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Prenatal management of disorders of Sex development

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Abstract Disorders of sex development (DSD) rarely present prenatally but, as they are very complex conditions, management should be directed by highly specialised medical teams to allow consideration of all aspects of diagnosis, treatment and ethical issues. In this brief review, we present an overview of the prenatal presentation and management of DSD, including the sonographic appearance of normal genitalia and methods of determining genetic sex, the prenatal management of pregnancies with the unexpected finding of genital ambiguity on prenatal ultrasound and a review of the prenatal management of pregnancies at high risk of DSD. As this is a rapidly developing field, management options will change over time, making the involvement of clinical geneticists, paediatric endocrinologists and urologists, as well as fetal medicine specialists, essential in the care of these complex pregnancies. The reader should also bear in mind that local social, ethical and legal aspects may also influence management.

Introduction

Disorders of sex development (DSD) are very challenging conditions requiring management by highly specialised medical teams to allow consideration of all aspects of diagnosis, treatment and ethical issues. Although rare, DSD is usually identified at the first physical examination after birth. However, in recent years, DSD has become more and more

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a prenatal medical issue. Probably the most common circumstance is a pregnancy presenting with a family history of an inherited form of DSD. Less common is the discovery of abnormal genitalia by prenatal ultrasonography or, and very rarely, discordance between the genetic sex determined by karyotyping, performed because of an increased risk of aneuploidy, and the phenotypic sex observed by ultrasonography. Ideally, these patients should be referred to a specialised multidisciplinary team including a pediatric endocrinologist, geneticist, paediatric radiologist and paediatric urological surgeon. This team should also have access to expertise in hormonal profiling and molecular genetics.

In this paper we shall briefly review some aspects regarding the prenatal presentation and management of DSD. We shall start by describing the sonographic appearance of normal genitalia and methods of determining genetic sex before discussing the prenatal management of pregnancies with the unexpected finding of genital ambiguity on prenatal ultrasound. Finally, we will review the prenatal management of pregnancies at high risk of DSD, keeping in mind that this is a rapidly developing field, dependent not only on the experience of the medical team but also patient access to advanced technical imaging and molecular genetic analyses. Local social, ethical and legal aspects may also influence management.

Normal appearances and evaluation of fetal genitalia

The appearance of normal fetal genitalia and the accuracy of sonographic fetal sex assignment across gestation are well documented [1,2]. However, reliable identification of fetal genital dysmorphology requires an experienced operator. In early pregnancy the genital tubercle is identical in size in male and female fetuses. From 12 weeks' gestation the critical observation is variation in the angle or 'sagittal sign' of the tubercle (Fig. 1) which allows for highly accurate sonographic identification of fetal sex using either 2-D (>95%) [3] or 3-D [4] ultrasound when performed by a skilled sonographer. The downward, or more obtuse angle, represents a female fetus and the upward, or acute angle, a male fetus. Sonographic assignment of fetal sex before 12 weeks' gestation is highly inaccurate [1,2].

Later in pregnancy, assignment is based on direct visualisation of the genital anatomy, including the scrotum and midline raphe of the penis in males, and the three lines, representing the labial lines, and uterus in female fetuses. There are charts of fetal penile length available but their utility has yet to be proven and different publications give slightly different normal ranges [5,6]. Three-dimensional ultrasound is of limited use for sonographic sex determination in routine practice, but it may be useful in defining malformations of the external genitalia. Colour flow Doppler ultrasound is not useful in defining normality, but it can be helpful in defining the extent of hypospadias if the origin of micturition can be identified (Fig. 2). Whilst there are descriptions of normal genital anatomy defined by in-utero magnetic resonance imaging [7,8], this modality is really of use in the evaluation of abnormal genitalia when it may add useful information regarding the genital anatomy, internal mullerian structures and other anatomical parts [9].

