The Egyptian Journal of Medical Human Genetics (2012) **13**, 281–289

Ain Shams University

The Egyptian Journal of Medical Human Genetics

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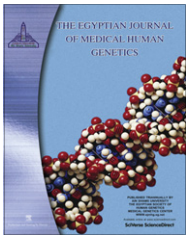

ORIGINAL ARTICLE

Cytogenetic and molecular study in intersex

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Received 8 May 2012; accepted 4 June 2012
Available online 6 July 2012

KEYWORDS

Developmental Sex Disorders;
SRY gene;
CYP21 gene;
Genetic counseling;
Cortisol;
17-Hydroxyprogesterone

Abstract "Disorders of Sex Development" (DSD) is the state of a person whose sex chromosomes, genitalia and/or secondary sex characteristics are determined to be neither exclusively male nor female. The aim of this work was the genetic study of patients with ambiguous genitalia and genetic counseling of these cases. This study was conducted in the period from November 2007 to February 2009. Cases were obtained from the Genetics and Endocrine Units, Pediatric department, Faculty of Medicine, Menoufiya University, Egypt. Thirteen cases with ambiguous genitalia were studied, 10 genetic female and 3 genetic male patients. Their ages ranged from the first week to eight years. All cases were subjected to the following: Detailed history, thorough clinical examination, routine investigations, hormonal, imaging studies, cytogenetic study and molecular study. Sequencing for both the SRY and the CYP21 genes and genetic counseling were performed. Study revealed that among our patients, cases number 2 and 11 were mainly presented as salt losing crisis. Cases 5 and 13 were presented with hirsutism of the external genitalia and upper and lower limbs. Eight cases (61.5%) had a positive consanguinity and Cases 3 and 4 were sisters. Hormonal study revealed that (adrenocorticotropic hormone) ACTH, was within the normal range with a mean 83.14 pg/ml ± 53.6 pg/ml for all patients. Serum Cortisol and 17-OHP (17-hydroxyprogesterone) were elevated in all patients. Karyotype using G-banding showed that there were no apparent anomalies in the sex chromosomes. We found four cases (30.8%) with different mutations in the SRY gene at codon Q57R and S143C. As for the CYP21 gene, we found a variety of deletions in size and site within the structure of the gene in 85.4% of cases leading to alteration in the function of the CYP21 gene which ultimately lead to congenital adrenal hyperplasia (CAH) in these cases.

1. Introduction

Genital anomalies are estimated to occur in 1 in 4,500 births. The most common cause of ambiguous genitalia in the newborn is congenital adrenal hyperplasia (CAH) – 1 per 15,000 live births. CAH appears to be more common in those of European Jewish, Hispanic, Slavic and Italian descent.

The ability to diagnose Developmental Sex Disorders (DSD) has advanced rapidly in recent years. In most cases today, clinicians can promptly make an accurate diagnosis

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Peer review under responsibility of Ain Shams University.

ELSEVIER Production and hosting by Elsevier

1110-8630
http://dx.doi.org/10.1016/j.ejmhg.2012.06.003

and counsel parents on therapeutic options [1]. The ultimate goal in any management strategy is to provide a framework that will allow the affected child to develop into a well-adjusted psychosocially stable individual who identifies with and is happy in the assigned sex [2]. Factors that influence sex assignment include diagnosis, genotype, genital appearance, surgical options, need for lifelong replacement therapy, potential for fertility, views of the family, and sometimes circumstances relating to cultural practices [2–4].

The aim of this work was the genetic study (both cytogenetics and molecular genetics) of DSD cases with special prominence of Karyotyping and DNA extraction, PCR, gel electrophoresis, SSCP and automated DNA sequencing for both the SRY and the CYP21 genes and genetic counseling of DSD cases and determination of the causes and/or risk factors in each case.

2. Patients and methods

2.1. Patients

The study was conducted on 13 children with ambiguous genitalia. They were selected from Menoufiya university hospitals, Genetics and Endocrine Unit, Pediatric department, Egypt, during the period from 2007–2009. They were 10 genetic females and 3 genetic males. Their ages ranged from the first week up to eight years.

