ISSN 2472-1972

Frequency of Ambiguous Genitalia in 14,177 Newborns in Turkey

Banu Kucukemre Aydin,[1,2] Nurcin Saka,[1] Firdevs Bas,[1] Evrim Kiray Bas,[3] Asuman Coban,[4] Sukran Yildirim,[4] Tulay Guran,[5,6] and Feyza Darendeliler[1]

[1]Department of Pediatrics, Pediatric Endocrinology Unit, Istanbul University, Istanbul Faculty of Medicine, Istanbul 34093, Turkey; [2]Pediatric Endocrinology Unit, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul 34303, Turkey; [3]Neonatology Unit, Sisli Etfal Training and Research Hospital, Instanbul 34360, Turkey; [4]Department of Pediatrics, Neonatology Unit, Istanbul University, Istanbul Faculty of Medicine, Istanbul 34093, Turkey; [5]Pediatric Endocrinology Unit, Zeynep Kamil Women's and Children's Hospital, Istanbul 34668, Turkey; and [6]Department of Pediatrics, Pediatric Endocrinology Unit, Marmara University, Faculty of Medicine, Istanbul 34890, Turkey

ORCiD numbers: 0000-0003-0859-1565 (B. K. Aydin); 0000-0001-9689-4464 (F. Bas); 0000-0001-5230-8718 (E. K. Bas); 0000-0003-2658-6866 (T. Guran).

Context: Limited data are available on the exact incidence of disorders of sex development (DSD) with genital ambiguity at birth.

Objective: To determine frequency of ambiguous genitalia in newborns.

Design: Prospective multicenter study.

Setting: Three tertiary care hospitals.

Patients or Other Participants: All 14,177 babies born during the study period were included.

Main Outcome Measures: All newborns were examined at birth; data on weeks of gestation, birth weight, and length were collected. A structured questionnaire was used for data collection. Quigley and Prader scales were used for phenotypic grading. Clinical and genetic investigations were performed.

Results: Eighteen babies with ambiguous genitalia were found among 14,177 newborns (1.3/1000). Fifteen newborns had 46,XY DSD, one had 46,XX congenital adrenal hyperplasia, and one had 45,X/46,XY mixed gonadal dysgenesis. Karyotype analysis was not done in one baby who died in the neonatal period. The ratio of prematurity was higher in the DSD group (44% vs 11%; $P < 0.001$) and the ratio of small for gestational age was also higher in the DSD group (22% vs 5%; $P = 0.007$). Eight babies with DSD had mothers who had additional medical conditions, such as preeclampsia, depression, insulin resistance, and gestational diabetes mellitus.

Conclusion: The frequency of ambiguous genitalia was higher than in previous studies, but, as with any experiment, the finding should be met with caution because this study was conducted in tertiary care hospitals. In addition, lower birth weight in the DSD group supports the hypothesis that early placental dysfunction might be important in the etiology of male genital anomalies.

Freeform/Key Words: ambiguous genitalia, disorders of sex development, androgen insensitivity, gonadal dysgenesis, preeclampsia, birth defects

Abbreviations: CAH, congenital adrenal hyperplasia; DSD, disorders of sex development; GDM, gestational diabetes mellitus; MCA, multiple congenital anomalies; PAIS, partial androgen insensitivity syndrome; SDS, SD score; SGA, small for gestational age.

Received 13 December 2018
Accepted 5 April 2019
First Published Online 24 April 2019

June 2019 | Vol. 3, Iss. 6
doi: 10.1210/js.2018-00408 | Journal of the Endocrine Society | 1185–1195

Sex development is a complex process that relies on a well-coordinated series of events at the chromosomal, gonadal, and tissue levels [1]. Disruption in this process can result in disorders of sex development (DSD). DSD constitute a heterogeneous group of conditions in which development of chromosomal, gonadal, or anatomic sex is atypical [2]. Clinical presentation is variable, with obvious ambiguous genitalia at birth in some cases and male or female external genitalia with discordant internal reproductive anatomy in others. Clinical recognition may be delayed until puberty or adulthood in the latter form [1].

Limited data are available on the exact incidence of DSD with genital ambiguity at birth, which has been estimated to be approximately 1 in 4500 to 5500 [3]. Moreover, most of the published data are coming from Western countries in which consanguinity rates are lower. A German study reported that ambiguous genitalia incidence was fourfold higher in infants of non-German origin compared with the general population [4], which they attributed to an increase in autosomal recessive forms of DSD due to higher rates of consanguinity in migrant populations. Other studies support this hypothesis. The incidence of ambiguous genitalia was reported as 1 in 2500 live births in Saudi Arabia [5] and 1 in 3000 live births in Egypt [6], rates higher than the reported frequency in European countries. Furthermore, in those studies many cases of ambiguous genitalia lacked an accurate diagnosis.

Our primary objective was to determine the frequency of ambiguous genitalia in a population of babies born in Istanbul, Turkey.

