A novel mutation 5′ to the HMG box of the *SRY* gene in a case of Swyer syndrome

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Abstract

We describe a novel mutation in the coding region of the *SRY* gene in a 46,XY female with Swyer syndrome. Analysis of *SRY* was carried out by direct sequencing of a 780-bp PCR product that included the *SRY* open reading frame (ORF). This revealed the presence of a point mutation, ins108A, in the coding region 5′ to the HMG box which results in a frame shift and premature termination of the encoded protein. No other mutation was found in the *SRY* ORF. We infer that sex reversal in this individual is a result of this insertion. In none of the 13 other 46,XY females that were studied was a mutation detected in *SRY*, confirming earlier findings that most cases of XY femaleness are due to causes other than mutation in *SRY*. These observations and those of others are discussed in relation to the aetiology of XY sex reversal.

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Introduction

In mammals the Y chromosome determines whether the bipotential gonads differentiate into testes or ovaries. In humans a gene called *SRY* (Sex-determining Region Y) has been localized to the minimum region of the Y short arm that is necessary for maleness (Sinclair *et al.* 1990). This gene exhibits all the properties of a testis determiner (reviewed in Greenfield and Koopman 1996). Among 46,XY females about 10–15% show mutations in *SRY*. Most of these mutations lie in a DNA-binding motif, the HMG (high mobility group) box (reviewed in Cameron and Sinclair 1997).

The 46,XY female type of gonadal dysgenesis (MIM 306100) is characterized by failure to develop secondary sexual characteristics at puberty, failure to menstruate, and the presence of streak gonads. Complete gonadal dysgenesis (Swyer syndrome) is characterized by the presence of fibrous ovary-like structures and no evidence of testicular differentiation. The term partial gonadal dysgenesis is used to describe individuals with incomplete testicular differentiation and ambiguous genitalia (Berkovitz and Seeherunvong 1998). We report here on the identification of a novel mutation in the coding region of *SRY* 5′ to the HMG box in a subject with complete gonadal dysgenesis from among a total of fourteen 46,XY cases of complete or partial sex reversal that were studied. To our knowledge only four other mutations have been reported in this part of the coding region (Veitia *et al.* 1997; Brown *et al.* 1998; Domenice *et al.* 1998; Scherer *et al.* 1998). We discuss the significance of this and other observations (reviewed in Cameron and Sinclair 1997) in the aetiology of XY sex reversal.

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Materials and methods

Chromosome banding and extraction of genomic DNA from whole blood was done using standard techniques (Dracopoli *et al.* 1995). Sequences XES10 and XES11 (Ogata *et al.* 1992) were used as primers to amplify a 778-bp fragment encompassing the *SRY* coding region. The amplifications were carried out in a reaction volume of 50 μl containing 100 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 0.25 mM of each primer and 2 units of Taq DNA polymerase (Pharmacia). Initial denaturation was carried out for 3 minutes at 94°C, following which amplification was performed for 35 cycles (1 minute each at 94°C and 60°C, and 2 minutes at 72°C), and a final extension of 10 minutes at 72°C. After PCR, the reaction mix was electrophoresed on a 1% agarose gel in 1× TBE buffer at 4 V/cm for 1 hour and the 778-bp band eluted using GeneClean II elution kit according to manufacturer's specifications. Sequencing reactions were carried out using the primers XES10, XES11, XES7 (Berta *et al.* 1990), IIIA (5′-GCCGAAGAATTGCA-GTTTGCTTCCC-3′) and EVB (5′-AGCTTCTCCGGAGA-GCGGGAATA-3′), and the Perkin-Elmer Anyprimer fluorescent-tagged DNA sequencing kit. The sequencing conditions were 96°C, 30 seconds; 53°C, 15 seconds; and 60°C, 4 minutes for 25 cycles. Electrophoresis was carried out on a 6% acrylamide gel (19 : 1 :: acrylamide : bis-acrylamide) containing 7.6 M urea in a Perkin-Elmer automated sequencer (ABI 310) and the gel image analysed using Sequence Scan software.

