



Multiscale analysis of SRY-positive 46,XX testicular disorder of sex development: Presentation of nine cases

Omer Salih Akar¹ | Sezgin Gunes² | Ummet Abur¹ | Engin Altundag¹ |
Ramazan Asci³ | Onur Emre Onat⁴ | Tayfun Ozelik⁴ | Gonul Ogur¹

¹Department of Medical Genetics, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

²Department of Medical Biology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

³Department of Urology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

⁴Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey

Correspondence

Sezgin Gunes, Department of Medical Biology, Faculty of Medicine, Ondokuz Mayıs University, 55139 Samsun, Turkey.
Email: sgunes@omu.edu.tr

Abstract

46,XX testicular disorder of sex development (46,XX TSD) is a relatively rare condition characterised by the presence of testicular tissue with 46,XX karyotype. The present study aims to reveal the phenotype to genotype correlation in a series of sex-determining region Y (SRY)-positive 46,XX TSD cases. We present the clinical findings, hormone profiles and genetic test results of six patients with SRY-positive 46,XX TSD and give the details and follow-up findings of our three of previously published patients. All patients presented common characteristics such as azoospermia, hypergonadotropic hypogonadism and an SRY gene translocated on the terminal part of the short arm of one of the X chromosomes. Mean \pm standard deviation (SD) height of the patients was 164.78 ± 8.0 cm. Five patients had decreased secondary sexual characteristics, and three patients had gynecomastia with varying degrees. Five of the seven patients revealed a translocation between protein kinase X (PRKX) and inverted protein kinase Y (PRKY) genes, and the remaining two patients showed a translocation between the pseudoautosomal region 1 (PAR1) of X chromosome and the differential region of Y chromosome. X chromosome inactivation (XCI) analysis results demonstrated random and skewed XCI in 5 cases and 1 case, respectively. In brief, we delineate the phenotypic spectrum of patients with SRY-positive 46,XX TSD and the underlying mechanisms of Xp;Yp translocations.

KEYWORDS

array-CGH, infertility, SRY-positive 46,XX male, X chromosome inactivation

1 | INTRODUCTION

46,XX testicular disorder of sex development (46,XX TSD) (MIM 400045) was first described by De la Chapelle, Hortling, Niemi, and Wennström (1964) and generally characterised by a male phenotype despite having a female karyotype. The incidence is estimated to be one in 20,000 male newborns. Patients with sex-determining region Y (SRY)-positive 46,XX TSD are usually asymptomatic males diagnosed in puberty or adulthood because of hypergonadotropic hypogonadism, microorchidism, and infertility due to azoospermia and Sertoli cells only (Delot & Vilain, 1993; Ropke & Tuttmann, 2017; Zenteno-Ruiz, Kofman-Alfaro, & Mendez, 2001). Until the early stages of puberty,

Sertoli and Leydig cells are functional in these patients. Sertoli cells produce anti-müllerian hormone (AMH), which is responsible for the regression of müllerian ducts, and Leydig cells synthesise testosterone and dihydrotestosterone to drive the differentiation of derivatives of the Wolffian duct in developing male fetuses. Therefore, these patients have normal internal and external male genitalia with an average gonadal size during childhood. Germ cell proliferation and growth in seminiferous tubules are triggered by the onset of puberty. During the adolescence and adulthood with the effect of the extra X chromosome, testicles remain smaller than normal and cause to spermatogenic failure because of the dramatic germ cell loss during meiosis. Absence of azoospermia factor (AZF)a, AZFb and AZFc regions results

in Sertoli cell-only syndrome (SCOS; Kamp et al., 2001), maturation arrest and azoospermia, respectively (Krausz & Riera-Escamilla, 2018). The probability of sperm retrieval is virtually zero in patients with complete AZFa and AZFb microdeletions; therefore, assisted reproductive techniques are not recommended to these patients (Grinspon & Rey, 2019). Although all patients generally have small testicular tissues, the external genitalia varies from normal virilised male to ambiguous genitalia depending on the presence and length of SRY gene. Hence, SRY-negative patients tend to be more frequently having genital ambiguities compared to SRY-positive ones. Approximately 80%–90% of 46,XX TSD patients have SRY gene which is usually translocated on the distal portion of the short arm of an X chromosome (Chen et al., 2019; Dauwerse, Hansson, Brouwers, Peters, & Breuning, 2006; Gunes & Esteves, 2020; Queralt et al., 2008; Ropke & Tuttelmann, 2017; Zenteno-Ruiz et al., 2001). Typical features of SRY-positive 46,XX TSD patients are female karyotype with completely normal male phenotype and virilised male external genitalia, small testes, azoospermia and hypergonadotropic hypogonadism (Akinsal, Baydilli, Demirtas, Saatci, & Ekmekcioglu, 2017; Ropke & Tuttelmann, 2017). Patients usually come to attention after puberty because of hypogonadism and infertility (Zenteno-Ruiz et al., 2001). Gynaecomastia, sparse body and/or pubic hair, cryptorchidism and/or hypospadias could also be detected in these patients (Majzoub et al., 2017).

