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A Gene Involved in XY Sex Reversal Is Located on Chromosome 9, Distal to Marker D9S1779

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Summary

The genetic mechanisms involved in sex differentiation are poorly understood, and progress in identification of the genes involved has been slow. The fortuitous finding of chromosomal rearrangements in association with a sex-reversed phenotype has led to the isolation of SRY and SOX9, both shown to be involved in the sex-determining pathway. In addition, duplications of the X chromosome, deletions of chromosomes 9 and 10, and translocations involving chromosome 17 have been reported to be associated with abnormal testicular differentiation, leading to male-to-female sex reversal in 46,XY individuals. We present the cytogenetic and molecular analyses of four sex-reversed XY females, each with gonadal dysgenesis and other variable malformations, and with terminal deletions of distal chromosome 9p, resulting from unbalanced autosomal translocations. PCR amplification and DNA sequence analysis of SRY revealed no mutations in the high-mobility-group domain (i.e., HMG box) in any of the four patients. Conventional and molecular cytogenetic analyses of metaphase chromosomes from each patient suggest that the smallest region of overlap (SRO) of deletions involves a very small region of distal band 9p24. Loss-of-heterozygosity studies using 17 highly polymorphic microsatellite markers, as well as FISH using YAC clones corresponding to the most distal markers on 9p, showed that the SRO lies distal to marker D9S1779. These results significantly narrow the putative sex-determining gene to the very terminal region of the short arm of chromosome 9.

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Introduction

Phenotypic females with an XY chromosome constitution, who exhibit ambiguous genitalia or complete gonadal dysgenesis, occur with a frequency of ~1/20,000 births (Robinson and Linden 1993) and often present as both medical and social emergencies. Not only do such abnormalities lead to recognition of other potentially serious birth defects and long-term medical problems, but the failure to promptly resolve the question of gender at birth may lead to a family psychosocial crisis relative to the sex of rearing. At puberty these individuals are typically deficient in secondary sex characteristics, have primary amenorrhea, and tend to be taller than XX females, yet, despite the presence of only a single X chromosome, they lack the stigmata of a Turner syndrome phenotype. Gynecological evaluation has shown that gonadal dysgenesis may present with a wide spectrum of findings, ranging from individuals with either testicular tissue on one side and a streak gonad on the other or bilateral streak gonads, although internal sex organs are most often female. In the presence of the Y chromosome, these individuals are at a significant (30%) risk for the development of malignant gonadoblastoma, and early gonadectomy is recommended (Sultana et al. 1995). XY sex reversal occasionally has been reported to occur in families in a manner suggestive of X-linked recessive inheritance, but sporadic occurrences are more common.

Progress in our understanding of sex differentiation and development, as well as the identification of genetic loci involved, has been slow. The identification of chromosomal rearrangements found in association with a sex-reversed phenotype has led to the isolation of two genes, SRY (sex determining region, Y chromosome) (Sinclair et al. 1990) and SOX9 (SRY-related box gene 9) (Foster et al. 1994; Wagner et al. 1994), both shown to be involved in the sex-determining pathway. Mutations in SOX9 have been observed in XY females with campomelic dysplasia (Foster et al. 1994; Wagner et al. 1994; Kwok et al. 1995; Meyer et al. 1997), and mu-

tations in SRY have been identified in a relatively small number (15%) of XY females (Berta et al. 1990; Jager et al. 1990; Hawkins et al. 1992; Affara et al. 1993; Cameron and Sinclair 1997). These findings suggest that sex reversal in the majority of cases involves alterations of other genetic loci.

