An autosomal or X linked mutation results in true hermaphrodites and 46,XX males in the same family

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Abstract
It is now well established that the differentiation of the primitive gonad into the testis during early human embryonic development depends on the presence of the SRY gene. However, the existence of total or partial sex reversal in 46,XX males with genetic mutations not linked to the Y chromosome suggests that several autosomal genes acting in association with SRY may contribute to normal development of the male phenotype. We report a family in which four related 46,XX subjects with no evidence of Y chromosome DNA sequences underwent variable degrees of male sexual differentiation. One 46,XX male had apparently normal male external genitalia whereas his brother and two cousins had various degrees of sexual ambiguity and were found to be 46,XX true hermaphrodites. The presence of male sexual development in genetic females with transmission through normal male and female parents indicates that the critical genetic defect is most likely to be an autosomal dominant mutation, the different phenotypic effects arising from variable penetrance. Other autosomal loci have been implicated in male sexual development but the genetic mechanisms involved are unknown. In this family there may be an “activating” mutation which mimics the initiating role of the SRY gene in 46,XX subjects.

Keywords: true hermaphrodite; 46,XX male; SRY gene; male sexual development

The development of the male phenotype in 46,XX subjects is a well established phenomenon.1,2 Many cases have been shown to result from the translocation of Y chromosome material containing the testis determining factor (SRY gene) to the X chromosome or one of the autosomes.3,4 However, familial occurrence of 46,XX males or 46,XX true hermaphrodites provides evidence that male development may occur because of autosomal factors, in the absence of the SRY gene. In some cases, this may be the result of mutations of recessive genes involved in the pathway of male development.5,6 However, a few rare families have been reported in which XX true hermaphrodites and XX males coexist with transmission through parents of both sexes.7,8 Two subsequent reports have confirmed this phenomenon arises in the absence of Y chromosome DNA.9,10 These familial cases have led to the suggestion that XX true hermaphrodites and XX males may represent alternative manifestations of the same genetic defect, most likely to be an autosomal testis determining factor.9 We report another family which supports this hypothesis and shows that a putative autosomal (or X linked) gene has variable phenotypic effects in XX subjects, including completely normal female development, true hermaphroditism with ambiguous genitalia, and XX males with ambiguous or normal genitalia. The significance of these findings is discussed in relation to the genetic control of male development.

Case reports
A 34 year old mother was referred for genetic counselling before reversal of sterilisation. Her 10 year old son had been born with ambiguous genitalia and subsequent investigation had shown him to be a true hermaphrodite. Further enquiry indicated that other members of the family were affected (fig 1).

**CASE 1**
Case 1 (fig 1, III.2) was born following a normal pregnancy apart from an episode of premature labour at 32 weeks’ gestation. His birth weight was 3500 g and he had ambiguous genitalia with a small penis, hypospadias, and a bifid scrotum with no palpable testes (fig 2). He was admitted to The Hospital for Sick Children at the age of 4 weeks and initial investigation showed normal plasma electrolytes...
and 17-hydroxyprogesterone and normal urinary oxosteroids. The HCG challenge test showed a partial but reduced testosterone response; however, the karyotype on two occasions was 46,XX. A urogenital sinogram showed a normal bladder outline with no vesicoureteric reflux and abdominal ultrasound confirmed normal kidneys, but the testes were not seen and no uterine structure was identified. He underwent an exploratory laparotomy which identified a right intra-abdominal gonad. A left gonadal remnant, small midline uterus, and vagina were all excised. Histologically, the left gonad contained interdispersed testicular tubules, Sertoli cells, and ovarian follicles (fig 2). These findings indicated that he was a true hermaphrodite and male gender was assigned because of the appearance of the external genitalia. The hypospadias was repaired in stages between the ages of 18 months and 3 years. The HCG test was repeated at 3 years and no rise in plasma testosterone levels was seen; therefore the right gonad was removed and histologically was also an ovotestis. At 7 years he was given testicular prostheses and at the age of 10 he was started on testosterone replacement. He is otherwise well with normal growth and development and attends a normal school.

CASE 2
Case 2 (fig 1, III.1) was the older bother of case 1 and at the time of referral there was no concern about him. He had been born following a normal pregnancy and because the external genitalia were entirely normal at birth, no investigations were undertaken. However, at 12 years his height was on the 10th centile and he showed no signs of pubertal development. Blood was taken for karyotyping and he was also found to be 46,XX. The HCG challenge test was unresponsive in this child and he was therefore given testosterone replacement. The mother of cases 1 and 2 (fig 1, II.2) had a normal female phenotype.