2.2. Methods

All studied cases were subjected to the following: A detailed history with stress on maternal exposure to androgens, virilization symptoms in the mother or the use of phenytoin and the presence of similar conditions in the family and family pedigree.

A thorough clinical examination including general examination of all systems, body hair distribution (hirsutism) and skin examination (signs of dehydration).

External genitalia examination with stress on: Phallus: size, degree of differentiation, length, ventral curvature, appearance of the foreskin and location of the urethral meatus. We also used Prader scoring system for this [5]. The scrotum or labia: were examined for separation and/or fusion between the two halves of the labioscrotal tissue. Scrotal rugea or labioscrotal folds with increased pigmentation were noted. Tanner staging was established and it included examination of the breast in females, the external genitalia in males and pubic hair in both.

Gonadal examination with stress on its site: (within the labioscrotal tissue or outside it), fully descended or not. Also anthropometric measurements were obtained.

Routine investigations included complete blood picture, serum electrolytes and glucose levels which were closely monitored in cases with salt losing congenital adrenal hyperplasia (CAH) especially sodium (Na) and potassium (K).

Hormonal investigations included serum levels of ACTH (Adrenocorticotropic hormone), Cortisol and 17-OHP (17-hydroxyprogesterone) [6,7] .

Imaging studies including genitogram and abdomino-pelvic ultrasound, CT and/or MRI were needed to determine the presence and/or absence of internal genital structures (such as undescended testes).

Cytogenetic study included chromosomal karyotype using G-banding [8].

Molecular study: focused mainly on the SRY gene and the CYP21 gene. The SRY gene is the main regulator of sexual differentiation, while the CYP21 gene is the main gene affected in CAH, the commonest form of Developmental Sex Disorders (DSD). The following steps were applied:

a. DNA extraction [9].
b. PCR (Polymerase Chain Reaction) [10].
c. Gel electrophoresis [11].
d. SSCP (Single-strand conformational polymorphism) [12].
e. Automated DNA sequencing [13].

And lastly genetic counseling was done to all families in order to prevent, avoid or ameliorate the disorder [14].

3. Results

The results of this study were illustrated in Tables 1–5 and Figs. 1–4.

Generally, cases number 2 and 11 were mainly presented as salt losing crisis in the form of severe, recurrent vomiting, diarrhea and dehydration.

Cases 5 and 13 were presented with hirsutism of the external genitalia and upper and lower limbs. While cases number 1, 3, 4, 6 to 10 and 12 were mainly presented as ambiguous genitalia. Case number 7 was presented by clitoromegaly with no palpable gonads (as shown in Fig. 1) and case number 9 was presented with micropenis with bilateral undescended testes (as shown in Fig. 2).

We must note that the patient number 13 was not subjected to the molecular study because the patient died before we could obtain a blood sample.

Demographic data of studied patients (Table 1) showed that eight cases (61.54%) of the 13 (cases number 1–7 and 11) had positive consanguinity. Cases 3 and 4 were sisters. Most of the cases had irrelevant maternal history.

Anthropometric measurements (Table 2) showed that 4 patients were less than the 5th centile (2 females, 2 males: cases 2, 8, 11 and 12), 6 patients were within the normal range (5 females, 1 male: cases 3–6, 9 and 10) and 1 female (case number 1) was above the 95th centile as regards weight, height and head circumference.

Table 3 showed that Tanner staging in most cases were in the 1st stage.

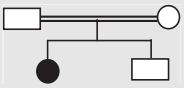
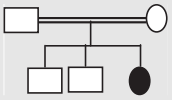
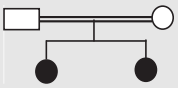
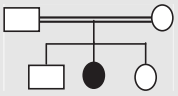
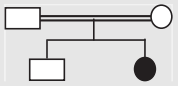
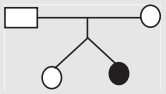
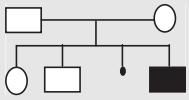
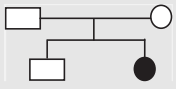
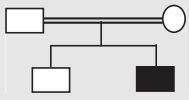
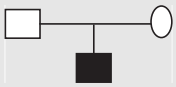
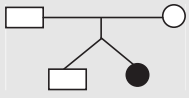
Case 1 was a female with a Tanner staging of B1, P3, and A1, while cases 5 and 13 were females with tanner staging of B1, P3 and A2. Also this table showed that clitoromegaly was the most common presentation in our patients (77%).

