International Archives of Medical Research
Volume 9, No.2, pp.51-60, 2017

ORIGINAL RESEARCH

108 patients with sex abnormality; cytogenetic and molecular analysis in Diyarbakır, Turkey

Diclehan Oral*, Selda Şimşek, Hilmi İsi, İlyas Yüce

Department of Medical Biology and Genetic, Medical Faculty, Dicle University, Diyarbakir, Turkey

Abstract

The relationship between abnormal chromosomal constitution and sex anomalies has been established since the development of cytogenetic and molecular methods. In order to investigate the relationship between sex dysplasia and sex-determining region Y (SRY) gene, 108 patients with sexual abnormality were analyzed by cytogenetic and molecular genetic methods. SRY gene was amplified by polymerase chain reaction (PCR) and its mutation was detected by direct sequencing. The results showed that among 108 patients, 49 were positive for SRY and the remaining negative for SRY. In the patients positive for SRY genes, with 45, X/46, XY 46, XXY,46,XX karyotype, In the patients negative for SRY, with 46,XY,46, XX karyotype It was concluded that SRY gene is strongly involved in male sex determination, while a sequence of other genes may be taken into account in sexual development. . It is also essential for defining genotypic and phenotypic correlation and understanding the basic mechanism involved in sex determination.

Keywords: Chromosome; Sry gene; sex abnormality

* Corresponding author: Diclehan Oral, Dicle University, Medical Faculty, Dept. of Medical Biology and Genetic, Diyarbakir, Turkey
e-mail: diclehan.oral@dicle.edu.tr

Introduction

The *SRY* (sex-determining region Y), which is normally located in the distal part of the short arm of the Y chromosome is a genetic 'master switch' of gonadal differentiation (1), the product of which is present in the male genital ridge before testis formation and is required for the regular development of male genitalia (2). *SRY* encodes a transcription factor that is a member of the high mobility group (HMG)-box family of DNA binding proteins and in mammals triggers the development of undifferentiated gonads towards a testicular phenotype (1,3). In humans, zygotes bearing mutations in SRY develop into XY females (4,5), while XX individuals with the presence of *SRY* typically show a normal male phenotype (6), but may occasionally show ambiguous genitalia (7). One example is testicular regression syndrome (XY gonadal regression syndrome), in which there are no gonads, and variable development of Mullerian and Wolfian ducts depending on the stage of fetal development at which the embryonic testis involutes; in most cases this happens before Mullerian tissues have regressed and before testosterone synthesis has started, the etiology is unknown but affected siblings have been reported (8).

However, the erroneous exchange between homologous but normally non-recombining regions can also occur resulting in the transfer of differing amounts of Y chromosomematerial, including the *SRY* gene, most often to the short arm of the X chromosome, leading to a 46,XX male phenotype (9,10).

XX maleness is a rare genetic condition affecting one in 24,000 newborn males (11). XX maleness is a genetically heterogeneous condition. The SRY gene is present in the majority of patients (12), although several studies have shown that approximately 10% of patients lack Y material, including the *SRY* gene (13,14). While the majority of XX males are carriers of an X/Y translocation, XX maleness may also arise as a consequence of a genetic mutation permitting testicular differentiation in the absence of *SRY*, or result from undetected mosaicism for a cell line carrying the Y chromosome (6,9, 15). Previously, we found a 46, XX male infant without the presence of the *SRY* gene, derived from intracytoplasmic sperm injection (ICSI) (16). Whether ICSI had enhanced the production of this 46, XX male is unclear.

The phenotype of the XX male varies greatly. Some XX males may have normal internal and external male gonads (17), whereas others may have small testes, abnormal secondary sexual characteristics (11), and hypospadias (18,19,20). Most men are diagnosed in adulthood due to infertility caused by azoospermia (11). A small proportion of *SRY* positive XX individuals are true hermaphrodites (7,21). It is not yet clear how one genotype can give rise to different phenotypes. It has been proposed that the variation in phenotype observed in SRY positive XX individuals is primarily dependent on X chromosome inactivation (XCI) pattern and the amount of Y material that has been translocated to the X chromosome, or a combination of both (7, 22,23)Therefore, a more masculine phenotype is expected when the Y bearing X chromosome preferentially escapes inactivation and when more Y chromosome material is present on the X, presumably protecting the *SRY* gene from the spread of inactivation (7,22,23). However, more recently, another mechanism, known as the position effect, has been proposed to delineate the observed phenotypic differences (24)Under this hypothesis, the phenotypic differences are dependent on the proximity of the breakpoint to the *SRY* gene as well as the presence or absence of cryptic rearrangements affecting the expression of the *SRY* gene(24).

