Disorders of Gonadal Differentiation in Men: True Hermaphroditism and XX Males

Medicine Grand Rounds

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Jean D. Wilson

Introduction

The gonads in the two sexes develop from common anlagen, and the commitment of the primordial gonad to differentiate into the ovary or a testis is the central event in sexual dimorphism. The chromosomal nature of sex determination was established, largely by T.H. Morgan and his colleagues, between 1910 and 1916, but even at a primitive level the nature of the genetic information responsible for gonadal differentiation has remained largely enigmatic. The basic work in this field was performed in Drosophila; in that species XX, XXX, and XXY flies are female, XY is male, and XO is a sterile male. (Reviewed in ref. 1). The findings in Drosophila were assumed to apply to all species; it was generally believed (and I was taught) that gonadal differentiation is a function of the X chromosomes, two X chromosomes causing female development and one X causing male development. In light of this relation in Drosophila, together with the failure for many years to demonstrate specific genes on the mammalian Y chromosome (holandric inheritance), the Y chromosome was believed to be a dummy or null chromosome and was not thought to carry any information with the possible exception of some factor that promoted fertility in males (2). The development of techniques for the karyotyping of the mammalian chromosome in the early 1960's resulted in a reformulation of the relation between the chromosomes and sex determination, when it became apparent that in man the Y chromosome specifies the development of the testis (subsequently shown to apply in other mammals as well). Namely, XO is female, and no matter how many X chromosomes are present, a testis will develop as long as a Y chromosome is present (as in XXY, XXYY, XXXY, XXXXY, etc.) (1).

Nevertheless, there are two exceptions to this general relation - the 46,XX male who develops bilateral testes and the 46,XX true hermaphrodite who develops some combination of ovaries and testes despite absence of a Y chromosome. These two disorders have constituted major obstacles to understanding the chromosomal control of gonadal development, and any comprehensive theory about testicular differentiation must explain how testicular development can occur in the absence of a Y chromosome.

What I propose to do this morning is to review the current understanding of the structure, composition, and function of the mammalian Y chromosome, to describe studies on the pathogenesis of the XX male syndrome and XX true hermaphroditism and to attempt to formulate a working model for understanding testicular differentiation.

The Y Chromosome

The human Y chromosome is the third smallest human chromosome, on average just larger than the group G chromosomes (chromosomes 20 and 21) (1). The short arm is consistent (invariable) in size whereas among normal men the long arm varies considerably in length. In 5% of normal newborn males the Y is longer than chromosome 20, and in 0.25% of normal men the long arms are barely detectable (Yq^-) or I(Yp).

FIGURE 1

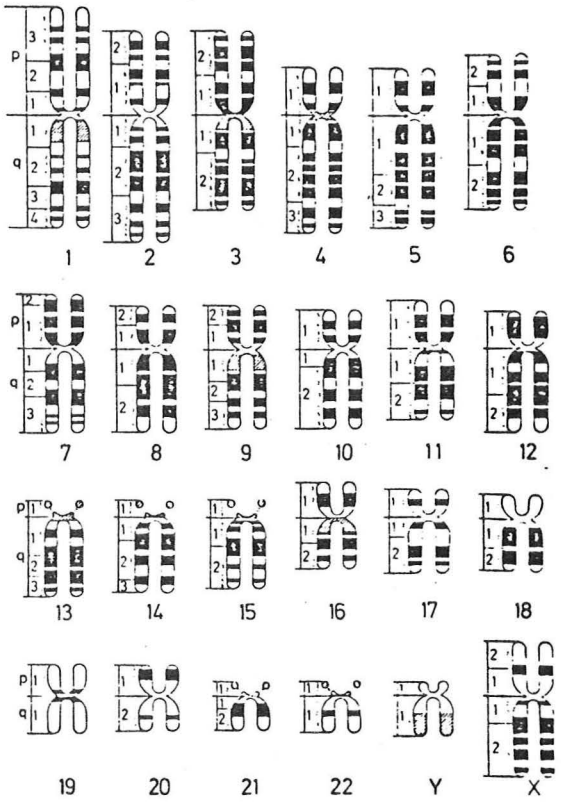


Fig. 1 Banding patterns according to the Paris nomenclature (G, Q, and R banding). Positive G and Q bands and negative R bands are *black*, variable regions are *hatched* (Paris Conference, 1971 [298])

The long arm exhibits brilliant distal fluorescence, two or three separate fluorescent bands being visible. This property can usually be identified in interphase nuclei as a bright dot about 0.3 μm in diameter.

FIGURE 2

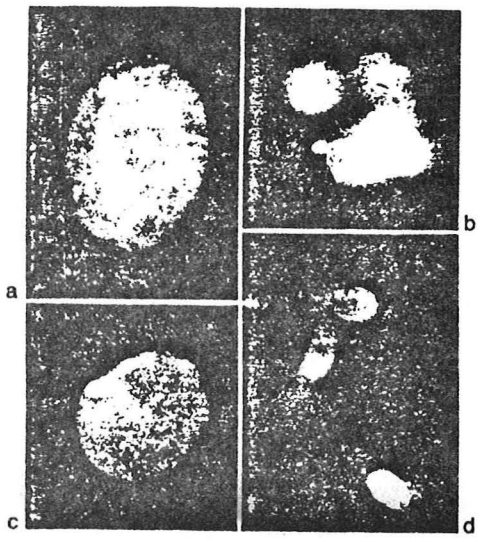


Fig. 2. a–d. Quinacrine mustard stainings of different cell nuclei of chromosomally normal men. **a** Buccal smear. The Y chromatin appears as a double structure. **b** Granulocyte from a blood smear. The Y chromatin is protruding as a small appendage. **c** Large lymphocyte from a blood smear. **d** Sperms. The Y chromatin is found near the border of the strongly fluorescent part of the sperm head (×2,400) (Schwarzacher and Wolf, 1974 [119])

The Y chromosomes of all species, like certain other chromosomes, contain satellites that are only visible under certain staining conditions. In the

human Y, satellite DNA is present both within a distal heterochromatin segment and in the centromeric region. The satellite DNA on the human Y chromosome is quite old in an evolutionary sense (its estimated age is around 18 million years), but it does not hybridize with chimpanzee DNA, implying that the human form differs from that of other species (1). The function, if any, of the satellite DNA is unknown.

Pairing of the X and Y Chromosomes

Other features of the Y chromosome are critical to understanding its role in normal and abnormal testicular differentiation. One is that pairing of the X and Y chromosomes is essential to ensure that they segregate during meiosis. The mechanism by which the two X chromosomes in XX females pair and segregate during the prophase of the first meiotic division of oogenesis is identical to that of pairing of the autosomes, in that the attachment occurs at the centromere. But males have one X and one Y chromosome, which must be paired during the first meiosis of spermatogenesis if the sperm are to contain either one X or one Y and not both or neither. The necessary pairing does not occur at the centromere but is instead made possible by a small region of homology between the short arm of the X and the short arm of the Y that enables them to join by base pairing during the first prophase. In this way they duplicate and partition evenly on the spindle; then at the second meiotic division only two types of sperm are produced, those containing X and those containing Y chromosomes (2).

There is considerable variability in the manner in which pairing is described by different authors. This is probably due both to uncertainty about the details and to true variation. As some authors formulate it the pairing of X and Y is side by side; others formulate it as occurring end to end. In the hamster where this has been studied extensively there is considerable variability among species (3).

Figure 3

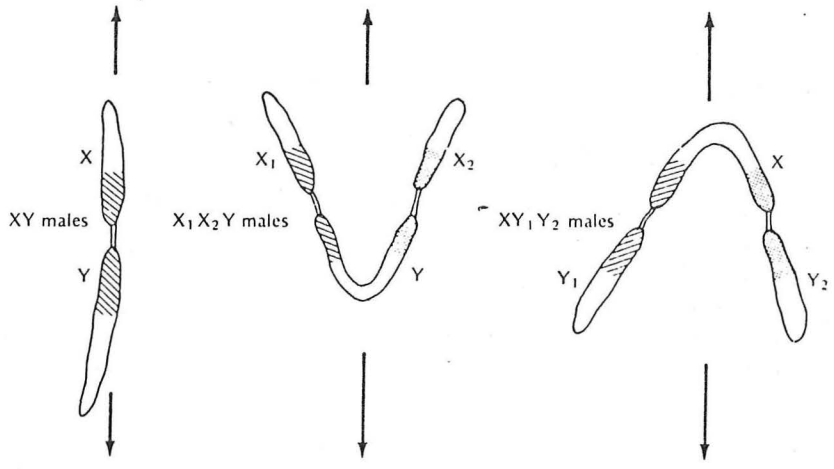
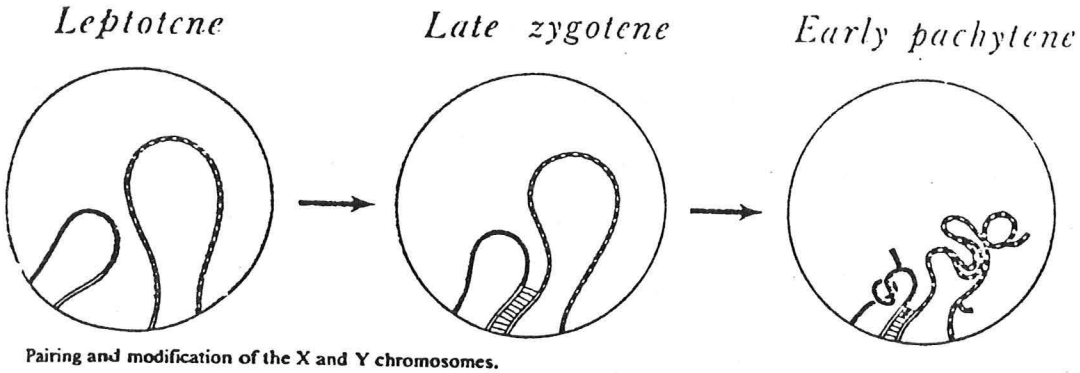


Figure 3

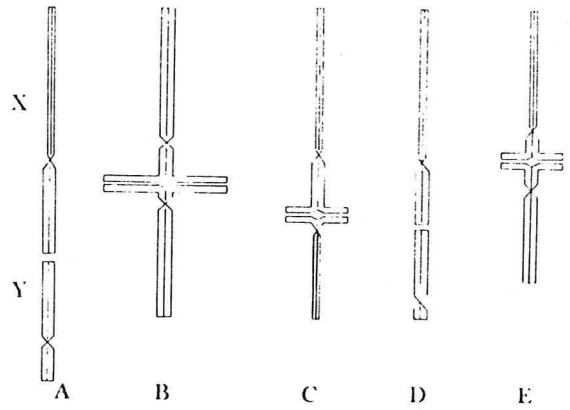
Reductional division of sex chromosomes in males with different sex chromosome constitutions. Hatched and shaded areas represent pairing segments.

