Gonadal development in T16H/XSxr hermaphrodite mice*

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Summary. Sexual development was studied in 25 hermaphrodite mice with the genotype T16H/XSxr. The majority of the animals had a male phenotype similar to that seen in XXSxr males. A few, however, had feminized external genitalia and were classified as females. Examination of the gonads and reproductive tracts of the male hermaphrodites revealed a strong tendency for the left gonad to be more masculine than the right. Most of the gonads in male and female hermaphrodites appeared to be ovaries or testes rather than ovotestes.

Introduction

Sex-reversed (*Sxr*) is a spontaneous mutation which causes XX mouse embryos to develop as phenotypic males (Cattanach *et al.*, 1971). In 1982, *Sxr* was shown to be located at the distal tip of the Y chromosome (Evans *et al.*, 1982; Singh & Jones, 1982) and appeared to be a duplication of the testis-determining region. During male meiosis, a single obligatory crossover between the X and Y chromosomes allows the transfer of *Sxr* from the Y to the X chromosome (Burgoyne, 1982; Eicher, 1982), resulting in the development of XXSxr males. As a result of the presence of two X chromosomes in their germ line, XXSxr males are invariably sterile (Burgoyne, 1978; McLaren, 1983).

The X-autosome translocation, T(X;16)16H (hereafter referred to as T16H), disrupts the random pattern of X inactivation found in most adult XX mammals (Lyon, 1961). In females heterozygous for this translocation, the translocated X chromosome appears always to be the active one in the adult, so that the normal X chromosome is rarely if ever expressed (Lyon *et al.*, 1964). In mice with the genotype T16H/XSxr, it is the *Sxr*-carrying X chromosome which is inactivated. If the attached *Sxr* sequence were also to be inactivated, mice with this genotype should develop as normal fertile females. Although some do develop as females, sterile males and hermaphrodites are also produced (Cattanach *et al.*, 1982; McLaren & Monk, 1982). Presumably the *Sxr* sequences are inactivated in some cells only, producing a mosaic population of male-determining and female-determining cells. Sexual phenotype would then depend on the mosaic composition of the gonadal primordium. All T16H/XSxr mice, whatever their sexual phenotype, express H–Y antigen (Simpson *et al.*, 1984).

All the hermaphrodites described by McLaren & Monk (1982) and by Cattanach *et al.* (1982) had a male phenotype. The gonads of the hermaphrodites reported by Cattanach *et al.* (1982) were described as testes covered with blood clots. Histological sections revealed areas of disorganized tubules which contained oocytes. McLaren & Monk (1982) described three hermaphrodites which had a testis on one side and an ovotestis on the other. The ovotestes were very small and contained oocytes, sometimes in well developed follicles, within the disorganized testis tubules. In neither report was it stated whether the feminized gonad was on the left or the right, nor were any ovary-like gonads described.

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The present paper describes the external genitalia and gross morphology and distribution of the gonads of T16H/XSxr hermaphrodite mice. The histology of the gonads forms the subject of a separate paper (Ward *et al.*, 1988).

Materials and Methods

The origin of the stocks of mice used, and the breeding system, were as described by McLaren (1986) and McLaren & Monk (1982). Twenty-five T16H/XSxr hermaphrodite mice were available for the study, derived from matings of T16H/X or T16H/XSxr females with X/YSxr, XSxr/Y or XSxr/YSxr males. Details of the breeding behaviour of these different crosses are given in McLaren (1986). 'Male' hermaphrodites were initially classified as males; they were identified as hermaphrodites at autopsy by the gross appearance of the gonads and ducts, confirmed by gonadal histology. 'Female' hermaphrodites were initially classified as females; they were identified as hermaphrodites by their sterility and some ambiguity of the external genitalia, confirmed by gonadal histology. All animals were sexually mature at the time of autopsy. They were killed by cervical dislocation, and the gonads and ducts were then examined and fixed for histology.

Results

The external appearance and gross morphology of the ducts and gonads

Of the 10 animals for which detailed records of appearance at autopsy were kept, 4 had a male phenotype, although in one case the genital papilla was small and pointed rather than the usual square shape. These 'male' hermaphrodites had obvious scrotum development and no nipples were evident. The remaining 6 animals were classified as 'female' hermaphrodites although they all had ambiguous external genitalia. The genital papilla in this latter group was abnormal with redness on the undersurface, which resembled hypospadias in man. The extent of nipple and scrotum development in this group varied; one animal had distinct nipples but no scrotum whilst two others showed faint nipple development but had some scrotum formation. One had an almost normal female appearance; the genital papilla was of the female type although the vagina was not patent, the nipples were well developed and there was no sign of a scrotum.

