Three patients with a 45,X/46,X,psu dic(Xp) karyotype

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Abstract
Few cases of isochromosomes for the short arm of the X have been reported and all are dicentric with variable portions of the long arms interposed between the two centromeres. This paper reports three cases of complete short arm duplication of one X chromosome in unrelated female patients. All patients also have a 45,X cell line and present with some characteristic features of Turner syndrome. We used conventional cytogenetics, in situ hybridisation, and molecular genetics to describe all three structurally abnormal chromosomes and the parental origin of two of them. We briefly discuss the “inactivation enhancement” theory; however, any genotype-phenotype correlation is complicated by the presence of the 45,X cell line.

Keywords: psu dic(Xp); Turner syndrome

Although isochromosomes for the long arm of the X chromosome are the most common structural abnormality of the X in Turner syndrome patients,1,2 isochromosomes for the short arm of the X are rare. Indeed monocentric constitutional i(Xp)s with no long arm material appear not to exist, presumably because of the lack of the XIST locus and their consequent failure to inactivate. Isochromosomes can originate either in meiosis or in mitosis. Wolff et al. suggested that the majority of i(Xq)s arise not by centromere misdivision but via breakage and reunion of susceptible regions of the short arm. The resulting i(Xq)s would be dicentric, leaving unstable acentric Xp fragments to disperse. It has also been postulated that an isodicentric Xp is only viable if the XIST locus on q13 is retained in at least one of the deleted long arms.3 Indeed our patients and all the published cases we reviewed have Xq breakpoints either in or distal to q13 (table 1).

Case reports
CASE 1
At the time of clinical examination the patient was 9 years old; her mother and father were 29 and 50 years old respectively when she was born.

Physical findings
Her height was 120.9 cm (<3rd centile but 75th-90th centile for Turner syndrome). She had received growth hormone since the age of 6 years. Her weight was 22.5 kg (5th centile).

She had a triangular face with severe bilateral ptosis and wide set eyes (inner canthal distance 35 mm, 97th centile), hypermetropia, low posterior hairline, and low set, posteriorly rotated ears. She had one hairy pigmented naevus. She also had bilateral clinodactyly and broad thumbs but normal metacarpal lengths. Other clinical features included oesophageal reflux, constipation, and eczema.

Psychological findings
She was tested on the Wechsler Intelligence Scale for children when she was 6 years 11 months old. Her outcome was: verbal IQ=80, performance IQ=82, and full IQ=79.

The following information was obtained from interviewing her parents. At school she has special educational needs. She has violent temper tantrums and runs away from her parents. Owing to her oppositional behaviour she has been seen as a child psychiatrist. She is a very disinhibited child, with little sense of danger and an impulsive pattern of behaviour. She is over friendly with strangers and has little regard for her own safety. Her parents are attempting to obtain a statutory assessment for her. She lacks the appropriate use of social smiling, but has some use of other facial expressions. She dislikes change within an established environment, for example, a chair moved from its usual place.

CASE 2
The second patient was 16 years old at the time of clinical examination; her mother and her father were 21 and 26 years old respectively when she was born.

Physical findings
Her height was 150.5 cm (3rd centile but 90th-95th centile for Turner syndrome). She had started growth hormone treatment at the age of 13. Her weight was 63.5 kg and her body mass index was 42.2 (non-obese is <27.7). She had a broad face with hypertelorism (75th-97th centile), a right sided unilaterial epicanthic fold, wide set eyes, high arched palate, and low posterior hairline. She had an excess of pigmented naevi. She had oedema of the fingers and toes which appeared at the age of 8 years and was not present at birth. All digits were long with ulnar deviation of the distal phalanx of the middle fingers. Her chest was broad with widely spaced, pale nipples. She had one small ovary shown on pelvic ultrasonography. Menarche occurred at the age of 15 after two years of ethinyl oestradiol treatment. She subsequently suffered menorrhagia, which was controlled with transdermal...
She suffers from hypertension but has no evidence of structural cardiac, renal, or vascular disease.

**Psychological findings**

She was tested on the Wechsler Adult Intelligence Scale when she was 16 years 9 months old. Her outcome was: verbal IQ=103, performance IQ=86, and full IQ=94.

The following information was obtained from interviewing her parents. At school she has a statement of special educational needs.

