Letters to the Editor

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A De Novo Mutation (Gln2Stop) at the 5′ End of the SRY Gene Leads to Sex Reversal with Partial Ovarian Function

To the Editor:

In mammals, the development of the embryonic gonads into either testes or ovaries determines gender. Sex reversal in XY females results from the failure of the indifferent gonad to develop into a testis. There are an unknown number of genetic loci in the testis-determining pathway, but there is ample evidence that the Y-chromosome gene that is essential for testicular development is the testis-determining gene, SRY (Goodfellow and Lovell-Badge 1993). Deletions of and inactivating mutations of SRY are among the known causes of XY sex reversal. Of XY females with pure gonadal dysgenesis, 10%–15% have been found to have mutations in the SRY gene (Hawkins et al. 1992*b;* Affara et al. 1993; Hawkins 1994). To date, 22 XY females with SRY mutations have been reported, and all but two of the mutations have been in the highly conserved high-mobility–group portion of the gene (i.e., the "HMG box"). Of the two mutations not in the HMG box, one was a deletion 5′ to the coding region of the gene (McElreavy et al. 1992*a*), and the other was a point mutation 3′ to the HMG box (Tajima et al. 1994). Patients with SRY deletions or mutations are, in general, normal females with complete gonadal dysgenesis. We describe a female patient with premature ovarian failure, an XY karyotype, and a presumably inactivating mutation of her SRY gene. We believe that this is the first reported case of an XY female whose sex reversal is due to an SRY mutation and in whom gonadal dysgenesis is incomplete. In addition, this patient's SRY gene mutation is itself unusual, in that it creates a stop in the second codon of the gene. A third unusual feature of this case is that tubules suggestive of early testicular development are seen in the ovary.

The patient is a 28-year-old West Indian woman who presented to one of us (L.S.), at the age of 28 years, with the primary complaint of infertility. She reported menarche and normal breast development at age 13–14 years and had had regular monthly menses until age 17 years, when she electively began oral contraceptives, which she continued until age 25 years. She reports that irregular menses (every 28–45 d) resumed with the discontinuation of the oral-contraception and that for the 2 subsequent years she attempted to get pregnant. Prior to our evaluation, she had been treated with clomiphene citrate, for presumed anovulation. When she presented to us, she was still having irregular menses. She appeared normally feminized, and her physical exam, including gynecological exam, was entirely normal, except for the fact that she was 193 cm tall. Specifically, she was not hirsute and had no stigmata of Turner syndrome. Breasts and pubic hair were Tanner stage 4. The following hormone levels were found: progesterone, 0.5 ng/ml; FSH, 44 mUI/ml; LH, 36 mUI/ml; prolactin, 12.8 ng/ml; testosterone, 0.25 ng/ml; and estradiol 2, 40 pg/ml. These values all suggested nonfunctional ovaries. A chromosome analysis showed a 46,XY karyotype. At laparoscopy, the gonads appeared to be white fibrous streaks and were removed without difficulty. The excised tissue was formalin fixed and routinely processed.

As noted, the patient's history, physical exam, and laboratory analysis were all suggestive of early ovarian failure. Because of her oral-contraception regimen, we cannot be sure about when her ovarian failure occurred; however, the patient's history of menses for 3 years after the cessation of an oral-contraception regimen is convincing. Standard G-banded chromosome analysis of peripheral lymphocytes showed a 46,XY karyotype in all 50 cells examined. Subsequently, fibroblasts cultured from a skin biopsy gave the same result. Two-color interphase FISH analysis of 100 nuclei by means of X and Y centromere probes (obtained from Oncor) failed to detect any cells with either two X chromosomes or without a Y chromosome. The excised gonad from the right side consisted entirely of fibroadipose tissue. The left gonad contained a small amount of ovarian stromalike tissue. No follicles were seen; however, a cluster of tubular structures was present. One of the tubules was ciliated, reminiscent of epididymis (fig. 1), and it was concluded that the structures were suggestive of embryonic male type. Fibroblastlike cells cultured from both gonadal-tissue samples also had a pure XY karyotype.

Because of the possibility of an SRY gene mutation, the entire coding region of the SRY gene was amplified from peripheral lymphocyte DNA, by means of primers