Figure 4



Pairing and modification of the X and Y chromosomes.

Figure 5



Patterns of association between the X and Y chromosomes in different hamster species. The uncondensed regions are drawn thinner. A, Golden hamster. B, Chinese hamster. C, European hamster. D, Hungarian hamster. E, Armenian hamster.

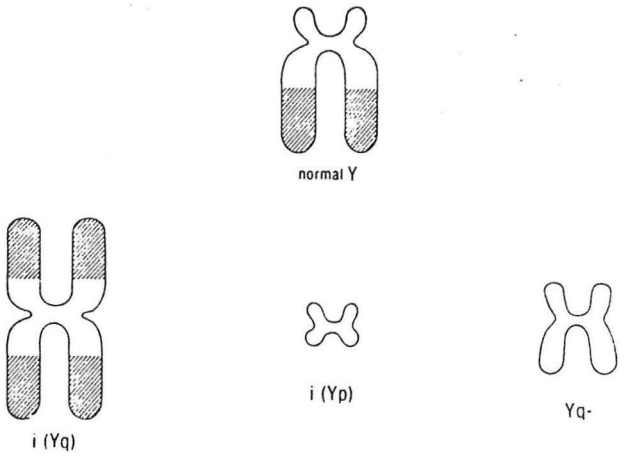
In any event, the net effect is the formation of the XY body that is identifiable in nucleoli between zygotene and mid pachytene. The XY body is largely composed of the condensed chromatin of the fused X and Y chromosomes (3).

Location of the Testis Determining Genes

The testis determining genes are not on the distal portion of the long arm and not on the most distal part of the short arm. This deduction is based on studies that correlate the chromosomal karyotype and the phenotype in men with variations in the structure of the Y chromosome. Loss of the fluorescent segment of the long arm has no effect on male development, and formation of a Y ring chromosome (which requires a loss of the distal-most segment of both long and short arms) also does not interfere with the formation of normal testis in men. Most evidence indicates that the testis determining genes are situated near the centromere on the long arm or (more probably) on the short arm

(possibly on both). Indeed, isochromosomes of the long arm of the Y involving loss of Yp have been detected in fertile women (5).

Figure 6



Idiogram of quinacrine banding patterns in normal and abnormal human Y chromosomes. Crosshatching indicates quinacrine-bright region of the long arm (Yq). Three abnormal types are depicted. Left: isochromosome of the long arm, i(Yq). Center: isochromosome of the short arm, i(Yp). Right: fragment arising as a result of loss of distal Yq.

Several types of sex-specific DNA sequences have been identified in addition to the Satellite DNA and to the testis-determining (TD) genes themselves.

1.) About 70% of the DNA of the human Y chromosome consists of repeated sequences of DNA located on the long arm (6). These sequences are related in an evolutionary sense to sequences on the human X, and hence no detectable phenotypic effect results when the long arm of the Y becomes translocated onto autosomes. The long repetitive fragments of DNA of the Y chromosome in many (or all) species including Drosophila, the mouse, and the human are composed of two repeating base quadruplets (GATA and GACA). It is almost certainly these repetitive sequences that are responsible for fluorescence of the distal arm of the Y, and variability.

2.) Homologous single copy regions of the DNA of the human X and Y chromosome have been identified with the use of restriction endonuclease techniques, but these fragments appear to be unique to the human (e.g., different than the homologous regions in other species), and hence these regions like the major repeat segments are not thought to play a role in sexual differentiation (7). More importantly, the homologous regions appear to be on the tips of the short arms of the X and Y chromosomes (8) and are presumed to be the segments responsible for pairing of the two chromosomes during meiosis rather than to contain genes that specify gonadal development.

3.) A third type of sex specific DNA (termed BMK) was originally described in the W chromosome of snakes and is now known to be conserved in the males of all vertebrate species (7A). In the mouse these sequences are concentrated on the short arm of the Y chromosome (e.g., adjacent to the site thought to contain

the determinants of testicular differentiation), and these regions of DNA are transcribed into a male-specific RNA of 1250 - 1400 bases (9, 10). Thus, it would in theory fulfill the requirement for a testes determining factor, but the protein product of this RNA has yet to be identified.

X Chromosome Inactivation During Spermatogenesis

Several types of evidence suggest that the X chromosome of the testis is not active during spermatogenesis. The formation of the XY body in pachytene is associated both with late replication of the X chromosome (and presumably Y chromosome as well) (12) and decreased incorporation of radioactive uridine into messenger-like RNA (13). Furthermore, studies of the enzyme composition of the testis support the view that the X chromosome is largely inactivated during spermatogenesis; in a number of mammals including man an X-linked form of phosphoglycerate kinase is expressed in all cells except the testis, which contains an isozyme specified by an autosomal locus (14). (This finding implies that X inactivation may involve all tissue components of the testis not just the germ cells). In support of the view that the presence of a functional X chromosome is not essential for testicular differentiation, Ohno et al have demonstrated that in some voles the X chromosome is eliminated (by non-disjunction) during spermatogenesis so that the germ cell lines in the male are OY in composition (15); this suggests that an X chromosome is not necessary for the formation of fertile sperm. Additional evidence in support of this view has come from studies of translocations of X chromosomes to autosomes in the mouse; some such translocations impair spermatogenesis presumably by impairing X inactivation in primary spermatocytes (16-20). The mechanism of X chromosome inactivation during spermatogenesis is fundamentally different than the random X-chromosome inactivation that occurs in the autosomes of XX females; the latter type of X chromosome inactivation is associated with chemical modification of the DNA (21) whereas the X-chromosome DNA from sperm is functional in in vitro transcriptional assays and presumably not modified (22).

The HY Antigen Hypothesis

The molecular basis by which gene(s) on the Y chromosome act to promote testicular differentiation is not known. A leading theory, namely that the Y chromosome either specifies or regulates the production of a differentiative antigen that causes transformation of the sexually indifferent gonad into a testis, was a biproduct of transplantation biology. Eichwald and Silsmer (CF 23) documented in 1955 that in certain inbred strains of mice intrastrain skin grafts are rejected when the donor is a male and the recipient is a female; in the same strains males cannot reject female grafts. This phenomenon was subsequently shown to be the consequence of a Y situated minor histocompatability gene that was named the HY antigen by Billingham and Silvers. (In this sense antigen is used solely as a descriptive term; the mechanism(s) by which the protein could exert biological effects is unknown). The designation "minor histocompatibility" gene means that immune responses against such antigens can only be demonstrated if the donor and recipient are identical with regard to the major histocompatibility (MHC) antigens.

Following the development in 1971 of an assay for the HY antigen that does not depend on the transplantation of skin two major theses were advanced -- that the H-Y antigen is the determinant of testicular differentiation (not merely a

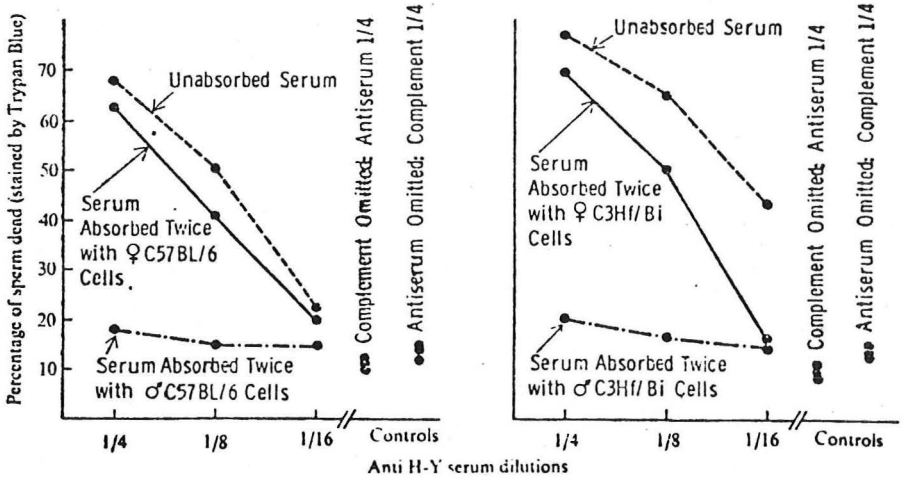
manifestation of male development) and that the measurement of H-Y antigen in humans with abnormal sexual development can be used to determine if the testis determining portion of the Y chromosome is present. Both of these theses have come under major challenge in the recent past.