In humans, several visible cytogenetic alterations have been associated with various sex-reversal syndromes, indicating not only the presence of other sex-determining genes, but also clues as to their location. Duplications of band Xp21 have been shown to be associated with XY-female development (Bernstein et al. 1980), suggesting the presence, on the X chromosome, of a region called "DSS" (dosage-sensitive sex reversal), mapped to a 160-kb region at Xp21 (Bardoni et al. 1994). In addition to SOX9, mapped to 17q24.3-q25.1 (Tommerup et al. 1993), two other autosomal loci also have been implicated in XY sex reversal. Deletions of loci on chromosomal regions 10q (Wilkie et al. 1993) and 9p (Jotterand and Julliard 1976; Fryns et al. 1986; Hoo et al. 1989; Crocker et al. 1988; Magenis et al. 1990; Bennett et al. 1993; McDonald et al. 1997; Ogata et al. 1997) also can result in XY females with gonadal dysgenesis, suggesting that these chromosomes also harbor genes involved in sex determination. Of eight previously reported cases of 9p−, six showed concurrent loss of 9p and duplication of different chromosomal regions, resulting from familial or de novo chromosome translocations (Jotterand and Julliard 1976; Fryns et al. 1986; Crocker et al. 1988; Hoo et al. 1989; Magenis et al. 1990; McDonald et al. 1997). Two cases showed terminal de novo deletions (Bennett et al. 1993; Ogata et al. 1997). In most cases, the patients exhibit sex reversal with normal-appearing female external genitalia in addition to varying degrees of gonadal dysgenesis and other phenotypic malformations. Among those individuals, the most proximal breakpoint reported is at 9p21, and the most distal is at 9p24. These data suggest that a gene(s) located within band 9p24 is involved in the production of a normal male testis. In this study, we have obtained lymphoblastoid cell lines from two previously reported patients (Hoo et al. 1989; McDonald et al. 1997) and two previously unreported patients with complete or partial sex reversal and 9p monosomy, who also have duplications of other autosomal regions. Conventional and molecular cytogenetic characterization of these cell lines, in addition to loss of heterozygosity (LOH) studies using 17 highly polymorphic microsatellite markers, have localized the smallest region of overlap (SRO) distal to marker D9S1779 within band 9p24.3. These results further define the minimum 9p critical region that harbors a gene involved in male sex determination.

Patients and Methods

Patients and Cytogenetic Analysis

Case 7, a normal female at birth, with no immediate physical or developmental findings, was referred for evaluation of a previously identified familial chromosomal translocation. Family history revealed recurrent miscarriages. Karyotype analysis of the proband's father and his twin brother showed a balanced translocation between chromosomes 8 and 9. Their karyotypes were designated 46,XY,t(8;9)(p21;p24). The clinical and cytogenetic evaluation of this patient has been reported elsewhere (McDonald et al. 1997). In brief, at 3 years of age she had no noticeably dysmorphic features but demonstrated mild developmental delays in major motor development and expressive-language development. Routine G-band analysis revealed a male 46,XY chromosome complement, in addition to a derivative chromosome 9 (der[9] chromosome) in which the distal short arm of one homologue appeared to include additional genetic material of undefined origin. Her karyotype was determined to be 46,XY,der(9)t(8;9) (p21;p24)pat, indicating that she is monosomic for the distal portion of chromosome 9p24 and trisomic for the p arm of chromosome 8 bands p21-pter. FISH analysis, using a chromosome 9–specific paint probe, confirmed these findings in both the proband and her father (McDonald et al. 1997). In view of her XY sex-chromosome constitution, she was evaluated by pelvic ultrasound. Examination showed female internal structures including a uterus and vagina; gonads were not identified. Levels of both follicle-stimulating hormone and luteinizing hormone were elevated, suggesting that she has ovarian dysgenesis. She has since undergone a complete gonadectomy because of an increased risk for the development of gonadoblastoma.

Case 8 was a full-term infant ascertained for tracheoesophageal fistula and complex congenital heart disease. Physical examination revealed multiple anomalies, including short palpebral fissures, broad nasal bridge with telecanthus, mild micrognathia, short neck with redundant skin, and choanal atresia. The patient's chest was asymmetrical, with the right side larger than the left and with slight pectus excavatum. The heart showed a ventricular septal defect, coarctation of the aorta, and a hypoplastic left ventricle. She also had bilateral hydronephrosis with hydroureters, normal female external genitalia, and a uterus. Other findings included syndactyly of the third and fourth toes, hyperextensibility of the joints, and mild hypotonia. Chromosome analysis revealed both a der(9) chromosome showing loss of distal 9p and additional material thought to be derived from chromosome 15. Her karyotype was designated

46,XY,der(9),t(9;?15)(p22;?q22.3). Subsequent FISH analysis using a chromosome 9–specific paint probe confirmed that the translocation involved chromosome 9, but no signal was observed on the der(9) chromosome when a chromosome 15 paint probe was used.