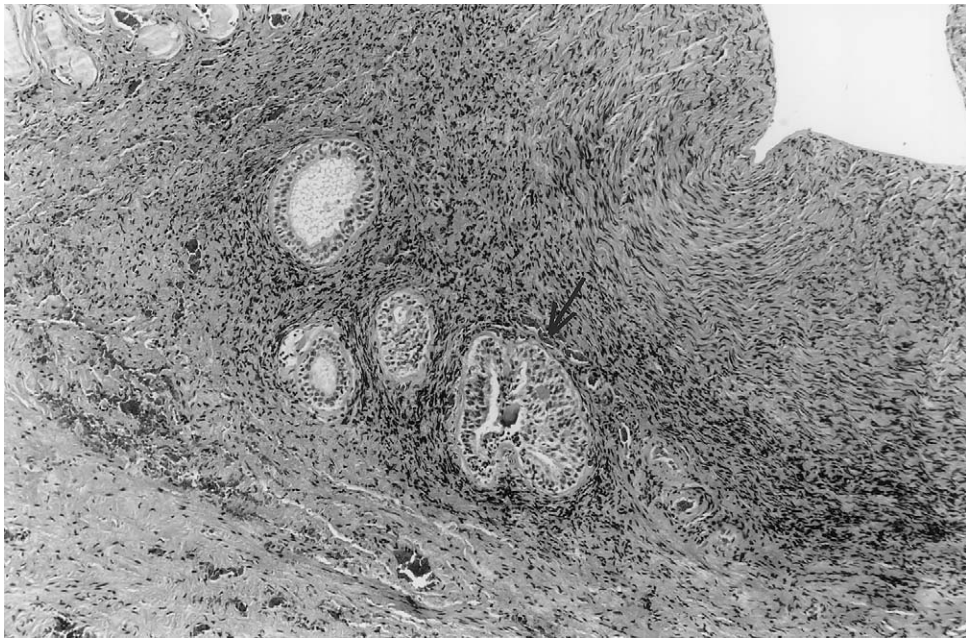

Figure 1 H- and E-stained section of the ovary, showing a ciliated tubule reminiscent of epididymis.

XES10 and XES11, as described elsewhere (Hawkins et al. 1992*a*), and were cloned into a plasmid. Two independent cloned PCR products showed the same C→T transition in the second codon of the gene. This was the only consistent deviation from the published sequence. Subsequent direct sequencing of pooled PCR products showed the same C→T transition (fig. 2). This C→T transition is predicted to create a stop codon (Gln2stop) at the second codon of the gene. The SRY gene was amplified and sequenced from both ovaries, and the same C→T mutation was found to be present bilaterally. Thus, there was no evidence of mosaicism for the mutation. The patient's father's SRY gene was sequenced and was shown to be entirely normal. In addition, paternity was proved by the normal segregation of polymorphic PCR-based markers (data not shown).

A stop mutation in the second codon of a gene is unusual, and it is unclear how a eukaryotic ribosome would respond to a stop placed immediately after the initiating methionine. Either initiation would never proceed to elongation, resulting in complete absence of protein product, or a low level of readthrough would occur with a reduced level of an otherwise normal protein. In order to investigate whether this mutant stop completely destroyed protein synthesis, hemagglutinin (HA)-tagged constructs of both the normal and mutant SRY genes were prepared. These constructs were then cloned into a eukaryotic expression vector so that the HA-tagged SRY gene was placed downstream from the cytomegalovirus promoter/enhancer. Normal and mutant constructs were transiently transfected into the BOSC 23 cell line by calcium phosphate coprecipitation, as described by Pear et al. (1993). SRY-HA proteins from lysates of transfected BOSC 23 cells were analyzed by immunoblotting by means of methods described elsewhere (Munsterberg et al. 1995). A representative result is shown in figure 3; the higher-molecular-weight band in the left-hand lane corresponds to the protein product

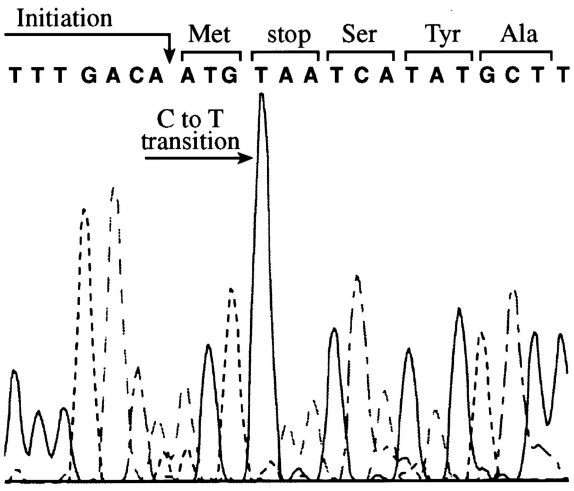

Figure 2 Electropherogram showing mutant SRY sequence. Sequencing was done directly from the PCR product, by use of the forward primer.

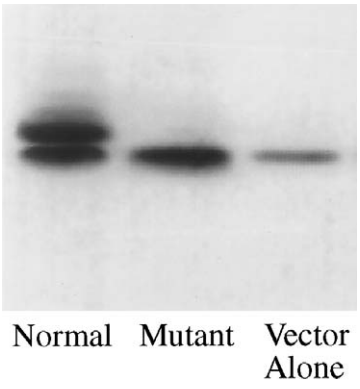

Figure 3 Western blot analysis of transiently transfected HA-tagged SRY constructs. The upper band in the left-hand lane arises from the transfected SRY construct, the lower band, common to all lanes, is due to nonspecific binding of the anti-HA antibody.

from a normal SRY gene, whereas no such band is detectable with the mutant SRY allele (middle lane). The constant band is due to nonspecific binding of the anti–HA-tag antibody. Although only one representative example is shown, this experiment was repeated a total of four times, with identical results. We conclude from this analysis that, if the mutant protein is expressed, it must be at a level below the sensitivity of our system, which we estimate to be ~1%.