In the human, there are normally 46 chromosomes, two sex chromosomes and 22 **chromosome** pairs for which one copy is inherited from each parent at conception. The sex chromosomes are called the X and the Y chromosome. Everyone needs at least one X chromosome to survive. Females normally have two X chromosomes whereas males typically have one X and one Y chromosome. In the absence of a Y chromosome, babies will develop as females. When the Y chromosome is present, they will develop as males. As a result, it is obvious that for SRY, deletions/mutations may cause sex reversal in humans.

Materials and methods

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. The karyotypic descriptions were according to the ISCN recommendations.

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 5Õ-GAATATTCCCGCTCTCCGGAG-3Õ, SRY R 5Õ- ACCTGTTGTCCAGTTGCACT-3Õ 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 MgCl2 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).

Twenty metaphases from the patients and from each control were examined cytogenetically. 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.

Results

Combined results of cytogenetic and molecular studies of patients

Number of patient	Diagnosis	Karyotype	Sry gene
1	Ambigious genitalia	46,XY	+
2	Ambigious genitalia	46,XY	+
3	Ambigious genitalia	46,XY	+
4	Ambigious genitalia	46,XY(27)/45,X(3)	+
5	Ambigious genitalia	46,XY	+
6	Ambigious genitalia	46,XY	+
7	Ambigious genitalia	46,XY	-
8	Ambigious genitalia	47,XXY	-
9	Ambigious genitalia	46,XY	+
10	Ambigious genitalia	46,XX	-
11	Ambigious genitalia	46,XX	-
12	Ambigious genitalia	46,XX	-
13	Ambigious genitalia	46,XX	-
14	Ambigious genitalia	46,XY	+
15	Ambigious genitalia	46,XY	+
16	Ambigious genitalia	46,XY	+
17	Ambigious genitalia	46,XY	+
18	Ambigious genitalia	46,XX	-
19	Ambigious genitalia	46,XY	+
20	Ambigious genitalia	46,XY	+
21	Ambigious genitalia	46,XY	-
22	Ambigious genitalia	46,XY	-
23	Hypogonadizm	47,XXY	+
24	Hypogonadizm	46,XY	+
25	Hypogonadizm	46,XX	+
26	Hypogonadizm	46,XX	+
27	Primer amenore	46,XX	-
28	Primer amenore	46,XX	-
29	Primer amenore	46,XX	-
30	Primer amenore	46,XX	-
31	Primer amenore	46,XX	-
32	Primer amenore	46,XX	-
33	Primer amenore	46,XX	-
34	Primer amenore	46,XX	-
35	Primer amenore	46,XX	-
36	Primer amenore	46,XX	-
37	Primer amenore	46,XX	-
38	Primer amenore	46,XX	-
39	Primer amenore	46,XX	-
40	Primer amenore	46,XX,inv(9)(p13q13)	-
41	Primer amenore	46,XX	-
42	Primer amenore	46,XX	-
43	Primer amenore	46,XX	-
44	Primer amenore	46,XX	-
45	Primer amenore	46,XX,1qh+	-
46	Primer amenore	46,XX	-
47	Primer amenore	46,XX	-
48	Primer amenore	46,XX	-
49	Primer amenore	46,XX,9qh+	-
50	Primer amenore	46,XX,9qh+	-
51	Primer amenore	46,XY,1qh+	+
52	Primer amenore	46,XX	-
53	Primer amenore	46,XX	-
54	Primer amenore	46,XX	-
55	Primer amenore	46,XX	-
56	Primer amenore	46,XX	-
57	Primer amenore	46,XX	-
58	Primer amenore	46,XY	+
59	Primer amenore	46,XX	-
60	Primer amenore	46,XX	-
61	Primer amenore	46,XX	-
62	Primer amenore	46,XY	+
63	Klinefelter syn.	46,XY	+
64	Klinefelter syn.	46,XY	+
65	Klinefelter syn.	46,XY	+
66	Klinefelter syn.	47,XXY	+
67	Klinefelter syn.	47,XXY	+
68	Klinefelter syn.	47,XXY	+
69	Klinefelter syn.	46,XY	+
70	Klinefelter syn.	46,XY	+
71	Klinefelter syn.	46,XY,1qh+	+
72	Klinefelter syn.	46,XY	+
73	Azospermi	46,XY	+
74	Azospermi	46,XY	+
75	Azospermi	46,XY	+
76	Azospermi	46,XY	+
77	Azospermi	46,XY	+
78	Azospermi	46,XY	+
79	Azospermi	46,XY	+
80	Azospermi	46,XXY	+
81	Azospermi	46,XY	+
82	Azospermi	46,XY	+

