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Differential Diagnosis of Disorders of Sex Development in Egypt

I. Mazen[a] O. Hiort[b] R. Bassiouny[a] M. El Gammal[a]

[a]Clinical Genetics Department, National Research Center, Cairo, Egypt; [b]Department of Pediatrics, University of Luebeck, Luebeck, Germany

Key Words
Disorders of sex development · Genetics · Androgen insensitivity · 5α-Reductase deficiency · Ambiguous genitalia

Abstract
Background: Disorders of sex development (DSD) with birth of an infant with ambiguous genitalia require medical attention to elucidate the differential diagnosis. This group of disorders is not uncommon in Egypt (1:3,000 livebirths). ***Aims:*** We want to provide an extensive review of a patient collective with ambiguous genitalia from 6 years experience at the Department of Clinical Genetics at the National Research Center in Egypt. ***Methods:*** 208 patients with ambiguous genitalia were recruited from the genetic clinic from 2000 to 2005. They were subjected to history taking, pedigree analysis. Full clinical examination, cytogenetic study, hormonal, radiological investigations, and molecular studies were performed where possible. ***Results:*** 46,XY DSD was more common than 46,XX DSD constituting 65.9% of total cases. Consanguinity was high with 61% in the affected families; however, only 21 cases had a positive family history. There was preference of male sex of rearing (regardless of karyotype), despite a severe degree of ambiguity. ***Conclusion:*** Disorders of sex development have a broad range of underlying causes also in Egypt with some preference of rare monogenic disorders. For improving diagnostic standards, the provision of centers of tertiary pediatric care is recommended for patients with DSD even in developing countries.

Introduction

Disorders of sex development (DSD) resulting in the birth of an infant with ambiguous genitalia are very disturbing for parents and families and may require immediate medical treatment in some cases, e.g. congenital adrenal hyperplasia. This group of disorders, previously termed as intersexuality, is not an uncommon entity in Egypt. A previous study has reported an incidence of one newborn with ambiguous genitalia per 3,000 livebirths [1]. Thus, DSD comprises a majority of heritable monogenic diseases in this country.

The newborn with abnormal genital development presents a difficult diagnostic and treatment challenge for the pediatrician providing care, as well as for life-time counseling [2]. As DSD represents a group of entities, heterogeneous in their etiopathogenesis and clinical manifestations, it is important that a definitive diagnosis be determined as quickly as possible so that the appropriate treatment plan can be established to minimize medical, psychological and social complications [2].

Olaf Hiort is an active member of the ESPE.

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Prof. Dr. Olaf Hiort
Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics
University of Lübeck, Ratzeburger Allee 160
DE–23538 Lübeck (Germany)
Tel. +49 451 500 2191, Fax +49 451 500 6867, E-Mail hiort@paedia.ukl.mu-luebeck.de

A previous study on 61 patients with ambiguous genitalia had shown that the 46,XX DSD diagnosis of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase (21OH) deficiency was the most common DSD-underlying etiology among Egyptians [4] as well as worldwide [5]. In Egypt, androgen insensitivity syndrome and 5α-reductase deficiency (5αRD) have been the two most frequently reported 46,XY DSD etiologies constituting 64% among all reported cases [4].

The purpose of the present study was to provide an extensive review of the clinical characteristics as well as endocrine and genetic aspects of Egyptian patients with ambiguous genitalia presenting to a single institution. Moreover, this study aimed at showing the great magnitude of disorders of abnormal sex development in Egypt.

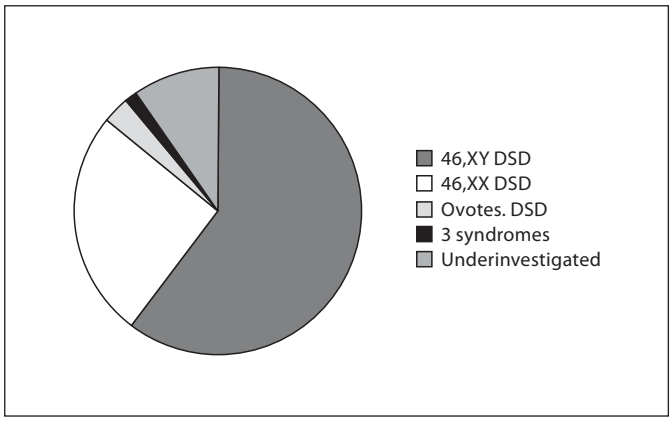

Fig. 1. Prevalence of different entities of DSD in this series.

