Autosomal XX Sex Reversal Caused by Duplication of SOX9

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SOX9 is one of the genes that play critical roles in male sexual differentiation. Mutations of SOX9 leading to haploinsufficiency can cause campomelic dysplasia and XY sex reversal. We report here evidence supporting that SOX9 duplication can cause XX sex reversal. A newborn infant was referred for genetic evaluation because of abnormal male external genitalia. The infant had severe penile/scrotal hypospadias. Gonads were palpable. Cytogenetic analysis demonstrated a de novo mosaic 46,XX,dup(17)(q23.1q24.3)/46,XX karyotype. Fluorescent in situ hybridization (FISH) with a BAC clone containing the SOX9 gene demonstrated that the SOX9 gene is duplicated on the rearranged chromosome 17. The presence of SRY was ruled out by FISH with a probe containing the SRY gene and polymerase chain reaction with SRY-specific primers. Microsatellite analysis with 13 markers on 17q23-24 determined that the duplication is maternal in origin and defined the boundary of the duplication to be approximately 12 centimorgans (cM) proximal and 4 cM distal to the SOX9 gene. Thus, SOX9 duplication is the most likely cause for the sex reversal in this case because it plays an important role in male sex determination and differentiation. This study suggests that extra dose of SOX9 is sufficient to initiate testis differentiation in the absence of SRY. Other SRY-negative XX sex-reversed individuals deserve thorough investigation of SOX9 gene. Am. J. Med. Genet. 87:349–353, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: sex determination; sexual differentiation; 17q duplication; FISH; microsatellite analysis

INTRODUCTION

Human sex determination and differentiation are complex processes with the SRY (sex determining region, Y chromosome) gene considered the major determinant of testis development [Simpson et al., 1987; Berta et al., 1990; Jager et al., 1990; Koopman et al., 1991]. Female-to-male sex reversal in karyotypic XX individuals is most frequently due to the presence of SRY, either due to an X-Y translocation or low-level XXY/XX mosaicism [de la Chapelle et al., 1990; Fechner et al., 1993]. However, approximately 10–20% of the XX males do not have any detectable Y chromosome material [de la Chapelle et al., 1990]. These observations, along with the observations of XY females with an intact, nonmutated SRY gene, suggest the presence of autosomal genes in the male sexual differentiation pathway initiated by SRY. One of the genes in the sexual differentiation and developmental cascade, SOX9, was identified in individuals with campomelic dysplasia and XY sex reversal [Foster et al., 1994; Wagner et al., 1994]. SOX9 mutations identified in these individuals result in loss-of-function products, suggesting that reduced gene dosage (haploinsufficiency) plays a role in altering the male developmental pathway. A dominant negative role of the mutated SOX9 protein was ruled out recently when a deletion of SOX9 was reported in an XY sex reversed individual with campomelic dysplasia [Olney et al., 1999].

SOX9 haploinsufficiency can cause XY sex reversal. Supporting the hypothesis that SOX9 duplication can cause XX sex reversal, we report here SOX9 duplication in a phenotypically male infant with XX karyotype. To our knowledge, this is the first demonstration of XX sex reversal caused by an autosomal gene.
MATERIALS AND METHODS

Clinical History

An infant boy was referred due to abnormal external genitalia. The infant had severe penile/scrotal hypospadias. The phallus was 0.5 cm in diameter and 1.2 cm long. The opening of the urethral meatus was at the base of the phallus. The perineal surface was closed between the urethral meatus and the anal opening. The scrotum was bifid with palpable gonads. Endoscopic or surgical visualization of the internal genitalia has not been done. Ultrasound examination did not identify a uterus. Full-body skeletal radiographs were normal. The remainder of the physical findings were normal. Congenital adrenal hyperplasia was not found as the serum electrolytes; 11-deoxycortisol and 17-hydroxyprogesterone were normal. Gonad biopsy was not performed at this time because of the young age.