As regards laboratory investigations (Table 4), hormonal assay showed that adrenocorticotropic hormone (ACTH) was within the normal range with a mean 83.14 pg/ml ± 53.6 pg/ml for all the patients. Serum Cortisol and 17-OHP were elevated in all the patients:

- Cortisol mean levels (±SD) were *A.M* sample: 59.8 μg/dl (±62.45 μg/dl) and

  P.M sample: 40.58 μg/dl (±11.8 μg /dl).

Table 1 Demographic data of the studied cases.

Serial no.	Age	Gender (by Karyotype)	Birth order	Maternal age (years)	Paternal age (years)	Relevant maternal history	Relevant family history	Pedigree
1	5 yrs	♀	1st of 2	23	25			
2	4.5 mth	♀	3rd of 3	28	30			
3	5 years	♀	1st of 2	24	30	Toxoplasmosis	Younger Sister	
4	2.5 yrs	♀	2nd of 2				Older sister	
5	5 yrs	♀	2nd of 3	24	37			
6	1.5 yrs	♀	2nd of 2	28	33			
7	6 days	♀	3rd of 3	27	31			
8	1 day	♀	2nd of twin	30	39	PET & PROM > 36 h		
9	8 day	♂	3rd of 3	35	45	Gestation D.M & PROM (10 days)		
10	3 days	♀	2nd of 2	26	27	Rheumatic Fever and B.A		
11	1 mth	♂	2nd of 2	21	29			
12	1 mth	♂	1st born	20	24	Recurrent fever due to tonsillitis treated by antibiotic under doctor supervision	One cousin: hydrocele. – Another cousin:umbilical and inguinal hernia	
13	8 yrs	♀	2nd of twin	32	40	Graves' disease (treated during pregnancy)		

B.A: Bronchial Asthma; mth: months; yrs: years; PROM: Premature rupture of membranes; PET: Pre-eclamptic toxemia; ♀: female; ♂: male.

Table 2 Anthropometric measurements of the studied cases.

Serial no.	Weight		Height		BMI (kg/m^2)	U.S (cm)	L.S (cm)	U/L ratio	Arm span (cm)	Head circ.	
	kg	%	cm.	%						cm	%
1	25	>95	119.5	>95	17.5	59.5	60	0.99	120	51	>95
2	3	<5	50	<5	12	31.5	18.5	1.71	50	35	<5
3	21	90	110	50–75	17.35	58	52	1.12	110.5	51	50–75
4	14.5	75–90	95	75–90	16	54	41	1.32	94.5	50	75
5	20	75–90	114	90	15.4	60	54	1.11	114	51.5	75
6	12	75–90	83	75	17.42	49.5	33.5	1.48	84	49	90
7	3	5–25	55	75–95	11.09	31	24	1.29	53	35	50
8	2.2	<5	40	<5	13.75	21	19	1.11	41	32	<5
9	4	75–95	48	5–25	17.36	28	20	1.4	47	33	<5
10	3.5	50–75	53	75–95	12.46	31	22	1.41	51	33	10
11	2	<5	50	5–25	8	30	20	1.5	46	29	5–10
12	3	<5	47	<5	13.58	30	17	1.76	48	35	<5
13	21	5–10	125	25–50	13.44	63	62	1.02	125	51	10–25

cm: centimeters; kg: kilograms.

- 17-OHP mean levels (±SD) were *Neonates:* 1.017 ng/ml (±0.61 ng/ml) (cases 7–12) and *Child:* 15.71 ng/ml (±2.024 ng/ml) (cases 1–6 and13).