CASE 3
Following these surprising results some further details of the family history (which had not been forthcoming initially because of loss of contact) were obtained from hospital records. The mother of cases 1 and 2 (II.2) had a half brother (II.5) who had two sons who were also born with abnormal external genitalia. Case 3 (III.3) had been referred to The Hospital for Sick Children in 1973 at the age of 8 months for investigation. He was born following a normal pregnancy with a birth weight of 2900 g. The phenotype resembled case 1 with a small penis and hypospadias (fig 2). Investigations had shown normal plasma electrolytes and 17-hydroxyprogesterone and urinary oxosteroids. Gonadal biopsy confirmed the presence of bilateral ovotestes and HCG challenge showed a partial testosterone response. He had been initially assigned female gender because his karyotype was 46,XX, but this was subsequently changed to male. He had a bilateral gonadectomy and was given testicular prostheses and testosterone replacement treatment. There were no other dysmorphic features and he attended a normal school.

CASE 4
Case 4 (III.4), the younger brother of case 3, also had ambiguous genitalia at birth with a smaller penis than his brother and severe hypospadias (fig 2). The results of biochemical and endocrine investigations were as for cases 1 and 3. He also had a normal female karyotype and bilateral ovotestes. His clinical course and management was similar to his brother and his neurodevelopment was normal.

Molecular studies
DNA methods
DNA was extracted by previously described methods. Ten different Y specific and one X specific primers were used to detect the presence of Y sequences in cases 1 and 2 and
their parents. Primer sequences for the loci were SRY (primer pair XES10 5' GTGGT-GAGGGCGAGAATGCG and XES11 5' GTAGCCTATGTACCGATTGTC, 778 bp Yp specific product, 68°C annealing6), ZFX/Y (primer set ZFX 5' AGACACAC-TACTGAGCAGAATGCG and ZF1 5' ATTTGTCTAATGCGCATAT-TCTCT, 488 bp X specific and 340 bp Yp specific products, 60°C annealing7), DYS32 (primer pair GMGYX6F 5' ATTATCTC-TTGCATTCG and GMGYX6R 5' TCACAGGGCCTGATAAAAA, 150 bp Yp specific product, 55°C annealing8), DYS21Y (primer pair GMGYX2F 5' TACAGTCTC-CAGGTTCCAG and GMGYX2R 5' CGGT-GCTGACATTTGGGTATA and ZFX/Y were shown to amplify ZFX and ZFY sequences but both the brothers showed a pattern identical to their mother, indicating the absence of any different Y chromosome material. These findings exclude the possibility of a small cytogenetically undetectable Y or Y-autosome translocation in the brothers.

**Discussion**

**MODE OF INHERITANCE**

The family we present is similar to those previously reported in 46,XX true hermaphrodites and 46,XX males coexist with evidence of autosomal dominant or X linked inheritance with incomplete penetrance (table 1). Evaluation of the pedigrees of these families suggests that the responsible genetic factor(s) can be transmitted to males through apparently phe-notypically normal 46,XY fathers 9 and normal males tested by DNA methods, Y chromosome DNA sequences were completely absent (*). NR indicates that the result was not recorded.

### Table 1: Summary of previously reported familial cases of 46,XX males and 46,XX true hermaphrodites which show autosomal dominant inheritance.