1. Material and Methods

We conducted this study in three tertiary care hospitals (Istanbul University, Istanbul Faculty of Medicine Hospital, Zeynep Kamil Women's and Children's Hospital, Sisli Etfal Training and Research Hospital) in Istanbul, Turkey. All 14,177 babies born in these three hospitals during an 18-month period were included in this study. All newborns were first examined at birth (within 2 days). Examinations were done in warm conditions with the child in supine position. Appearance, symmetry or asymmetry of the genitalia, size of the penis or clitoris, appearance of the labioscrotal folds, location of the urethral opening, and presence or absence and location of palpable gonads were examined, along with a detailed physical examination. Diagnostic criteria for DSD were ambiguous genitalia and/or mass at inguinal region or labium (predominantly female phenotype) and penoscrotal hypospadias, bilateral nonpalpable testes, and micropenis (predominantly male phenotype). Discordance between prenatal or postnatal karyotype and phenotype and discordance between internal and external genitalia were other criteria. Babies with bilateral nonpalpable testes were evaluated for congenital adrenal hyperplasia (CAH) and other rare disorders, but if they had normal male internal genitalia, they were not included in our ambiguous genitalia group. Data on weeks of gestation, birth weight, and birth length were available for 6201 babies. Birth weight and birth length were expressed as SD score (SDS) according to national standards for gestational age and for the same karyotype [7]. Small for gestational age (SGA) was defined as birth weight less than −2 SDS for gestational age. Prematurity was defined as birth before 37 weeks of gestation. Babies suspected of having DSD were referred to the pediatric endocrinology clinics at the three hospitals.

A. Evaluation of Babies With Ambiguous Genitalia

A structured questionnaire was used for data collection, including history focused on the time just before and during the pregnancy, specifically referring to medications and exposure to hormonal agents. A detailed family history, including consanguinity, birth defects, atypical genital development, and unexplained infant deaths, was gathered from the parents. A comprehensive physical examination, including evaluation for dysmorphic features, was done to identify other associated anomalies. We used Quigley and Prader scales for phenotypic grading [8, 9]; external genital appearance of all babies with ambiguous genitalia

is described in Table 1. The levels of serum electrolytes, gonadotropins, anti-Müllerian hormone, sex hormones, and their precursors were measured and karyotype analysis was done. Pelvic ultrasonography was performed to look for the presence and symmetry of Müllerian structures and the location and structural characteristics of intra-abdominal gonads. Genetic analysis of single candidate genes based on the information gathered from previous phenotypic investigations was the last step of the diagnostic process. However, some patients were lost to follow-up and some did not accept genetic testing.

The Istanbul University Review Board approved this study, and parents of patients with ambiguous genitalia signed informed consent forms.

B. Data Analyses

Statistical analysis was performed by using SPSS Statistical Package, version 15 (IBM Inc., Chicago, IL). Data are presented as the mean ± SDS. Comparisons were made by using independent samples *t*-test, Mann-Whitney *U* test, or χ^2 test. Statistical significance was defined as $P \leq 0.05$.

2. Results

The study included 14,177 newborns, 18 of whom (1.3/1000) had ambiguous genitalia. One newborn was diagnosed with 46,XX CAH, one was diagnosed with 45,X/46,XY mixed gonadal dysgenesis, and 15 were diagnosed with 46,XY DSD (Table 1). Karyotype analysis was not done in one baby who had multiple congenital anomalies (MCA) and died in the neonatal period (Table 1). Of the 15 babies with 46,XY DSD, 9 were diagnosed with partial androgen insensitivity syndrome (PAIS), 3 with 5 α-reductase deficiency, 2 with MCA, and 1 with persistent Müllerian duct syndrome (Table 1). Among all 18 patients with DSD, only 1 had a genetically confirmed diagnosis (patient 3, with a genetic diagnosis of PAIS) (Table 1).

Two patients with MCA died in the newborn period. Sex of rearing was male in all surviving patients except one patient with CAH (Table 1). One patient with genetically confirmed PAIS had ectopic left kidney (Table 2).

Eight babies with DSD were born to a mother who had additional medical conditions during pregnancy, such as preeclampsia, depression, insulin resistance, and gestational diabetes mellitus (GDM) (Table 2).

Four babies with DSD were born to a mother with a history of medication use during the first trimester of pregnancy. Those medications were levonorgestrel, paroxetine, methyldopa and metformin (Table 2).

Consanguinity was found in three families (Table 2), and two families had other members with DSD (families 12 and 17).

Mean gestational age was lower in babies with DSD, and the ratio of prematurity was higher in the DSD group (Table 3). Mean birth weight SDS was lower in babies with DSD, and the SGA ratio was higher in the DSD group (Table 3). All the SGA babies had 46,XY karyotype (Table 1). Birth length SDS did not significantly differ between babies with and those without DSD (Table 3).

