# Turner syndrome: the case of the missing sex chromosome

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***Turner syndrome is the phenotype associated with the absence of a second sex chromosome in humans. Recent observations support the hypothesis that the phenotype results from haploid dosage of genes that are common to the X and Y chromosomes and that escape X inactivation. A goal of current studies is the identification of these 'Turner' genes.***

In 1938, Henry Turner addressed the Association for the Study of Internal Secretions[1]. While we have no record of the audience's response, the syndrome described by the University of Oklahoma endocrinologist has piqued the interest of biologists ever since. The seven women studied by Turner had all failed to develop secondary sexual characteristics. He noticed that they also had in common the now classic features of Turner syndrome (TS): short stature, webbed neck and cubitus valgus, a deformity of the elbow. Although it was not recognized at the time, Otto Ullrich had earlier published a description of a girl with the same physical features[2]; hence the condition is sometimes called Ullrich-Turner syndrome.

TS joined the realm of genetic disorders in 1959, when Charles Ford and colleagues described a patient with the karyotype 'XO' (now denoted 45,X)[3]. This woman had 45 chromosomes rather than the usual 46; she lacked a second sex chromosome. Cytogeneticists have since found that TS is almost always associated with sex chromosome aberrations, and girls with pubertal delay or somatic Turner characteristics are now routinely karyotyped. Clinicians are reluctant to make a diagnosis of TS if the karyotype is normal. Nevertheless, the Turner phenotype is occasionally seen in individuals with an apparently normal karyotype. For the purposes of this review, we consider TS to denote the phenotype, irrespective of the genotype. There is confusion in the literature on this point, especially because another disorder, Noonan syndrome, shares some phenotypic features with TS. Unlike TS, Noonan syndrome occurs in both sexes, usually without obvious karyotypic abnormalities, and may show autosomal dominant inheritance. Despite common features, the Turner and Noonan phenotypes are differentiated by most clinicians.

## The Turner phenotype

In the last few decades, careful clinical studies of many patients (see, for example, Ref. 4) have refined the description of the phenotype associated with monosomy X (Box 1). Like many aneuploidies, monosomy X is associated with diminished viability. However, the life expectancy of 45,X children who survive infancy is not severely reduced. In liveborn individuals, the cardinal features are short stature, ovarian failure and the variable presence of specific somatic abnormalities. Short stature reflects a generalized growth defect that is first manifest *in utero*, and not simply the absence of the pubertal growth spurt. In the USA, the adult height of women with TS rarely exceeds 150 cm. The absence of secondary sexual characteristics is now known to be caused by prepubertal ovarian failure and the consequent lack of ovarian sex steroid production. The ovaries initially develop normally, but at about six months of gestation massive oocyte loss occurs (XX females also undergo oocyte loss *in utero*, but to a lesser extent). Generally, by early infancy only small streaks of fibrous tissue remain. It is not unusual for girls with TS to first seek medical help because they fail to begin menstruating. Occasionally, enough ovarian function remains for spontaneous puberty and, very rarely, pregnancy; infertility is the rule in TS.

**Box 1. The phenotype of monosomy X**

- Poor viability *in utero*
- Short stature
- Prepubertal ovarian failure
- Anatomical abnormalities
  - Webbed neck
  - Increased carrying angle of the elbow (cubitus valgus)
  - Congenital swelling of the hands and feet (lymphedema)
  - Aortic narrowing (coarctation)
  - High arched palate
  - Low posterior hairline
  - Low set ears
  - Kidney and urinary tract anomalies
  - Short fourth metacarpals
  - Multiple pigmented nevi (moles)
  - Fingernail and toenail deformities
- Other features
  - Glucose intolerance
  - Hypothyroidism
  - Cognitive deficit in the ability to analyse visual–spatial relationships

Many somatic abnormalities in addition to those noted by Turner are now known to be associated with monosomy X (Box 1). It must be emphasized that the phenotype is highly variable, and that only a subset of the characteristic somatic features is present in a typical individual with monosomy X. There is no mental retardation in TS, unlike other human chromosomal disorders, but specific deficits in the ability to analyse spatial relationships occur frequently.

