RCP mutations reported by Nathans et al. (1989) to cause reduced visual acuity but with normal fundus. In fact, figure 2 in the article by Nathans et al. (1989) shows a fundus photograph from a patient with "progressive bilateral central retinal degeneration" (p. 832). Other patients are described as having macular lesions and atrophy, on ophthalmic examination. Therefore, a phenotype such as the one Bergen and Pinckers observed in this family evidently could result from a mutation or combination of mutations in the red- and green-pigment genes. In these circumstances, exclusion of these genes is essential before a new locus is assigned.

Bergen and Pinckers do indeed describe a multipoint analysis using markers DXS8103 and DXS8069, apparently spanning the RCP and GCP genes, that excludes these genes. However, this evidence is given as "data not shown," no LOD scores for these markers are included in table 3, and no reference is given to a published map proving that these markers span the genes in question. Furthermore, the authors' assertion that "Southern blot analysis with an RCP/GCP cDNA probe…did not reveal any structural abnormalities" (Bergen and Pinckers 1997, p. 1,472) surely is insufficient, since abnormalities at this locus can result from point mutations or from rearrangements 4-kb upstream of the red cone–pigment gene and 43-kb upstream of the green cone–pigment gene. Therefore, although data excluding the RCP/GCP locus in this family may exist, this could not be proved on the basis of the results presented.

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Swyer Syndrome and 46,XY Partial Gonadal Dysgenesis Associated with 9p Deletions in the Absence of Monosomy-9p Syndrome

To the Editor:

Sex determination in humans depends on the function of the *SRY* (sex-determining region, Y) gene. This gene consists of a single exon located on the short arm of the Y chromosome and encodes a protein that has a conserved domain shared by the high-mobility–group nuclear proteins and various transcription factors (Sinclair et al. 1990). The SRY protein has DNA-binding and DNA-bending activities, suggesting that it may be a transcription factor that controls the expression of downstream genes involved in sex determination and/or differentiation (Pontiggia et al. 1994). Swyer syndrome is characterized by a female phenotype and gonadal dysgenesis leading to a streak gonad, in an individual with a 46,XY chromosome complement (MIM 306100). Affected individuals have normal stature and do not have Turner stigmata (German 1970). Approximately 15% of these cases have mutations in the *SRY* gene. Duplications of the locus *DSS* (dosage-sensitive sex reversal) at Xp21.3 also are associated with 46,XY gonadal dysgenesis; however, duplications of Xp are uncommon in 46,XY females (Veitia et al. 1997*a*). The etiology of the majority of cases is unknown.

Partial monosomy of 9p (MIM 158170) is associated with a syndrome characterized by mental retardation; trigonocephaly; upward-slanting palpebral fissures; short, broad, and webbed neck; flat nasal bridge; anteverted nostrils; and long philtrum (Alfi et al. 1973; Huret et al. 1988). The presence of ambiguous genitalia has been reported in 70% of XY individuals monosomic for 9p (De Grouchy and Turleau 1982, pp. 162–167; Schinzel 1984). In addition, an increasing number of reports describe 46,XY partial or complete gonadal-dysgenesis cases associated with deletions of 9p and presenting with dysmorphic features due to either the deletion syndrome or the presence of a trisomic segment (Bennett et al. 1993; Ogata et al. 1997; Veitia et al. 1997*b*; MIM 273350). In three of these cases, the breakpoints have been defined at a molecular level, with the smallest deleted region distal to *D9S144* (Veitia et al.

1997*b*) and *D9S168* (Ogata et al. 1997), which map ~21 cM from the telomere. Here, we describe the molecular analysis of two cases of 46,XY gonadal dysgenesis associated with deletions of 9p. One of them had a cytogenetically detectable 9p deletion, and the other had an apparently normal 9p.