She is very immature for her age. Her spoken language has no melody and she speaks very loudly, which can be embarrassing in public places. She lacks the appropriate use of social smiling but she uses other facial expressions, but not always appropriately. She does not use conventional gestures. She sets the agenda for conversation and resists changes in the topic. She has a tendency to repeat back what others say exactly as it was spoken. When younger she did not play imaginative games, either alone or with friends. She has difficulty making friends.

### Table 1  Review of reported psu dic (Xp) cases

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Age</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Menstrual status</th>
<th>General features</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,psu dic(X)(q13) [60]</td>
<td>21</td>
<td>143.5</td>
<td>51</td>
<td>Obese</td>
<td>Breast and external genitalia consistent with Tanner stage III development. Large hypopigmented macula of irregular shape on forehead. Bone age 14. Small uterus, ovaries could not be identified. Triangular face with bilateral, severe ptosis and wide set eyes, hypertelorism, low posterior hairline, and low set, posteriorly rotated ears. One hairy pigmented naevoid. Bilateral clinodactyly and broad thumbs. Eczema and oesophageal reflex. Special education needs. Psychological problems</td>
</tr>
<tr>
<td>45,X[98]/46,psu dic(X) (pter−q13.3:q13.3−pter) [32] Case 1</td>
<td>9</td>
<td>120.9</td>
<td>22.5</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>46,psu dic(X) (pter−q13.2:q13.2−pter) [28/16] L/F?</td>
<td>28</td>
<td>142</td>
<td>48 Slightly</td>
<td>Obese</td>
<td>Slightly breast development, infantile mammary. Sparse pubic hair. Infantile external genitalia, streak ovaries, no uterus</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter)</td>
<td>17</td>
<td>157</td>
<td>Slightly</td>
<td>Obese</td>
<td>Gonadal infantilism. Facial pigmented naevoid, short neck, broad chest with hypoplastic, widely spaced nipples. Brachymetacarpus and brachymetatarsy IV-V. Discrete oedema of dorsum of the hands</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>16</td>
<td>146</td>
<td>50</td>
<td></td>
<td>Slight webbing of the neck. Scaity axillary and pubic hair. Poor breast development. Shield chest with widely spaced nipples. External genitalia infantile. Small uterus, bilateral streak gonads. Hypertensive for 2 years</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>16</td>
<td>144</td>
<td>52 Obese</td>
<td></td>
<td>High, rounded palate, short, webbed neck, low posterior hairline. Bilateral lymphoedema, puffiness of lateral nail folds. Mild cubitus valgus. Subaortic stenosis, bicuspid aortic valve, coarctation of the aorta. Eczema</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>11</td>
<td>128.3</td>
<td>53.8</td>
<td></td>
<td>Broad face with hypertelorism, unilateral epicanthic fold, wide set eyes, high arched palate, low posterior hairline. Excess of pigmented naevoid. Oedema of the fingers and toes. Broad chest, widely spaced, pale nipples. Only one small ovary. Hypertension.</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>11</td>
<td>150.5</td>
<td>63.5 Obese</td>
<td></td>
<td>Menarche age 15, almost regular menstrual cycles. Secondary sexual development normal but laparoscopy showed normal uterus and bilateral streak gonads. Normal intelligence</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>30</td>
<td>157</td>
<td>75 Obese</td>
<td>SA from age 28</td>
<td>Absent axillary hair, scanty pubic hair, infantile external genitalia, no palpable ovaries, barely palpable uterine. Slight breast development</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>16</td>
<td>155</td>
<td>56.5 Slightly</td>
<td>Obese</td>
<td>Large feet and hands. No breast development. Scanty pubic and axillary hair, normal female genitalia but small cervix and retroverted uterus. Streak gonad on right side, no gonadal tissue on left side</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>17</td>
<td>171.5 Large</td>
<td>71.7</td>
<td></td>
<td>Broad shoulders and small breasts. Pubic and axillary hair normal. Barely palpable uterine. Neurological findings normal; verbal IQ 64, performance IQ 50, total IQ 54</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>40</td>
<td>168</td>
<td>82 Obese</td>
<td></td>
<td>No Turner stigmata present. Small antverted uterus. No gonads palpable</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>21</td>
<td>182</td>
<td></td>
<td></td>
<td>No breast tissue. Scanty axillary and pubic hair. Minor external ear anomaly. Small uterus, streak gonads</td>
</tr>
<tr>
<td>46,diric(X)(pter−q2102−pter) [45/5%]</td>
<td>23</td>
<td>173</td>
<td>106 Obese</td>
<td></td>
<td>Pale stria on abdomen skin. No axillary hair and pubic hair sparse. Little mammary tissue. Normal external genitalia, uterus and ovaries not palpable</td>
</tr>
</tbody>
</table>

L - lymphocytes; F - fibroblasts; PA - primary amenorrhoea; SA - secondary amenorrhoea.
inhibition and appears over friendly or over familiar on first acquaintance. She plays imaginative games alone but not with her peers. She has some friends but her friendships appear immature for her age.

