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Cytogenetic evidence of autosomal chromosomal aberrations in disorders of sex development from an Iraqi cohort

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Abstract

Background: Disorders of sex development (DSD) are congenital conditions involving atypical chromosomal, gonadal, or anatomical sex development. Although sex chromosome abnormalities are well recognized, autosomal alterations remain insufficiently studied, especially in underrepresented populations. Objective: This study aimed to describe cytogenetic findings related to clinical phenotypes, particularly autosomal rearrangements, in an Iraqi cohort with DSD. Methods: A total of 81 patients with clinically suspected DSD were subjected to cytogenetic analysis using standard G-banding techniques. Typically, 25 - 30 metaphases were analyzed per case whenever culture quality permitted, with additional metaphases examined when necessary. Chromosomal abnormalities were classified as numerical or structural and involved sex chromosomes and/or autosomes. Descriptive statistics were applied, and associations between chromosomal abnormalities and clinical phenotypes were explored.
Results: Chromosomal abnormalities were identified in 64% (53 / 81) of the patients. Both sex chromosomes and multiple autosomes were involved, with recurrent abnormalities observed mainly on chromosomes 19, 2, 5, 12, and 16. The short arm of chromosome 19 (19p) was the most frequently affected region. Notably, several patients exhibited discordance between their sex and chromosomal constitution, often in association with complex autosomal rearrangements. Autosomal abnormalities were most frequent in ambiguous genitalia and primary amenorrhea.
Conclusion: These findings suggest that autosomal chromosomal abnormalities contribute to the phenotypic diversity of DSD. Recurrent chromosome 19p involvement in female patients may indicate a broader role in sex development beyond male-specific pathways. Further molecular studies are needed. These results emphasize the importance of comprehensive cytogenetic evaluation beyond sex chromosomes in DSD patients.

Keywords
chromosomal abnormalities, chromosome 19, DSD, Iraqi patients, G-banding

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Introduction

Disorders of sex development (DSD) are a heterogeneous group of congenital conditions that manifest as unusual chromosomal, gonadal, or anatomical sex development[1,2]. They are characterized by high clinical and genetic variability, reflecting the molecular complexity of human sex determination and differentiation[3,4]. Despite much progress in terms of cytogenetics and molecular diagnostics, the underlying genetic mechanism remains unresolved in a significant proportion of DSD cases[4].

Historically, the genetics of DSD are largely attributed to sex chromosome abnormalities, especially alterations involving the Y chromosome and the sex-determining region Y (SRY) gene, as well as numerical aberrations of the X chromosome [5,6]. While these may account for some of the manifestations of DSD, increasing evidence suggests that autosomal genes play critical roles in regulating and modulating gonadal development, steroidogenesis, and phenotypic sex differentiation [7-9].

Consequently, structural or numerical rearrangements affecting autosomes may result in DSD phenotypes even in individuals with an apparently normal 46, XX or 46, XY karyotype.

Several autosomal chromosomes harbor genes with established roles in sex determination and differentiation, including HHAT on chromosome 1 [10,11], SRD5A2 on chromosome 2 [12], MAP3K1 on chromosome 5 [13,14], DMRT1/2 on chromosome 9 [15,16], AMHR2 on chromosome 12 [15], and SOX9 on chromosome 17 [15,17,18]. Because it contains the genes AMH and INSL3, which are essential for Müllerian duct regression and testicular descent, respectively, chromosome 19 is particularly noteworthy among these genes [15,19,20]. Therefore, disruption of this chromosomal region may contribute to the genotype-phenotype in DSDs and has significant implications for sex development.

Although the contribution of autosomal abnormalities to DSD is increasingly recognized , few studies have focused exclusively on autosomal chromosomal abnormalities, especially in underrepresented populations [9]. Insight into populations with specific genetic structure and genotype-phenotype associations is limited by the paucity of data from Middle Eastern groups, especially those in Iraq.

A thorough cytogenetic examination of Iraqi individuals with disorders of sex development was conducted in this study, paying particular attention to the prevalence and range of autosomal chromosomal abnormalities to describe cytogenetic findings in relation to clinical phenotypes and investigate the possible contribution of recurrent autosomal rearrangements, namely, the involvement of chromosome 19 in the pathophysiology of DSD.

Materials and Methods:

Cytogenetic analysis

Each sample was collected from peripheral blood in a heparinized tube (5 ml), after which the lymphocytes were cultured with PHA (0.1 ml) in RPMI media according to standard protocols. After being treated with KCL hypotonic solution (0.075) and fixed with glacial acetic acid and methanol solution (1:3), the metaphases were analyzed at a resolution of 450-550 bands per haploid set using Trypsin GTG banding. Cytogenetic abnormalities were considered clonal when observed in at least three metaphase cells [21]. All the karyotypes were independently reviewed by three experienced cytogeneticists and reported according to the International System for Human Cytogenetic Nomenclature (ISCN2020) [22]. The number of metaphases with each abnormality is indicated in brackets in the karyotype descriptions in Table 1. Imaging of metaphases and karyotyping were performed via CytoVision (Leica Microsystems Crop). Chromosomal abnormalities were categorized as numerical or structural and involved either sex chromosomes or autosomes.

The study of patients referred for karyotyping and the study protocol were approved by the scientific and ethics committee of the Iraqi Center for Cancer and Medical Genetics Research (No. 1 on January 25, 2023), and informed consent was obtained from all participants or their legal guardians prior to inclusion in the study. The clinical and morphological data of all patients were retrieved from patient medical records.