Case 9 presented, at birth, with ambiguous external genitalia and respiratory distress. Dysmorphology evaluation revealed hypotonia, micrognathia, microsomia, long philtrum, short nose with prominent alae, a short neck, and wide-set nipples. The external genitalia consisted of hyperpigmented, fused labioscrotal folds, extreme microphallus, and small palpable testes. There was a small urethral opening at the base of the phallus, with a normal anus. A pelvic examination showed normal kidneys, adrenals, and bladder, with no uterine structures. Karyotype analysis revealed a 46,XY,der(9)t(9;11)(p24;q14.2) chromosome complement. Subsequent cytogenetic studies of parental blood samples showed that the mother is a carrier of a balanced reciprocal translocation involving chromosomes 9 and 11. The proband's karyotype was designated 46,XY,der(9)t(9;11)(p24;q14.2)mat. FISH analysis with chromosome 9–specific and chromosome 11–specific paint probes confirmed involvement of these two chromosomes.

Case 4 has been published elsewhere (Hoo et al. 1989). The clinical details on this child have not been described completely, although she was reported to have multiple congenital anomalies, including normal female external genitalia and the presence of a vagina. Previous analysis of the patient's chromosomes revealed a 46,XY,der(9),t(2;9)(p21;p24) karyotype. This cell line was obtained through the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository (Camden, NJ) (repository number GM12896). G-banding and FISH with chromosome 2–specific and chromosome 9–specific paint probes confirmed the cytogenetic findings.

SRY Analysis

Genomic DNA samples from each patient and from normal male and female controls were used as templates to PCR-amplify the entire high-mobility group (HMG) domain of SRY. Primer sets used in the PCR have been reported elsewhere (Affara et al. 1993) and include XES2 and XES7, which synthesizes a 609-bp fragment that spans both the entire HMG domain of the SRY gene and flanking sequences. For each DNA sample, duplicate PCR reactions were performed at 94°C for 5 min, followed by 35 cycles each of 94°C for 1 min, 68°C for 2 min, and 72°C for 3 min. PCR products were analyzed on 1% agarose gels. Amplified fragments subsequently were isolated, purified, and sequenced by means of M13 forward and reverse nested primers, SRY3 and SRY6, which flank a 269-bp region of the HMG domain of the SRY gene. The resulting double-strand DNA templates were sequenced directly from both ends by means of the dsDNA Cycle Sequencing System (Gibco/BRL). Sequence reactions were run on 6% denaturing polyacrylamide gels and were autoradiographed overnight. Patient SRY sequences were compared with those of normal male control samples.

FISH

Hybridization protocols have been reported elsewhere (Flejter et al. 1993, 1995). YAC clones corresponding to markers D9S1858, D9S1779, and D9S1813 were hybridized to metaphase cells from case 7 and her father. A chromosome 9–specific centromere probe was used as a control for each hybridization. Slides were visualized on a standard fluorescence microscope equipped with the appropriate epifluorescence optics. Photographs were taken with an Olympus microscope equipped with a Photometrics CCD camera. Images were produced by Vysis Imaging Software.

Microsatellite Studies

Amplification of genomic DNA from each patient was performed according to the specific methods reported for each oligonucleotide pair. Primer sequences and the PCR conditions are as described in the references given in table 1. Typically, one primer was end-labeled with [^{32}P]-dATP, and PCR was performed for 25–35 cycles, with 30–50 ng of DNA in a 25-μl reaction volume under recommended amplification conditions. A portion of each amplified DNA sample was denatured and separated on 6%–8% denaturing polyacrylamide gels at 200–300 V for 3–6 h, depending on the fragment size. Gels were exposed to Kodak XAR-5 film for 4–6 h. Each set of reactions also contained DNA from three CEPH individuals (K884-01, K1347-8408, and K1362-8574), as standard controls.

Results

Conventional and molecular cytogenetic analyses of the cell lines derived from each of four sex-reversed individuals showed breakpoints in chromosome 9p, spanning deletions ranging from band 9p22 to band 9p24, indicating that the smallest region of deletion—and, hence, the putative sex-determining gene—lies within band 9p24 (table 2).

