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HUMAN GENETICS

# Molecular Analysis of the Y Chromosome in XX Sex-Reversed Patients

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**Abstract**—Molecular genetic analysis was performed for 26 phenotypically male patients lacking the Y chromosome in the karyotype. The *sex*-determining region Y (*SRY*) gene was found in 77% of the patients. PCR analysis of Y-specific loci in the 17 *SRY*-positive patients revealed Yp fragments varying in size in 16 cases and cryptic mosaicism (or chimerism) for the Y chromosome in one case. The frequencies of class I, II, and III (Yp+)XX sex reversals were 18.75, 25.25, and 56%, respectively. All of the class III (Yp+)XX sex-reversed patients had a 3.5-Mb paracentric inversion flanked by inverted repeats 3 (IR3) on the short arm of the Y chromosome.

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## INTRODUCTION

Distorted sex determination and/or differentiation is one of the most common causes of sexual maldevelopment [1]. The male or intersexual phenotype and the presence of testicular tissue in individuals lacking a cytogenetically identifiable Y chromosome or its fragments (karyotypes 46,XX; mos 45,X/46XX; rarely, 47,XX; and extremely rarely, 45,X) can be due to XX sex reversal or true hermaphroditism [2–6]. In a few cases, such individuals display chimerism or cryptic mosaicism, including gonadal mosaicism, which is characterized by the presence of a cell clone carrying the Y chromosome [3].

The *sex-determining region Y* (*SRY*) gene codes for the testis-determining factor (TDF) and is the key regulator determining male gonadal differentiation in humans [1, 7–9]. The *SRY* gene belongs to the *SOX* gene family and occurs in a single copy in the distal part of the short arm of the Y chromosome (Yp11.3), close to the boundary between pseudoautosomal region 1 (PAR1) and the male-specific region of the Y chromosome (MSY) [7]. TDF is involved in regulating transcription of the genes that are responsible for Sertoli cell differentiation and the development of testes from indifferent (bipotential) gonads [1, 9].

The *SRY* gene is found in 70–80% of all patients with the de la Chapelle (XX-male) syndrome (MIM 278850) and only in 10% of all true hermaphrodites, lacking the Y chromosome in the karyotype [10]. As Ferguson-Smith [11] assumed, most cases of XX sex reversal are caused by translocation of a fragment containing the *SRY* gene from the short arm of the Y chromosome to the X chromosome as a result of ectopic X–Y recombination during spermatogenesis in the father [11–13].

Molecular genetic studies have shown that the size of the Y-chromosome fragment varies among XX sex-reversed patients, suggesting a difference in the breakpoint location and the mechanisms of sex reversal [14–16]. All Y-positive XX males preserve the *SRY* gene, which is commonly translocated to the X chromosome [10, 13, 17]. Vergnaud et al. [14] have constructed a deletion map of the Y chromosome and have divided all cases of XX sex inversion into three classes differing in the size of the preserved region of the Y-chromosome short arm and, accordingly, having a breakpoint in different deletion intervals of Yp. A mapping of the breakpoints in the short arm of the Y chromosome has revealed that the positions of gonosome breakpoints depend on the loci involved in X–Y recombination [16]. The most common class is class 3 of Yp-positive (Yp+) XX sex inversion, resulting from extopic X–Y recombination between the *PRKX* and *PRKY* homologous genes [18–20].

## MATERIALS AND METHODS

We examined 26 XX sex-inversed patients (15 children and 11 adults) aged from 1 to 52 years. All patients were earlier identified as males. The adults were subjected to medical genetic examination because of hypogonadism and/or infertility due to a severe spermatogenic disorder (secretory azoospermia or severe oligozoospermia). The children displayed sexual maldevelopment in the form of poor masculinization, varying in extent from isolated hypospadia to dual