Results

A total of 81 patients diagnosed with disorders of sex development (DSD) were included in this study, comprising 61 females and 20 males, with ages ranging from 2 months to 27 years. All patients were referred by clinicians on the basis of the initial diagnosis of clinical manifestations suggestive of DSD, including ambiguous genitalia, primary amenorrhea (PA), secondary amenorrhea (SA), hypogonadism, Klinefelter syndrome, and Turner syndrome.

Cytogenetic analysis was successfully performed in 53 of the 81 patients and revealed a broad spectrum of chromosomal abnormalities affecting both autosomes and sex chromosomes. The remaining of the patients presented normal karyotypes. Structural and numerical aberrations were identified in chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 12, 14, 15, 16, 17, 18, 19, 22, X and Y (Table 1).

The results revealed numerical and structural abnormalities, and the most frequent structural abnormalities observed were either deletions or derivative chromosomes, which underwent breakage and reunion. Structural rearrangements are shown in Figs. 1-5. On the other hand, the distribution of chromosomal involvement varied across cases, and recurrent autosomal chromosomal involvement was most frequently observed in chromosome 19 (28 cases) (as a deletion at the short arm 19p13.2), followed by chromosome 2 (as a deletion in region 2) (p21-p23) (21 cases)), chromosome 5 (20 cases) showed abnormalities involving three regions (5) (p15) or (5) (q11-q14) or/and (5) (q3) 1, and chromosome 12 (19 cases) involving regions (12) (q13) or (12) (q22-23). Chromosome 16 (16 cases) had a 16q23-terminal deletion. Less frequent abnormalities involved chromosomes (1) (p34.3), (1) (p36.12), (1) (q25) and inversion (1) (q42-32). Chromosome 9 involved

the regions (p24), (q13), and (q34). Chromosome 14 (5 cases each) involved regions associated with either (14) (q11.2) or (q32), whereas chromosome 22 showed the lowest frequency of involvement in the region involved in del (12) (q12-terminal), which was identified only in one patient.

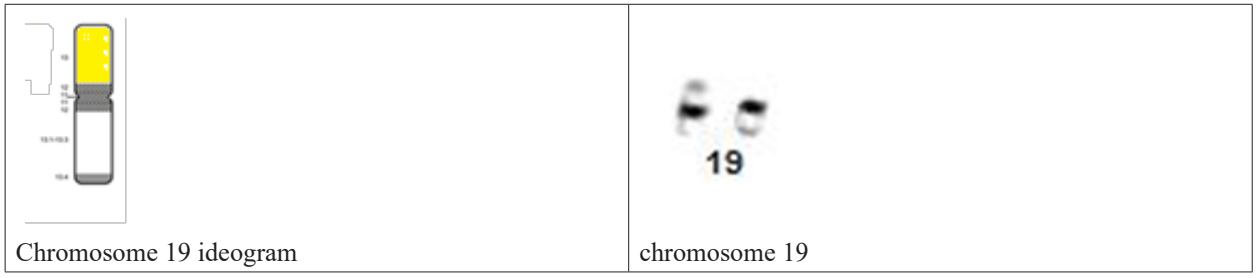


Fig. 1. Representative chromosome 19 deletions identified in DSD patients. Chromosome 19 was the most frequently affected autosome in the cohort. Yellow highlighted regions in the ideograms indicate the affected chromosomal regions [22].

