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Short Report

M. Emin ERDAL[1]
I. Omer BARLAS[2]

Detection of the SRY Gene in a 46,XY Phenotypic Female by the PCR-SSCP Method

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[1]Department of Medical Biology and Genetics, Faculty of Medicine, Mersin University, TR-33160 Mersin-Turkey
[2]Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University, TR-27310 Gaziantep-Turkey

Abstract: The short arm of the human Y chromosome determines maleness, initiating the development of the testes. Mutations and deletions in this region affect gonadal differentiation, causing primary sex reversal of 46,XY. Cytogenetic analysis was performed and the sex-determining region of the Y chromosome (SRY) sequence was amplified by the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method. The patient showed a 46,XY chromosome arrangement while she was a phenotypic female. Also a shifted band was detected by the SSCP method.

Key Words: PCR-SSCP; Sex reversal; SRY; 46,XY Female.

The sex-determining region of the human Y chromosome (SRY) encodes a testes-determining factor (TDF) which initiates male sex determination (1-4). But this does not exclude the possible effects of the other sex genes on the Y chromosome. If they exist, these genes must be located adjacent to the pseudoautosomal boundary, close to SRY (1). SRY is hypothesized to function as a transcription factor, triggering a cascade of gene interactions that induce the bipotential fetal gonads to develop into testes, leading to Sertoli cell differentiation with subsequent production of Müllerian-inhibiting substance and regression of the Müllerian ducts (5, 6). The demonstration of SRY provides evidence that the female phenotype in the presence of sex chromosome XY may result from alterations in another part of the sex-determining pathway or downstream from SRY (1, 3, 7). Deletions/mutations of SRY on the Y chromosome, during meiotic recombination with X, causes failure of testis determination or differentation, when the gene should be expressed at the expected stage of male gonadal development (3). As a result, it is obvious that for SRY, deletions/mutations may cause sex reversal in humans. In this study, we described sex reversal in a phenotypic female with a 46,XY karyotype and a shifted SSCP band. Deletions/mutations in this region may create 46,XY leading to the female phenotype.

The patient was a 22-year-old woman who was referred for primary amenorrhea, rudimentary uterus, unknown inguinal particle named "ovo-testis", clitoromegaly and lack of secondary sexual characterictics for genetic analysis, thought to have testicular feminization syndrome. Endocrinological study showed normal results except for PGN and E2, which have high values and hCG, which was lower. No other cases were reported in her family. Cytogenetic analysis was performed on peripheral blood from the patient and from a fertile male and a fertile female (controls). Lymphocytes were cultured according to the standard method. Banding patterns were analyzed using GTG techniques. Genomic DNA was extracted with phenol and chloroform from peripheral leukocytes for PCR. SRY gene sequences (8) were amplified by PCR from the DNA of the patient, as well as from the fertile XX female and fertile XY male controls. SRY primer sequences were as follows:

SRY F 5'-GAATATTCCCGCTCTCCGGAG-3', SRY R 5'-ACCTGTTGTCCAGTTGCACT-3' were used to amplify a 418 bp fragment. The PCR mixture consisted of 0.2 µg of genomic DNA, 2.5 U of Taq polymerase (MBI Fermentas Inc.), 0.5 µmol/L of each primer, 100 mmol/L dNTPs, 3.0 mM $MgCl_2$ and 1X PCR buffer in a final volume of 50 µl. The PCR conditions were 2 min at 94°C for pre-heating, 35 cycles of 94°C for 30 seconds, 57 °C for 30 seconds and 72°C for 1 min, and 72°C for 10 min

post-extension in an automated thermal cycler (Techne Genius). Reaction products were electrophoresed on 1.7% agarose-TBE gels containing 0.5 µg/ml ethidium bromide and documented with an gel electrophoresis visualizing system (Vilber Lourmat). SSCP analysis was used to screen SRY gene mutations according to a modified protocol as described previously (9). The SSCP gels were developed using a silver staining procedure (10).

Twenty metaphases from the patient and from each control were examined cytogenetically. The karyotypes revealed 46,XY; 46,XY and 46,XX respectively. The use of specific primers for SRY sequence allowed us to detect the characteristic band corresponding to the amplified 418 bp DNA fragment in the XY male and in the patient by PCR analysis (Figure 1). Both yielded a 418 bp product as expected, while no detectable product was obtained in the 46,XX female. Although no shifted SSCP bands were detected for the SRY gene in the fertile male, slow migrating bands were detected in the patient, suggesting that point mutations/polymorphisms may exist in this region (Figure 2).

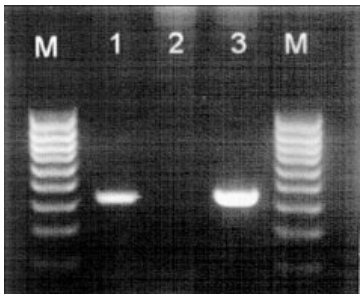

Figure 1 PCR amplification of the SRY gene in a fertile male, female and 46,XY phenotypic female. Lane 1: fertile XY male; lane 2: fertile XX female; lane 3: patient; M, 100bp DNA ladder.

