Journal of Endocrinological Investigation (2021) 44:145–151
https://doi.org/10.1007/s40618-020-01284-8

ORIGINAL ARTICLE

Disorders of sexual development with XY karyotype and female phenotype: clinical findings and genetic background in a cohort from a single centre

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Received: 23 March 2020 / Accepted: 28 April 2020 / Published online: 6 May 2020

Abstract

Purpose 46, XY disorders (or differences) of sex development (DSD) are a group of clinical conditions with variable genetic background; correct diagnosis is often difficult, but it permits to optimize the management. The aim of this study is to identify clinical and genetics features of a group of women with 46, XY DSD to define some issues characterizing people with 46, XY DSD in Italy.

Methods Retrospective analysis of girls and women with 46, XY DSD and female phenotype evaluated between year 2000 and 2016, performed by anonymised database, focusing on the clinical features and management, including presentation, first diagnostic suspect, gonadal surgery and molecular diagnostic delay.

Results A total of 84 records were collected (mean age at clinical presentation: 9.1 ± 7.9 years; mean age at definitive diagnosis: 20.1 ± 15.0 years). Complete androgen insensitivity syndrome was the most common diagnosis (60%). Only 12 patients (14.3%) did not receive a molecular diagnosis. Early misdiagnoses frequently occurred; diagnostic delay was 10.2 ± 11.2 years, being reduced in patients presenting from 2007 to 2016. The discordance between genotypic and phenotypic sex during pregnancy or at birth determined early reason for referral in a considerable percentage (4.9%).

Conclusion Misdiagnosis and long diagnostic delays are present in females with 46, XY DSD in Italy, but the new genetic techniques permit faster right diagnoses in the last years. The centralization in dedicated third level units permits to reduce the number of patients without a molecular diagnosis, allowing better clinical management and appropriate genetic counselling.

Keywords Disorders of sexual development · Complete androgen insensitivity syndrome · Gonadal dysgenesis · Sex differentiation · Sex determination

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Abbreviations

CAIS Complete androgen insensitivity syndrome
CGD Complete gonadal dysgenesis
DHH Desert Hedgehog
DSD Disorders (or differences) of sexual development
HRT Hormonal replacement therapy
NIPT Non-invasive prenatal testing
PAIS Partial androgen insensitivity syndrome
PGD Partial gonadal dysgenesis
SF1 Steroidogenic factor-1
SRY Sex determining region on the Y chromosome
WT1 Wilms' tumour 1

Introduction

In the embryo, sex development is a multi-step process that involves a complex network of genetic and hormonal factors. Usually, in presence of an XY karyotype, the *SRY* (*sex determining region on the Y chromosome*) and related gene network promote the formation of a functional testis (sex determination) [1]. Then the hormones produced by the testis guide the development of the male genital phenotype (sex differentiation) [2]. Disorders (or differences) of sex development (DSD) are defined as congenital conditions featured by the alteration of genetic, gonadal or phenotypic sex [3, 4].

The 46, XY DSD group includes a wide spectrum of conditions due to genetic variants, altered hormonal secretion or abnormal peripheral sensitivity to testicular hormones that are able to change the usual foetal development of male genitalia to cause different grades of under-virilization in newborns with 46, XY [1, 3, 4]. The impact of 46, XY DSD on the quality of life of the affected women is remarkable, as these conditions are featured by a complex clinical, endocrinological and psychological management, regarding sex assignment and eventual re-assignment, decisions about gonadal removal, hormone replacing therapy from adolescence onward, long-term monitoring of bone health and risk of gonadal neoplasia. Therefore, the care of the women with 46, XY DSD requires a strict cooperation between different specialists. While correct diagnosis is a key factor for optimizing management and better quality of life also in adulthood [5, 6], true diagnoses may be not reached jeopardizing long-term outcome, thus carrying significant clinical and psychological consequences.

In this study, we report on a relatively large cohort of patients with 46, XY DSD and female phenotype from a single centre, focusing on the clinical presentation and the mistakes of diagnosis to provide useful issues to improve the management of people with 46, XY DSD.

Patients and methods

Patient selection

The clinical records of 107 patients with 46 XY DSD and assigned female sex (Sinnecker grade 4 or 5) [7], evaluated in our Department from the year 2000 to 2016, were reviewed.