Subjects and Methods

Two hundred and eight Egyptian patients with ambiguous genitalia presented at the genetic clinic of the National Research Center during the period 2000–2005. They were subjected to extensive questioning with history taking, age of initial presentation, family history including consanguinity, ethnic origin of parents, and reasoning for sex of rearing. Full clinical examination included assessment of associated dysmorphism or congenital anomalies. The genital examination and ambiguity were classified according to Quigley et al. [6]. Cytogenetic studies were performed including the G-banding technique with 50 metaphases counted. Most prepubertal patients with 46,XY DSD were subjected to hormonal and biochemical assays of both basal and post-hCG stimulation of serum testosterone, Δ4-androstenedione, and dihydrotestosterone. Ratios of androgens were calculated for assessment of androgen biosynthesis enzyme defects. In patients with 46,XX karyotype, serum levels of electrolytes, cortisol, ACTH, 17α-OH-progesterone, dehydroepiandrosterone sulfate, and progesterone were investigated. Imaging examinations included pelvic ultrasound and genitography. Laparoscopy, laparotomy and gonadal biopsy were performed in selected cases. Molecular genetic analysis of the coding region of the SRD5A2 gene, the AR gene and the 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) gene was done in 25 cases of 46,XY DSD with suspicion for these disorders from clinical and laboratory assessment.

Results

Fifty-three patients had a 46,XX DSD (28%), and 124 had a 46,XY DSD (65.9%), 3 cases had a complex syndrome with associated malfomations, 6 cases had ovotesticular DSD, and 20 cases were examined for ambiguity, but could not be classified because they did not complete investigations due to its high cost (table 1; fig. 1).

Their ages at presentation were 1 month to 2 years in 120 cases (57.7%), 2–10 years in 52 cases (25%), and 10–29 years in 35 cases (16.8%). One case presented unexpectedly late at the age of 46 years. Familial cases were reported with 4 cases from two families with 46,XX DSD, and 17 cases from 7 families with 46,XY DSD.

The 208 patients were classified with regard to consanguinity and origin of parents (table 1). The consanguinity rate was 62.8% among all presented cases with DSD (72% in 46,XX DSD and 59.5% in 46,XY DSD; table 1). 46,XY DSD was more common than 46,XX DSD constituting 65.9% of the total cases (fig. 1).

46,XY DSD was more common in patients coming from upper Egypt followed by lower Egypt and Giza (table 1). Moreover, there was a preference of male sex of rearing despite severe degree of ambiguity (Quigley 3–4) (table 2).

Among 46,XX DSD, 40 cases were diagnosed as CAH due to 21-hydroxylase deficiency (75.4%) suggested by increased testosterone and its precursors if measured. Salt losing cases were noted in 13 cases. Other enzyme deficiencies (e.g. of 11β-hydroxylase or 3β-hydroxysteroid dehydrogenase) were not reported. These diagnoses may have been missed because of lacking appropriate biochemical analysis. Ten cases (18.8%) had no definitive diagnosis, they had a 46,XX karyotype and virilization of the external genitalia. The pelvic ultrasound revealed mullerian derivatives, but unfortunately no hormonal assessment was done as it was unaffordable for the patients. These may be the patients with above deficiencies. Three cases were classified as 46,XX ovotesticular DSD based on histological findings (5.6%).

