An XXY sex chromosome constitution in a house musk shrew (*Suncus murinus* L.) with testicular hypoplasia

M. B. Rogatcheva^{1,2}, S-I. Oda², A. I. Zhelezova¹ and P. M. Borodin¹

¹Institute of Cytology and Genetics, Novosibirsk, 630090, Russia; and ²Laboratory of Animal Management, School of Agricultural Sciences, Nagoya University, Nagoya 464-01, Japan

An adult male house musk shrew with an XXY sex chromosome constitution was found in a laboratory-bred colony. Maternal origin of the additional X chromosome was demonstrated. The external appearance of the animal was normal, but the testes were small and displayed a high density of interstitial cells. The seminiferous tubules were narrow and contained only Sertoli cells.

Introduction

The XXY condition has been reported for man (Klinefelter syndrome: Ferguson-Smith *et al.*, 1960; Hamerton *et al.*, 1975; Abyholm and Stray-Pedersen, 1981) and many species of mammal: tamar wallaby (Sharman *et al.*, 1970), common shrew (Searle, 1984), mouse (Cattanach, 1961), black rat (Yosida, 1979), Chinese hamster (Ivett *et al.*, 1978), wood lemming (Gropp *et al.*, 1976), pig (Breeuwsma, 1968; Hancock and Baker, 1981), bull (Scott and Gregory, 1965), sheep (Bruere *et al.*, 1969), goat (Bhatia and Shanker, 1992), horse (Kubien *et al.*, 1993), cat (Centerwall and Benirschke, 1975; Hageltorn and Gustavsson, 1981) and dog (Clough *et al.*, 1970). In all these species, the XXY condition results in a male phenotype with small testes that lack germ cells (Cattanach, 1974).

The present paper describes an adult house musk shrew (*Suncus murinus* L.) with an XXY sex chromosome constitution found in a laboratory colony.

Materials and Methods

The XXY male was detected during cytogenetic screening of the laboratory SK stock of *Suncus murinus*. This stock had been established by crossing two strains of *S. murinus*: KAT and SRI. The KAT strain was derived from a wild population of Katmandu, Nepal (Oda *et al.*, 1992) and has been shown to have $2n = 40$ (Rogatcheva *et al.*, 1996). The SRI strain was derived from the animals captured on the West coast of Sri Lanka (Ishikawa *et al.*, 1989) and differs from the KAT strain in five Robertsonian fusions Rb(8.17), Rb(9.13), Rb(10.12), Rb(11.16), Rb(14.15) and an insertion of heterochromatin at chromosome 7. Sex chromosomes of the SRI strain differ substantially from those of the KAT strain and can be identified unambiguously. The SRI X chromosome (X^S) has an additional C-band at the p-arm compared with the KAT X chromosome (X^K). The SRI Y chromosome (Y^S) is much longer than the KAT Y chromosome (Y^K) and differs in both the G- and C-banding patterns. All these variant chromo-

somes are present in the hybrid SK stock (Rogatcheva *et al.*, 1997).

Metaphase chromosomes were prepared from a primary culture of fibroblasts and bone marrow cells as described by Rogatcheva *et al.* (1996). G-band staining was performed according to the method of Seabright (1971), as modified by Grafodatsky and Radjabli (1988). Slides were treated with a 0.025% (w/v) trypsin solution in isotonic saline for 1–2 min at room temperature, rinsed in $2 \times$ SSC and stained for 5 min in 4% (w/v) Giemsa solution.

The fresh weight of paired testes was scored in the XXY male and his two half-brothers (control males). The genitalia were fixed in formalin. Standard histological sections (5 μ m thick) were prepared from the left testis and stained with haematoxylin and eosin. The diameter of the seminiferous tubules was measured with a micrometer eyepiece on 10 tubules.

Results

Karyotype

The karyotype of the XXY male is shown (Fig. 1). All the G-banded spreads of fibroblasts (11) and bone marrow cells (50) scored for karyotype had a chromosome composition of 40XXY and contained Rb(11.16) in heterozygous state. The chromosome preparations provided no indication of sex chromosome mosaicism.

The father of the XXY male was a representative of the SK stock and had a karyotype of 35XY, Rb(8.17)/+, Rb(9.13)/+, Rb(11.16)/Rb(11.16), Rb(14.15)/+. He was crossed with six females of KAT strain and produced five daughters and six sons, all of which were Rb(11.16)/+. The X and Y chromosomes of the father of the XXY male were classified as the SRI type (X^S , Y^S) according to Rogatcheva *et al.* (1997). The mother of the XXY male was representative of the KAT strain and had 40XX. Her X chromosomes were typical X^K .

