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Ambiguous Genitalia: What Prenatal Genetic Testing Is Practical?

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Concern for ambiguous genitalia or chromosome-phenotype discordance detected in a prenatal setting has increased over the last two decades. Practitioners faced with this prenatal finding have a variety of genetic tests available to them; however, it is unclear to what extent prenatal testing for disorders of sex development (DSD) is useful or practical. We undertook a retrospective review of the medical records of 140 individuals evaluated through the DSD clinic at Seattle Children's Hospital with birthdates from 01/01/1994 through 08/16/2011 to determine the rate of prenatal detection of ambiguous genitalia in individuals with DSD, what prenatal diagnostic workup was undertaken, and the postnatal outcome, including whether a postnatal genetic diagnosis was confirmed. Of all 140 subjects, 34 (24%) were identified prenatally. The most common postnatal diagnoses were penoscrotal hypospadias with transposition of the scrotum with no known genetic cause (24/140; 17%) and 21-hydroxylase deficiency (20/140; 14%). Apart from these, no single diagnosis comprised more than a few cases. Prenatal diagnostic testing varied widely, from no tests to multiple molecular tests with amniotic fluid hormone concentrations. In the absence of other fetal anomalies or growth retardation on ultrasound, prenatal karyotype with fluorescence in situ hybridization for the *SRY* gene is the most useful test when ambiguous genitalia is suspected. Further prenatal testing for Smith-Lemli-Opitz syndrome in 46,XY individuals and congenital adrenal hyperplasia in 46,XX individuals may be considered. However, targeted molecular testing for rare DSD conditions in the absence of a family history of DSD has a low yield.

Key words: ambiguous genitalia; disorders of sex development; prenatal diagnosis; intersex; hypospadias

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INTRODUCTION

The frequency of any type of genital anomaly in newborns is estimated to be as high as 2%, with 1 to 2 per 1,000 individuals undergoing "corrective" genital surgery [Blackless et al., 2000]. The birth of a child with ambiguous genitalia is distressing to the family and to the medical team caring for the child, who feel the need to make a timely and appropriate sex-assignment. Ideal postnatal management of ambiguous genitalia is outlined elsewhere [Houk et al., 2006; Hughes et al., 2006; Parisi et al., 2007] and includes a team of experts from multiple disciplines assessing the child through careful physical examination, laboratory studies, imaging studies, and/or gonadal biopsy. Perhaps even more stressful is the prenatal detection of ambiguous genitalia or a fetal karyotype that is not congruent with the prenatal ultrasound images of the genitalia (so called "chromosome-phenotype discordance"). In these situations, the practitioner is not able to closely examine the external and internal fetal genitalia and has limited additional diagnostic studies (such as gonadal pathology and fetal hormone concentrations) that can be done prior to birth. Furthermore, the parents are often faced with the difficult decision of whether to terminate or continue the pregnancy. The spectrum of outcomes

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ranges from mild, such as low grade hypospadias, to severe, such as a genetic syndrome with associated multiple anomalies and cognitive disability [Parisi et al., 2007; Hughes, 2010a].

In 1981, a multidisciplinary Gender Assessment Team was established at Seattle Children's Hospital to evaluate individuals with disorders of sex development (DSD). A review of this team and their experience over a 25-year period was published previously [Parisi et al., 2007]. However, that review did not address the prenatal diagnostic evaluation of presumed ambiguous genitalia or the most common postnatal outcomes. To this effect, we have undertaken a retrospective review of the medical records of individuals evaluated through the DSD clinic at Seattle Children's Hospital over a 17-year period to determine if a prenatal diagnosis of a DSD was suspected, what prenatal diagnostic work-up was undertaken, and the postnatal outcome, including whether a postnatal genetic diagnosis was confirmed. We discuss how prenatal diagnostic testing for ambiguous genitalia has increased over time and we review the utility of such testing. We highlight the importance of a multidisciplinary team approach beginning in the prenatal setting.