Patient 1, of Portuguese origin, was born to unrelated parents and presented at birth with a normal somatic phenotype and clitoridomegalia, mild hypotonia, and a left clubfoot. Birthweight was 2,900 g, and height was 49 cm. At age 7 years the patient had affective disorders but a normal IQ. At age 10 years weight was 40 kg, and height was 145 cm. At this time, internal genitalia consisted of a normal vaginal cavity, uterus, and fallopian tubes and of bilateral streak gonads. Karyotype analysis indicated a 46,XYdel(9)(p23) chromosome complement. Hormonal evaluation at age 9 years was consistent with partial gonadal failure: plasma follicle-stimulating hormone 33 mU/ml; luteinizing hormone 21 mU/ml; testosterone 46.9 ng/dl (normal 0–30 ng/dl) and, after hCG stimulation, 68.7 ng/dl; and dihydrotestosterone 12 ng/dl (normal 3–10 ng/dl) and, after hCG stimulation, 20 ng/dl. The gonads consisted of a fibrous stroma without primary follicles (fig. 1). Small islands of Leydig-like cells were observed. On the basis of the combined endocrinology, histology, and phenotype of the patient, the diagnosis of Swyer syndrome was made. The analysis, by SSCP, of 4 kb around the *SRY* gene failed to show the presence of mutations.

A group of 10 completely or partially 46,XY sex-reversed patients (for whom the DNA of the parents was available) also were analyzed, for the presence of a loss of heterozygosity (LOH) at 9p. These cases previously had been screened for mutations in the *SRY* and *DSS* regions (Veitia et al. 1997*a*). In one case, a small deletion was found (for details of molecular analysis, see below). This individual, patient 2, of French origin, presented at birth (birthweight 2,000 g, height 49 cm) with ambiguous external genitalia (Prader 3) and one gonad palpable in the right genital fold. At laparatomy, normal uterus, vagina, fallopian tubes, and bilateral rudimentary gonads were observed. Wolffian structures such as the epididymis were also present. Gonad histology showed the presence of syncytial Sertolian cords and some spermatogonia. The patient had no dysmorphic features, and there was no evidence of psychomotor retardation, except for learning difficulties and affective disorders. High-resolution karyotype analysis indicated an apparently normal 46,XY chromosome complement. Final height, at age 19 years, was 163 cm. The patient was diagnosed as having partial gonadal dysgenesis.

The breakpoints were mapped, in both cases, by analysis of the segregation of polymorphic microsatellite alleles. The markers used in this study were, from telomere to centromere, *D9S1779, G10023, D9S1858, VLDLR* trinucleotide repeat, *D9S1813, D9S1810, D9S937, G10183, D9S286, D9S144, D9S168, D9S256, D9S267,* and *D9S254*. Primer sequences for all markers were obtained from Genome Database and Whitehead Institute Database. The PCR reactions were performed under standard conditions for 30 cycles, in the presence of a fluorescently labeled dUTP derivative (R6G; Perkin Elmer, Applied Biosystems). Analysis was performed with an ABI370A automatic sequencer provided with GeneScan software (ABI), according to the manufacturer's instructions.