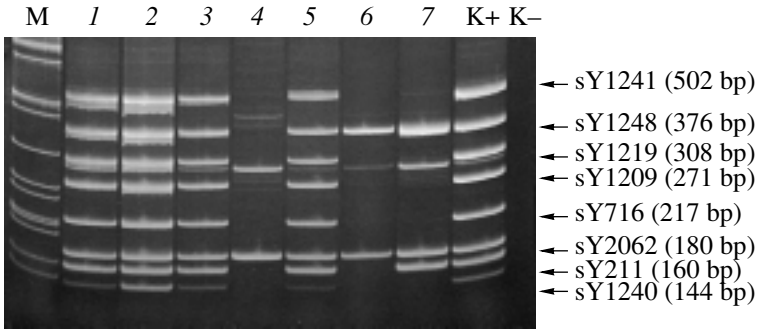


**Fig. 1.** Electrophoresis of the multiplex PCR products obtained with primers to several loci of the short arm of the Y chromosome. M, molecular weight markers. Lanes *1–7*, DNAs of seven *SRY*-positive XX sex-reversed patients; C+, positive control (DNA of a fertile male); C–, negative control (reagent mixture without DNA). Specimens *1–3* and *5* contain all of the loci examined; specimens *4, 6*, and *7* lack some loci of the Y-chromosome short arm.

development (sex-indefinite structure) of the sex organs. Of all patients, 22 had the 46,XX karyotype and 3 were 45,X/46,XX mosaics. In one case, the karyotype was initially identified as 45,X and then more precisely determined as mos 45,der(X)ins(X;Y)(p22.3;p11.3q12)/45,X. This case has been described in detail previously [21].

*Molecular analysis.* DNA was isolated from peripheral blood lymphocytes by the standard technique. To detect various sequences of the X and Y chromosomes in genomic DNA, the corresponding loci were amplified in the polymerase chain reaction (PCR). At the first step, we tested genomic DNA for the *SRY* and *AMELX* sequences of the Y-chromosome short arm, using the latter as an internal control. If the *SRY* gene was detected, DNA was tested for Yq sequences (second step) and the breakpoint in the short arm of the Y chromosome was localized by examining several Yp-specific loci (third step). The analysis included three multiplex PCRs addressing *SRY* (sY14), *AMELY* (sY70), *ZFY*, and 15 Y-specific STSs (sY2062, sY1248, sY211, sY1240, sY716, sY1241, sY1219, and sY1209 for Yp and sY84, sY86, sY615, sY127, sY134, sY254, and sY255 for Yq). Amplification with primers directed to the X-specific sequences *AMELX* and *ZFX* was used as an internal control.

The reaction mixture contained 20 ng of genomic DNA, 0.25 μM each primer, and 250 μM each dNTP in 25 μl of 1× PCR buffer (67 mM Tris–HCl, pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 0.01% Tween-20). The mixture was supplemented with 1.5 units of thermophilic DNA polymerase and 20–30 μl of mineral oil. Amplification included initial denaturation at 95°C for 2 min; cycling with 94°C for 45 s, 65°C for 45 s, and 72°C for 45 s; and last synthesis at 72°C for 7 min. The products were separated in 7% PAAG and stained with 0.1 μg/ml ethidium bromide in 1× TBE.

## RESULTS

Y-specific sequences were found in 20 (77%) out of the 26 patients examined (Fig. 1). The *SRY* gene was detected in 11 (73.3%) patients aged below 18 and in nine (81.8%) adults. These cases included 16 out of 22 patients with karyotype 46,XX, all three X/XX mosaics, and the boy with karyotype 45,X (Fig. 1). The *SRY* gene was not found in two phenotypically normal adult males and four boys varying in extent of hypogonadism and masculinization deficit. One patient with karyotype 46,XX and a severe gonadal masculinization deficit (cryptorchism and urogenital sinus) had all of the Yp- and Yq-specific loci under study. It is clear that the male phenotype in this case is determined by cryptic mosaicism or chimerism and the presence of a clone carrying the Y chromosome. A lack of the *AMELY* gene and Yq-specific sequences was observed in all of the other 19 *SRY*-positive cases, suggesting the translocation or Yp+ form of XX sex reversion.