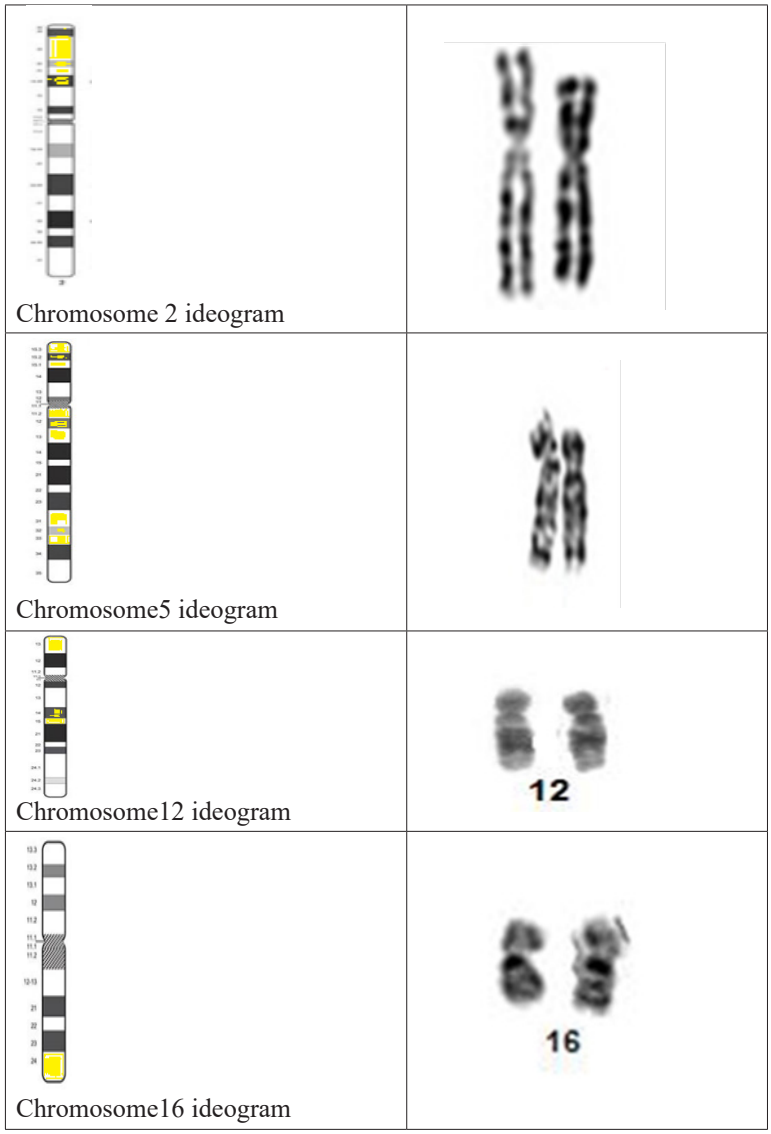


Fig. 2. Representative recurrent autosomal chromosomal abnormalities involving chromosomes 2, 5, 12, and 16 in DSD patients. Yellow highlighted regions in the ideograms indicate the affected chromosomal regions [22].

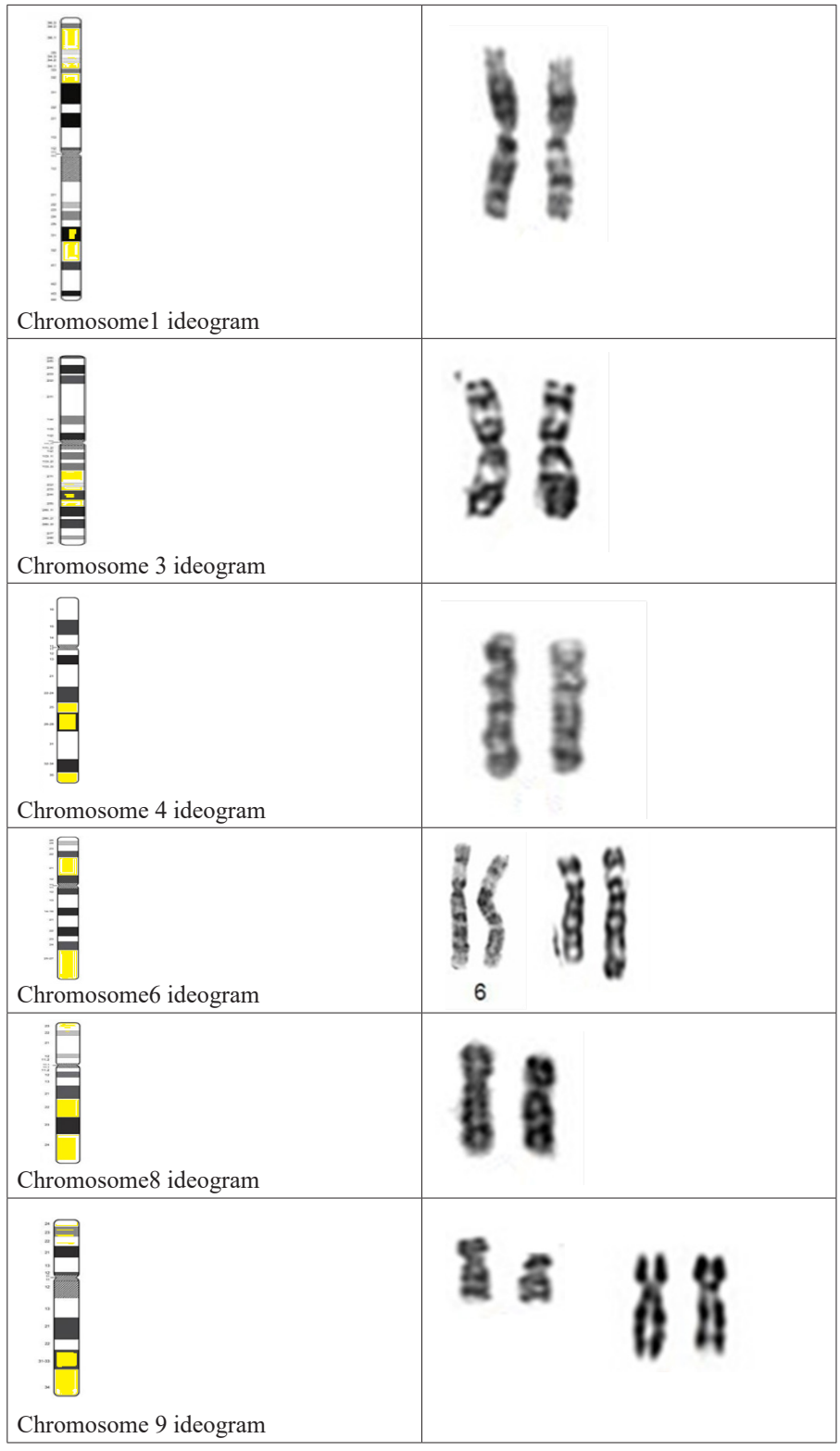


Fig. 3. Representative structural chromosomal rearrangements involving chromosomes 1, 3, 4, 6, 8 and 9, including deletions, derivative chromosomes and chromosomal break points, are shown. Yellow highlighted regions in the ideograms indicate the affected chromosomal regions [22].

