Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# Xp Duplications and Sex Reversal [and Discussion]

Elena Zanaria, Barbara Bardoni, Branka Dabovic, Vladimiro Calvari, Marco Fraccaro, Orsetta Zuffardi, Giovanna Camerino, J. A. M. Graves and A. McLaren

*Phil. Trans. R. Soc. Lond. B* 1995 **350**, 291-296
doi: 10.1098/rstb.1995.0164

**Email alerting service**

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1995 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

# Xp duplications and sex reversal

ELENA ZANARIA[1], BARBARA BARDONI[1], BRANKA DABOVIC[1], VLADIMIRO CALVARI[1], MARCO FRACCARO[1], ORSETTA ZUFFARDI[1] AND GIOVANNA CAMERINO[1,2]*

[1] *Biologia Generale e Genetica Medica, Università di Pavia, via Forlanini 14, 27100 Pavia, Italy*
[2] *Istituto di Istologia ed Embriologia, Università di Sassari, via S.Pietro 43/B, Sassari, Italy*

## SUMMARY

Male to female sex reversal has been observed in individuals with duplications of the short arm of the X chromosome. The study of Xp duplicated patients demonstrated that sex reversal results from the presence of two active copies of the DSS (dosage sensitive sex reversal) locus. A double dosage of DSS disrupts testis formation whereas its absence is compatible with a male phenotype, suggesting a role for DSS in ovarian development and as a link between ovary and testis formation.

DSS was localized to a 160 kb region of Xp21, overlapping the adrenal hypoplasia congenita locus. The search for expressed sequences in the DSS critical region led to the identification of two types of genes: the DAM family and DAX-1, an atypical member of the nuclear receptor superfamily. Although no function is currently known for DAM genes, functional deficiency for DAX-1 has been shown to be responsible for adrenal hypoplasia congenita and hypogonadotropic hypogonadism. The search for the DSS gene(s) is still open and both the DAM genes and DAX-1 represent DSS candidate genes.

## 1. INTRODUCTION

Phenotype analysis in sex chromosome aneuploidies (for example, 45,X Turner individuals or 47,XXY Klinefelter individuals) suggests that the number of X chromosomes present in a zygote, although important for normal sex differentiation, does not influence gonadal sex determination. Only one of the X chromosome is, however, genetically active in mammalian cells and most X-linked genes are subject to X-inactivation. The sex phenotype of sex chromosome aneuploidies thus simply indicates that the dosage of X-specific genes escaping X-inactivation is not important in gonadal sex determination. On the other hand, activity of more than one X chromosome and true hermaphroditism is common in triploid 69,XXY foetuses (reviewed in Petit *et al.* 1992). Furthermore, sex reversal has been observed in 46, XY individuals carrying a duplication of the short arm of the X chromosome (Bernstein *et al.* 1980; Scherer *et al.* 1989; Stern *et al.* 1990; May *et al.* 1991; Ogata *et al.* 1992; Arn *et al.* 1994; Bardoni *et al.* 1994), a condition involving obligatory double dosage of the Xp duplicated genes. The dosage of X-specific gene(s) subject to X-inactivation might thus be important in the determination of gonadal sex. In this paper we describe the mapping of one Xp locus, DSS (dosage sensitive sex reversal), involved in sex determination and the search for the DSS candidate gene(s).

## 2. THE DSS LOCUS: GENE DOSAGE AND GONADAL DIFFERENTIATION

Sex reversal has been observed in 12 of the 17 dup(Xp) genetic males described (reviewed in Arn *et al.* 1994; Bardoni *et al.* 1994; Ogata & Matsuo 1994). Phenotype-karyotype correlation suggested that the duplication of an Xp21-p22 locus is responsible for male to female sex reversal (Arn *et al.* 1994; Ogata & Matsuo 1994)

Molecular analysis of Xp duplicated patients confirmed that sex reversal results from the presence of two active copies of an Xp21 locus that was called DSS (Bardoni *et al.* 1994). The DSS locus was initially mapped to a region of approximately 15 Mb of Xp21.2-p22.1 by comparing the extent of the duplications in four sex reversed patients and in four patients with normal testis differentiation (see figure 1*a*).