Results
CYTOGENETIC ANALYSIS
Conventional cytogenetic preparations were made from PHA stimulated peripheral blood lymphocytes and, in order to study late replication, 30 μg of BrdU was added to some cultures six hours before harvest.

Analysis of G banded metaphases from cases 1, 2, and 3 all showed the presence of two cell lines, one with a 45,X complement and one with 46 chromosomes with a normal X and a pseudodicentric chromosome. This appeared to consist of two complete short arms of the X chromosome separated by long arm material. Standard C banding technique confirmed in all three cases that the abnormal chromosome was dicentric with only one primary constriction (presumably the active centromere) (fig 1A, B, C).

The karyotypes were interpreted as 45,X[p15]/46,X,psu dic(X)(pter→q13::q13→pter) [32] for case 1, as 45,X[73]/46,X,psu dic(X)(pter→q22.3::q22.3→pter) [27] for case 2, and as 45,X[4]/46,X,psu dic(X)(pter→q22.1::q22.1→pter) [96] for case 3. All three different pseudodicentric chromosomes were shown to be late replicating when metaphases from BrdU cultures were analysed.

MOLECULAR CYTOGENETICS
In situ hybridisation using standard techniques19 was applied to each of the abnormal chromosomes. Probe DXZ2, which specifically hybridises to the X centromere alaphoid repeat sequences and cosmid c100H0130 which maps to the XIST locus on proximal Xq, were used on the three patients. On the abnormal chromosome from case 1, two signals were seen for probe DXZ2 but only one strong signal for the XIST probe, indicating the presence of only one XIST locus or alternatively of two XIST loci very close together (fig 2A). However, two clear signals were seen for both probe DXZ2 and the XIST probe (fig 2B, F) on the abnormal chromosome in patients 2 and 3.

In addition, the following probes, 811-d-11, 657-e-12, and 923-d-3, which map to Xp22, Xq22.3, and Xq24 respectively, have been applied to cases 2 and 3 to confirm conventional cytogenetic breakpoints (fig 2G, D, E, G).

MOLECULAR ANALYSIS
DNA was extracted by a salt precipitation technique19 from peripheral blood from probands 1 and 2 and from buccal cells from their parents. The parental origin of the normal X chromosome was determined by PCR amplification of polymorphic microsatellite repeat sequences using standard PCR conditions.20 For primers KAL and DXS451 one primer of each set was radioactively end labelled, the products separated on a 6% polyacrylamide gel,
Figure 2  In situ hybridisation on structurally abnormal chromosomes. (FISH). Normal X on the left of each picture. Centromere probe (DXZ2) green, all other probes red. (A) Patient 1 shows a strong signal with probe c100H0130 (XIST locus), indicating the presence of one locus or two loci close together. With the same probe (B) patient 2 and (F) patient 3 show two signals indicating the presence of two XIST loci. (C) Patient 2 and (G) patient 3 both show two signals with probe 811-d-1 (Xp22) at each end of the pseudodicentric chromosome, confirming the presence of a duplicated Xp region. (D) Patient 2 shows a signal with probe 657-e-12, indicating that the Xq22.3 region is retained on the abnormal chromosome, while (E) patient 2 shows a signal with probe 923-d-3 (Xq24 locus) only on the normal X chromosome, indicating that the breakpoint for patient 2 is between Xq22.3 and Xq24.

and the results visualised by autoradiography. For primers DXS548, FRAXAC1, FRAXAC2, and DXS1691 one primer of each set was fluorescently labelled and the result analysed using an ABI 373 automated DNA sequencer. Details of the primer sets used are available on the GDB.