To rule out the possibility that sex reversal in our patients is not due to alterations in the conserved region of SRY but, rather, results from a deletion on distal 9p, mutation analysis of the SRY HMG domain was performed. In these studies, genomic DNA from each 9p− cell line and from normal male and female controls was

Table 1

Microsatellite Analysis of Sex-Reversed 9p− Patients

Locus	Genotype of[a]						No. of Alleles	Hetero-zygosity	Reference[b]
	Case 7	Father	Mother	Case 8	Case 9	Case 4			
D9S1858	2,2	1,2	1,2	2,2	2,2	2,2	3	58	Dib et al. (1996)
D9S1779	2,–	3,4	2,4	4,4	1,1	2,2	8	63	Dib et al. (1996)
D9S1813	4,6	4,6	2,6	1,1	2,2	5,5	13	83	Dib et al. (1996)
D9S1871	4,6	4,6	2,6	1,1	2,2	5,5	7	68	Dib et al. (1996)
D9S143	1, 2	1, 1	1,2	2,2	1,1	1,1	8	54	Furlong et al. (1992)
D9S230	2,3	2,5	1,3	5,5	4,4	2,2	4	67	Wilkie et al. (1992)
D9S54	1,2	2,2	1,5	1,1	1,1	1,1	8	54	UMDG
D9S178	1,2	1,2	1,2	1,1	3,3	2,2	4	68	Dib et al. (1996)
D9S286	3,6	1,6	3,4	3,5	1,5	2,6	12	87	Dib et al. (1996)
D9S168	1,2	2,2	1,2	2,2	3,3	1,4	9	75	Dib et al. (1996)
D9S235	2,2	2,2	1,2	1,2	1,2	1,2	2	63	Dib et al. (1996)
D9S285	2,2	2,2	2,2	1,3	2,2	4,5	9	79	UMDG
D9S156	1,2	2,2	1,2	1,4	2,3	1,2	15	79	Dib et al. (1996)
D9S162	1,7	7,7	1,2	1,2	2,2	2,2	10	72	Dib et al. (1996)
D9S171	1,6	…				…	9	79	Dib et al. (1996)
D9S770	2,3	3,4	1,2	1,3	1,2	1,4	2	10	UMDG
D9S232	1,4	2,4	1,3	1,4	1,2	3,4	7	63	UMDG

[a] Underlining denotes that LOH is suggested.
[b] UMDG = The Utah Marker Development Group (1995).

used as template to PCR-amplify both the entire HMG domain and flanking sequences of SRY. Gel electrophoresis revealed an expected 609-bp product from the DNA of each patient and from that of normal male controls. No product was seen in normal female control lanes. Sequence analyses, using nested PCR primers flanking the HMG domain spanning 269 bp, revealed no mutations in either the SRY HMG domain or immediate flanking sequences, in any of the four cell lines analyzed, compared with normal male HMG sequences (data not shown).

To define the SRO and to identify resources for mapping studies, each patient DNA sample was characterized for LOH, by means of 17 highly polymorphic markers previously mapped to 9p21-pter (table 1). Parental DNA samples were available for case 7 only. The genetic and physical maps of distal 9p are shown in figure 1. Map locations are based on recent data compiled from Généthon and CHLC databases and from mapping reports published elsewhere (Dib et al. 1996; Povey et al. 1997). Results of the analyses reveal that case 7 showed LOH for marker D9S1779, with neither of the paternal alleles present in her DNA (table 1 and fig. 2). For marker D9S1858, both parental samples showed alleles 1 and 2, whereas the proband showed only an allele 2 (fig. 2). Since this marker lies distal to D9S1779, it is likely that this result is consistent with heterozygous loss, rather than homozygosity, for allele 2.

Parental samples were not available for cases 4, 8, and 9. Analyses of DNA from each of these individuals, by means of all 17 microsatellite markers, revealed a single allele for eight consecutive markers ranging from D9S1858 through D9S178 (table 1). Although we cannot distinguish definitively the difference between homozygosity and heterozygous loss in these individuals, it is extremely unlikely that all eight consecutive markers would be homozygous in each of the three individuals by chance alone. If these individuals are in fact hemizygous for these markers, the data suggest that their deletions are ~11 cM larger than that seen in case 7 (fig. 1).

