2.0
Hells	helicase, lymphoid specific	19	-2.1	-7.0
Ris2	retroviral integration site 2	8	-2.1	-2.6
Gadd45b	growth arrest and DNA-damage-inducible 45 beta	10	-2.1	-2.5
Edn1	endothelin 1	13	-2.1	-2.3
Stk4	serine/threonine kinase 4	2	-2.1	-2.3
Galnt7	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7	8	-2.1	-2.0
Rarres2	retinoic acid receptor responder (tazarotene induced) 2	6	-2.1	-2.0
Zcchc3	zinc finger, CCHC domain containing 3	2	-2.0	-3.2
Ctnnal1	catenin (cadherin associated protein), alpha-like 1	4	-2.0	-2.3
Plagl1	pleiomorphic adenoma gene-like 1	10	-2.0	-2.3
1300007B12Rik	RIKEN cDNA 1300007B12 gene	1	-2.0	-2.1
4930588M11Rik	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
Xist	inactive X specific transcripts	X	48.5	27.9
Klk16	kallikrein 16	7	24.3	7.5
Asb12	ankyrin repeat and SOCS box-containing protein 12	X	17.1	2.5
Sult1e1	sulfotransferase family 1E, member 1	5	14.9	7.0
Klk6	kallikrein 6	7	14.9	4.0
Col9a3	procollagen, type IX, alpha 3	2	13.0	4.6
Rhbg	Rhesus blood group-associated B glycoprotein	3	12.1	3.7
Klk27	kallikrein 27	7	9.8	4.9
Myh6	myosin, heavy polypeptide 6, cardiac muscle, alpha	14	9.8	3.0
D16Bwg1494e	DNA segment, Chr 16, Brigham & Women's Genetics 1494 expressed	16	9.2	4.0
FHOS2	formin-family protein FHOS2	18	9.2	3.2
Snrpn	small nuclear ribonucleoprotein N	7	9.2	2.8
Klk24	kallikrein 24	7	8.6	7.0
Cps1	carbamoyl-phosphate synthetase 1	1	8.0	8.6
Stom	stomatin	2	8.0	2.3
Klk22 /// Klk9	kallikrein 22 /// kallikrein 9	7	7.5	6.5
Svs5	seminal vesicle secretion 5	2	7.5	3.2
Htatip2	HIV-1 tat interactive protein 2, homolog (human)	7	7.5	2.3
Glb1	galactosidase, beta 1	9	7.0	2.5
Klk21	kallikrein 21	7	6.5	6.5
Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus)	7	6.5	4.9
Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	5	6.5	2.8
RIKEN cDNA 1100001H23	1100001H23Rik	6	6.5	6.5
Slc39a8	solute carrier family 39 (metal ion transporter), member 8	3	6.1	4.3
Akr1c12	aldo-keto reductase family 1, member C12	13	6.1	2.0
Spp1	secreted phosphoprotein 1	5	5.7	5.3
Gpx7	glutathione peroxidase 7	4	5.7	2.3
Anxa3	annexin A3	5	5.7	2.0
Asb9	ankyrin repeat and SOCS box-containing protein 9	-	5.3	2.5
Masp1	mannan-binding lectin serine peptidase 1	16	4.9	2.6
Klk1	kallikrein 1	7	4.6	5.7
A930025J12Rik	RIKEN cDNA A930025J12 gene	5	4.6	4.0
Hsd17b3	hydroxysteroid (17-beta) dehydrogenase 3	–	4.0	2.1
Ephx1	epoxide hydrolase 1, microsomal	1	3.7	2.3
Rcn1	reticulocalbin 1	2	3.7	2.1
LOC544986	similar to hypothetical protein LOC67055	14	3.5	3.2
Kit	kit oncogene	5	3.5	2.0
Vnn1	vanin 1	10	3.2	4.0
Mbp	myelin basic protein	18	3.2	2.8
Hsd3b1	hydroxysteroid dehydrogenase-1, delta<5>-3-beta	3	3.2	2.5
Akr1c13	aldo-keto reductase family 1, member C13	13	3.2	2.3
Itih2	inter-alpha trypsin inhibitor, heavy chain 2	2	3.2	2.3
Pcolce	procollagen C-endopeptidase enhancer protein	5	3.2	2.0
Rdh11	retinol dehydrogenase 11	12	3.0	3.7
Wnt5a	wingless-related MMTV integration site 5A	14	3.0	2.8
4933407N01Rik	RIKEN cDNA 4933407N01 gene	11	3.0	2.3
Plxnd1	Plexin D1 (Plxnd1), mRNA	6	3.0	2.3
Txk	TXK tyrosine kinase	5	3.0	2.3
Zfp185	zinc finger protein 185	X	3.0	2.0
Tnfrsf12a	tumor necrosis factor receptor superfamily, member 12a	17	2.8	2.8
Tcn2	transcobalamin 2	11	2.8	2.6
Plp1	proteolipid protein (myelin) 1	X	2.8	2.5
MGI:1889205	plasma glutamate carboxypeptidase	15	2.8	2.1
Cd36	CD36 antigen	5	2.8	2.0
Slc39a8	solute carrier family 39 (metal ion transporter), member 8	3	2.6	5.7
Synpo	synaptopodin	18	2.6	3.5
Bscl2	Bernardinelli-Seip congenital lipodystrophy 2 homolog (human)	19	2.6	3.0
Frzb	frizzled-related protein	2	2.6	2.5
Car4	carbonic anhydrase 4	11	2.6	2.1
Cyp2d22	cytochrome P450, family 2, subfamily d, polypeptide 22	–	2.6	2.1
Pcolce	procollagen C-endopeptidase enhancer protein	5	2.6	2.1
Txndc5	thioredoxin domain containing 5	13	2.6	2.1
2310016C16Rik	RIKEN cDNA 2310016C16 gene	13	2.6	2.0
Myadm	myeloid-associated differentiation marker	7	2.6	2.0
Pld3	phospholipase D family, member 3	7	2.6	2.0
Olig1	oligodendrocyte transcription factor 1	16	2.5	2.6
Hfe	hemochromatosis	13	2.5	2.5
Tceal3	transcription elongation factor A (SII)-like 3	X	2.5	2.5
4632428N05Rik	RIKEN cDNA 4632428N05 gene	10	2.5	2.3
Ctps2	cytidine 5′-triphosphate synthase 2	X	2.5	2.3
Trp53inp1	transformation related protein 53 inducible nuclear protein 1	4	2.5	2.3
Sesn3	sestrin 3	9	2.5	2.1

Ndrg4	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