A phenotypic female patient with primary and secondary sexual abnormalities presented in this study

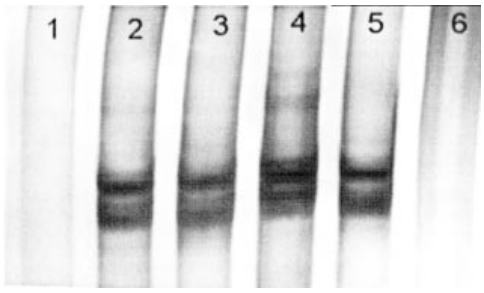

Figure 2. SSCP analysis of the SRY gene. Lane 1,6: fertile XX female; lane 2,3,5: fertile XY male; lane 4: patient.

revealed a 46,XY karyotype and a shifted SSCP band in the SRY gene. Our findings in this phenotypic female were consistent with previous reports of XY females in which de novo point mutation or frame-shift mutation in this region produces "non-functional" TDF protein (1, 4, 11-13). But this does not exclude the possibility of other alterations in another part of the sex-determination pathway leading to a female phenotype. In additon, this and other reported cases prove that mutations outside the SRY gene do occur and should be looked for in the setting of sex reversal (14, 15). The presence of a cytogenetically normal Y chromosome is not an absolute barrier to ovarian function in humans. Although it is unlikely that many women with premature ovarian failure will have a Y chromosome, it is still desirable to karyotype all such woman (16). The female phenotype in the presence of 46,XY may result from de novo deletions/mutations in the SRY region.

Correspondence author:

M. Emin Erdal

Department of Medical Biology and Genetics,

Medical Faculty, University of Mersin,

TR-33160 Mersin, TURKEY

References

1. Berta P, Hawkins JR, Sinclair AH, Taylor A, Griffiths BL, Goodfellow PN, Fellous M. Genetic evidence equating SRY and the testis-determining factor. Nature 348:448-450, 1990.
2. Lobaccaro JM, Medlej R, Berta P, Belon C, Galifer RB, Guthmann JP, Chevalier C, Czernichow P, Dumas R, Sultan C. PCR analysis and sequencing of the SRY sex determining gene in four patients with bilateral congenital anorchia. Clin Endocrinol 38:197-201, 1993.
3. Hawkins JR. Sex determination. Hum Mol Genet 3 :1463-1467,1994.
4. Imai A, Horibe S, Fuseya T, Takagi H, Takagi A, Tamaya T. Detection of SRY in 45,X/47,XYY mosaicism leading to phenotypic female. Clin Genet 51:124-126, 1997.

5. Carlisle C, Winking H, Weichenhan D, Nagamine CM. Absence of correlation between SRY polymorphisms and XY sex reversal caused by the M. m. domesticus Y chromosome. Genomics 33:32-45, 1996.

6. MacLean HE, Warne GL, Zajac JD. Intersex disorders: shedding light on male sexual differentiation beyond SRY. Clin Endocrinol 46:101-108, 1997.

7. Hines RS, Tho SPT, Zhang YY, Plouffe L, Hansen KA, Khan I, McDonough PG. Paternal somatic and germ-line mosaicism for a sex-determining region on Y (SRY) missens mutation leading to recurrent 46,XY sex reversal. Fertil Steril 67:675-679, 1997.

8. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240-244, 1990.

9. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci 86:2766-2770, 1989.

10. Oto M, Miyake S, Yuasa Y. Optimization of nonradioisotopic single strand conformation polymorphism analysis with a conventional minislab gel electrophoresis apparatus. Anal Biochem 213:19-22, 1993.

11. Jager RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. Nature 348:452-454, 1990.

12. Hawkins JR, Taylor A, Berta P, Levilliers J, Van der Auwera B, Goodfellow PN. Mutational analysis of SRY: nonsense and missense mutations in XY sex reversal. Hum Genet 88:471-474, 1992.

13. McElreavey KD, Vilain E, Boucekkine C, Vidaud M, Jaubert F, Richaud F, Fellous M. XY sex reversal associated with a nonsense mutation in SRY. Genomics 13:838-840, 1992.

14. Mertin S, McDowall SG,Harley VR. The DNA-binding specifity of SOX9 and other SOX proteins.Nucleic Acids Res. 27:1359-1364, 1999.

15. Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K, Jaruzelska J, Reinberg Y, Flejter WL, Bardwell Vj, Hirsch B, Zarkower D. A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. Hum Mol Genet 8:989-996, 1999.

16. Brown S, Yu CC, Lanzano P, Heller D, Thomas L, Warburton D, Kitajewsky J, Stadtmauer L. A de novo mutation (Gln2Stop) at the 5' end of the SRY gene leads to sex reversal with partial ovarian function. Am J Hum Genet 62:189-192, 1998.