The identification of a submicroscopic duplication in a 46,XY sex reversed female patient (BI, Bardoni *et al.* 1994), allowed us to refine the mapping of the DSS critical region to a 160 Kb region of Xp21 (see figure 1*a*), overlapping to the AHC locus and approximately 0.4 and 1 Mb distal to the glycerol kinase deficiency (GK) and Duchenne muscular dystrophy (DMD) genes, respectively (see figure 1*b*, Bardoni *et al.* 1994; Zanaria *et al.* 1994).

Interestingly, the DSS critical region maps to a region of Xp21 where a number of deletions have been described in 46,XY individuals. These deletions are associated with a contiguous gene syndrome (see below) but are not lethal. The circumscribed mapping of the DSS locus allowed us to analyse the effect of duplications and deletions of the DSS critical region on gonadal differentiation in 46,XY and 46,XX individuals:

*Phil. Trans. R. Soc. Lond.* B (1995) **350**, 291–296
*Printed in Great Britain*



© 1995 The Royal Society

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions: Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org



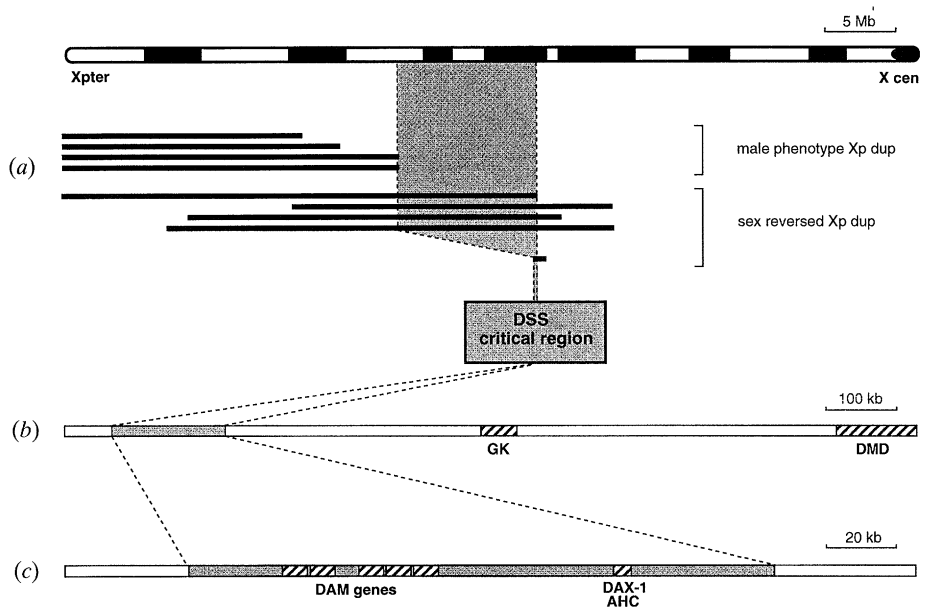


Figure 1. Mapping of the DSS locus and search for the DSS gene. (*a*) Mapping of the DSS locus by phenotype-genotype correlation in patients with microscopic and submicroscopic Xp duplications. (*b*) The DSS critical region maps approximately 0.4 and 1 Mb distal to the glycerol kinase deficiency (GK) and Duchenne muscular dystrophy (DMD) genes, respectively. (*c*) Localization of DAX-1 and DAM genes within the DSS critical region.

### (*a*) *Duplication of DSS in genetic males*

The internal and external genitalia of published sex reversed Xp duplicated patients are described in table 1.

The phenotype of the patients indicates that two doses of DSS are sufficient to disrupt normal testis development in the presence of a functional SRY gene. Interestingly, apparently normal ovarian development was described in one dup(Xp) foetus at 20 weeks of gestation (Bernstein *et al.* 1980, see case 2).

Sex reversed, Xp duplicated patients show different degrees of gonadal dysgenesis, ranging from incompletely differentiated testes to the presence of a single streak gonad. Phenotypic variability was described in the sex reversal syndromes associated with mutations in SRY (Berta *et al.* 1990; Harley *et al.* 1992), in the Wilm's tumour gene (Hastie 1992) or in the SOX9 gene (Foster *et al.* 1994; Wagner *et al.* 1994).

Patient BI (Bardoni *et al.* 1994), who carries a submicroscopic Xp21 duplication, has normal female external genitalia and an almost complete form of gonadal dysgenesis. This indicates that the variability in sexual differentiation is not related to the size of the duplications and suggests that additional Xp genes are not involved in sex reversal.