To confirm the microsatellite findings and to further define the smallest region of deletion, we obtained YAC clones corresponding to the three most distal markers, for FISH analysis of case 7 and her father: two YACs represent D9S1858, two are for marker D9S1779, and three are for D9S1813. The results of the hybridizations are shown in table 3. To insure that any observed loss was due to deletion and was not the result of suboptimal hybridization, an alpha-satellite probe was used as a control marker for the centromere of chromosome 9. The normal chromosome 9 also served an internal control. The failure to observe a hybridization signal on the distal p arm of one chromosome 9 in multiple cells, for any given probe, should be detected when it is located within the site of deletion, indicating LOH. The presence of a signal on both chromosome 9p regions indicates that the deletion does not involve that locus. The data show that case 7 is deleted for the two YACs corresponding to marker D9S1858. In these hybridizations, signal was observed on the normal chromosome 9 only. Cells from the proband's father showed a signal both on the normal chromosome 9 and on the der(8) chromosome. These data confirm the microsatellite re-

sults—that is, that there is LOH for this marker, as opposed to homozygosity of this locus (fig. 3*A*). Hybridization of YAC 747A1, corresponding to marker D9S1779, to metaphase cells from the proband showed hybridization both to the normal chromosome 9 and to the der(9) chromosome (fig. 3*B*). Hybridization of the same probe to metaphase cells from her father revealed hybridization to the normal chromosome 9, to the der(9) chromosome, and to the der(8) chromosome, indicating that this clone crosses the translocation breakpoint (fig. 3*C*). The second D9S1779 YAC clone, 904H6, showed hybridization both to the normal chromosome 9 and to the der(9) chromosome, in both the proband and her father. Each of the three YAC clones corresponding to marker D9S1813 showed hybridization both to the normal chromosome 9 and to the der(9) chromosome, in both the proband and her father. These results coincide with the microsatellite LOH data.

Discussion

To date, 10 cases, including our patients, who have 9p− found in association with XY sex reversal have been reported (table 2). Cases 1–5 and 7–9 each have concurrent duplication of different DNA segments, resulting from unbalanced chromosomal translocations. Cases 6 and 10 have de novo deletions of 9p. Of the total 10 cases, 7 exhibit normal female external genitalia, and 3 have ambiguous external genitalia. All patient's have varying degrees of internal female structures and mixed gonadal dysgenesis. Conventional cytogenetic studies indicate that the 9p breakpoints range from band 9p21 to band 9p24, with the SRO spanning 9p24-pter. These findings suggest that the monosomic region common to the 10 patients contains a gene(s) responsible for defective testis formation and, as a result, varying degrees of impaired male sex development.

Previous studies have shown that mutations in SRY are found in only a small portion (15%) of sex-reversed XY females. With few exceptions (McElreavy et al. 1992; Cameron and Sinclair 1997), the majority of mutations have occurred in the conserved HMG domain of the SRY gene. Therefore, it has been postulated that the majority of XY females who lack mutations in SRY have either mutations in or loss of other genes involved in the testis-determining pathway. To rule out the possibility that sex reversal in our patients is not due to alterations

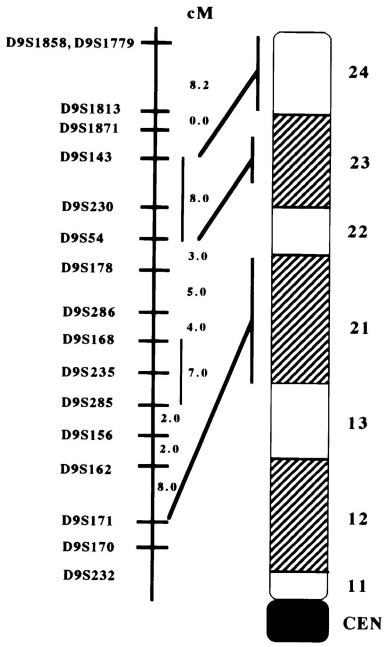

Figure 1 Physical and genetic maps of distal 9p. Previously mapped microsatellite markers, with both estimated distance between markers and map locations, are shown on the left. The map is based on information from Genome Database and CHLC and on other previously published data.