Results and discussion

We report here on mutational analysis of *SRY* ORF in 14 males with apparently normal 46,XY karyotypes. Clinical data on these individuals are summarized in table 1. A 778-bp region encompassing the *SRY* ORF was amplified from each of the 14 cases and sequenced (figure 1). A single-base-pair insertion mutation (A/T) was detected 108 nucleotides downstream of the major transcription start site in individual Y18, a typical case of Swyer syndrome (figure 2a). The position of the mutation specified above is in relation to the major transcription start site of *SRY* as identified by Clepet *et al.* (1993). This mutation is in the coding region 5′ to the HMG box as a result of which there

Table 1. Clinical features of 46,XY individuals included in this study.

Patient	Age at presentation	External genitalia	Gonads	Reproductive tracts	Other features
Al	3 days	Ambiguous with small phallus and labioscrotal folds	Small palpable bilateral masses seen in the inguinal canal	Small uterus present on ultrasound	Infant was delivered prematurely at 32 weeks gestation
A2	12 years	Ambiguous, phallus enveloped by labioscrotal folds	Small palpable bilateral masses (~1.5 cm in diameter) seen in the labioscrotal folds	Uterus absent	
A4	16 years	Normal female	Not palpable	Information not available	Primary amenorrhea
A5	17 years	Normal female	Not palpable	Information not available	Primary amenorrhea
Y4	9 months	Ambiguous	Small gonads palpable in the scrotal folds	No uterus seen on ultrasound; vagina absent	
Y8	28 years	Normal female	Gonads not palpable	Uterus and vagina absent	Haemophilia A
Y10	11 years	Female: clitoromegaly	Gonads palpable in labioscrotal folds	Information not available	
Y11	4 years	Ambiguous	Gonads not palpable	Small uterus, fallopian tubes seen on both sides; vagina absent	
Y13	11 months	Female: clitoromegaly	Gonads present on ultrasound	Uterus present on ultrasound; vagina absent	
Y14	17 years	Normal female	Gonads not palpable	Uterus absent on ultrasound; vagina present	Poor secondary sexual characters
Y16	19 years	Normal female	Small testes? Gonads not seen on ultrasound	Uterus absent; vagina present	Poor secondary sexual characters
Y17	14 years	Female: clitoromegaly	Undescended gonads	Small uterus present on ultrasound; vagina absent	Poor secondary sexual characters
Y18	17 years	Normal female	Dysgenetic gonads present on ultrasound	Uterus absent; small vagina	Poor secondary sexual characters
T1297	18 years	Normal female	Gonads palpable	Uterus present on ultrasound; blind vagina	Scant pubic and axillary hair

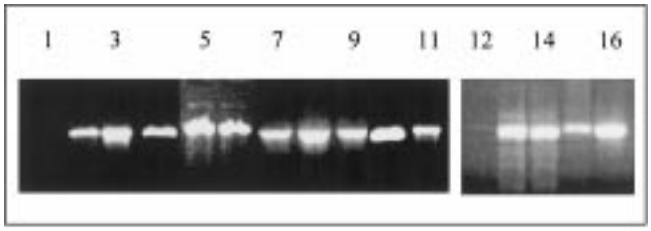

Figure 1. PCR of *SRY* using XES10 and XES11: detection of the 778-bp product on an ethidium bromide-stained 2% agarose gel in 1× TBE buffer. Lanes 1–16 are PCR products from, respectively, negative control (DNA from XX fertile female), A1, A2, Y8, Y10, Y14, Y16, Y17, Y18, T1297, positive control (DNA from XY fertile male), negative control, A4, A5, Y11 and Y13.