### (*b*) *Deletion of DSS in genetic males*

Male individuals carrying deletions in Xp21 are affected by a contiguous gene syndrome that may include AHC, hypogonadotropic hypogonadism (HHG), mental retardation (MR), glycerol kinase deficiency (GK), Aland Island eye disease (AIED), hearing loss and Duchenne muscular dystrophy (DMD) (Walker *et al.* 1992; Worley *et al.* 1992, 1993). Amongst the 46,XY patients affected by the complex AHC, GK, DMD deletion syndrome, many are entirely deleted for the DSS critical region (Muscatelli *et al.* 1994; Zanaria *et al.* 1994). Although these patients may show HHG and cryptorchidism (see DAX-1 mutations below), they have male external genitalia and no evidence for Mullerian derivatives. Altogether these data provide strong evidence that 46,XY, del(DSS) patients undergo normal male gonadal sex determination and suggest that DSS is not essential for testicular development.

### (*c*) *Heterozygous deletion or duplication of DSS in genetic females*

In some of the Xp duplication cases the duplication was inherited from the mother. Similarly, in a few cases of Xp deletion encompassing the DSS locus, the deletion was maternally inherited (Bardoni *et al.* 1994). Genotypic females carrying a duplication or a deletion at the DSS locus can thus be fertile. Although it is likely that an increased DSS dosage does not interfere with ovary formation, the effect of its deletion in XX females is difficult to assess, due to X-inactivation. Accordingly, only a mosaic of normal and dysgenetic gonadal cells would be found in heterozygous carriers of a deletion, even if DSS is essential for ovarian development (see below) and acts cell autonomously.

Double dosage of DSS disrupts testis formation whereas its absence is compatible with a male phenotype. This paradox could be explained if DSS is a link between the ovarian and testicular pathways. Different genes must be activated and/or repressed to differentiate an ovary or a testis. Although it is likely that absence of the SRY gene is sufficient to avoid activation of the testicular pathway in normal females, it is not clear how the reciprocal function (i.e. repression of the ovarian pathway) is achieved in normal males. It is possible that DSS is an ovarian differentiation gene with an important function in the



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





Downloaded from rstb.royalsocietypublishing.org

Table 1. *Gonadal dysgenesis in dup(Xp) patients*

| reference, case (age at examination) | dup (X) | external genitalia | Mullerian derivatives | Wolffian derivatives | gonads |
|---|---|---|---|---|---|
| Bernstein *et al.* 1980 case 1 (5 years) | p21-pter | normal female | normal vagina, hypoplastic uterus and Fallopian tubes | tubular structures resembling immature epididymes adjacent to Fallopian tubes | small area of ovarian stroma with scant early primoridial follicle formation, many showing degenerative changes |
| Bernstein *et al.* 1980 case 2 (20 weeks foetus, sib of case 1) | p21-pter | normal female | normal | absent | normal fetal ovarian tissue with ovarian stroma and numerous early primordial follicles, no testicular tissue |
| Stern *et al.* 1990 (child) | p21-p22.3 | female, hypoplasia of labia minora | normal (?) | absent (?) | ? |
| May *et al.* 1991 (birth) | p11.4-pter | normal female | normal (?) | absent (?) | ? |
| Ogata *et al.* 1992 (2 years) | p21-p22.3 | normal female | normal | absent | streaks gonads with ovarian stroma and gonadoblastoma |
| Arn *et al.* 1994 case 1 (birth) | p21.2-p22.1 | ambiguous: fusion of labioscrotal folds, 1.5 cm cleft phallus with meatus at the base | absent (laparatomy) (large prostatic utricle or Mullerian remnant) | vasa deferentia | gonads (in the internal ring): right fibrovascular tissue and smooth muscle with few embryonal tubular rests; left structures resembling epididymis and vas deferens |
| Arn *et al.* 1994 case 2 (2 months, sib of case 1) | p21.2-p22.1 | ambiguous: prominent clitoris with a uretra at the base and fused labia minora | absent | well-defined epididymis and vas deferens | prominent fibroconnective tissue and multiple nests of seminiferous tubules, no Leydig cells, no germ cells |
| Scherer *et al.* 1989; Bardoni *et al.* 1994 case FR (2 years) | p21.2-p22.3 | hypoplastic female | normal vagina | ? | ? |
| Scherer *et al.* 1989; Bardoni *et al.* 1994 case BG (7 years) | p21.2-p22.2 | normal female | normal (laparascopy) | absent | right streak gonad with primordial sex cords and multifocal gonadoblastoma; absent left gonad |
| Bardoni *et al.* 1994 case PT (4 years) | p21.2-p22.1 | female, slight clitoromegaly | rudimentary right tube, normal left tube | vasa deferentia and epididymes | intra-abdominal testes with seminiferous tubules focally hypotrophic and well represented rete testis |
| Bardoni *et al.* 1994 case RR (21 years) | p21.3-pter | ambiguous: perineal hypospadias, bifid empty scrotum | hypoplasic, adjacent to left testis | ectasic vasa deferentia and epididymes; bilateral epididymal cystis | intra-abdominal testes with atrophic tubules and Leydig cell hyperplasia |
| Bardoni *et al.* 1994 case BI (14 years)i | > 1 Mb | normal female | rudimentary uterus, thin and long tubes with incomplete ampulla | glandular structures resembling epididymes | ovarian streaks; cortical ovarian tissue with rare glandular structures resembling rete testis; no seminiferous tubules |