Table 2

Summary of Patients with Sex Reversal and Deletions of 9p

Case	Reference	Karyotype[a]	Origin[b]
1	Jotterand and Juillard (1976)	46,XY,der(9),t(9;13)(p21;q21)	Maternal
2	Fryns et al. (1986)	46,XY,der(9),t(3;9)(p21.33;p22.1)	Maternal
3	Crocker et al. (1988)	46,XY,der(9),t(7;9)(q31.1;p23)	Paternal
4[c]	Hoo et al. (1989)	46,XY,der(9),t(2;9)(p21;p24)	De novo
5	Magenis et al. (1990)	46,XY,der(9),t(4;9)(?;p24)	Paternal
6	Bennett et al. (1993)	46,XY,del(9)(p23)	De novo
7[c]	McDonald et al (1997)	46,XY,der(9),t(8;9)(p21;p24)	Paternal
8[c]	Present study	46,XY,der(9),t(9;?15)(p22;?q22.3)	Unknown
9[c]	Present study	46,XY,der(9)t(9;11)(p24;?q14.2)	Maternal
10	Ogata et al. (1997)	46,XY,del(9)(p23)	De novo

[a] Underlining denotes chromosome 9 breakpoints.

[b] "Maternal" denotes that the mother is a carrier of the balanced chromosome, and "Paternal" denotes that the father is a carrier of the balanced chromosome.

[c] Cell line is currently available for study.

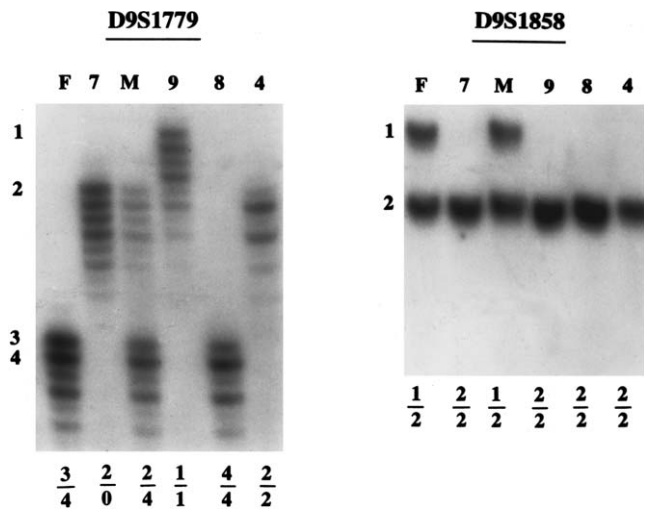

Figure 2 Microsatellite analyses of four 9p− cases (lane numbers denote case numbers) and the father (lanes F) and mother (lanes M) of case 7. Genotypes for each individual are listed under their respective lanes. Marker D9S1779 shows LOH in case 7 and a single allele in cases 4, 8, and 9. Marker D9S1858, mapping distal to D9S1779, shows a single allele for each patient.

in the HMG domain of SRY but, rather, results from a deletion of distal 9p, sequence analysis of the HMG domain in each individual was performed. No mutations were found in any of the four patients. These results support our hypothesis that either mutation in or loss of a gene(s) located on chromosomal region 9p is likely responsible for the XY-female phenotype—and that the latter is not the result of either mutations in the HMG domain or loss of function of the SRY gene product.