Short stature and infertility, with the associated lack of secondary sexual characteristics, are generally the most distressing aspects of TS for patients. Therapy is therefore directed toward these symptoms. Although their short stature is not caused by growth hormone deficiency, treatment of TS women with exogenous hormone, alone or in combination with sex steroids, may increase their final height; clinical trials are ongoing. Hormonal replacement therapy with estrogen and progesterone is used to induce the development of secondary sexual characteristics and to maintain cyclic menses. With recent advances in fertilization

*in vitro*, and by the use of donor embryos or oocytes, women with TS (who would otherwise be infertile) can even become pregnant.

## Pathogenesis

The pathogenesis of the developmental abnormalities seen in TS is poorly understood. Simpson and LeBeau speculated that a generalized cellular defect underlies growth retardation (and perhaps other phenotypic features), after observing that fibroblasts with partial or complete monosomy X have an increased cell doubling time *in vitro*[5]. However, the results of other such studies are discrepant.

Ovarian failure may have multiple causes. Burgoyne and Baker proposed that incomplete chromosomal pairing in meiosis may cause germ cell death[6]. Consistent with this hypothesis, a recent study found that ovarian failure was frequent not only with monosomy X but also with partial autosomal aneuploidies[7]. Other studies (reviewed in Ref. 8) have reported ovarian failure associated with interstitial X chromosome deletions that are presumed not to affect meiotic pairing, suggesting that the deficiency of specific gene products is important in oocyte loss.

Little is known about the embryology of somatic abnormalities in TS. 45,X fetuses often show lymphedema, or swelling due to accumulation of interstitial fluid. This may be due to malformation and obstruction of lymphatic vessels. Painstaking anatomical studies of fetuses with TS indicated that, at six to eight weeks' gestation, normal connections between the developing lymphatic and venous systems in the neck were absent[9]. This observation has led to the idea that distension of blind lymphatic vessels and overlying skin results in webbing of the neck. Congenital lymphedema of the hands and feet is presumably also a result of lymphatic obstruction, and nail malformation may be a secondary deformity. Interestingly, there is an association between aortic malformation and webbed neck in TS, suggesting that these abnormalities may have a common etiology[10,11].

## Epidemiology and cytogenetics

Because there are no strict diagnostic criteria for TS, and the diagnosis may be missed at birth, the true incidence of TS is not known. The best estimates come from epidemiological studies of sex chromosome abnormalities. Overall, the incidence of all abnormalities that include partial or complete monosomy X is about 1 in 5000 live female births[12]. Monosomy X is much more frequent among spontaneous abortuses, and accounts for nearly 10% of all miscarriages and perhaps as many as 1–2% of all human conceptions[13]. It is estimated that 99% of 45,X fetuses do not survive to term; most die by 28 weeks of gestation.

The mechanism of chromosome loss in monosomy X is not known. In most cases, meiotic nondisjunction is probably not the cause[13]. Unlike common human trisomies such as Down syndrome (trisomy 21), the risk of TS does not increase (and indeed may slightly decrease) with increasing maternal age. No significant differences in the incidence of TS among different populations have been observed. Among both live-born individuals and spontaneous abortuses with monosomy X, the single X chromosome is of maternal origin in approximately 75% of cases, and of paternal origin in the remaining 25%. As there are no phenotypic differences associated with the parental origin of the remaining X chromosome, imprinting does not appear to play a role in TS (Ref. 14).

**TABLE 1. Karyotypes observed in Turner syndrome[a]**

| Karyotype[b] | Frequency (%) |
|---|---|
| **Nonmosaic** | |
| 45,X | 58 |
| 46,X,i(Xq) | 6 |
| 46,X,r(X) | 5 |
| 46,XXp- | 1 |
| 46,XXq- | 1 |
| 46,XYp- | <1[c] |
| Total | 71% |
| **Mosaic** | |
| 45,X/46,X,i(Xq) | 10 |
| 45,X/46,XX | 8 |
| 45,X/46,XY | 6 |
| 45,X/47,XXX | 1 |
| 45,X/46,XXq- | 1 |
| 45,X/46,XXp- | <1 |
| Others | 2 |
| Total | 29% |

[a]Data are from Ref. 4.
[b]Nomenclature:
i(Xq), X isochromosome with deletion of short arm and duplication of long arm;
r(X), ring X chromosome;
Xp-, X chromosome lacking a terminal portion of the short arm;
Xq-, X chromosome lacking a terminal portion of the long arm.
[c]Fifteen cases total in the literature and our unpublished data.