Cytogenetic and Fluorescence In Situ Hybridization Studies

A peripheral blood specimen was collected at age 2 days and a skin biopsy specimen at age 1 month. Lymphocyte culture, skin fibroblast culture, harvest, and GPG banding were performed using standard methods. Fluorescence in situ hybridization (FISH) studies with a chromosome 17 painting probe (Vysis, Downers Grove, Illinois) were performed according to the manufacturer’s protocol. FISH studies with a probe containing SRY (Genzyme Genetics) and a BAC clone (RPCI-11 BAC clone 84E24, Research Genetics, Huntsville, Alabama) containing the SOX9 gene were also performed following standard protocol. The presence of SOX9 in this BAC clone was confirmed by polymerase chain reaction (PCR) with SOX9 specific primers [Foster et al., 1994] and by verifying the sequence of the clone (GenBank accession #AC007461).

Molecular Studies

To further determine whether SRY gene is present in this patient, multiplex PCR for SRY and β-actin were performed on DNA isolated from skin fibroblasts of the patient and blood of parents. The β-actin gene serves as internal positive control for PCR. SRY primers are as described [Lo et al., 1998]. Sequences of the forward and reverse primers of β-actin are ATCGTGATG-GACTCCGGTGAC and GCTGATCCACATCTGCG-TGGA, respectively. PCR was performed in a Perkin-Elmer GeneAmp 2400 thermal cycler in a volume of 25 μl consisting of 10 pmol of each of the SRY and β-actin primers, 100 ng of genomic DNA, 200 μM of dNTPs, 1 U of AmpliTaq DNA polymerase in buffer containing 2 mM MgCl₂, 50 mM KCl, and 10 mM Tris (pH 8.3). Following initial denaturing at 94°C for 3 min, amplifications were performed for 30 cycles of 94°C denaturing for 15 sec, 55°C annealing for 15 sec, and 72°C for 30 sec. PCR products were electrophoresed in 2% agarose gel, and bands were visualized under ultraviolet light after ethidium bromide staining.

Microsatellite PCR analyses were performed on the DNA isolated from skin fibroblasts of the patient and the blood specimens from both parents with 13 microsatellite markers on 17q23-24. The markers studied are listed in Table I. The primer sequences, genetic map position, and distance were described previously [Foster et al., 1994; Dib et al., 1996]. PCR and denaturing polyacrylamide gel electrophoreses were performed as described previously [Christian et al., 1995].

RESULTS

Cytogenetic and FISH Analysis

Cytogenetic analysis demonstrated a mosaic 46,XX,dup(17)(q23.1q24.3)/46,XX karyotype. The 17q duplication was found in 34% (17/50) of the lymphocytes and 78% (39/50) of the skin fibroblasts. The chromosome 17 homologs are illustrated in Figure 1. Both parents had normal chromosomes.

FISH with a chromosome 17 painting probe (Vysis) hybridized to the entire length of both chromosome 17 homologs (data not shown), confirmed that the duplicated region is chromosome 17 in origin. The SRY probe did not hybridize to any of the chromosomes (data not shown). FISH with the BAC clone containing

TABLE I. Microsatellite Analyses of the Duplicated Region

<table>
<thead>
<tr>
<th>Microsatellite markers</th>
<th>Map distance (cM)a</th>
<th>Genotypes</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Father</td>
<td>Mother</td>
<td>Child</td>
</tr>
<tr>
<td>D17S787</td>
<td>7.2</td>
<td>33</td>
<td>12</td>
<td>23</td>
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<tr>
<td>D17S1604</td>
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<td>12</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>D17S794</td>
<td>10.6</td>
<td>12</td>
<td>12</td>
<td>112</td>
</tr>
<tr>
<td>D17S840</td>
<td>1.0</td>
<td>11</td>
<td>12</td>
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</tr>
<tr>
<td>D17S1350</td>
<td>&lt;1.4b</td>
<td>12</td>
<td>22</td>
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</tr>
<tr>
<td>D17S970</td>
<td>&lt;1.4b</td>
<td>22</td>
<td>12</td>
<td>Homozygote 2</td>
</tr>
<tr>
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<td>112</td>
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<td>D17S785</td>
<td>0</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

aSex-averaged distance to the next marker.

bD17S970 is mapped between D17S1350 and D17S1351.

Sex-averaged distance between D17S1350 and D17S1351 is 1.4 cM.