Microsatellite analyses of markers spanning the distal short arm of chromosome 9 demonstrate that the gene necessary for male sex development lies distal to marker D9S1779. Among our patients, case 7 exhibits the smallest deletion associated with a sex-reversed phenotype. LOH studies show that this patient is hemizygous for both marker D9S1779 and marker D9S1858. FISH analyses using two YAC clones corresponding to marker D9S1858 confirmed loss of this locus in case 7. Although, on the basis of microsatellite analysis, this patient showed a single allele for D9S1779, FISH hybridization signals for two YAC clones corresponding to this marker were seen both on the normal chromosome 9 and on the der(9) chromosome, suggesting that the locus is present. Hybridization of the same two YAC clones to DNA from the proband's father showed that one clone, YAC 757A1, hybridized to the normal chromosome 9, to the der(9) chromosome, and to the der(8) chromosome. The second clone, 904H6, was on the normal chromosome 9 and the der(9) chromosome only. These data indicate that clone 757A1 crosses the translocation breakpoint and therefore defines the proximal 9p boundary of the sex-determining region. The discrepancy between the microsatellite data and the FISH results for this marker, in case 7, may indicate that the D9S1779 repeat sequence is not present on either of the YAC clones used in the FISH studies. Over time, YACs have been shown either to recombine out repetitive sequences or to delete random regions of insert DNA. If the repeat lies telomeric to 9p, at the translocation breakpoint, and is not present in the YAC DNA, hybridization signals would be expected on both chromosomes 9. Since YAC 904H6 does not cross the breakpoint in the father and is present on both copies of chromosome 9 in the proband, this clone may lie proximal on chromosome 9 and may remain unaffected by the translocation breakpoint. A second possibility is that the YAC crosses or is very close to the translocation breakpoint. Characterization of this locus by means of additional physical-mapping clones is needed, to clarify these results.

The mechanism by which monosomy 9p gives rise to a female phenotype in a 46,XY chromosome background is not clearly understood. In four of the reported cases, including two of our patients, the deletions were the result of familial balanced chromosomal translocations. In three cases the translocation was derived from the mother, and in three cases the translocation was paternal in origin. This makes the possibility of imprinting unlikely. In these instances, it is not known whether sex reversal is due to monosomy for dosage-sensitive genes or whether the deletions reveal recessive mutations. Hoo et al. (1989) have postulated that, since the majority of patients with "9p− syndrome" do not show complete sex reversal, there may be, on 9p, a recessive gene that is important in the early development of the testis. The lack of this gene product in 9p− syndrome patients is

Table 3

Chromosome 9–Specific YACs Hybridized to Metaphase Chromosomes from Case 7 and from Her Father

	Status in[a]	
Marker	Father	Case 7
D9S1858:		
765H2	+−	+−
816E6	+−	+−
D9S1779:		
757A1	++[b]	++
904H6	++	++
D9S1813:		
803F2	++	++
763G5	++	++
822E7	++	++

[a] ++ = Hybridization signals on both the normal chromosome 9 and the der(9) chromosome, +− = hybridization signal only on the normal chromosome 9.

[b] YAC crosses the translocation breakpoint.