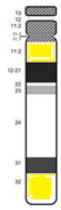
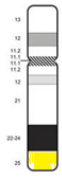
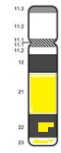
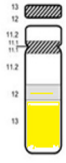
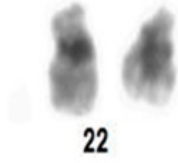
 Chromosome 14 ideogram	
 Chromosome15 ideogram	
 Chromosome17 ideogram	
 Chromosome18 ideogram	
 Chromosome 22 ideogram	

Fig. 4. Representative rare or less frequent chromosomal abnormalities associated with DSDs involving chromosomes14,15,17,18 and 22. Yellow highlighted regions in the ideograms indicate the affected chromosomal regions [22].

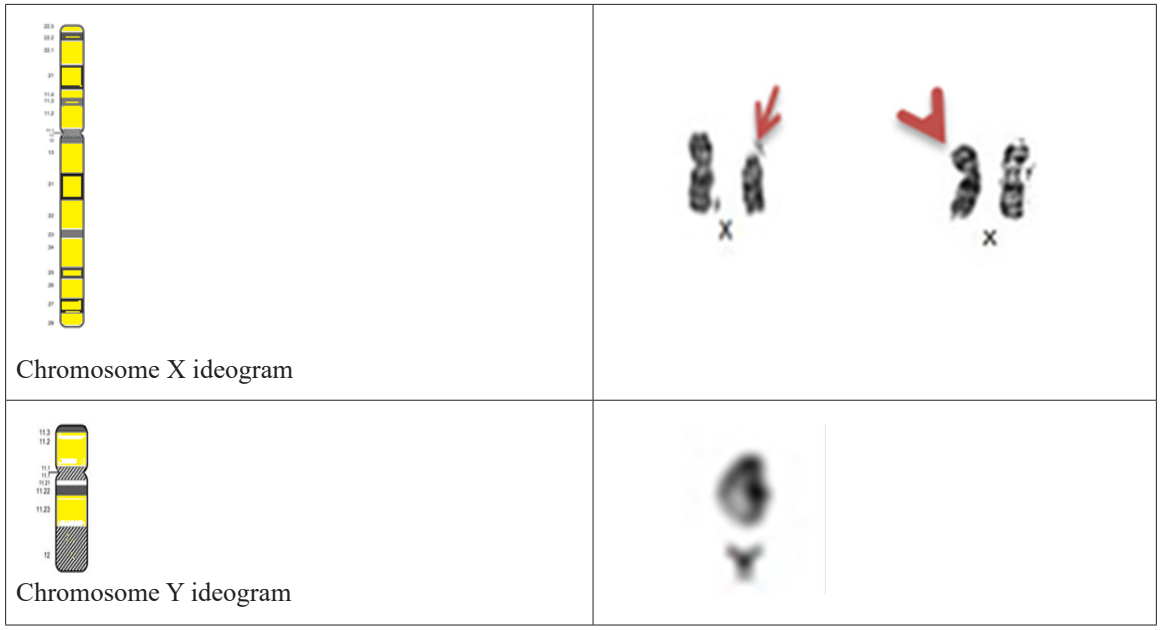


Fig. 5. Representative sex chromosome abnormalities involving chromosomes X and Y identified in DSD patients. Yellow highlighted regions in the ideograms indicate the affected chromosomal regions[22].

On the basis of clinical presentation, DSD patients were classified into seven diagnostic categories and further subdivided according to their phenotypic sex into female and male phenotype groups (Table 1).

Table 1: Distribution of DSD cases among primary amenorrhea, secondary amenorrhea, Turner syndrome Klinefelter syndrome, ambiguous genitalia and hypogonadism cases and their karyotype formulas

Female Clinical category	**No. of cases**	**Karyotype(s)**
Primary amenorrhea	17	• 46, XY.{5} • 46, XX, del(19)(p22-ter).{3} • 46, XX, der (19), der(X). {1} • 46, XX 50%,45, X 50%. {1} • 46, XY, der (9), der (12), der (18), del (19).{2} • 46, X, der(X). {1} • 46, XX, der (1), der (3). {1} • 46, X, der (2), der(X). {2} • 46, XX, der (5), der(X) [16];46, XX, der (3), der(X) [12]. {1}
Secondary amenorrhea	1	• 45, X . {1}
Turner syndrome	1	• 46, X, del(Xp). {1}

Ambiguous genitalia	21	• 46, XY, der (4) der (8), der (9), del(16q), der (18), del (19). {1} • 46, XX, der (2), der (5), der (6) (q25-27), der (12), der (16), del(16q), del (19), der(X). {1} • 46, XX, der (8), der (15), del(16q), der (18), del(19p), der(X). {1} • 46, XX, der (1), der (3), der (X)[26] 46, XX, der (1), der (3), der (5), der(X) [12]. {1} • 46, XX, der (2), der (5), der (6), del(9p), der (12), del(16q), der (18), del(19p). {2} • 46, XX, del(X)(q25-28). {11} • 46, XY, der (2), der (5), der (12), der (15), del (16), del (19). {2} • 46, XX, der (2) der (5), der (8), der (9), der (14), del(15q), der (17), der (18), del(19p). {1} • 46, XX, der (8), der (9), der (12), der (18), del(19p), del (Xp). {1}
Total	**40**	