Tables 5 and 6 showed that true hermaphrodite can be diagnosed in two cases (case number 2 and 12) (15.5%) and the other 10 cases (84.5%) were diagnosed as CAH.

Fig. 3 demonstrates that the SRY gene was not detected in all the female patients except for case number 2 in which the SRY gene was detected but in an abnormal position along the run (at 900 bp). While in the male patients, the SRY gene was detected in all of them at 650 bp i.e. normal position. But, in case number 11 it was in an abnormal position (at 900 bp). PCR products were scanned for mutations in the SRY gene using single strand conformational polymorphism (SSCP) analysis. All four cases (case 2, 9, 11 and 12) showed aberrant migration in the SSCP assay (as shown in the Fig. 4).

By direct sequencing of PCR products, we have identified three different point mutations in the SRY gene. The first point mutation was the replacement of glutamine with an arginine residue at amino acid 57 in the open reading frame, just outside and upstream of conserved DNA-binding motif called HMG box in patients 9, 11 and 12. The second mutation was the replacement of a tyrosine residue with a phenylalanine residue at amino acid 127 within the HMG box in patients 2, 9, 11 and 12. The third point mutation was the replacement of the serine residue at codon 143 with a cysteine residue, just outside but downstream of the HMG box sequence in patients 2, 9, 11 and 12. The Y127F variant was found in patients 2, 11 and 12. These data suggest that the Y127F variant is a mutation and not a common polymorphism. No nucleotide substitution mutations were found in our preliminary SRY sequencing from 5 normal males excluding the possibility of a polymorphism.

The CYP21 gene is located on the short arm of chromosome 6 (6p21.3). CYP21 had a variety of deletions within the structure of the gene in cases 2, 4, 6, 9, 11 and 12. Other cases had substitution mutations (cases 1–8) where Valine 282 → Leucine. Only one case (case 10) had a normal structure of the CYP21gene.

4. Discussion

In our study, 13 cases were studied of which 10 were confirmed as females and 3 as males cytogenetically. This is consistent with the finding of Anhalt [15] who declared that the most common presentation of DSD was females (70% of cases). The maternal history was irrelevant to the disease, as none of the mothers had a disease before or during the pregnancy. As for the family history, it was only relevant in one case where both the sisters were affected by DSD. This was consistent with the approach taken by Bidarkar [16] who recommended that the doctor should obtain a full history from the parents and/or the patient, with the revision of the medical background of the family, both paternal and maternal side. Also, he recommended that a family pedigree should be obtained and structured. Positive consanguinity was found in 8 cases of our 13 (61.54%). Also, cases 4 and 5 were sisters. Shawky et al. [17] found a consanguinity rate of 35.3% in Egypt which helps to accumulate deleterious genes in families. This means that the high prevalence of consanguinity in our cases is a risk factor in DSD cases which was also reported by Hashem [18]. All of this is constant with the fact that CAH (the commonest type of DSD) is autosomal recessive [19,20].

In this study, the presenting manifestation was salt-losing crisis in two cases (one apparent female, and one apparent male), hirsutism in another two apparent female cases and the remaining nine cases were presented as ambiguous external genitalia. All these presentations were in agreement with the findings of Hendren [21] who declared that the signs of DSD vary from the more obviously apparent, as ambiguous external genitalia, to the outwardly invisible, as in cases with salt-losing crisis. As for the anthropometric measurements of the cases, 4 patients were less than the 5th centile, 6 patients were within the normal range and 1 female was above the 95th centile. This was constant with the findings of Boehmer [22] who recommended serial anthropometric measurements to insure that salt wasting crisis did not cause the child to suffer from stunted growth. As regards the Tanner staging, most cases were in the 1st stage except for 3 cases. That meant that most cases were obtained in the early onset of the disease. This was in

