Ovotestes in B6-XXSxr Sex-Reversed Mice

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The sex-reversed mutation Sxr results in XX males. In the absence of any other mutations, testis differentiation in XXSxr fetuses is essentially normal and only one report of an XXSxr fetus with ovotestes is in the literature. We report that 84% (21/25) of 13 days postcoitum XXSxr fetuses on the B6 inbred genomic background have ovotestes. Ovotestes were found in fetuses from both Sxr\textsuperscript{a} and Sxr\textsuperscript{b} variants. Examination of fetuses older than 13 dpc suggests that the presence of ovotestes is transient in most fetuses. However, one overt hermaphrodite was identified after birth. The development of ovotestes is associated with the inbred background and is exacerbated by the dominant spotting oncogene allele Kit<sup>W-42</sup>. We propose that spreading of X-inactivation into the Sxr region resulting in loss of Sry expression is more extensive in B6-Sxr strains.

INTRODUCTION

The sex reversed mutation Sxr (Tp(Y)1Ct) (Cattanach et al., 1971) originated from the duplication of Y Chromosome (Chr) short arm sequences and the transposition of these sequences distal to the pseudoautosomal region of the Y Chr long arm (Ashley et al., 1995; McLaren et al., 1988; Roberts et al., 1988). The transposed “Sxr region” contains several genes, including, from proximal to distal, the testis-determining gene sex-determining region of the Y (Sry), zinc-finger protein on Y-2 (Zfy2), selected mouse cDNA on Y (Smcy), Y-linked ubiquitin-activating enzyme E1 homologue (Ube1y), and zinc-finger protein on Y-1 (Zfy1) (Bishop and Mitchell, 1996). Two Sxr variants exist: Sxr\textsuperscript{a} and Sxr\textsuperscript{b} (McLaren et al., 1984). Sxr\textsuperscript{b} was derived from Sxr\textsuperscript{a} and differs by having a \textgreek{>900-kb} deletion between its Zfy2 and Zfy1 loci. The deletion removed most of Zfy2 and all of Ube1y and Smcy (Bishop and Mitchell, 1996), generating a Zfy2/Zfy1 fusion gene comprised of the Zfy2 promoter through first coding exon joined to intron sequences 5′ of the second coding exon of Zfy1 (Simpson and Page, 1991).

XXSxr males are a result of meiosis in males carrying Sxr (XY Sxr). During prophase I, a Y Sxr and X chromatin pair and recombine within the pseudoautosomal region, transcribing the Sxr region from the Y to the X chromatin. Four types of sperm are produced that differ with regard to their sex chromosome (X, Y, X Sxr, Y Sxr) and these give rise to normal XX females, normal XY males, XXSxr males, and XY Sxr carrier males, respectively.

Because XXSxr males have two X Chr, random X-inactivation should result in XXSxr fetuses being mosaic for the expression of Sry. Ovotestes are not uncommon in XX-XY chimeric and XO/XY and XO/XY/XY mosaic mice (Bradbury, 1987; Eicher et al., 1980; McLaren et al., 1972; Myszkowska and Tarkowski, 1970; Whitten et al., 1991). For XO/XY and XO/XY/XYY mosaic mice, it was estimated that their gonads develop some ovarian tissues if XO cells comprise more than 24% of the fetus (Eicher et al., 1980). Therefore, one would predict many XXSxr fetal gonads should develop as ovotestes since X-inactivation would result in 50% of the fetal cells not expressing Sry. In fact, the literature reports that testis differentiation in XXSxr fetuses is essentially normal (Spolar and Drews, 1978) and no overt hermaphrodites have been found in over 900 XXSxr mice when examined postnatally (Cattanach, 1987; Drews et al., 1974). This paradox is explained by the phenomenon of position effect variegation which results in variable inactivation of the Sxr region and Sry (Cattanach, 1987; Cattanach et al., 1982; McLaren and Monk, 1982). We now report that 84% of XXSxr fetuses in the strains C57BL/6-Sxr\textsuperscript{a} (B6-Sxr\textsuperscript{a}) and C57BL/6-Sxr\textsuperscript{b} (B6-Sxr\textsuperscript{b}) develop ovotestes at 13 days postcoitum (dpc). Although the ovarian regions regress with subsequent fetal development such that most B6-XXSxr

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mice present as males at birth, one B6-XXSxr
to verification of the Sxr region was complete in B6-XXSxr strains.

