

## Cytogenetics and etiology of ambiguous genitalia in 120 pediatric patients

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**Background:** A newborn with ambiguous genitalia needs prompt evaluation to detect life-threatening conditions (e.g., salt-losing crisis in congenital adrenal hyperplasia [CAH]) and gender assignment. Sex assignment in these children continues to be a challenging diagnostic and therapeutic problem. We studied the causes and characteristics of ambiguous genitalia in children who were referred to a cytogenetic laboratory.

**Patients and Methods:** We retrospectively reviewed a total of 120 medical records of patients with a primary indication of ambiguous genitalia that were referred to the cytogenetic lab for karyotyping during the period of 1989 to 1999. Diagnosis was based on a clinical impression from the primary physician, who was primarily a staff pediatrician, endocrinologist and/or pediatric urologist.

**Results:** CAH was the underlying cause of ambiguous genitalia in 41 of 63 patients with ambiguity due to endocrine causes; 39 of these patients showed a 46,XX karyotype and 2 cases were 46,XY (both the 46,XY patients had 3  $\beta$ -hydroxylase deficiency). In 57 patients, ambiguous genitalia were due to congenital developmental defects. The most common endocrine case of ambiguous genitalia was 21-OH deficiency. Seven patients were classified as idiopathic with six showing the 46,XY and one the 46,XX karyotype. Gender was reassigned at birth or at diagnosis in 15 patients.

**Conclusion:** The etiology of ambiguous genitalia is variable. The physician managing these families could minimize the trauma of having a child with unidentified sex by providing appropriate genetic counseling so that the parents can make an early decision. Prenatal DNA testing in at-risk families should be considered and appropriate therapy offered to minimize or prevent genital ambiguity.

**Key words:** Ambiguous genitalia, karyotyping, gender reassignment, congenital adrenal hyperplasia

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Sexual ambiguity is a complex and often confusing medical problem in the newborn. Determining the appropriate sex in these patients is an urgent matter, particularly in congenital adrenal hyperplasia (CAH).<sup>1</sup> On rare occasions, the cause of ambiguity remains unexplained despite extensive studies. The general appearance of the external genitalia is seldom diagnostic of the underlying disorder, but palpable gonads as a general rule exclude female pseudo-hermaphrodites. Treating a child with ambiguous genitalia continues to be one of the most challenging diagnostic as well as therapeutic problems. A multidisciplinary approach involving pediatric endocrinologists, neonatologists, pediatric surgeons, psychiatrists and social workers, is needed. Essential in the satisfactory management of these children is early diagnosis and gender assignment, which may have positive effects on the well-being of both child and family.<sup>2,3</sup> However, recent reports of long-term follow-up of children whose gender was reassigned after surgical correction of their genital organs showed negative socio-psychological effects.<sup>4</sup>

This study describes the underlying causes for ambiguous genitalia in children seen over a ten-year period (1989-1999) at King Faisal Specialist Hospital and Research Centre, a tertiary care facility in Saudi Arabia.

### Patients and Methods

We retrospectively reviewed a total of 120 medical records of patients with a primary indication of ambiguous genitalia that were referred to the cytogenetic lab for karyotyping during the period of 1989 to 1999. Diagnosis was based on karyotyping and on a clinical impression from the primary physician, who was primarily a staff pediatric endocrinologist and/or pediatric urologist. The majority of patients underwent extensive hormonal evaluations, including basal hormonal assay and stimulation using adrenocorticotropic hormone (0.25 mg cosyntropin) intravenously or human chorionic gonadotropin (hCG) 3000 IU/m<sup>2</sup> subcutaneously on alternate days for 7 doses or 4 to 5 doses daily. From these data, the precursor-to-product ratios were calculated for enzyme deficiencies of testoster-

one, dehydrotestosterone, dehydroepiandrosterone and androstenedione pathways. Genitogram and ultrasound were also done on some of the patients.

**Results**

Ambiguity attributed to endocrine causes was found in 63 patients (Table 1). CAH was the underlying cause in 41 of 63 patients, 39 of whom showed a 46,XX karyotype. Two cases were 46,XY (both 3 β-hydroxylase deficiency). Six patients had a clinical picture of vanishing testicular

syndrome (VTS); 5 patients showed Leydig cell hypoplasia (LCH); androgen insensitivity syndrome (AIS) was seen in another 5 patients, 5 α-reductase deficiency was found in 3 patients, gonadal dysgenesis (histologically proven) in 2 patients, and panhypopituitarism in one patient. All of these patients showed a 46,XY karyotype.