We also compared the anthropometric measurements of newborn boys and girls without ambiguous genitalia. Healthy newborn boys (n = 3178) were heavier (3170 ± 650 g vs 3044 ± 604 g; $P < 0.001$) and taller (n = 3005; 48.6 ± 3.3 cm vs 48.1 ± 3.1 cm; $P < 0.001$) than the girls. Gestational age did not differ between sexes (38.1 ± 2.5 weeks vs 38.2 ± 2.5 weeks; $P = 0.08$).

3. Discussion

This Turkish study reports on ambiguous genitalia frequency in a large population of newborns. We found a higher rate of ambiguous genitalia than in previous studies, which were mostly from Western countries with a consanguineous marriage rate <5% [10]. However, in Turkey this rate is around 20% to 25%, causing a higher incidence of autosomal

Table 1. Clinical and Laboratory Features of Patients With Ambiguous Genitalia

Patient	BW SDS	GW	Description of External Genitalia	Prader/ Quigley Scale	Karyotype	Relevant Laboratory Results	Working Clinical Diagnosis	Genetic Diagnosis	Sex of Rearing
1	0.1	38	Phallus dorsal 1.8 cm, ventral 1.2 cm, chordee +, scrotal hypospadias, bifid scrotum, right gonad nonpalpable, left gonad palpable 1 mL	4	45,X/46, XY	Pelvic and scrotal USG: Müllerian structures+, right streak gonad, left gonad in the scrotum 12 × 8.3 mm	MGD	Nnot studied	M
2	1.5	38	Phallus dorsal 2 cm, ventral 1.5 cm, penoscrotal hypospadias, bilateral nonpalpable gonads, scrotal hyperpigmentation+	4[a]	46,XX	17OHPG: 62.5 ng/mL, ACTH: 297 pg/mL	CAH	Not studied	F
3	−1.0	33	Phallus dorsal 1.4 cm, ventral 1 cm, chordee +, scrotal hypospadias, bifid scrotum, bilateral gonads palpable 1 mL	4	46,XY	2 mo old Unstimulated LH: 6.8 mIU/mL, FSH: 1.4 mIU/mL, T: 1.2 ng/mL	PAIS	*AR* gene mutation +	M
4	0.2	34	Phallus dorsal 2.3 cm, ventral 1.6 cm, chordee +, penoscrotal hypospadias, right gonad 2 mL, left gonad 1 mL palpable	3	46,XY	hCG stimulation test T: 4.14 ng/mL, DHT: 0.25 ng/mL, T/DHT: 16.56	5-ARD	Not studied	M
5	−2.5	36	Phallus dorsal 1.5 cm, ventral 1 cm, chordee +, penoscrotal hypospadias, bifid scrotum, bilateral gonads palpable 0.5 ml	3	46,XY	15 d old Unstimulated T: 1.45 ng/mL, DHT: 0.39 ng/mL, T/DHT: 3.72	PAIS	No mutation in *AR* or *SRD5A2* genes	M
6	1.5	38	Phallus dorsal 1.7 cm, ventral 1 cm, chordee +, scrotal hypospadias, bifid asymmetric scrotum, bilateral gonads palpable right 3 mL, left 1 mL	4	46,XY	hCG stimulation test T: 2.32 ng/mL, DHT: 0.59 ng/mL T/DHT: 3.93	PAIS	No mutation in *AR* or *SRD5A2* gene	M

(*Continued*)

Table 1. Clinical and Laboratory Features of Patients With Ambiguous Genitalia (Continued)

Patient	BW SDS	GW	Description of External Genitalia	Prader/ Quigley Scale	Karyotype	Relevant Laboratory Results	Working Clinical Diagnosis	Genetic Diagnosis	Sex of Rearing
7	−1.5	34	Phallus dorsal 2.2 cm, ventral 1.4 cm, chordee +, penoscrotal hypospadias, asymmetric scrotum, right gonad palpable 2 mL, left gonad nonpalpable hydrocele+	3	46,XY	hCG stimulation test T: 6.86 ng/mL, DHT: 0.87 ng/mL, T/DHT: 7.89	PAIS	No mutation in *AR* or *SRD5A2* gene	M
8	−0.8	38	Phallus dorsal 2.5 cm, ventral 1.5 cm, chordee +, penoscrotal hypospadias, bifid scrotum, bilateral gonads palpable 2 mL	3	46,XY	1 mo old Unstimulated T: 2.15 ng/mL, DHT: 0.14 ng/mL, T/DHT: 15.36	5-ARD	Not studied	M
9	−2.2	37	Phallus dorsal 2.5 cm, ventral 1.5 cm, chordee +, penile hypospadias, bilateral gonads palpable 1 mL	2	46,XY	1 mo old Unstimulated T: 2.25 ng/mL, DHT: 0.61 ng/mL, T/DHT: 3.69	PAIS	Not studied	M
10	0.1	38	No erectile tissue, one urogenital opening, scrotum was formed, bilateral nonpalpable gonads	4	46,XY	USG: No Müllerian structures, bilateral testes were in inguinal canal	MCA	Not studied	Deceased
11	−4.3	33	Phallus dorsal 1.2 cm, ventral 0.7 cm, chordee + penoscrotal hypospadias, bifid scrotum, bilateral nonpalpable gonads	3	46,XY	1.5 mo old Unstimulated T: 1.85 ng/mL, DHT: 0.11 ng/mL, T/DHT: 16.81	5-ARD	Homozygote polymorphisms in SRD5A2, no mutation in AR gene	M
12	−0.9	36	Phallus dorsal 1.8 cm, ventral 1 cm, chordee +, scrotal hypospadias, bifid scrotum, bilateral gonads palpable 1 mL	4	46,XY	hCG stimulation test T: 3.58 ng/mL, DHT: 0.59 ng/mL, T/DHT: 6.06	PAIS	not studied	M
13	−1.5	28	Phallus dorsal 0.9 cm, ventral 0.6 cm, chordee+, penoscrotal hypospadias, bifid scrotum, bilateral nonpalpable gonads	3	46,XY	hCG stimulation test T: 2.83 ng/mL, DHT: 0.37 ng/mL, T/DHT: 7.65	PAIS	Not studied	M