MATERIALS AND METHODS

Individuals who were evaluated through the multidisciplinary DSD clinic at Seattle Children's Hospital (formerly Children's Hospital and Regional Medical Center) with birthdates from 01/01/1994 through 08/16/2011 were included in this study. Therefore, a subset of individuals in this report (those evaluated between 1994 and 2005) were also included in the report by Parisi et al. [2007], which focused primarily on postnatal diagnoses and management. Individuals with birthdates prior to 01/01/1994 were excluded due to the difficulty in obtaining their medical records. The medical records were reviewed for the following: whether a prenatal diagnosis of ambiguous genitalia was suspected based on either prenatal imaging studies alone or imaging studies that did not correspond to prenatal karyotype; what prenatal diagnostic studies were completed; and postnatal outcome, including whether a specific genetic diagnosis was made. We did not review actual prenatal ultrasound images in most cases, as many of the families in our cohort received prenatal care at outside facilities throughout the greater Pacific Northwest area. Therefore, an ultrasound report that stated "ambiguous genitalia" was accepted as such, without re-review of the images.

The medical records of 189 individuals were reviewed. Of these, 49 individuals were excluded due to insufficient records detailing their prenatal findings. Postnatal diagnosis was accomplished through our multidisciplinary Disorders of Sex Development team, which includes a geneticist, an endocrinologist, an urologist, a psychologist, and a genetic counselor. Individuals were placed into clinical diagnostic categories using previously defined terminology [Hughes et al., 2006; Parisi et al., 2007] based on the results of external and internal genital examination, gonadal pathology (when available), cytogenetic studies, endocrinologic studies, and specific molecular genetic studies (when available). This study was deemed exempt by the Institutional Review Board at Seattle Children's Hospital.

RESULTS

Of the 140 individuals included in this study, 34 (24%) were identified as having ambiguous genitalia or chromosome-phenotype discordance on prenatal ultrasound examination. As not all conditions that cause DSD are readily identifiable in the neonatal period, such as complete androgen insensitivity syndrome (AIS; OMIM # 300068), our cohort of individuals was divided into those who had conditions in which concern for a DSD was apparent in infancy and those who came to attention in childhood/adolescence (Tables I and II, respectively). Clearly, prenatal diagnosis for conditions that typically present outside of infancy are expected to have a low prenatal detection rate, unless a pregnancy is evaluated in light of a specific known family history, which was the case for one family in which a prenatal diagnosis of complete AIS was made.

To evaluate whether the prenatal detection of ambiguous genitalia has improved over time and to analyze whether more prenatal diagnostic tests were offered over time, we arbitrarily divided our cohort into three birth date categories: category 1 included birth dates between 01/01/1994 and 12/31/1999 (33 individuals); category 2 included birth dates between 01/01/2000 and 12/31/2005 (50 individuals); and category 3 included birth dates between 01/01/2006 and 08/16/2011 (57 individuals). The findings in the 34 individuals identified prenatally as having ambiguous genitalia or chromosome-phenotype discordance are summarized in Table III, and include 3 individuals in category 1, 8 individuals in category 2, and 23 individuals in category 3.

Table III also outlines the prenatal diagnostic studies that were completed in the 34 instances of prenatal diagnosis of ambiguous genitalia or chromosome-phenotype discordance. In the chromosome-phenotype discordance group, prenatal karyotype was performed for advanced maternal age in 3 and for an abnormal maternal serum screen in 2; in none of these patients was karyotype performed because of a prenatal ultrasound concerning for ambiguous genitalia. In 15/34 instances (44%), prenatal diagnostic studies were declined, including 2 instances in birth date category 1, 1 instance in birth date category 2, and 12 instances in birth date category 3. In the remaining 19 patients, a karyotype was done as the sole diagnostic test in 11 instances (58%), including the 5 with chromosome-phenotype discordance. If the 5 chromosome-phenotype discordance patients are removed from this group, a total of 6/14 (43%) had a karyotype as the only prenatal evaluation for ambiguous genitalia detected on imaging. This included 1 patient in birth date category 1, 2 patients in birth date category 2, and 3 patients in birth date category 3. In 4 patients, fluorescence in situ hybridization (FISH) for *SRY* was done in conjunction with a karyotype and in 1 patient FISH for the Y-centromere probe was done in conjunction with a karyotype. Four out of these 5 patients fell within birth date category 3, while 1 fell into birth date category 2. In 2 patients, testing for Smith-Lemli-Opitz syndrome (MIM # 270400) by measurement of amniotic fluid 7-dehydrocholesterol concentration was performed in addition to karyotype (1 patient) or in addition to karyotype and FISH for *SRY* (1 patient). In one instance karyotype, FISH for *SRY*, *SRY* sequencing, *AR* (androgen receptor) sequencing, and amniotic fluid 7-dehydrocholesterol concentration was completed prenatally. All of these remaining patients were within birth date category 3.

