Deletion 9p and sex reversal

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Abstract

We report a case of a female infant with a de novo deletion of the short arm of chromosome 9, sex reversal, and an apparently intact SRY gene. Sex reversal has been reported in a number of subjects with a normal Y chromosome and a deletion of the terminal segment of the short arm of chromosome 9. The factors controlling early development of the male testes are unknown. There are likely to be many genes involved and we present additional evidence that one of these is situated on the end of the short arm of chromosome 9.

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The primary event in the determination of male and female sex is dependent on the presence or absence of the sex determining region of the Y chromosome (SRY).¹ Sex reversal has been reported in a number of subjects with a Y chromosome and a terminal deletion of the short arm of chromosome 9.²⁻⁶ These cases involved translocation of chromosome 9 with other chromosomes and therefore they were trisomic for part of another chromosome in addition to being monosomic for terminal 9p. We report a case of a sex reversed female infant with a de novo deletion of the distal short arm of chromosome 9, sex reversal, and an apparently intact SRY gene.

Methodology for sequencing the SRY gene

POLYMERASE CHAIN REACTION AMPLIFICATIONS DNA was amplified from genomic DNA extracted by standard methods.⁷ Amplifications were performed with the primers XES10 and XES11 flanking the SRY open reading frame of sequence pY53.3¹ generating a 778 bp fragment. Amplifications were performed with 0.2 µg genomic DNA, 200 µmol/l each dNTP, 0.5 µmol/l each primer, 1.2 U of *Taq* polymerase (Promega) using the manufacturer's buffer in a reaction volume of 40 µl. After an initial incubation of two minutes at 94°C, reactions were cycled for 80 seconds at 94°C and one minute at 54°C for 32 cycles. Primer sequences are:

XES10: 5'-GAGCTCGAGAATTCGGTG-TTGAGGGCGGAGAAATGC-3'.
XES11: 5'-GAGCTCGAGAATTCGTAG-CCAATGTTACCCGATTGTC-3'.

SUBCLONING OF PCR PRODUCTS

The amplified DNA was phenol extracted and ethanol precipitated. Resuspended DNA was digested with *Eco*RI to cleave the 5' of the

primers. Digested DNA was electrophoresed on a 0.6% 'Sea Plaque' agarose gel (FMC). The DNA fragments were excised from the gel and ligated into the *Eco*RI site of pUC19. Ligated plasmids were transformed into *E coli* DH5α.

DNA SEQUENCING

The insert of one recombinant plasmid was sequenced as double stranded DNA by the dideoxy chain termination method.⁸ Template DNA was isolated using the method of Xhou *et al.*,⁹ and DNA sequenced on one strand using synthetic oligonucleotide primers and Sequenase (USB) by the method of Hsiao.¹⁰

Case report

The proband is the first child of unrelated parents. The mother had a flu-like illness at 16 weeks of pregnancy and gestational diabetes was diagnosed at 33 weeks. The baby was born at 34 weeks' gestation by vaginal delivery, birth weight 2550 g. The external genitalia were those of a normal female. She required resuscitation at birth. She was noted to have microcephaly and widely spaced nipples.

At 6 months of age she presented with bronchiolitis and convulsions which required treatment with ACTH. Investigations showed a raised CSF and plasma lactate. A diagnosis of a mitochondrial respiratory chain defect was pursued but a muscle biopsy showed no structural or histochemical abnormality and assay of respiratory chain enzymes was normal.

At 9 months of age she was admitted to hospital for failure to thrive, gastro-oesophageal reflux, and severe constipation. A rectal biopsy was normal.

At the age of 2 years 8 months she has been walking for seven months, says 17 words, and is learning Makaton sign language and knows six signs. On examination her head circumference (45.2 cm), height (79.5 cm), and weight (10.0 kg) are below the 3rd centile. She has no dysmorphic features and no abnormal neurological signs.

Cytogenetic and molecular analysis

Chromosome analysis, performed on cultured cells from a skin biopsy taken as part of the investigation for a mitochondrial respiratory chain defect, showed a male karyotype with an abnormal chromosome 9. A more detailed chromosome analysis of cultured lymphocytes with high resolution banding showed that the abnormal chromosome 9 has a terminal deletion of the short arm, 46,XY,del(9) (p2305) (fig 1). The parental karyotypes were normal.

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Figure 1 Partial G banded metaphase to show deleted chromosome 9 with apparently terminal deletion of the short arm *del(9)* (p2305).

Frozen fibroblasts from the proband are banked at Guy's Hospital, reference 90/3077.

The SRY gene was amplified by the polymerase chain reaction, cloned, and sequenced and no mutations found.¹

Endocrine investigations

Endocrine investigation was consistent with gonadal failure with a raised FSH of >40 mU/l (normal range <2 mU/l). The LH rose markedly after LHRH stimulation from 11 IU/l to >50 IU/l (resting normal range 1–6 IU/l). The testosterone rose only minimally from 0.9 to 1.5 nmol/l (resting normal range ≤ 1.0 nmol/l) after three injections of HCG (1000 units $\times 3$).