(*Continued*)

doi: 10.1210/js.2018-00408 | Journal of the Endocrine Society | 1189

Table 1. Clinical and Laboratory Features of Patients With Ambiguous Genitalia (Continued)

Patient	BW SDS	GW	Description of External Genitalia	Prader/ Quigley Scale	Karyotype	Relevant Laboratory Results	Working Clinical Diagnosis	Genetic Diagnosis	Sex of Rearing
14	−1.3	33	Phallus dorsal 0.7 cm, ventral 0.5 cm, penoscrotal hypospadias, bifid scrotum, bilateral palpable gonads in the inguinal canal	3	Not studied	Not studied	MCA	Not studied	Deceased
15	−1.2	38	Phallus dorsal 2.2 cm, ventral 1 cm, chordee + penoscrotal hypospadias, bilateral palpable gonads in the inguinal canal	3	46,XY	hCG stimulation test T: 4.3 ng/mL, DHT: 0.65 ng/mL, T/DHT: 6.62	PAIS	Not studied	M
16	1.0	31	Phallus dorsal 1.2 cm, ventral 0.8 cm, chordee +, penoscrotal hypospadias, scrotal hypoplasia, bilateral nonpalpable gonads	3	46,XY	2 mo old Unstimulated T: 1.8 ng/mL, DHT: 0.33 ng/mL, T/DHT: 5.46	PAIS	Not studied	M
17	−0.0	37	Phallus dorsal 3.7 cm, ventral 3.7 cm, no hypospadias, bilateral nonpalpable gonads	1	46,XY	Pelvic USG: Uterus and bilateral fallopian tubes+, bilateral intra-abdominal gonads. Biopsy: Bilateral testicular tissue AMH <0.1 ng/mL, inhibin B: 69 pg/mL	PMDS	Not studied	M
18	−2.4	39	Phallus dorsal 2 cm, ventral 1.4 cm, chordee +, penoscrotal hypospadias, scrotal hypoplasia, bilateral nonpalpable gonads	3	46,XY	Pelvic USG: No Müllerian structures, bilateral abdominal gonads	MCA	Not studied	M

Abbreviations: 5-ARD, 5 α-reductase deficiency; 17OHPG, 17-hydroxyprogesterone; ACTH, adrenocorticotropic hormone; AMH, anti-Müllerian hormone; AR, androgen receptor; BW, birth weight; DHT, dihydrotestosterone; F, female; GW, gestational week; hCG, human chorionic gonadotropin; M, male; MGD, mixed gonadal dysgenesis; PMDS, persistent Müllerian duct syndrome; *SRD5A2*, steroid 5 α-reductase 2; T, testosterone; USG, ultrasonography; UTR, untranslated region.
[a]Prader staging.

Table 2. Concomitant Conditions, Mothers' Disease, Medication Status, and Consanguinity in Patients With Ambiguous Genitalia

Patient	Concomitant Conditions	Mother's Disease	Mothers Medication	Consanguinity
1	No	Depression	No	No
2	No	No	No	No
3	Ectopic kidney	Preeclampsia	No	No
4	No	Preeclampsia	No	First degree
5	No	HT, GDM	Methyldopa	No
6	No	No	No	No
7	No	Preeclampsia	No	No
8	No	No	No	Second degree
9	No	No	No	No
10	CHD, polydactyly	No	No	No
11	No	Preeclampsia	No	No
12	No	No	No	No
13	No	No	No	No
14	URA, anal atresia	Insulin resistance	Metformin	No
15	No	No	Levonorgestrel	No
16	No	No	No	No
17	No	Depression	Paroxetine	First degree
18	Dysmorphic features, CCA	No	No	No

Abbreviations: CCA, corpus callosum agenesis; CHD, congenital heart disease; HT, hypertension; URA, unilateral renal agenesis.

recessive diseases [11]. In our study group, the consanguineous marriage rate does not seem to be very high, but in populations with a high consanguineous marriage rate, the actual relationship coefficient between the couples is much higher than the one calculated based on the information given [12]. In addition, previous studies were retrospective or used registries with a low capture rate [4–6], but ours was a prospective study. However, our study was conducted in three tertiary care hospitals with higher frequency of complicated pregnancies, which may explain higher rates of DSD with prematurity, SGA, and coexistence of maternal morbidities.

