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

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Abstract

We report on a sex reversed Japanese child with a 46,X,Yp + karyotype, minor dysmorphic features, and no testicular development. The Yp + chromosome was derived by translocation of an Xp fragment (Xp21→Xp22.3) to Yp11.3. This has resulted in deletion of distal part of the Y chromosome pseudoautosomal region (DXYS1-telomere) and duplication of the X specific region (DXS84-PABX) and proximal part of the pseudoautosomal region (MIC2-DXYS17). No deletion of the Y specific region was detected nor was any mutation found in SRY. Cytogenetic analysis suggests that the proximal part of the Xp fragment is the most distal part of the short arm of the Yp + chromosome (Xp21→Xp22.3::Yp11.3→Yptel). No chromosomal mosaicism was detected.

These results are similar to previous reports of sex reversal in four subjects with a 46,Y,Xp + karyotype. We conclude that the sex reversal is a direct, or indirect, consequence of having two active copies of the distal part of Xp and may indicate the presence of a gene(s) which acts in the testis determination or differentiation pathway.

Sex determination in man, and other mammals, is chromosomally based: males have an X and a Y chromosome, females have two X chromosomes. Correlation between phenotype and karyotype in subjects with unusual sex chromosome constitutions has shown that the Y chromosome carries a gene, TDF (testis determining factor), essential for testis formation and male sex determination. Recent molecular analysis of the genomes of XX males and XY females has provided strong evidence that the Y located gene SRY is TDF, and this has been confirmed by transgenic mice experiments. However, not all cases of sex reversal can be explained by alterations in SRY and it would be predicted that both 'gain of function' and 'loss of function' mutations in other genes in the sex determination pathway may cause sex reversal. Another theoretical possibility is that dosage of critical genes may affect sex determination. In this report, we describe a patient with sex reversal and an extra fragment of Xp, which is translocated to Yp.

Case report

A phenotypic female child was born to non-consanguineous parents at 40 weeks of gestation after an uncomplicated pregnancy and delivery. The parents and older sister were clinically normal. The birth length was 48 cm and weight 2900 g. From birth, she was admitted frequently to a local hospital with recurrent fever.

At 2 years 3 months of age, the patient was referred to Keio University Hospital because of high fever, lymphadenopathy, and erythematous rashes. Physical examination showed a weak child with marked hypotonia. External genitalia were feminine and there was no clitoromegaly or labial fusion. Dysmorphic features included frontal bossing, antimongoloid slant, large, low set ears with thick auricular folds, and cleft palate. Psychomotor development was severely retarded. Laboratory studies showed anaemia (Hb 9.5 g/dl), thrombocytopenia (7.1×10^5/μl), positive antinuclear antibody (2560 X, homogeneous pattern) and anti-DNA antibody (320 X), decreased complement level (C3 0.3 g/l), and immunoglobulin A (IgA) deficiency (<0.01 g/l). After a diagnosis of systemic lupus erythematosus (SLE),
The copy number of each locus.

<table>
<thead>
<tr>
<th>Locus (probe)</th>
<th>Enzyme</th>
<th>RFLP</th>
<th>Patient</th>
<th>Sister</th>
<th>Father</th>
<th>Mother</th>
<th>Reference</th>
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<tr>
<td>DXYS14 (29C1)</td>
<td>TaqI</td>
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<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>9</td>
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<tr>
<td>DXYS15 (13D)</td>
<td>TaqI</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>DXYS17 (601)</td>
<td>TaqI</td>
<td>+</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>11</td>
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<tr>
<td>M12C (19B)</td>
<td>TaqI</td>
<td>-</td>
<td>3 (1-47)</td>
<td>2 (1-08)</td>
<td>2 (1-05)</td>
<td>2 (0-98)</td>
<td>12</td>
</tr>
<tr>
<td>PABX (H0.2)</td>
<td>SstI</td>
<td>-</td>
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<td>2 (1-24)</td>
<td>1 (0-65)</td>
<td>2 (1-47)</td>
<td>13</td>
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<tr>
<td>DXS143 (Duc56)</td>
<td>BclI</td>
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<tr>
<td>DXS9 (RCS)</td>
<td>TaqI</td>
<td>-</td>
<td>2 (1-05)</td>
<td>2 (1-20)</td>
<td>1 (0-60)</td>
<td>2 (1-02)</td>
<td>15</td>
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<td>DXS43 (pD2)</td>
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<td>-</td>
<td>2 (0-85)</td>
<td>2 (0-80)</td>
<td>1 (0-38)</td>
<td>2 (0-78)</td>
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<td>2 (1-10)</td>
<td>1 (0-49)</td>
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<td>ZFX (pPB, pMPF-1)</td>
<td>EcoRI</td>
<td>-</td>
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<td>2 (1-19)</td>
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<td>-</td>
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<td>1 (0-09)</td>
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<td>-</td>
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<td>OTc (cDNA probe)</td>
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<td>DXS7 (L1.28)</td>
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<td>2 (0-37)</td>
<td>1 (0-74)</td>
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</table>