In Vitro Assay of H-Y Plasma Membrane Antigen

Investigation of the biological significance of this or any other antigen depends upon a reproducible means of producing antibodies to the H-Y antigen and of detecting the antigen with such antibodies. The H-Y antigen was defined on the basis of rejection of skin transplantation, e.g., a transplantation antigen, whereas the serological tests examine for the presence of an antigen on the surface of cells. It is difficult in most instances to determine whether the two tests measure the same property, and the the gene that determines the H-Y plasma membrane antigen and the gene that specifies H-Y transplantation antigen may not be identical. Furthermore, in the mouse and rat (where the H-Y antigen was first described) as in other species skin rejection is MHC restricted, namely rejection of intrastrain transplantation of male skin into female occurs only in strains carrying certain MHC compositions; for example, one human anti-H-Y antibody only reacts with male cells that carry HLA-A2 Class 1 MHC antigen (23). For this reason virtually all studies of H-Y antigen utilize mouse antisera (polyclonal or monoclonal) and target cells from diverse strains rather than studies of skin transplantation.

The fundamental serological test that was the basis of the H-Y hypothesis was developed in 1971 by Goldberg et al at the Sloan-Kettering Institute (24) and is illustrated schematically in Figure 7.

Figure 7



Specificity of sperm lysis by anti H-Y serum($\frac{1}{4}$). Titration of cytotoxic anti H-Y serum absorbed twice with either male or female cells, and then tested on C57BL/6 sperm. First experiment (left): absorption with C57BL/6 cells; second experiment (right): absorption with C3Hf/Bi cells.

Anti H-Y serum was obtained from C57BL/6 female mice 5-14 days after they had rejected a fourth or fifth skin graft from C57BL/6 males. When this serum was reacted with C57BL/6 sperm it killed 50% or more of the sperm. This killing capacity could be prevented by absorption of the antiserum with male (but not female) cells of the same or different strain of mice. Similar results were obtained if the recipient cells were epithelial skin cells but not with most other male cells (such as lymphocytes). From the very first there have been a multitude of problems with this assay: 1) The antibody is almost invariably present in low titers, rarely demonstrable at dilution greater than 1:16; such sera commonly contain interfering antibodies; 2) The amount of killing of target cells rarely exceeds 50 percent even with undiluted sera so that the killing curves are inevitably shallow; 3) The assessment of cell death involves a trypan blue assay, which may be difficult to interpret reproducibly. 4) The tests are frequently poorly reproducible even within the same laboratory. 5) More importantly, sera have been produced in different laboratories with different techniques and varying standards of rigor as to adequate absorption. Ohno has pointed out that in his laboratory only a small percentage of female mice of the appropriate strains that are suitably transplanted develop usable H-Y antibodies (25). As a consequence of these various problems H-Y serology has come under serious attack; the question has been raised as to whether the reported differences are in fact significant (25 - 29) and indeed whether the entire theory is based on an invalid assay.

A second assay - a cytotoxic assay using lymphocytes - is so cumbersome that it is rarely used (30, 31). Other assays have been developed, none as yet receiving widespread application (CF 5). Furthermore, while monoclonal antibodies can be used in greater dilution than unpurified antisera, the maximal degree of killing is usually less so that the reading of the endpoint is not necessarily more reliable (32). This implies that the polyclonal antibodies that appear in female plasma following transplantation of male skin are composed of antibodies with a variety of idiotypes.

Is HY the Testis Determinant?

While H-Y serology may have been blurred by too many practitioners and too little rigor it does not necessarily follow that H-Y antigen is not the testis determining (Tdy) gene. The fundamental work in the problem was done by Ohno, who was impressed by evidence suggesting the evolutionary conservation of the H-Y antigen and wondered if H-Y could itself be the testis inducer. He therefore performed an often quoted experiment. Embryonic mouse testes were dissociated with trypsin and then exposed to H-Y antibody or to control sera and then placed in roller cultures under conditions that promote reaggregation of tubule structures. The cells that were exposed to control serum reformed structures resembling seminiferous tubules whereas the cells exposed to H-Y antibody developed structures said to resemble follicles (33). Similar results were reported in the rat by others (34). These findings were interpreted as indicating that prevention of the expression of H-Y antigen allowed the natural tendency of the gonad to develop into an ovary to proceed.

The histological interpretation that seminiferous tubules reform in the control has come under challenge, and it is not established that any effects were due to specific anti H-Y antibodies rather than to other properties of the antisera. For example, antisera to MHC can cause the same phenomenon, whereas

monoclonal antibody to H-Y antigen does not have the same effect (CF 4). Nevertheless, it is possible that failure to reproduce the phenomenon is due to the failure to control conditions and antisera adequately.

Initial efforts to do the opposite experiment namely to convert the ovary to a testis with H-Y antigen were unsuccessful, but Ohno prepared an H-Y concentrate from cultured Daudi cells and exposed bovine fetal ovaries to the preparation. After 3 days the ovaries were said to be transformed into testes as evidenced by the appearance of seminiferous cords (35).

These two types of circumstantial evidence were in keeping with the postulated role for H-Y antigen as the testis inducer. In the ensuing years several doubts have arisen as to whether this relation is in fact one of cause and effect (25 - 29).

One of the complicating features of the thesis that H-Y is the testis determining gene has been the recognition that in species such as birds in which the female rather than the male is heterogametic (e.g., ZW/ZZ species) the female rather than the male is H-Y antigen positive. In contrast, as pointed out by Ohno (33) as mentioned for the case of the male-specific RNA (9, 10) it would be more straightforward to suppose that a testis inducer that is conserved throughout evolution would be present in homogametic as well as heterogametic males. The presence of H-Y antigen in female chickens is even more puzzling in view of the fact that administration of estradiol to ZZ chicks causes a change in phenotype from $H\text{-}Y^-$ to $H\text{-}Y^+$ (5). A second problem is that 45,X women with gonadal dysgenesis are said to by H-Y antigen positive (5), a finding that also does not fit with the original thesis.

In summary, caveats have been constructed to support the identify of H-Y antigen and the testis determining genes. None of the criticisms - either by themselves or considered together - disprove the thesis. Even if the two properties are not the same, it is possible that the structural gene that specifies H-Y antigen (or a regulatory gene that controls expression of the antigen) is located on the short arm of the Y chromosome near the genes for the testis determinants. If so, accurate measurement of the H-Y antigen would be clinically useful if its presence corresponds in most instances to the presence of the testis determinant.

THE XX MALE

More than 150 men who lack a Y chromosome have now been described (36, 37). The incidence of the 46,XX karyotype in phenotypic males is approximately 1 in 20,000 to 24,000 male births. The phenotype resembles that of the 46,XXY Klinefelter syndrome. The testes are small and firm (generally less than 2 ml), and the Wolffian duct derivatives are male in character. The penis is normal to small in size. Gynecomastia is common; azoospermia and hyanalinization of the seminiferous tubules are invariably present. Mean plasma testosterone levels are low, the plasma estradiol level is normal to high, and gonadotropin levels are increased (38, 39). 46,XX men differ from typical Klinefelter subjects in that the average height is less than in normal men, the frequency of mental deficiency is not increased, and the incidence of hypospadism is increased (40).

Case Report 46,XX Male

J.S. This boy was noted at birth to have bilateral cryptorchidism that was surgically corrected at age 4. He was thought to be normal otherwise except for the fact that he is shorter than his brother. Virilization began at around age 14, but did not progress to completion in that beard growth was scanty and body hair was immature. Spontaneous erections began about age 15. At the age of 17 he was referred to UT San Antonio and subsequently to us because of late puberty. His academic performance has been good. He was 63" in height and weighed 207 pounds. Axillary and pubic hair were within normal limits. No gynecomastia was present. The penis was 4 cm in stretched length. The right testis was 3 ml in volume, the left 2 ml. Both were firm.

The routine laboratory workup was normal as were thyroid and adrenal function tests. Bone age was the same as chronological age. Pooled values for androgens and gonadotropins were as follows:

Testosterone	2.36 ng/ml	(N 4 - 10)
LH	40 MIU/ml	(N 5 - 20)
FSH	86 MIU/ml	(N 4 - 20)

Chromosomal analysis was performed on peripheral lymphocyte and on cultured skin fibroblasts. The karyotype was 46,XX, and there was no evidence of a Y chromosome translocation. G and Q banding patterns were normal, and no abnormalities were observed in the number or shape of chromosomes. In a buccal smear a single Barr body was demonstrated, and no Y-fluorescence was observed, in keeping with the 46,XX karyotype. He was placed on a weight reduction regimen and begun on testosterone cypionate 200 mg every 3 weeks. Over the next six months he lost weight (to 190 pounds) and developed a mature male beard and extensive body hair.

Four theories have been proposed to explain the pathogenesis of the disorder: 1) Mosaicism in some cell lines for a Y-containing cell line or early loss of a Y chromosome; 2) A Mendelian gene mutation on an autosome; 3) Deletion or inactivation of an X-chromosome gene that normally has a negative regulatory effect on testis development; 4) Interchange of a Y chromosome gene with the X chromosome (37). The etiology of the condition may be heterogeneous, but two types of evidence support the fourth possibility, namely there is indirect genetic evidence for X-Y interchange in human XX males (41), and some XX males have Y chromosome DNA fragments in the genome (42), probably as the result of translocation to an X chromosome.

The Sex Reversal Mouse

The sex reversal mutation in the mouse (Sxr) was described by Cattanach and coworkers in 1971 as a form of male infertility (43). The phenotype is similar to the human XX male syndrome in that affected mouse resemble XXY mice phenotypically. This mutation was originally assumed to be autosomal in character.