A varied picture was also presented when the ducts and gonads were examined at autopsy. The testes were very small, sometimes measuring only about 1 mm in maximum anterior-posterior length, compared with about 10 mm in control males, and they were usually found either in the inguinal region or in a position in the abdominal cavity more typical of ovaries. The ducts associated with the testes were almost always epididymides, either rudimentary or quite well developed. In contrast, the ovaries observed were often similar in size, position and appearance to normal ovaries. The associated uteri were usually very swollen in the region of the cervix. In 2 animals an epididymis as well as a uterus and oviduct was associated with an ovary.

The gross morphology of the external genitalia, gonads and ducts of the animals used in the study are summarized in Tables 1 and 2.

Analysis of the distribution of the gonads

Mice 1-3, killed in mid-1982 and included in McLaren & Monk (1982), had 3 ovotestes out of a total of 6 gonads. Our later series, killed between February 1984 and September 1985 (see McLaren, 1986), had significantly fewer ovotestes (4/41, $\chi^2_{(1)} = 6.7$, $P < 0.01$). The overall incidence of testes and ovaries was very similar and the most common pairing arrangement was one ovary and one testis.

In males, the left gonad was significantly more masculine than the right ($P < 0.002$). The distribution between sides was significantly different in the two sexes (Table 3; $\chi^2_{(1)} = 4.4$, $P < 0.05$).

Table 1. The external genitalia and gross morphology of the ducts and gonads of T16H/XSxr hermaphrodite mice

Animal no.	External genitalia	Macroscopic appearance of ducts and gonads
19	Abnormal papilla, redness on underside; faint nipple formation	L: ovary, uterus with swelling near cervix R: tiny testis with rudimentary duct
20	Abnormal papilla, redness on underside; faint nipple formation	L: no gonad or ducts found R: ovotestis in inguinal region
21	Abnormal papilla, redness on underside; some scrotum and nipple development	L: tiny gonad, ambiguous ducts R: tiny gonad, swollen uterus with poor vasculature
22	Abnormal papilla, redness on underside; distinct nipple formation	L: testis with rudimentary duct R: ovary, uterus
23	Male papilla and scrotum	L: small testis, epididymis R: ovary, uterus with swelling near cervix
24	Abnormal papilla, redness on underside; some scrotum development; faint nipple formation	L: small inguinal testis, rudimentary epididymis R: large ovary with visible follicles, swollen uterus
25	Male papilla, scrotum development	L: tiny gonad (< 1 mm a-p length), rudimentary uterus R: abnormal ovary, uterus with swelling near cervix
26	Female papilla, nipple formation	L: ovary, uterus with some swelling R: tiny streak gonad, male ducts
29	Male papilla, small scrotum	L: ovary with small uterus R: small testis
30	Male papilla	L: small inguinal testis, epididymis R: inguinal ovary, swollen fluid-filled uterus with poor vasculature

Discussion

The majority of animals in this study had a male phenotype, as did those hermaphrodites described by Cattanaach *et al.* (1982) and McLaren & Monk (1982). Externally these animals appeared similar to XXSxr males, with a male genital papilla and a scrotum. They were only identified as hermaphrodites at autopsy. A small number of animals, however, were originally classified as female. On reaching maturity they showed a greater range in development than did the male hermaphrodites; most had an abnormal genital papilla and varied amounts of nipple formation. Since all the hermaphrodites, by definition, possess some testicular tissue, one would expect some masculinization of the external genitalia as a result of the effects of androgens produced by the fetal testis. The influence of androgens on the external genitalia was apparent in the female hermaphrodites since only one of them had a normal female genital papilla.

Most of the animals examined were alternate hermaphrodites with a testis and male duct on one side and an ovary and uterus on the other. This implies that each gonad must have developed independently according to the proportion of cells with *Sxr* active in the gonadal primordia. That a uterus was always associated with an ovary implies that anti-Müllerian hormone produced by the contralateral testis only acted locally. Androgens, however, may have acted systemically since some ovaries had both uterus and epididymis associated with them.