Male Clinical category	**No. of cases**	**Karyotype(s)**
Klinefelter syndrome	3	• 47, XXY 50%; 46, XY 50%. {1} • 47, XXY 70%;46, XY 30%. {2}
Ambiguous genitalia	9	• 46, XY, del (1p-) {1} • 46, XY 50%; 45, X 50%. {1} • 46, XX. {1} • 46, XX, del(X)(pter)10% ; 46, der (Xq) 20% ,46, XX, del(Xpter), der(X) 60%.{1} • 46, XY, der(3), der(X)[32];46, XY, der(3), der(5), der(X) [15] 46, XY, der(2), der(3), der(X.) {1} • 46, XY, der(4), der(5), der(6)(p21.3), der(14), der(15), der(16), del(19p). {1}
Hypogonadism	1	• 46, XY, der(2), der(4), der(5), der(12), der(14), der(15), del(16q), der(17), der(18), der(19). {2} • 46, XY, der(1), der(2), der(5), der(14), del(19p), der(X), del(19q), der(22). {1} • 46, XY, del(1p-) , del (Y)
Total	13	

Note: Numbers in curly brackets { } indicate the number of patients carrying the abnormality, whereas the number in square brackets [ ] indicates the number of metaphases exhibiting the abnormality. where % indicates the percentage of abnormalities in which one cell line appeared in a person.

First, the female phenotype group included 40 patients who presented with primary amenorrhea, secondary amenorrhea, Turner syndrome, or ambiguous genitalia. Among patients with primary amenorrhea, most exhibited abnormal karyotypes, including five patients with a male karyotype (46, XY) despite a female phenotype. Recurrent chromosomal abnormalities involved chromosome 19 in twenty-four female patients, either as terminal deletions (19)(p22-ter) or as derivative chromosomes, frequently in association with abnormalities of the X chromosome. Mosaicism was detected in some patients, including a 46XX/45X mosaic, as well as in patients with multiple abnormal cell lines involving both autosomes and sex chromosomes. The single case of secondary amenorrhea demonstrated monosomy X (45X), whereas the Turner syndrome patients exhibited a structurally abnormal X chromosome (46, X, der(X)).

Patients with ambiguous genitalia within the female phenotype group showed marked karyotypic heterogeneity. Some patients exhibited complex rearrangements involving multiple autosomes as well as abnormalities in sex chromosomes and recurrent deletions affecting chromosomes 19p and 16q. Familial recurrence was identified in two sets of sisters sharing identical karyotypes, suggesting a potential hereditary component in selected cases (Table 1).

The second group consisted of male patients with Klinefelter syndrome, ambiguous genitalia and hypogonadism, as shown in Table 1. Klinefelter syndrome patients were characterized by mosaic karyotypes consisting of 47,XXY and 46,XY cell lines. A review of the findings revealed heterogeneous chromosomal defects, including autosomal deletions, derivative chromosomes and mosaicism of sex chromosomes, among patients with ambiguous genitalia. The patient with hypogonadism had a complex karyotype involving autosomal imbalance and deletion of the Y chromosome.

In contrast, several patients demonstrated discordance between their phenotype and sex chromosome constitution (Table 1). Five individuals with a female phenotype exhibited a 46, XY karyotype, whereas multiple patients with ambiguous genitalia presented with either 46, XX or 46, XY karyotypes in association with complex autosomal rearrangements. These findings underscore the substantial contribution of autosomal chromosomal abnormalities to the phenotypic variability in DSD independent of sex chromosome constitution.

Discussion

In this study, the cytogenetic spectrum of disorders of sex development in an Iraqi cohort was investigated, with a particular emphasis on the contribution of autosomal chromosomal abnormalities. To our knowledge, the present study is the first to investigate chromosomal abnormalities in Iraqi DSD patients. This population remains underrepresented in global genomic databases. On the one hand, the genetic backgrounds of each population, inbreeding patterns, and environmental exposures may contribute to the distinct cellular and genetic landscape observed in this group. The inclusion of these populations is essential for broadening the global understanding of sexual development disorders and reducing origin-related bias in medical genetics research. The findings of this study demonstrate that autosomal rearrangements were more frequent than isolated sex chromosome abnormalities, which explains the marked phenotypic heterogeneity observed in DSD [23,24,25].

Autosomal chromosomal abnormalities in DSD

While DSD has traditionally been associated with abnormalities of the X and Y chromosomes, particularly SRY, accumulating evidence indicates that autosomal genes play indispensable roles in sex determination and differentiation[26]. In agreement with recent large-scale genomic studies, frequent structural and numerical abnormalities involving multiple autosomes were observed, most notably chromosomes 19, 2, 5, 12, and 16 [21, 27]. These findings suggest that disruption of dosage-sensitive regions or regulatory elements on autosomes can significantly influence sex development, even in the presence of an apparently normal sex chromosome complement [27].

Chromosome 19p as a recurrently affected region

Among all the autosomes, chromosome 19p emerged as the most recurrently affected region in our cohort and was involved in 28 cases. This region harbors important genes implicated in sex differentiation, including *AMH* and *INSL3*. AMH, which is secreted by Sertoli cells, is essential for Müllerian duct regression, whereas *INSL3* plays a critical role in gubernacular development and testicular descent [19,27].

Interestingly, structural alterations involving 19p were also identified in female patients, particularly those presenting with primary amenorrhea, ambiguous genitalia, and discordant genotype-phenotype patterns. Both 46, XY and 46, XX individuals with atypical genital development exhibited recurrent changes encompassing the *AMH/INSL3* locus [19]. Chromosome 19p may harbor regions of interest that warrant further investigation in DSD.

Although *AMH* is best known for its role in male sexual differentiation, the gene is also expressed in the ovary, where it regulates follicular development and is widely used as a biomarker of ovarian reserve. Therefore, the recurrent involvement of chromosome 19 in female patients in our cohort raises the possibility that structural alterations affecting this chromosome may have broader implications for gonadal and reproductive development beyond its established a causal relationship, and further molecular and functional studies are required to clarify the contribution of chromosome 19 genes to DSD phenotype.