SRY-positive 46,XX TSD results from an aberrant Y to X translocation during the paternal meiosis. Either the nonallelic homologous recombination (NAHR) between the identical sequences X- and Y chromosome or spontaneous errors during the replication-based mechanisms could mediate the Xp;Yp translocations in 90% of cases (Giglio et al., 2002).

Clinical pictures of the patients with 46,XX TSD are heterogeneous ranging from infertile men seeking fertility with normal male internal and external genitalia to the child attending to urology and child health clinics at an early age due to the ambiguous genitalia or micropenis (Majzoub et al., 2017). Therefore, the present study aimed to reveal the clinical and genetic findings of this heterogeneous group of patients in the view of our nine patients with SRY-positive 46,XX-DSD. Additionally, we aimed to evaluate the molecular basis of this disorder based on the results of array comparative genomic hybridisation (array-CGH) analysis.

2 | MATERIALS AND METHODS

2.1 | Clinical evaluation of patients

Nine patients with SRY-positive 46,XX TSD out of 1,300 consecutive infertile men (548 severe oligozoospermic and 752 azoospermic patients) attending to Urology Clinics of the Ondokuz Mayıs University between 2004 and 2017 were enrolled in the present study. The study was approved by the Ondokuz Mayıs University Clinical Research Ethical Committee (Approval No: 2018/353), and patients signed informed consent before participating in the study. The medical and family history, detailed physical examination

including measuring of height, weight and body mass indexes, an inspection of the external genitalia and assessment of secondary sex characteristics were evaluated. Testicular volumes of these patients were calculated using the formula [length (L) × width (W) × height (H) × 0.52 with the dimensions] obtained by testicular ultrasound.

Serum levels of follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin (PRL), oestradiol (E2) and total testosterone (TT) were measured using a radioimmunoassay in all patients. Semen samples were processed within 1 hr after the collection and liquefaction and then analysed according to the World Health Organization (WHO) guidelines in the year of investigation (1999 or 2010) by the same laboratory technician.

The clinical data, karyotype and fluorescence in situ hybridisation (FISH) findings, and X chromosome inactivation (XCI) patterns of three of our patients had been reported previously (Gunes et al., 2013). However, array-CGH data have not been analysed and published in our previous publication. Therefore, array-CGH and follow-up findings of these cases after publication have also been included in the present study.

2.2 | Cytogenetic analysis

Peripheral blood samples were obtained from all patients for chromosomal analysis. Blood lymphocytes were cultured using modified methotrexate–thymidine synchronisation method and GTG banding as previously described (Abur et al., 2019; Rooney, 2001). Chromosomal analyses were assessed with CytoVision software (version 3.93; Applied Imaging).

2.3 | Fluorescence in situ hybridisation analysis

FISH analyses were conducted on both metaphase spreads and interphase nuclei with (SRY/CEPX; Vysis; Gunes et al., 2013). Image analyses were evaluated using CytoVision software (version 3.93; Applied Imaging) with Olympus BX51 microscope equipped with Progressive Scan Video Camera.

2.4 | DNA extraction

The genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Blood Mini Kit (Qiagen GmbH) according to the manufacturer's instruction (Abur et al., 2019).

2.5 | Array comparative genomic hybridisation analysis

Array-CGH analysis was carried out in 7/9 patients using a 60K oligonucleotide microarray (Agilent Technologies) as described

TABLE 1 Clinical, hormonal and semen analysis results of patients with SRY-positive 46,XX TDSD