Table 1. Classification of ambiguous genitalia in correlation to consanguinity and origin of parents

Classification	Total	Origin of parents				Consanguinity	
		UE	Giza	LE	Sinai	+	%
46,XXDSD	50	17	12	20	1	36	72
46,XX testicular DSD	3	1	2	–	–	3	100
46,XY DSD	94	34	28	29	3	56	59.5
Gonadal regression	4	–	3	1	–	1	25
Ovotesticular DSD	6	1	4	1	–	2	33.3
Sex chromosome DSD	2	1	1		–	1	50
Syndromes	3	2	–	1	–	2	66.6
Unclassified	26	4	12	10	–	13	50
Under investigation	20	7	6	7	–	11	52.3
Total	208	67	68	69	4	125	60

UE = Upper Egypt; LE = lower Egypt.

One hundred and twenty-nine patients had 46,XY DSD. Forty-nine patients did not complete the investigations and did not receive a definitive diagnosis. Of the remaining 75 patients, 45 had suspected disorders in androgen synthesis or action. Thirty patients were diagnosed by hormonal assay, while molecular study of the SRD5A2 (table 3) and AR genes (table 4) were performed in the remaining 15 patients and revealed different mutations in either gene. Interestingly, the measured values of testosterone as well as the T/DHT ratios did not reveal the underlying diagnosis of an AR or SRD5A2 gene defect in all cases. 5α-Reductase deficiency was found to be the most prevalent cause of disorders in androgen synthesis or action, and G34R mutation is a relatively common mutation in Egyptian patients with 5α-reductase deficiency (table 3).

Twenty-six cases with 46,XY DSD remained unclassified. There were no significant molecular genetic findings in 10 of these patients after sequencing of both AR and SRD5A2 gene. Only 1 patient was proven with 17β-hydroxysteroid dehydrogenase deficiency, as confirmed by sequencing analysis of the HSD17B3 gene with a homozygous mutation in exon 1 inducing a leucine to proline exchange in codon position 14. We would suspect testicular dysgenesis in the remaining patients, but gonadal regression was diagnosed and confirmed by laparoscopy only in 4 cases. Testicular tumors have not been reported in any case.

Sex chromosome DSD with mixed gonadal dysgenesis was found in 2 cases. One patient was reared as a boy and carried a mosaic karyotype 45,X/46,XY, the second pa-

Table 2. Phenotype in correlation with sex of rearing in 188 cases of DSD of this series

Quigley score (1995)	XX karyotype (total n = 59)	XY karyotype (total n = 129)	Sex of rearing	
			M:F	uncertain
1	4	4	8:0	–
2	2	8	9:1	–
3	19	57	54:19	3
4	25	39	31:29	4
5	7	10	1:14	2
6	2	11	0:13	–

Table 3. Clinical, endocrine, and molecular genetic results of patients with SRD5A2 mutations

Case No.	Age	Phenotype	T, ng/ml	T/DHT	Mutation
1	4 months	Gr 6	3.5	22.6	G34R
2	4 months	Gr 6	2.7	9	N160D
3	5 months	Gr 4	3.5	ND	G34R
4	9 months	Gr 6	1.5	98.5	Y235F
5	4 years	Gr 4	3.8	ND	G34R
6	5.4 years	Gr 4	4.8	35.6	G34R
7	14 years	Gr 4	3.6	160	N160D
8	16 years	Gr 3	16	17.7	G34R
9	20 years	Gr 4	5.2	78	A62E
10	28 years	Gr 3	4.6	6.7	G196S

Note the highly variable T/DHT ratio, which was suspicious of 5α-reductase deficiency only in 5 of 8 cases, where laboratory analysis was possible.

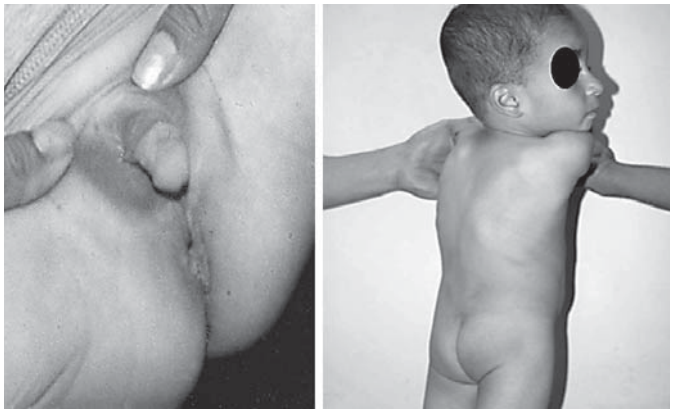

Fig. 2. A 2-year-old patient with vertebral anomaly and ambiguous genitalia (VATER association).