Table 1

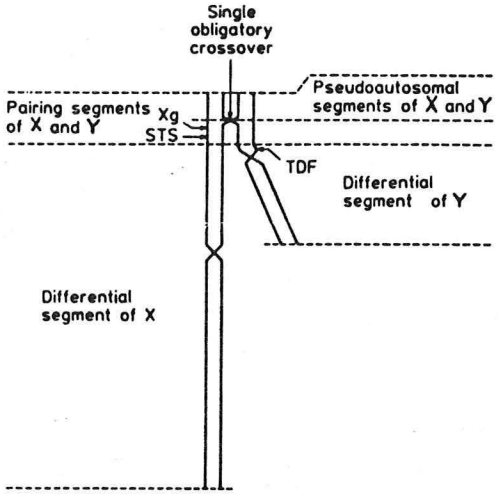
Table 1 The inheritance of *Sxr*

Cross	X/X ♀	×	X/Y *Sxr* ♂
Progeny	X/X ♀		X/Y ♂
	X/X *Sxr* ♂		X/Y Sxr ♂

The sex-reversed (*Sxr*) factor causes chromosomal females to develop as phenotypically normal, but sterile males[5]. Transmission is through X/Y carrier males and, hence, all X/Y males, whether carrying *Sxr* or not, normally have the same Y chromosome. The mode of inheritance appears to be that of an autosomal dominant, but all attempts to locate *Sxr* on an autosome have failed[7]. A location in an homologous region of the X and Y chromosomes could give the same pattern of inheritance, however[2]. The observed segregation ratios accord well with expectation, the X/X and X/Y classes of progeny usually being distinguished with the aid of an X chromosomal marker gene. Stock maintenance is by crossing X/Y *Sxr* males × F_1 hybrid (C3H/HeH ♀ × 101/H ♂) females.

However, extensive studies in several laboratories failed to detect autosomal linkage. The suggestion was made by Burgoyne (44) that where X and Y pairing takes place crossing over occurs between the paired segment. One consequence of this model would be that any gene located distal to the crossover point would be inherited like an autosomal gene (e.g. a pseudoautosomal gene).

Figure 8

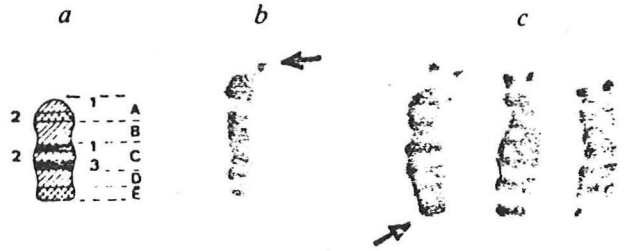


Burgoyne's X-Y crossover model, as exemplified from the human X-Y chromosome pair (from Burgoyne [3])

Burgoyne pointed out that the Sxr mutation is a candidate for such a mutation, and in fact two types of evidence indicate that this is in fact the case. Singh and Jones (45) found that a DNA probe to the Y chromosome hybridized with XX_{Sxr} male DNA fragments but not with DNA fragments from XX females and that the hybridization is localized to one end of an X chromosome in XX_{Sxr} metaphrase chromosome. This has been interpreted to indicate that in XY_{Sxr} carrier males the testes determining gene has duplicated to give an extra fragment attached to

the end of the Y. During male meiosis this fragment is transferred to the X chromatid by crossing over (Fig. 8). Indeed, such a fragment has now been identified at the distal end of the Y chromosome in XY_{Sxr} carrier males and at the distal end of one X in XX_{Sxr} males (46).

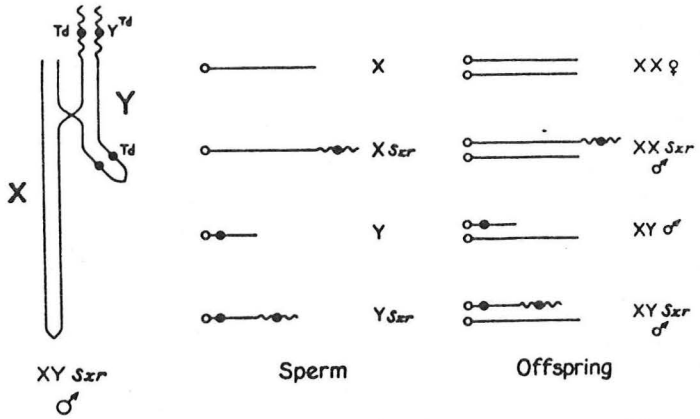
Figure 9



a, The accepted standard idiogram of the mouse Y chromosome[12]. *b*, G-banded, elongated Y from a bone marrow cell of a normal (control) XY male mouse, showing evidence of a short arm (arrowed) and further bands on the long arm. Control mice are derived from the 101/H, Ju/FaCt and Q (Falconer) strains among which no clear Y chromosomal differences were found. *c*, G-banded Y chromosomes from bone marrow cells of an X/Y *Sxr* mouse. Left: in early metaphase, showing the additional, heavily stained, distal body (arrowed). Centre: in mid-metaphase, showing the equivalent staining of proximal and distal ends. Right: in late metaphase, showing the fainter staining of the distal body with mitotic progression.

Thus, in the Sxr mouse and at least some human XX males, it is now possible to explain testicular differentiation in the absence of a Y chromosome (47). Translocation of a fragment of the Y chromosome carrying the testis determining genes is sufficient to cause development of a testis on the background of an XX chromosome composition but not to convey fertility. In actuality this is a form of the 47,XXY Klinefelter syndrome involving a Y fragment rather than an intact Y chromosome.

Figure 10



Sxr, interpreted on Burgoyne's X-Y crossover model as the addition of a duplicate of the Y-linked testis-determining region Y(Td) to the distal tip of the Y 'pseudoautosomal' segment. A similar model has been proposed by Eicher.

TRUE HERMAPHRODITISM

True hermaphroditism is a condition in which both an ovary and a testis or a gonad with histologic features of both ovary and testis (ovotestis) is present (48, 49, 50). The disorder is rare, something more than 400 cases having been reported. To justify the diagnosis both types of gonadal epithelium must be present, the presence of ovarian stroma without oocytes not being sufficient, although overlap syndromes do occur. Three categories are recognized: (1) a fifth are bilateral -- testicular and ovarian tissue (ovotestes) on each side; (2) two fifths are unilateral -- an ovotestis on one side and an ovary or a testis on the other; and (3) the remainder are lateral -- a testis on one side and an ovary on the other.

The external genitalia display all gradations of the male-to-female spectrum. Sixty percent are sufficiently masculinized to be reared as males. However, less than a tenth have normal male external genitalia; most have hypospadias, and more than half have incomplete labioscrotal fusion. Two-thirds of phenotypic females have an enlarged clitoris, and most have a urogenital sinus. Commonly, differentiation of the internal ducts corresponds to the adjacent gonad. Although an epididymis usually develops adjacent to a testis, development of the vas deferens is complete in only a third. Only one duct is usually present next to an ovotestis. On histological exam about two-thirds of the ducts next to an ovotestis have the characteristics of a fallopian tube, and one-third have the characteristics of a vas deferens. The more testicular tissue present in an ovotestis, the more likely the adjacent duct will be a vas deferens. A uterus is usually present although it may be hypoplastic or unicornuate; the latter is characteristic of the lateral type of true hermaphroditism with absence of the horn on the side of testis. The ovary usually occupies the normal position, but the testis or ovotestis may be found at any level along the route of embryonic testicular descent, frequently associated with an inguinal hernia. Testicular tissue is present in the scrotum or the labioscrotal fold in a third, in the inguinal canal in a third, and in the abdominal area in a third.

At puberty signs of variable feminization and virilization develop; three-fourths develop significant gynecomastia, and about half menstruate. In phenotypic men menstruation presents as cyclic hematuria. Ovulation occurs in approximately a fourth and is more common that spermatogenesis. In phenotypic men ovulation may present as testicular pain. Fertility has been reported in women following removal of an ovotestis and in a man who fathered two children. Congenital malformation of other systems are rare.

Although rare as a clinical entity, the disorder is more common in abortuses, namely an incidence of 1.08 percent of 1525 human embryos (51). There are at least two reasons for this apparent discrepancy. First, the disorder may be commonly associated with other mutations or abnormalities that are lethal, as in the case in gonadal dysgenesis in which there is also a discrepancy between the frequency in embryos and newborn. However, unlike gonadal dysgenesis, there is no increased frequency of extragenital anomalies among the survivors. Second, as developed below, a significant proportion of the embryonic cases may be due to mixed gonadal dysgenesis in which the ovarian follicles have not undergone atresia to form the unilateral streak gonads characteristic of the postnatal state.

The clinical syndrome termed true hermaphroditism is actually several different disorders (52). About two-thirds of subjects have a 46,XX karyotype, a tenth have a 46,XY karyotype, and the remainder are chromosomal mosaics either 46,XX/46,XY chimeras, or 45,X/46,XY mosaics. Because this is actually a group of diseases, we will describe the individual types separately.

46,XX True Hermaphroditism

The karyotype most often detected among true hermaphrodites is 46,XX. Hermaphroditism in the presence of an XX karyotype, like the XX male syndrome, is an apparent contradiction to the axiom that a Y chromosome is involved in testicular differentiation. Possible explanations include (1) undetected loss of a Y chromosome after initiation of testicular development or undetected chromosomal mosaicism or chimerism, (2) translocation of testicular determinants from the Y to the X chromosome or to an autosome, or (3) a single gene mutation. In all instances in which it has been reported to date, 46,XX true hermaphrodites are H-Y antigen positive, including the XX form of true hermaphroditism in the dog (53 - 55). However, the mean titer of the H-Y antigen in these instances is intermediate between that of normal men and women (56, 57).