Since the normal X chromosome is present in 100% of cells and the structurally abnormal X in a proportion of cells (25% in case 1, 27% in case 2), it should be possible to determine the parental origin of the normal X chromosome using primers located on Xp. The allele represented on the normal X chromosome should be detectable at twice the level of that on the structurally abnormal X chromosome. In case 1, the increased dosage of one allele at locus DXS451 allowed the assignment of parental origin of the normal X. However, in case 2 the difference in dosage between the two alleles detected at the two loci, KAL and DXS451, was not convincing and further loci, DXS548, FRAXAC1, FRAXAC2, and DXS1691, located at Xq27-q28 were tested. These loci are not present on the structurally abnormal X and the allele detected in the proband represents the normal X chromosome. In both cases the normal X was of maternal origin.

Discussion
In our three patients, as in most of the published cases, the dicentric chromosomes on the short arm of the X chromosome are pseudodicentric,
with only one active centromere (table 1). De la Chapelle et al. first suggested that some isochromosomes may be mitotically unstable and therefore give rise to a 45,X cell line. The degree of instability would depend on the amount of material between centromeres, so that a stable i(X) would be possible only when the centromeres are very close and acting in coordination or when, if relatively distant, one is rendered effectively inactive. Wolff et al. observed in i(Xp) a direct correlation between the distance between centromeres and the presence of a 45,X cell line, suggesting that a structurally dicentric i(X) would originally include two functionally active centromeres. These can form an anaphase bridge causing the isochromosome to break during early division of the zygote, thus giving rise to a 45,X cell line.

The remaining cells which still contain the isochromosome would survive only if one of the centromeres became inactive, thus conferring stability.

Table 1 summarises the published reports of pseudodicentric i(Xp) together with data on our patients. All patients, except two of ours who have not yet been examined in this respect, have gonadal dysgenesis. This may be because of the presence of a 45,X cell line in the gonads or the absence of a region of Xq that is critical for ovarian development. Tharapel et al. assumed a locus for ovarian development on Xq26-27, while it has often been proposed that a region between Xq13 and Xq23 contains genes essential for normal ovarian function. However, it is well established that genes on Xp are also required for ovarian development and recently Jones et al. suggested that the DFFRX locus on Xp11.4 may be a candidate for gonadal dysgenesis. The general difficulty of pairing in the early stages of meiotic prophase and the consequent degeneration of the oocytes may be an alternative underlying cause of gonadal dysgenesis in cells containing single or structurally abnormal chromosomes.

The data in table 1 seem to suggest a degree of correlation between short stature and position of Xq breakpoints, with people with more distal breakpoints being quite tall. Geerkens et al. in analysing Xq deletions observed the same trend.

Some authors support the idea that the deletion of a portion of the long arm of the X chromosome can cause alteration of chromatim structure and induce spreading of the inactivation process to those genes on the inactive chromosome which are usually active. It has been postulated that the level of "inactivation enhancement" is correlated with the size of the deletion, with larger deletions being more effective in determining the degree of inactivation which might reach the pseudautosomal region. Geerkens et al. further hypothesised that this additional inactivation along the X chromosome is not obligatory but can vary among patients with the same variable pattern of the spreading of X inactivation as seen in X-autosome translocations.

The silencing of those regions of the X chromosome which are usually active would effectively cause monosity for genes supposedly needed in double dose, for example, the "Turner gene(s)"); however, for any imprinted gene the effect would depend on the parental origin of the abnormal X chromosome. If the imprinted gene was only expressed from the paternal X chromosome, the enhanced inactivation of an abnormal X' chromosome would cause nullisomy for the gene. However, enhanced inactivation of an abnormal X' chromosome would cause no change because the gene would be expressed from the normal X' chromosome.

Our cases 1 and 2 support the hypothesis: both patients are of relatively short stature, in spite of having in one cell line, three pseudoautosomal regions and therefore being trisomic for the stature gene in distal Xp, and they have some Turner syndrome features. The pseudodicentric X chromosome in patients 1 and 2 is of paternal origin but the origin in patient 3 is unknown. All our patients showed severe emotional and behavioural problems, suggesting that genes governing some aspects of behaviour may be deleted or silenced on the structurally abnormal chromosome.

However, the presence in our patients, as in most other cases, of a 45,X cell line makes any correlation with the "inactivation enhancement" hypothesis difficult. We could postulate that although our patients are trisomic for those portions of the X chromosome which normally remain active on the inactive X and disomic for any imprinted gene, it is the 45,X cell line and its distribution among tissues at different developmental stages that explains the Turner phenotype. Furthermore, two doses of an imprinted gene may have an adverse dosage related effect. However, it would be instructive to measure the level of expression of known active genes along the arm of the abnormal X chromosome to verify their inactivation status and thus to test the "inactivation enhancement" theory.