agreement with the findings of Emans [23] which is an indicator of the hormonal effect on secondary sex characteristics. The external genital manifestations of the female patients were mainly as clitoromegaly (76.9%) with no palpable gonads and three of them (30%) had abnormal growth of pubic hair. Also, as for the imaging study of the studied group using pelvi-abdominal ultrasound and MRI, 9 of the 10 females (90%) had small infantile internal genitalia, while in the remaining one (10%) no uterus or testis was found. But on the other hand, this case had a structure like the root of the penis on pelvi-abdominal ultrasound. All these findings were consistent with the findings of Bidarkar [16]. Bidarkar explained that the main external findings in any apparent female case of DSD may present with a wide range of presentations beginning from clitoromegaly, fused labia, a concealed vagina or even an abnormal position of the urethra can be demonstrated. As for the male patients, the first male patient had a micropenis with bilateral undescended testis. The second male patient had a severe hypospadias with a urethral opening at the tip of the phallus with bilateral descended testis. In both patients, pelvi-abdominal ultrasound revealed that there were no internal female genitalia. All of these previous findings were consistent with the findings of Nicolino [24]). Nicolino elucidated that the external genital findings in any apparent male case of DSD may present with an extensive variety beginning from unrecognizable male external genitalia, undescended testes, micropenis, small scrotum with separation or a misplaced urethral opening. The third male had a micropenis with 2 urethral openings one at the tip of the phallus and the other dorsally at the base. Also, there was a direct inguinal hernia with hydrocele, more on the right side with palpable gonads. The pelvi-abdominal ultrasound revealed a uterine-like structure which was confirmed to be a uterus via MRI. When both female and male genitalia are detected, the patient should be regarded as true hermaphrodite [7]. ACTH was within the normal range, Serum Cortisol and 17-OHP were above the normal range in all the cases. The measurement of these three hormones especially 17-OHP is crucial for the construction of the diagnosis of CAH [6,7].

The genetic study was divided into two main categories: karyotyping and molecular study. Karyotyping of the cases revealed that all the cases were either apparent females with 46,XX chromosomes or apparent males with 46, XY chromosomes i.e., there were no apparent anomalies in the sex

Table 3 Clinical findings in the studied group.

Serial no.	Genitalia	Tanner			N.B
		Breast/ Penis	Pubic hair	Axillary hair	Hair distribution
1	Clitoromegaly with a single opening and pubic hair growth	B1	p3	A1	Temporal recession
2	Clitoromegaly	B1	P1	A1	
3	Clitoromegaly with a single opening and no palpable gonads	B1	P1	A1	
4	Clitoromegaly with a single opening and no palpable gonads	B1	P1	A1	
5	Clitoromegaly with a single opening with hair growth over the mons veneris, no palpable gonads	B1	P3	A2	
6	Clitoromegaly with persistent urogenital sinus and no palpable gonads	B1	P1	A1	
7	Clitoromegaly with no palpable gonads	B1	P1	A1	
8	Clitoromegaly with fusion of labia minora and no alpable gonads	B1	P1	A1	
9	Micropenis with bilateral descended testis	P1	P1	A1	
10	Clitoromegaly with no palpable gonads	B1	P1	A1	
11	Severe hypospadias with no palpable gonads				
12	Direct inguinal hernia with hydrocele (more on the right side) with a micropenis with 2 openings (one on the tip of the phallus and the other at the root, posteriorly). Scrotal sac contains testis (at least one on the right side was felt by palpation)	P1 P1	P1 P1	A1 A1	
13	Clitoromegaly with sparse hair growth	B1	P3	A2	Hirsutism of the UL, LL & mustache area

B 1: Breast papillae elevation only;
P1: Either prepubertal penis or prepubertal pubic hair (Villus hair, no coarse or pigmented hair.);
P2: Minimal, coarse, pigmented hair at base of penis or on the labia;
P3: Coarse, dark curly hair spread over the pubis or over the mons pubis;
A1: No axillary hair;
A2: Scanty growth of axillary hair.

Table 4 A summary of the findings in the female patients.