With the advent of improved cytogenetic techniques, it is now clear that many Turner women do not have a 45,X karyotype. A variety of other karyotypes, which have in common at least partial sex chromosome monosomy, have also been observed in TS patients (Table 1). A substantial fraction of patients are mosaic, having two or more karyotypically distinct cell types. Mosaicism is thought to result from chromosome loss after fertilization; it is much more frequent in Turner syndrome than in other human chromosomal disorders, which are generally the result of errors in meiosis. Mosaic Turner females often have one cell type with a normal karyotype, and this is associated with enhanced fetal viability and a less severe phenotype. Some authors have even suggested that no truly nonmosaic 45,X human conception is viable, but this hypothesis cannot readily be tested.

## Turner syndrome and the X chromosome

Most investigators believe that the Turner phenotype is the result of a deficiency of specific 'Turner' genes, as yet unidentified, rather than a consequence of monosomy X *per se*. Abnormalities in TS are thought to be due to the haploid dosage of these genes, rather than the complete absence of gene

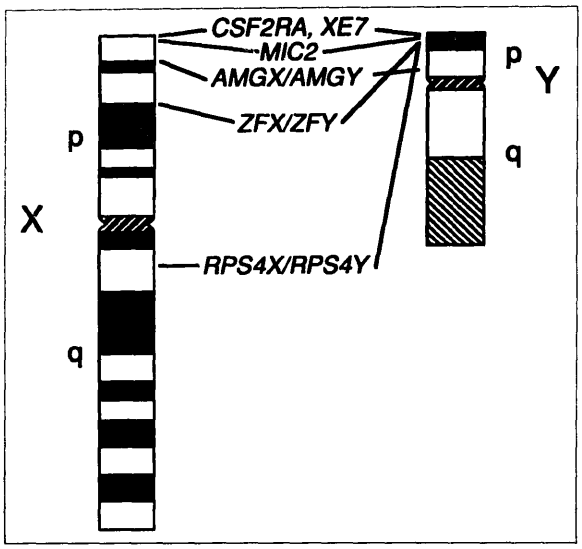


*FIG* 1

Human X-linked genes that have functional Y homologs. *CSF2RA*, encodes a GM-CSF receptor subunit; *XE7*, function unknown; *MIC2*, encodes a glycoprotein involved in T cell adhesion; *AMGX/AMGY*, encodes amelogenin, a tooth-bud protein; *ZFX/ZFY*, encodes a zinc finger protein; *RPS4X/RPS4Y*, encodes ribosomal protein S4. *CSF2RA*, *XE7* and *MIC2* are pseudoautosomal. *XE7*, *MIC2*, *ZFX* and *RPS4X* have been shown to escape X inactivation. The inactivation status of *CSF2RA* and *AMGX* has not been determined.

products or the presence of abnormal products. The task of identifying Turner genes is complicated by the phenotypic variability among individuals of the same karyotype. Despite this, there have been attempts to map X-linked Turner loci by correlating phenotypic features with deletions of portions of the X chromosome. There are several caveats to consider when interpreting these studies. Earlier workers used cytogenetic techniques of limited resolution. Karyotypes were generally prepared using cells from only one or, at most, two tissues, so somatic mosaicism may not have been detected. Even in apparently nonmosaic cases, similar phenotypes have been observed in patients with different, well-documented deletions of either Xp (the short arm) or Xq (the long arm). Nevertheless, there is general agreement on several points. There is probably a locus important for stature on distal Xp in the pseudoautosomal region[15]. Xq appears to carry one or more genes important for fetal viability, since the 46,X,i(Xq) karyotype (isochromosome with Xq duplication and Xp deletion) is much more frequent in liveborn infants than in abortuses[12]. Both Xp and Xq appear to contain loci necessary for normal ovarian function[8].

TS is the only complete human monosomy that is compatible with postnatal life. This is probably because of the peculiar expression of X-linked genes in mammals: in XX females, one X chromosome undergoes condensation and transcriptional silencing (X inactivation) very early in embryogenesis. This process compensates for the different dosage of X-linked genes in XY and XX individuals. Thus, regardless of the number of X chromosomes present, only one X chromosome is active during most of development.