Downloaded from rstb.royalsocietypublishing.org



sexual determination network. In normal male individuals ovarian development and DSS function are repressed allowing testis formation. In 46, XY individuals with deletions of DSS, the ovarian pathway is inactive, due to the absence of functional DSS gene(s) and testis differentiation takes place. The double dosage of DSS in individuals with Xp duplications and a functional SRY gene, however, hampers repression of the ovarian pathway, leading to gonadal dysgenesis and phenotypic sex reversal.

## 3. SEARCH FOR THE DSS GENE

The DSS critical region maps to a 160 kb region of Xp21, overlapping to the AHC locus and approximately 0.4 and 1 Mb distal to the glycerol kinase deficiency (GK) and Duchenne muscular dystrophy (DMD) genes, respectively (see figure 1*b*, Bardoni *et al*., 1994). Among the disease-genes of this region (AHC, HHG, MR, GK, AIED and DMD), only those responsible for DMD and GK were isolated when we mapped the DSS locus to Xp21. The DSS-AHC region was, however, partially cloned in YAC contigs (Walker *et al*. 1992; Worley *et al*. 1992, 1993).

To isolate expressed sequences from the region, we subcloned two overlapping YACs and constructed a 230 kb phage contig covering the DSS-AHC critical intervals. We then analysed the YACs by PFGE, subcloned the single CpG rich region and used it to screen cDNA libraries (Zanaria *et al*. 1994). In addition, we used the minimum phage overlap of the region to select cDNAs (Korn *et al*. 1992) from an adult testis cDNA library. Several overlapping cDNA clones, corresponding to three genes that we called DAX-1, DAM6 and DAM 10, were isolated (see figure 1*c*).

### (*a*) *DAM genes*

By using the direct cDNA selection approach, we identified two DAM genes (for *DSS*/*A*HC critical interval genes, belonging to the *M*AGE superfamily) expressed in adult testis, DAM10 and DAM6 and demonstrated that at least five DAM genes are clustered in 50 kb of the DSS critical region. DAM6 and DAM10 are approximately 82% identical at the DNA level and their predicted protein products are 66% identical and 82% similar. Multiple forms of DAM10 mRNA, derived from alternative splicing, were detected in adult testis. The mouse DAM family, as judged by Southern blotting experiments, has the same complexity as the human counterpart (Dabovic *et al*. 1995).

DAM predicted proteins show a highly significant and continuous similarity to the MAGE family of tumour-associated antigens (van der Bruggen *et al*. 1991; De Plaen *et al*. 1994). The best characterized gene of this family, MAGE1, encodes for a tumour antigen recognized on a melanoma by autologous cytolytic T lymphocytes (van der Bruggen *et al*. 1991). MAGE genes are not expressed in normal adult tissues (with the exception of testis and placenta) but are expressed at a high level in tumours. A very significant similarity was also found between the C-terminal portions of the DAM predicted proteins and mouse necdin (Maruyama *et al*. 1991), a brain-specific nuclear protein. The biological function of MAGE and necdin proteins is presently unknown. It has been hypothesised that MAGE proteins play a role during foetal differentiation (De Plaen *et al*. 1994). DAMs, MAGEs and necdin define a new superfamily of genes, the MAGE superfamily, probably derived by duplication of a common ancestor. Interestingly, while DAM genes are clustered in Xp21, MAGE genes have been reported to cluster in Xq26–28 (De Plaen *et al*. 1994, Wang *et al*. 1994).