83	Azospermi	46,XY	+
84	Azospermi	46,XY	+
85	Azospermi	46,XY	+
86	Azospermi	46,XY	+
87	Azospermi	46,XXY	+
88	Azospermi	46,XY	-
89	Azospermi	46,XY	-
90	Azospermi	46,XY	-
91	Azospermi	46,XY	-
92	Azospermi	46,XY	-
93	Azospermi	46,XY	-
94	Azospermi	46,XY	-
95	Azospermi	46,XY	-
96	Azospermi	46,XY	-
97	Azospermi	46,XY	-
98	Azospermi	46,XY	-
99	Azospermi	46,XY	+
100	Azospermi	46,XY	-
101	Azospermi	46,XY	+
102	Azospermi	46,XY	+
103	Azospermi	46,XY	+
104	Turner syn.	45,X	-
105	Turner syn.	45,X/46,XY	+
106	Turner syn.	46,XY	+
107	Turner syn.	46,XX	-
108	Turner syn.	46,X,i(X)	-

A total of 108 patients were studied. Of those, 31 were azoospermic men,22 baby with Ambigious genitalia ,5 Turner syndrome,10 Klinefelter syndrome ,4 with hypogonadism,36 with Primer amenore . The results of cytogenetic analysis in 108 patients are shown in Table 1. Molecular analysis of some patients are shown in Figure 1.

22 baby with Ambigious genitalia :5 baby 46,XX karyotype and sry gene negative,15 baby 46,XY and 10 sry gene pozitif ,5 sry gene negative , , One baby who had mos46,XY(27)/45,X(3)mosaicism, and sry gene pozitif,one baby 47,XXY karyotype consistent with Klinefelter syndrome,

4 patient with hypogonadizm 2 of them 46,XX karyotype and sry gene pozitif ,one patient 46,XY and sry gene pozitif,one patient 47,XXY 46,XX,inv(9)(p13q13)

36 patient with primary amenorrhea 29 normal karyotype and sry gene negative, one patient 46,XX, inv(9)(p13q13) karyotype and sry gene negative,one patient 46,XX,1qh+ karyotype and sry gene negative, one patient 46,XY,1qh+(testicular feminization) karyotype and sry gene pozitif,two patient 46,XX,9qh+ karyotype and sry gene negative

39 patient with azospermia 23 normal karyotype (46,XY) and sry gene pozitif,12 normal karyotype and sry gene negative, 5 cases showed 47,XXY karyotype consistent with Klinefelter syndrome and sry gene positive,one patient 46,XY,1qh+ karyotype and sry gene pozitif.

5 patient with turner syndrome ;2 patient normal karyotype and sry gene negative, one patient normal karyotype(46,XY) sry gene pozitif, one patient 46,X,i(X) karyotype and sry gene negative,one patient 45,X karyotype and sry gene negative.

Seven cases showed 47,XXY karyotype consistent with Klinefelter syndrome, One patients who had 46,XY(27)/45,X(3) mosaicism, One patient 46,XX,inv(9)(p13q13) ,3 patient 46,XX,9qh+and .