In 56 patients ambiguous genitalia were due to congenital developmental defects (CDD) (Table 2). Ten patients had cloacal abnormality. Among these, 6 patients were 46,XY, and 4 patients showed 46,XX karyotype. Another ten patients had congenital local genital malformation; 9 of these cases showed a 46,XY karyotype and the remaining a 46,XX complement. Hypospadias was seen in 11 patients, and all of these showed 46,XY karyotype. Twenty-six patients were classified differently for various reasons. Eight patients had ambiguity as part of their syndrome, including trisomy 13(2 cases), split notchord syndrome (2 cases), Opitz syndrome (1 case), CHARGE association (1 case), Prader-Willi syndrome (1 case), and Sanjad-Sakati syndrome (1 case). Nine patients with ambiguity had dysmorphic features of non-specific syndromes, with associated metabolic disease in 2 cases (Table 2). The karyotype analysis in these cases is shown in Table 2. Seven of these cases were classified as idiopathic and of these, 6 showed 46,XY and one showed 46,XX karyotype. The cause of ambiguous genitalia could not be identified despite extensive clinical and laboratory investigations.

**Table 1.** Patients with ambiguous genitalia due to endocrine causes (n=63).

Type	46,XY	46,XX
Congenital adrenal hyperplasia		
21-OH deficiency		33
11β-OH deficiency		6
3β-OH deficiency	2	
5 α-Reductase deficiency	3	
Gonadal dysgenesis	2	
Panhypopituitarism	1	
VTS	6	
Leydig cell hypoplasia	5	
Androgen insensitivity syndrome	5	
<b>Total</b>	<b>24</b>	<b>39</b>

**Table 2.** Patient with ambiguous genitalia due to congenital defects.

Type	No. of cases	46,XY	46,XX
Cloacal abnormality	10	6	4
Congenital local genital malformation	10	9	1
Hypospadias	11	11	–
Metabolic Defect <sup>1</sup>	2	2	–
Syndromes	8	7	1
Dysmorphic feature <sup>2</sup>	9	7	2
Unidentified (idiopathic)	7	6	1
<b>Total</b>	<b>57</b>		

<sup>1</sup>Multiple carboxylase deficiency (MLD) (one case), organic acidemia (one case)

<sup>2</sup>Single palmer crease (one case); skeletal dysplasia, inverted genitalia with growth hormone insensitivity (one case); dysmorphic, short stature, micropenis, bilateral orchidism (one case); skeletal deformations, congenital malformation at birth (one case); CHD, small mandible, cloacal abnormalities (one case); dysmorphic features and ambiguous genitalia (one case); dysmorphic features and ambiguous genitalia (one case); dysmorphic, IUGR, inversion of genitalia, empty scrotum (one case); micropenis, dysmorphic, CHD (one case)

Age of presentation, consanguinity and family history are shown in Table 3 for patients with endocrine defects and in Table 4 for patients with CDD. Age of presentation in both categories was less than 18 months in more than 85% of patients. Consanguinity, mainly first cousin, ranged between 60% and 100% in various types of endocrine and CDD defects. A family history of ambiguous genitalia in endocrine defects was present in all patients except in cases with 5 α-reductase deficiency and vanishing testicular syndrome. In CDD, a family history of ambiguous genitalia was reported only in patients with hypospadias.

Table 5 summarizes data on the 15 patients whose gender was reassigned at birth or at diagnosis. Eight patients with CAH due to 21-hydroxylase deficiency presented as males, four of whom were reassigned as females based on 46,XX karyotype. The parents of the other patients declined to change the sex of their child. One male patient with 11 β-hydroxylase deficiency was reassigned as a female (46,XX) and another female patient with 3 β-hydroxylase deficiency declined to have the gender reassigned as male based on the presence of an XY karyotype. Of 3 female patients with 5 α-reductase deficiency, two were reassigned as 46,XY males, and the other declined. One female patient with partial AIS was reassigned as a male and, another male patient with cloacal anomaly as a female.

**Table 3.** Age of presentation, consanguinity and family history in patients with endocrine related ambiguous genitalia.

Disorder (No. of cases)	Age at presentation		Consanguinity*	Family history of ambiguous genitalia
	< 18 months	>18 months		
CAH				
(i) 21-OH (33)	30	3	23	10
(ii) 11 $\beta$ -OH (6)	3	3	4	3
(iii) 3 $\beta$ -OH (2)	2		2	1
5 $\alpha$ -Reductase (3)	3		3	0
Vanishing testis syndrome (6)	5	1	5	0
Leydig cell hypoplasia (5)	4	1	5	5
Androgen insensitivity Syndromes (5)	4	0	5	1
Gonadal dysgenesis (2)	0	2	0	2
Panhypopituitarism (1)	1	0	1	1
Total (63)	52	11		

\*Majority were first cousins.