Furthermore, the presence of uterine remnants or abnormal internal genital development in some individuals carrying 19p rearrangements may indicate that altered expression of genes within this region could also contribute to abnormalities in female reproductive tract development.

These observations are consistent with previous reports describing autosomal forms of DSD in individuals with intact SRY sequences but impaired gonadal development resulting

from alterations in autosomal genes such as *NR5A1, SOX9, GATA4,* and *MAP3K1*[23,19,20,13]. The present study extends these observations by highlighting recurrent cytogenetic alterations involving chromosome 19p within an Iraqi DSD cohort.

Role of other autosomal regions

In addition to chromosome 19, recurrent rearrangements involving chromosomes 1, 2, 5, 12, 16, 17, 18, and 22 were also identified. These chromosomes harbor genes involved in gonadal development and steroidogenesis, including *HHAT, SRD5A2, MAP3K1, DMRT1/2, AMHR2, SOX9, DMC1* and *SOX10* [14,15,17,19,23,]. Dysregulation of these loci may contribute to abnormal gonadal differentiation, altered androgen metabolism, and atypical internal or external genital development. The role of each region that underwent structural aberration in this study is illustrated, and candidate genes previously reported to be associated with DSD and located within the affected chromosomal regions are summarized in Table 2.

Recurrent structural rearrangements involving chromosome 18 were of particular interest, as similar abnormalities have been repeatedly reported in patients with ambiguous genitalia [28,29]. However, the current evidence does not establish a direct mechanistic relationship between chromosome 18 abnormalities and sex development disorders. These findings suggest that structural alterations of chromosome 18 may contribute to DSD pathogenesis, although further molecular and functional studies are needed to clarify their precise role in sex differentiation and related developmental abnormalities.

Table -2. Candidate genes previously reported to be associated with DSD and located within the affected chromosomal regions.

Chromosome	Regions which involved in the chromosomal aberrations	Genes located at the involved regions	Role in DSD	Ref.
1	(1)(p36.12) (1)(p34.3) (1)(q31.3) (1)(q32.3)	*WNT4* *RSPO1* *LHX9* *HHAT*	Testicular/ovatesticular/DSD Testicular/ovatesticular/DSD Testicular dysgenesis Testicular dysgenesis	11 12 13 14
2	(2)(p21-p16) (2)(p23.1) (2)(q35)	*FSHR* *SRD5A2*	Follicular activation, development and maturation, hormonal support. Male differentiation of the urogenital sinus, the genital tubercle, the urogenital fold, and labioscrotal folds	15
3	(3)(q23)	*FOXL2*	Ovary development Antitestis	16
4	-	-	-	-
5	(5)(q11.2) (5)(q31)	*MAP3K1* *GDF9*	Testicular dysgenesis Primaryamenorrhea/ovary development Follicular activation, development and maturation, cell division	17 18
6	(6)(p21.3) (6)(p25.3) del (6)(q25-27)	Estrogen receptor		19,20
8	(8)(p22.3) (8)(q23.3)	*ZFPM2* *GATA4*	Testicular/ovatesticular Testicular dysgenesis	19,20

9	(9)(p24.3) (9)(q33)	*DMRT1/DMRT2* *NR5A1*	Ovarian dysgenesis Testicular dysgenesis Ovarian dysgenesis/ovatesticular/DSD/ Gonadogenesis-oogenesis	19,20 23
12	(12)(q13.12) (12)(q23.3)	*AMHR2,DHH* *SART*	Testicular dysgenesis Testicular dysgenesis	24 25
14	(14)(q13.2) (14)(q23.2-q23.3)	*PPP2R3C* *ESR2*	complete gonadal dysgenesis Ovarian Dysgenesis	26
15	(15)(q21) (15)(q26.2)	*CYP19A1* *NR2F2*	Sexual differentiation Testicular DSD	19,20
16	(16)(q24)		Hypospadias	26
17	(17)(q24.3) (17)(q25.3)	*SOX9* *CBX2*	Testicular dysgenesis/Ovarian dysgenesis	19
18	unknown	-	Ambiguous genitalia	28,29
19	(19)(p13.3)	*AMH* *INSL3*	Persistent Müllerian duct syndrome type I Cryptorchidism	19,20
22	Del (22)(q12-ter,(22)(q13)	*SOX10* (SRY (sex determining region Y)-box 10) *DMC1*	Normal male sexual development Meiosis/primary amenorrhea	24
X	(X)(p13) (X)(p21.2) (X)(p22.13) (X)(q11.3) (X)(q25) (X)(q27.1) (X)(q28)	*ATRX* *DAX1 -NROB1* *ARX* *AR* *XPNEP2* *SOX9* *MAMLD1*	Testicular dysgenesis Hypogonadism Testicular dysgenesis Normal male sexual development Premature ovarian failure candidate gene Testicular dysgenesis Hypospadias/DSD	19,20
Y	(Y)(p11.3)	*SRY*	Testicular/Ovatesticular	19,20

On the other hand, this study has several limitations, particularly the absence of molecular analyses that could provide functional validation of the observed chromosomal rearrangements, such as next-generation sequencing or copy number variation profiling [30]. Therefore, further studies using high-resolution genomic approaches are needed to validate the candidate regions identified in this cohort, identify additional genes potentially involved in DSD, and clarify the molecular mechanisms underlying the contribution of autosomal abnormalities to sex development disorders.