MATERIALS AND METHODS

Animals and Tissues

C57BL/6-Sxr (B6-Sxr) was obtained from Dr. Anne McLaren (Wellcome/CRC Institute, UK). C57BL/6J, C57BL/6j-Sxr (B6-Sxr), and C57BL/6J-Kitw-42, B6CBA/Ca A+/A Ta/0 females, and CBA/Caj were purchased from the Jackson Laboratory (Bar Harbor, ME). The B6-Sxr and B6-Sxr strains were maintained by backcrossing to B6/ J females; no coat color markers were used.

Fetuses were obtained from timed pregnant matings (day of plug = day 0 postcoitum, dpc). Fetuses at 11 dpc were staged by counting tail somites starting from the base of the genital tubercle (Nagamine and Carlisle, 1996). Testis lengths of newborn mice were measured under a dissecting microscope with the aid of an eyepiece micrometer.

Genotyping

For B6-Sxr, XXSxr were identified among XY and XY Sxr littermates by probing dot blots of genomic DNAs with the murine Y-repetitive probe Y353/B as previously described (Nagamine et al., 1987a). XXSxr DNAs do not hybridize with Y353/B whereas XY and XY Sxr DNAs hybridize strongly (Fig. 1A).

For B6-Sxr, XXSxr, XY Sxr, and XY genotypes were identified by PCR for Zfy1 and Zfy2 as previously described (Nagamine et al., 1990). The Zfy primers (sense: 5'-AAG-ATA-AGT-TTA-CAT-AAT-CAC-ATG-GA-3'; antisense: 5'-CCT-ATG-AAA-TCC-CTT-TTG-GCT-GCA-CAT-GT-3') were designed to 1) amplify a fragment encoding part of the Zfy1 and Zfy2 zinc finger domains, 2) to recognize the Zfy1 and Zfy2 genes equally and, and 3) to flank a Zfy2 18-bp deletion (nt 1438-1455) thereby allowing one to discriminate Zfy1 (618 bp) and Zfy2 (600 bp) amplified products. The PCR amplified products were electrophoresed through a 4% polyacrylamide gel and the fragments visualized by ethidium bromide staining and UV transillumination.

PCR of XXSxr DNA results in a single 618 bp fragment derived from the Zfy2/1 fusion gene in the Sxr region (Fig. 1B). XXSxr genotypes have a Zfy1 and Zfy2 gene on their Y Chr plus a Zfy2/1 fusion gene within the Sxr region; PCR gives a 618 bp band (Zfy1 + Zfy2/1) that fluoresces with approximately twice the intensity of the 600 bp band (Zfy2). XY genotypes give 618 bp band (Zfy1) and 600 bp (Zfy2) bands that fluoresce with equal intensity.

Newborn Kitw-42 heterozygotes were identified among wild-type littermates by the absence of pigmented hair follicles in their dermis (Nagamine and Carlisle, 1996).

RNA Isolation, cDNA Synthesis, and RT-PCR

Gonadal primordia, mesonephros, and associated Wolfian ducts from individual 11 dpc fetuses were placed in 5 μl phosphate buffered saline in 0.5 ml microcentrifuge tubes, frozen on dry ice, and stored at −85°C. Total RNA was prepared from individual pairs of urogenital ridges using RNase easy kits (Qiagen, Inc.). RNA samples were treated with RQ1 DNase (Promega Corp.) (37°C, 30 minutes), phenol extracted, ethanol precipitated, then resuspended in 11 μl of diethyl pyrocarbonate-treated water containing 1.0 U/μl of rRNasin (Promega Corp.) and 1.5 mM dithiothreitol. To confirm the absence of genomic DNA, 1 μl of the RNA preparation was PCR amplified using primers specific for Sry. The remaining 10 μl of RNA were reverse transcribed using Superscript II (GIBCO/BRL). One microscop of the cDNA was used for PCR.

Murine Sry transcripts are of two types: a linear transcript and a circular transcript that lacks a cap and poly(A) tail (Capel et al., 1993). Amplification of linear Sry cDNAs was ensured by designing the sense primer (5'-AGC-TCT-TAC-AGT-TTA-GAC-CTC-TCG-AAG-TGT-3') from a site 145-bp upstream of the splice acceptor that generates the circular transcript. The antisense primer (5'-GCA-GCT-GTA-CTC-CAG-TCT-GTC-GCA-CAT-CGC-3') was from a region 3' of the HMG box. PCR conditions were previously described (Carlisle et al., 1996) and result in a 612-bp fragment. Hprt RT-PCR served as a positive control (sense: 5'-AAG-GAC-CTC-TGG-ATG-TGG-TGG-ATA-3', antisense: 5'-GCA-TCT-GTA-AGG-AAA-CTAC-TTG-TGA-CAA-CG-3') (Nagamine et al., 1990).