In contrast to other forms of true hermaphroditism several family aggregates of 46,XX true hermaphroditism have been reported (58 - 63). In five of these families multiple siblings are affected in one generation only. However, in one family the disorder was present in first cousins (64). The available data in the familial cases are compatible with either autosomal recessive inheritance, new autosomal dominant mutations, or Y translocation in a paternal line. In no family has parental consanguinity been described, a finding that is against a a rare autosomal recessive mutation. In addition, two families have been reported in which one affected member was a 46,XX male and the other was a 46,XX true hermaphrodite (64, 65). In general, the clinical manifestations in the familial subgroup are similar to those in the sporadic variety of 46,XX true hermaphroditism, except that a uterus is absent in most familial cases whereas it is almost invariably present in the sporadic disorder and bilateral ovotestes are somewhat more common in the familial than in the sporadic disorder. Most 46,XX true hermaphrodites appear to have no affected relatives.

Case Report - 46,XX True Hermaphrodite

This 14-year-old black man was born at term to a 23-year-old mother whose pregnancy was normal. At birth, the urethral orifice was located at the base of the phallus. The hypospadias was repaired in two stages when he was 14 months and 4½ years of age. He functioned without problems, until 14 years of age. At this time he developed a swollen, painful left hemiscrotum from which 350 ml. of blood were aspirated percutaneously in the Parkland Emergency Room. Two weeks later the swelling in the left hemiscrotum recurred so that the penile shaft was deviated to the right and soft tissue swelling extended into the inguinal canal. A 12-year-old brother and a 16-year-old sister, who had predictable cyclic menses, have no genital ambiguity. There is no known family member with genital ambiguity, gynecomastia, or infertility in four generations.

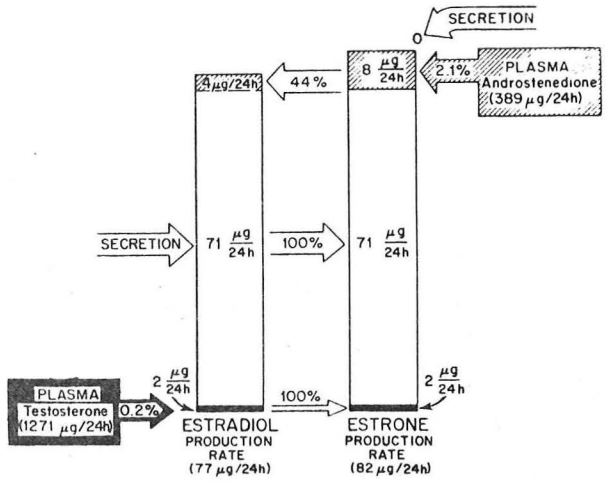
On physical examination, his blood pressure was 112/86; height 152 cm; and weight 55.5 kilograms. There was no facial, axillary, or pubic hair but

gynecomastia (Tanner, Stage II-III) was present. Physical findings were otherwise normal except for the genitalia. The penis was approximately 8 cm. long, and there was coronal hypospadias, i.e., the urethral meatus opened 1 cm. from the distal tip of the penis. Scrotal rugae were present, and the scrotum was normal except for the swelling on the left side. The gonad on the left was not palpable because of the interscrotal hematoma. The right gonad measured 1 by 1 by 2 cm. An epididymis and a vas deferens were not palpable on the right, and no prostate gland was felt by rectal examination. A uterus was not felt. There were no inguinal hernias. The enlarged and indurated left gonad and attached cord were removed.

Barr bodies were present in epithelial cells of the buccal mucosa and the leukocyte karyotype was 46,XX (21 cells analyzed). In both the ovarian and testicular components of the left gonad the karyotype was also 46,XX (15 cells from each tissue were analyzed). Duplication of the left renal pelvis and ureter was found on intravenous pyelography. The collecting system of the upper pole of the left kidney drained ectopically into the neck of the bladder, near the urethrovesical junction. This orifice and the orifices of the two ureters entering the bladder at the trigone were visualized by cystoscopy; however, the examiner could not visualize an orifice suggestive of an entrance into a vaginal canal. Thirty days after left gonadectomy, the right gonad and cord and the breast tissue were removed. With testosterone replacement, he now functions as a normal male.

By histologic examination, each gonad was found to be an ovotestis containing ovarian follicles adjacent to areas of seminiferous tubules that contained only Sertoli cells. Normal-appearing insterstitial cells were present in both gonads, but no spermatogenic epithelium was observed in either ovotestis. In the left gonad a hemorrhagic corpus luteum was the source of the scrotal hematoma. Within the cord of tissue excised with the left gonad, a Fallopian tube was present but no wolffian duct structures were found. No uterus was present. The endocrine findings are summarized in the figure below.

Figure 11



Production rates and sources of estradiol and estrone in a true hermaphrodite, Subject A. The production rate of estrone, 82 μg per 24 hours, was computed from the specific activity of urinary [^{3}H]estrone after the intravenous infusion of [6,7-^{3}H]estrone. The production rate of estradiol, 77 μg per 24 hours, was computed from the specific activity of urinary [^{3}H]estradiol after the intravenous infusion of [6,7-^{3}H]estradiol. The sources of estrone and estradiol formation were computed from the production rates of precursors and the transfer constants of conversion to estrone and to estradiol.

(From Aiman, J., Hemsell, D.L.,and MacDonald, P.C.: Production and origin of estrogen in two true hermaphrodites. Am. J. Obstet. Gynecol. 132:401-409, 1978.

46,XY True Hermaphroditism

Approximately half of the approximately 40 reported cases of 46,XY true hermaphroditism are Japanese, and this appears to be the most common form of the disorder in Japan in contrast to its infrequency elsewhere. On average, the external genitalia appear to be toward the male end of the spectrum, and the majority are reared as males. A uterus is present in virtually all, and that gonadal tumors may be more frequent in this group than in the 46,XX disorder. The presence of oocytes in 46,XY true hermaphrodites could result from undetected chimerism or mosaicism or from a mutant gene. The lack of familial aggregation and the lack of parental consanguinity in the 46,XY disorder do not support the presence of a single gene mutation, and Simpson has suggested that undetected mosaicism is the common cause (52). While this should be very easy to detect in ovarian tissue, relatively few detailed cytogenetic studies have been performed in the disorder.

46,XX/46,XY True Hermaphroditism

More than 30 documented 46,XX/46,XY true hermaphrodites have been reported, e.g., it is about as common worldwide as the 46,XY variety and in France may be as common as the 46,XX variety. It is of interest in this regard that XX/XY chimerism in the mouse does not cause hermaphroditism but either a male or female phenotype (67).

This chromosomal composition could arise by chimerism or by post fertilization nondisjunction, as for example by the loss of a Y chromosome from a 47,XXY/46,XY mosaic. The facts that true hermaphroditism can occur in chromosomal mosaics (46,XX/47,XXY,45,X/46,XY) and that true hermaphroditism in mice can result from nondisjunction (68) imply that the 46,XX/46,XY chromosome composition can arise form chromosomal non-disjunction, but the common cause of the 46,XX/46,XY karyotype in the human is thought to be chimerism, the presence in a single individual of cells derived from different zygotes. Such chimerism could result from (1) fertilization of an ovum and its polar body, (2) fertilization of two ova within a single binucleated follicle, (3) fertilization of ova from different follicles, following by fusion. In one such patient studied carefully no evidence of double fertilization could be obtained (69) whereas in another the karyotype arose from the fertilization of one ovum and its polar body by two sperm (70).

Whole body chimerism with a 46,XX/47,XY karyotype is not invariably associated with true hermaphroditism in the human (and almost never in mice); the relative frequency of normal males or females versus true hermaphrodites in this disorder is not clear because there is an ascertainment bias in favor of making the diagnosis in true hermaphrodites because of the common association with ambiguous genitalia, gynecomastia, etc.

The phenotype in this form of true hermaphroditism is not distinctive. Whereas the external genitalia are usually male, ovulation is more common than spermatogenesis. A uterus and one or more fallopian tubes are usually present.

45,X/46,XY True Hermaphroditism

Most subjects with the 45,X/46,XY chromosome composition have the syndrome of mixed gonadal dysgenesis (a testis on one side, a streak gonad on the other, a variable degree of male development, and some stigmata of gonadal dysgenesis); e.g., in most no ovarian follicles are demonstrable. However, eight patients have been described with ovarian follicles, usually under conditions in which a testis was present on one side and an ovary was present on the other.

The X/XY karyotype in the mouse is invariably associated with true hermaphroditism. In view of the fact that the ovaries human 45,X subjects contain follicles in utero but undergo an accelerated atresia to eventuate in streak gonads by the time of infancy or later, it is reasonable to assume that in some humans with the 45,X/46,XY karyotype the atresia is incomplete so that the histological criteria of true hermaphroditism are met, namely the presence of both ovarian and testicular tissue. However, since gonadotropins are in the castrate range (71) and since fertility has not been described in this type of true hermaphroditism (in contrast to the forms described above) some authors consider this a form of mixed gonadal dysgenesis rather than a variant of true hermaphroditism (72).

Case Report - 45,X/46,XY True Hermaphrodite [BB (CMC 142389)]

This child was born with ambiguous genitalia on 11-23-69 at St. Paul Hospital after an uneventful pregnancy and uncomplicated delivery and was transferred at one day of age to Childrens Medical Center for evaluation. The physical examination was normal except for an enlarged hooded clitoris with partial fusion of the labia minora, a single urogenital sinus opening, and a nodular mass in the right hernial region. Pregnanetriol excretion was normal, and the chromosomal karyotype was 45,X(45 cells)/46,XY(5 cells). When the karyotype was repeated by Dr. Howard-Peeples in 1982 she was found to be 45,X/46,XdicY/47,XYdicY/46,XY. Cystography and vaginography revealed a normal cervix and vagina and a single fallopian tube. The remainder of the urogenital tract was normal. A decision was made to raise the child as a female. At 12 weeks of age she had surgery which included a bilateral inguinal herniorrhaphy, excision of a right ovotestis located in the right inguinal hernia sac, and biopsy of the left gonad which was an ovary (containing cortical stroma, primary follicles, and ova) that had luxated into the left inguinal canal. In 1974 an uncomplicated clitoroplasty was performed. Her growth rate was consistently more than two standard deviates below normal so that by age 12 years her height was 49 3/4 inches. No other stigmata of gonadal dysgenesis were identified, and she was sexually infantile. In 1982 she was seen by Z. Chakmakjian and found to have a normal growth hormone response to levodopa (0.8 ng/ml at 0 time, 7.5 ng/ml at 30 minutes, and 11.6 ng/ml at 60 minutes), and it was felt that the diminished rate of growth was most compatible with gonadal dysgenesis. LH was 6.7 MIU/ml, FSH was 31.6 MIU/ml, and estradiol was less than 10 pg/ml.