Conclusion

The findings of this study suggest that autosomal chromosomal abnormalities may represent important contributors to the etiology and phenotypic variability of disorders of sex development. While the role of chromosome 19p in male sex differentiation is supported by the presence of key genes such as *AMH* and *INSL3*, its recurrent involvement in female patients in this cohort may indicate a broader regulatory influence that extends beyond male-specific pathways. These observations should be considered preliminary, and molecular studies are

needed to determine the specific genes and mechanisms involved. These results emphasize the value of comprehensive cytogenetic evaluation beyond sex chromosomes, particularly in underrepresented populations.

Author declarations

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Author Contributions

Asmaa Amer Almukhtar: Conceptualization, methodology, investigation, cytogenetic analysis, data curation, formal analysis, interpretation of results, and preparation of the original manuscript draft. Amal Mohammed Ali: Methodology, cytogenetic analysis, supervision, critical revision of the manuscript, and validation of the scientific content. Noor Hashim Ismail: Cytogenetic analysis, data interpretation, manuscript review and editing, and validation. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval

The study protocol was approved by the Scientific and Ethics Committee of the Iraqi Center for Cancer and Medical Genetics Research (Approval No. 1, January 25, 2023) and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Informed Consent

Written informed consent was obtained from all participants or from their legal guardians before enrollment in the study.

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Supplementary Data

Supplementary table:

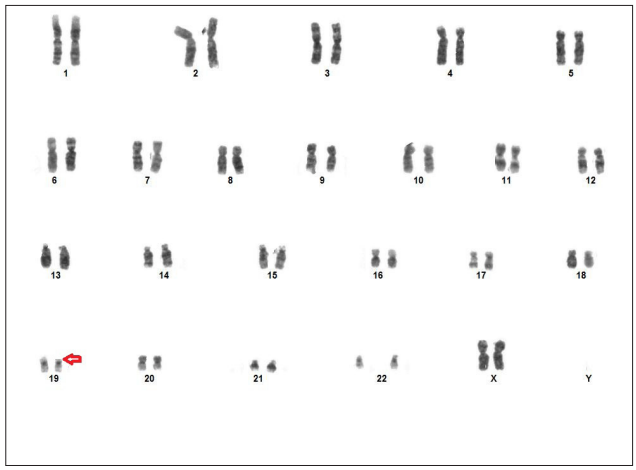
No.	Sex	Clinical diagnosis	Karyotype	Age	Notes
1	♀	Primary amenorrhea	46, XY	18Y	
2	♀	Primary amenorrhea	46, XY	16Y	
3	♀	Primary amenorrhea	46, XY	16Y	
4	♀	Primary amenorrhea	46, XY	17Y	
5	♀	Primary amenorrhea	46, XY	17Y	
6	♀	Primary amenorrhea	46, XX, del(19)(p22-ter)	20Y	
7	♀	Primary amenorrhea	46, XX, del(19)(p22-ter)	16Y	
8	♀	Primary amenorrhea	46, XX, del(19)(p22-ter)	19Y	
9	♀	Primary amenorrhea	46, XX, der (19), der(X).	15Y	
10	♀	Primary amenorrhea	46, XX 50%,45, X 50%.	17	
11	♀	Primary amenorrhea	46, XY, der (9), der (12), der (18), del (19).	15Y	
12	♀	Primary amenorrhea	46, XY, der (9), der (12), der (18), del (19).	14Y	
13	♀	Primary amenorrhea	46, X, der(X)	20Y	
14	♀	Primary amenorrhea	46, XX, der (1), der (3).	16Y	
15	♀	Primary amenorrhea	46, X, der (2), der(X).	14Y	sisters
16	♀	Primary amenorrhea	46, X, der (2), der(X).	15Y	
17	♀	Primary amenorrhea	46, XX, der (5), der(X) [16];46, XX, der (3), der(X) [12]	16Y	
18	♀	Secondary amenorrhea	45, X	15Y	
19	♀	Turner syndrome	46, X, del(Xp).	17Y	
20	♀	Ambiguous genitalia	46, XY, der (4,) der (8), der (9), del(16q), der (18), del (19).	7Y	
21	♀	Ambiguous genitalia	46, XX, der (2), der (5), der (6) (q25-27), der (12), der (16), del(16q), del (19), der(X).	5Y	
22	♀	Ambiguous genitalia	46, XX, der (8), der (15), del(16q), der (18), del(19p), der(X).	5Y	