Histology

Gonads were fixed in 4% paraformaldehyde in phosphate buffer (pH 7.2), embedded in Historesin (Reichert-Jung) according to manufacturer's instructions, and sectioned at 2 μm on an ultramicrotome. Slides were stained in Lee's methylene blue-basic fuschin (Bennett et al., 1976).
FIG. 2. (A–E) 13 dpc fetal gonads. (A) XX/Sxr<sup>a</sup> testis, (B) XX ovary, (C and D) B6-XX/Sxr<sup>b</sup>, and (E) B6-XX/Sxr<sup>a</sup> gonads with undifferentiated regions at cranial and/or caudal poles (arrows). (F) Internal genitalia of 1-week-old B6-XX/Sxr<sup>b</sup> overt hermaphrodite. t, testis; vd, vas deferens; ut, uterus.

FIG. 3. Internal genitalia of newborn XX/Sxr<sup>b</sup> Kit<sup>W-42J</sup>/<sup>+/+</sup> hermaphrodites. (A) Overt hermaphrodite with ovary (ov) and contralateral testis (t). ut, uterus; vd, vas deferens. (B) Hermaphrodite with persistent Mullerian ducts (dark arrows). Open arrows point to the vasa deferentia.
XXSxr Fetuses Have Abnormal Testis Differentiation

In the laboratory mouse, testicular cords are first visible under the dissecting microscope at 12.5 dpc (normal gestation period = 19-20 days). Examination of nine B6-Sxr<sup>a</sup> and eleven B6-Sxr<sup>b</sup> litters at 13 dpc revealed that the developing testes from 8/41 (19.5%) male fetuses from B6-Sxr<sup>a</sup> and from 14/62 (22.5%) male fetuses from B6-Sxr<sup>b</sup> were abnormal with undifferentiated regions present at the cranial and/or caudal poles (Fig. 2). This phenotype is characteristic of ovotestes in the mouse (Biddle and Nishioka, 1988; Bradbury, 1987; Eicher et al., 1982; Nagamine et al., 1987b). The degree of virilization varied, ranging from gonads with only 2-5 testicular cords (13 dpc testes average 10-12 tubules, n = 40 testes) (Figs. 2C, D) to those with only a small region of undifferentiated tissue at one or both poles (Fig. 2E). All female fetuses were normal.

Genotyping revealed 21/22 fetuses scored as having abnormal testes were XXSxr (Table 1). In total, 21/25 (84%) of B6-XXSxr<sup>a</sup> and B6-XXSxr<sup>b</sup> fetuses had ovotestes. Our past observations of B6-XXSxr<sup>a</sup> and B6-XXSxr<sup>b</sup> 15 dpc fetuses, newborns, and adults have not revealed any obvious abnormalities of their testes. We surmise that ovarian tissues regress with further fetal development and the gonads present as testes at birth, as previously suggested for XX-XY chimeric gonads (Bradbury, 1987). One notable exception,

FIG. 4. Histological sections of the gonads of hermaphrodite in Fig. 3B. (A) Left testis. Open arrow points to testicular cords shown at higher magnification in (D). (B) Right ovotestis with ovarian (ov) and testicular (t) regions separated by connective tissue (arrowhead). (C) Merging of ovarian and testicular tissues in a subsequent serial section. Open arrow points to the testicular cord shown at higher magnification in E. Closed arrow points to the ovarian region shown at higher magnification in F. Bar = 50 µm in A–C and 20 µm in D–F.
had an ovary on the right and a contralateral testicular gonad. We conclude that presence of ovotestes in B6-XXSxr fetuses is usually transient. However, in rare B6-XXSxr mice ovarian tissues are not lost and ovotestes and/or ovaries are present at birth.