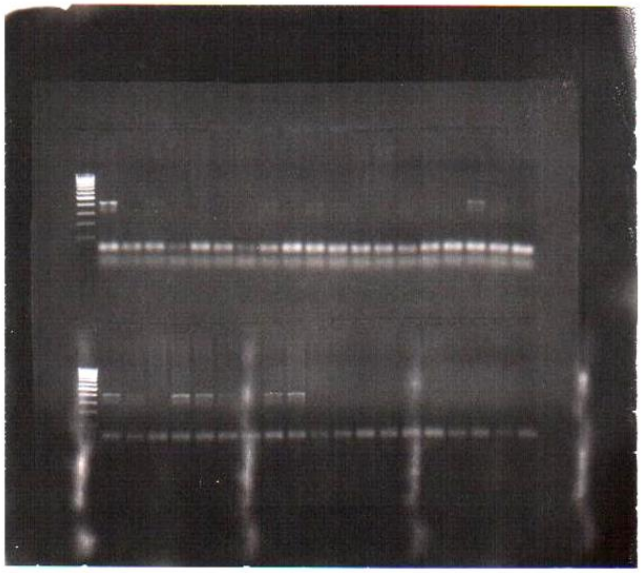

Figure 1 : PCR amplification of the SRY gene

Discussion

Human males with a 46,XX karyotype are sterile males with normal female chromosomes. The reported incidence varies from 1 in 9000 (25) to 1 in 20000 (26) in newborn males. Most cases are sporadic, that is, they are without familial clustering (11). The majority of cases are due to interchange of a fragment of the short arm of the Y chromosome containing the region that encodes the testes determining factor (TDF) with the X chromosome (27,28,2930). Other less likely causes include mutation in an autosomal or X chromosomal gene, which permits testicular determination in the absence of TDF, and undetected mosaicism with a Y-bearing cell line. It is now possible to identify two forms of this syndrome: Y DNA positive and Y DNA negative. The Y DNA positive males result from a X;Y translocation with a low recurrence risk; the Y DNA negative males are due to a mutation with a high recurrence risk (31).

All males with discordant phenotype/sex chromosomal pattern are azoospermic due to absence of the long arm of the Y chromosome containing the azoospermia factor (AZF) gene, which is necessary for normal spermatogenesis (32,33,34). The majority of cases have normal external genitalia, but 10–15% of XX males shows various degrees of hypospadias (35). Molecular studies have detected Y chromosome material in 75% of XX males (36), which explains their testicular development. On the other hand, many theories have been put forward to explain how patients who are Y-negative, as in this case, can have testes, despite complete absence of the Y chromosome. Researchers (29,37) suggested the presence of other mutations (autosomal or X-linked) which could be responsible for testicular determination in the absence of Y sequences. The presence of hidden mosaicism with a Y-bearing cell line was proposed (38). More recently, the reporting of a Mexican family in which two siblings without genital ambiguities were SRY negative (39), suggested that an inherited loss of function mutation in a gene participating in the sex-determining cascade could induce normal male sexual differentiation in the absence of SRY gene. This would strengthen what has been reported (12), that although the absence of Y-specific DNA generally results in incomplete musculinization, exceptions do occur.

A total of 60% of true hermaphrodites have a 46,XX karyotype (40). Although the majority of 46,XX true hermaphrodites are negative for the Y-DNA sequence, including SRY gene (41,42,43), a minority could be positive (44,45,46,47,48). Nevertheless, this diagnosis was unlikely in this patient because of the presence of normal male external genitalia, absence of gynaecomastia and no pelvic abnormality detected on examination. Translocations involving the human sex chromosomes are rarely reported. One of the best-known consequences of such exchanges is sex reversal in 46,XX males and some 46,XY females, due to exchange in the paternal germ line of terminal portions of Xp and Yp, including the SRY gene . The presence of the *SRY* gene in the normal male zygote leads to the development of male genital organs and the absence of this gene leads to the development of the female genitalia. Patients who carry a structural abnormality of the X chromosome and ambiguous genitalia have provided opportunities to elucidate the genotype/phenotype correlation in relation to the X and Y chromosome content and X chromosome inactivation

We detected the SRY gene in peripheral blood leucocytes of 49 patients with 46,XY, 45,X/46,XY46,XXY, 46,XX karyotype, whereas all other patients were SRY negative. This incidence of SRY-positive results in sex abnormality patients agrees with the results of other studies [49,50,51].