Most of our patients had 46,XY DSD; only one newborn was diagnosed with 46,XX CAH and one with 45,X/46,XY mixed gonadal dysgenesis. Unlike our results, ~50% of all cases with DSD have been reported to be due to 46,XX DSD or sex chromosome DSD [3, 4, 13–15]. The worldwide incidence of CAH has been estimated to be 1 in 14,000 to 15,000 live births [16], and the frequency of testicular or mixed gonadal dysgenesis is estimated at 1:10,000 [3]. However, DSD incidence has been reported to be 1 in 20,000 among patients with 46,XY karyotype [3]. In our study, frequency of 46,XY DSD was 1.1/1000 among all births. One 46,XX baby with CAH and ambiguous genitalia in 14,177 babies is consistent with the literature frequency values [16, 17] because studies on CAH frequency were done with blood screening; however, we screened newborns with physical examination and could identify only the virilized females. With this result, we estimated 100% capture rate in our study.

Table 3. Comparison of Clinical Characteristics of Babies With or Without Ambiguous Genitalia

Variable	With Ambiguous Genitalia (n = 18)	Without Ambiguous Genitalia (n = 6183)	*P* Value
Birth weight SDS	−0.85 ± 1.6	0.18 ± 1.4	0.009
Birth length SDS	−0.97 ± 2.0	−0.23 ± 1.4	0.4
SGA, %	22.2	4.7	0.007
Gestational age, wk	35.5 ± 3.1	38.2 ± 2.5	<0.001
Prematurity, %	44.4	11.1	<0.001

Values are means ± SDs unless otherwise stated.

Unfortunately, we had a very low rate of genetic confirmation in our study group. That made definitive diagnosis impossible in most cases. Although important advances in our knowledge have been achieved over the last decade, only a limited number of genes involved in the DSD process are available for clinical testing [3]. During the study period, the standard for genetic diagnosis was sequencing a small number of known genes chosen as likely candidates based on disease phenotype, and with this method genetic etiology remained unclear in most cases [3, 18]. A recent position paper about molecular genetic diagnosis of DSD recommended a new parallel approach [19]. In this integrative approach, clinical phenotype is considered in parallel with the biochemical and genetic data. Additionally, the authors recommended using a high-throughput sequencing panel or whole exome sequencing for the analysis of candidate genes [19]. Whole genome sequencing can be used as one of the firstline tests for diagnosis in the near future, but currently there are difficulties, including long turnaround times, high costs, a lack of national health care system coverage, and difficulties in the interpretation of the results [3].

Cox *et al.* [20] reported that SGA was observed in 23% of 46,XY DSD cases with associated conditions. Similarly, in our study, mean birth weight SDS was lower in babies with 46,XY DSD, and the SGA ratio was 22% in the DSD group. The association between SGA and DSD has long been recognized [21]. Furthermore, although the cause of birth weight difference is unknown, boys are heavier than girls at birth, and we showed the same result in our healthy cohort. It has been speculated that *in utero* androgen action may alter prenatal growth [22], and some previous studies have supported this hypothesis [23]; however, others reported no association between prenatal androgens and birth weight [24, 25]. Alternative hypotheses for the origins of low birth weight in 46,XY DSD cases include genetic factors, early placental dysfunction, and endocrine disrupters [22, 26, 27]. Because fetal masculinization occurs between gestational weeks 8 and 14 and during the first 14 weeks of gestation placental human chorionic gonadotropin controls fetal testosterone secretion, a dysfunctional placenta may lead to growth retardation and hypospadias [26, 27]. Therefore, placental insufficiency can mimic PAIS, causing severe undermasculinization with normal testicular androgen production [24]. We could find only one androgen receptor gene mutation among four patients who were tested; genetic analysis was not done in five patients. This is why some of our patients with a clinical diagnosis of PAIS may have ambiguous genitalia due to early placental dysfunction.

Epidemiological studies have also identified an association between hypospadias with some risk factors tied to SGA, including prematurity, preeclampsia, and placental insufficiency [28]. Huisma *et al.* [28] reported that a cohort of SGA boys had an increased ratio of severe to mild hypospadias. In addition, the prevalence of prematurity, maternal hypertension, and oligohydramnios was higher among those with severe vs mild hypospadias. Similarly, in our study, mean gestational age was lower and the ratio of prematurity was higher in babies with DSD. Sekaran et al. [29] reported that prematurity and intrauterine growth restriction are significantly associated with proximal hypospadias and undescended testis.