**Table 4.** Age of presentation, consanguinity and family history in patients with congenital developmental defects.

Disorder (No. of cases)	Age at presentation		Consanguinity*	Family history of ambiguous genitalia
	< 18 months	>18 months		
Cloacal abnormality (10)	9	1	2	0
Congenital local genital anomaly (10)	9	1	2	0
Hypospadias (11)	10	1	4	2
Metabolic defects (2)	2	1	2	0
Syndromes (8)	7	1	4	0
Dysmorphic features (9)	8	1	8	0
Idiopathic (7)	6	1	3	0
Total (57)	51	6		

\*Majority were first cousins.

**Table 5.** Gender assignment at and after diagnosis.

Disorder	At presentation		Reassigned as		Decline	Assigned after diagnosis (XX)
	Female	Male	XX	XY		
Congenital adrenal hyperplasia						
21-OH deficiency		8	4		4	9
11-BOH deficiency		1	1			
3 $\beta$ -OH deficiency	1				1	
5 $\alpha$ -reductase	3			2	1	
Androgen insensitivity	1			1		
Cloacal anomaly		1	1			

## Discussion

A genetic female with sexual ambiguity-also known as female pseudohermaphroditism (FPH)-accounts for 50% to 70% of endocrine causes of sexual ambiguity.<sup>4,5</sup> These individuals possess ovaries with masculinized genitalia, generally due to CAH and rarely as a result of maternal ingestion of androgenic compounds or a virilizing tumor during pregnancy. In our series, 62% of the cases with ambiguous genitalia were FPH, and CAH was diagnosed in all of these patients (21 $\beta$ -hydroxylase deficiency was present in 84.6% and 11  $\beta$ -hydroxylase deficiency in 15.4% of cases). Previous studies from Saudi Arabia reported a higher frequency of 11  $\beta$ -hydroxylase (25.6%).<sup>6</sup> The difference between our study and the previous study might be due to sample size (41 patients in the present study versus 86 patients in the previous study). Both studies were hospital-based and may not reflect the general population incidence or prevalence of CAH and its cause; however, our data on FPH and CAH is similar to previous reports in the literature.<sup>4,5,7-10</sup> All patients with CAH who were expected to be a female were found to have a 46,XX karyotype, except in two patients with 3  $\beta$ -hydroxylase deficiency, who were found to have 46,XY karyotype.

Male pseudo-hermaphrodites (MPH), or genetic males characterized by the presence of female or ambiguous genitalia, have testes and their sex chromosomes are XY. They do not have mullerian duct structures. The undervirilization of the external genitalia in these patients is due to inadequate exposure to androgen during the first trimester. Patients with 5  $\alpha$ -reductase deficiency, LCH due to inadequate or absent testosterone, and AIS are examples of MPH in this study. VTS followed by LCH and AIS constituted the majority of MPH cases in this study. VTS was found in the prepubertal boy with bilateral cryptorchidism, which is diagnosed by the finding of abnormally elevated serum concentrations of gonadotropins and an absence of testosterone production following HCG stimulation. The condition is confirmed at surgery.<sup>11</sup> All of the six cases in our study were more than a year old and were diagnosed in this manner.

LCH is inherited as a rare autosomal recessive condition. Affected males with 46,XY karyotype develop as females and remain sexually immature at the time of expected puberty. These individuals express low basal and hCG-stimulated levels of serum testosterone as well as elevated levels of luteinizing hormone (LH). The underlying defect is due to failure of fetal Leydig cell differentiation.<sup>12-15</sup> Diagnosis is made by demonstrating elevated gonadotropin and low plasma testosterone levels, pre-and post-hCG stimulation in female patients with 46,XY karyotype.<sup>16-18</sup> Molecular diagnostic methods for LH receptor abnormalities can also be utilized. The 5 cases of LCH presenting in this study were all females with 46,XY karyotype, and diagnosis was established by hCG stimulation of serum testosterone and LH. These cases are the first reported from Saudi Arabia.