In this context, TS poses a paradox. If only one X chromosome is active, why is the absence of the second X chromosome of any phenotypic consequence? In the case of ovarian failure, as discussed previously, incomplete chromosomal pairing could cause oocyte loss. The observation that the inactive X chromosome is reactivated during oogenesis suggests another hypothesis: oocytes may require diploid dosage of one or more X-linked genes. Yet, since reactivation of the silent X chromosome is known to occur only in oocytes, other possible explanations for the somatic features of TS must be considered. First, there may be X-linked genes, expressed early in embryogenesis and before X inactivation, that are required in diploid dosage for normal somatic development. However, X inactivation in humans occurs during the first few weeks of gestation[16], while the earliest recognized TS anatomical abnormality, defective lymphatic development, is not evident until several weeks later. A second possible explanation is based on the observation that in humans, X inactivation is incomplete. Early cytogenetic data suggested that regions of the X do not undergo inactivation, and it has recently been shown that several X-linked genes, both in these regions and elsewhere, are expressed from both the active and the inactive X chromosome (reviewed in Ref. 17). It seems likely that the genes involved in Turner syndrome are X-linked genes that escape inactivation.

## The Ys have it

If an X-linked gene is required in diploid dosage for normal development, how can we account for the situation in males, who develop normally yet have only a single X chromosome? One possibility is the presence of a functionally equivalent gene on the Y chromosome. Indeed, several X-linked genes that escape X inactivation have functional Y homologs (Fig. 1). Turner genes are probably among the set of X-linked genes that both escape inactivation and have functional Y homologs, as Ferguson-Smith argued long before any such genes were discovered[18].

Support for this notion comes serendipitously from studies of sex-reversed XY females. These individuals fall into two classes. The vast majority have a Y chromosome that is grossly intact. These XY females have ovarian failure but none of the somatic features of TS. In most cases the reason for sex reversal is not known, but a few have point mutations in *SRY*, the sex-determining gene on the distal short arm of the Y chromosome (Yp). The second, much rarer, class of XY females have deletions of part of Yp; they develop as females because the deletions include *SRY*. Among over a dozen such women studied, a striking phenotype has emerged: almost all have one or more somatic features of TS. In these patients, congenital lymphedema is the most consistent feature (Refs 19, 20 and references therein; D. Page, unpublished). The most likely explanation of the phenotype of these XY females is that the Yp deletions include not only *SRY*, but also the Y-linked copy of an X–Y homologous Turner gene important in lymphatic development.

Careful characterization of these deletions using

molecular probes indicates that the sex-determining and Turner loci are genetically separable, and that a 90 kb region of Yp (interval 1A1B) is likely to contain a Turner gene[21]. One gene, *RPS4Y*, has been identified in this region[22]. Its X-linked homolog, *RPS4X*, escapes inactivation. These loci encode functionally interchangeable isoforms of ribosomal protein S4, a component of the 40S subunit (A. Zinn, unpublished). These findings are all consistent with a role for *RPS4* deficiency in the Turner phenotype. However, Just *et al.* have argued that *RPS4* deficiency plays no part in TS, because cell lines from some patients with structurally abnormal X chromosomes have two or more active copies of *RPS4X*[23]. Unfortunately, the phenotypes of these patients were not described in detail. In addition, structurally abnormal X chromosomes are associated with a high incidence of 45,X mosaicism, and it is possible that the presence of Turner features in these individuals is due to occult mosaicism. Further genetic and molecular biological studies should clarify whether *RPS4* deficiency contributes to the Turner phenotype.

## XO mice

Although the X chromosomes of all eutherian mammals appear to have approximately the same complement of genes[24], there are few good animal models of TS. A few other XO mammals have been described, some of which had TS-like abnormalities (reviewed in Ref. 25). An XO cat that died shortly after birth had aortic coarctation, and an XO pig with skeletal defects was discovered in a search for animals with abnormal karyotypes. Horses with monosomy X have been recognized that are small, infertile and have hind leg deformities[26]. However, the best characterized XO animal, the mouse, is fertile and has no anatomical abnormalities. In the mouse the only phenotypic consequences of monosomy X appear to be slight retardation of growth *in utero* and mild diminution of the reproductive life span[27,28]. The striking difference in the phenotype of monosomy X between humans and mice can be explained if only a single dose of the Turner homologs is required for normal development in the mouse. Consistent with this hypothesis, the mouse *Zfx* and *Rps4* genes have recently been shown to undergo X inactivation[29–31], unlike their human counterparts. Moreover, there appears to be no functional Y-linked or autosomal *Rps4* homolog in the mouse. Thus one active copy of the *Rps4* gene is apparently sufficient for normal development in mice.