Removal of the ovary was recommended by the pediatric surgeon who had performed the previous surgery, and she was referred here in March of 1983 because the family wanted another opinion as to the advisability of the ovarian removal. Rereview of the original ovarian biopsy confirmed the presence of a few primary follicles, and repeat gonadotropin measurements on three pooled blood samples revealed FSH of 64 and LH of 12 MIU/ml. She was short (still 50

inches), had abnormal carrying angles of the arms, and had no axillary or pubic hair, immature nipples with no palpable breast tissue, and an immature introitus. Dr. Melvin Grumbach was consulted, and it was his feeling that this phenotype is a variant of mixed gonadal dysgenesis rather than of true hermaphroditism, and since the ovaries are invariably nonfunctional he concurred with the decision to remove it and institute cyclic estrogen therapy. (Although the potential for malignancy in ovarian tissue in these phenotypic females is small, he, felt that a dysgenetic gonad is best removed.) This was subsequently done, and the child has been begun on cyclic estrogen therapy.

Other Types of True Hermaphroditism

46,XX/47,XXY true hermaphroditism probably arises as a result of nondisjunction of a 47,XXY zygote. (This is about as frequent as the 45,X/46,XY disorder). Rarely other karyotypes have been described such as 45,X/46,XX, 46,XX/47,XYY, 46,XX/46,XT/47,XXY, etc. None of these disorders have been characterized in great detail.

Pathogenesis of 46,XX True Hermaphroditism

The pathogenesis of those forms of true hermaphroditism in which a Y containing cell line is present is beyond the scope of this discussion; those instances associated with mosaicism are generally assumed to the clonal in origin, the X or XX cell clone giving rise to ovarian cell lines the Y containing cell lines giving rise to testicular elements. Furthermore, on the basis of studies in the mouse (73), the 46,XY variety is assumed to be due to a mutation in the testis determining gene(s) that interacts abnormally with the X chromosome and hence causes inconsistent testicular development.

The most baffling problem is to explain the ordinary 46,XX form of true hermaphroditism and to account both for testicular development in the absence of a Y and for the fact that this disorder is compatible with fertility, e.g., some 46,XX true hermaphrodites can produce both sperm and ova, whereas all human 46,XX males to date have been infertile. Once again, insight into the 46,XX true hermaphrodite has come from studying the Sxr mouse.

To provide a background for these studies, it is necessary to consider briefly the role of the sex chromosomes in ovarian differentiation. According to the Lyon hypothesis all extragonadal cells in the female have only one active X chromosome, and any additional X chromosomes are inactivated and form the sex chromatin bodies characteristic of the nuclei of female cells (74). In normal women, the inactivation is random and occurs early in embryonic life so that in individual cells only the maternal- or paternal-derived X chromosome remains active (74). This random inactivation is believed to be operative in all cell lines of the female, but in the ovary X inactivation differs from that in other tissues. By studying women heterozygous for electrophoretic variants of the X-linked enzyme glucose-6-phophate dehydrogenase, it was shown that oocytes both from adult and from fetal ovaries (75, 76) express both X-linked alleles. Furthermore, both X chromosomes appear to be active in germ cells prior to entering meiosis (77). However, Kratzer and Chapman (78) have shown that only one X chromosome is active in the embryonic mouse ovary up to day 10 of embryogenesis and that the inactive X is reactivated as germ cells enter meiosis. Thus, it appears that random X inactivation occurs in the ovary as in

other tissues of the female and that reactivation of the X occurs only in the oogonia themselves. To summarize, in normal testicular development, the single X chromosome appears to be inactivated as a part of the XY body, whereas in oogenesis one of two X chromosomes is active during organogenesis of the ovary and both X chromosomes are active during oogenesis itself. As pointed out above random X chromosome inactivation of the Lyon type involves chemical modification of the DNA and hence occurs by a different mechanism than that responsible for X chromosome inactivation in the XY body (21, 22).

McLaren and Monk then devised an ingenious experiment in the Sxr mouse. They crossed XY_{Sxr} mice with mice that carry Searle's X-autosome translocation T(X;16)H (79). In this mouse the translocated X is protected against inactivation so that the normal X is preferentially inactivated and hence genes on that X fail to be expressed (80). This abnormal X will be designated X_I (for isochrome) for this discussion.

Table 2

Progeny (only non-recombinant types are listed) of matings between females heterozygous for the X-autosome translocation T16H and males carrying *Sxr*. Data from McLaren and Monk [29]

	From male			
	Y	Y *Sxr*	X	X *Sxr*
From female				
X	24♂	11♂	23♀	13♂
T16H	36♂		18♀	5♂ 2♀ 3♂

Of the 10 X_IX_{Sxr} young 5 were sterile males, two were fertile females, and 3 were hermaphrodites (a testis on one side and an ovotestis on the other) (79). The observation that some X_IX_{Sxr} individuals develop as females, some as males, and some as hermaphrodites can most simply be explained by assuming that the testis determining Sxr region of the X chromosome is inactivated in some cells but not others. Inactivation is conceived as spreading to a variable extent from the inactive X chromatin to the Sxr fragment, and it is assumed that the extent of inactivation is an hereditable characteristic determined at the time of X chromosome inactivation during embryogenesis. Such 46,XX individuals then are mosaics, some cell lines expressing Sxr and some not. The sex of the gonads (and hence the aggregate sex of the individual) depends on the proportion of cells expressing Sxr in the gonad primordia.

Figure 12

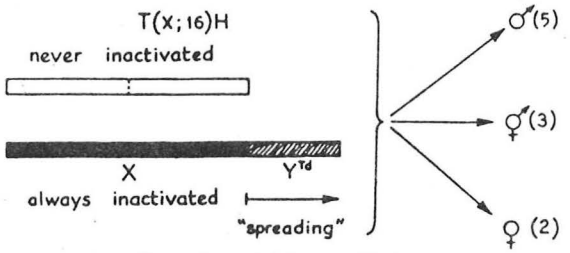


Fig. 3. The effect of combining an X chromosome carrying Y(Td), i.e. *Sxr*, with the preferentially active X-autosome translocation T(X;16)H. The numbers of males, intersexes and females identified by McLaren and Monk [29] are given in parentheses

Provided 30% of cells in a gonadal primordia are XY, a normal testis will develop. When the X chromosome to which the Sxr gene is attached is opposite an X_I, the probability of inactivation will be around 0.5. Hence X_IX_{Sxr} females and hermaphrodites will be unusual but not really rare. However, when the X Chromosome to which Sxr is attached is opposite a normal X chromosome, half of cells will express Sxr; hence, the majority of individuals will be sterile males, and female and hermaphrodites will be rare (a relationship that is in keeping with the rare occurrence of XX males and XX true hermaphrodites in some families.

In summary, at a theoretical level, then, the same type of disorder, namely duplication of the male determinant gene(s) on the Y chromosome and translocation of such genes onto the X chromosome could explain the development of either the XX male or the XX true hermaphrodite, depending on the completeness and frequency of the inactivation of such testis determining genes during the embyrogenesis of the XX individual carrying such a translocation. It has yet to be proven that this model can explain the potential fertility of the XX true hermaphrodite (both maternity and paternity occurring on occasion). However, this model has two predictable and testable features for the human disorder; (1) It implies that testis determining genes and hence Y chromosome fragments will be detectable in cells from 46,XX true hermaphrodites, and (2) If the translocation is to an X chromosome (as in the case of Sxr) and not to an autosome, then the XX male should be more common than the XX true hermaphrodite.

THE KLINEFELTER SYNDROME

The presence of a second X chromosome (as in XXY or XX_{Sxr}) does not appear to affect sexual differentiation at the gonadal level; e.g., either a Y chromosome or a fragment of a Y chromosome that contains the testis determining genes is capable of inducing testicular development, no matter how many X chromosomes are present. However, if an XX line is present, spermatogenesis is ordinarily impaired (except in XX true hermaphroditism). The most likely explanation for the infertility in the XXY state is that inactivation of the X chromosome in the formation of the XY body during normal spermatogenesis is essential for fertility. In other words, some X-coded gene product must prevent male germ development (82). This concept is in keeping with the fact that some

balanced translocations of X chromosomes to autosomes in mice (20) and in humans (83) are associated both with male infertility and with incomplete inactivation of X chromosomal genes. If the situation in the creeping vole (15) is applicable to the human, then it can be assumed that function of the X chromosome is not essential either for testicular differentiation or spermatogenesis. It is of interest in this regard that $X_{Sxr}O$ male mice do undergo more advanced spermatogenesis than do XX_{Sxr} males but are nevertheless infertile (43). It is easiest to assume that the fragment of translocated Y contains sufficient genes to dictate testicular differentiation but insufficient to result in male fertility. [The latter supposition is in keeping with the fact that Y-linked genes for height are not included in the translocated Yp fragments that have been characterized to date (84).] In the XX_{Sxr} testes, both X chromosomes become reactivated in prospermatogenia, but the prospermatogenia cannot progress beyond the G_1 phase of the cell cycle, e.g., they cannot undergo the final DNA synthesis or enter meiosis (47).