In conclusion, our results strongly indicate that X inactivation spreading into a translocated Yp region containing the sex determining SRY gene and the selection of such a cell line could be the major mechanism causing phenotypic sexual ambiguity, including the presence of ovarian tissue in XX (SRY+) subjects. It was concluded that SRY gene is strongly involved in male sex determination, while a sequence of other genes may be taken into account in sexual development.

Reference

1. 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. 1990;**346**:240–244. doi: 10.1038/346240a0.*
2. *Clépet C, Schafer AJ, Sinclair AH, Palmer MS, Lovell- Badge R, Goodfellow PN. The human SRY transcript. Hum Mol Genet. 1993;**2**:2007–2012*
3. *Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. Nature. 1991;**351**:117–121. doi: 10.1038/351117a0*
4. *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. 1992;**88**:471–474. doi: 10.1007/BF00215684.*
5. *Iida T, Nakahori Y, Komaki R, Mori E, Hayashi N, Tsutsumi O, Taketani Y, Nakagome Y. A novel missense mutation in the HMG box of the SRY gene in a patient with XY sex reversal. Hum Mol Genet. 1994;**3**:1437–1438.*
6. *Fechner PY, Marcantonio SM, Jaswaney VJ, Stetten G, Goodfellow PN, Migeon CJ, Smith KD, Berkovitz GD, Amrhein JA, Bard PA. The role of the sex determining region Y gene (SRY) in the etiology of 46,XX maleness. J Clin Endocrinol Metab. 1993;**76**:690–696. doi: 10.1210/jc.76.3.690.*

7. *Kusz K, Kotecki M, Wojda A, Szarras-Czapnik M, Latos-Bielenska A, Warenik-Szymankiewicz A, Ruszczynska-Wolska A, Jaruzelska J. Incomplete masculinisation of XX subjects carrying the SRY gene on an inactive X chromosome. J Med Gene. 1999;**36**:452–456.*
8. *Affara NA, Ferguson-Smith MA, Magenis RE, Tolmie JL, Boyd E, Cooke A, Jamieson D, Kwok K, Mitchell M, Snadden L. Mapping the testis determinants by an analysis of Y-specific sequences in males with apparent XX and XO karyotypes and females with XY karyotypes. Nucleic Acids Res. 1987;**15**:7325–7342.*
9. *Ferguson-Smith MA. X-Y chromosomal interchange in the aetiology of true hermaphrodism and of XX Klinefelter's syndrome. Lancet. 1966;2:475–6. doi:10.1016/S0140-6736(66)92778-4.*
10. *Weil D, Wang I, Dietrich A, Poustka A, Weissenbach J, Petit C. Highly homologous loci on the X and Y chromosomes are hot-spots for ectopic recombinations leading to XX maleness. Nat Genet. 1994;7:414–9. doi:10.1038/ng0794-414*
11. de la Chapelle A. The etiology of maleness in XX men. Hum Genet. 1981;58:105–16. doi:10.1007/BF00284157.
12. Boucekkine C, Toublanc JE, Abbas N, Chaabouni S, Ouahid S, Semrouni M, Jaubert F, Toublanc M, McElreavey K, Vilain E, Fellous M. Clinical and anatomical spectrum in XX sex reversed patients: relationship to the presence of Y specific DNA sequences. Clin Endocrinol (Oxf). 1994;40:733–42. doi:10.1111/j.1365-2265.1994.tb02506.x.
13. Palmer MS, Sinclair AH, Berta P, Ellis NA, Goodfellow PN, Abbas NA, Fellous M. Genetic evidence that ZFY is not the testis-determining factor. Nature. 1989;342:937–9. doi:10.1038/342937a0.
14. Abbas NE, Toublanc JE, Boucekkine C, Toublanc M, Affara NA, Job JC, Fellous M. A possible common mechanism of "Y-negative" human XX males and XX true hermaphrodites. Hum Genet. 1990;84:356–60. doi:10.1007/BF00196234.
15. Page DC, Brown LG, de la Chapelle A. Exchange of terminal portions of X- and Y-chromosomal short arms in human XX males. Nature. 1987;328:437–40. doi:10.1038/328437a0.
16. Ma S, Tang SS, Yuen BH, Bruyere H, Penaherrera M, Robinson WP. Cytogenetic and molecular study of a premature male infant with 46,XX derived from ICSI: case report. Hum Reprod. 2003;18:2298–301. doi:10.1093/humrep/deg462.
17. Abdelmoula NB, Portnoi MF, Keskes L, Recan D, Bahloul A, Boudawara T, Saad A, Rebai T. Skewed X-chromosome inactivation pattern in SRY positive XX maleness: a case report and review of literature. Ann Genet. 2003;46:11–8. doi:10.1016/S0003-3995(03)00011-X
18. Lopez M, Torres LM, Mendez JP, Cervantes A, Perez-Palacios G, Erickson RP, Alfaro G, Kofman-Alfaro S. Clinical traits and molecular findings in 46,XX males. Clin Genet. 1995;48:29–34
19. Castineyra G, Copelli S, Levalle O. 46,XX male: clinical, hormonal/genetic findings. Arch Androl. 2002;48:251–7.
20. Grigorescu-Sido A, Heinrich U, Grigorescu-Sido P, Jauch A, Hager HD, Vogt PH, Duncea I, Bettendorf M. Three new 46,XX male patients: a clinical, cytogenetic and molecular analysis. J Pediatr Endocrinol Metab. 2005;18:197–203.
21. Quiepo G, Zenteno JC, Pena R, Nieto K, Radillo A, Dorantes LM, Erana L, Lieberman E, Soderlund D, Jimenez AL, Ramon G, Kofman-Alfaro S. Molecular analysis in true hermaphroditism: demonstration of low-level mosaicism for Y-derived sequences in 46,XX cases. Hum Genet. 2002;111:278–83. doi:10.1007/s00439-002-0772-9.