The breakpoints of the Y chromosome were localized in 15 (Yp+)XX males and the patient with 45,der(X)ins(X;Y)(p22.3;p11.3q12)/45,X mosaicism. The patients varied in the size of the retained Y-chromosome fragment (Fig. 2). Eight out of the 15 adult males had all but one (*AMELY*) locus specific for deletion intervals 1–3 of the Y chromosome [14]. The other seven patients had STSs mapping to deletion intervals 1 and 2 of the Y chromosome. The breakpoint was between subintervals 1A1B and 1A2 in two cases, 1A2 and 2A in one case, and 2A and 3A (subintervals 2B–2C) in the other four cases [15]. In the patient with mosaicism for the rearranged X chromosome containing a fragment of region Yp11.3, the breakpoint was between subintervals 1A2 and 2A (1B–1E), as characteristic of class I (Yp+)XX sex reversal.

The males with class I (Yp+)XX sex reversal differed in the location of the Y-chromosome breakpoint (Fig. 2). The breakpoint was proximal of the *RPS4Y1* gene between the sY2062 and sY1248 loci in two cases and in the *TGIF2LY* gene region between the sY1248 and sY211 loci in the other two cases. In all of the four cases with class II (Yp+)XX sex reversal, the breakpoint was between the sY211 and sY1240 loci and, possibly, distal of the *PCDH11Y* gene. All of the