The molecular characterization of DAM genes provides very little information on their function. Several 46,XY patients, partially or completely deleted for the DSS critical region and adjacent portions of Xp21 have been described (Walker *et al*. 1992; Worley *et al*. 1992, 1993). The complexity and poor clinical description of the phenotype associated with these deletions, however, currently hampers correlation between DAM genes loss and a specific pathological phenotype.

### (*b*) *The DAX-1 gene*

The DAX-1 gene (DSS-AHC critical region on the X, gene 1) encodes a protein product of 470 amino acids that can be divided in two portions: the N-terminal portion, which contains four incomplete repeats of a new structural motif and may define a novel DNA-binding domain, and the C-terminal part, which shows approximately 50% continuous similarity to the ligand binding domain of the nuclear hormone receptor superfamily. DAX-1 is a nuclear protein that binds efficiently a retinoic acid (RA) responsive element and acts as a down regulator of the RA-dependent transcriptional activation elicited by different RA receptors (Zanaria *et al*. 1994).

DAX-1 is responsible for the X-linked form of adrenal hypoplasia congenita. Adrenal hypoplasia congenita is an inherited disorder of adrenal gland development. The X-linked form, (AHC, MIM 300200) (McKusick 1992) is characterized by the absence of the permanent zone of the adrenal cortex and by a structural disorganization of the glands. The disorder, which is lethal if untreated, results in adrenal insufficiency early in infancy, with low serum concentration of glucocorticoids, mineralcorticoids and androgens, and failure to respond to ACTH stimulation. Hypogonadotropic hypogonadism (HHG) is commonly associated with the X linked form of the disease and is generally noted at the expected time of pubertal maturation. It is not clear if this form of HHG is of pituitary or hypothalamic origin (reviewed in Kletter *et al*. 1991).

DAX-1 is expressed in adrenal glands and adult testis, and is deleted in all AHC deletion patients previously used to define the AHC critical interval (Walker *et al*. 1992; Worley *et al*. 1992, 1993). Furthermore, mutations that disrupt the integrity of the DAX-1 protein were identified in three unrelated AHC patients with no detectable deletions or rearrangements in this region (Zanaria *et al*. 1994). In



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



a more comprehensive screening, we identified point mutations, small insertions or deletions within the coding portion of the DAX-1 gene, in 15 out of 21 (71%) unrelated AHC patients (Muscatelli *et al.* 1994).

It was not clear if mutations causing AHC are also responsible for HH or if rearrangements involving two closely linked genes cause AHC and HHG, respectively. We demonstrated that patients carrying point mutations in the DAX-1 gene and no detectable deletions or rearrangement in Xp21, can be affected by both AHC and HHG. These data definitively establish that the absence of an intact DAX-1 gene product is responsible for both AHC and HHG (Muscatelli *et al.* 1994; Zanaria *et al.* 1994). Further studies will allow a better clarification of the role of DAX-1 in the hypothalamus/pituitary/ steroidogenic axis.

## 4. CONCLUSIONS

We have localized DSS, a locus involved in gonadal sex differentiation, to 160 kb of Xp21. Duplications of DSS in 46,XY individuals results in male to female sex reversal, although DSS nullisomy is compatible with a male phenotype. We propose that DSS has a role in ovarian development and functions as a switch between ovary and testis formation.

Search for expressed sequences in the 160 kb DSS critical region led to the identification of two types of genes: the DAM family and DAX-1, an atypical member of the nuclear receptor superfamily. Although no function is currently known for DAM genes, functional deficiency for DAX-1 has been shown to be responsible for adrenal hypoplasia congenita and hypogonadotropic hypogonadism.