<table>
<thead>
<tr>
<th>Report</th>
<th>Case</th>
<th>Genitalia</th>
<th>Genotype</th>
<th>Genital duct derivatives</th>
<th>Karyotype</th>
<th>Diagnosis</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kassian et al</td>
<td>1</td>
<td>Small penis, urogenital sinus, bilateral ovo testes</td>
<td>Bilateral ovo testes</td>
<td>Unilateral vas deferens, vagina</td>
<td>46,XXm</td>
<td>TH</td>
<td>M</td>
</tr>
<tr>
<td>Skorodis et al</td>
<td>2</td>
<td>Hypospadias with chordee</td>
<td>Bilateral testes, azoospermia</td>
<td>Normal male</td>
<td>46,XXm</td>
<td>XY male</td>
<td>M</td>
</tr>
<tr>
<td>Skorodis et al</td>
<td>3</td>
<td>Normal</td>
<td>Bilateral ovaries</td>
<td>Uterus and fallopian tubes, vagina</td>
<td>46,XX</td>
<td>TH</td>
<td>F</td>
</tr>
<tr>
<td>Skorodis et al</td>
<td>4</td>
<td>Small penis, hypospadias, bilateral scrotum</td>
<td>Descended ovo testes</td>
<td>Unilateral vas deferens, round ligaments</td>
<td>46,XX</td>
<td>TH</td>
<td>M</td>
</tr>
<tr>
<td>Kuhlin et al</td>
<td>II.1</td>
<td>Cliteromegaly, urogenital sinus</td>
<td>Uterus and fallopian tubes, vagina</td>
<td>Unilateral vas deferens, round ligaments</td>
<td>46,XX</td>
<td>TH</td>
<td>M</td>
</tr>
<tr>
<td>Kuhlin et al</td>
<td>II.2</td>
<td>Cliteromegaly, urogenital sinus</td>
<td>Unilateral ovary, unilateral ovo testes</td>
<td>Unilateral vas deferens, round ligaments</td>
<td>46,XX</td>
<td>TH</td>
<td>M</td>
</tr>
<tr>
<td>Ramos et al</td>
<td>I.3</td>
<td>Normal penis, small testes</td>
<td>Bilateral ovo testes</td>
<td>Uterus and fallopian tubes</td>
<td>46,XX</td>
<td>TH</td>
<td>M</td>
</tr>
<tr>
<td>Present report</td>
<td>1</td>
<td>Small penis, hypospadias, bilateral scrotum</td>
<td>Normal genitalia</td>
<td>Normal</td>
<td>NR</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Present report</td>
<td>2</td>
<td>Normal penis, hypospadias, bilateral scrotum</td>
<td>Normal</td>
<td>Normal</td>
<td>NR</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Present report</td>
<td>3</td>
<td>Small penis, hypospadias, bilateral scrotum</td>
<td>Small uterus and vagina</td>
<td>Small uterus and vagina</td>
<td>46,XX</td>
<td>TH</td>
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<td>4</td>
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<td>46,XX</td>
<td>TH</td>
<td>M</td>
</tr>
</tbody>
</table>
mal 46,XX mothers. This provides evidence for the existence of an autosomal testis determining factor or T DFA, which may play a role in the initiation and regulation of male development. However, in our family an X linked factor cannot be excluded with certainty.

SUMMARY OF CLINICAL PHENOTYPES
Comparison of these cases highlights several important findings. There is a wide range of structural effects on both the external genitalia and genital tracts in 46,XX subjects. In the index cases, the external genitalia were usually ambiguous, although some cases resembled normal males and in others there were female external genitalia with only clitoromegaly. Some subjects had evidence of either müllerian or wolffian derivatives, while in others both persisted and underwent variable degrees of differentiation. The gonads were frequently ovotestes and, although some biopsies only appeared to contain testicular tissue, it is impossible to exclude true hermaphroditism unless the whole gonad was examined histologically. Although case 2 in this report appeared to be a normal male, he had never had a gonadal biopsy and could in fact also be a true hermaphrodite. All affected subjects tested had a 46,XX karyotype: those who had molecular studies showed no evidence of Y sequences. This suggests that in these subjects male development must be initiated and maintained to a variable degree by one or more genes not linked to the Y chromosome, distinct from SRY, and yet able to mimic its role in early human sexual differentiation. Current knowledge suggests that 46,XX males are infertile because they lack the Yq spermatogenesis factors. It has also been shown in the mouse model that the presence of two X chromosomes prevents normal spermatogenesis. It is not obvious why mutation(s) in the putative T DFA gene(s) may be non-penetrant in the mothers of affected subjects, but presumably in the fathers with a Y chromosome the effects are “overridden” by the normal role of SRY. From this family, as has previously been pointed out, it seems likely that there is no phenotypic effect in 46,XY gene carriers.

ROLE OF SRY IN MALE DEVELOPMENT
Present understanding (fig 4) suggests that male development is an active process which is initiated in the indifferent embryonic gonad at approximately 6 weeks after conception, but only in the presence of SRY. SRY acts as a trigger to the expression of a cascade of other proteins which direct and maintain further differentiation of the testes. The Leydig cells in the testes produce testosterone, which is converted to the more potent metabolite dihydrotestosterone, which promotes further development of the testes, wolffian ducts, and external genitalia. The regression of müllerian structures is mediated by müllerian inhibiting factor (MIF) produced by the Sertoli cells in the testes. In the absence of SRY and related proteins this does not occur and the indifferent gonads develop into ovaries and the müllerian ducts into the uterus, fallopian tubes, and upper part of the vagina.