TABLE I. Total Infants With DSD (n = 123)

	Prenatally detected	Total
Abnormal genitalia with Y chromosome material present (n = 68)		
Hypospadias		
Penoscrotal hypospadias with transposition	8	24
Mixed gonadal dysgenesis (testis and streak)	2	9
Otherwise normal 46,XY SGA male	1	2
Ovotesticular DSD (both ovarian and testicular tissue)	1	2
5-alpha reductase deficiency	1	2
46,XX testicular DSD (*SRY*+)	0	2
Uncomplicated/unknown	1	7
Micropenis (without hypospadias)		
Vanishing testis syndrome	0	5
PAIS	1	3
Known sex chromosome abnormality (excluding 45,X/46,XY)	0	3
Aphallia	0	2
LH/FSH deficiency	0	1
Unknown	3	6
Abnormal genitalia with 46,XX karyotype and no Y chromosome (n = 28)		
CAH	1	20
Clitoromegaly/labial anomalies	2	3
46,XX testicular DSD (no Y chromosomal material)	1 (detected due to family history)	2
Cloacal malformation	1	2
Vaginal anomaly	1	1
Infants with disorders of chromosome-phenotype discordance (n = 8)		
45,X/46,XY with normal male phenotype	4	5
45,X with Y chromosomal material	1	2
45,X/46,XY with ambiguous genitalia	0	1
Other (n = 19)		
MCA	3	10
Other chromosome anomaly (not sex chromosome related)	1	3
46,XX/46,XY chimerism in twins	0	2 (1 twin pair)[a]
Campomelic dysplasia	0	1
NF-1 with plexiform NF of vaginal area	0	1
SLOS	0	1
WAGR (11p-)	0	1

DSD, disorders of sex development; SGA, small for gestational age; *SRY*, sex-determining region on the Y chromosome; PAIS, partial androgen insensitivity syndrome; LH, luteinizing hormone; FSH, follicle-stimulating hormone; CAH, congenital adrenal hyperplasia; MCA, multiple congenital anomalies; NF-1, neurofibromatosis type 1; NF, neurofibroma; SLOS, Smith-Lemli-Opitz syndrome; WAGR, Wilms tumor-Aniridia-Genitourinary anomaly-mental retardation syndrome.

[a] Souter VL, Parisi MA, Nyholt DR, Kapur RP, Henders AK, Opheim KE, Gunther DF, Mitchell ME, Glass IA, Montgomery GW. 2007. A case of true hermaphroditism reveals an unusual mechanism of twinning. Hum Genet 121:179–185.

TABLE II. Presentation in Childhood/Adolescence (n = 17)

	Prenatally detected	Total
Disorders with chromosome-phenotype discordance (n = 8)		
Complete AIS	1 (based on family history)	8
Mullerian agenesis (n = 4)		
MRKH	0	4
Micropenis (n = 5)		
Hypogonadotropic hypogonadism	0	5

AIS, androgen insensitivity syndrome; MRKH, Mayer-Rokitansky-Kuster-Hauser syndrome.

In 23/34 (68%) of the prenatally identified individuals with ambiguous genitalia or chromosome-phenotype discordance, the final clinical diagnostic category was assigned based on imaging studies, gonadal pathology (if available), karyotype, and hormonal studies, with no specific molecular diagnosis found (Table III). Both individuals with mixed gonadal dysgenesis had postnatal evidence of a mosaic sex chromosome abnormality. In the one instance of 5-alpha reductase deficiency (OMIM #607306), a homozygous mutation in *SRD5A2* confirmed the clinical diagnosis. The one instance of congenital adrenal hyperplasia (CAH) (OMIM # 201910) due to 21-hydroxylase deficiency was also molecularly confirmed with heterozygous mutations detected in *CYP21A2*. In 4/5 instances of chromosome/phenotype discordance, a postnatal mosaic sex chromosome anomaly was confirmed; in the 5th patient,