22. Numabe H, Nagafuchi S, Nakahori Y, Tamura T, Kiuchi H, Namiki M, Kohda N, Yoshimitsu Fukushima Y, Fuse H, Kusano M, Arai T, Matsuzaki Y, Fukutani K, Isurugi K, Kuroki Y, Ikeuchi T, Yoshida M, Minowada S, Nakagome Y. DNA analysis of XX and XY hypospadic males. Hum Genet. 1990;90:211–4

23.McElreavey K, Salas-Cortes L. X-Y tranlocations and sex differentiation. Semin Reprod Med. 2001;19:133–9. doi:10.1055/s-2001-15393

24. Sharp A, Kusz K, Jaruzelska J, Tapper W, Szarras-Czapnik M, Wolski J, Jacobs P. Variability of sexual phenotype in 46,XX(SRY) patients: the influence of spreading X inactivation versus position effects. J Med Genet. 2005;42:420–7. doi:10.1136/jmg.2004.022053.

25. de la Chapelle, A. (1972) Analytic review: nature and origin of males with XX sex chromosomes. Am. J. Hum. Genet., 24, 71–105.

26.Nielsen, J. and Sillesen, I. (1975) Incidence of chromosome aberrations among 11 148 newborn children. Hum. Genet., 30, 1–12.

27.Muller, U., Donlon, T., Schmid, M. et al. (1986) Deletion mapping of the testis determining locus with DNA probes in 46,XX males and in 46,46XY and 46,X,dic(Y) females. Nucleic Acids Res., 14, 6489–6505.

28. Page, D.C., Mosher, E., Simpson, E. et al. (1987) The sex determining region of the human Y chromosome encodes a finger protein. Cell, 51, 1091–1104.

29. Ferguson-Smith, M.A., Cook, A., Affara, N.A. et al. (1990) Genotype–phenotype correlation in XX males and the bearing on current theories of sex determination. Hum. Genet., 84, 198–202.

30. Sinclair, A.H. (1998) Human sex determination (review). J. Exp. Zool., 28, 501–505.

31. Van Dyke, D.C., Hansom, J.W., Moor, J.W. et al. (1991) Clinical management issues in males with sex chromosomal mosaicism and discordant phenotype/sex chromosomal patterns. Clin. Pediatr. (Phil.), 30, 15–21.