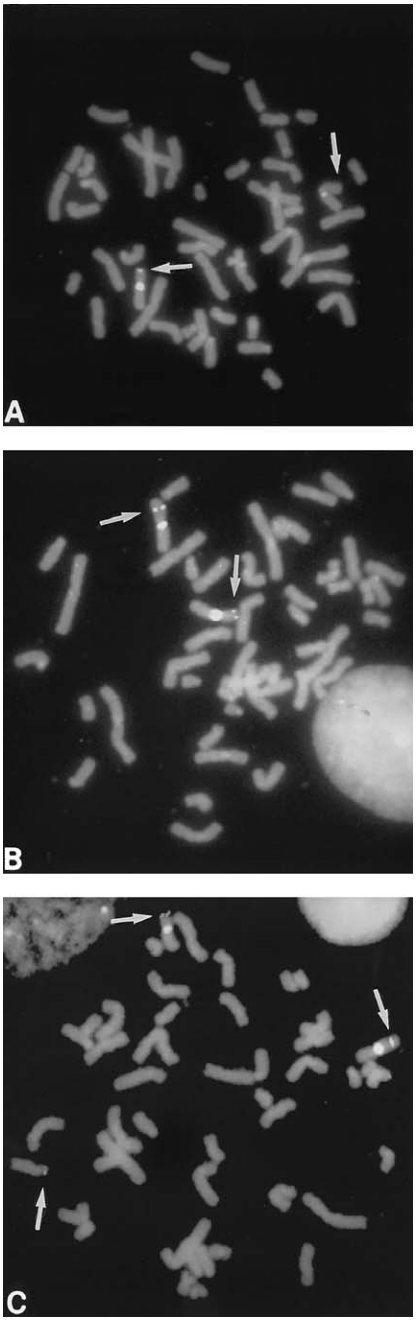

Figure 3 Characterization of case 7 and her father, by FISH. *A*, Hybridization of YAC 765H2, representing marker D9S1858, to a metaphase cell from case 7. A hybridization signal is seen on the normal chromosome 9 only. *B* and *C*, Hybridization of YAC 747A1, representing marker D9S1779, to a metaphase cell from case 7 (*B*) and from her father (*C*). In the proband, signals are seen on both the normal chromosome 9 and on the der(9) chromosome. In her father, signals are seen on the normal chromosome 9, on the der(9) chromosome, and on the der(8) chromosome, indicating that the YAC crosses the translocation breakpoint. In all cells, an alpha-satellite probe marks the chromosome 9 centromere.

sufficient to cause a delayed and incomplete testicular formation, resulting in the absence of influence of male hormones during the crucial period of sexual differentiation. In contrast, 46,XY sex-reversed individuals may carry a defective gene on their apparently normal intact chromosome 9, concurrent with deletion of the healthy allele on the translocated 9p, resulting in complete sex reversal. Haploinsufficiency is an alternative explanation, and a dominant mode of expression cannot be ruled out. Variable penetrance and expressivity could account for the low frequency of sex reversal due to the deletion, and for the phenotypic differences between patients. This has been proposed as a mechanism whereby some 46,XY patients with campomelic dysplasia have normal sex development whereas others (75%) have some degree of ambiguous genitalia or sex reversal (Meyer et al. 1997). One other hypothesis is that sex reversal may be dependent on whether deletions are interstitial or terminal (Magenis et al. 1990). It has been suggested that phenotypic features in monosomy 9p patients—and the associated sex reversal—are part of a contiguous-gene syndrome. Varying clinical findings may be dependent on which 9p segment is missing, with a critical segment, leading to testis formation of male genitalia, being in the most distal region. The vast majority of deletions in 9p− syndrome patients are de novo, and deletions must be interstitial, since, in theory, the chromosome end must be capped by the telomere. In those cases, it is possible that the sex-determining gene, located in close proximity to the telomere, remains intact and that sex reversal is not seen even though other malformations may be present. In contrast, in all but one individual with sex reversal and 9p−, the deletions are the result of reciprocal familial translocations. In those cases, the deleted chromosome 9 is capped by the telomere of the alternate chromosome involved in the translocation. The chromosome 9 telomere, as well as the putative sex-determining gene from the der(9) chromosome, presumably is translocated to the second chromosome involved in the translocation, which does not segregate to the unbalanced probands. In these cases, the patients have lost of one copy of the sex-determining gene, which results in gonadal dysgenesis. This hypothesis would be consistent with the data presented, which suggest a terminal location of the putative sex-determining gene, distal to marker D9S1779.

Few previous studies have defined the location and physical size of the 9p deletion involved in XY sex reversal. Ogata et al. (1997) have mapped the deletion in one 9p− patient to the region distal to marker D9S168, ~28 Mb from the 9p telomere. Veitia et al. (1997) have mapped the smallest deletion in their patients as being distal to marker D9S144, ~14–21 cM from the telomere, and Guioli et al. (1997) have mapped the smallest deletion in their panel of patients as being between markers

D9S1779 and D9S1810, with an estimated physical size of ~8–10 cM. In the present study, FISH and LOH studies of four patients suggest that the smallest common region is defined by case 7. The deletion breakpoint in this patient is defined by the balanced translocation in her father and lies within or distal to marker D9S1779 at band 9p24.3. This defines the proximal locus for further physical-mapping efforts. Since the distance of marker D9S1779 from the 9p telomere is not known, our efforts to define the exact size of the deletion in case 7—and to identify the putative sex-determining gene—will rely on the construction of a physical map spanning this smallest region of deletion. Not only will the identification of this gene(s) be of significance in the clinical diagnosis and management of patients with ambiguous or incomplete gonadal development, but it also will provide insight into the genetics of sex determination. Together, these data can be integrated with the cell biology of gonadal differentiation, to produce a more complete understanding of this mammalian developmental system.

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