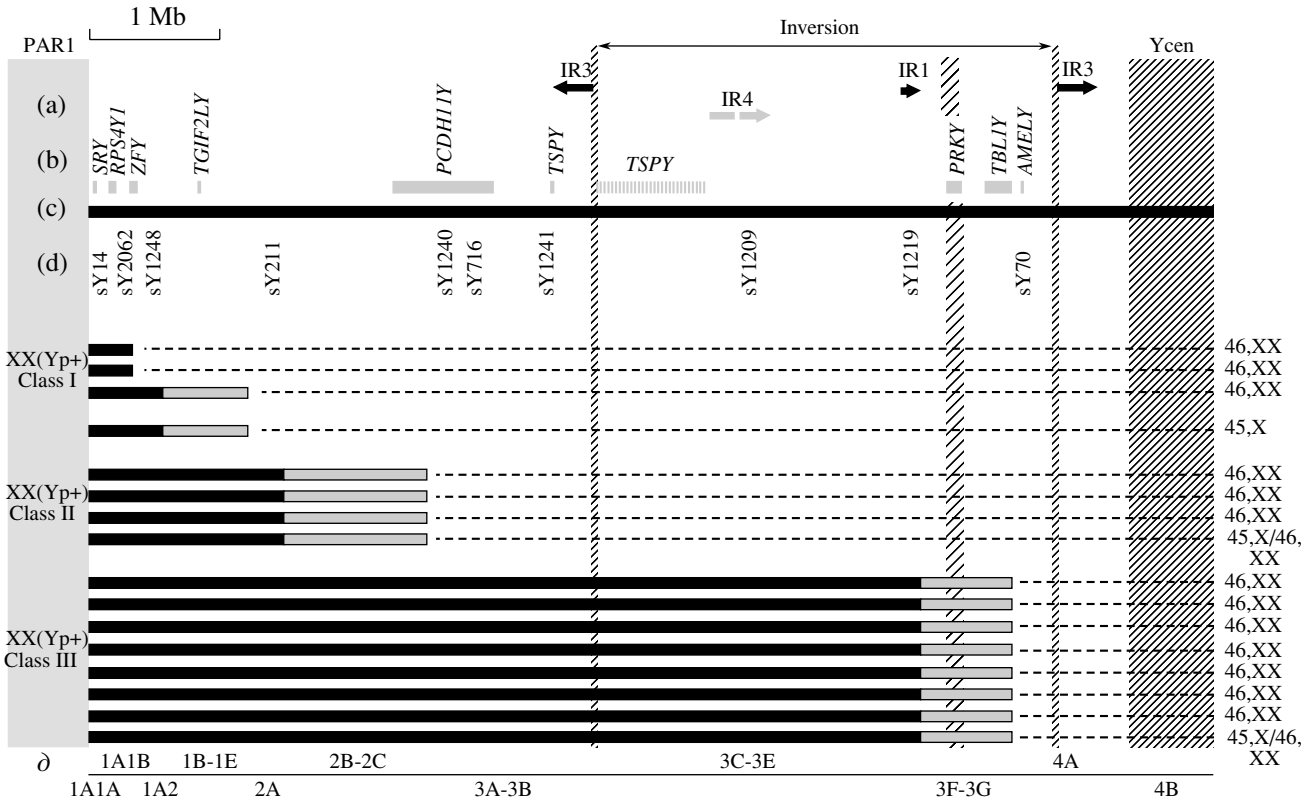


**Fig. 2.** Map of the short arm of the Y chromosome. The positions are shown for (a) intrachromosomal repeats, (b) some genes, (d) STSs, (c) the MSY region and its fragments detected in 16 *SRY*-positive patients, and (d) deletion intervals. The paracenric inversion flanked by inverted repeats 3 (IR3) is shown at the top. Classes I–III of XX sex reversal, differing in size of the present Yp region, are indicated on the left. The karyotypes of the patients are shown on the right. Black bar, present Y-chromosome fragment; dashed line, absent Y-chromosome fragment; gray bar, fragment whose presence or absence is questionable. Two thin crosshatched lines indicate the boundaries of the paracentric inversion. A thick crosshatched line, passing through the *PRKY* gene region, shows the breakpoint hot spot of the short arm of the Y chromosome in patients with class III (Yp+)XX sex reversal [18–20]. PAR1, pseudoautosomal region 1 (its size and genes are not shown); Ycen, pericentric region of the Y chromosome.

patients with class III (Yp+)XX sex reversal displayed an interruption of the MSY region according to the modern map of the Y chromosome [22, 23]. This is explained by an extended paracentric inversion between inverted repeats 3 (IR3) in the proximal region of the short arm of the Y chromosome. We did not detect a size variation of the Y-specific region present in the patients. The breakpoints were between the sY1219 and *AMELY* loci, probably, in the *PRKY* gene region (subintervals 3F–3G) (Fig. 2).

## DISCUSSION

Recombination between the X and Y chromosomes is normally restricted to the pseudoautosomal regions. In rare cases, the recombination region extends proximally of the PAR1 boundary so that a Yp fragment containing the *SRY* gene can be translocated to the X chromosome. The Y-chromosome fragment is translocated to the short arm of the X chromosome in most of such cases and, extremely rarely, to its long arm. Such ectopic X–Y recombination events are facilitated by numerous homologous sequences, various repeats (e.g., *Alu*), inversions, and other microstructural rearrangements [13]. The difference in the positions of the X- and Y-chromosome breakpoints among XX males suggests different mechanisms of microstructural rearrangements [16, 24]. Xp–Yp translocations are associated with breakpoints arising in Xp22.3, proximal to the PAR1 boundary in most cases or, more rarely, within the pseudoautosomal region; in the latter case, the PAR1 genes are partly triplicated in the affected XX males [25].

According to the pooled data of several large studies aimed at detail molecular analysis of the Y chromosome in XX males, the frequencies of class I, II, and III XX sex reversals are 18, 26, and 56%, respectively [14, 15, 24]. Similar frequencies were obtained for our sample: 18.75, 25.25, and 56%, respectively. These data indicate that ectopic X–Y recombination most commonly involves the regions that are far away from the *SRY* gene.

In contrast to the class I and II cases, class III (Yp+)XX sex reversal was characterized by a high similarity in breakpoint location. The lack of the *AMELY* locus was observed in all of our patients with class I–III

(Yp+)XX sex reversal. In published XX sex-reversed cases with the preserved *AMELY* locus, the breakpoints are distal of deletion interval 3, which may be associated with cryptic mosaicism for the Y chromosome [15]. The MSY fragment was interrupted in all of our patients with (Yp+)XX sex reversal because the translocation occurred in the presence of a paracentric inversion of the short arm of the Y chromosome [23]. The inversion polymorphism is found in about one-third of the total Caucasoid male population [22, 26]. It is clear that the 3.5-Mb inversion flanked by IR3 was present in our cases of class III (Yp+)XX sex reversal [22, 23, 27]. The breakpoints arising in such cases are close to the *PRKX* and *PRKY* homologous genes [6, 19, 20].