23	♀	Ambiguous genitalia	46, XX, der (1), der (3), der (X) [26] 46, XX, der (1), der (3), der (5), der(X) [12].	9Y	
24	♀	Ambiguous genitalia	46, XX, der (2), der (5), der (6), del(9p), der (12), del(16q), der (18), del(19p).	1Y	
25	♀	Ambiguous genitalia	46, XX, del(X)(q25-28).	2Y	
26	♀	Ambiguous genitalia	46, XX, del(Xp)	.mon 2	
27	♀	Ambiguous genitalia	46, XX, del(Xp)	1Y	
28	♀	Ambiguous genitalia	46, XX, del(Xp)	3Y	
29	♀	Ambiguous genitalia	46, XX, del(Xp)	.mon 6	
30	♀	Ambiguous genitalia	46, XX, del(Xp)	11Y	
31	♀	Ambiguous genitalia	46, XX, del(Xp)	18Y	
32	♀	Ambiguous genitalia	46, XX, del(Xp)	18Y	
33	♀	Ambiguous genitalia	46, XX, del(Xp)	12Y	
34	♀	Ambiguous genitalia	46, XX, del(Xp)	14Y	
35	♀	Ambiguous genitalia	46, XX, del(Xp)	13Y	
36	♀	Ambiguous genitalia	46, XX, del(Xp)	15Y	
37	♀	Ambiguous genitalia	46, XY, der (2), der (5), der (12), der (15), del (16), del (19).	13Y	sisters
38	♀	Ambiguous genitalia	46, XY, der (2), der (5), der (12), der (15), del (16), del (19).	15Y	
39	♀	Ambiguous genitalia	46, XX, der (2,) der (5), der (8), der (9), der (14), del(15q), der (17), der (18), del(19p).	3 .Mon	
40	♀	Ambiguous genitalia	46, XX, der (8), der (9), der (12), der (18), del(19p), del (Xp).	1Y	
41	♂	Klinefelter syndrome	47, XXY 50%; 46, XY 50%	17Y	
42	♂	Klinefelter syndrome	47, XXY 70%;46, XY 30%.	19Y	
43	♂	Klinefelter syndrome	47, XXY 70%;46, XY 30%.	27Y	
44	♂	Ambiguous genitalia	46, XY, del (1p-)	14Y	
45	♂	Ambiguous genitalia	46, XY 50%; 45, X 50%.	11Y	
46	♂	Ambiguous genitalia	46, XX.	13Y	
47	♂	Ambiguous genitalia	46, XX, del(X)(pter)10% ; 46, der (Xq) 20% ,46, XX, del(Xpter), der(X) 60%.{	12Y	
48	♂	Ambiguous genitalia	46, XY, der(3), der(X)[32];46, XY, der(3), der(5), der(X) [15] 46, XY, der(2), der(3), der(X.)	6Y	
49	♂	Ambiguous genitalia	46, XY, der(4), der(5), der(6) (p21.3), der(14), der(15), der(16), del(19p).	15Y	

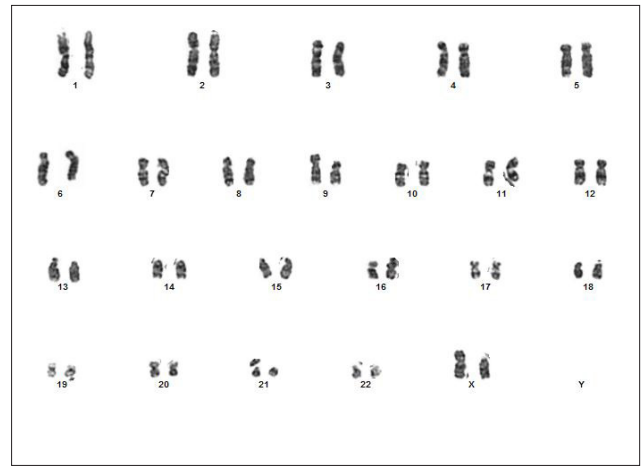
50	♂	Ambiguous genitalia	46, XY, der(2), der(4), der(5), der(12), der(14), der(15), del(16q), der(17), der(18), der(19).	17Y	relatives
51	♂	Ambiguous genitalia	46, XY, der(2), der(4), der(5), der(12), der(14), der(15), del(16q), der(17), der(18), der(19).	13Y	
52	♂	Ambiguous genitalia	46, XY, der(1), der(2), der(5), der(14), del(19p), der(X), del(19q), der(22).	6Y	
53	♂	Hypogonadism	46, XY, del(1p-) del (Y).	13Y	

Supplementary Figures:

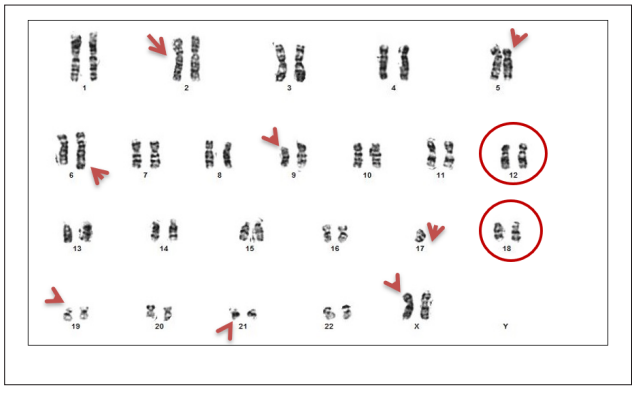
Supplementary Figures: Representative abnormal karyotypes identified in the study cohort.



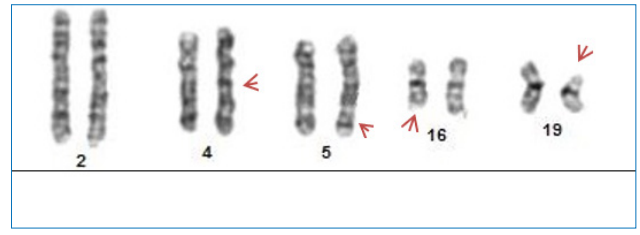
S 1: Representative karyotype from a female patient with primary amenorrhea showing a deletion involving the short arm of chromosome 19 [46,XX,del(19p)].



S 2:Karyotype showing deletion of the short arm of the X chromosome [46,XX,del(Xp)].



S 3 : Complex karyotype demonstrating multiple chromosomal abnormalities, including a derivative X chromosome.



S 4 : partial karyotype from a patient with DSD and ambiguous genitalia showing multiple autosomal abnormalities [46,XY,der(2),der(4),der(5),del(16q),del(19p)]