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Mean \pm SD
Clinical findings										
Age (years)	34	27	25	32	25	27	30	22	16	26.44 \pm 5.4
Height (cm)	161 (-2.0 SDS)	168 (-0.9 SDS)	160 (-2.1 SDS)	169 (-0.7 SDS)	171 (-0.4 SDS)	175 (0.2 SDS)	155 (-2.9 SDS)	172 (-0.2 SDS)	152 (-3.4 SDS)	164.78 \pm 8.0
Weight (kg)	74 (0.8 SDS)	74 (0.8 SDS)	62 (-0.4 SDS)	68 (0.2 SDS)	75 (0.9 SDS)	96 (0.8 SDS)	70 (0.4 SDS)	95 (3 SDS)	65 (0 SDS)	75.00 \pm 12.2
BMI (kg/cm ²)	28.5	26.2	24.2	23.8	25.6	31.3	27.3	32.4	24.1	26.82 \pm 3.3
Onset of puberty (years)	15	15	13	14	16	12	14	15	14	14.33 \pm 1.2
Secondary sexual characteristics (Marshall & Tanner staging)	Stage 4	Stage 4	Stage 3	Stage 3	Stage 3	Stage 4	Stage 3	Stage 4	Stage 2	
Gynaecomastia	+	-	-	-	-	+	+	+	-	
Testicular volume (R/L) (ml)	8/8	NA/5	3/3	4/8	3/3	6/8	4/1	5/5	3/3	5.22 \pm 2.5/4.88 \pm 1.7
Stretched penile length (cm)	10	11	12	12	14	13	14	13	12.5	12.39 \pm 1.3
Serum hormones										
FSH (mIU/ml) (1.5-12.4)	28	51.1	38	28.3	23.7	20.6	40	35	41.1	33.73 \pm 9.5
LH (mIU/ml) (1.7-8.6)	23	33.2	30	17.1	16.4	9.6	20.1	17.1	14.6	20.00 \pm 7.5
TT (ng/ml) (2.8-8.0)	2.8	1.5	2.4	1.9	2	2.8	0.6	2.3	2.2	2.03 \pm 0.7
E2 (pg/ml) (7.6-43.0)	20.9	15.1	36.1	39.9	40.4	40.6	21.2	30.6	32	30.38 \pm 10.1
Semen analysis										
Sperm count	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia	Azoospermia
Semen volume (ml)	4	2	1.6	2.8	2.5	2	0.2	3	1.7	2.22 \pm 1.1
Pellet test	-	-	-	-	-	-	-	-	NA	

Note: Clinical and laboratory findings of the patients Cases 7-9 have previously published by Gunes et al. (2013). SDS = (observed value - median value of the reference population)/standard deviation value of reference population.

Abbreviations: BMI, body mass index; E2, oestradiol; FSH, follicle-stimulating hormone; L, left; LH, luteinising hormone; NA, not available; NOA, nonobstructive azoospermia; R, right; SD, standard deviation; SDS, standard deviation score; TDSD, testicular disorder of sex development; TT, total testosterone.

TABLE 2 Cytogenetic, molecular cytogenetic and molecular results of the patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Karyotype	46,XX	46,X,der(X) ^a [29]/47,X,der(X) ^a ,der(X) ^a [1]	46,X,der(X) ^a	46,XX	46,XX	46,XX	46,XX	46,X,der(X) ^a	46,XX
FISH SRY-CEPX ^b	SRY*1,CEPX*2	SRY*1,CEPX*2[243]/SRY*2,CEPX*3[4]/SRY*0,CEPX*1[6]/	SRY*1,CEPX*2	SRY*1,CEPX*2	SRY*1,CEPX*2	SRY*1,CEPX*2	SRY*1,CEPX*2	SRY*1,CEPX*2	SRY*1,CEPX*2
X inactivation pattern	NI	NI	Random	Random	NI	Random	Random	Skewed	Random
Array-CGH									
Xp breakpoint	Within PRKX	Within PAR1 (DHRSX)	Within PAR1 (CRLF2)	NA	Within PRKX	Within PRKX	Within PRKX	Within PRKX	NA
Yp breakpoint	Within inverted PRKY	Proximal to RSP4Y1	Within PCDH11Y	NA	Within inverted PRKY	Within inverted PRKY	Within inverted PRKY	Within inverted PRKY	NA

Note: Karyotype, FISH and array-CGH findings of the patients. Karyotype and FISH analysis findings of Cases 7–9 have previously published by Gunes et al. (2013).

Abbreviations: CEP, chromosome enumeration probe; CGH, comparative genomic hybridisation; CRLF2, cytokine receptor-like factor 2; NA, not available; NI, not informative; PAR1, the Xp/Yp pseudoautosomal region-1; PCDH11Y, protocadherin 11 Y-linked; PRKX, protein kinase X; PRKY, protein kinase Y; SRY, sex-determining region Y; STS, sequence tagged site.

^ader(X)t(X;Y)(p22.3;p11.3).

^bCases 1,3,4,5,6,7,8 & 9:46,XX,ish der(X)t(X;Y)(p22.3;p11.3) (DXZ1x2)(SRY+); Case 2:46,XX, nuc ish(DXZ1x1)[6]/(DXZ1x2.SRYx1)[243]/(DXZ1x3.SRYx2)[4].

elsewhere (Pinkel et al., 1998). DNA samples of Case 4 and Case 9 were inadequate; therefore, we could not perform array-CGH analysis to two of these patients. Human male genomic DNA was used as reference DNA for the array-CGH analysis. Labelling with Cy3 and Cy5, washing and scanning steps were performed following a standard protocol. Data extraction was carried out using Agilent Feature Extraction Software, and the data analysis was assessed on Agilent Cytogenomics Software (v.2.0.6.0; Agilent Technologies). Data included imbalances with at least three consecutive probes with abnormal log2 ratios. The probe sequences and gene annotations were based on GRCh37/hg19 assembly

Karyotype results, FISH and array-CGH analyses were described according to the International System for Human Cytogenetic Nomenclature 2016.