Serial no.	Provisional diagnosis	Age	Gender (Karyotype)	Hormonal assay			Imaging study		Final diagnosis
				ACTH (pg/ml)	Cortisol (μg/dl)	17-OHP (ng/ml)	Abdominopelvic U/S	Others	
1	Ambiguous Genitalia	5 Y	♀(46,XX)	45	AM = 28.5 PM = 31	16.2	Normally developed female organs		*CAH*
2	Ambiguous Genitalia	4.5 M	♀(46,XX)	37.5	AM = 32 PM = 36.6	15	- Female organs cannot be detected. Structure like the root of penis with no detectable testis		*True hermaphrodite*
3	Ambiguous Genitalia	2.5 Y	♀(46,XX)	33	AM = 29.8 PM = 42.9	13.4	Well defined retrovasical structure (1.8 × 1 cm) seems to be a uterus	*MRI:* small structure with in the pelvis, but no definite uterus or testicles was found.	*CAH*
4	Ambiguous Genitalia	5 Y	♀(46,XX)	36	AM = 160.6 PM = 72	16.5	Normally developed uterus but small in size	*MRI:* infantile uterus with visualized both ovaries	*CAH*
5	Hirsutism	5 Y	♀(46,XX)	39.5	AM = 30 PM = 32.4	14.9	Visible uterus and ovaries	*Genitogram:* visualized uterus and vagina.	*CAH*
6	Ambiguous Genitalia	1.5 Y	♀(46,XX)	29.8	AM > 50 PM > 50	> 20	Visible uterus and ovaries	*MRI:* infantile uterus (0.5 × 2.5 × 0.5 cm).	*CAH*
7	Ambiguous Genitalia	6 D	♀(46,XX)	146	AM = 32 PM = 35.4	2	Normal infantile uterus		*CAH*
8	Ambiguous Genitalia	1 D	♀(46,XX)	152	AM = 29 PM = 7.1	0.5	Normal infantile uterus		*CAH*
9	Ambiguous Genitalia	3 D	♀(46,XX)	156	AM = 37.2 PM = 39	1.4	Normal infantile uterus		*CAH*
10	Hirsutism	8 yrs	♀(46,XX)	35	AM = 231.2 PM = 51.9	14	Normally developed but small female organs		*CAH*

ACTH: adrenocorticotropic hormone, 17-OHP: 17 hydroxyprogesterone.
U/S: ultrasound, CAH: congenital adrenal hyperplasia.
MRI: magnetic resonance imaging.

chromosomes. Karyotyping is essential for provisional diagnosis and classification of the DSD cases [25].

As regards the molecular studies, the main genes that we have focused on were the SRY and the CYP21 genes. That is because the male genital differentiation mainly relies on the SRY gene and the most common cause of DSD in females is CAH (congenital adrenal hyperplasia) which is mainly caused by a defect in the CYP21 gene. We found four cases with different mutations in the SRY gene at codon Q57R and S143C. Both these mutations lie just outside the highly conserved HMG box. Also, we found that the polar neutral amino acid, glutamine, is replaced with a polar charged arginine in patients 9, 11 and 12. It is possible that with this mutation in place, the mutated SRY protein may not be able to enter the nucleus to elicit the male gene expression or it may have disrupted the nuclear localization signal necessary to perform male gene expression. The polar neutral amino acid serine is found to be replaced with a neutral and non-polar Cysteine at codon 143 in four patients (2, 9, 11 and 12). This change may result in an altered SRY protein which may have lost some of its stabilizing potential [27]. Any mutation in the SRY gene may cause a defect in its function and that it is hypothesized that the regions outside the HMG box might be required to stabilize protein binding and to generate specificity by helping to discriminate between the protein–protein interactions [26,27]. As for the case number 2, she was an apparent female with clitoromegaly and with no internal female or male genitalia except for a structure like the root of penis that was detected with abdomino-pelvic U/S. Her karyotyping was 46,XX and yet she carried the SRY gene which was found to have several mutations as described above. The importance of the SRY gene appears when reciprocal translocation of the SRY gene between Y and X chromosome takes place which may result in 46,XX with a male genitalia [28].