Data collection

For each patient, the following data were collected in an anonymized database: clinical signs rising the suspect of a DSD, age at first presentation, first diagnosis (term meaning the diagnostic "tag" of each patient before our evaluation), features at first presentation, clinical management, including the occurrence of gonadal removal, definitive diagnosis, age at definitive diagnosis, the occurrence of sex re-assignment, and hormone substitutive therapy (when appropriate); hormonal treatments were largely prescribed from personal physicians of each woman.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the QIAsymphony Instrument (QIAGEN, Italy). PCR amplification of single genes was done from 2000 to 2010 basing on clinical phenotype as well as available endocrine and histological data. Purified PCR products were bidirectionally sequenced (BigDye Terminator v3.1 Cycle sequencing Kit; Life Technologies, Italy) and analyzed on a 3130Xl Genetic Analyzer (Applera, Italy). A targeted NGS panel for DSD was introduced in the Laboratory of Medical Genetics from 2015 onward. Briefly library preparation was initially performed by a customised TruSeq Custom Amplicon (TSCA, Illumina Inc) 17-gene panel and more recently by a SureSelectXT Custom system (Agilent) 20-gene panel; sequencing was performed on an Illumina MiSeq system. In silico analysis was performed using the commonly used bioinformatics tools such as Mutation Taster, Polyphen, SIFT, FATHMM, HumanSplicingFinder. Direct Saenger sequencing was performed to confirm genetic variants individuated by NGS.

Statement of ethics

The study was conducted according to the Declaration of Helsinki and the standard protocol of investigation of people with 46, XY in our Department. The parents of children aging less than 18 years or directly adults had

given their informed written consent before any clinical and genetic investigation.

Statistical analysis

Data are presented as the mean value and the standard deviation (SD) in case of normal distribution. Otherwise, the data are presented as the median value and min/max. An exact Fisher test or a Chi square test was used to compare data about categorical variables from two groups. A non-parametric Mann–Whitney test was used to compare the data about continuous from two groups of patients; a p value of < 0.05 was considered significant.

Table 1 Molecular diagnoses in females wit 46, XY DSD

Disorders of sex determination	*n*	Disorders of sex differentiation	*n*
SF1 deficiency	3	CAIS	51
		PAIS	1
SRY deficiency	1	5α-reductase 2 deficiency	9
DHH mutation	1	17β-hydroxysteroid dehydrogenase deficiency	3
		17,20-lyase deficiency	1
WT1 mutation	1	Leydig's cell hypoplasia	1
Total	6	Total	66

CAIS complete androgen insensitivity syndrome, *PAIS* partial androgen insensitivity syndrome, *SF1* steroidogenic factor 1, *SRY* sex determining region on the Y chromosome, *DHH* Desert hedgehog, *WT1* Wilms' tumor

Results

From the total sample, patients with insufficient clinical documentation ($n = 7$) and patients with initial virilisation degree ≤ of Sinnecker grade 3 at revision of clinical records were excluded ($n = 16$).

The final sample consisted of 84 females with 46, XY DSD. Mean age at first presentation was of 9.1 ± 7.9 years and the mean age at definitive diagnosis was of 20.1 ± 15.0 years. Thus, the diagnostic delay was about 10 years (10.2 ± 11.5 years). In our cohort, 63patients presented to medical attention from the year 2000 to 2006, and 21 patients from the year 2007 to 2016.

A molecular diagnosis was reached in the majority of the patients ($n = 72$; 85.7%) as reported in Table 1 Molecular diagnosis remained unknown in 12 females with gonadal dysgenesis (14.3%). In this subgroup of patients, the clinical diagnosis of gonadal dysgenesis was made according to hormonal, imaging and histological investigations. Clinical data regarding patients without a molecular diagnosis are summarized in Table 2.

Complete androgen insensitivity syndrome (CAIS) was the main subgroup, representing more than 60% of all diagnoses (Table 1). In this cohort, eight couples of siblings were present. Within the CAIS subgroup, the mean age at clinical presentation was 12.7 ± 7.1 years, and the mean age at definitive genetic diagnosis was 23.4 ± 14.0 years (mean diagnostic molecular delay 11.2 ± 11.7 years). In the majority of these females (Table 3), CAIS was correctly the first diagnostic suspect, but endocrine and/or genetic tests to confirm the diagnostic suspect were often delayed. Primary amenorrhea was the main cause of consultation ($n = 34$; 66.6%).