In our study group there were four mothers with preeclampsia and one mother with hypertension and antihypertensive use in the first trimester. It is believed that early placental dysfunction creates immune alteration and vascular disorder and causes preeclampsia [30]. In a Danish Nationwide Cohort Study, Arendt *et al.* [31] reported that boys of mothers with preeclampsia had the highest occurrence of cryptorchidism and hypospadias, increasing with preeclampsia severity. They proposed that preeclampsia and genital anomalies share common etiologic factors and that placental dysfunction and androgen deficiency in early pregnancy are important in the etiology of male genital anomalies. Similarly, Caton *et al.* [32] reported an association between hypertension, antihypertensive medication use, and the risk of severe hypospadias, particularly when medication use began late in pregnancy. Because genital masculinization occurs in early pregnancy, they suggested that hypertension and its morphologic/physiologic precursors play an etiologic role, perhaps via compromised uteroplacental perfusion.

One mother had GDM (patient number 5) and another one had insulin resistance and metformin use during the first trimester in our study group (patient 14). García-Patterson

et al. [33] reported that severity of GDM is a predictor of congenital malformations, with a 0.3% hypospadias rate in those patients. Metformin is an oral glucose-lowering agent that acts mainly by improving the sensitivity of peripheral tissue and the liver to insulin, thus opposing insulin resistance [34]. In a meta-analysis of eight studies, Gilbert *et al.* [35] concluded that first-trimester exposure to metformin is not associated with an increased risk for major malformations. More recently, Andrade [36] reported that metformin exposure during the first trimester does not seem to be associated with major congenital malformations, and metformin reduces the risk for early pregnancy loss, preeclampsia, preterm delivery, and GDM in women with polycystic ovarian disease.

Two mothers had depression in our study, and one of them had also used paroxetine. Pregnancy is a period of relatively high risk for depressive episodes. Psychiatric disorders are present in 14% of pregnant women and major depression may affect nearly 5% of patients [37]. Reis and Källén [38] reported that the congenital malformation rate was increased with the use of tricyclic antidepressants and verified an association between use of paroxetine and hypospadias. However, it was not clear how much of this was due to medication or underlying pathology.

One mother in our study stated that she had used levonorgestrel as an emergency contraceptive. Progestogens may act as weak androgens or antiandrogens based on the structural similarity with the receptors [34]. Using data from the National Birth Defects Prevention Study, Carmichael *et al.* [39], reported that the rate of hypospadias was 8.4% in babies whose mothers used progestins from 4 weeks before conception to 14 weeks after and 2.4% in controls. However, that study had no information on the dose and method of administration for most women and did not specify the type of progestin and indication. De Santis *et al.* [40] reported that the failure of levonorgestrel as an emergency contraceptive was not associated with an increased risk for major congenital malformations, prepartum or peripartum complications, or an adverse pregnancy outcome.

Three babies in our study had MCA and one had PAIS and ectopic kidney. Similar to our results, the frequency of concomitant conditions in patients with DSD has been reported at around 23% to 38% [4, 20, 24]. Although DSD and renal anomalies are often encountered together in patients with gonadal dysgenesis, rare cases with androgen insensitivity syndrome and renal anomalies have been described previously [20]. Human sex development is closely linked to the development of the urological system. Moreover, in mice, the kidney is one of the most androgen-sensitive organs, with a close correlation between kidney mass and androgen levels [41].

Two of our patients with MCA died in the newborn period. Thyen et al. [4] reported a 10% mortality rate in patients with ambiguous genitalia and all but one of those infants had complex syndromes like our cases.

The current study may be of interest because it is a Turkish study reporting ambiguous genitalia frequency in a large newborn population. Many previous studies used registries with a low capture rate, but this was a prospective study. We found a very high rate of DSD compared with the literature, and this would be an important element for comparison in future epidemiologic studies. This study has some limitations, however. It was performed in tertiary care hospitals, which would cause a selection bias because complicated pregnancies were referred to these centers. Additionally, we did not have a genetic diagnosis in most of our patients.

In conclusion, DSD frequency in this study was 1.3 in 1000 births, which was higher than previous studies. We found that mean birth weight SDS was lower and SGA ratio higher in babies with 46,XY DSD. Preeclampsia was a common concomitant condition in those pregnancies. These findings support the hypothesis that early placental dysfunction and androgen deficiency might be important in the etiology of male genital anomalies.

Acknowledgments

Correspondence: Banu Kucukemre Aydin, MD, Department of Pediatrics, Pediatric Endocrinology Unit, Istanbul University, Istanbul Faculty of Medicine, Cocuk Sagligi ve Hastaliklari AD, Capa, Fatih, Istanbul 34093, Turkey. E-mail:bkucukemre@yahoo.com.

Disclosure Summary: The authors have nothing to disclose.