## Conclusions

TS is a human haploinsufficiency: the phenotype is due to the presence of one rather than two copies of a gene or genes required in diploid dosage for normal development. Ironically, for a disorder of women so strongly associated with the X chromosome, clues to the molecular basis of developmental defects in TS may first come from study of the Y chromosome and X–Y homologous genes.

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## References

1 Turner, H.H. (1938) *Endocrinology* 23, 566–574
2 Ullrich, O. (1930) *Z. Kinderheilk* 49, 271–276
3 Ford, C.E. *et al.* (1959) *Lancet* i, 711–713
4 Lippe, B. (1991) *Endocrinol. Metab. Clin. North Am.* 20, 121–152
5 Simpson, J.L. and Le Beau, M.M. (1981) *Am. J. Obstet. Gynecol.* 141, 930–940
6 Burgoyne, P.S. and Baker, T.G. (1984) *Symp. Soc. Exp. Biol.* 38, 349–362
7 Cunniff, C., Jones, K.L. and Benirschke, K. (1991) *Hum. Genet.* 86, 552–556
8 Simpson, J.L. (1988) in *Turner Syndrome* (Rosenfeld, R.G. and Grumbach, M.M., eds), pp. 65–77, Marcel Dekker
9 van der Putte, S.C.J. (1977) *Virchows Arch. Abt. A Pathol. Anat. Histopathol.* 376, 233–246
10 Clark, E.B. (1984) *Teratology* 29, 355–361
11 Miyabara, S. *et al.* (1989) *Am. J. Med. Genet.* 34, 489–501
12 Hook, E.B. and Warburton, D. (1983) *Hum. Genet.* 64, 24–27
13 Hassold, T.J. (1986) *Trends Genet.* 2, 105–110
14 Mathur, A. *et al.* (1991) *Am. J. Hum. Genet.* 48, 682–686
15 Baliabio, A. *et al.* (1989) *Proc. Natl Acad. Sci. USA* 86, 10001–10005
16 Glenister, T.W. (1956) *Nature* 177, 1135–1136
17 Davies, K. (1991) *Nature* 349, 15–16
18 Ferguson-Smith, M.A. (1965) *J. Med. Genet.* 2, 142–155
19 Blagowidow, N., Page, D.C., Huff, D. and Mennuti, M.T. (1989) *Am. J. Med. Genet.* 34, 159–162
20 Levilliers, J., Quack, B., Weissenbach, J. and Petit, C. (1989) *Proc. Natl Acad. Sci. USA* 86, 2296–2300
21 Page, D.C., Fisher, E.M., McGillivray, B. and Brown, L.G. (1990) *Nature* 346, 279–281
22 Fisher, E.M. *et al.* (1990) *Cell* 63, 1205–1218
23 Just, W., Geerkens, C., Held, K.R. and Vogel, W. (1992) *Hum. Genet.* 89, 240–242
24 Ohno, S. (1967) *Sex Chromosomes and Sex-linked Genes*, Springer-Verlag
25 Lyon, M.F. (1974) *Proc. R. Soc. London Ser. B. Biol. Sci.* 187, 243–268
26 Bowling, A.T., Millon, L. and Hughes, J.P. (1987) *J. Reprod. Fertil. Suppl.* 35, 149–155
27 Lyon, M.F. (1973) *Genet. Res.* 21, 185–194
28 Burgoyne, P.S., Evans, E.P. and Holland, K. (1983) *J. Reprod. Fertil.* 68, 381–385
29 Adler, D.A. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 4592–4595
30 Ashworth, A., Rastan, S., Lovell-Badge, R. and Kay, G. (1991) *Nature* 351, 406–408
31 Zinn, A.R. *et al.* (1991) *Genomics* 11, 1097–1101

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