2.6 | X chromosome inactivation analysis

The polymorphic region of exon 1 of the androgen receptor (AR) gene was used to assess the XCI patterns of the patients. The genomic DNA was cleaved using methylation-sensitive restriction enzyme *Hpa*II (MBI Fermentas) which digests the active X chromosome (unmethylated) fragments. After amplification of AR gene exon 1 using PCR, amplicons were separated on 8% denaturing acrylamide: bisacrylamide (29:1) gel for 4 hr. Then, the densitometric analysis of the alleles was carried out with MultiAnalyst version 1.1 software (Bio-Rad; Gunes et al., 2013).

2.7 | Data analysis

Data were analysed using SPSS Statistics Version 22.0. Results were expressed as mean and standard deviation (SD) for continuous variables. Standard deviation scores (SDS) of height and weight were calculated using the following formula:

$$\text{SDS} = (\text{observed value} - \text{median value of the reference population}) / \text{standard deviation value of reference population}$$

3 | RESULTS

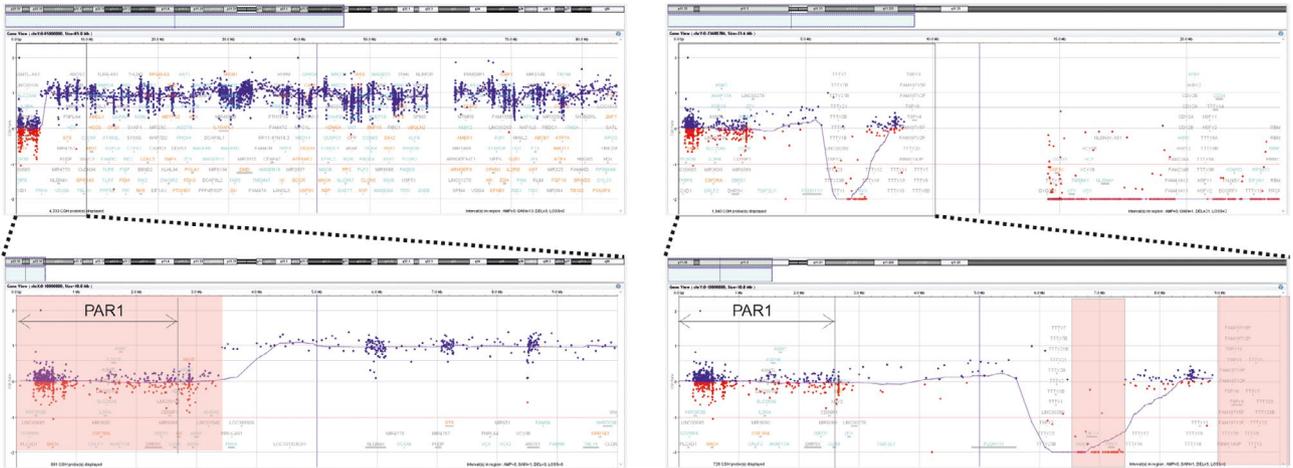
3.1 | Clinical findings

Nine male patients were diagnosed with SRY-positive 46,XX TDSD. All cases demonstrated male phenotype with similar clinical findings, including small testes, hypergonadotropic hypogonadism and pellet-negative azoospermia. Clinical and laboratory findings of our six new patients and three of our previously published patients were presented in Table 1. Mean \pm SD height of the patients was calculated to be 164.78 ± 8.0 cm, and these mean adult heights are apparently shorter than the normal adult Turkish men (164.78 ± 8.0 vs. 174.3 ± 4.9 cm) (Aslan et al., 2011; Soylemez et al., 2012). Five out of 9 (55%) patients had decreased secondary sex characteristics.