Both X chromosomes of the XXY male were of the KAT type (X^K). The Y chromosome was of the SRI type (Y^S). These observations indicate unambiguously that the XXY male inherited both X chromosomes from his mother.

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Fig. 1. G-banded chromosome spread of the XXY *Suncus murinus*. The arrows indicate the sex chromosomes.

Studies of the reproductive system

The XXY male had a normal male phenotype, as indicated by its external appearance and the size of its accessory reproductive structures. The epididymis, vas deferens and prostate glands were of a size and appearance similar to those observed in the control males. However, the testes of the XXY male were very small in comparison with those of normal adult males. The left testis of the XXY male weighed 5.0 mg, while those of his half-brothers weighed 117 ± 15 mg (mean $\pm$ SE).

The histological examination revealed considerable differences in the structure of testes of the XXY male and the control males (Fig. 2). The seminiferous tubules of the XXY male were smaller (average diameter $\pm$ SE was 50 ± 6 μ m) and less complex than those of the control males (200 ± 19 μ m), which displayed normal spermatogenic activity. Only one type of cell, located near the basal membrane, was found within the tubules of the XXY male. These cells resembled the Sertoli cells of control males on the basis of size, shape and staining of nuclei.

There was also a remarkable difference in the interstitial tissue from the XXY and control males. In the XXY male, most of the testis volume consisted of interstitial tissue (Fig. 2a), whereas in the control males, there were only small groups of interstitial cells between the seminiferous tubules (Fig. 2b).

Discussion

The phenotypic manifestation of the XXY condition in *S. murinus* agrees well with that found in other mammals. As in other species, there is a male phenotype in terms of external appearance and accessory reproductive structures, while the presence of small testes appears to be a general feature (Cattanach, 1974).

The histology of the testis of the XXY *S. murinus* resembles that observed in other adult mammals with the XXY condition. In all XXY animals in which the testes have been examined histologically, narrow and simplified tubules lined with Sertoli

Fig. 2. Histological section of the left testis of (a) the XXY *Suncus murinus* showing the narrow seminiferous tubules and high density of the interstitial cells and of (b) a control shrew showing normal spermatogenesis. Arrows indicate seminiferous tubules. Scale bars represent 50 μ m.

cells have been reported. Interstitial cell hyperplasia has also been noted as a characteristic of the XXY condition in many species. In the XXY male shrew, most of the testis volume consists of interstitial tissue.

Klinefelter syndrome is probably the most common sex chromosomal aberration found in humans. In random surveys, it has been found in about 1 in every 500–1000 liveborn males (Epstein, 1986; Chandley, 1988). In the work presented here, one XXY male was found among 143 karyotyped males of *S. murinus*.

Parental origin of the additional sex chromosome in humans has been examined in several studies (Jacobs *et al.*, 1988; Lorda-Sanchez *et al.*, 1992; MacDonald *et al.*, 1994). The proportion of paternally and maternally derived cases was close to 1:1. With regard to the cell division of origin,

MacDonald *et al.* (1994) found that among 107 maternally derived aneuploids 73 were the result of an MI error, 24 the result of a MII error and 10 the result of a post-zygotic mitotic error involving the maternal X chromosome. In the present case, the additional X chromosome of the XXY shrew was shown to be of maternal origin. However, it was not possible to determine whether the error occurred during maternal MI or MII because the mother of XXY shrew was an X^KX^K homozygote. Female *S. murinus* have a rather high rate of premature desynapsis of X chromosomes at diplotene (Borodin *et al.*, 1994) and this may cause a non-disjunction at MI. We consider that post-zygotic mitotic error, as the cause of aneuploidy, is unlikely because both tissues studied contained XXY cells only.

It is believed that multiple heterozygosity for chromosome rearrangements increases the risk of non-disjunction of heteromorphic and homomorphic chromosomes (de Boer and de Jong, 1989; Speed, 1989). As the stock examined in the present work was of hybrid origin, the majority of SK shrews were multiple heterozygotes for different autosome and gonosome rearrangements (Rogatcheva *et al.*, 1997). Paradoxically, the XXY shrew received the additional X chromosome from his mother of the KAT strain, which was homozygous for all standard chromosomes, and not from his father, which was heterozygous for as many as three Robertsonian translocations.

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