TABLE III. Summary of Prenatally Diagnosed Cases by Indication

Postnatal clinical diagnosis	Prenatal diagnostic studies	Birth date category*	Postnatal genetic diagnosis confirmed?
Ambiguous genitalia noted on ultrasound (n = 28)			
Penoscrotal hypospadias with transposition (n = 8)	46,XY, *SRY*+	3	No
	46,XY, *SRY* +, nl AF 7-dehydrocholesterol, *SRY* sequencing nl, *AR* sequencing nl	3	No
	46,XY, *SRY*+	3	No
	46,XY	3	No
	46,XY, *SRY* +, SLOS −	3	No
	46,XY	3	No
	None	3	No
	None	3	No
MCA (n = 3)	46,XY	2	No
	None	3	No
	None	3	No
Unknown micropenis (n = 3)	46,XY, *SRY*+	2	No
	46,XY, *SRY*+	3	No
	46,XY,sattelited Y chromosome that was paternally inherited, nl AF 7-dehydrocholesterol	3	No
Clitoromegaly/labial anomalies (n = 2)	None	1	No
	46,XX	3	No
Mixed gonadal dysgenesis (n = 2)	None	2	45,X/46,XY
	45,X with centromeric Y FISH +	3	45,X/46,X,isoYp
5-Alpha reductase deficiency (n = 1)	None	3	Homozygous P212R mutations in *SRD5A2*
CAH (n = 1)	None	3	Q318X/Intron 2 G heterozygous mutations in *CYP21A2*
Cloacal malformation (n = 1)	None	3	No
Other chromosome anomalies (n = 1)	None	3	47,XX,+21
Otherwise normal 46,XY SGA male (n = 1)	None	3	No
Ovotesticular DSD (n = 1)	None	3	No
PAIS (n = 1)	None	1	No
46,XX testicular DSD (n = 1)	None	3	No
Uncomplicated hypospadias (n = 1)	46,XY	2	No
Vaginal anomaly (n = 1)	None	3	No
Chromosome-phenotype discordance revealed secondary to maternal factors (n = 5)			
Mosaic sex chromosome abnormality	45,X/46,XY	1	45,X/46,XY
	45,X	1	45,X[83]/46,X,dic(Y;14)(p11.32;p11.2)[19] in skin
	45,X/46,XY	2	45,X/46,XY
	45,X/46,XY	2	45,X/46,XY
	45,X/46,XY	3	46,XY
Family history of DSD (n = 1)			
Complete AIS	46,XY	1	No molecular testing performed

SRY, sex-determining region on the Y; "+", present; nl, normal; AF, amniotic fluid; *AR*, androgen receptor; SLOS, Smith-Lemli-Optiz syndrome; MCA, multiple congenital anomalies; FISH, fluorescence in situ hybridization; CAH, congenital adrenal hyperplasia; SGA, small for gestational age; DSD, disorders of sex development; PAIS, partial androgen insensitivity syndrome; AIS, androgen insensitivity syndrome.
*Category 1: 01/01/1994 to 12/31/1999, Category 2: 01/01/2000 to 12/31/2005, Category 3: 01/01/2006 to 08/16/2011.

a postnatal blood karyotype found only 46,XY cells. Further tissue testing on this individual was not pursued. Lastly, one individual in the "Other" category was found to have 47,XX,+21 (OMIM #190685) postnatally, in addition to ambiguous genitalia. Given that Down syndrome is not typically associated with genital anomalies, this may represent an unrelated finding.

In 2/34 (6%) instances, pregnancy was terminated based on the prenatal finding of ambiguous genitalia. In the first instance, fetal autopsy demonstrated hypospadias, hypoplastic "shawl" scrotum, no Mullerian remnants, and normal testicular tissue histologically. No other physical anomalies were found and no molecular diagnosis was made. The fetus appeared to fall within the clinical

diagnostic category of penoscrotal hypospadias with transposition. In the second instance, postmortem examination demonstrated micropenis (phallus measuring <2 mm at gestational age of 20 weeks), no Mullerian structures, and bilateral testes with Leydig cell hyperplasia. There was a question as to whether this could represent partial androgen insensitivity syndrome (PAIS; OMIM # 312300), however no molecular diagnosis was pursued. Therefore, this fetus was placed into the ''Unknown Micropenis'' clinical category for the purposes of this study.