AIS is considered a common cause of ambiguity in a genetic male, but it manifests in a wide spectrum of defects in male sexual development, ranging from complete female phenotype to a phenotypically normal infertile male. In prepubertal children, LH secretion is physiologically decreased; therefore, testosterone production is assessed after HCG stimulation, which results in a rise in testosterone concentration.<sup>19</sup> Five cases presenting in this study were diagnosed by routine methods since molecular analysis was not available. This disorder is the result of mutations of the androgen receptor gene. Molecular screening using single-strand conformation polymorphism is currently used in the diagnosis.<sup>20</sup> Recently, Holterhaus et al<sup>21</sup> reported that the same mutation in the androgen receptor gene (AR) is responsible for variable external genitalia in different patients. Furthermore, when the mutation showed in a family of four members displaying variable external genitalia, the AR gene function could be switched from subnormal to normal in the presence of a physiological concentration range of testosterone. Based on their observations, they concluded that the variability observed in AIS patients is due to the time-dependant variation in testosterone concentration in early fetal development and its impact on the mutant AR gene function.<sup>21</sup>

Hypospadias is found commonly in newborn boys and is seen in approximately 8.2/1000 live births.<sup>22</sup> It may be inherited as a Mendelian defect, but is mostly an isolated finding. In our series, eleven patients presented with hypospadias, including two who gave a positive family history of hypospadias. In cases with severe hypospadias but fully descended testes, a testosterone biosynthetic defect has been reported in approximately 5%.<sup>23,24</sup> In one study, 50% of boys with proximal hypospadias and fully descended testes were shown to have evidence of a testosterone biosynthetic defect with a high incidence of 3  $\beta$ -hydroxysteroid dehydrogenase and 17,20 lyase deficiency.<sup>25</sup> Since endocrine evaluation in our patient was not done, the question of testosterone biosynthetic defects in our population remains unanswered.

Cloacal anomaly may be present as ambiguous genitalia, generally associated with a higher predilection for extrophy and epispadias, in males more than in females (2:1). In our series, the ratio was 1.5:1. Patients often show variable mal-function of the cloaca, and generally present with a difficult reconstructive challenge for the pediatrician, urologist and surgeon. In boys, the penis tends to be short and stubby with dorsal curvature, whereas girls have a bifid clitoris. Some patients with cloacal anomalies might have additional defects, including myelomeningocele, hydrocephalus, cardiac, renal, gastrointestinal and limb defects. The diagnosis should be suspected in any female fetus presenting with bilateral hydronephrosis, a poorly visualized bladder and a cystic lesion arising from the pelvis antenatally.<sup>26</sup> In seven patients in our study with ambiguous genitalia, endocrine defects could not be found. Six of these cases turned out to

be 46,XY karyotype and one patient was 46, XX karyotype. Single gene defects may be a cause in these cases.

Reassignment of sex in patients with ambiguous genitalia is a very sensitive matter. The topic has been debated extensively and specific guidelines are proposed to manage cases of traumatized or ambiguous genitalia.<sup>27,28</sup> These guidelines take into consideration how the patient will develop post-puberty and adapt as a sexually active person. Accordingly, rearing as males is recommended in 1) 46,XY patients with partial AIS, 2) hypospadias, 3) micropenis with testes, because these children should develop a satisfactory male gender identity and sexual function;<sup>29</sup> and 4) 5  $\alpha$ -reductase deficiency. Rearing as females is recommended in 1) XY patients with complete AIS; 2) XX or XY patients with gonadal dysgenesis; 3) 46,XX patients with CAH with mild or severe virilization although some of these girls have a significantly masculinized brain.<sup>30,31</sup> However most of these cases seem to have a female gender identity with varying degree of masculinized behavior.<sup>32</sup> Even in cases of severe virilized 46,XX CAH, it is highly recommended to raise them as female because they are potentially fertile and sexually functional,<sup>33</sup> although they require a reconstructive procedure. In our study, 15 patients were considered for sex

reassignment after evaluating clinical, physical, and laboratory data. Before attempting the sex reassignment, parents were counseled and a decision was taken only after their approval. Retrospectively, the criteria used for sex assignment in our patients are more or less similar to the guidelines discussed above.

In our series, 9 of 15 cases (60%) accepted reassignment of sex. Interestingly, 5 of 10 patients with CAH agreed for sex reassignment from male to female. This trend is similar to a previous report from Saudi Arabia in which male sex assignment was preferred over female.<sup>28,34</sup> Late referral and sociocultural circumstances seem to be the contributing factors in these cases.

In conclusion, newborns with ambiguous genitalia pose a difficult emotional situation for parents in deciding their future sexual orientation. Moreover, these parents also face a social nightmare explaining to their relatives and friends, the gender reassignment. The physician managing these families could minimize the trauma of having a child of unidentified sex by providing appropriate genetic counseling so that the parents can make an early decision. Prenatal DNA testing in at-risk families should be considered, and appropriate therapy offered to minimize or prevent genital ambiguity.

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