SOX9 gene is located between D17S970 and D17S1351.

mat, maternal in origin.
the SOX9 gene showed one signal on the normal chromosome 17 and two signals on the rearranged chromosome 17 (Fig. 2), demonstrating that the SOX9 gene is duplicated.

**Molecular Studies**

PCR with SRY specific primers failed to amplify the DNA from the patient and the mother but did amplify the SRY fragment from the father (Fig. 3). Therefore, the XX sex reversal in this case is unlikely to be caused by the presence of SRY.

**DISCUSSION**

XX sex reversal cases are relatively rare and mostly caused by the presence of the SRY gene [de la Chapelle et al., 1990; Fechner et al., 1993]. Most SRY negative XX males have a high incidence of genital ambiguity or hypospadias, which are not common in SRY positive XX males. XX males and XX true hermaphrodites may exist in the same family, and the inheritance pattern leading to XX males is consistent with either X-linked, autosomal dominant, or both modes of inheritance [Kasdan et al., 1973; Skordis et al., 1987; de la Chapelle et al., 1990]. These findings suggest the presence of autosomal sex determining genes “down-stream” to SRY that can lead to masculinization in XX individuals.

Human sex development is a complex process. Male sex differentiation requires delicate dosage balance and interaction among multiple genes including SRY, SOX9, DAX-1, SF-1, and WT-1, and female sex differentiation acts as a ‘default’ pathway in the absence of SRY [Capel, 1998; Parker and Schimmer, 1998; Parker et al., 1999]. Several lines of evidence suggest that SOX9 plays an important role in male sex differentiation. Its expression is up-regulated in developing testes but absent in developing ovaries [Morais da Silva et al., 1996]. By direct interaction with SF-1 gene, SOX9 is also involved in the regulation of Sertoli cell-specific expression of anti-Müllerian hormone, which is required for Müllerian duct regression and phenotypic male differentiation [De Santa Barbara et al., 1998]. More importantly, SOX9 haploinsufficiency caused by mutations, balanced chromosome rearrangements, and deletions can cause campomelic dysplasia and XY
male-to-female sex reversal [Foster et al., 1994; Wagner et al., 1994; Olney et al., 1999].

In this report, we identified an SRY-negative female to male sex reversal patient with a duplication of chromosome band 17q23-24 including the SOX9 gene. Because of its important role in testis determination and differentiation, SOX9 duplication apparently is the most likely cause of the sex reversal in this case. However, not all XX individuals with a duplication of 17q23-24 have sex reversal, and several XX female patients with larger 17q duplications due to unbalanced translocations have been reported [Feldman et al., 1982; Lenzini et al., 1988; Caine et al., 1989]. The lack of sex reversal in these previously reported 17q duplication cases exemplifies the complex nature of mammalian sex determination and differentiation. The same phenomena have been observed in the other published findings of sex reversal cases. For example, not all XY patients with SOX9 haploinsufficiency have sex reversal [Foster et al., 1994; Schafer et al., 1996]. In fact, an identical mutation in SOX9 was found in two XY campomelic dysplasia patients, one with a male phenotype and the other with sex reversal [Kwok et al., 1995]. In another similar situation, duplication of DAX-1 or DSS gene was demonstrated to cause XY sex reversal [Bardoni et al., 1994; Zanaria et al., 1994], but only a portion of XY patients with DAX-1 duplication have sex reversal [Bardoni et al., 1994]. In a transgenic mouse study, it was found that high dose of Dax1 caused complete male to female sex reversal only in mice with weak Sry alleles [Swain et al., 1998].

If replicated in other SRY-negative XX sex-reversal patients, our study has the following significance. First, it demonstrates that duplication of SOX9 can cause XX sex reversal in absence of SRY and suggests that an extra dose of SOX9 is sufficient to initiate testis differentiation. Second, as DAX-1 is the first gene found to cause XY sex reversal when duplicated, SOX9 is the first gene identified that may cause XX sex reversal when duplicated. Third, in the present case, the duplication of chromosome area 17q23-24 is present at a much lower percentage in lymphocytes than that in skin fibroblasts. This finding warrants study of other cell types besides peripheral blood lymphocytes in search for SOX9 duplication in SRY negative XX sex reversal cases.

ACKNOWLEDGMENTS

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REFERENCES