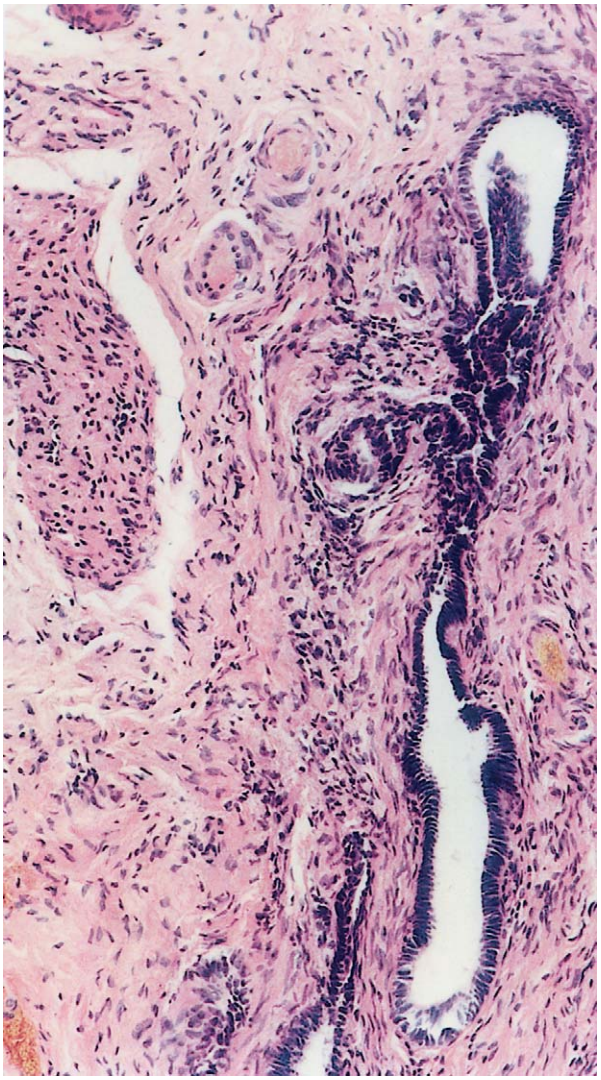

Figure 1 Detail of a hematoxylin-and-eosin–stained section of the right gonad of patient 1. Both gonads consisted of a fibrous stroma without primary follicles. Rare agglomerations of Leydig-like cells also were observed. Full clinical, histological, and molecular data are available on request.

Patient 1 carried a deletion of one chromosome 9,

involving 9p23 (distal-middle) to pter. The rearranged chromosome 9 was found to be of paternal origin, and its breakpoint was located between *D9S144* and *D9S267* (fig. 2). In spite of carrying a large deletion, the patient did not present with the specific stigmata of the monosomy-9p syndrome. The hypotonia in this and other 9p-deleted patients could be caused by an alteration of the high-affinity glutamate-transporter (excitatory amino acid carrier) gene (*SLC1A1*), which maps to the monosomic region (Smith et al. 1994). Monosomy-9p syndrome is due to the heterozygous deletion of a critical region mapping between *D9S286* and *D9S267* (Schwartz et al. 1997). However, Ogata et al. (1997) have described a sex-reversed patient carrying a pure 9p deletion who presented with a phenotype compatible with the 9p-deletion syndrome. The breakpoint was located distal to *D9S168* (20 cM from the telomere), suggesting that the monosomy-9p critical region must lie distal to this marker. Our results suggest that this critical region is proximal to *D9S144,* because the absence of the 9p distal portion including this marker in patient 1 is not associated with the characteristic dysmorphology. Taken together, these data indicate that the minimum region responsible for the syndrome may be located between *D9S144* and *D9S168*. Notably, both markers have been unambiguously mapped to YAC 784-B-4 (Whitehead Institute Database), which may contain genes involved in brain, skeletal, and craniofacial development. These data also indicate that the 9p deletion–syndrome locus and the sex-reversal locus are distinct entities and that some 46,XY females (or even normal individuals) without somatic anomalies may harbor 9p deletions. Consistent with this hypothesis, we identified an individual (patient 2) with an apparently normal 9p who was hemizygous for markers *D9S1858* and *VLDLR* (fig. 2). All alleles present were of paternal origin. The most distal heterozygous marker was *D9S1813,* which maps to a point 8 cM from the telomere. Since *VLDLR* resides in the contig WC1422, ~2 cM telomeric with respect to *D9S1813* (the distal end of contig WC844), the breakpoint in this patient may lie 6–8 cM from the telomere. The presence of a deletion was confirmed by PCR amplification of somatic-cell hybrids containing the rearranged chromosome 9. This in-