Table 4. Phenotype, biochemical and molecular results of 6 patients diagnosed with androgen insensitivity syndrome

Case No.	Age	Phenotype	T ng/ml	T/DHT	Mutation
1	6.5 years	Gr 4	3.8	17	R840S
2	7 months	Gr 4	4.9	8.9	P817A
3	2 weeks	Gr 6	14	100	F804L
4	5 months	Gr 6	10.7	4.3	frame shift codon 241 (deletion of C) stop 256 in exon 1
5	2.2 years	Gr 3	3.3	50	A596T

Again, as seen in table 3, in 2 patients the T/DHT ratio was rather suspicious of 5α-reductase deficiency.

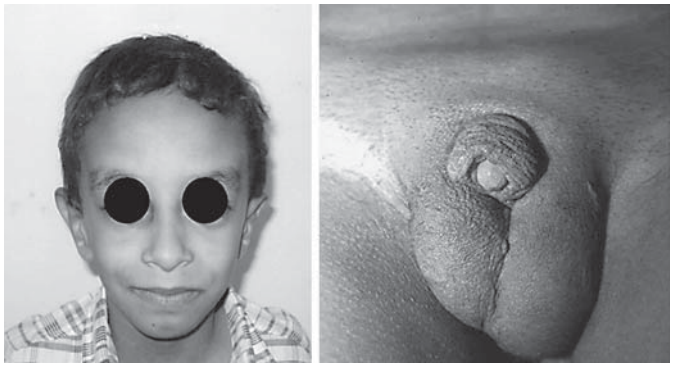

Fig. 3. A 5-year-old male with triangular face, hypertelorism, high forehead and ambiguous genitalia diagnosed with Aarskog syndrome.

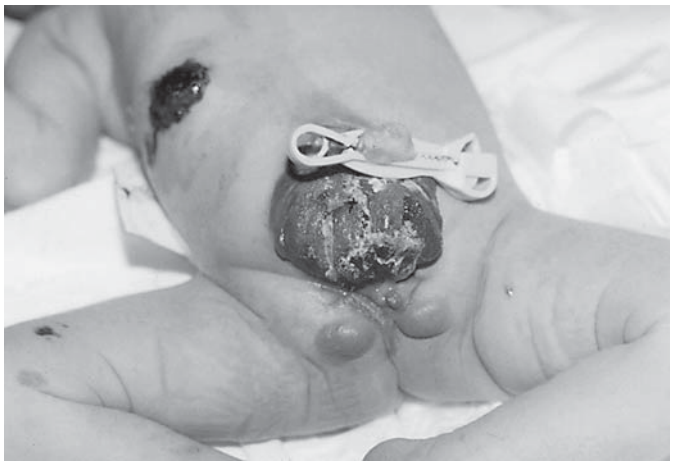

Fig. 4. A 46,XX newborn with ambiguous genitalia associated with imperforate anus and ectopia vesica.

tient was reared as a girl and had a 45,X/46,XY/47,XXY karyotype. Three patients were diagnosed with multiple congenital anomalies and classified as having syndromes. They were clinically diagnosed as VATER association (fig. 2), Aarskog syndrome (fig. 3), and ambiguous genitalia associated with imperforate anus and ectopia vesica (fig. 4). In addition, ovotesticular DSD was found in 6 cases (3.1%).

Discussion

Human sex development is a highly complex process under the control of multiple genes and hormones. Both male and female sexual differentiation follow a timetable of events with predictable development of the gonads, internal genital ducts, and the external genitalia. Completion of sexual maturation occurs during the pubertal years. Abnormalities of sexual differentiation may occur at any step along the way [3].

