

by which isolates could be scored as mutator or non-mutator with >95% confidence.

Complementation tests. Strains were transformed with plasmids carrying wild-type alleles for *mutH* (plasmid pGW1899)²³, *mutL* (pGW1842)²³, *mutS* (pGW1811)²³, *uvrD* (pGT26)²⁴, *mutT* (pSK25)²⁵, *dnaQ* (pMM5)²⁶ and *dnaE* (pMK9)²⁷, according to a standard protocol²⁸. Fluctuation tests were conducted as described above, except that all strains were propagated in LB medium (containing 60 µg ml⁻¹ of ampicillin where the strain was plasmid bearing), and five parallel cultures were used per fluctuation test.

Analysis of fluctuation test data. A local computer program using a Luria–Delbrück distribution-generating algorithm²⁹ was used to calculate maximum-likelihood mutation rates from fluctuation test data. Approximate 95% confidence intervals for the mutation rates illustrated in Fig. 1 were calculated from formulae³⁰. Approximate 95% confidence limits for the mutation rates illustrated in Fig. 3 are based on the theoretical variance of the maximum-likelihood estimate of $\ln(m)$, assuming normality, where m is the expected number of mutations per culture.

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Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function

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Turner's syndrome is a sporadic disorder of human females in which all or part of one X chromosome is deleted¹. Intelligence is usually normal² but social adjustment problems are common³. Here we report a study of 80 females with Turner's syndrome and a single X chromosome, in 55 of which the X was maternally derived (45,X^m) and in 25 it was of paternal origin (45,X^p). Members of the 45,X^p group were significantly better adjusted, with superior verbal and higher-order executive function skills, which mediate social interactions⁴. Our observations suggest that there is a genetic locus for social cognition, which is imprinted⁵ and is not expressed from the maternally derived X chromosome. Neuro-psychological and molecular investigations of eight females with partial deletions of the short arm of the X chromosome⁶ indicate that the putative imprinted locus escapes X-inactivation⁷, and probably lies on Xq or close to the centromere on Xp. If expressed only from the X chromosome of paternal origin, the existence of this locus could explain why 46,XY males (whose single X chromosome is maternal) are more vulnerable to developmental disorders of language and social cognition, such as autism, than are 46,XX females⁸.

An increasing number of mammalian genes are known to be subject to genomic imprinting, defined as parental origin-specific differential gene expression⁵. No imprinted gene has yet been described on the X chromosome in humans⁹, although the *Xist* gene has been shown to be imprinted in the mouse¹⁰. We considered that it should be possible to identify the effects of an X-linked imprinted locus by comparing classes of females with Turner's syndrome. In this chromosomal disorder all, or a substantial part, of one X chromosome is missing as a result of non-disjunction (chromosome loss during gametogenesis or early cleavage of the zygote). In 70% of monosomic (45,X) Turner-syndrome females, the single X chromosome is maternal in origin¹; in the remainder it is paternal. Normal females (46,XX) possess both a maternally derived X chromosome (X^m) and a paternally derived X chromosome (X^p), one of which is randomly inactivated in any given somatic cell⁷. In monosomy X, the single chromosome is never inactivated. Differences in physical or behavioural phenotype between 45,X^p and 45,X^m Turner-syndrome subjects might therefore indicate the existence of an imprinted genetic locus.

Impaired social competence and adjustment are frequent in Turner's syndrome³, but a minority have good social skills¹¹. Intelligence is usually normal in monosomic (45,X) cases². We wished to test the hypothesis that 45,X^p females would be distinguishable from 45,X^m females by their social behaviour.

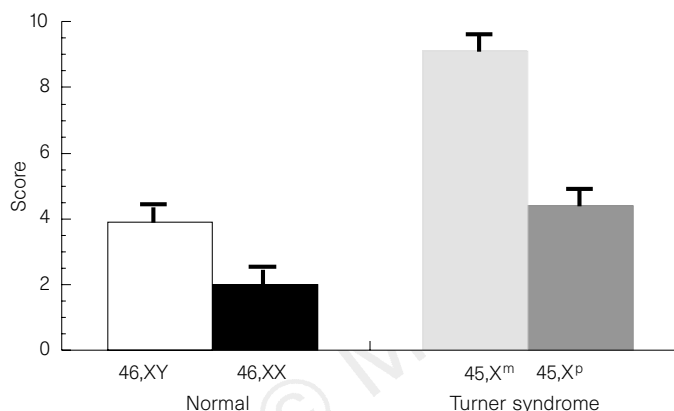
We karyotyped 80 monosomic (45,X) females and eight with deletions of the short arm of one X chromosome (46,XXp-). The