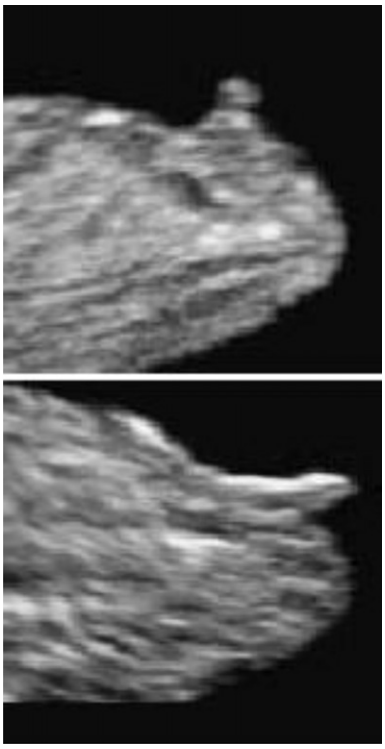

Figure 1 Showing the different angles of the phallus from 12 weeks gestation in a male (upper image) and female (lower image). Note the size of the phallus is equivalent in both, but it is the difference in angle that is diagnostic — acute in males and more obtuse in females.

Determination of genetic sex

Traditionally, determination of genetic sex has been performed by karyotype, fluorescent in-situ hybridisation

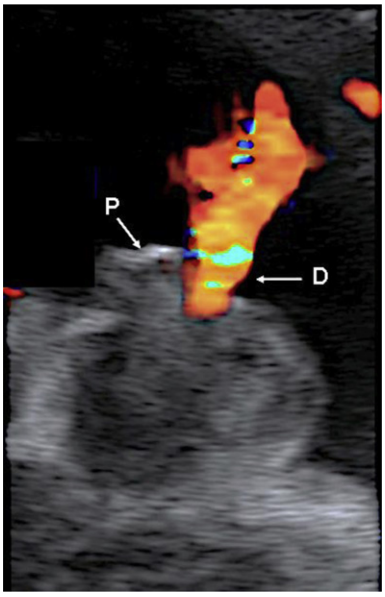

Figure 2 Doppler ultrasound showing urinary flow from a proximally placed urinary orifice (D) at the base of the short phallus (P). (Courtesy F Ushakov, London).

(FISH) or quantitative fluorescent polymerase chain reaction (qfPCR) analysis of amniocytes or chorionic villi following invasive techniques such as amniocentesis or chorionic villus sampling (CVS), both of which carry a risk of miscarriage of around 0.5–1% in experienced hands [10]. The identification of cell free fetal DNA (cffDNA) circulating in maternal blood has offered the potential for non-invasive prenatal testing (NIPT) of fetal genetic material [11]. This cffDNA is present in maternal blood from 4 weeks' gestation, but as the majority of cell free DNA in maternal blood emanates from the mother herself, there are significant technical challenges when using cffDNA for genetic diagnosis [12]. Cell free fetal DNA represents up to 10% of total circulating cell free DNA [13,14], increases with gestation and is very rapidly cleared from plasma at delivery [15]. As such, this is increasingly being used to identify genes or alleles in maternal plasma that are not present in the mother but are in the fetus because they have been inherited from the father or arisen *de novo* at conception. Current applications include fetal sex determination [16], the diagnosis of single gene disorders such as achondroplasia [17] and the determination of fetal Rhesus D status in RhD negative mothers [18].