Differences were noted between the size and position of ovaries and testes and their associated ducts. Ovaries were usually of normal size and in their normal position. This is to be expected since, for ovaries to develop, *Sxr* must have been inactivated in the majority of cells. In other words, cells

Table 2. Phenotype and sex of the gonads and ducts of T16H/*Sxr* hermaphrodite mice

Animal No.	Phenotype	Left duct	Left gonad	Right duct	Right gonad
1	Male	Epididymis	Testis	Epididymis	Ovotestis
3	Male	Not known	Ovotestis	Epididymis	Ovotestis
5	Male	Epididymis, uterus	Ovary	Epididymis	Testis
6	Male	Epididymis	Testis	Uterus	Ovary
7	Male	Epididymis	Testis	Uterus	Ovary
8	Male	Epididymis	Testis	Epididymis, uterus	Ovary
9	Male	Not known	Testis	Epididymis	Ovotestis
11	Male	Not known	Testis	Not known	Ovotestis
12	Male	Not known	Testis	Uterus	Ovary
13	Male	Epididymis	Testis	Uterus	Ovary
18	Male	Epididymis	No gonad found	Uterus	Ovary
19	Female	Uterus	Ovary	Epididymis	Testis
20	Female	Not known	No gonad found	Epididymis	Ovotestis
21	Female	Epididymis	Testis	Uterus	Ovary
22	Female	Epididymis	Testis	Uterus	Ovary
23	Male	Epididymis	Testis	Uterus	Ovary
24	Female	Epididymis	Testis	Uterus	Ovary
25	Male	Uterus (rudimentary)	Testis	Uterus	Ovary
26	Female	Uterus	Ovary	Epididymis	Testis
27	Male	Epididymis	Testis	Uterus	Ovary
28	Female	Uterus	Ovary	Epididymis	Testis
29	Male	Uterus	No gonad found	Epididymis	Testis
30	Male	Epididymis	Testis	Uterus	Ovary
2*	Male	Epididymis, testis			
		Uterus, ovary			
14*	Male	(Duct not identified), testis			
		Epididymis, ovotestis			

*Animals for which the side of origin of the gonads was not recorded.

Table 3. Distribution of gonads between sides (phenotype of left gonad is given first)

Phenotype	Testis/ovotestis	Testis/ovary	Ovary/testis	Ovotestis/ovotestis
Male	3	9	1	1
Female	0	4	3	0

comprising an ovary were effectively XX and should, therefore, have developed normally. The associated uteri were similar to normal uteri except that, in some cases, there was a swelling in the cervical region. This was due to the normal uterine secretions being unable to escape to the exterior.

Unlike ovaries, testes differed considerably from normal testes both in size and position. They were always very small, due in part to the absence of germ cells, and they were located at various points along the normal pathway of descent from a position typical of ovaries to one in the inguinal region. It appears that they were descending but must have been prevented from reaching the scrotum in some way. Two possible factors which could have affected the descent of the testes include the effects of female steroid hormones produced by the contralateral ovary and insufficient androgen production owing to *Sxr* being inactivated in a portion of the testicular cells. That the epididymides varied from being relatively well developed to merely rudimentary is probably a reflection of the concentration of androgens present in individuals during development.

The most striking feature of the distribution of the gonads was that in male hermaphrodites the left gonad showed a strong tendency to be more masculine than the right. This is the opposite to the situation found in human alternate hermaphrodites in which the testis is usually found on the right side (Simpson, 1976; van Niekirk & Retief, 1981; Polani, 1981). This is true also for hermaphrodite pigs (Breeuwsma, 1970; Hunter *et al.*, 1982, 1985). Mittwoch & Mahadevaiah (1980) have found that human fetal testes are larger than ovaries, and in both sexes right gonads are larger than left. They postulate that testicular development depends upon increased growth rate. Mittwoch and Buehr (1973) failed to find any right-left size differences in fetal mouse gonads, although testes were larger than ovaries at all stages examined.

Most gonads in the T16H/XSxr hermaphrodites examined were either testes or ovaries with very few ovotestes (see Ward *et al.*, 1988). This pattern of development is to be expected since, with reference to XX/XY chimaeras, McLaren (1984) states that sexual differentiation tends to follow "a majority vote rather than proportional representation" to produce a testis or an ovary rather than an ovotestis. In mammals and birds, the 'voting system' is biased in favour of the heterogametic sex. This latter statement is supported in T16H/XSxr hermaphrodites by the observation that the testis was the single most common type of gonad.

It is difficult to explain the occurrence of phenotypically female animals which possessed a testis, since testis differentiation normally precedes ovarian differentiation and a hormonally competent testis usually directs development of the external genitalia along a male pathway (see Baker, 1972). Most phenotypic 'females' had rather ambiguous external genitalia and so it is conceivable that, in these animals, the testis was unable to produce sufficient androgens to masculinize the external genitalia. As there seemed to be a link between the phenotype and the left gonad it is possible that the left gonad may have become functional before the right.

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