Box 1 Scale measuring social cognition

Complete the following section by circling 0 if the statement is not at all true of your child, 1 if it is quite or sometimes true of your child, and 2 if it is very or often true of your child:

- * lacking an awareness of other people's feelings
- * does not realise when others are upset or angry
- * is oblivious to the effect of his/her behaviour on other members of the family
- * behaviour often disrupts normal family life
- * very demanding of people's time
- * difficult to reason with when upset
- * does not seem to understand social skills: e.g., interrupts conversation
- * does not pick up on body language
- * unaware of acceptable social behaviour
- * unknowingly offends people with behaviour
- * does not respond to commands
- * has difficulty following commands unless they are carefully worded

Internal consistency for set of 12 questions: Standardised item alpha 0.94.



parental origin of the normal X chromosome was determined by comparing proband and parental DNA polymorphisms located on distal Xp, in a region that was deleted in both 45,X and 46,XXp-patients. Of the 80 45,X females, 25 were 45,X^p and 55 were 45,X^m, with ages from 6 to 25 years. Clinical records did not reveal any significant group differences in terms of physical phenotype or in the provision of hormone-replacement therapy¹². From a first-stage screening survey¹³ of parents and teachers, using standardized instruments¹⁴⁻¹⁶, we discovered that 40% of 45,X^m girls of school age had received a statement of special educational needs, indicating academic failure, compared with 16% of 45,X^p subjects ($P < 0.05$); the figure in the general population is just 2%. We also found that clinically significant social difficulties affected 72.4% of the 45,X^m subjects over 11 years of age (21 of 29), compared with 28.6% of 45,X^p females (4 of 14) ($P < 0.02$).

Such phenotypic variability between the two classes of monosomy X subjects could indicate the existence of an imprinted genetic locus, at which gene(s) that influence social adjustment are expressed only from the paternally derived X chromosome. On the maternally derived X chromosome, the corresponding locus would be silenced. This could account for the excess of social and learning difficulties among 45,X^m females compared with the 45,X^p variant. Pilot interviews and observations showed that 45,X^m females in particular lacked flexibility and responsiveness in social interactions. We therefore devised a questionnaire relevant to social cognition to summarize the main features of their behaviour (Box 1). Parents of our sample of Turner's syndrome females and the parents of age-matched normal male and female comparisons

Table 1 Neuropsychological test results

	Turner's syndrome		Normal	
	45,X ^m (mean ± s.d.)	45,X ^p (mean ± s.d.)	46,XX (mean ± s.d.)	46,XY (mean ± s.d.)
IQ				
Verbal	96.2 ± 15.9	106.4 ± 14.4	100.1 ± 16.7	98.6 ± 17.1
Non-verbal	79.5 ± 18.8	82.1 ± 15.9	-	-
Executive function tasks				
Behavioural inhibition	8.7 ± 7.1	5.7 ± 4.8	5.3 ± 4.1	6.8 ± 3.4
Planning ability	6.1 ± 1.7	7.4 ± 1.7	6.6 ± 1.6	7.2 ± 1.4

The 45,X^p females have significantly higher verbal IQ than 45,X^m subjects ($P < 0.02$), but neither Turner group differs significantly from the normal female comparisons. Non-verbal IQ was measured only in Turner-syndrome subjects and does not significantly distinguish the subgroups; it incorporates tests of visuospatial abilities, which are known to be specifically impaired in this condition²⁹. In all analyses using the executive function measures, age has been covaried because, unlike conventional IQ measures, these tests are not yet standardized for age. Behavioural inhibition scores (Same-Opposite World) are measured in seconds, higher scores indicating more difficulty completing the task accurately. The 45,X^m females are less competent than either 45,X^p subjects ($P < 0.02$) or normal females ($P < 0.03$). Males are less competent than normal females ($P < 0.03$). On the planning task (Tower of Hanoi), neither the two Turner subgroups nor normal males and females are significantly distinguished from one another by the mean highest level achieved.