It was earlier believed that the presence of the *SRY* gene and the size of the Y-chromosome fragment play a key role in determining the extent of masculinization in XX sex-reversed patients [28]. However, distinct genotypic–phenotypic correlations were not found [29]. There are reports of phenotypically normal (apart from infertility) males and male fetuses lacking the *SRY* gene, as well of a substantial difference between sibs (XX males and true hermaphrodites) with the same sizes of the preserved Y-chromosome regions [17, 30–32]. The lack of the Yq genes (the total AZF region), controlling male germline cell differentiation, leads to an almost complete absence of the germinative epithelium in seminal ducts and, consequently, nonobstructive azoospermia and infertility in all XX males [2–4]. Our patients similarly did not display an association between the size of the present Y-chromosome fragment and the phenotype. Three *SRY*-positive cases (two children with sexual maldevelopment and one infertile adult male) displayed X/XX mosaicism, which was probably due to a postzygotic alteration of sex chromosome conjugation and/or sex chromosome nondisjunction, since one of the X chromosomes was rearranged as a result of an X–Y translocation. We believe that such gonadal mosaicism and, especially, the proportion of the clone with the 45,X karyotype and the ratio between the X chromosomes having or lacking the *SRY* gene are one of the most important factors determining the character of gonad differentiation. It is possible that some other patients also had cryptic 45,X/46,XX mosaicism.

Recent studies have focused on the effects of selective X-chromosome inactivation in XX sex-reversed patients. It is important that the X chromosome carrying the *SRY* gene remains active [33, 34]. The extent of X inactivation has been associated with the size of the Y-chromosome fragment [24]. It is possible that the X lionization pattern depends to a substantial extent on the breakpoint location in the rearranged X chromosome, which is determined by the mechanism of ectopic X–Y recombination.

The development of the reproductive system and the extent of masculinization strongly depend on the activity of the main genes regulating gonadal differentiation (*SRY, WT1, DAX1, SF1, SOX9*, etc.), which are located on both sex chromosomes and several autosomes. *SRY*-negative cases of XX sex reversal clearly demonstrate that testicular tissue can develop even in the absence of all Y-chromosomal genes. Such a situation is observed upon duplication of the *SOX9* gene, 22q duplication, or Xp deletion [35, 36]. In addition, *SRY*-negative XX sex reversal is associated with several genetic syndromes [37]. In some cases, the causes of the de la Chapelle syndrome remain unknown in spite of comprehensive molecular analysis [32].

The key cell events initiating the male developmental pattern of the gonads is differentiation of Sertoli cells from bipotential precursors (supporting cells) and proliferation of epithelial cells [1, 9]. It is probable that hyperactivity and/or disbalance of the key genes regulating these processes disturb gonadal differentiation, in particular, in *SRY*-negative XX sex reversal. Further molecular genetic studies are necessary for clarifying the causes and mechanisms of testicular development in the presence or absence of the *SRY* gene, as well as analysis of the genetic interactions taking place during the morphogenesis of the male reproductive system.

## REFERENCES

1. Chernykh, V.B. and Kurilo, L.F., Genetic Control of Sexual Differentiation in Humans, *Russ. J. Genet.*, 2001, vol. 37, no. 10, pp. 1103–1114.
2. Kurilo, L.F., Kozlov, G.I., Fedotov, V.P., et al., Clinical and Cytogenetical Examination a Patient with Karyotype 46,XX, Azoospermia and Infertility, *Probl. Endokrinol.* (Moscow), 1994, vol. 40, no. 2, pp. 50–51.
3. Osipova, G.R., *SRY* Gene Study in Relation to Some Aberrations of Sex Determination (XY Pure Form of Gonadal Dysgenesis, Turner Syndrome, XX-Sex Inversions), *Cand. Sci. (Med.) Dissertation*, Moscow: Medical Genetic Research Center, 1997.
4. de la Chapelle, A., The Etiology of Maleness in XX Men, *Hum. Genet.,* 1981, vol. 58, no. 1, pp. 105–116.
5. Muller, U., Latt, S.A., and Donlon, T., Y-Specific DNA Sequences in Male Patients with 46,XX and 47,XXX Karyotypes, *Am. J. Med. Genet.*, 1987, vol. 28, no. 2, pp. 393–401.
6. Weil, D., Wang, I., Dietrich, A., et al., Highly Homologous Loci on the X and Y Chromosomes Are Hot-Spots for Ectopic Recombinations Leading to XX Maleness, *Nat. Genet.*, 1994, vol. 7, pp. 414–419.
7. Sinclair, A.H., Berta, P., Palmer, M.S., et al., A Gene from the Human Sex-Determining Region Encodes a Protein with Homology to a Conserved DNA-Binding Motif, *Nature,* 1990, vol. 346, pp. 240–244.
8. McElreavey, K., Vilain, E., Abbas, N., et al., A Regulatory Cascade Hypothesis for Mammalian Sex Determination: *SRY* Represses a Negative Regulator of Male Development, *Proc. Natl. Acad. Sci. USA,* 1993, vol. 90, pp. 3368–3372.
9. Koopman, P., Gubbay, J., Vivian, N., et al., Male Development of Chromosomally Female Mice Transgenic for *SRY*, *Nature*, 1991, vol. 51, pp. 117–121.