SUMMARY

1.) Gonadal Differentiation in the human is determined by both the X and Y chromosome. The Y chromosome can dictate testicular development despite the presence of two or more X chromosomes. Initially, during ovarian development one X chromosome is functional, but for oogenesis both X chromosomes become functional. For initial testicular development the usual single X chromosome is inactivated and any additional X chromosome would be inactivated by the Lyon mechanism, but the presence of two or more X chromosomes prevents normal spermatogenesis.

2.) The Y chromosome contains several identifiable types of DNA.

A. Long repetitive sequences on the long arms responsible for fluorescence (function unknown).

B. Satellite DNA (function unknown)

C. A region on the tip of the small arm that is homologous to a region on the short arm of the X chromosome. This region is essential for pairing of the X and Y chromosomes during meiosis.

D. A region on the short arm adjacent to the region that contains the TD genes and that is transcribed into male specific RNA. Such RNA is present in all vertebrate males including chicken.

E. The testis determining region on the short arm of the Y chromosome. This region is probably not the same as region D.

3. Some unidentified region of the Y chromosome outside the TD region contains one or more genes that influence height, tooth size, and skeletal size.

4. In the mouse translocation of the TD region of the Y chromosome to an X chromosome causes development of a testis in an XX individual; e.g., this is the functional equivalent of the XXY male. This mechanism is probably the commonest cause of the XX male in humans.

5. True hermaphroditism that occurs in the presence of a Y chromosome (46,XY, 46,XX/46,XY or 45,X/46,XY) is best explained by mosaicism, some cell lines containing functional Y chromosomes and some not containing functional Y chromosomes so that ovarian and testicular cell lines can arise in the same individual. In the case of the 46,XY true hermaphrodite, such mosaicism presumably arises by chromosome nondisjunction following fertilization of the zygote.

6. True hermaphroditism of the XX variety in the mouse is best understood in terms of the Sxr translocation. In the vast majority of instances the X chromosome that is the recipient of the Y translocation is functionally suppressed during embryogenesis so that the TD genes cause testicular development and hence the XX male. Rarely, however, the translocated X is incompletely suppressed, so that some XX cell lines can persist and result in ovarian development. Whether this mechanism can explain human 46,XX true hermaphroditism remains to be established.

REFERENCES

1. Vogel, F., and Motulsky, A.G. Human Genetics. Berlin. Springer-Verlag, 1979.

2. Stern, C.: The problem of complete Y-linkage in man. Am. J. Hum. Genet. 9:147-165, 1957.

3. Solari, A.J.: The behavior of the XY pair in mammals. Int. Rev. Cytol. 38:273-317, 1974.

4. Rasmussen, S.W., and Holm, P.B.: Mechanics of Meiosis. Hereditas 93:187-216, 1980.

5. Wachtel, S.S.: HY antigen and the biology of sex determinants. Grune and Stratton, New York, 1983.

6. Cooke, H.J., Fantes, J., and Green, D.: Structure and evolution of human Y chromosome DNA. Differentiation 23:S48-S55, 1983.

7. Page, D., de Martinville, B., Barker, D., Wyman, A., White, R., Francke, U., and Botstein, D.: Single-copy sequence hybridizes to polymorphic and homologous loci on human X and Y chromosomes. Proc. Natl. Acad. Sci., USA 79:5352-5356, 1982.

7A. Jones, K.W.: Evolutionary conservation of sex specific DNA sequences. Differentiation 23:S56-S59, 1983.

8. Goodfellow, P., Banting, G., Sheer, D., Ropers, H. H., Caine, A., Ferguson-Smith, M.A., Povey, S., and Voss, R.: Genetic evidence that a Y-linked gene in man is homologous to a gene on the X chromosome. Nature 302:346-349, 1983.

9. Singh, L., Purdom, I.F., and Jones, K.W.: Sex chromosome associated satellite DNA: Evolution and conservation. Chromosoma (Berl.) 79:137-157, 1980.

10. Epplen, J.T., McCarrey, J.R., Sutou, S., and Ohno, S.: Base sequence of a cloned snake W-chromosome DNA fragment and identification of a male-specific putative mRNA in the mouse. Proc. Natl. Acad. Sci., USA 79:2798-3802, 1982.

11. Epple, J.T., Cellini, A., Shorte, M., and Ohno, S.: On evolutionarily conserved simple repetitive DNA sequences: Do "sex-specific" satellite components serve any sequence dependent function? Differentiation 23:S60-S63, 1983.

12. Odartchenko, N. and Pavillard, M.: Late DNA replication in male mouse meiotic chromosomes. Science 167:1133-1134, 1970.

13. Monesi, V.: Synthetic activities during spermatogenesis in the mouse. RNA and protein. Exp. Cell Res. 39:197-224, 1965.

14. VandeBerg, J.L., Cooper, D.W., and Close, P.J.: Mammalian testis phosphoglycerate kinase. Nature New Biol. 243:48-50, 1973.

15. Ohno, S., Jainchill, J., and Stenius, C.: The creeping vole (Microtus oregoni) as a gonosomic mosaic. The OY/XY constitution of the male. Cytogenetics 2:232-239, 1963.

16. Lifschytz, E.: X-chromosome inactivation: An essential feature of normal spermiogenesis in male heterogametic organisms. In: Proc. Int. Symp. The Genetics of the Spermatozoon. Edinburgh, Aug. 16-20, 1971. R.A. Beatty and S. Gluecksohn-Waelsch (eds). Edinburgh and New York, 1971 pp. 223-232.

17. Lifschytz, E. and Lindsley, D.L.: The role of X-chromosome inactivation during spermatogenesis. Proc. Natl. Acad. Sci. USA 69:182-186, 1972.

18. Russell, L.B., and Montgomery, C.S.: Comparative studies on X-autosome translocations in the mouse. I. Origin, viability, fertility, and weight of five T(X,I)'S. Genetics 63:103-120, 1969.

19. Forejt, J., and Gregorova, S.: Meiotic studies of translocations causing male sterility in the mouse. I. Autosomal reciprocal translocations. Cytogenet. Cell Genet. 19:159-179, 1977.

20. Forejt, J.: Meiotic studies of translocations causing male sterility in the mouse. II. Double heterozygotes for Robertsonian translocations. Cytogenet. Cell Genet. 23:163-170, 1979.

21. Yen, P.H., Patel, P., Chinault, A.C., Mohandas, T., and Shapiro, L.J.: Differential methylation of hypoxanthine phosphoribosyltransferase genes on active and inactive human X chromosomes. Proc. Natl. Acad. Sci. USA 83:1759-1763, 1984.

22. Venolia, L., Cooper, D.W., O'Brien, D.A., Millette, C.F., and Gartler, S.M.: Transformation of the Hprt gene with DNA from spermatogenic cells. Chromosoma 90:185-189, 1984.

23. McLaren, A., Simpson, E., Tomonari, K., Chandler, P., and Hogg, H.: Male sexual differentiation in mice lacking H-Y antigen. Nature 312:552-555, 1984.

24. Goldberg, E.H., E.A. Boyse, D. Bennett, M. Scheid, and E.A. Carswell: Serological demonstration of H-Y (male) antigen on mouse sperm. Nature 232:478-480, 1971.

25. Crichton, D.N., and C.M. Steel: Serologically detectable H-Y ('male') antigen: Mr or myth? Immunol. Today 6:202-3203, 1985.

26. Wolfe, J. and P.N. Goodfellow: The elusive testis-determining factor. Trends Genet. 1:3-4, 1985.

27. Gore-Langston, R.E., P.S. Tung, and I.B. Fritz: The absence of specific interaction of Sertoli-cell-secreted proteins with antibodies directed against H-Y antigen. Cell 32:289-301, 1983.

28. Silvers, W.K., D.L. Gasser, and E.M. Eicher: H-Y antigen, serologically detectable male antigen and sex determination. Cell 28:439-440, 1982.

29. Differentiation 23:(Suppl 1) 1983.

30. Hurme, M., P.R. Chandler, C.M. Hetherington, and E. Simpson: Cytotoxic T-cell responses to H-Y: Correlation with the rejection of syngeneic male skin graft. J. Exp. Med. 147:768-775, 1978.

31. Koo, G.C., and A. Varano: Inhibition of H-Y cell-mediated cytolysis by monoclonal H-Y-specific antibody. Immunogenetics 14:183-188, 1981.

32. Koo, G.C., N. Tada, R. Chaganti, and U. Hammerling: Application of monoclonal anti-HY antibody for human H-Y typing. Hum. Genet. 57:64-67, 1971.

33. Ohno, S, Y. Nagai, S. Ciccarese: Testicular cells lipostripped of HY antigen organize ovarian follicle-like aggregates. Cytogenet. Cell. Genet. 20:351-364, 1978.

34. Zenzes, M.T., U. Wolf, E. Guenther, and W. Engel: Studies on the function of HY antigen dissociation and reorganization experimental on rat gonadal tissue. Cytogenet. Cell. Genet. 20:365-372, 1978.

35. Ohno, S., Y. Nagai, S. Ciccarese, and H. Iwata: Testis-organizing HY antigen and the primary sex determining mechanism of mammals. Rec. Prog. Horm. Res. 35:449-476, 1979.

36. De la Chapelle, A.: Nature and origin of males with XX sex chromosomes. Am. J. Hum. Genet. 24:71-105, 1972.

37. De la Chapelle, A.: The etiology of maleness in XX men. Hum. Genet. 58:105-116, 1981.

38. Perez-Palacios, G., Medina, M., Ullao-Aguirre, A., Chavez, B.A., Villareal, G., Dutrem, M.T., Cahill, L.T., and Wachtel, S.: Gonadotropin dynamics in XX males. J. Clin. Endocrinol. Metab. 53:254-257, 1981.