Figure 1 Subscale scores (mean + s.e.) of questionnaire on social-cognitive impairment (Box 1). Higher scores indicate poorer social cognitive skills. The 45,X^m Turner-syndrome females score higher than 45,X^p females and both normal groups ($P < 0.0001$). Normal males score higher than normal females ($P < 0.001$); the effect size of this difference is 0.58, implying that the upper 50% of females score higher than approximately 72% of males. The ratios of mean social-dysfunction scores male:female and 45,X^m:45,X^p are very similar 2.2:1 and 2.1:1, respectively). The overall higher scores for the Turner-syndrome subjects, compared with normal females, may reflect the contribution made by visuospatial abilities to social cognition¹¹. These abilities are impaired equally in both monosomic groups. No information regarding parental origin of the normal X chromosome was made available to parents, their consultants, or members of the research team gathering these or other data.

completed the questionnaire. The results for subjects aged from 6 to 18 years of age confirm there are significant differences between 45,X^m and 45,X^p females in the predicted direction (Fig. 1). We found that 45,X^m subjects obtained significantly higher scores than 45,X^p females on our measure of social-cognitive dysfunction. Normal boys also obtained significantly higher scores on the questionnaire than did normal girls, indicating poorer social cognition (Fig. 1). The magnitude and direction of this difference are compatible with the hypothesis that there is an imprinted locus on the X chromosome that influences the development of social cognitive skills (although the finding is of course also compatible with other explanations of gender differences in behaviour¹⁷). If the putative locus only expressed a gene (or genes) from the X chromosome of paternal origin (X^p), we would expect a tendency for normal females to have superior social cognitive skills than males. Because males (46,XY) invariably inherit their single X chromosome from their mothers, the genetic locus would be silenced. In contrast, the gene(s) would be expressed from X^p in approximately half of the cells of normal females if it were inactivated, and from all cells if it escaped X-inactivation⁷.

We hypothesized that an imprinted X-linked locus, either without a Y-linked homologue or with a Y homologue showing a lower level of expression, could also explain why males are markedly more vulnerable than females to pervasive developmental disorders affecting social adjustment and language, such as autism⁸. Accordingly, 45,X^m Turner females should be exceptionally vulnerable to disorders of language and social adjustment that are usually more common in males. Consistent with this hypothesis, we identified

Table 2 Cytogenetic and molecular information

Patient	Karyotype	Breakpoints between	
		proximal	distal
85/5142	46,X,del(X)(qter → p22:)	DXS8036	DXS1053
95/4894	46,X,del(X)(qter → p21.2:)	DXS1036	DXS985
95/4247	45,X[33]/46,X,del(X)(qter → p21.2:)[17]	DXS1036	DXS985
95/3557	45,X[32]/46,del(X)(qter → p22.11:)[68]	DXS8039	DXS8049
91/219	46,X,del(X)(qter → p11:)	DXS1208	DXS1055
96/863	46,X,del(X)(qter → 11.2:)	DXS8062	DXS1003
96/8266	46,X,del(X)(qter → p11.23:)	DXS423E	UBE1
96/5509	45,X[9]/46,X,der(X)del(X)(p21.2 → pter)inv(X)(p21.2 q22.1)[166]	DXS1067	DXS8039

Cytogenetic analysis was performed by standard techniques and the chromosomes were examined in a minimum of 100 cells. To map the position of the breakpoints in the deleted X chromosomes, DNA from probands and parents was tested using PCR amplification of polymorphic microsatellite repeat sequences located along the length of Xp. For DXS423E and UBE1, the status of the locus in the proband was determined by fluorescence *in situ* hybridization (FISH) using metaphase chromosome spreads. The breakpoints lie between the most distal non-deleted marker and the most proximal deleted marker.

three Turner-syndrome females with autism (meeting ICD-10 criteria)¹⁸, out of the total unbiased sample assessed personally in the course of this investigation (3.75%). All three females had retained a normal maternal X chromosome. The population prevalence of autism is less than 1 per 10,000 females⁸.

We then sought corresponding differences in the results of neuropsychological testing of both normal and Turner-syndrome subjects. We found that 45,X^P females were significantly superior in verbal intelligence to 45,X^m females (Table 1). Verbal IQ was negatively correlated with the social dysfunction score in the sample as a whole ($r = -0.41$, $P < 0.002$). Both monosomic Turner subgroups were equivalently impaired in non-verbal (including visuospatial) abilities.

We subsequently performed several more focused neuropsychological assessments. First, although we had found that verbal abilities were moderately good predictors of social cognition, we hypothesized that higher-order cognitive skills would be better predictors. These are not measured directly by conventional intelligence tests. The executive functions of the prefrontal cortex⁴ exert an important influence on social interactions, and include skills that allow for the development of strategies of action and the inhibition of distracting impulses when striving towards a goal. Developmental disorders of social adjustment and language are associated with impairment in measures of executive function¹⁹. Many monosomic Turner-syndrome subjects have executive-function deficits². We also predicted that 45,X^P females would perform better than 45,X^m females in tests of executive function skills. Finally, we predicted that abilities in which 45,X^P subjects were superior to 45,X^m subjects would also distinguish normal females from males, and in the same direction.