Figure 2 Streak gonad showing wavy, whorled ovarian type stroma, devoid of ovarian follicles.

Clinical investigations

In view of the male karyotype, a sinogram was performed and showed a smooth walled bladder with a vagina situated behind the bladder. Bilateral gonadectomy was performed because of the high risk of gonadal malignancy.¹¹ At operation a normal uterus and fallopian tubes with dysplastic gonads were found.

The histology of the gonads showed streak gonads associated with a few epididymal tubules, islands of interstitial cells, and Wolffian duct remnants. There was no evidence of tumour (fig 2).

Discussion

The sex determining Y chromosome gene (SRY) has recently been identified.¹ The product of this gene appears to determine whether the primitive gonad will develop into a testis or ovary. In this case the entire SRY open reading frame (ORF), along with 62 base pairs 5' to the ORF, were sequenced and found to be normal. It remains a possibility, however, that a mutation could lie outside the region sequenced, that is, at the 5' end or in the control elements of SRY which are as yet uncharacterised.

The production of the male phenotype is dependent on the production of two hormones from the testes, anti-Müllerian hormone (AMH) also known as Müllerian-inhibiting substance or factor, a glycoprotein produced by the Sertoli cells,¹² that causes regression of the paramesonephric ducts, and dihydrotestosterone, a steroid derived from testosterone produced by the Leydig cells that results in virilisation of the external genitalia.¹³ The gene for AMH has been mapped to the short arm of chromosome 19.¹⁴

The presence of testicular tissue with normal female external and internal genitalia suggests that the fault lies in the development of the primitive gonads before the production of AMH and testosterone. The two substances are produced from two different cell types within the gonads and therefore the fault must be in a common pathway leading to their production. It has been suggested that the testis determining gene leads to Sertoli cell differentiation and the production of AMH directs the further differentiation of the testes.¹⁵ In some males with persistent Müllerian duct syndrome there is no AMH produced but the testes are normal.¹⁶

The association of 9p deletion with ambiguous genitalia has been previously reported.^{17,18} There are five recorded cases of females with a 46,XY karyotype and a chromosome 9p deletion.^{2–6} In all the cases reported except one⁵ they were products of a familial translocation. In one case the father had a questionable chromosome 9 and chromosome 'painting' showed a 4;9 translocation.⁶ The most distal breaks reported are at 9p24^{5,6} and the most proximal at 9p21³ (table). This would suggest that the 9p24 band is involved in production of the male phenotype.

The mechanism by which the loss of one copy of a gene by chromosomal deletion can

Summary of patients with sex reversal and deletions of the distal short arm of chromosome 9.

Reference	Year	Karyotype
Jotterand and Juillard ²	1976	46,XY,-9,der(9),t(9;13)(p21;q21)mat
Fryns <i>et al</i> ³	1986	46,XY,-9,der(9),t(3;9)(p21.33;p22.1)mat
Crocker <i>et al</i> ⁴	1988	46,XY,-9,der(9),t(7;9)(q31.1;p23)pat
Hoo <i>et al</i> ⁵	1989	46,XY,-9,der(9),t(2;9)(p21;q24)
Magenis <i>et al</i> ⁶	1990	46,XY,-9,der(9),t(4;9)(?;p24)pat

lead to the complete loss of function of the gene product is not clear. In the four reported cases involving a familial translocation, the translocation was derived from the mother in two cases^{2,3} and the father in two cases.^{4,6} This makes imprinting unlikely.¹⁹

A second possibility is that the deletion has unmasked a recessive allele on the other chromosome 9. This mechanism has been used to explain the development of non-ketotic hyperglycinaemia in a patient with a 9p deletion²⁰ and is the mechanism suggested by Hoo *et al*⁵ to explain sex reversal in the reported cases. There are two known autosomal recessive syndromes associated with sex reversal. Campomelic dysplasia is a skeletal dysplasia associated with female phenotype and male karyotype.²¹ Gonadal dysgenesis has been reported in a family with parental consanguinity²² and separately in three sisters with a normal brother and no details about the parents.²³ Mutations in the Wilms' tumour suppressor gene (WT1) have been reported in the Denys-Drash syndrome (renal failure, pseudohermaphroditism, and Wilms' tumour) suggesting that WT1 has a role in human sexual development.²⁴ De la Chappelle²⁵ suggested that there is an autosomal dominant testis determining gene, TDFA. This suggests that autosomal genes are involved in early sex determination. There are no candidate genes on the tip of the short arm of chromosome 9.²⁶ However, Affara *et al*²⁷ identified by in situ hybridisation a ZFY related DNA sequence that mapped to 9pter-9p22.

The factors controlling early development of the male testes are unknown. There are likely to be many genes involved and one of these seems likely to be situated on the end of the short arm of chromosome 9 being located in 9p24. Further study is in progress to identify the gene(s) involved.

The following doctors have been involved in the care of this child: Miss M Agrawal, Consultant Paediatric Surgeon, Guy's Hospital, and Mr Drake, Consultant Paediatric Surgeon, The Hospital for Sick Children, Great Ormond Street, London.

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