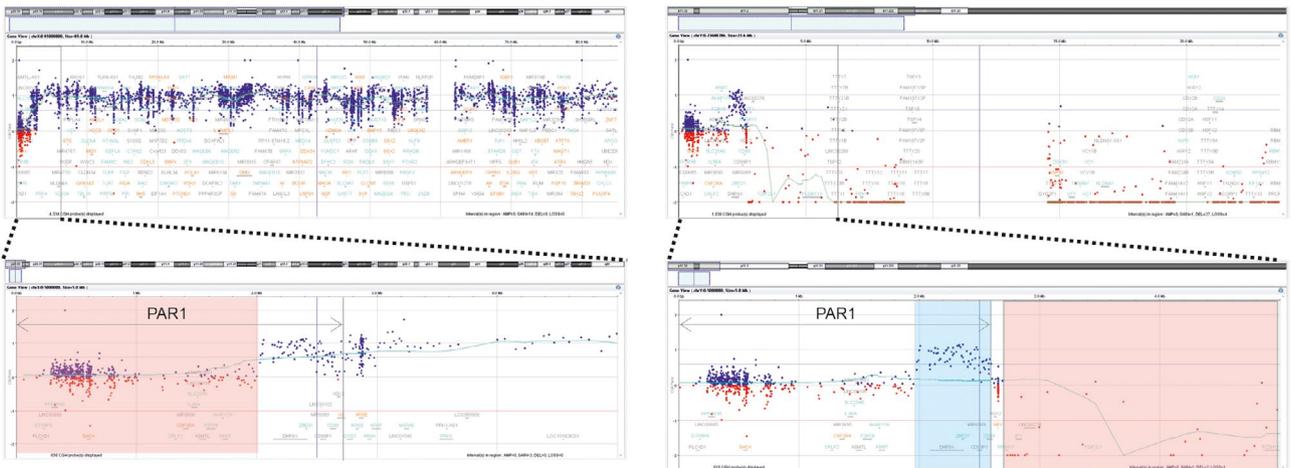
X chromosome

Y chromosome

Case 1, 5, 6, 7 & 8



Case 2



Case 3

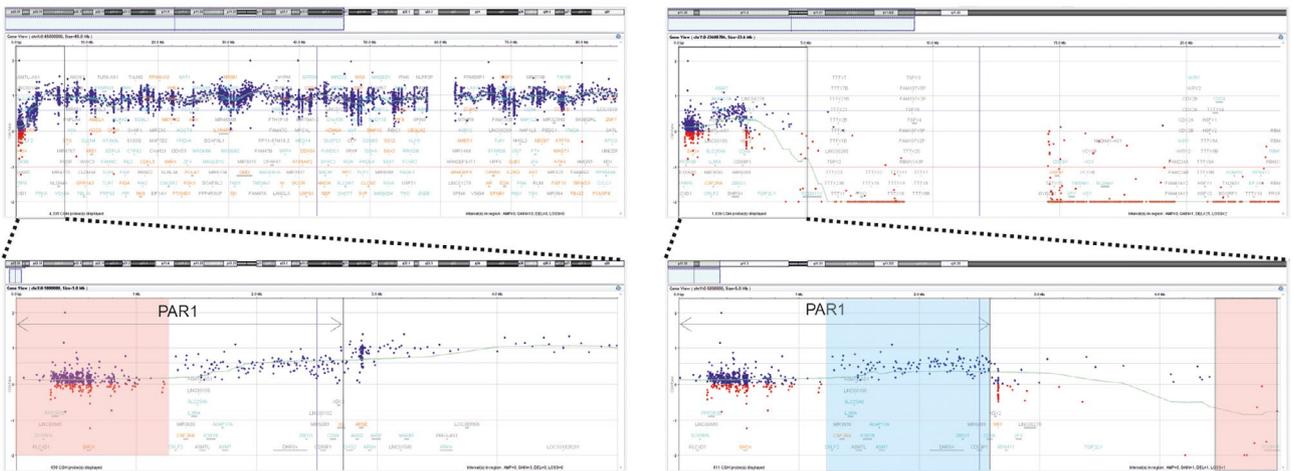


FIGURE 1 Representative results of the array CGH analysis. The segments highlighted with red and blue rectangles represent the decreased and increased copy numbers, respectively. PAR1 regions of X and Y chromosomes are indicated with the lines.

Stretched penile length mean \pm SD of our patients was found to be 12.39 ± 1.3 cm.

Additionally, clinical follow-up of our three previously published cases (Case 7–9) was included in the present study. Case 9 has been diagnosed 12 years ago. After his diagnosis with 46,XX TSD, he started to receive intermittent testosterone replacement treatment. His height increased 12 cm after starting of testosterone replacement therapy. He is still single, and his gynaecomastia increased depending on the testosterone treatment. His erectile function is normal with testosterone treatment. Case 8 is still single as well and his erectile function, libido and ejaculation are normal under testosterone therapy. He received antidepressant therapy for a time to overcome the depression symptoms. Case 7 is still married, and his sexual functions are normal.