Table 2 Clinical features of the patients without molecular diagnosis

Patient	First visit (years)	Presentation sign	First diagnosis	Gonadal removal (years)	Genetic approach
1	17	Male pubertal delay	CAIS	24	Single genes
2	8	Virilization	CAIS/ 5α-reductase 2 deficiency	11	Single genes
3	0	Genital ambiguity	Leydig's cell hypoplasia	1	Single genes
4	0	Genital ambiguity	CGD	2	NGS
5	17	Primary amenorrhea	CAIS	18	Single genes
6	5	Incidental	Prader Willy	7	Single genes
7	0	Incidental	CAIS	12	Single genes
8	0	Genital ambiguity	5α-reductase 2 deficiency	1	NGS
9	0	Genital ambiguity	5α-reductase 2 deficiency	0.5	NGS
10	14	Primary amenorrhea	CAIS	–	Single genes
11	15	Primary amenorrhea	CAIS	–	NGS
12	0	Genital ambiguity	CGD	1	Single genes

CAIS complete androgen insensitivity syndrome, *NGS* next generation sequencing, *Single genes* on the basis of the clinical/endocrinologial phenotype (AR, SRY, SF1, SRD5A2 and others)

Table 3 Correct first diagnosis, misdiagnosis and diagnostic delay in 46, XY DSD women

Condition	Correct first diagnosis	Misdiagnosis	Diagnostic delay (mean)
CAIS	47/51 (92%)	4/51 (8%)	11.2 ± 11.7 years
5α reductase deficiency	4/9 (44.4%)	5/9 (55.6%)	8.2 ± 11.3 years
PAIS	0/1	1/1	0 year
Leydig's cell hypoplasia	0/1	1/1	13 years
17,20-lyase deficiency	0/1	1/1	18 years
17β-hydroxysteroid dehydrogenase deficiency	0/3	3/3	2.3 ± 2.5 years
SF1 mutation	0/3	3/3	4.7 ± 7.2 years
WT1 mutation	0/1	1/1	1 month
SRY mutation	0/1	1/1	2 years
DHH mutation	0/1	1/1	47 years

Table 4 Signs of presentation in 51 females with CAIS

Sign	*N*	%
Primary amenorrhea	34	66.6
Inguinal hernia containing testes	11	21.6
Discordance genotypic/phenotypic sex[a]	3	5.9
Delayed puberty	2	3.9
Early puberty	1	1.9

[a]At amniocentesis

Table 5 Hormonal replacement therapy in a cohort of females with 46, XY DSD

HRT	*N*	%	Good adherence
Oral 17βestradiol	6	13.3	6/6
Oral ethynilestradiol	2	4.4	2/2
Transdermal 17βestradiol	23	51.1	19/23
Conjugated estrogens	3	6.7	3/3
Oral contraceptives	7	15.6	5/7
Testosterone	2	4.4	2/2
Unavailable	2	4.4	–

HRT hormonal replacement therapy

The other causes of presentation are summarized in Table 4. Bilateral inguinal hernia was experienced by more than 40% of the sample (22/51). The majority ($n = 36$) underwent gonadal removal at the mean age of 15.7 ± 10.8 years; 30 patients (70.6%) underwent surgery before the molecular diagnosis. Postpubertal women with removed gonads were on substitutive hormonal therapy with various formulations of estrogens (Table 5); two women were on testosterone therapy. Data on adherence to therapy are summarized in Table 5, too. During follow-up, one woman died for sudden cardiac attack and another one for gastric cancer.

Table 6 summarizes the data of females with non-CAIS 46, XY DSD, subgrouping patients as gonadal dysgenesis (disorders of sex determination) or defect in androgen metabolism (disorders of sex differentiation). Altogether, gonadal surgery was performed before the definitive diagnosis in ten patients (30.3%). Patients of Table 6 had a significantly higher rate of misdiagnosis at first presentation in comparison with females with CAIS ($p < 0.0001$). Disorders of sex differentiation had an earlier presentation compared to patients with disorders of sex determination (2.6 ± 4.0 versus 5.2 ± 6.8 years, $p = 0.55$), while the diagnostic delay is higher for patients with disorders of sex determination (8.8 ± 14.7 versus 5.3 ± 6.7, $p = 0.50$). Sex re-assignment occurred in four patients with gonadal dysgenesis, all without a definitive molecular diagnosis.

Out of the 21 patients with clinical presentation from 2007 to 2016, 19 (90.5%) received a molecular diagnosis. Diagnostic delay was markedly higher for patients referred from year 2000 to 2006 compared to patients evaluated for the first time from 2007 to 2016 (13.5 ± 11.54 versus 0.6 ± 1.25 years, $p < 0.0001$).