10. McElreavey, K., Rappport, R., Vilain, E., et al., A Minority of 46,XX True Hermaphrodites Are Positive for the Y-DNA Sequence Including *SRY*, *Hum. Genet.*, 1992, vol. 90, pp. 121–125.
11. Ferguson-Smith, M.A., XY Chromosomal Interchange in the Etiology of True Hermaphroditism and of XX Klinefelter's Syndrome, *Lancet*, 1966, vol. 2, pp. 475–477.
12. Anderson, M., Page, D.C., and de la Chapelle, A., Chromosome Y-Specific DNA Is Transferred to the Short Arm of X Chromosome in Human XX Males, *Science*, 1986, vol. 233, no. 4765, pp. 786–788.
13. Page, D.C., Brown, L.G., and de la Chapelle, A., Exchange of Terminal Portions of X- and Y-Chromosomal Short Arms in Human XX Males, *Nature*, 1987, vol. 328, pp. 437–440.
14. Vergnaud, G., Page, D.C., Simmler, M.C., et al., A Deletion Map of the Human Y Chromosome Based on DNA Hybridization, *Am. J. Hum. Genet.,* 1986, vol. 38, pp. 109–124.
15. Vollrath, D., Foote, S., Hilton, A., et al., The Human Y Chromosome: A 43-Interval Map Based on Naturally Occurring Deletions, *Science*, 1992, vol. 258, pp. 52–59.
16. Petit, C., Levilliers, J., and Weissenbach, J., Long-Range Restriction Map of the Terminal Part of the Short Arm of the Human X Chromosome, *Proc. Natl. Acad. Sci. USA,* 1990, vol. 87, no. 10, pp. 3680–3684.
17. Abbas, N., McElreavey, K., Leconiat, M., et al., Familial Case of 46,XX Male and 46,XX True Hermaphrodite Associated with a Paternal-Derived *SRY*-Bearing X Chromosome, *C. R. Acad. Sci. III*, 1993, vol. 316, no. 4, pp. 375–383.
18. Wang, I., Weil, D., Levilliers, J., et al., Prevalence and Molecular Analysis of Two Hot Spots for Ectopic Recombination Leading to XX Maleness, *Genomics*, 1995, vol. 28, no. 1, pp. 52–58.
19. Klink, A., Schiebel, K., Winkelmann, M., et al., The Human Protein Kinase Gene *PKX1* on Xp22.3 Displays Xp/Yp Homology and Is a Site of Chromosomal Instability, *Hum. Mol. Genet.,* 1995, vol. 4, no. 5, pp. 869–878.
20. Schiebel, K., Winkelmann, M., Mertz, A., et al., Abnormal XY Interchange between a Novel Isolated Protein Kinase Gene, *PRKY*, and Its Homologue, *PRKX,* Accounts for One Third of All (Y+)XX Males and (Y–)XY Females, *Hum. Mol. Genet.*, 1997, vol. 6, pp. 1985–1989.
21. Chernykh, V.B., Vyatkina, S.V., Antonenko, V.G., et al., A Unique Case of Mosaicism with X;Y Insertion in a Newborn Male Infant with Congenital Ichthyosis, Hydrocephaly and Frontal Holoprosencephaly, *Med. Genet.*, 2006, vol. 5, no. 7.
22. Tilford, C.A., Kuroda-Kawaguchi, T., Skaletsky, H., et al., A Physical Map of the Human Y Chromosome, *Nature,* 2001, vol. 409, no. 6822, pp. 943–945.
23. Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P.J., et al., The Male-Specific Region of the Human Y Chromosome Is a Mosaic of Discrete Sequence Classes, *Nature*, 2003, vol. 423, pp. 825–837.