CANDIDATES FOR T DFA
The identity of T DFA in the families described remains unknown. The pedigrees discussed are too small for linkage analysis and there is the added problem of identifying gene carriers who may have no obvious clinical features, so that only obligate carriers could be used in molecular genetic studies. However, it is of interest that a number of different autosomal loci have been associated with sexual ambiguity in 46,XY subjects. Sex reversal has been noted in cases of campomelic dysplasia which results from mutations in the HMG box gene SOX9 and has >60% homology in the HMG box domain with SRY. Mutations in the zinc finger domains of WT1, the Wilms tumour suppressor gene, have been identified in cases of Denys-Drash syndrome who are 46,XY but have female or ambiguous genitalia, indicating that WT1 has a role in male development. The SF-1 gene (encoding a steroidogenic nuclear orphan receptor) has also been shown in the mouse to be essential for gonad formation. In addition to these specific genes, some other chromosomal regions have been implicated in male sex differentiation. Terminal deletion of 10q26.1-qter has been associated with various phenotypic effects ranging from complete sex reversal to micropenis and cryptorchidism, implying that a critical gene may lie in this region. Normal female development has been described in a 46,XY infant with a deletion of chromosome 9p but with an intact SRY gene sequence. The mechanisms of sex reversal in these different situations is unclear, but the chromosome deletions suggest the possibility of reduced gene dosage.

By contrast, there is no known molecular basis for sex reversal in 46,XX subjects. Seaver et al reported three 46,XX subjects who
lacked Y specific DNA sequences but developed male external genitalia. These infants also had incomplete development of female internal genitalia and complex malformations of the urinary and gastrointestinal tracts. A fourth case with ambiguous genitalia had the karyotype 46,XX,del(10)(q25.3-qter). It was suggested that these cases could be explained by the abnormal expression of genes (such as PAX2) which are normally regulated by androgens.

A similar mechanism has been proposed to explain affected sibs in a single generation. They suggested that an autosomal negative regulator of male development present in the homozygous state normally prevents male development in 46,XX females; in 46,XY males the SRY gene suppresses this effect (fig 5).

Recessive mutations in this regulator could explain other reported instances of affected sibs with very similar phenotypes, in contrast to the family described here with coexistence of different phenotypes. The families summarised in table 1 provide evidence for a dominant gene, which could operate either by gain of function ("activating" mutation) (fig 5), or by a dominant negative effect. In the family we report, an X linked mutation cannot be ruled out. There are several genes on the X chromosome which have been implicated in male to female sex reversal. 46,XY subjects with duplications of Xp led to the discovery of a dosage sensitive locus at Xp21(DSS), which when present in duplicate caused the female phenotype. As deletions of DSS usually have no effect on male development, it has been concluded that DSS may be involved in ovarian development. However, in one case of a 46,XX subject with a distal Xp deletion associated with ambiguous genitalia and ovotestes, it was suggested that the male development may be caused by a loss of function mutation in DSS. Sex reversal in 46,XY subjects has also been reported in a thalassaemia-mental retardation syndrome (ATRX), resulting from deletions of the ATRX gene on Xp. If an X linked gene is responsible for sex reversal in our family, the coexistence of different phenotypes could be explained by skewed X inactivation within the gonad. Mutations of the androgen receptor gene have been described in 46,XY subjects with a female phenotype. No reports of activating mutations in the androgen receptor gene are known to exist, but this remains a theoretical explanation of 46,XX maleness. A final possible mechanism in the family described is of an unstable mutation present in a premutation form in the mother, grandmother, and maternal half uncle, expanding to a full mutation in the affected subjects who are all in the third generation.

Clearly, many different genes at different loci in the genome are involved in normal male sexual development, although their exact roles, interrelationships, and mechanisms of action remain unclear at the present time.

GENETIC COUNSELLING

The existence of dominant mutations in TDFAs genes presents difficult problems in genetic counselling. In this family, as the mother must be a gene carrier, there would be a 50% risk of a child inheriting the mutation. In this instance, if the child were a 46,XY male, then present evidence from other families suggests that even if he were a carrier of the mutation there would be no harmful effects, although there would be risks in the subsequent generation. If the child were female, it is anticipated that a spectrum of possible phenotypes could result, ranging from a normal female to a 46,XX subject with complete male development. It has been suggested that most cases present the intermediate phenotype of a true hermaphrodite or an XX male with ambiguous genitalia, as was the case in this family. The opportunity of prenatal diagnosis with fetal karyotyping followed by detailed ultrasonography of the external genitalia has been discussed in detail with the parents. Other difficult issues arise in which affected relatives who would be at 50% risk of carrying the mutation with no definite test. It is hoped that further elucidation of the molecular mechanisms involved in testes determination and male development will enable these problems to be overcome in the future.

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