32. Tiepolo, L. and Zuffardi, O. (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum. Genet., 34, 119–124.

33. Grumbach, M.M. and Conte, F.A. (1992) Disorders of sex differentiation. In Wilson, J.D. and Foster, D.W. (eds) Williams textbook of endocrinology. Saunders, Philadelphia, pp. 853–951.

34. Vogt, P.H., Edelmann, A., Kirsch, S. et al. (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum. Mol. Genet., 5, 933–943.

35. Lopez M., Torres, L., Mendez, J.P. et al. (1995) Clinical traits and molecular findings in 46, XX males. Clin. Genet., 48, 29–34.

36. Muller, U., Latt, S.A., Donlon, T. (1987) Y-specific DNA sequences in male patients with 46,XX and 47,XXX karyotypes. Am. J. Med. Genet., 28, 393.

37. Vilain, E., Le Fiblee, B., Morichon-Delvallez, N. et al. (1994) SRY- negative XX fetus with complete male phenotype. Lancet, 343, 240–241.

38. Fechner, P.Y., Marcantonio, S.M., Jaswaney, V. et al. (1990) The role of the sex-determining region Y gene in the aetiology of 46,XX maleness. J. Clin. Endocrinol. Metab., 76, 690–695.

39 .Zenteno, J.C., Lopez, M., Vera, C. et al. (1997) Two SRY-negative XX male brothers without genital ambiguity. Hum. Genet., 100, 606–610

40..Van Niekerk, W.A. and Retief, A.E. (1981) The gonads of human true hermaphrodites. Hum. Genet., 58, 105–116.

41.Ranie J., Robertson M.E., Malcolm, S. et al. (1989) Absence of Y specific DNA sequences in two siblings with 46XX hermaphroditism. Arch. Dis. Child., 64, 1185–1187.

42 .Pereira, E.T., de Almeida, J.C., Gunha, A.C. et al. (1991) Use of probes for ZFY, SRY, and the Y pseudoautosomal boundary in XX males, XX true hermaphrodites, and an XY female. J. Med. Genet., 28, 591–595.

43 .Sadi, A.M., Toda, T., Kiyuna, M. et al. (1996) A true hermaphrodite with bilateral ovotestes: a case report. J. Obstet. Gynaecol. Res., 22, 247–251.

44 .McElreavey, K., Rappaport, R., Vilain, E. et al. (1992) A minority of 46,XX true hermaphrodites are positive for the Y-DNA sequence including SRY. Hum. Genet., 90, 121–125.

45. Abbas, N., McElreavey, K., Leconiat, M. et al. (1993) 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, 316, 375–383.

46. Copelli, S.B., Bergada, C., Billerbeck, A.E. et al. (1996) Molecular analysis of sex determination in sex-reversed and true hermaphroditism. Braz. J. Med. Biol. Res., 29, 743–748.

47. Ramos, E.S., Moreira-Filho, C.A., Vicente, Y.A. et al. (1997) SRY-negative true hermaphrodites and an XX male in two generations of the same family. Hum. Genet., 97, 596–598.

48. Kojima, Y., Hayashi, Y., Asai, N. et al. (1998) Detection of the sex-determining region of the Y chromosome in 46,XX true hermaphroditism. Urol. Int., 60, 235–238.

49. Binder G, Koch A, Wajs E, Ranke MB. Nested polymerase chain reaction study of 53 cases with Turner's syndrome: is cytogenetically undetected Y mosaicism common? J Clin Endocrinol Metab 1995; 80(12): 3532-3536

50. Fernandez Garcia R, Garcia Doval S, Costoya S, Pasaro E. Analysis of sex chromosome aneuploidy in 41 patients with Turner syndrome: a study of 'hidden' mosaicism. Clin Genet 2000; 58(3): 201-208.

51.. Medlej R, Lobaccaro JM, Berta P, Belon C, Leheup B, Toublanc JE, Weill J, Chevalier C, Dumas R, Sultan C. Screening for Y-derived sex determining gene SRY in 40 patients with Turner syndrome. J Clin Endocrinol Metab 1992; 75(5): 1289-1292.