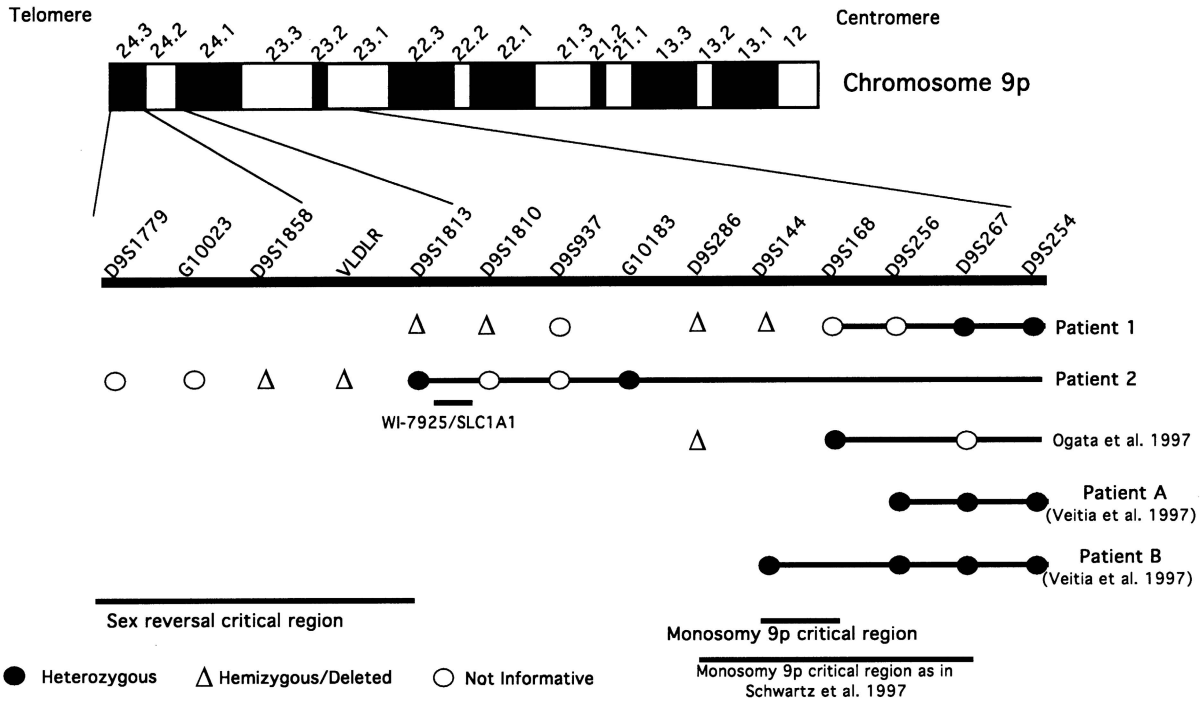

Figure 2 LOH analysis of monosomy-9p sex-reversed individuals (patients 1 and 2). The loci studied for each patient are represented by circles connected by bars representing the undeleted regions of the abnormal chromosome 9p. The positions of the sex-reversing locus and of the region putatively associated with monosomy-9p syndrome are indicated. The molecular findings for the patients described by Ogata et al. (1997) and Veitia et al. (1997*b*) also are shown, for clarity. Patients A and B (Veitia et al. 1997*b*) presented with complex phenotypes due to the presence of unbalanced chromosomal segments in addition to the deletion of 9p. Notice that most breakpoints shown map between D9S286 and D9S267. This suggests that the region contained within these markers is prone to ectopic recombination. The approximate position of SLC1A1 (WI-7925), proximal to D9S1813, is shown.

dividual did not present with hypotonia, and, consistent with the hypothesis described earlier, the marker *WI-7925* (*SLC1A1*), mapping proximal to *D9S1813* (Whitehead Institute Database), could be amplified from the hybrids containing the deleted chromosome 9. The molecular analysis of this patient confirmed that a deletion of 9p may be associated with 46,XY sex reversal in the absence of monosomy-9p syndrome and that the critical region for the sex-reversing locus is distal to *D9S1813*. Although, given the small number of samples studied, no statistical conclusions can be drawn from this screening it is remarkable that 1 of 10 patients carried a deletion. These deletions may be undetectable by ordinary cytogenetic techniques, because of their very-terminal character (Huret et al. 1988). Recently, a case of sex reversal associated with a familial translocation with a breakpoint at 9p24 has been described; however, the position of the breakpoint has not been defined at a molecular level (McDonald et al. 1997). Elsewhere, we also have described a patient with a complex 9p rearrangement associated with 46,XY partial gonadal dysgenesis (Ion et al. 1998). This patient had an inverted duplication of 9p, with a distal breakpoint proximal to the *SNF2* gene, at 9p24.1. In this case, we failed to identify any rearrangement distal to this gene, suggesting that the phenotype may be caused by a position effect.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://www.gdb.org
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for Swyer syndrome [MIM 306100] and monosomy-9p syndrome [158170])
Whitehead Institute Database, http://carbon.wi.mit.edu

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Molecular Analysis of 9p Deletions Associated with XY Sex Reversal: Refining the Localization of a Sex-Determining Gene to the Tip of the Chromosome

To the Editor:

Mammalian sexual development is dependent on chromosomal constitution. The Y chromosome acts as a dominant inducer of testis formation. The choice between testis and ovary equates with the choice of male or female development (sex determination). The differentiation of internal and external genitalia and secondary characteristics are regulated by endocrine functions associated with the gonads (sex differentiation). Any defect along the male and female pathway can result in inconsistencies between karyotypic and phenotypic sex (sex reversal) (Ford et al. 1959; Jacobs and Strong 1959; Jost et al. 1973).