3.2 | Karyotype and FISH analysis

Six out of nine patients revealed 46,XX karyotype and the remaining three showed 46,X,der(X)t(X;Y)(p22.3;p11.3) karyotype. The karyotype of one out of these three patients with derivative X chromosome demonstrated low level somatic chromosomal mosaicism [46,X,der(X)(29)/47,X,der(X),der(X)(1)]. FISH analysis of this patient showed that *SRY* gene region was translocated on the distal terminal of the short arm of one of the X chromosomes and mosaicism of 45,X/46,X,der(X)/47,X,der(X),der(X) cell lines. Low level of 45,X-bearing cell lines detected by cytogenetic analysis in this patient, but could not be in FISH analysis (Table 2).

3.3 | Array-CGH analysis

Array-CGH analysis findings of seven out of nine patients were presented in Figure 1, and these results are summarised in Table 2. Five out of seven patients had two copies of the pseudoautosomal

region 1 (PAR1), an approximately 1.0 Mb deletion in X-differential region with breakpoints on protein kinase X (*PRKX*), gene and ~7 Mb deletion in Yp-differential region, including an ~0.6-Mb interstitial deletion with the breakpoint on protein kinase Y (*PRKY*). These findings were compatible with an Yp to Xp translocation resulting from a common ~3.5 Mb paracentric Yp inversion. The remaining two patients had three copies of the proximal and two copies of the distal part of the PAR1, indicating Xp terminal deletion in the PAR1 region and presence of the Yp terminal segments including PAR1 region and a very distal portion of Y-differential region. These results were in concordance with the Xp;Yp translocation associated with the breakpoints inside of the X-PAR1 and inside of the Y-differential region (Figure 2). Additionally, array-CGH analysis also revealed the absence of AZFa, AZFb and AZFc in the patients.

3.4 | XCI analysis

The methylation analysis of the *AR* gene yielded random and skewed XCI in five patients in one patient respectively (Figure 3). A single polymorphic allele was observed in exon 1 of *AR* gene for Case 1, Case 2 and Case 5; therefore, the XCI analyses of these patients were accepted noninformative. These findings could be explained by the presence of two alleles with the same repeat number or alleles do not amplify under the same conditions (Table 2).

4 | DISCUSSION

In the present study, seven of our patients applied to our clinic with infertility complaints, one with intermittent scrotal pain in adulthood and one with hypogonadism in puberty. All our patients had hypogonadism and azoospermia with 46,XX or 46,X,der(X) karyotypes with translocated *SRY* locus onto Xp. None of them had genital ambiguity consistent with literature data.

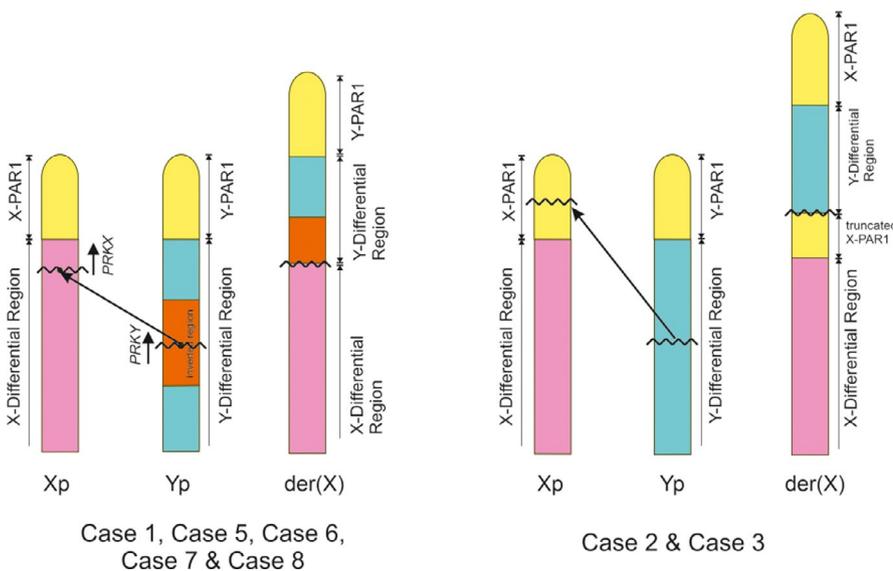


FIGURE 2 Schematic representation of the aberrations. The yellow, the pink, the blue and the green segments denote the PAR1, the X-differential region, the Y-differential region and the Y-differential inverted region, respectively.

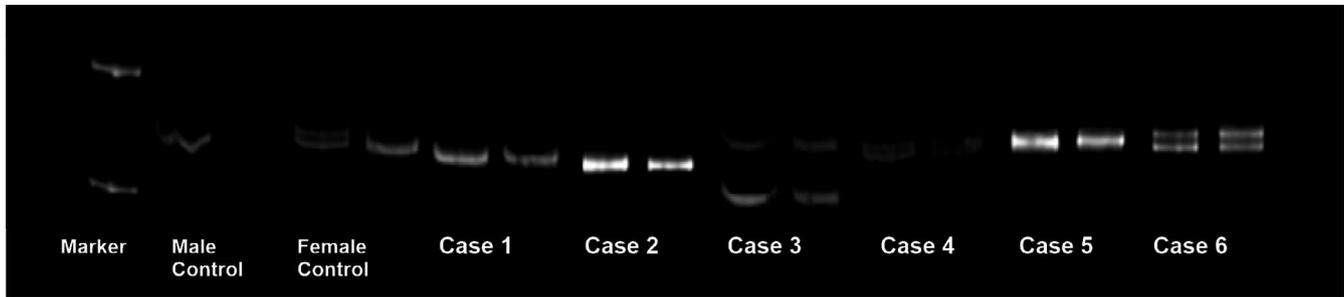


FIGURE 3 XCI patterns of Cases 1–6, PCR products of undigested and *HpaII*-digested DNA from peripheral blood. Line 1: marker 242- and 331-bp fragments are visible; line 2 and 3: normal male control; line 4 and 5: normal female control; line 5 and 6: case 1; line 6–17 shows the results of the cases as the first line is ‘undigested’ and the second line as ‘*HpaII*-digested’ DNA for each patient.

Testosterone synthesis was found to be impaired and decreased with an increase in FSH and LH levels after puberty (Anik, Catli, Abaci, & Bober, 2013; Vorona, Zitzmann, Gromoll, Schuring, & Nieschlag, 2007). It was proposed that testosterone levels may be normal during adolescence but decreased in adulthood (Velasco, Savarese, Sandorfi, Jimenez, & Jabbour, 2011). Additionally, the testosterone-to-oestradiol ratio was observed to be related to gynaecomastia, and poor axillary and pubic hair after puberty. Variations were observed in the secondary sexual characteristics among the patients. These variations have been suggested to be associated with the length of the translocated *SRY* gene region (Nakashima et al., 2014; Sharp et al., 2005). However, we have not found any significant correlation between the length of translocated *SRY* region and secondary sexual characteristics. Testosterone replacement therapy has been reported to cause the gynaecomastia as a result of the aromatisation of exogenous androgen (Rhoden & Morgentaler, 2004). Therefore, the increase in gynaecomastia with testosterone replacement treatment in Case 9 may be explained with the aromatisation of exogenous androgen. We did not observe under-masculinised external genitalia among the patients, but the stretched penile length mean of our patients was found to be slightly shorter than normal healthy Turkish men (12.39 ± 1.3 vs. 13.7 ± 1.6 and 13.98 ± 1.6) (Aslan et al., 2011; Soylemez et al., 2012).

One of the characteristic clinical findings of the patients was short stature which is compatible with the literature. The mean height of our patients was apparently shorter than the mean height of normal adult Turkish men (164.78 ± 8.0 cm vs. 173.2 cm, 174.79 ± 5.44 , 174.3 ± 4.9) (Aslan et al., 2011; Ozer, 2008; Soylemez et al., 2012), and the height of the patients has closely been resembled the mean height Turkish women (164.78 ± 8.0 cm vs. 158.9 ± 6.4 cm and 161.4 cm) (Ozer, 2008). The impaired testosterone-driven pubertal growth is proposed to be the primary cause of short stature in these patients. Case 9 had been diagnosed at puberty, although his height increased 12 cm with the testosterone replacement therapy, he still is shorter than normal adult Turkish men (162.0 cm vs. 173.2 cm, 174.79 ± 5.44 , 174.3 ± 4.9) (Aslan et al., 2011; Ozer, 2008; Soylemez et al., 2012). Despite the fact that the patients with Klinefelter syndrome also show both hypogonadism and testosterone deficiency, they present tall stature compared to those of *SRY*-positive 46,XX

TSD patients (Kirsch, Weiss, Zumbach, & Rappold, 2004). This discrepancy could partly be explained by the absence of Y chromosome material except *SRY* gene in these patients. Therefore, the Y chromosome could be suggested to play a role in the control of growth and development of men and have an impact on the male stature. Furthermore, it has also been hypothesised that the expression of short stature homeobox (*SHOX*) gene has an impact on the growth of these patients (Rappold et al., 2002).

SRY-positive 46,XX TSD results from translocation of Yp to Xp with various mechanisms and several breakpoint locations. The array-CGH analysis showed two main mechanisms leading to Xp;Yp translocations in our patients. The first mechanism is NAHR, which occurs between the homologous sequences of short arms of X- and Y chromosomes, and the second one is replication-based mechanisms takes place between the nonhomologous sequences. NAHR causes to Xp;Yp translocation in Cases 1, 5, 6, 7 and 8. The most common paracentric inversion of the Yp, about 3.5-Mb length, results in the displacement of *PRKY* and *PRKX* genes in the same direction. These genes are located outside of the PAR1 of both in X- and Y chromosomes and show a high sequence similarity, but they are orientated in two opposite directions. Therefore, the homology of these sequences may lead to the crossover errors between the X- and Y chromosomes and creates an ectopic recombination during paternal meiosis (Jobling et al., 1998; Nakashima et al., 2014; Wang et al., 1995). Replication-based mechanism seems to be responsible for the translocations between the nonhomologous parts of Xp and Yp in Case 2 and Case 3. Uniform translocations of the terminal part of the Yp-differential region to Xp-PAR1 have been reported previously (Jobling et al., 1998; Nakashima et al., 2014; Rouyer, Simmler, Page, & Weissenbach, 1987; Sharp et al., 2005). The fork stalling, template switching, microhomology-mediated break-induced replication and nonhomologous end-joining (NHEJ) mechanisms could be the cause of such translocations and deletions (Giglio et al., 2002; Nakashima et al., 2014; Simmons, Carvalho, & Lupski, 2012). There was no significant variation between these mechanisms with respect to the clinical findings of the patients. Also, the loss of very distal part of Xp-differential region in Cases 1, 5, 6, 7, 8 and duplication of the proximal PAR1 in Case 2 and Case 3 seem to have no significant effect on the clinical picture.

The results of the XCI analysis of patients with 46,XX TSD are controversial in the literature. Although some authors found skewed XCI patterns among these patients, the others could not confirm these results (Bouayed Abdelmoula et al., 2003; Gunes et al., 2013; Nakashima et al., 2014; Vorona et al., 2007). Despite one of our patients had skewed XCI pattern, the remaining five patients had random XCI pattern in the present study. The patient with skewed XCI pattern had paracentric Yp inversion-mediated Xp;Yp translocation. There were no significant differences between the patients with skewed XCI and the others based on clinical findings, especially regarding to the external genitalia. It has been proposed that XCI pattern is essential for the formation of external genitalia. Nakashima et al. (2014) reported that patients with random XCI had a normal male external genitalia; however, the patients with skewed XCI pattern that lead to inactivation of the expression of derivative X chromosome-bearing SRY gene cause to hypogonadism including micropenis, microphallus, cryptorchidism and penoscrotal hypospadias. Our single patient with skewed XCI pattern had normal external male genitalia. However, we could not be able to identify whether the derivative X chromosome was inactivated. Additionally, no correlation was found between the XCI status and the mechanism of translocation among our patients. These data are consistent with the findings of Nakashima and colleagues (Nakashima et al., 2014).

Case 8 demonstrated low level of X-chromosomal mosaicism, 46,X,der(X)/47,X,der(X),der(X)/45,X, and the clinical picture was not distinct from the diploid cases. X-chromosomal mosaicism in SRY-positive 46,XX TSD has been reported in the literature previously, and mitotic nondisjunction has been suggested to be responsible for mosaicism (Chernykh et al., 2009; Macia Bobes, Alonso Troncoso, Botas Cervero, Castano Fernandez, & Fau Cubero, 2002). Patients with mosaicism have a clinical variability ranging from the true hermaphrodites to complete masculinisation based on the proportion of cell lines (Chernykh et al., 2009). In our patient, the 46,XX cell line was predominant, and very low level of 45,X and 47,X,der(X),der(X) mosaicism was detected and having complete masculinisation is consistent with the previous studies.

5 | CONCLUSION

In brief, this study summarises the clinical and molecular data of a rare but important cause of male infertility. This report serves us to delineate the complex structure of the Xp;Yp translocations caused by NAHR or replication-based mechanisms and the outcomes of the derived chromosome. Further studies on this clinically and genetically heterogeneous disorder could clarify the genomic basis of Xp;Yp translocations in 46,XX TSD and their refined consequences. Finally, testicular sperm extraction is not recommended in these patients and adoption or in vitro fertilisation with a sperm donor should be considered.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Omer Salih Akar  <https://orcid.org/0000-0001-5686-2185>
 Sezgin Gunes  <https://orcid.org/0000-0002-3103-6482>
 Ummet Abur  <https://orcid.org/0000-0002-4811-9321>
 Engin Altundag  <https://orcid.org/0000-0001-8841-1426>
 Ramazan Asci  <https://orcid.org/0000-0002-2119-8963>
 Onur Emre Onat  <https://orcid.org/0000-0002-7105-1572>
 Tayfun Ozcelik  <https://orcid.org/0000-0001-5937-1082>
 Gonul Ogur  <https://orcid.org/0000-0002-9944-4423>

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