The copy number of each locus was determined by the presence of RFLP (DXYS14, DXYS15, DXYS17, and DXS143) or by the comparison of band intensity (other loci). The values in parentheses represent the ratio of the band intensity between each locus and autosomal TK gene. The loci are arranged from telomere to centromere.

she received corticosteroid therapy. At 2 years 6 months of age, a human chorionic gonadotrophin test (3000 IU/m²/dose i.m for three consecutive days) was done, yielding no testosterone response (< 0.5 → < 0.5 nmol/l). At three years of age, she exhibited persistent SLE-like symptoms and died of cachexia.

Macroscopic examination of the internal genitalia at necropsy showed that Mullerian duct derivatives (fallopian tubes, uterus, and upper portion of vagina) were normally developed and Wolffian duct derivatives were absent. Streak gonads were observed in the place of ovaries. Other organs were normal. Microscopic examination of the gonads showed ovarian stroma and gonadoblastoma (fig 1). Testicular development and ovarian germ cells were absent. In the extraglandular organs, severe necrotising vasculitis characteristic of polyarteritis nodosa was observed.

Mutational analysis of SRY

SRY of this patient was subjected to both single strand conformational polymorphism (SSCP) analysis2526 and DNA sequencing (fig 2). For SSCP analysis, polymerase chain reaction (PCR) amplifications were performed with primers XES10 and XES11 flanking the SRY open reading frame,1 generating a 778 bp fragment. Amplifications were performed with 0.5 to 1.0 μg genomic DNA under standard conditions in a reaction volume of 50 μl. After an initial incubation of two minutes at 94°C, reactions were cycled for 80 seconds at 94°C, 1.5 minutes at 60°C, and 2.5 minutes at 71°C for 32 cycles. Primer sequences were: XES10 5'-GAGCTCGAGAATTG- GTGTTGAGGGCGAGAATGC-3' and XES11 5'-GAGCTCGAGAATTGTGACCAATGGTTACCAGGATTGC-3'. Two-fifths of the amplified DNA was fractionated on a 0.6% agarose gel. The amplified fragment was excised from the gel and melted and 1 μl of this DNA was reamplified with primers XES7 and XES2, generating a 609 bp fragment. PCR

Figure 2. A schematic map of SRY. The boxed region represents the open reading frame of SRY extending from nucleotide positions 354-1022 of the genomic clone of pY313. The positions of oligonucleotide PCR primers are indicated by horizontal arrows. The positions of the restriction endonuclease sites DdeI (D), HpaII (Hp), XbaI (X), HinfI (H), TaqI (T), and PstI (P) are marked between the primers XES7 and XES2. The positions of these sites are: D, 336 and 672; Hp, 495; X, 648; H, 654; T, 798; and P, 874.
Figure 3  The X and Yp+ chromosomes of the patient by high resolution G banding.

Figure 4  Southern blot analysis (P = patient, S = sister, F = father, M = mother).  (A) TaqI digests probed with 29C1. The paternal DXYS14 locus has not been inherited by the patient, though the maternal locus is present. (B) SstI digests probed with H033. The patient has both the X specific 4.5 kb band and the Y specific 3.2 kb band, with a band intensity ratio approximating 2:1. (C) EcoRI digests probed with pY53.3. The patient is positive for SRY. (D) BclI digests probed with ds56. RFLP is shown for the patient as well as her sister and mother, showing the presence of two copies of this locus. (E) PeuII digests probed with pERT87-1, 754, and probes for OTG, Tk, and F8C, respectively (same filter). The patient has two copies of DXS164, DXS84 and TK, and one copy of OTG and F8C.