DISCUSSION

Prenatal detection of ambiguous genitalia has increased over time, as evidenced by the fact that in our study many more individuals ascertained prenatally were from birth date category 3 (birth date after 01/01/2006). While there were overall more individuals in birth date category 3 compared to categories 1 and 2 (57 vs. 33 and 50, respectively), the percentage of prenatal detection of ambiguous genitalia in category 1 was 3/33 (9%), in category 2 was 8/50 (16%), and in category 3 was 23/57 (40%). This could be due to several factors: (1) improved ultrasound technology and operator experience over the last 17 years; (2) improved access to a tertiary care center for further evaluation and confirmation of fetal anomalies, including ambiguous genitalia; and (3) more referrals to our DSD center due to improved publicity.

Recently, some centers have adopted 3-dimensional (3D) ultrasonography for further evaluation of the external genitalia. However, a comparison by Hackett et al. [2010] of the accuracy of 2-dimensional versus 3-dimensional ultrasound in determining postnatal sex did not find a statistically significant difference between these two imaging modalities. In addition, several articles have highlighted the finding that 3D ultrasound images can actually lead to incorrect fetal sex assignment [Cafici and Iglesias, 2002; Verwoerd-Dikkeboom et al., 2008; Abu-Rustum and Chaaban, 2009]. Therefore, in our center, 3D ultrasonography for suspected ambiguous genitalia is used with extreme caution, if at all.

Within the literature, prenatal diagnostic studies for ambiguous genitalia differed, and included offering fetal karyotype alone, fetal karyotype with FISH for *SRY*, or fetal karyotype with FISH for *SRY* and amniotic fluid hormone studies [Mandell et al., 1995; Cheikhelard et al., 2000; Pinhas-Hamiel et al., 2002; Mazza et al., 2003]. Prenatal diagnostic testing offerings also varied widely within our cohort. As FISH testing did not become clinically available until the mid to late 1990s, it is not surprising that this test was not used routinely until the last decade. Within our cohort of fetuses with prenatally diagnosed ambiguous genitalia, no FISH testing was done on those born in birth date category 1, 1 FISH test was used in birth date category 2, and 6 FISH tests were used in birth date category 3. Amniotic fluid hormonal testing was limited in our cohort to 7-dehydrocholesterol concentration and was performed in three patients, all of whom were from birth date category 3.

In this study, only about one-fourth (9/34; 26%) of individuals prenatally diagnosed with ambiguous genitalia or chromosome-phenotype discordance ultimately received a specific genetic diagnosis postnatally. Of these individuals, 6/9 (67%) had chromosome anomalies that were detected on karyotype analysis and did not require further molecular testing. Within the literature, a specific molecular diagnosis is identified postnatally in only approximately 20% of gonadal differentiation defects [MacLaughlin and Donahoe, 2004; Houk et al., 2006]. While our understanding of the multitude of genes responsible for the vast group of DSD conditions is increasing steadily, as evidenced by recent publications in which duplications within the *SHOX* gene were identified as one cause of Mayer-Rokitansky-Kuster-Hauser syndrome (MIM #277000) [Gervasini et al., 2010] and mutations in *MAP3K1* were found to lead to some cases of 46,XY DSD [Pearlman et al., 2010], our postnatal genetic testing is still low yield. Until we are able to fully understand the genetic causes of the clinical diagnoses made after birth, our ability to molecularly diagnose these conditions prenatally will remain elusive.

The most common clinical diagnosis identified prenatally in our cohort was penoscrotal hypospadias with transposition of the scrotum and no other congenital malformations (8/34; 24%). All were assigned a male sex of rearing with cautiously positive outcomes to date, although none has yet reached adulthood. Interestingly, the most common postnatal diagnosis was also penoscrotal hypospadias with transposition of the scrotum (24/140; 17%). Although the most common cause of virilization in the presence of a 46,XX karyotype was 21-hydroxylase deficiency (20/28; 71%) representing 20/140 (14%) of the entire cohort, this was rarely diagnosed prenatally in a female at a low a priori risk (1/28; 3.5%).