**Kit^{W-42}** Exacerbates Abnormal Testis Differentiation in XXSxr

XY_DOM sex reversal occurs when certain M. m. domesticus Y Chrs are introduced into B6 (Eicher et al., 1982). In the consomic strain B6-Y_TIR, which has a Y Chr from mice trapped in Tirano, Italy, testis differentiation is severely compromised (Carlisle et al., 1996; Nagamine et al., 1987b; Nagamine et al., 1987c). About a third of XY fetuses develop male internal and external genitalia and bilateral testicular gonads. However, histological studies reveal that these testicular gonads are in fact ovotestes and the “males” are in fact cryptic hermaphrodites (Nagamine et al., 1987b; Nagamine et al., 1987c). A third of the XY fetuses develop as overt hermaphrodites, and the remaining third develop as XY females with bilateral ovaries.

The dominant spotting gene Kit (=W) encodes a cell surface receptor tyrosine kinase whose ligand is the mast cell growth factor (= Steel factor, stem cell factor, Kit ligand). We recently showed that the Kit^{W-42} allele exacerbates XY_DOM sex reversal resulting in almost all B6-Y_TIR Kit^{W-42/+} fetuses developing female phenotypes (Nagamine and Carlisle, 1996). To ascertain if Kit^{W-42} also exacerbates abnormal testis differentiation in XXSxr mice, we introduced Kit^{W-42} into B6-Sxr^{a} and examined the internal genitalia of newborn mice. Nine litters were obtained resulting in 18 XXSxr^{b} mice (9 +/+ , 9 Kit^{W-42/+}). No hermaphrodites were identified among XXY or XY Sxr^{a} (+/+ or Kit^{W-42/+}) or XXSxr^{b} +/+ progeny. Among XXSxr^{b} Kit/+ mice, 2/9 (22%) were hermaphrodites. The first was an overt hermaphrodite with a testis and contralateral ovary (Fig. 3A). The second had internal genitalia that appeared to be testes with associated epididymides and vasa deferentia. However, persistent Müllerian ducts were present bilaterally (Fig. 3B).

Histological sections revealed the left gonad to be a testis devoid of germ cells (Figs. 4A, D). In contrast, the right gonad was an ovotestis (Figs. 4B, C). The ovarian and testicular regions of the ovotestes were separated by connective tissue in one region of the gonad but were found to merge in subsequent serial sections (Figs. 4C–4F). Partial segregation of ovarian and testicular regions also occur in ovotestes from XY_DOM sex reversed and in XX-XY chimeric mice (Nagamine et al., 1987b; Tarkowski, 1964).

**XXSxr Fetal Gonads Express Sry**

Sry is first expressed in XY gonads on late 10 dpc, reaches a peak on 11 dpc then quickly drops to low levels by 13 dpc (Hacker et al., 1995). RT-PCR for Sry was performed on individual 11 dpc XY (n = 4), XXSxr^{a} (n = 1), and XXSxr^{b} (n = 2) fetal gonads obtained from 2 litters. Sry transcripts were detected in both XXSxr^{a} samples suggesting that abnormal testis development is not due to absence of Sry transcripts at this age (Fig. 5).

**Development of Ovotestes Associated with Inbred Background**

In crosses designed to test the role of X-inactivation in the development of ovotestes, XX females of a mixed B6 and CBA/Ca genetic background (B6CBA/Ca A^{+/A} Ta/O or B6CBA/Ca A^{+/A} Ta/O × B6-Y^{AKR} or B6-Y^{W-42J}/+O) were mated to B6-XY Sxr^{b} males and the fetuses examined at 13-14 dpc for the presence of ovotestes. All XY (n = 11), XXSxr^{a} (n = 10), and XXSxr^{b} (n = 3) fetuses had normal testes. Among the XXSxr^{b} fetuses only 50% (6/12) were found with ovotestes. This is in contrast to 84% of XXSxr^{a} fetuses with both ovotestes and ovaries in B6-Sxr strains. The number of fetuses with ovotestes in the two XXSxr populations was statistically significant (Fisher’s exact test, two-tailed P value = 0.046).

We posited that the reduction in number of fetuses with ovotestes resulted from the XO females being on a mixed genetic background. To test this hypothesis we mated CBA/CaJ females to B6-Sxr^{b} males and examined the fetuses at 13 dpc. Five (CBA/CaJ × B6-Sxr^{b})F_{1} XXSxr^{b} 13 dpc fetuses were obtained; all had normal testes (Fig. 6). If ovotestes had developed in the F_{1} XXSxr fetuses at the same frequency as in the B6-XXSxr strains, 4 of the 5 F_{1} XXSxr fetuses should have had ovotestes. The difference in number of XXSxr fetuses with ovotestes in the (CBA/CaJ × B6-Sxr^{b})F_{1} versus B6-Sxr populations was statistically significant (Fisher’s exact test, two-tailed P value = 0.0009). The cumulative data suggest that the B6 inbred genetic background contributes to the development of ovotestes in XXSxr fetuses.