Discussion
Our results suggest that the paternal distal Xp segment (Xp21→p22.3) was translocated to Yp11.3 and inverted to form the Yp+ chromosome (Xp21→Xp22.3→Yp11.3→Yqter) (fig 6). Although the Y chromosome is missing the distal part of the PAR, no deletion of the Y specific region was detected nor was any mutation found in SRY. This strongly indicates that the impaired testis formation and the resultant female development of our patient occurred in the presence of SRY.

Results
Cytogenetic Studies
The patient’s karyotype was 46,XY,Yp+ in all of the 50 cells examined. On the elongated Yp, four extra dark bands were visible by high resolution G banding, the most distal band being the largest (fig 3). The karyotypes of the older sister and the parents were normal.

Southern Blot Analysis
Representative results are shown in fig 4 and summarised in the table. In the patient, only a single copy was detected for DXYS14 and DXYS15 in the distal part of the pseudoautosomal region (PAR), whereas three copies were found for DXYS17 and MIG2 in the proximal part of the PAR. The X specific loci from PABX to DXS84 were present in two copies. The Y specific loci were present in a single copy as expected. Paternity was confirmed by the minisatellite analysis.

Mutational Analysis of SRY
In the SSCP analysis, none of the three different restriction enzyme digests gave an abnormal banding pattern as compared with normal male controls (fig 5). The DNA sequence was also completely normal (data not shown).
This patient is similar to four non-mosaic sex reversed patients with a 46,XY,Xp+ karyotype. In spite of the presence of a morphologically normal Y chromosome, two sibs with 46,Y,dup(X)(p11→pter) and two patients with 46,Y,dup(X)(p11.2→p22.2) and 46,Y,dup(X)(p11.2→p22.3) respectively exhibited a female phenotype. Furthermore, the two sibs were examined for gonadal structure and confirmed to lack testis formation. In contrast to the four patients, all other reported non-mosaic patients with a partial X chromosome duplication between Xp21.2 and Xqter showed male sex development in the presence of a normal Y chromosome.40-45

The four sex reversed patients and our patient have a similar duplication of an active X specific segment encompassing distal Xp21 and proximal Xp22 (the translocated Xp segment in our patient lacks the inactivation centre19 and the duplicated X chromosome segments in karyotypically male patients have been reported to escape inactivation20). Thus, it appears logical to assume that the same mechanism inhibiting testis formation is operating in the four patients with dup(Xp) and in our patient with Yp+. Although latent mosaicism in the gonad or a position effect on SRY might be possible in our patient, there is no evidence for either mechanism (the associated alteration of PAR is unlikely to affect sexual phenotype, since neither monosomy nor trisomy of the PAR influences testis formation21-23). Similarly, although it might be possible in the four patients with dup(Xp) that latent mosaicism or a mutation of SRY existed, or that the breakage of Xp caused a gene disruption which acted as a dominant inhibitor for testis formation, such a mechanism also remains speculative.

If a causal relationship exists between two active doses of the Xp distal region and impaired testis formation, this implies that a gene or genes subject to X inactivation, involved in testis formation, exist in this region and two active copies of the gene(s) hinder the testis determination or differentiation process. Under this hypothesis, patients with only one active copy of the gene(s), for example, 47,XXY and 48,XXXXY, masculinise like normal 46,XY males, whereas patients with two active copies of the gene(s), for example, 46,Y,dup(Xp) and 46,X,Yp+, result in sex reversal. Because no evidence for global developmental disruption was found in our patient, it appears that this gene(s) functions mainly, if not exclusively, in the gonad. Furthermore, it is possible that some SRY positive XY females may be explained by cryptic duplications of the gene(s) proposed here. It is also possible that other types of alteration in the gene(s) would cause sex reversal. Although Bernstein et al26 ascribed defective testis formation of two sibs with dup(Xp) to absent H-Y antigen, and Scherer et al27 regarded two copies of ZFX as the cause of sex reversal in two patients with dup(Xp), both hypotheses are untenable at present for the following reasons. (1) It has been shown that H-Y antigen is not required for testis determination.40-41 (2) ZFX has been shown to escape inactivation,42 so that if two copies of ZFX result in sex reversal, Klinefelter patients should develop as females.

In the present case, polyarteritis nodosa (autoimmune inflammatory disease) and IgA deficiency were observed. Interestingly, the association between sex chromosome aberrations and immune related diseases has been described previously.43-45 However, it is uncertain at this time whether the immune related complications of our patient were directly related to the Yp+ chromosome.

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1 Sinclair AH, Berta P, Palmer MS, et al. A gene from the human sex-determining region encodes a protein with