We chose tests of both planning ability (Tower of Hanoi) and behavioural inhibition (Same/Opposite World). The results were consistent with the initial hypothesis. Although the social-cognitive impairment score correlated independently with verbal IQ and with both measures of executive function, only planning ability ($r = -0.4$; $P < 0.006$) and behavioural inhibition ($r = -0.37$; $P < 0.015$) retained significance when the three variables were forced into a multiple regression analysis. The second prediction was partly confirmed: there were significant differences between 45,X^P and 45,X^m subjects in terms of the behavioural inhibition task, although not on the test of planning ability. The third prediction was fully confirmed (Table 1): there was no significant difference in the highest level achieved on the planning task between normal males and females. However, on the behavioural-inhibition task, normal females were superior to males and their mean scores were very similar to those of the 45,X^P subjects. Previous reports have noted gender differences on inhibition tasks, and they have been conceptually linked to corresponding differences in social behaviour²⁰.

We then attempted to map the putative imprinted locus, provisionally, by studying eight females, ascertained as part of the Turner-syndrome project, who had large terminal deletions of the short arm

of the paternally derived X chromosome (a 46,X^mX^P p-karyotype). These deletions all extended to a point proximal to the *MLS* gene at Xp22.3 (Table 2); the paternal X chromosome was consequently preferentially inactivated⁶. On examination, no-one in this series was found to have any significant learning difficulties. The mean verbal intelligence score for the group was 103.4 (s.d., 13.7), and their mean non-verbal IQ was 93 (s.d., 11.2). Their mean social dysfunction score was 3.75 (s.d., 3.1), a value very similar to that of the 45,X^P subjects (Fig. 1). We drew the following conclusions from these data. First, the imprinted locus had not been deleted on the structurally abnormal paternal X chromosome, and so it must lie on Xq or on Xp closer to the centromere than, *UBE1* at Xp11.23. Second, the imprinted locus was not subject to X-inactivation, or the preferentially inactivated, partly deleted chromosome would not have expressed it. We already know of several genes that escape X-inactivation²¹.

We expected that isochromosomes of the long arm of the X chromosome [i(Xq)] would help in the mapping of the putative imprinted locus. However, virtually all i(Xq) chromosomes have been shown to contain proximal Xp sequences²². Duplication and consequent trisomy of the long arm of the X chromosome further complicate the interpretation of correlations between phenotype and genotype. Accordingly, this approach did not provide unambiguous evidence to assist in the deletion mapping of the locus.

An imprinted locus is not the only possible explanation for our findings. Among the 45,X^P females, there may have been a greater degree of cryptic mosaicism (with a normal 46,XX cell line) than among those who were 45,X^m. Some degree of mosaicism in apparently monosomic females may be essential for the fetus to avoid spontaneous abortion²³. We examined both blood and cheek cells, tissues of mesodermal and ectodermal origin, respectively, and found two cryptic mosaics, but both were from the 45,X^m group.

Males are substantially more vulnerable to a variety of developmental disorders of speech, language impairment and reading disability, as well as more severe conditions such as autism⁸. Our findings are consistent with the hypothesis that the locus described, which we propose to be silent both in males and 45,X^m females, acts synergistically with susceptibility loci elsewhere on the genome to increase the male-to-female prevalence ratio of such disorders. Our data on normally developing children suggest it may also exert an effect on social and cognitive abilities in the normal range. These preliminary findings could thus provide evidence for the evolution of an imprinted X-linked locus that underlies the development of sexual dimorphism in social behaviour¹⁷. □

Method

Subjects. This study, which was approved by the local hospital ethics committee, involved 88 females with Turner's syndrome (80 monosomic and 8 partial X-chromosome deletions; age range, 6–25 years). They were selected from a national survey of Turner's syndrome and from records of the Wessex Regional Laboratory. The mean age of the 45,X^m females was 162.3 months (s.d., 57.6), that of the 45,X^P females was 164.5 months (s.d., 57.7), and that of

the Xp- females was 185.8 months (s.d., 74.9). All subjects were healthy, with no significant neurological disease. Females with an Xp- chromosome were all referred for investigation because of short stature in middle childhood, with one exception who was karyotyped at birth. Neuropsychological test results are presented for subjects with verbal IQs ≥ 65 (three 45,X^m subjects and one 45,X^p subject had verbal IQs that fell out of range). Parents rated 70 normal males and 71 normal females (age range, 6–18 years) on the social-cognition scale. The neuropsychological test battery was used to assess 68 normal males and 91 normal females (age range, 6–25 years). Verbal IQs were in the range 65–151. All normal comparison subjects were recruited from urban and suburban schools (6–18 years) and from hospital staff (18–25 years).