**DISCUSSION**

The mammalian gonad is bipotential and capable of developing into a testis or ovary. Current dogma is that ovary differentiation is the default pathway. In XY karyotypes, SRY preempts ovary determination by triggering testis differentiation. Mutations that affect Sry expression or SRY protein can cause XY sex reversal. Alternatively, the presence of Sry in XX karyotypes either through transgenesis (Eicher et al., 1995; Koopman et al., 1991), the Sxr region, or abnormal X-Y chromatid exchange in humans (de la Chapelle et al., 1984; Guellaen et al., 1984; Sinclair et al., 1990) result in XX sex reversal.

Because XXSxr mice have two X Chrs, random X-inactivation should result in 50% of their cells having the Xsxr Chr inactivated. Two reports suggest that X-inactivation may
adversely affect testis differentiation in XXSxr. First, about 83% of XXSxr 15-17 dpc fetuses have gonads with meiotic germ cells, a hallmark of oogenesis, and 29% of 8-18 days postpartum XXSxr mice have testicular gonads with enlarging oocytes (Mclaren, 1980; McLaren, 1981). No meiotic germ cells were found in the testes of five XO Sxrα fetuses suggesting that X-inactivation is necessary for anomalous oogenesis in XXSxr gonads (Mclaren, 1981). It should be emphasized that ovarian somatic tissues were not observed in these gonads so it is unclear if the presence of meiotic germ cells is indicative of abnormal testis differentiation, as suggested by (Burgoyne, 1989), or abnormal germ cell development. Second, if one ensures that the X Chr is inactivated in all cells by introducing the reciprocal X-autosomal translocation T(X;16)16H (abbreviated T16H), some T16H/XSxr gonads develop into ovaries (Cattanach et al., 1982; McLaren and Monk, 1982). XO/XY and XO/XY/XYY mosaic fetuses possess ovo- testes if the XO component is between 24-44% (Eicher et al., 1980; Whitten et al., 1991). Similarly, XX-XY chimeric fetuses have ovo- testes at 12-14 dpc (Bradbury, 1987). Given that XO/XY and XO/XY/XYY mosaic and XX-XY chimeric fetuses can develop into overt hermaphrodites, one would predict the same for XXSxr. In fact, the literature suggest testis differentiation is essentially normal in XXSxr. In XYDDom sex reversal, the development of ovo- testes is usually heralded by a delay in the initiation of testis cord formation (Carlisle et al., 1996; Taketo et al., 1991). It is logical to assume that a similar delay in testis differentiation would also be seen in XXSxr fetuses if ovo- testes normally develop. However, based on a study of ten 12 dpc XXSxrα fetuses, Spoljar and Drews (1978) concluded that XXSxrα fetuses were normal both in the timing of testis differentiation and in testis morphology. Moreover, only a single 15 dpc XXSxrα fetus with ovo- testes comprised of distinct ovarian and testicular somatic tissues is in the literature (Mittwoch and Buehr, 1973). Postnataally, XXSxr overt hermaphrodites have not been observed in over 900 XXSxr mice, even when the XSxr Chr is preferentially inactivated in the majority of cells by heterozygosity for strong Xce alleles (Cattanach, 1987; Drews et al., 1974). Therefore, this report demonstrating that ovo- testes are found at high frequency in B6-XXSxr fetuses and that one B6-XXSxrα and two B6-XXSxrα KitW-42J hermaphrodites were identified postnataally is noteworthy.

One explanation as to why XXSxr fetuses do not normally develop ovo- testes is that X-inactivation of the Sxr region is incomplete (Cattanach, 1987). In T16H/XSxr mice, the XSxr Chr is inactivated in most, if not all, cells. If the neighboring Sxr region (and Sry) was also inactivated, all T16H/XSxr fetuses should develop as females. Although female phenotypes are indeed observed, 22-65% of T16H/XSxr develop as males and 9-13% develop as overt hermaphrodites suggesting that complete inactivation of the Sxr region does not occur (Cattanach et al., 1982; McLaren and Monk, 1982). It was suggested that, analogous to position effect variegation in X-autosomal translocations (Cattanach, 1974), inactivation of the attached Sxr region is variable resulting in some cells expressing Sry (Cattanach et al., 1982; McLaren and Monk, 1982). Whether the Sry locus is inactivated depends on modifying loci, one being linked to the X Chr (Mclaren, 1986). The difficulty in inactivation of the Sxr region may reflect a property of the Sxr region itself, its distance from the X-inactivation center, or the fact that the Sxr region is distal to the pseudoautosomal region which escapes inactivation (Keltges and Gartler, 1986; Salido et al., 1996). Inactivation of Sry within the Sxr region can be ranked, from