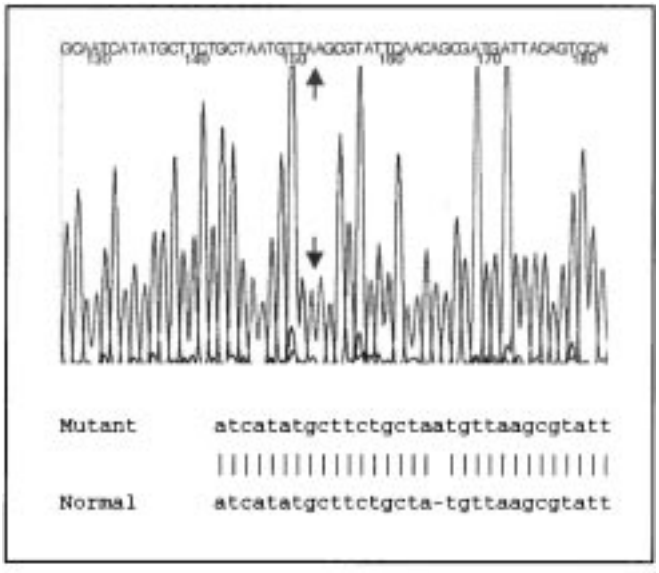

Figure 2. Sequence of *SRY* in Y18: (a) Insertion of an extra A is shown by arrows in red colour and (b) this insertion leads to a frame shift from codon 8 onwards and premature termination of the protein (shown in red).

is a +1 frame shift starting from the eighth codon (figure 2b). This apparently leads to premature termination after another seven amino acids. Studies of cloned *SRY* in a similar case of apparent premature termination have shown that the protein is not synthesized (Brown *et al.* 1998). Since no such nucleotide change has so far been reported from individuals with XY gonadal dysgenesis or from normal males, it is unlikely that this insertion is a rare polymorphism. Mutations in this region of the ORF have been reported in only four other cases. Three of these lead to premature termination (Veitia *et al.* 1997; Brown *et al.* 1998; Scherer *et al.* 1998) and the fourth is a Ser–Asn amino acid substitution (Domenice *et al.* 1998). Since all the four cases mentioned above had been diagnosed as having Swyer syndrome, we infer that this insertion is the cause of sex reversal in the present case (Y18). However, in the absence of samples from other tissues, specifically the gonads, it is not possible to say whether the mutation had occurred post-zygotically in the index case or was transmitted by the father. Further, owing to lack of paternal samples, it is not possible to distinguish between paternal mosaicism for the mutation and variable penetrance.

In the remaining 13 cases, we did not detect any mutation in the 778-bp fragment containing the *SRY* ORF. Thus, in this study, only one out of 14 cases of XY individuals with sex reversal or ambiguous genitalia was found to carry a mutation in *SRY.* The proportion of XY females in whom an *SRY* mutation was detected is slightly lower in the present study than in other investigations (reviewed in Cameron and Sinclair 1997). The proportion of individuals with mutations in *SRY* is higher in cases of complete gonadal dysgenesis (20–30%) (McElreavey *et al.* 1996; Scherer *et al.* 1998). The present study includes four cases of complete gonadal dysgenesis (see table 1), Y18 being one of them.

A majority of sex-reversed cases do not show a mutation in the *SRY* structural gene. What, then, could be the cause of sex reversal in these individuals? One possibility is that the regions flanking the ORF may have mutations leading to ectopic expression of *SRY* or its silencing. Only three instances of nucleotide changes have been reported from the flanking regions. In one study (Kwok *et al.* 1996), the region extending to 2 kb upstream of the *SRY* structural gene was investigated in 49 XY females and XY intersex individuals. A single-base-pair substitution was discovered at position −2027 in one case. In another study (Poulat *et al.* 1997), a point mutation at position −75 was detected in one out of 22 XY females. In a third study (Veitia *et al.* 1997), which included 26 cases of XY gonadal dysgenesis, three kilobases upstream and one kilobase downstream of the *SRY* ORF were investigated. A single-base-pair change at 1097 was detected 3′ of the ORF and another at −2627 upstream of the ATG. However, in all three cases the nucleotide changes were present in the fathers as well. Hence, in these cases too, it was not clear whether these nucleotide changes represent rare polymorphisms or were responsible for sex reversal in the index cases. If the mutation was indeed responsible for the sex reversal, then it must not have been penetrant in the fathers. Thus it appears that mutations in regions immediately flanking the structural gene are not a major cause of XY sex reversal. Deletions in regions away from the ORF have been reported in two sex-reversed individuals, suggesting that regions quite away from the ORF can affect the sexual phenotype. One of these deletions