As for the CYP21 gene, we found that our studied cases had a variety of deletions in size and site within the structure of the gene (cases 2, 4, 6, 9, 11 and 12) which was mainly in the terminal part of the gene. Other cases had substitution mutations (cases 1 to 8) where Valine 282 → Leucine. Only one case (case 10) had a normal structure of the CYP21 gene. These deletions in the gene caused alteration in the function of the CYP21 gene which ultimately caused CAH in these cases. The commonest type of CAH is the deficiency of 21 hydroxylase (21-OH) which is carried on two genes – CYP21 (which is the active gene) and CYP21P – and that any malfunction in CYP21 will cause hyperfunction and hyperplasia of the adrenals, leading to CAH [7,25].

As for Genetic counseling, the patients and their families were advised of the consequences and nature of the disorder, the probability of developing or transmitting it. The options were open to them in management and family planning in order to prevent, avoid or ameliorate DSD. This complex process was seen from the diagnostic and the supportive aspects. The culture of the Middle Eastern families greatly influenced our work. This was evident during obtaining the material for this study. The parents' main concern was the identification of the child's gender, not for the relief of this condition, but for the fear of the stigma associated with gender re-assignment. The spectrum of DSD can pose a diagnostic dilemma and

Table 5 A summary of the findings in male patients.

Serial no	Provisional diagnosis	Age	Gender (Karyotype)	Hormonal assay			Imaging study		Final diagnosis
				ACTH (pg/ml)	Cortisol (μg/dl)	17-OHP (ng/ml)	Abdominopelvic U/S	Others	
9	Ambiguous Genitalia	8 D	♂(46,XY)	135	AM = 42 PM = 35	0.7	No internal female genitalia were detected.		*CAH*
111	Undescended Testes	1 M	♂(46,XY)	115	AM = 31 PM = 33.8	1.1	No internal female genitalia were detected.		
112	Ambiguous Genitalia & Severe Dehydration	1 M	♂(46,XY)	121	AM = 44.1 PM = 40.5	0.4	Uterine like structure	*Chest CT*: Dextrocardia with right lung hypoplasia	*True hermaphrodite*

ACTH: adrenocorticotropic hormone, 17-OHP: 17 hydroxyprogesterone.
U/S: ultrasound, CT: computerized tomography.
CAH: congenital adrenal hyperplasia.

Figure 1 External genital appearance of case 9 demonstrating micropenis with bilateral undescended testes.

Figure 2 External genital appearance for case 7 demonstrating Clitoromegaly with no palpable gonads.

there will be strong pressures, especially from the parents, to provide a rapid gender determination with limited information [29,30]. We found this to be true in all of our 13 cases with no discrimination between the attitudes of the male or female patients' families. The parents of the patients were anxious to know their children's condition. A dispute that we had to face was the parents urge that we would reach a diagnosis as soon as possible. This was met from our side with assurance that the condition can be diagnosed only after completion of the appropriate and full anatomical, biochemical and multidisciplinary evaluation before rushing to an arbitrary gender assignment. It was a challenge to convince the parents with the importance of these tests and evaluations before rushing into any decision making. Hughes [4] also recommended that we should inform the parents that the gender chosen by the parents and health care providers on behalf of the patient or against his or her will may not be the same gender later chosen by the patient. This was informed to the patients and their parents as well as the other members of the multidisciplinary team. Our multidisciplinary team consisted of a neonatologist, a geneticist, an endocrinologist and a pediatric surgeon. A psychological consultation was provided when necessary. All these were tried to remain foremost focused on the child's