FIG. 5. Sry RT-PCR on 11 dpc XY, XY Sxrα, XX Sxrβ, and XX gonads. Specificity of the reaction is demonstrated by the absence of amplified product in the XX sample. Hprt RT-PCR reveals cDNA in all samples. (--, negative control with water substituting for cDNA.

FIG. 6. Gonads from (CBA/CaJ × B6- Sxrβ)F1 XXSxr (A) and XY Sxr (B) 13 dpc fetuses demonstrating normal testis differentiation.
minimal to maximal, as: XXSxr (outbred) < B6-XXSxr < T16H/Sxr.

Past studies used strains with a mixed genetic background. We hypothesized that the high frequency of ovotestes in B6-XXSxr fetuses and the identification of an overt hermaphrodite postnatally is linked to Sxr being on the B6 inbred genetic background. Our studies demonstrating a reduction in the percentage of XXSxr fetuses with ovotestes on a mixed B6-CBA/Caj genetic background and the absence of ovotestes in (CBA/Caj × B6-Sxr)F1. XXSxr fetuses are in keeping with this hypothesis. It remains to be determined if introducing Sxr into an inbred strain other than B6 will also result in a high frequency of ovotestes during fetal development. If the B6 genetic background is essential for development of ovotestes, candidate modifying genes for this effect are testis-determining, autosomal-1, 2, or 3 which modify \( XY^{DOM} \) sex reversal (Biddle et al., 1994; Eicher et al., 1996).

It has been proposed that one or more of the B6 testis-determining, autosomal genes is actually an early-acting, ovary-determining gene(s) (Burgoyne and Palmer, 1991; Palmer and Burgoyne, 1991). According to this “timing-mismatch” hypothesis, the B6 genetic background predisposes XXSxr fetuses to develop ovotestes because the ovary-determining program is initiated before Sry reaches sufficiently high titers to block its activity.

Kit mutations are characterized by their pleiotropic effects on melanocytes, hematopoietic cells, and germ cells (Morrison-Graham and Takahashi, 1993). Kit\(^{W^{-}}\), one of the most severe of the Kit alleles (Geissler et al., 1981), exacerbates \( XY^{DOM} \) sex reversal (Nagamine and Carlisle, 1996). The mechanism by which Kit\(^{W^{-}}\) inhibits testis differentiation is currently being investigated but is not due to retardation of early fetal growth, reduction of testis size, or a substantial delay in initiation of Sry expression. It is formally possible that Kit\(^{W^{-}}\) reduces Sry titers at a critical stage of testis differentiation or adversely affects testis differentiation downstream of Sry. The present results suggest that Kit\(^{W^{-}}\) heterozygosity also exacerbates abnormal testis differentiation in B6-XXSxr\(^{b} \). We conclude that Kit\(^{W^{-}}\)‘s detrimental effect on testis differentiation is not unique to \( XY^{DOM} \) sex reversal.

In humans, XX sex reversal is estimated to occur in 1:20,000–1:25,000 newborn males (de la Chapelle, 1981). The majority of XX sex reversed males result from an abnormal X-Y interchange that transfers Y-specific sequences, including SRY, to the X Chr (de la Chapelle et al., 1984; Guellaen et al., 1984). Although SRY+, XX sex reversed patients usually present as phenotypic males, 6 cases of true hermaphroditism have been reported (Fechner et al., 1994; Jäger et al., 1990; McElreavey et al., 1992; Nakagome et al., 1991; Palmer et al., 1989). One explanation for SRY+, XX true hermaphroditism is preferential inactivation of the X Chr bearing SRY (Fechner et al., 1994; McElreavey et al., 1992). The Sxr data suggest that X Chr or autosomal genes that enhance spreading of X-inactivation into the translocated SRY locus and/or adversely affect testis differentiation (e.g., Kit\(^{W^{-}}\)) may also contribute to true hermaphroditism.

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