These abnormalities may result in abnormal differentiation of the gonads, the internal genital ducts, or the external genitalia. The end result of these abnormalities produces predictable clinical syndromes. While many of these defects of sexual differentiation are evident at birth, others will not be identified until puberty at which time the patient may manifest aberrant external maturation or may remain sexually infantile [2]. Genital ambiguity in a newborn represents a true social and psychological as well as sometimes also medical urgency. Establishing a precise diagnosis in DSD is just as important as in other chronic medical conditions that have lifelong consequences.

In the series reported here, the majority of infants were 46,XY DSD, more common than 46,XX DSD. While in 46,XX DSD, we suspect congenital adrenal hyperplasia to be most common, the differential diagnosis in many cases of 46,XY DSD remains elusive. Despite the high consanguinity rate, monogenic autosomal-recessive disorders could only be proven in a minority of the cases. This may be attributed to the lack of biochemical and molecular genetic assessment, but could also reflect the overall diversity of the underlying disorders. In only 23 patients could a strong familiar background suggestive of an inherited disease be established. This raises the question if environmental factors or other unknown genetic and epigenetic factors are involved in the magnitude of DSD.

In our study, 46XY DSD was more common than 46,XX DSD while Al-Mutair et al. [7] in Saudia Arabia retrospectively reviewed a total of 120 medical records of patients with a primary indication of ambiguous genitalia during the period 1989–1999. CAH was the underlying cause of ambiguous genitalia in 41 of 63 patients with ambiguity due to endocrine causes. In 57 patients, ambiguous genitalia were due to congenital developmental defects. The low rate of 46,XX DSD reported here for Egypt may be due to a selection bias of patients presenting to the National Research Center. One assumption may be that patients with severe 46,XX DSD due to CAH could have died from a crisis being misdiagnosed as an infectious gastroenteritis before coming to medical attention.

Also in other studies, a relatively higher incidence of 46,XY DSD was reported [8–11]. However, this may again be due to selection criteria. The high number of patients of our study in a relatively short period (6 years) compared to the study done by Al-Agha et al. [8] (51 patients in 17 years, Nimkarn et al. [9] (109 patients in 22 years) and Alvarez-Naza et al. [10] (214 patients in 26 years) reflects the great magnitude of genital abnormalities in Egypt and its impact on the society.

Therefore, we recommend study of the actual prevalence of DSD in Egypt and other developing countries. To serve these patients and their families, diagnostic algorithms have to be established, and the initiation of early neonatal screening for CAH (17α-hydroxyprogesterone) should be considered. Also, regular molecular genetic analysis needs to be established in suspected androgen insensitivity versus 5α-reductase type 2 deficiency. These disorders were not distinguishable on the basis of the biochemical laboratory measurements, as T/DHT ratios were highly variable despite stimulated testosterone values (tables 3, 4). In 5α-reductase deficiency, the G34R mutation was again proven to be relatively common [11].

The age at diagnosis was late in most cases, one case presented at 46 years old with ultimate psychological problems. To our knowledge, it is rare to seek medical advice at such a late age; this is mainly due to social pressure, religious impact and inexperienced medical service.

In Egyptian society, female infertility precludes marriage, which also affects employment prospects. This drives the family of the patient to rather choose the male sex to create economic independence. This assumption was supported in our study by the preference of male sex of rearing despite the severity of genital ambiguity (table 2). A structured clinical study as currently performed in Western societies could assess the quality of life and well-being of patients with DSD also in societies with different cultural and religious as well as economic backgrounds [12–14].

Early and expedient diagnosis is essential to avoid life-threatening crises, to determine sex-of-rearing, to provide for appropriate immediate and long-term treatment, and to assure that the family is provided with adequate genetic counseling. To provide this adequate treatment, we recommend providing centers of tertiary pediatric care with a consistent and standardized model of care for patients with DSD even in developing countries as Egypt.

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