A similar retrospective study of prenatally diagnosed ambiguous genitalia over a 4-year period by Mandell et al. [1995] found comparably diverse diagnoses, with no single diagnosis comprising more than a handful of cases. The most common diagnosis in a 46,XX fetus was congenital adrenal hyperplasia. However, the majority of their patients were 46,XY, and at least 5/17 (29%) were found to have hypospadias and/or penoscrotal transposition, although several also had other associated birth defects, such as imperforate anus or orofacial clefting. The changing terminology pertaining to DSD conditions [Hughes et al., 2006; Aaronson and Aaronson, 2010; Hughes, 2010a,b; Pasterski et al., 2010] over time along with the lack of specific clinical definitions in the older literature make a direct comparison of our data to their data difficult.

A more recent study from Cheikhelard et al. [2000] of 53 cases of DSD ascertained both prenatally and postnatally found that of 23 males with ambiguous genitalia, the most common diagnosis was male pseudohermaphroditism or as it is now termed 46,XY DSD (17/23; 74%), which they defined as posterior hypospadias with chordee and scrotal anomaly with or without cryptorchidism. Within the karyotypic females, the most common finding was female pseudohermaphroditism or 46,XX DSD (6/11; 55%), which they define as clitoromegaly with or without vulvar or vaginal anomalies.

The diversity of postnatal diagnoses in our cohort and in those reported in the literature underscores the importance of a multidisciplinary team approach to the evaluation and management of DSD conditions. Ideally this should begin with counseling during the pregnancy utilizing a team with expertise in DSD, including genetics, endocrinology, urology, and OB/GYN. Discussions with the family should center around prenatal testing options, most

likely diagnoses, plan for delivery, and plan for immediate postnatal evaluation. Prenatal testing should focus on excluding conditions that lead to multiple anomalies and/or cognitive disabilities. When isolated ambiguous genitalia is suspected prenatally, it would seem reasonable to offer karyotype with FISH for *SRY*. Prenatal assessment for Smith-Lemli-Optiz syndrome (SLOS) via amniotic fluid hormone testing or molecular testing is also reasonable, particularly in the presence of a 46,XY fetal karyotype, because this condition is typically associated with significant cognitive disability and further ultrasound markers for SLOS may be absent. In 46,XX fetuses, molecular testing for *CYP21A2* for 21-hydroxylase deficiency is the highest yield, although amniotic fluid hormone testing could be pursued and might identify rarer types of steroid biosynthesis disorders. Identifying 21-hydroxlyase deficiency prenatally allows planning for the medical management of the child immediately after birth. While our cohort is small, making widespread conclusions about the yield of prenatal molecular testing for such genes as *SRY*, *AR*, or *SRD5A2* difficult, the utility of such testing is questionable, as affected individuals typically do not have intellectual disability or congenital anomalies outside of the genitourinary tract. Furthermore, the low yield of such testing postnatally when evaluations of the external and internal genitalia, hormone concentrations, and gonadal pathology are available, makes prenatal testing for such conditions impractical.

In the presence of other congenital anomalies or growth abnormalities, a targeted prenatal chromosomal microarray should be considered, to maximize the opportunity of identifying cryptic microdeletions or microduplications that may underlie a multiple congenital anomaly syndrome. However, as chromosomal microarray is not yet as sensitive at detecting chromosomal mosaicism as routine karyotype with adequate numbers of cells analyzed [Miller et al., 2010], a karyotype study to detect mosaic sex chromosome anomalies should remain a component of the evaluation in fetuses with ambiguous genitalia. Certain DSD conditions that present primarily with findings in adolescence, as listed in Table II, are difficult to detect prenatally in the absence of a known family history, highlighting the role of genetic consultation and a careful family history in the evaluation of those with DSD conditions and/or suspected fetal ambiguous genitalia.

Our data are biased in that, with the exception of a few cases, only those with a known DSD postnatally were ascertained. In addition, the number of cases in which the parents elected termination without consultation with the DSD team is unknown. Therefore, we were not able to determine the frequency of prenatally suspected ambiguous genitalia with true anomalies or normal/mild genital anomalies. In order to improve counseling about postnatal outcomes, prospective studies on the sensitivity and specificity of prenatal ultrasound at different gestational ages to diagnose ambiguous genitalia and correlation of prenatal findings with the final postnatal diagnosis should be undertaken.

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REFERENCES

Aaronson IA, Aaronson AJ. 2010. How should we classify intersex disorders? J Pediatr Urol 6:443–446.