39. Schweikert, H.U., Weissbach, L., Leyendecker, G., Schwinger, E., Wartenberg, H., and Kruck, F.: Clinical, endocrinological, and cytological characterization of two 46,XX males. J. Clin. Endocrinol. Metab. 54:745-752, 1982.

40. Roe, T.F., and Alfi, O.S.: Ambiguous genitalia in XX male children: Report of two infants. Pediatrics, 60:55-59, 1977.

41. De la Chapelle, A, Tippett, P.A., Wetterstrand, G., and Page, D.: Genetic evidence of X-Y interchange in a human XX male. Nature 307:170-171, 1984.

42. Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M., and Weissenbach, J.: Human XX males with Y single-copy DNA fragments. Nature 307:172-173, 1984.

43. Cattanach, B.M., Pollard, C.E., and Hawkes, S.G.: Sex-reversed mice XX and XO males. Cytogenetics 10:318-337, 1971.

44. Burgoyne, P.S.: Genetic homology and crossing over in the X and Y chromosomes of mammals. Hum Genet 61:85-90, 1982.

45. Singh, L., and Jones, K.W.: Sex reversal in the mouse (Mus musculus) is caused by a recurrent non-reciprocal crossover involving the X and the aberrant Y chromosome. Cell 28:205-216, 1982.

46. Evans, E.P., and M.D. Burtenshaw: Meitoic crossing-over between the X and Y chromosomes of male mice carrying the sex-reversing (Sxr) factor. Nature 300:443-445, 1982.

47. McLaren, A.: Sex reversal in the mouse. Differentiation 23:S93-S98, 1983.

48. van Niekerk, W.A., and Retief, A.E.: The gonads of human true hermaphrodites. Hum. Genet. 58:117-122, 1981.

49. van Niekert, W. A.: True Hermaphroditism: Clinical, Morphologic, and Cytogenetic Aspects, Harper and Row, Hagerstown, MD, 1974.

50. van Niekert, W.A.: True hermaphroditism. Pediatr. Adolesc. Endocrinol. 8:80-99, 1981.

51. Lee, S.: High incidence of true hermaphroditism in the early human embryos. Biol. Neonate 18:418-425, 1971.

52. Simpson, J.L.: True hermaphroditism: Etiology and phenotypic considerations. Birth Defects 14:9-35, 1978.

53. Saenger, P., Levine, L.S., Watchel, S.S., Korth-Schutz, S.,Doberne, Y., Koo, G.C., Lavengood, R.W. Jr., German, J.L. III, and New, M.I.: Presence of H-Y antigen and testis in 46,XX true hermaphroditism, evidence of Y-chromosomal function. J. Clin. Endocrinol. Metab. 43:1234-1239, 1976.

54. Boucekkine, C., Menasria, A., Chouiter, A., Benelkadi, N., and Benmiloud, M.: H-Y positive 46 XX true hermaphroditism with intrascrotal uterus. Clin. Endocrinol. 15:529-535, 1981.

55. Selden, J.R., Wachtel, S.S., Koo, G.C., Haskins, M.E., and Patterson, D.F.: Genetic basis of XX male syndrome and XX true hermaphroditism: Evidence in the dog. Science 201:644-646, 1978.

56. Moreira, C.A., Otto, P.G., Mustacchi, Z., Frota-Pessoa, O., and Otto, P.A.: H-Y antigen expression in a case of XX true hermaphroditism. Hum. Genet. 55:309-314, 1980.

57. Winters, S.J., Wachtel, S.S., White, B.J., Koo, G.C., Javadpour, N., Loriaux, D.L., and Sherins, R.J.: H-Y antigen mosaicism in the gonad of a 46,XX true hermaphrodite. N. Engl. J. Med. 300:745-749, 1979.

58. Clayton, G.W., Smith, J.D., and Rosenberg, H.S.: Familial true hermaphroditism in pre- and postpubertal genetic females. Hormonal and morphologic studies. J. Clin. Endocrinol. Metab. 18:1349-1358, 1958.

59. Mori, Y., and Mitzutani, S.: Familial true hermaphroditism in genetic females. Jpn. J. Urol. 59:857-864,1968.

60. Rosenberg, H.S., Clayton, G.W., and Hsu, T.C.: Familial true hermaphrodism. J. Clin. Endocrinol. Metab 23:203-206, 1963.

61. Gallegos, A.J., Guizar, E., Cortes-Gallegos, V., Cervantes, C., Bedolla, N., and Parra, A.: Familial true hermaphrodism in three siblings: Plasma hormonal profile and in vitro steroid biosynthesis in gonadal structures. J. Clin. Endocrinol. Metab. 42:653-660, 1976.

62. Lowry, R.B., Honore, L.H., Arnold, W.J.D., Johnson, H.W., Kliman, M.R., and Marhsall, R.H.: Familial true hermaphroditism. Birth Defects 11:105-113, 1975.

63. Armendares, S., Salamanca, F., Cantu, J.M., del Castillo, V., Nava, S., Dominguez-de-la-Piedra, E., Cortes-Gallegos, V., Gallegos, A., Cervantes, C., and Parra, A.: Familial true hermaphrodism in three siblings. Clinical, cytogenetic, histological and hormonal studies. Humangenetik 29:99-109, 1975.

64. Kasdan, R., Nankin, H.R., Troen, P., Wald, N., Pan, S., and Yanaihara, T.: Paternal transmission of maleness in XX human beings. N. Engl. J. Med. 288:539-545, 1973.

65. Berger, R., Abonyi, D., Nodot, A., Vialatte, J., and Lejeune, J.: Hermaphrodisme vrai et "Garcon XX" dans une fratrie. Rev. Eur. Etud. Clin. Biol. 15:330-333,1970.

66. Nihoul-Fekete, C., Lortat-Jacob, S., Cachin, O., and Josso, N: Preservation of gonadal function in true hermaphroditism. J. Pediatr. Surg. 19:50-55, 1984.

67. Mystkowska, E.T., and Tarkowski, A.K.: Observations on CBA-p/CBA-T_6T_6 mouse chimeras. J. Embryol. Exp. Morphol. 20:33-52, 1968.

68. Eicher, E.M., Beamer, W.G., Washburn, L.L., and Whitten, W.K.: A cytogenetic investigation of inherited true hermaphroditism in BALB/cWt mice. Cytogenet. Cell Genet. 28:104-115, 1980.

69. De Marchi, M., Carbonara, A.O., Carozzi, F., Massara, F., Belforte, L., Molinatti, G.M., Bisbocci, D., Passarino, M.P., and Palestro, G.: True hermaphroditism with XX/XY sex chromosome mosaicism: Report of a case. Clin. Genet. 10:265-272, 1976.

70. Dewald, G., Haymond, M.W., Spurbeck, J.L., and Moore, S.B.: Origin in chi46,XX/46,XY chimerism in a human true hermaphrodite. Science 207:321-323, 1980.

71. Gerli, M., Biagioni, M., Bruschelli, G.M., Gerrarese, R., and Rosi, G.: A case of true hermaphroditism with 45X/46XY mosaicism. Hum. Genet 34:93-97, 1976.

72. Grumbach, M.M., and Conte, F.A.: Disorders of Sexual Differentiation. In: Williams Textbook of Endocrinology, J.D. Wilson, and D.W. Foster (eds), W. B. Saunders Co., Philadelphia, PA, pp 312-401, 1985.

73. Eicher, E.M., Washburn, L.L., Whitney III, J.B., and Morrown, K.E.: Mus poschiavinus Y chromosome in the C57BL/6J murine genome causes sex reversal. Science 217:535-537, 1982.

74. Lyon, M.F.: Sex chromatin and gene action in the mammalian X-chromosome. An. J. Hum. Genet. 14:135-148, 1962.

75. Gartler, S.M., Liskay, R.M., Campbell, B.K., Sparkes, R., and Gant, N.: Evidence for two functional X chromosomes in human oocytes. Cell Differ. I:215-218, 1972.

76. Gartler, S.M., Liskay, R.M., and Gant, N.: Two functional X chromosomes in human fetal oocytes. Exp. Cell Res. 82:464-465, 1973.

77. Migeon, B.R., and Jelalian, K.: Evidence for two active X chromosomes in germ cells of female before meiotic entry. Nature 269:242-243, 1977.

78. Kratzer, P.G.,and Chapman, V.M.: X chromosome reactivation in oocytes of Mus caroli. Proc. Natl. Acad. Sci USA 78:2093-2097, 1981.

79. McLaren, A., and Monk, M.: Fertile females produced by inactivation of an X chromosome of 'sex-reversed' mice. Nature 300:446-448, 1982.

80. Lyon, M.F., Searle, A.G., Ford, C.E., and Ohno, S.: A mouse translocation suppressing sex-linked variegation. Cytogenetics 3:306-323, 1964.

81. Falconer, D.S., and Avery, P.J.: Variability of chimaeras and mosaics. J. Embryol. Exptl. Morphol. 43:195-219, 1978.

82. McLaren, A., and Monk, M.: X chromosome activity in the germ cells of sex-reversed mouse embryos. J. Reprod. Ferility 63:533-537, 1981.

83. Madan, K.: Balanced structural changes involving the human X: Effect on sexual phenotype. Hum. Genet 63:216-221, 1983.

84. Zuffardi, O., Maraschio, P., Lo Curto, F., Muller, U., Giarola, A., and Perotti, L.: The role of Yp in sex determination: New evidence from X/Y translocations. Am. J. Med. Genet. 12:175-184, 1982.