The molecular-genetic analysis of primary (gonadal) sex-reversal syndromes has led to the identification of several sex-determining genes: *SRY,* the only Y-linked gene needed for testis formation; *DSS,* the X-linked dosage-sensitive sex-reversal locus; and the campomelic dysplasia (CD) gene *SOX9*. Functional studies performed in the mouse have identified other genes involved in gonadogenesis, such as the Wilms tumor gene *WT-1,* and the gene encoding for the steroidogenic factor 1 *SF-1* (reviewed by Schafer and Goodfellow 1996). The genes identified to date cannot account for all the sex-reversal cases found in the literature. The reports of both familial forms of gonadal dysgenesis and sporadic forms associated with autosomal anomalies implicate the existence of other loci (Schafer and Goodfellow 1996). Male-to-female primary sex reversal has been reported in patients with terminal deletions of the short arm of chromosome 9: unbalanced translocations and terminal deletions in which breakpoints range from 9p21 to 9p24 (Bennett et al. 1993; Begleiter et al. 1995; McDonald et al. 1997; Ogata et al. 1997; Veitia et al. 1997) (MIM 273350). The patients have normal female or ambiguous external genitalia and varying grades of mixed gonadal dysgenesis, ranging from fibrous streak gonads associated with Wolffian duct remnants to immature hypoplastic testicles. The lack of a correlation between the size of the deletions and the degree of impaired male development excludes the possibility that the deletion has a position effect on the differentiation of the sexual phenotype. It is likely that the interval in the 9p24 region deleted in all patients contains gene(s) involved in the differentiation of the male phenotype.

To better define the chromosome 9 region associated with sex reversal, we have performed a deletion-mapping analysis, by looking for the loss of heterozygosity in a panel of sex-reversed patients with translocations/deletions of chromosome 9p. This study involves four patients (SV, B, RB, and TO) who previously had been reported in the literature (Hoo et al. 1989; Bennett et al. 1993; Begleiter et al. 1995; Ogata et al. 1997) and an additional case (GG). These individuals are monosomic for varying segments of the chromosome 9 short arm. The extent of the sex reversal is variable among them, ranging from an ambiguous intersex to a nearly complete female phenotype. Karyotypes and clinical details are summarized in table 1.

The extent of the 9p deletions in the sex-reversed patients was analyzed by the typing of 20 microsatellite markers localized to an interval of 27.1 cM in 9p23-9pter. The heterozygosity of the markers tested is >70%. Their relative order on the chromosome was derived from an integrated map of 9p23-9pter, which combines genetic-mapping data on the above-mentioned loci with radiation hybrid–mapping data (fig. 1) (Bouzyk et al. 1996; Dib et al. 1996).

Loss of heterozygosity at the microsatellite loci was scored as potential loss of one allele. For patients TO, GG, and SV, it was possible to genotype the parents. This allowed the ascertainment of true hemizygosity for some of the markers tested. The results from this analysis are summarized in table 2. The microsatellite analysis allowed us to determine the potential maximum size of all the deletions but did not give any information on the minimum size of the deletions in patients RB, B, and SV. In the case of SV, the haplotype analysis of the parents' DNA for the potentially deleted markers was not informative. In the case of RB and B, parental DNA was not available. To better define the breakpoints in patients SV, RB, and B, FISH analysis also was performed, with genomic clones from 9p24 (for details, see the legend to fig. 1). All the results obtained from microsatellite typing and the FISH experiments were analyzed in relation to the integrated map of 9p23-9pter and were converted into a map that represents the maximum deletions potentially present in each patient (fig. 1, *bars on right side*):

Patient GG.—The maximum deletion extends from the telomere to D9S256-D9S269. The proximal break-