Behavioural and cognitive measures. Initial screening was conducted by postal questionnaires using a well-standardized set of instruments^{14–16}. These were completed by parents, teachers and the Turner-syndrome subjects themselves (11 years and over). The social cognition questionnaire (Box 1) was completed by parents only. In a survey of 175 Turner-syndrome subjects for whom we obtained parental ratings on two occasions, a mean of 2.7 years apart, the intraclass correlation coefficient was 0.81 ($P < 0.01$). Scores correlate with the self-rated social problem subscale of the YSR¹⁶ 0.58 ($P < 0.002$), with the teacher rating on the TRF¹⁵ 0.54 ($P < 0.001$), and with the parent-rated CBCL¹⁴ 0.69 ($P < 0.001$). The range of scores was 0–23 in the Turner-syndrome sample and 0–21 in the normal sample (maximum score of 24). The CBCL¹⁴ was completed by 70 parents, the YSR¹⁶ was completed by 40 subjects over 11 years of age, and the TRF¹⁵ was completed by 45 teachers. Clinical significance of social problems was estimated according to clinical *T* scores^{14–16}. Measures of cognition included the Wechsler Intelligence Scales for Children (WISC III-UK)²⁴ and the Wechsler Adult Intelligence Scales-Revised (WAIS-R)²⁵. The behavioural inhibition task was the Same-Opposite World subtest from the Test of Everyday Attention for Children²⁶. This yields a time measure that ascertains the difference in latency for a subject responding to a series of stimuli on a task of sequential responses, which are named both as they appear and then opposite to their appearance. The subject reads a random series of numbers (1 and 2) saying 'one' to 1, and 'two' to 2. The subjects then repeat the task on a new series, but this time they have to inhibit the prepotent response and instead say 'two' to 1, and 'one' to 2, correcting any errors before proceeding. Test-retest reliability on a sample of 70 normal children gave an intraclass correlation coefficient of 0.62 ($P < 0.001$). The Tower of Hanoi task was based on the procedure described previously²⁷. It was scored according to the most complex level of the problem the child could solve reliably. Test-retest reliability gave an intraclass correlation coefficient for the highest level achieved of 0.45 ($P < 0.001$), which is in line with expectations for a test that makes novel demands of this nature²⁸.

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Molecular evidence for an ancient duplication of the entire yeast genome

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Gene duplication is an important source of evolutionary novelty^{1,2}. Most duplications are of just a single gene, but Ohno¹ proposed that whole-genome duplication (polyploidy) is an important evolutionary mechanism. Many duplicate genes have been found in *Saccharomyces cerevisiae*, and these often seem to be phenotypically redundant^{3–7}. Here we show that the arrangement of duplicated genes in the *S. cerevisiae* genome is consistent with Ohno's hypothesis. We propose a model in which this species is a degenerate tetraploid resulting from a whole-genome duplication that occurred after the divergence of *Saccharomyces* from *Kluyveromyces*. Only a small fraction of the genes were subsequently retained in duplicate (most were deleted), and gene order was rearranged by many reciprocal translocations between chromosomes. Protein pairs derived from this duplication event make up 13% of all yeast proteins, and include pairs of transcription factors, protein kinases, myosins, cyclins and pheromones. Tetraploidy may have facilitated the evolution of anaerobic fermentation in *Saccharomyces*.

We searched systematically for duplicated regions^{6,7} in the complete yeast genome⁸ by using BLASTP⁹ amino-acid sequence similarity searches of all yeast proteins against one another, and plotted the results on dot matrices. Duplicate regions are visible as a diagonal series of 'hits' with conserved gene orientation. In the example shown in Fig. 1, three separate diagonals indicate three distinct regional duplications between chromosomes X and XI. Within each region the homologues are interspersed with genes that are not now duplicated. We propose that this is the result of random deletion of individual duplicated genes from one or other chromosome subsequent to the initial duplication of the whole region.

In the whole genome, 55 duplicate regions were identified