24. Sharp, A., Kusz, K., Jaruzelska, J., et al., Variability of Sexual Phenotype in 46,XX ($SRY^+$) Patients: The Influence of Spreading X Inactivation versus Position Effects, *J. Med. Genet.,* 2005, vol. 42, no. 5, pp. 420–427.
25. Ross, N.L.J., Yang, J., Sargent, C.A., et al., Triplication of Several *PAR1* Genes and Part of the *Homo sapiens* Specific Yp11.2/Xq21.3 Region of Homology in a 46,X,T(X;Y)(p22.33;p11.2) Male with Schizophrenia, *J. Med. Genet.*, 2001, vol. 38, pp. 710–719.
26. Vogt, P.H., Affara, N., Davey, P., et al., Report of the Third International Workshop on Y Chromosome Mapping 1997 (Heidelberg, Germany, April 13–16, 1997), *Cytogenet. Cell Genet.*, 1997, vol. 79, nos. 1–2, pp. 1–20.
27. Jobling, M.A., Williams, G.A., Schiebel, G.A., et al., A Selective Difference between Human Y-Chromosomal DNA Haplotypes, *Curr. Biol.*, 1998, vol. 8, pp. 1391–1394.
28. Ferguson-Smith, M.A., Cooke, A., Affara, N.A., et al., Genotype–Phenotype Correlations in XX Males and Their Bearing on Current Theories of Sex Determination, *Hum. Genet.*, 1990, vol. 84, no. 2, pp. 198–202.
29. Boucekkine, C., Toublanc, J.E., Abbas, N., et al., Clinical and Anatomical Spectrum in XX Sex Reversed Patients: Relationship to the Presence of Y Specific DNA-Sequences, *Clin. Endocrinol.*, 1994, vol. 40, no. 6, pp. 733–742.
30. Ramos, E.S., Moreira-Filho, C.A., Vicente, Y.A., et al., *SRY*-Negative True Hermaphrodites and an XX Male in Two Generations of the Same Family, *Hum. Genet.,* 1996, vol. 97, no. 5, pp. 596–598.
31. Sharp, A., Kusz, K., Jaruzelska, J., et al., Familial X/Y Translocations Associated with Variable Sexual Phenotype, *J. Med. Genet.*, 2004, vol. 41, pp. 440–444.
32. Rajender, S., Rajani, V., Gupta, N.J., et al., *SRY*-Negative 46,XX Male with Normal Genitals, Complete Masculinization and Infertility, *Mol. Hum. Reprod.*, 2006, vol. 12, no. 5, pp. 341–346.
33. Kolon, T., Ferrer, F.A., and McKenna, P.H., Clinical and Molecular Analysis of XX Sex Reversed Patients, *J. Urol.,* 1998, vol. 160, pp. 1169–1172.
34. Kusz, K., Kotecki, M., Wojda, A., et al., Incomplete Masculinisation of XX Subjects Carrying the *SRY* Gene on an Inactive X Chromosome, *J. Med Genet.*, 1999, vol. 36, pp. 452–456.
35. Tar, A., Solyom, J., Gyorvari, B., et al., Testicular Development in an *SRY*-Negative 46,XX Individual Harboring a Distal Xp Deletion, *Hum. Genet.*, 1995, vol. 96, no. 4, pp. 464–468.
36. Huang, B., Wang, S., Ning, Y., et al., Autosomal XX Sex Reversal Caused by Duplication of *SOX9*, *Am. J. Med. Genet.,* 1999, vol. 87, pp. 349–353.
37. Vernole, P., Terrinoni, A., Didona, B., et al., An *SRY*-Negative XX Male with Huriez Syndrome, *Clin. Genet.*, 2000, vol. 57, pp. 61–67.

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