The search for the DSS candidate gene is still open. It is possible that the DSS locus corresponds to one or more of the genes we have isolated or to still unidentified gene(s) from the 160 kb critical interval. Although we failed to detect gross rearrangements in the DAX-1 gene or in DAM genes in approximately 30 46,XY females, some evidence indicate a role for DAX-1 in gonadal development. We have shown that DAX-1 is a transcription factor which is involved in adrenal development (Zanaria *et al.* 1994). As the steroidogenic components of the gonads and the adrenal glands have a common embryological origin, it is not unlikely, therefore, that DAX-1 may play an important role in the development of both organs. Accordingly, disruption of the Ftz-F1 gene, an orphan member of the nuclear hormone receptor superfamily, prevents the development of both the adrenal glands and the gonads (Luo *et al.* 1994). *In situ* experiments in developing mouse embryos indicates that DAX-1 is indeed expressed in the genital ridge during the critical period for gonadal sex determination (A. Swain *et al.*, unpublished data). Further experiments, including the construction of animals carrying additional copies of DAX-1 or DAM genes and the study of the pattern of expression of DAM genes in the developing mouse will allow to test if any of these genes is involved in sex determination/gonadal differentiation.

The financial support of Telethon-Italy, Progetto Finalizzato 'Ingegneria Genetica' of the Consiglio Nazionale delle Ricerche, Regione Lombardia, Commission of the European Communities is gratefully acknowledged.

## REFERENCES

Arn, P., Chen, H., Tuck-Muller, C. M. *et al.* 1994 SRVX, a sex reversing locus in Xp21.2–p22.11. *Hum. Genet.* **93**, 389–393.

Bardoni, B., Zanaria, E., Guioli, S. *et al.* 1994 A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. *Nature Genet.* **7**, 497–501.

Bernstein, R., Jenkins, T., Dawson, B. *et al.* 1980 Female phenotype and multiple abnormalities in sibs with a Y chromosome and partial X chromosome duplication: H-Y antigen and Xg blood group findings. *J. med. Genet.* **1**, 291–300.

Berta, P., Hawkins, J. R., Sinclair, A. H. *et al.* 1990 Genetic evidence equating SRY and the testis-determining factor. *Nature, Lond.* **348**, 448–450.

Dabovic, B., Zanaria, E., Bardoni, B. *et al.* 1995 A family of rapidly evolving genes from the sex reversal critical region in Xp21. *Mammalian Genome.* (In the press.)

De Plaen, E., Arden, K., Traversari, C. *et al.* 1994 Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Imunogenetics* **40**, 360–369.

Foster, J. W., Dominguez-Steglich, M. A., Guioli, S. *et al.* 1994 Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature, Lond.* **372**, 525–529.

Harley, V. R., Jackson, D. J., Hextall, P. J. *et al* 1992 DNA binding activity of recombinant SRY from normal males and XY females. *Science, Wash.* **255**, 453–456.

Hastie, N. D. 1992 Dominant negative mutations in the Wilms tumour (WT1) gene causes Denys-Drash syndrome: proof that a tumour suppressor gene plays a crucial role in normal genitourinary development. *Hum. molec. Genet.* **1**, 293–295.

Kletter, G. B., Gorski, J. L. & Kelch, R. P. 1991 Congenital adrenal hypoplasia and isolated gonadotropin deficiency. *Trends Endocrinol. Metabol.* **2**, 123–128.

Korn, B., Sedlacek, Z., Manca, A. *et al.* 1992 A strategy for the selection of transcribed sequences in the Xq28 region. *Hum. molec. Genet.* **4**, 235–242.

Luo, X., Ikeda, Y. & Parker, K. L. 1994 A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* **77**, 481–490.

Maruyama, K., Usami, M., Aizawa, T. & Yoshikawa, K. 1991 A novel Brain-specific mRNA encoding nuclear protein (necdin) expressed in neurally differentiated embryonal carcinoma cells. *Biochem. Biophys. Res. Com.* **178**, 291–296.

May, K. M., Grinzaid, K. A. & Blackston, R. D. 1991 Sex reversal and multiple abnormalities due to abnormal segregation of t(X;16)(p11.4;p13.3). *Am. J. Hum. Genet.* **49**, 19. (Suppl.)

McKusick, V. A. 1992 *Mendelian inheritance in man*, edn 10, pp. 1841–1843. Baltimore: Johns Hopkins University Press.

Muscatelli, F., Strom, T., Walker, A. P. *et al.* 1994 Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature, Lond.* **372**, 672–676.