Abu-Rustum RS, Chaaban M. 2009. Is 3-dimensional sonography useful in the prenatal diagnosis of ambiguous genitalia? J Ultrasound Med 28: 95–97.

Blackless M, Charuvastra A, Derryck A, Fausto-Sterling A, Lauzanne K, Lee E. 2000. How sexually dimorphic are we? Review and synthesis. Am J Human Biol 12:151–166.

Cafici D, Iglesias A. 2002. Prenatal diagnosis of severe hypospadias with two- and three-dimensional sonography. J Ultrasound Med 21:1423–1426.

Cheikhelard A, Luton D, Philippe-Chomette P, Leger J, Vuillard E, Garel C, Polak M, Nessmann C, Aigrain Y, El-Ghoneimi A. 2000. How accurate is the prenatal diagnosis of abnormal genitalia? J Urol 164:984–987.

Gervasini C, Grati FR, Lalatta F, Tabano S, Gentilin B, Colapietro P, De Toffol S, Frontino G, Motta F, Maitz S, Bernardini L, Dallapiccola B, Fedele L, Larizza L, Miozzo M. 2010. *SHOX* duplications found in some cases with type I Mayer- Rokitansky-Kuster-Hauser syndrome. Genet Med 12:634–640.

Hackett LK, Tarsa M, Wolfson TJ, Kaplan G, Vaux KK, Pretorius DH. 2010. Use of multiplanar 3-dimensional ultrasonography for prenatal sex identification. J Ultrasound Med 29:195–202.

Houk CP, Hughes IA, Ahmed SF, Lee PA. the Writing Committee for the International Intersex Consensus Conference Participants. 2006. Summary of consensus statement on intersex disorders and their management. Pediatrics 118:753–757.

Hughes I. 2010a. How should we classify intersex disorders? J Pediatr Urol 6:447–448.

Hughes IA. 2010b. The quiet revolution. Best Pract Res Clin Endocrinol Metab 24:159–162.

Hughes IA, Houk C, Ahmed SF, Lee PA. LWPES Consensus Group. ESPE Consensus Group. 2006. Consensus statement on management of intersex disorders. Arch Dis Child 91:554–563.

MacLaughlin DT, Donahoe PK. 2004. Sex determination and differentiation [published correction appears in N Engl J Med 2004; 351:306]. N Engl J Med 350:367–378.

Mandell J, Bromley B, Peters CA, Benacerraf BR. 1995. Prenatal sonographic detection of genital malformations. J Urol 153:1994–1996.

Mazza V, Ottolenghi C, Di Monte I, Baldassari F, Rivasi F, Volpe A, Forabosco A. 2003. Early prenatal diagnosis of recurrent 46, XY partial gonadal dysgenesis. Prenat Diagn 23:716–721.

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. 2010. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86:749–764.

Parisi MA, Ramsdell LA, Burns MW, Carr MC, Grady RE, Gunther DF, Kletter BG, McCauley E, Mitchell ME, Opheim KE, Pihoker C, Richards GE, Soules MR, Pagon RA. 2007. A gender assessment team: Experience with 250 patients over a period of 25 years. Genet Med 9: 348–357.

Pasterski V, Prentice P, Hughes IA. 2010. Impact of the consensus statement and the new DSD classification system. Best Pract Res Clin Endocrinol Metab 24:187–195.

Pearlman A, Loke J, Le Caignec C, White S, Chin L, Friedman A, Warr N, Willan J, Brauer D, Farmer C, Brooks E, Oddoux C, Riley B, Shajahan S,

Camerino G, Homfray T, Crosby AH, Couper J, David A, Greenfield A, Sinclair A, Ostrer H. 2010. Mutations in *MAP3K1* cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination. Am J Hum Genet 87:898–904.

Pinhas-Hamiel O, Zalel Y, Smith E, Mazkereth R, Aviram A, Lipitz S, Achiron R. 2002. Prenatal diagnosis of sex differentiation disorders: The role of fetal ultrasound. J Clin Endocrinol Metab 87:4547–4553.

Verwoerd-Dikkeboom CM, Koning HJ, Groenenberg IAL, Smit BJ, Brezinka C, Van Der Spek PJ, Steegers AP. 2008. Using virtual reality for evaluation of fetal ambiguous genitalia. Ultrasound Obstet Gynecol 32:510–514.