References and Notes

1. Kutney K, Konczal L, Kaminski B, Uli N. Challenges in the diagnosis and management of disorders of sex development. *Birth Defects Res C Embryo Today*. 2016;**108**(4):293–308.
2. Lee PA, Houk CP, Ahmed SF, Hughes IA; International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. *Pediatrics*. 2006;**118**(2): e488–e500.
3. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, Baratz Dalke K, Liao LM, Lin-Su K, Looijenga LH III, Mazur T, Meyer-Bahlburg HF, Mouriquand P, Quigley CA, Sandberg DE, Vilain E, Witchel S; Global DSD Update Consortium. Global disorders of sex development update since 2006: perceptions, approach and care [published correction appears in *Horm Res Paediatr*. 2016;85(3):180]. *Horm Res Paediatr*. 2016;**85**(3):158–180.
4. Thyen U, Lanz K, Holterhus PM, Hiort O. Epidemiology and initial management of ambiguous genitalia at birth in Germany. *Horm Res*. 2006;**66**(4):195–203.
5. Abdullah MA, Katugampola M, al-Habib S, al-Jurayyan N, al-Samarrai A, Al-Nuaim A, Patel PJ, Niazi M. Ambiguous genitalia: medical, socio-cultural and religious factors affecting management in Saudi Arabia. *Ann Trop Paediatr*. 1991;**11**(4):343–348.
6. Mazen I, Hiort O, Bassiouny R, El Gammal M. Differential diagnosis of disorders of sex development in Egypt. *Horm Res*. 2008;**70**(2):118–123.
7. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu MM, Akın MA, Çoban D, Gökoğlu S, Baştuğ O. Body weight, length and head circumference at birth in a cohort of Turkish newborns. *J Clin Res Pediatr Endocrinol*. 2012;**4**(3):132–139.
8. Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocr Rev*. 1995;**16**(3):271–321.
9. von Prader A. Der genitalbefund beim pseudohermaproditismus femininus des kongenitalen adrenogenitalen syndroms morphologie hausfigkeit entwicklung und vererbung der verschiedenen genitalformen. *Helv Paediatr Acta*. 1954;**9**:231–248.
10. Hamamy H. Consanguineous marriages: preconception consultation in primary health care settings. *J Community Genet*. 2012;**3**(3):185–192.
11. Tunçbilek E. Clinical outcomes of consanguineous marriages in Turkey. *Turk J Pediatr*. 2001;**43**(4): 277–279.
12. Zlotogora J. What is the birth defect risk associated with consanguineous marriages? *Am J Med Genet*. 2002;**109**(1):70–71.
13. Ocal G, Berberoğlu M, Sıklar Z, Ruhi HI, Tükün A, Camtosun E, Savaş Erdeve S, Hacıhamdioğlu B, Fitöz S. The clinical and genetic heterogeneity of mixed gonadal dysgenesis: does "disorders of sexual development (DSD)" classification based on new Chicago consensus cover all sex chromosome DSD? *Eur J Pediatr*. 2012;**171**(10):1497–1502.
14. Erdoğan S, Kara C, Uçaktürk A, Aydın M. Etiological classification and clinical assessment of children and adolescents with disorders of sex development. *J Clin Res Pediatr Endocrinol*. 2011;**3**(2):77–83.
15. Ocal G, Berberoğlu M, Siklar Z, Bilir P, Uslu R, Yağmurlu A, Tükün A, Akar N, Soygür T, Gültan S, Gedik VT. Disorders of sexual development: an overview of 18 years experience in the pediatric Endocrinology Department of Ankara University. *J Pediatr Endocrinol Metab*. 2010;**23**(11):1123–1132.
16. Pang SY, Wallace MA, Hofman L, Thuline HC, Dorche C, Lyon IC, Dobbins RH, Kling S, Fujieda K, Suwa S. Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics*. 1988;**81**(6):866–874.
17. Guran T, Tezel B, Gurbuz F, Selver Eklioglu B, Hatipoglu N, Kara C, et al. Neonatal screening for congenital adrenal hyperplasia in Turkey: a pilot study with 38,935 infants. *J Clin Res Pediatr Endocrinol*. 2019;**11**(1):13–23.
18. Heeley JM, Hollander AS, Austin PF, Merritt DF, Wesevich VG, Amarillo IE; Washington University School of Medicine in St Louis DSD Team. Risk association of congenital anomalies in patients with ambiguous genitalia: A 22-year single-center experience. *J Pediatr Urol*. 2018;**14**(2):153.e1–153.e7.
19. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM, Greenfield A, Bashamboo A, Hiort O, Wudy SA, McGowan R; The EU COST Action. Genetics in endocrinology: approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol*. 2018;**179**(4):R197–R206.