Discussion

The clinical spectrum of 46, XY DSDs is extremely variable as well as their genetic background [3, 4]. The present cohort of females with 46, XY DSD confirms that CAIS represents the most common cause. Anyway, the present series shows a higher percentage of females with CAIS in comparison with previous studies, in which they represent less than 40% of patients with 46, XYDSD [8, 9]. This elevated proportion of patients with CAIS may be due to a higher prevalence of this DSD in our country or a centre-selection bias, also related to the collaboration with the Italian patient support group (AISIA, www.aisia.org). Only large Italian multicentre epidemiological studies would highlight this aspect.

In the total group, first erroneous diagnoses were relatively frequent. The misdiagnoses were even higher in the other subgroups of patients in comparison to CAIS

Table 6 Clinical phenotype and disease management in patients of the non-CAIS group with a molecular diagnosis

Disease	No.	First visit (years)	Presentation sign	First diagnosis	Age at true diagnosis	Gonadal removal
Disorders of sex determination						
SF1 mutation	3	0	Genital ambiguity (3)	5 a reductase deficiency (1) PGD (2)	4.7 ± 7.2	2
WT1 mutation	1	0	Incidental	CGD (1)	0.2	0
SRY mutation	1	14	Primary amenorrhea	Ovarian insufficiency	16	1
DHH mutation	1	7	Inguinal hernia	CAIS	54	1
Disorders of sex differentiation						
5α reductase deficiency	9	1.7 ± 2.4	Inguinal hernia (5) Genital ambiguity (4)	CAIS (5) 5α reductase deficiency (4)	9.9 ± 10.9	6
PAIS	1	0.1	Incidental	CAIS	0.2	0
Leydig's cell hypoplasia	1	15	Primary amenorrhea	CAIS	28	1
17,20-lyase deficiency	1	5	Inguinal hernia	CAIS	23	1
17β-hydroxysteroid dehydrogenase deficiency	3	1.3 ± 1.5	Inguinal hernia (2) Inguinal hernia and clitordomegaly	CAIS (3)	3.0 ± 1.7	2

subgroup. Most of the females presenting with primary amenorrhea or inguinal hernia were suspected to have CAIS, while 5α-reductase deficiency was often the first diagnostic suspect in the patients presenting with some degrees of virlization, as mild clitoromegaly. Taken together, these data suggest a poor knowledge of the variable background of 46, XY DSD. In addition, first diagnosis was often based on clinical features more than initial fully accurate endocrine and genetic investigation. Anyway, the oldest women were evaluated several years ago, when pathogenetic background of DSD was less known and genetic techniques were less developed; then patients' "diagnostic tags" were never updated before our evaluation.

In the present cohort, the majority of the females underwent gonadal removal before a clear molecular diagnosis, suggesting that surgery was performed according to old criteria of management. In some patients, gonadal removal was done during surgery for inguinal hernia, while in other patients, early gonadal removal was performed to prevent gonadal cancer even in girls with low risk in the first decades of life [13, 14]. Gonadal removal in females with CAIS may determine poorer bone health in comparison with those with intact gonads [15]. At this regard, it may be considered that adequate oestrogen administration did not improve femoral and total body bone mineral density at a follow-up lasting until 7 years in women with CAIS [16]. Taken together, these findings suggest that better knowledge in this field must be shared and that irreversible interventions should be not done before expert evaluation and clear explanations to the people with DSD and their parents [4, 17].

Some girls came to early medical attention for discordance between the phenotypic and genotypic sex during pregnancy, as already reported [18, 19]. The growing use of prenatal genetic tests and high-resolution ultrasound allows an early detection of fetuses with genotype/phenotype sex mismatch during pregnancy or in neonatal period [20, 21]. Prenatal genetic tests, such as amniocentesis, chorionic villus sampling and non-invasive prenatal testing cell-free fetal DNA testing allow to discover chromosomal sex; ultrasound examination can permit the assessment of external genitalia features with an accuracy of 59% at 11 weeks and > 98% from 14 gestational weeks onward [22, 23]. The management of these conditions is an arising counselling problem. Specific genetic investigations could be performed prenatally—when possible—to provide parents with better information on postnatal outcome, but a complete evaluation is mandatory after delivery to reach a correct diagnosis and program a rationale management and follow-up.