(McElreavey *et al.* 1992) was a 33–60-kb *de novo* deletion with a breakpoint 1.8 kb 5′ to the *SRY* ORF. In the other (McElreavey *et al.* 1996), there was a *de novo* 3–8-kb deletion with one breakpoint 2–3 kb 3′ to the putative *SRY* polyadenylation site. These two deletions may have either removed regulatory sequences or interfered with *SRY* expression by bringing other regulatory sequences closer to the structural gene. Thus mutations outside *SRY* ORF are not only rare but it is also difficult to causally relate them to the observed sex reversal.

In the remaining 13 cases without mutations in *SRY*, another possibility is that mutations in genes other than *SRY* may be the cause of sex reversal. Six loci—*DSS* at Xp21, *WT1* at 11p13, *SOX9* at 17q24, and one each at 9p24, 10q and 1p—show such properties. *DSS* is defined by a 160-kb duplication which is associated with a female phenotype in an XY background (Bardoni *et al.* 1994). In the study of Bardoni *et al.* (1994) only one out of 27 XY females, and in another study (Veitia *et al.* 1997) none out of 20 XY cases had a detectable duplication of the *DSS* region. Lovell-Badge and coworkers (Swain *et al.* 1998) have shown that mice transgenic for two copies of *Dax1*, the human equivalent of which resides in the *DSS* region, undergo sex reversal in the presence of weak alleles of *Sry*. Association of sex reversal with partial monosomy of the 9p24 region has been reported in approximately 20 cases (Flejter *et al.* 1998; Guioli *et al.* 1998; Veitia *et al.* 1998 and references therein). Similarly, an association of deletion 10q with partial or complete sex reversal has been reported in a few cases (Wilkie *et al.* 1993). Recently, *de novo* duplication of 1p involving a locus at 1p31 has been detected in two XY females (J. Garcia-Heras, N. Corley, M. F. Garcia, M. K. Kukolich, K. G. Smith and D. W. Day 1998 De novo duplications of 1p. Report of 2 new cases in females without anomalies in genital development. *Am. J. Hum. Genet.* **63** (suppl.), A135 (abstract)). These 13 cases, which are apparently normal for *SRY*, showed no detectable chromosomal abnormalities. However, submicroscopic duplications/deletions of these loci cannot be ruled out. In addition, mutations in *WT1* at 11p13 (reviewed in Coppes *et al.* 1993) and *SOX9* at 17q24 (Foster *et al.* 1994) have also been implicated in sex reversal associated with Wilms' tumour and campomelic dysplasia, respectively. However, none of our cases show renal or bone disorders associated with Wilms' tumour or campomelic dysplasia. Since sex reversal without such abnormalities is not known to be associated with mutations in *WT1* (Nordenskjold *et al.* 1995) or *SOX9* (Meyer *et al.* 1997), these genes were not investigated.

In most cases of XY sex reversal there is a change in gene dosage, either from two to one, as with *SOX9*, 9p24 and 10q; from one to two, as in *DSS*; or from two to three, as in the case of 1p duplications. These examples, and the dominant inheritance of syndromes such as Denys–Drash and campomelic dysplasia, which involve *WT1* and *SOX9* respectively, and an increase in *SOX9* dosage from 2 to 3 in an XX male (B. Huang, S. Wang, Y. Ning, A. N. Lamb and J. Bartley 1999 Autosomal XX sex reversal caused by duplication of *SOX9*. *Am. J. Hum. Genet.* **65** (suppl.), abstract 20), provide further support for the view that dose-dependent mechanisms underlie human sex determination (Chandra 1985).

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