20. Cox K, Bryce J, Jiang J, Rodie M, Sinnott R, Alkhawari M, Arlt W, Audi L, Balsamo A, Bertelloni S, Cools M, Darendeliler F, Drop S, Ellaithi M, Guran T, Hiort O, Holterhus PM, Hughes I, Krone N, Lisa L, Morel Y, Soder O, Wieacker P, Ahmed SF. Novel associations in disorders of sex development: findings from the I-DSD Registry. *J Clin Endocrinol Metab*. 2014;**99**(2):E348–E355.
21. Akre O, Lipworth L, Cnattingius S, Sparén P, Ekbom A. Risk factor patterns for cryptorchidism and hypospadias. *Epidemiology*. 1999;**10**(4):364–369.
22. Hughes IA, Northstone K, Golding J; ALSPAC Study Team. Reduced birth weight in boys with hypospadias: an index of androgen dysfunction? *Arch Dis Child Fetal Neonatal Ed*. 2002;**87**(2): F150–F151.
23. de Zegher F, Francois I, Boehmer AL, Saggese G, Müller J, Hiort O, Sultan C, Clayton P, Brauner R, Cacciari E, Ibáñez L, Van Vliet G, Tiulpakov A, Saka N, Ritzén M, Sippell WG. Androgens and fetal growth. *Horm Res*. 1998;**50**(4):243–244.
24. Poyrazoglu S, Darendeliler F, Ahmed SF, Hughes I, Bryce J, Jiang J, Rodie M, Hiort O, Hannema SE, Bertelloni S, Lisa L, Guran T, Cools M, Desloovere A, Claahsen-van der Grinten HL, Nordenstrom A, Holterhus PM, Kohler B, Niedziela M, Krone N. Birth weight in different etiologies of disorders of sex development. *J Clin Endocrinol Metab*. 2017;**102**(3):1044–1050.
25. Miles HL, Gidlöf S, Nordenström A, Ong KK, Hughes IA. The role of androgens in fetal growth: observational study in two genetic models of disordered androgen signalling. *Arch Dis Child Fetal Neonatal Ed*. 2010;**95**(6):F435–F438.
26. Fujimoto T, Suwa T, Kabe K, Adachi T, Nakabayashi M, Amamiya T. Placental insufficiency in early gestation is associated with hypospadias. *J Pediatr Surg*. 2008;**43**(2):358–361.
27. Kalfa N, Philibert P, Sultan C. Is hypospadias a genetic, endocrine or environmental disease, or still an unexplained malformation? *Int J Androl*. 2009;**32**(3):187–197.
28. Huisma F, Thomas M, Armstrong L. Severe hypospadias and its association with maternal-placental factors. *Am J Med Genet A*. 2013;**161A**(9):2183–2187.
29. Sekaran P, O'Toole S, Flett M, Cascio S. Increased occurrence of disorders of sex development, prematurity and intrauterine growth restriction in children with proximal hypospadias associated with undescended testes. *J Urol*. 2013;**189**(5):1892–1896.
30. Zárate A, Saucedo R, Valencia J, Manuel L, Hernández M. Early disturbed placental ischemia and hypoxia creates immune alteration and vascular disorder causing preeclampsia. *Arch Med Res*. 2014; **45**(7):519–524.
31. Arendt LH, Henriksen TB, Lindhard MS, Parner ET, Olsen J, Ramlau-Hansen CH. Hypertensive disorders of pregnancy and genital anomalies in boys: a Danish nationwide cohort study. *Epidemiology*. 2018;**29**(5):739–748.
32. Caton AR, Bell EM, Druschel CM, Werler MM, Mitchell AA, Browne ML, McNutt LA, Romitti PA, Olney RS, Correa A; National Birth Defects Prevention Study. Maternal hypertension, antihypertensive medication use, and the risk of severe hypospadias. *Birth Defects Res A Clin Mol Teratol*. 2008; **82**(1):34–40.
33. García-Patterson A, Erdozain L, Ginovart G, Adelantado JM, Cubero JM, Gallo G, de Leiva A, Corcoy R. In human gestational diabetes mellitus congenital malformations are related to pre-pregnancy body mass index and to severity of diabetes. *Diabetologia*. 2004;**47**(3):509–514.
34. Elizur SE, Tulandi T. Drugs in infertility and fetal safety. *Fertil Steril*. 2008;**89**(6):1595–1602.
35. Gilbert C, Valois M, Koren G. Pregnancy outcome after first-trimester exposure to metformin: a meta-analysis. *Fertil Steril*. 2006;**86**(3):658–663.
36. Andrade C. Major malformation risk, pregnancy outcomes, and neurodevelopmental outcomes associated with metformin use during pregnancy. *J Clin Psychiatry*. 2016;**77**(4):e411–e414.
37. Gentile S. Selective serotonin reuptake inhibitor exposure during early pregnancy and the risk of birth defects. *Acta Psychiatr Scand*. 2011;**123**(4):266–275.
38. Reis M, Källén B. Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. *Psychol Med*. 2010;**40**(10):1723–1733.
39. Carmichael SL, Shaw GM, Laurent C, Croughan MS, Olney RS, Lammer EJ. Maternal progestin intake and risk of hypospadias. *Arch Pediatr Adolesc Med*. 2005;**159**(10):957–962.
40. De Santis M, Cavaliere AF, Straface G, Carducci B, Caruso A. Failure of the emergency contraceptive levonorgestrel and the risk of adverse effects in pregnancy and on fetal development: an observational cohort study. *Fertil Steril*. 2005;**84**(2):296–299.
41. Berger FG, Watson G. Androgen-regulated gene expression. *Annu Rev Physiol*. 1989;**51**(1):51–65.