Ogata, T., Hawkins, J. R., Taylor, A., Matsuo, N., Hata, J. & Goodfellow P. 1992 Sex reversal in a child with a 46,X,Yp+ karyotype: support for the existence of a gene(s), located in distal Xp, involved in testis formation. *J. med. Genet.* **29**, 226–230.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



Ogata, T. & Matsuo, N. 1994 Testis determining gene(s) on the X chromosome short arm: chromosomal localization and possible role in testis determination. *J. med. Genet.* **31**, 349–350.

Petit, P., Moerman, P. & Fryns, J. P. 1992 Full 69,XXY triploidy and sex-reversal: a further example of true hermaphroditism associated with multiple malformations. *Clin. Genet.* **41**, 175–177.

Scherer, G., Schempp, W., Baccichetti, C. *et al.* 1989 Duplication of an Xp segment that includes the ZFX locus causes sex inversion in man. *Hum. Genet.* **81**, 291–294.

Stern, H. J., Garrity, A. M., Saal, H. M., Wangsa, D. & Disteche, C. M. 1990 Duplication Xp21 and sex reversal: Insight into the mechanism of sex determination. *Am. J. Hum. Genet.* **47**, 41. (Suppl.)

van der Bruggen, P., Traversari, C., Chomez, P. *et al.* 1991 A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science,Wash.* **254**, 1643–1647.

Wagner, T., Wirth, J., Meyer, J. *et al.* 1994 Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX-9. *Cell* **79**, 1111–1120.

Walker, A. P. , Chelly, J., Love, D. R. *et al.* 1992 A YAC contig in Xp21 containing the adrenal hypoplasia congenita and glycerol kinase deficiency genes. *Hum. molec. Genet.* **1**, 579–585.

Wang, M. G., Zakut, H., Yi, H., Rosenberg, S. & McBride, O. W. 1994 Localization of the MAGE1 gene encoding human melanoma antigen to chromosome Xq28. *Cytogenet. Cell Genet.* **67**, 116–119.

Worley, K. C., Ellison, K. A., Zhang, Y. -H. *et al.* 1993 Yeast artificial chromosome cloning in the glycerol kinase and adrenal hypoplasia congenita region of Xp21. *Genomics* **16**, 407–416.

Worley, K. C., Towbin, J. A., Zhu, X. M. *et al.* 1992 Identification of three new markers in Xp21 between DXS28 (C7) and DMD. *Genomics* **13**, 957–961.

Zanaria, E., Muscatelli, F., Bardoni, B. *et al.* 1994 An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature, Lond.* **372**, 635–641.

### *Discussion*

J. A. M. Graves (*La Trobe University, Melbourne, Australia*). In wood lemmings there are X*Y females with a modified X chromosome which evidently suppresses testis determination. Does X* have a duplicated DAX-1?

G. Camerino. We have compared the DAX-1 Southern blotting hybridization profile (and intensity) in XX, X*Y and XY wood lemmings cell lines. We did not find evidence for duplication or other gross abnormalities in the DAX-1 gene.

A. McLaren (*Wellcome/CRC Institute, Cambridge, U.K.*). X-chromosome inactivation has not been discussed much at this meeting, but it is clear that gene dosage has an important part to play in sex determination, as evidenced by the male to female sex reversal brought about by a double dosage of the DSS locus and the greater incidence of female to male sex reversal when the *Sry*-containing transgene is in a homozygous rather than hemizygous condition. For a twofold difference in concentration of a gene product to switch development in a system as efficiently canlized as sex determination, with so few intersexual individuals, implies a sharp concentration threshold. This could well interact with developmental time: for example, a twofold increase in concentration of the DSS gene product could reach a critical threshold and thus switch development into the ovarian pathway earlier than normal, rendering *Sry* ineffective

G. Camerino. I am not so sure I agree with the idea that sex determination is efficiently canalized. Most human mutations in genes involved in sex determination are somehow leaky: patients with the same mutation in WT-1, or with DSS duplication, may show very different degrees of gonadal dysgenesis; the same SRY mutations have been found in XY sex reversed patients and in their fathers. On the other hand, I agree that gene dosage data suggest a sharp concentration threshold for SRY and DSS gene products. Dosage effects may be due to, or enhanced by, protein-protein interactions; for example, it is not unlikely that DAX-1 may function as an heterodimer. The hypothesis that the concentration threshold may interact with developmental time is very appealing. However I do not think that, for the moment, we have enough information to construct a convincing model of gonadal sex determination.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES