Advancements in Genetic Engineering
ISSN: 2169-0111
OPEN ACCESS Freely available online
Research Article

Cytogenetic Profiles of a Large Cohort of Patients with Sexual Developmental Disorders; a 22-Year Single-Center Experience

Osman Demirhan*

Department of Medical Biology, Çukurova University, Adana, Turkey

ABSTRACT

Although Disorder of Sexual Developmental (DSD) is rare, complementary clinical, genetic and hormonal diagnoses are needed for the diagnosis of a baby born with ambiguous genitalia or uncertain whether it is a boy or a girl. This is the largest retrospective cytogenetic study of DSD patients in Turkey. The aim of this study is to determine the frequency and structure of Chromosomal Abnormalities (CAs) seen in patients with the clinical spectrum of Ambiguous Genitalia (AG), Hypogonadism (HG), Intersex (IS), Hypospadias (HS), Testicular Feminization (TF) and Vaginal Hypoplasia (VH) between 1990 and 2012. Chromosome analysis was performed on 117 patients who applied to our laboratory with the complaint of DSD. For this, heparinized peripheral blood samples were cultured and analyzed according to standard cytogenetic methods. Percentage rates of all patients having AG, HG, HS, IS, TF and VH irregularities were 53.8%, 27.4%, 8.5%, 5.1%, 3.4% and 1.7%, respectively. Of the patients, 64.9% had normal karyotype and 35.1% had abnormal chromosome setup. In 17 (15.3% of all) patients, the phenotypic sex did not match with the genotypic sex (46, XX; 46, XY). Sex-chromosome mismatch chimerism was found in 7 patients (6.0%) (46, XX/46, XY chimeric individuals). Sixteen (13.7% of all) patients had mosaicism of the sex chromosomes. Structural abnormalities were found in gonosomal and autosomal chromosomes in 8 patients (6.3%). Our current findings have shown that CAs play a role in approximately 39% of patients with DSD, and those patients with mosaic karyotype may actually have chimerism and it is difficult to predict the clinical outcome in these patients. Additional molecular and hormonal techniques should be applied in patients with genotype-phenotype mismatch.

Keywords: Chromosomal aberrations; Chimerism; Ambiguous Genitalia (AG); Hypogonadism (HG); Intersex (IS); Hipospadias (HS); Testicular Feminization (TF); Vaginal Hypoplasia (VH)

INTRODUCTION

DSD are a group of congenital disorders affecting the genitourinary system, usually involving the endocrine and reproductive systems. The genetic mechanisms controlling sex determination and differentiation are extremely complex. DSD disorders cause a wide variety of phenotypes and may have a common genetic etiology. DSD can also be described as congenital conditions characterized by atypical development of chromosomal, gonadal, or anatomical sex (mismatches in chromosomal, gonadal, and phenotypic sex determination processes). While most DSD phenotypes are rare, the genetic mechanisms controlling sex determination and differentiation are also extremely complex. CAs are more common in groups of patients with sexual ambiguity. CAs, such as a missing sex chromosome or an extra one, also can cause AG. Depending on the karyotype, DSD individuals are divided into three main types as 46XX, 46XY and mixed sex chromosomes [1]. In some cases, the cause of AG may not be determined. AG or mixed gonadal dysgenesis in a newborn baby is very distressing for the parents and determining the sex is very difficult for the managing medical team. Genetic testing can reveal changes that can cause disease. Therefore, rapid and precise diagnosis is essential, and a rigorous procedure for determining etiology will have to be implemented. The aim of this study is to retrospectively evaluate the frequency and types of CAs in patients with DSD.

MATERIALS AND METHODS

This retrospective study was conducted to evaluate the cytogenetic analyzes of 117 patients who were referred to the Çukurova University, Faculty of Medicine, Department of Medical Biology and Genetics, Cytogenetics Laboratory. Sex anomaly diagnoses such as AG, HG, IS, HS, TF and GH were made in the neonatal, pediatric, urology and obstetrics clinics between 1990 and 2012. The age of the analyzed population ranged between newborn-44 years and the average age was 9 years. The sex ratio (male/female) was 1.04. Sixty-seven of these patients were children (85%). None of them had environmental exposure, radiation exposure, or prescription

Correspondence to: Osman Demirhan, Department of Medical Biology, Çukurova University, Adana, Turkey, E-mail: osdemir@cu.edu.tr

Received: 24-Feb-2023, Manuscript No. MAGE-23-22096; **Editor assigned:** 28-Feb-2023, Pre QC No. MAGE-23-22096 (PQ); **Reviewed:** 14-Mar-2023, QC No. MAGE-23-22096; **Revised:** 21-Mar-2023, Manuscript No. MAGE-23-22096 (R); **Published:** 28-Mar-2023. DOI: 10.35248/2169-0111.23.12.208.

Citation: Demirhan O (2023) Cytogenetic Profiles of a Large Cohort of Patients with Sexual Developmental Disorders; a 22-Year Single-Center Experience. Advac Genet Eng.12:208.

drug usage that could account for their DSD. Informed consent was taken from the patients and donors prior to collection of heparinised blood samples. Heparinized peripheral blood samples were collected from patients for routine cytogenetic analysis, and lymphocyte cultures were established according to laboratory standardized technique. Lymphocyte cultures were collected and slides were prepared following the conventional procedure. The karyotype analysis was performed by GTG-banding according to standard methods. At least 30 cells were routinely analyzed; in cases of mosaicism, this number was increased to approximately 100 metaphases. The karyotypic descriptions were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) recommendations.

RESULTS

The distribution of all patients according to their clinical diagnosis, age, phenotypic sex and chromosomal sex is shown in Table 1. Percentage rates of 117 patients having AG, HG, HS, IS, TF and VH irregularities were 53.8%, 27.4%, 8.5%, 5.1%, 3.4% and 1.7%, respectively. Of the patients, 64.9% had normal karyotype and 35.1% had abnormal chromosome setup. Of all abnormalities, 92.9% are numerical, 7.1% are structural CAs. CAs were found in 38.1% (24/63) of patients with AG, 21.9% (7/32) of patients with HG, 50.0% (5/10) of patients with IS and 33.3% (2/6) of patients with HS. Any CA was found in one of 4 patients with TF, and one of 2 patients with VH. Phenotypic sex and genotypic sex did not match in 17 patients (15.3%). The phenotypic sex was not specified in 6 (5.1%) newborns. Sex chromosome mismatch chimerism was detected in seven patients (8.2%). Y chromosome rearrangement was reported in five patients and X chromosome rearrangement in 2 patients (2.4%). Both gonosomal and autosomal structural irregularities were detected in six patients (7.1%). Sex chromosome mismatch chimerism was detected in seven patients (5.9%). Sixteen (13.7% of all) patients had mosaicism of the sex chromosomes and one patient had 47, XXY karyotype. Y and X chromosome structural rearrangements were detected separately in 3 (2.6%) patients. Structural irregularities were detected in autosomal chromosomes in 3 patients (2.6%). In 17 (15.3% of all) patients, the phenotypic sex did not match with the genotypic sex (46,XX; 46,XY). In addition, despite detection of the genotypic sex in 4 newborns, the phenotypic sex cannot be determined in those cases. Therefore, genotype-phenotype correlations could not be made. Sex-chromosome mismatch chimerism was found in 7 patients (6.0%) (46,XX/46,XY chimeric individuals); 46,XX/46,XY; 46,XY/46,XX/46,Xi(Xp); 46,XY/46,XX; 46,XY/46,XX; 46,XX/46,XY/45,X; 46,XX/46,XY; 46,XY/46,XX. Y-chromosome numerical rearrangement in five patients (4.3%) [45,X/46,XY; 45,X/46,XY; 45,X/46,XY; 45,X/46,XY; 45,X/46,XY] and X-chromosome numerical rearrangements were reported in 2 patients (1.7%) [46,XX/45,X; 45,XX/45,X]. Structural abnormalities were found in gonosomal and autosomal chromosomes in 8 patients (6.3%) [46,XY/46,XX/46,Xi(Xp); 46,XYqh+, 22s+; 46,XY,rob(15;14); 46,X,del(Yp); 46,X,iso(Xq); 45,X/46,X,izo(Xq),dup(Xq11-q13); 46,XY, Inversion 9 (inv 9).

Table 1: Distribution of patients according to their clinical diagnosis, age, phenotypic sex and chromosomal sex.

Case no.	Clinical information	Age	Phenotypical sex	Chromosomal sex
1	Ambiguous genitalia	4 years	M	45,X/46,XY
2	Ambiguous genitalia	9 years	F	45,X/46,XX
3	Ambiguous genitalia	27 days	M	46,XX/46,XY
4	Ambiguous genitalia	1 year	M	46,XY/46,XX/46,Xi(Xp)
5	Ambiguous genitalia	3 years	M	45,X/46,XY
6	Ambiguous genitalia	2 years	M	46,XY
7	Ambiguous genitalia	10 years	F	46, XX
8	Ambiguous genitalia	42 years	F	46, XX
9	Ambiguous genitalia	44 years	M	46,XY
10	Ambiguous genitalia	12 years	F	46, XX
11	Ambiguous genitalia	18 years	M	46,XY
12	Ambiguous genitalia	4 months	F	46, XX
13	Ambiguous genitalia	11 months	M	46,XY
14	Ambiguous genitalia	18 years	F	46, XX
15	Ambiguous genitalia	20 days	F	46,XY/46, XX
16	Ambiguous genitalia	24 years	M	46,XY
17	Ambiguous genitalia	1 year	F*	46,XY
18	Ambiguous genitalia	3 months	M	46,XY
19	Ambiguous genitalia	24 years	M	46,XYqh+, 22s+
20	Ambiguous genitalia	5 years	F	46, XX
21	Ambiguous genitalia	5 years	M	46,XY
22	Ambiguous genitalia	2 years	F*	46,XY

23	Ambiguous genitalia	22 years	M	46,XY
24	Ambiguous genitalia	4 years	F*	46,XY
25	Ambiguous genitalia	3 years	M	46,XY
26	Ambiguous genitalia	15 years	M	46,XY
27	Ambiguous genitalia	1 month	F*	46,XY
28	Ambiguous genitalia	8 months	M	46,XY
29	Ambiguous genitalia	8 days	F*	46,XY
30	Ambiguous genitalia	3 years	F	46, XX
31	Ambiguous genitalia	28 years	F	46, XX
32	Ambiguous genitalia	38 years	F*	46,XY
33	Ambiguous genitalia	16 years	F	46,XY/46, XX
34	Ambiguous genitalia	2 months	M	46,XY
35	Ambiguous genitalia	1 year	F	46,XX
36	Ambiguous genitalia	3 years	M	46,XY
37	Ambiguous genitalia	41 years	M	45,X/46, XX/46,XY
38	Ambiguous genitalia	2 months	F	46,XX
39	Ambiguous genitalia	16 days	F	46,XX
40	Ambiguous genitalia	3 days	?	46,XX
41	Ambiguous genitalia	4 years	M	46,XY
42	Ambiguous genitalia	5 years	F*	46,XY
43	Ambiguous genitalia	5 days	?	46,XX
44	Ambiguous genitalia	7 days	M*	46,XX
45	Ambiguous genitalia	5 months	M	46,XY
46	Ambiguous genitalia	4 months	F	46,XX
47	Ambiguous genitalia	1 day	?	46,XX
48	Ambiguous genitalia	8 months	M	45,X/46,XY
49	Ambiguous genitalia	8 years	M	46,XY
50	Ambiguous genitalia	4 years	F*	46,XY
51	Ambiguous genitalia	2 months	F*	46,XY
52	Ambiguous genitalia	6 months	M	46,XY
53	Ambiguous genitalia	1 day	?	46,XX
54	Ambiguous genitalia	4 months	F	46,XY
55	Ambiguous genitalia	24 years	M	46,XY
56	Ambiguous genitalia	1 years	M*	46,XX
57	Ambiguous genitalia	23 years	F	46,XX
58	Ambiguous genitalia	16 years	F	46,XX
59	Ambiguous genitalia	7 days	?	46,XY
60	Ambiguous genitalia	8 days	F	46,XX
61	Ambiguous genitalia	25 years	F*	46,XY
62	Ambiguous genitalia	30 years	M	46,XY/46, XX
63	Ambiguous genitalia+cancer	15 months	M	46,XY/46, XX
64	Hypogonadism	9 years	M	46,XY, rob(15;14)
65	Hypogonadism	11 years	M	46,XY
66	Hypogonadism	22 years	F	46,XX
67	Hypogonadism	26 years	M	46,XY
68	Hypogonadism	25 years	F	46,XX

69	Hypogonadism	21 years	F*	46,X, del(Yp)
70	Hypogonadism	9 months	M	46,XY
71	Hypogonadism	11 years	M	46,XY
72	Hypogonadism	6 years	M	46,XY
73	Hypogonadism	6 months	M	46,XY
74	Hypogonadism	33 years	F	46,X, izo(Xq)
75	Hypogonadism	15 years	M	46,XY
76	Hypogonadism	18 years	M	46,XY
77	Hypogonadism	16 years	F	46,XX
78	Hypogonadism	10 years	M*	46,XX
79	Hypogonadism	21 years	M	46,XY
80	Hypogonadism	1 year	M	46,XY
81	Hypogonadism	4 years	F	46,XX
82	Hypogonadism	13 years	M	46,XY
83	Hypogonadism	14 years	M	46,XY
84	Hypogonadism	27 years	F	45, X/46, X, izo(Xq), dup(Xq11-q13)
85	Hypogonadism	17 months	M	46,XY
86	Hypogonadism	3 months	M	46,XY
87	Hypogonadism	16 years	M	46,XY
88	Hypogonadism	16 years	F	46,XX
89	Hypogonadism	3 years	M	46,XY
90	Hypogonadism	35 years	F	46,XX
91	Hypogonadism	3 months	M	46,XY
92	Hypogonadism	5 years	F	46,XX
93	Hypogonadism	12 years	F	46,XX
94	Hypogonadism	18 years	F	46,XX
95	Hypogonadism	16 years	M	47,XXY
96	Intersex	6 years	M	46,XYqh+
97	Intersex	2 years	M	46,XY
98	Intersex	7 days	M	45,X/46,XY
99	Intersex	3 year	F*	46,XY
100	Intersex	30 years	M	46,XY/46,XX
101	Intersex	19 months	M	46,XY
102	Intersex	22 months	F*	46,XY
103	Intersex	1 year	M	46,XY
104	Intersex	8 days	?	46,XX
105	Intersex	10 months	M	46,XY
106	Hipospadias	12 years	M	45, X/46,XY
107	Hipospadias	9 years	M	46,XY, inv(9)(p12;q13)
108	Hipospadias	26 years	F	46,XX
109	Hipospadias	5 months	M	46,XY
110	Hipospadias	6 years	M	46,XY
111	Hipospadias	1 years	M	46,XY
112	Testicular feminization	14 years	F*	46,XY
113	Testicular feminization	29 years	F	46,XX

114	Testicular feminization	5 years	M	46,XY
115	Testicular feminization	2 years	M	46,XY
116	Vaginal hypoplasia	16 years	F	45,X/46,XX
117	Vaginal hypoplasia	17 years	F	46,XX

Note: *Those whose phenotypic and genotypic sex do not match

DISCUSSION

In the present study, the sex ratios (male/female ratio) of the patients were equal. This shows that sex irregularities do not show phenotypic sex discrimination and affect male and female sex equally. Of all patients, 64.9% had a normal karyotype, their genotype and phenotypic sex were compatible, but 35.1% had abnormal chromosomes (92.9% numerical and 7.1% structural). The condition in patients with a normal karyotype may be due to a change in genes and how the body responds to sex hormones or both. In addition, DSD may occur as a part of some syndromes, or it may occur due to imbalances affecting autosome or X-linked genes involved in the sex development mechanism [2].

Patients presenting with Ambiguous Genitalia (AG)

AG patients, who constitute the largest group (53.8%) of our patient population, are a heterogeneous group formed due to different chromosome damages. These account for over 50% of all cases of genital ambiguity in the neonatal period [3]. The incidence of mixed gonadal dysgenesis worldwide is 1:10,000, but varies considerably among different populations [4,5]. While it usually results in the presence of a missing or extra sex chromosome in patients with AG, the cause cannot be determined in some cases. On the other hand, 61.9% of our AG patients showed a normal karyotype, and CA was found in 38.1% of these patients. In a similar study conducted in Morocco, it was reported that 78.31% of patients with AG had normal karyotype, and 7.1% of patients had CAs [6]. In other studies, CA was reported to be 4.4% in Saudi Arabia, 10.1% in Oman and 13.6% in Turkey [7-9]. According to the results of these studies and the phenotypic sex ratios of our patients, the male and female ratios were very close to each other and the CAs ratio was very high. The difference in ratios obtained from different studies may have changed according to clinical diagnostic criteria and analysis methods. Unfortunately, we could not interpret the genetic sex of 5 newborns as the phenotypic sex was uncertain. At the same time, phenotypic sex and genotypic sex were not compatible in 19.0% of 12 patients with AG. Incompatibility of genotypic and phenotypic sex is a very rare condition. It is estimated that 1 in every 20,000 to 30,000 men has the karyotype XX [10,11]. It is known that chromosomal irregularities, hormonal deficiency, gene transfer and gene mutations are among the reasons leading to such cases. In some cases, the cause of AG may not be determined. A deficiency of male hormones in a genetic male fetus results in AG, while exposure to male hormones during development results in AG in a genetic female.

XX sex reversal is a rare congenital condition in which an individual with a XX karyotype has phenotypically male characteristics. Translocation and expression of the *SRY* gene into female individuals with XX results in male or indeterminate genitalia. Mutations in the *SRY* gene are identified in approximately 15% XY females. Missense mutations lead to XY gonadal dysgenesis. Evaluation of those who are positive for *SRY* is more difficult due to the multitude of various etiologies. Congenital adrenal hyperplasia can also lead to a deficient XY fetus. Etiologies include 17 alpha-hydroxylase, 3 beta-hydroxysteroid dehydrogenase, steroid acute regulatory protein, P450 oxidoreductase, P450 side chain degrading enzyme deficiency. Other individuals can be identified by measuring the concentration of the Anti-Mullerian Hormone (AMH). *SRY* negative XX males, which may be caused by a mutation in the autosomal or X chromosomal gene, are less common and masculinization of XX males is variable [12]. The appearance of XX men can be men with normal internal and external genitalia, men with external ambiguities, and men with both internal and external genital ambiguities. Most XX men have small testicles, have an increased number of undescended testicles compared to normal XY men or the urethra opening on the underside of the penis (hypospadias). The degree of development of the male phenotype in XX males varies even among *SRY*-positive individuals. The sometimes the presence of the *SRY* gene may cause ambiguities in the internal and/or external genitalia [13]. It is thought that X inactivation in XX men may explain the genital ambiguities and incomplete masculinization seen in *SRY*-positive XX men [13,14]. The masculinization of *SRY*-negative XX males depends on which genes are mutated and at what stage of development these mutations occur [15]. Some XX males do not have the *SRY* gene and the male phenotype may be caused by another gene on one of the autosomal chromosomes.

Fertilization errors can result in the formation of more than one cell line (mosaicism) in an individual. We found different sex chromosome mosaicism in 11 (17.5%) patients with AG. However, 5 patients (5.9%) with AG, HG and IS had 46,XY/45,X karyotype and one patient (1.2%) had 45,X/46,XX karyotype. The 46,XY/45,X karyotype is the most common karyotype in newborn children with mixed gonadal dysgenesis. Most of these patients have dysgenetic testes, contralateral striated gonads [16]. Its pathogenesis may possibly be related to the expression of a defective gene that controls testicular differentiation [17]. Most 45,X/46,XY mosaic patients with mixed gonadal dysgenesis also have a structural abnormality of the Y chromosome. An increase in the long arm of the Y chromosome was detected in one of our patients with AG. In other studies, 45,X mosaicism and dicentric Y chromosome were found in 25% of patients with mixed gonadal dysgenesis, and a high frequency of Y chromosome microdeletions were found in male patients with 45,X/46,XY mosaicism [18,19]. This mosaicism is an important genetic factor for spermatogenesis failure, and the most common sex CA found in infertile men. Y chromosome mosaicism/chimerism can result in a broad range of reproductive phenotypes. Males with mixed gonadal dysgenesis have varying degrees of under-masculinization and infertility [20]. In addition, these patients are at great risk for gonadal tumors. We reported 45,X/46,XX mosaicism in two (2.4%) female patients with AG and GH. In other studies, 45,X/46,XX mosaicism was found in 64.89% of patients with Turner syndrome. TS patients had mosaicism (45,X/46,XX) with a rate of 15%-65% [21,22].

Patients with mixed gonadal dysgenesis may show a normal female phenotype, TS, male with hypospadias, and male or female having signs of pseudohermaphrodism.

In the present study, we cytogenetically found an unexpected sex chromosome mismatch chimerism (46,XX/46,XY) in 7 (6.0%) phenotypically abnormal individuals presented with AG and IS complaints. But we could not perform molecular genetic analysis. We also reported both 46,XY/46,XX/46,Xi(Xp) chimerism and short-arm isochromosome of the X chromosome in a male patient. Mosaicisms have various physiological roles and pathological consequences. The cell lines of most such cases show sex chromosome incompatibility with the 46,XX/46,XY karyotype. Sex chromosome mismatched chimerism 46,XX/46,XY is associated with mosaicism or chimerism and is rarely found in people with a phenotypically normal appearance. Chimera is defined as the fusion of two different zygotes in an embryo, while mosaic is caused by a mitotic error in a single zygote. It is very difficult to distinguish these two genetic conditions. The physical findings of chimeric patients are very variable and can range from normal male or female genitalia to varying degrees of ambiguous genitalia. Chimeric individuals with sex chromosome mismatch have been described mostly because of gonadal dysplasia and hermaphroditism and have been reported to be responsible for approximately 13% of true hermaphroditism cases [23,24]. Same-sex chimeras (XX/XX and XY/XY tetragametik chimeras) are rarely detected because they are not usually sex-chromosome mismatches and do not usually show a clinical effect [25]. It is difficult to determine the exact incidence of these rare chimeric cases. The phenotypic spectrum of these patients can range from normal male/female external genitalia to AG of varying degrees, and these patients are often infertile.

The physical findings of patients with chimerism are very variable because there are many different mechanisms involved in its formation. Some mechanisms have been suggested for the origin of whole-body chimerism; these can be either in the form of postzygotic fusion of two independently fertilized oocytes (tetragametic), or fertilization involving gametes undergoing parthenogenetic division, or fertilization of an oocyte and its first or second polar body by two spermatozoa followed by fusion of two zygotes [26-30]. Moreover, a mosaic 46,XX/46,XY karyotype that originates from a single zygote may also arise from either a diploid (46,XY) or an aneuploid cell (47,XXY or 48,XXYY) [31]. Chimerism can therefore be distinguished from mosaicism by the extent of genotypic differences. In some patients, the potential chimerism could be identified on the basis of cytogenetic findings. In addition, it is possible to determine the mechanism of chimerism by analysis of the genotype patterns on the SNP array. Chimeric individuals with sex chromosome mismatch have been described mostly because of gonadal dysplasia and hermaphroditism and have been reported to be responsible for approximately 13% of true hermaphroditism cases [23,24]. It has been reported that 10%-33% of patients with ovotesticular sexual development disorder have 46,XX/46,XY karyotype. [23,32,33].

Y chromosome structural rearrangements are higher in newborns. In the present study, we reported an increase in the long arm of the Y chromosome in 2 male patients with AG and IS, and a short arm deletion of the Y chromosome in a female patient with HG. The structural aberrations of the Y chromosome are often associated with the Y chromosome aneuploidy. Deletions can often cause severe congenital anomalies, significant mental and physical disabilities. Since genes on the long arm of the Y chromosome are responsible for spermatogenesis, microdeletions are associated with the complete absence of spermatozoa and reduced sperm count [34,35]. AG may also be associated with autosomal abnormalities. We also found both sex and autosomal structural anomalies in one patient with AG [46,XYqh+,22s+]. Double CAs involving the sex chromosomes and an autosomal chromosome have been reported previously. Dispermic chimerism has been reported in two cases with structural CAs. One was a twin pregnancy in which the carrier of a balanced translocation t (14;20) gave birth to a stillborn dispermic male with chimerism [36]. We also described Robertsion translocation (14;15) in one of our patients with HG. One of our male patients with AG had gonadal cancer. Some disorders of sex development are associated with an increased risk of certain types of cancer. The expression of *SRY* in other somatic tissues such as adipose, esophagus, thymus, adrenal glands, kidneys, as well as in some cancer cell lines suggests that it has functions beyond sex determination [37,38]. Mixed gonadal dysgenesis and partial androgen insensitivity are associated with a higher oncologic risk. The highest risk of gonadal tumors is found in testicular-specific protein Y positive gonadal dysgenesis and partial androgen insensitivity syndrome with intra-abdominal gonads, while the lowest risk is found in ovotestis and complete androgen insensitivity syndrome [39]. Men with mixed gonadal dysgenesis are at risk for gonadal tumors.

Patients presenting with hypogonadism

Hypogonadism can occur as a result of either the testicles or ovaries not working properly, or problems with the pituitary and hypothalamus (when the sex glands produce little or no hormones). This condition is responsible for the signs and symptoms observed in both men and women [40]. The most common genetic disorders that cause primary HG are TS and Klinefelter Syndrome (KS). The deficiency of sex hormones may result in defective primary or secondary sexual development or withdrawal effects in adults. Clinical features of XX dysgenesis or XY dysgenesis are primary amenorrhea, scanty or absent pubic hair and absence of breast development. Both XX and XY gonadal dysgenesis can occur individually, they are genetically heterogeneous and in fact most cases remain unexplained. We found CAs in 3 (4.8%) patients with HG. Two patients had structural irregularities of the sex chromosome and one patient had an autosomal Robersonian translocation. One patient also had the KS karyotype. Long-armed isochromosome of the X chromosome [46,X,i(Xq)] was found in one of our patients who was referred to us with the suspicion of HG. Also, both long-arm isomer and long-arm duplication of X chromosome were found in a 45,X/46,XX mosaic female [45,X/46,X,iso(Xq),dup(Xq11-q13)]. This karyotype is the most common Turner variant, and one of the most observed features of this condition is haplo-insufficiency of the SHOX gene [41]. Rarely, 0.3%-0.9% of males with X chromosome polysomies or X-Y translocations cause KS with isochromosome Xqi(Xq) [42,43]. KS is also the most common cause of HG and infertility in males. We reported a rob (14;15) translocation in a patient with HG. Robertsonian translocations occur with a prevalence of about 1/1000 in the general population [30]. Patients with reciprocal translocations have a significantly increased risk of infertility and miscarriage due to unbalanced gamete formation. The most common Robertsonian translocation observed in infertile males is t (13q;14q). Other D/D Robertsonian translocations are much less frequent and rob (14;15) can be expected to have risks [30].

Patients presenting with intersex

Intersex is the condition of abnormally having both male and female characteristics of genital/sex organs and reproductive structures. In this disease, there is a disruption of the sex-determining steps, a mismatch between the appearance of the external genitalia and the internal sex organs or genetic sex (XX or XY). We found CAs in 6 (60.0%) of 10 patients diagnosed as IS. Three of these patients had phenotypic and genotypic gender mismatch. In the other 3 patients; Yq+ was found in one, mosaic 45,X/46,XY in one, and chimerism 46,XY/46,XX in one. The 46,XX/46,XY karyotype was found in 10%-33% of patients with ovotesticular sexual developmental disorders [32,33]. This is a genetically heterogeneous clinical condition in which the gonads reveal both ovarian and testicular tissue [32]. IS is evaluated in four categories as 46,XX IS, 46,XY IS, true gonadal IS, and complex/ambiguous IS. One of our patients had XX intersex and two of them had XY IS. An XX IS person has female sex chromosomes and female ovaries, but has external genitalia that look like a male. This is usually due to exposure of a female fetus to excess male hormones before birth. The XY IS person has male chromosomes, but the external reproductive organs are not fully formed, indistinct, or clearly female, and the testicles may be normal, defective, or absent. This condition is also referred to as with undervirilization. An IS person may appear to be female on the outside and be born with a mostly male anatomy inside or, as in our patients, it can be born with genitals that appear between normal male and female types. Sometimes, a person may be born with mosaic genetics, as in our two patients, some cells have XX chromosomes and some have XY chromosomes. Although IS is a congenital condition, its anatomy may not always be revealed at birth. Sometimes, the person is not found until they have IS anatomy, reach puberty, become a sterile adult, and die or have an autopsy performed. One of our patients was 30 years old and had just been discovered. In fact, some people unwittingly live and die with IS anatomy.

Patients presenting with hypospadias

Hypospadias is one of the most frequent genital malformations in the male newborn, affecting about one of every 250 males at birth and results from an abnormal penile and urethral development [44]. It is thought to be due to a combination of genetic, endocrine and environmental factors. In about 90% of cases, the urethral opening is located on or near the glans penis. In most cases, the foreskin is less developed and does not wrap completely around the penis, leaving the underside of the glans uncovered [45]. We found 45,X/46,XY mosaicism in one of the two patients with HS and inv(9)(p12;q13) in the other. It may occur as a result of mutations in the genes involved in the interactions between the mesenchyme and the urothelium. Mutations in SOX9, DMRT1 and GATA4 genes may be associated with disorders of male sex differentiation, including severe HS, often associated with testicular dysgenesis [46,47]. The duplicated SOX9 gene can lead to the formation of penoscrotal HS in patients with mosaicism of 46,XX and 46,XX dup(17q) [48]. However, the finding of 46,XX male HS patients with or without the *SRY* gene suggests the presence of other virilizing genes. Gonosomal abnormalities have been found in approximately 7% of patients with HS. These include Klinefelter syndrome, 47,XXY, 48,XXYY and some mosaics (such as 45,X/46,XY; 45,X/46,XYq-; 45,X/46,X,idic(Yp) and 45,X/69,XXY). Abnormal genital development in these patients may be related to the dosage effect of the *SRY* gene [49]. Autosomal dominant forms of syndromic HS are caused by mutations in genes involved in early genital development. It may also be associated with various CAs, including gonosomal mosaicism and autosomal deletions.

Since there is neither gain nor loss of genetic material in inversions, they are said to have no pathological significance. The relationship between the inv(9) variant and clinical pathologies is still unclear. Some researchers believe it is a normal variant, while others associate it with various pathologies such as infertility and bad obstetric history. In the present study, we found inv(9)(p12;q13) karyotype in one patient with HS, but we do not know whether this inversion is the true cause of this genetic anomaly. In a similar study, it was reported that inv(9)(p12;q13) causes HS, spontaneous abortion and infertility in couples [50]. Also, the most widely recognized reversal is 46,XY,inv(9)(p11;q13) in the oligozoospermic infertile male. Inversion 9(p12;q13) causes spontaneous abortions and couple infertility [51,52].

Patients presenting with testicular feminization

In the present study, one of the two patients with TF was phenotypically female but with XY genotype, and the other was female in phenotype and genotype. TF is a rare inherited disorder in phenotypically normal women with adequate breast development, normal external genitalia, a vagina of variable depth, an uterus, sparse or absent pubic and axillary hair. This syndrome occurs with a frequency of approximately one in 20,000-62,400 individuals and occurs due to hormonal resistance and androgen receptor dysfunction [53,54]. These patients have a male karyotype (XY) and negative sex chromatin. Undescended testicles can be intraabdominal, inguinal, or labial [55]. In most cases, XX or XY gonadal dysgenesis remains genetically unexplained. Although the absence of the *SRY* gene is known in approximately 20 percent of those cases with 46 XX testicular sex disorder, the cause of the disorder remains unknown in most of such patients. It has been reported that individuals with *SRY*-negative 46,XX testicular irregularity are more likely to have AG than *SRY*-positive individuals [56]. Female patients with XY have primary amenorrhea, sexual ambiguity, facial dysmorphism, or psychomotor retardation. The cause of gonadal dysgenesis in these patients may be either loss of *SRY* or a mutated *SRY* gene.

Patients presenting with vaginal hypoplasia

We detected 46,XX/45,X mosaicism in a female patient with VH. This anomaly is a birth defect characterized by an underdeveloped or reduced size vagina, and occurs in about 1 in 5,000 to 7,000 women. Although this condition is present at birth, it often remains undiagnosed until a teenager reaches puberty and realizes they are not menstruating. Indeed, our patient was a 16-year-old girl. Among them, the frequency of menstruation reaches 20%, but this rate does not exceed 3% in pure Turner patients [57]. Our patient did not show the Turner phenotype.

CONCLUSION

This is the most comprehensive study based on a retrospective analysis of the karyotype results of patients with DSD in Turkey. Our results confirm the importance of karyotype/phenotype correlations and also demonstrate the importance of cytogenetic and molecular genetic analyses in addition to performing detailed clinical evaluation and hormonal testing in confirmation of the diagnosis of DSD patients. When CA cannot be detected in such

patients, other possible causes should be investigated. Our results demonstrate the importance of considering genetic chimerism in patients with signs of multiple cell lines (mosaisizm) and the difficulty of estimating the severity of the phenotype in these patients. It shows that some patients with DSD may have the rare condition of XX/XY chimerism. The status of XX/XY chimeras can be fully elucidated by genotyping for exactly saying that these patients are tetragametic or parthenogenetic chimeras. Y chromosome microdeletions should be examined in male patients with 45,X/46,XY mosaicism.

ACKNOWLEDGEMENTS

Thanks to all my colleagues, Dr. Dilara Süleymanova and Dr. Nilgün Tanrıverdi for helping in different phases of this study. Also, I would like to thank the personnel of Medical Biology and Genetics Department Cytogenetic Laboratory at Çukurova University for their assistance and contribution.

AUTHOR CONTRIBUTIONS

Osman Demirhan conceived study, analyzed data, and wrote the paper.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

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