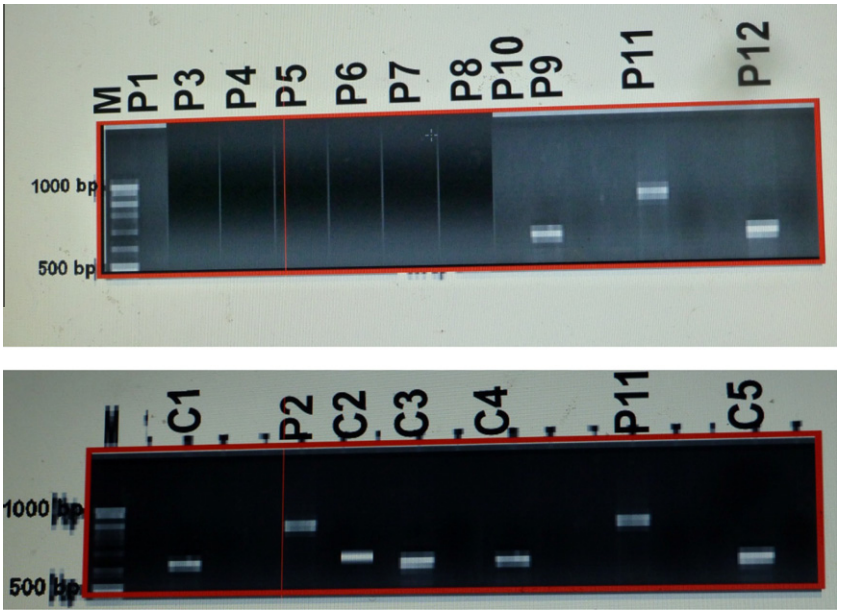


Figure 3 Gel electrophoresis of the PCR amplicons of the SRY gene. • *Lane M:* DNA ladder of 100 bp starting from 500 to 1000 bp. • *Lanes P1→P8 and P10*: SRY amplification for Egyptian female patients. • *Lanes P9, P11 and P12:* SRY amplification for the Egyptian male patients. • *Lanes C1→C5:* SRY amplification for a normal male.

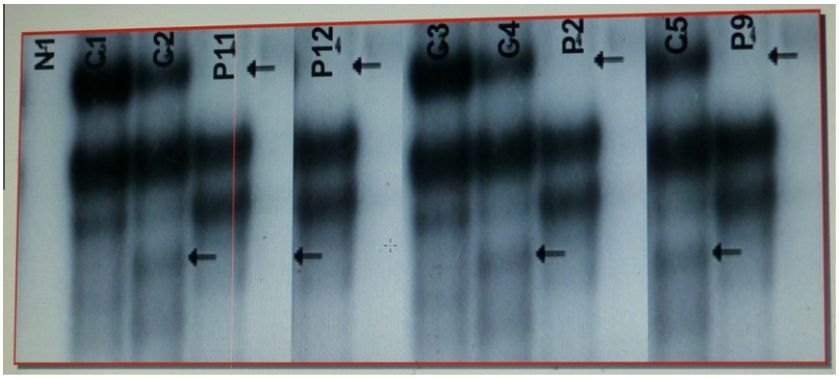


Figure 4 SSCP analysis of SRY gene in the studied cases. • *Lanes N1:* SRY negative control DNA (i.e., 46,XX) • *Lanes C1→C5:* SRY gene positive control DNA. • *P 2, 9, 11 and 12:* SRY gene with missing bands, shown with the upper arrows, indicating that these patients carry mutations in the SRY gene. Also, the lower arrows correspond to the missing area which was detected in the control.).

needs. Joel [27] also recommended the use of the multidisciplinary team but he added the use of counselors and ethicists. We replaced both with a psychiatrist, when needed, and, most importantly, our own ethics and moral values were our care guidance.

We recommend that determination of the sex is an emergency as it requires examination of the external genitalia during routine examination of the child. Assignment of sex is very crucial, yet, speed is not a part of the diagnosis. Early management of DSD is crucial to prevent the complications which are either the salt-losing crisis or the psychic impact of the condition on both the patient and the family. Management of DSD should not be decided under the influence of the parents or the doctors, but only under the influence of the child's welfare.

A communication system between Genetic and Endocrine Centers all over Egypt is a crucial and important part in the management of DSD and we are hopefully aiming toward the application of the SRY gene molecular study as a routine part of the investigations of DSD. Also, genetic counseling is a crucial part in the management of DSD cases which was addressed to both the patient and the family.

As a conclusion, our study focused on the importance of early diagnosis, early treatment and early counseling to all our studied cases. It was crucial to approach the families in a direct, yet simple, way. Once DSD is suspected, doctors should not hesitate to inform the parents about their child's condition and proceed to identify and diagnose the cause first then the gender.

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