Finally, only a small percentage of this series of patients have still not received a molecular diagnosis, while half of the patients of some previously cohorts has not a definitive diagnosis [10, 24–26]. Present results may be due several reasons, as large representation of females with CAIS, recent individuation of new genes involved in DSD, improvement during last years of genetic testing by the use of new techniques permitting to analyse simultaneously multiple causative genes [27]. At any rate, a long diagnostic delay occurred from presentation to definitive diagnosis in this as in other reports [8, 28, 29], underlining the difficulties in the diagnostic work-up of 46, XY DSD, mainly regarding the subgroup with gonadal dysgenesis likely related to abnormalities in the pathways of gonadal determination. The small group of present series with unknown molecular defects highlights the still incomplete knowledge about this wide spectrum of disorders. Our hypothesis is that these women with 46, XY DSD may be mostly affected by disorders of sex

determination with an unexplored genetic background. Anyway, some women of this group have been referred us before the availability of NGS techniques and they are actually lost at follow-up, thus not permitting new genetic investigations.

In our cohort, patients presenting from 2007 to 2016 had a higher rate of molecular diagnosis and a reduced diagnostic delay. This result is influenced not only by the abovementioned use of new genetic techniques, but likely by the centralization of patients in a dedicated unit. This effect may partly related to the diffusion of the conclusions of the Chicago consensus [4], that disseminated updated knowledge, true improvement for the management of people with DSD and better collaboration between doctors and patient support groups [30].

In adolescent and adult women under hormonal substitutive therapy, very heterogeneous schemes of treatments were recorded, and the compliance was not optimal in about 15% of the patients. Differences in the prescription of the oestrogen formulations and the route of administration were present, indicating that substitutive hormonal treatment largely based on personal experience of the various patients' physicians more than current available evidences. More physiological formulations, as micronized or transdermal estradiol should be preferred [31, 32]. In addition, transdermal estrogens provides a more physiologic delivery with increased bioavailability due to a reduce first-pass hepatic metabolism, while progestins prescribed to some women of the present sample should be not used in females without uterus as CAIS [33]. In addition, the use of estradiol has the advantage of measuring hormone levels in serum, differently from what happens when conjugated estrogens or ethinilestradiol are used. In a long-term therapy with estrogens having an idea of the amount of circulating estradiol is crucial for tailoring the treatment and for avoiding side-effects. Finally, the daily posology has to be individualised in each woman, and in this field, the management of women with DSD is particularly difficult, because specific trials for women with 46, XY remain to be developed on the basis of their specific genetic and endocrine background to optimize hormonal substitutive therapy. At any rate, to the choice of the therapeutic strategy has to be made considering the clinical picture and preference of each woman with 46, XY DSD to improve adherence. In addition, surveillance programs to investigate specific long-term benefits and risks of the various schemes should be done and clinical experiences among leading centres in DSD should be compared and shared to give better indications for practice on the various available treatment modalities [33].

In conclusion, the management of patients with 46, XY DSD should be made in selected centres to reduce misdiagnoses and to provide better care including full disclosure and expert psychological support [34]. Patients and their families must be clearly informed by an experienced team on their condition and on benefits and risks regarding the various aspects of management (as sex assignment, decisions regarding the gonadal removal, hormone replacing therapy). At this regard, reaching a molecular diagnosis may have benefits for understanding the natural history of a condition, identifying associated features, defining likely inheritance and chances of other family members being affected, and in the long term for understanding tumour risk [35]. Finally, it must be stressed that each newborn with the suspect of DSD must be evaluated in a specialized centre before sex assignment; inadequate and irreversible decisions may have considerable impact on the long-time quality of life of children and adolescents with DSD and their families.

Our study has some limitations, deriving from retrospective analyses of this case series over a long time period non-permitting a standardized prospective approach, at least for the oldest patients (see above), the unavailability of some clinical and endocrine data in females underwent gonadal removal before our evaluation and from the lack of a validation sample; however, it provides a significant overview of the management of people with 46, XY DSD in Italy, evidencing the main diagnostic and therapeutic concerns and some improvements in the last 20 years.

Acknowledgements The authors wish to thank the patients and their families involved in this paper and the Italian Androgen Insensitivity Support Group (AISIA) for precious and active collaboration. The endocrine nurse Sonia Stabilini (Pediatric Division, Azienda Ospedaliero- Universitaria Pisana, Pisa, Italy) must be acknowledged for her excellent clinical work and the good human qualities during follow-up of these special children and adults. Dr. A. Fogli (Laboratory of medical Genetics, Pisa) is also acknowledged for her helpful suggestions and advices.

Author contributions SB designed the study. GC, MC, BM and NT collected and interpreted the data, and drafted the manuscript. FB and MAC performed genetic analyses. SB and DP critically revisited and updated the manuscript. All authors gave final approval.

Funding None.

Data availability Data are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent to participate and publication The parents of children aging less than 18 years or directly adults had given their informed written consent before any clinical and genetic investigation.

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