Among patients with sex reversal and SRY-gene mutations, this woman is quite atypical, since she had normal menarche, normal secondary-sexual characteristics, and >3 years of at least partial ovarian function after the age of 25 years, when she discontinued an oral-contraception regimen. A total of ⩾22 SRY-gene mutations have been reported by several authors (Hawkins et al. 1992*a;* Jager et al. 1992; McElreavey et al. 1992*a;* McElreavy et al. 1992*b;* Affara et al. 1993; Zeng et al. 1993; Iida et al. 1994; Poulat et al. 1994; Tajima et al. 1994; Schmitt-Ney et al. 1995). In all of the reported cases, pure gonadal dysgenesis was present. This patient has a single-base-change mutation of her SRY gene, which is likely to have occurred either during spermatogenesis or very early postzygotically. This mutation creates a stop codon immediately following the initiating methionine and apparently inactivated her SRY gene. It is unusual, since all other known mutations of the SRY gene are either in the HMG-box region of the gene or 3′ to that region. It has been generally accepted that the reason that most of the reported mutations in the SRY gene are in the HMG box is that these are the mutations that disrupt the function of the gene and that other mutations are likely to be silent. However, premature termination upstream from the HMG box would also destroy function and would not be predicted to be silent. We considered the possibility that the unusual phenotype (i.e., partially functional gonads) in this case might be related to the unusual mutation. For instance, could a low level of SRY protein lead to the survival of germ cells? To this end, we sought to assess the possibility that this mutation is "leaky." Our results suggest that it is not.

Another possibility is that the unusual mutation in this patient has nothing to do with her unusual phenotype and that, with respect to the gonad, XY individuals with an SRY mutation are analogous to 45,X (Turner syndrome) patients. In 45,X, the presence of only one X chromosome is apparently not an absolute barrier to ovarian function, since ~5% of these girls will have some ovarian function (Palmer and Reichmann 1979; Hall et al. 1982). It has been hypothesized that this variation in the Turner-syndrome phenotype is due to a variable rate of decay of the oocytes (Hall et al. 1982; Page et al. 1990). Only 22 patients with XY karyotypes and SRY mutations have been described. If, as in 45,X, there is significant variability in gonadal function, then 22 patients is not enough from which to draw firm conclusions. As the number of such individuals increases, it may become apparent that a small percentage have partial ovarian function. This concept is supported by the observation that, in mice, the presence of only one X chromosome is associated with fertility and only a mild reproductive disadvantage (Epstein 1986). In addition, a mouse with a heritable mutation in the testis-determining gene has been described (Lovell-Badge and Robertson 1990). XY mice with this mutation are fertile females, although fertility is reduced, and their ovaries fail early, a picture quite similar to that of the patient whom we present.

In summary, we conclude that the patient whom we present is sex reversed because of a point mutation in her SRY gene. Further, we conclude that the presence of a cytogenetically normal Y chromosome is not an absolute barrier to ovarian function in humans. In addition, this and other reported patients prove that mutations outside the HMG box of the SRY gene do occur and should be looked for in the setting of sex reversal. Although it is unlikely that many women with premature ovarian failure will have a Y chromosome, it is still desirable to karyotype all such women.

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Stephen Brown,[1] C. C. Yu,[2] Patricia Lanzano,[1] Debra Heller,[3] L. Thomas,[4] Dorothy Warburton,[5] Jan Kitajewski,[1] and Laurel Stadtmauer[1]

[1]*Department of Obstetrics and Gynecology,* [2]*Department of Pathology, and* [3]*Department of Genetics and Development and Department of Pediatrics, Columbia University,* [4]*Presbyterian Hospital in the City of New York, and* [5]*Department of Pathology, Harlem Hospital, New York*

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Address for correspondence and reprints: Dr. Stephen Brown, Department of Obstetrics/Gynecology, PH 16, Columbia University, 630 West 168th Street, New York, NY 10032. E-mail: brown@cuccfa.ccc.columbia.edu

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Founder Effect, Seen in the British Population, of the 172 Peripherin/*RDS* Mutation—and Further Refinement of Genetic Positioning of the Peripherin/*RDS* Gene

To the Editor:

Peripherin/retinal degeneration slow (RDS) is a membrane-associated glycoprotein found in the outer segments of retinal rod and cone photoreceptor cells. It is thought to play a role in membrane structural stabilization, in conjunction with retinal outer segment membrane protein 1 (ROM1).

Mutations in the *RDS* gene give rise to retinal degenerations with a wide phenotypic spectrum. The majority of mutations result in macular dystrophies (reviewed in Keen and Inglehearn 1996). Specific mutations in the *RDS* gene may lead to a wide inter- and intrafamilial variability of phenotype, as seen in one family with retinitis pigmentosa, pattern dystrophy, and fundus flavimaculatus, in three different members with a deletion at codon 153/154. (Weleber et al. 1993)

Mutation analysis by heteroduplex and direct sequencing of PCR-amplified coding exons of the *RDS* gene was performed in 300 British patients with dominantly inherited macular dystrophies; 7.3% of this group had peripherin/*RDS* mutations, segregating with disease. One particular mutation accounted for 11 of