NIPT for fetal sex determination relies on detecting a signal from Y-chromosome sequences in the maternal plasma. If Y-chromosome sequences are detected, the fetus is predicted to be a male. If no Y-chromosome sequences are detected, the fetus is predicted to be female. This approach can use a variety of Y-chromosome sequences including *SRY*, *DYS14* and amelogenin [19]. In the context of DSD it is probably better to use *DYS14* than *SRY*, since abnormalities in the *SRY* gene can in itself cause a DSD. Indeed, in the case of negative detection of these markers, there is a need to ensure the presence of fetal DNA (as a control to demonstrate amplification of fetal DNA sequences). NIPT for fetal sex determination is increasingly used to determine fetal sex in pregnancies at increased risk of serious X-linked genetic disorders and congenital adrenal hyperplasia. In the UK and some other European countries it is now the standard of care in these situations [20]. It is valued by women and health professionals alike [21,22] as targeted use of NIPT for sex determination in these high risk pregnancies is highly accurate (>99%) when delivered by accredited molecular genetic laboratories, and can reduce the rate of invasive testing by around 45%, thereby avoiding uneccesary exposure to miscarriage risk [23]. In addition, it permits early cessation of steroid treatment pregnancies at risk of congenital adrenal hyperplasia (CAH) where the fetus is predicted to be male [23,24]. Indeed, in some European centres dexamethasone treatment is delayed until NIPT performed at 7 weeks' gestation suggests the presence of a female fetus, thereby completely avoiding steroid exposure for all male fetuses. Finally, an economic analysis, which took account of all aspects of the care pathway, showed that when used in pregnancies at high risk of serious X-linked conditions (where parents might elect to terminate an affected pregnancy) and those at risk of CAH, NIPT was no more expensive than invasive diagnostic testing as the savings in invasive tests and uneccesary steroid treatment more than covered the laboratory costs of NIPT [25].

The management of fetuses presenting with the unexpected finding of genital ambiguity

Genital abnormalities are a rare finding on prenatal ultrasound but can be seen when detailed examination is performed following detection of another structural abnormality or, and more commonly, when the genitalia are examined because of parental curiosity. The aetiology of these unexpected genital anomalies is broad and includes an isolated anomaly, an underlying genetic syndrome, intra-uterine fetal growth restriction (IUGFR), chromosomal abnormalities and, although very rare, anomalies of steroid biosynthesis or androgen insensitivity, with the most common association being an error in early fetal development which results in bladder or cloacal exstrophy (Table 1).

The varied aetiology requires a structured approach to diagnosis and subsequent management, which should commence with a detailed ultrasound scan and evaluation of maternal and fetal dopplers. Clitoromegaly in a female fetus or hypospadias in a male fetus are very difficult to differentiate sonographically (Fig. 3), although 3-D ultrasound may be useful to distinguish the two (Fig. 3). Determination of genetic sex is almost always required in this situation. This can be done by analysis of cffDNA in maternal blood in most cases but in some situations full karyotyping following amniocentesis is required to exclude other chromosomal abnormalities (Table 1).

Once chromosomal, urinary tract and syndromal aetiologies are excluded, consideration should be given to steroid profiling or, in a 46XY fetus, sequencing of the androgen receptor gene to exclude Androgen Insensitivity Syndromes (AIS). These investigations are best directed by the DSD team. Genetic consultation is advisable in most cases without urinary tract aetiology. A suggested pathway for investigation is given in Fig. 4.

At present, relevant targeted molecular testing for rare DSD conditions in the absence of a family history has a low yield [27]. However, with the development of next generation sequencing it is likely that it will soon become possible to test for a wide range of known genetic mutations in cases presenting *de novo*.

Prenatal management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency (CAH)

Amongst the rare adrenal steroidogenesis biosynthesis disorders that lead to DSD, CAH due to 21-hydroxylase deficiency (CYP 21 CAH) is less rare than 11-β-hydroxysteroids dehydrogenase deficiency. Despite half a century of experience of CYP 21 CAH, the paediatric management of this condition remains challenging and the prenatal management even more so. Neonatal screening for CYP 21 CAH, based on 17-hydroxyprogesterone (17OHP) measurement from blood collected early after birth on filter paper, identifies affected infants [28–30]. Here, we summarise the general principles and suggested pathways for the prenatal diagnosis of CYP 21 CAH and briefly discuss approaches to prenatal management.

Table 1 Review of all cases with sonographic genital abnormalities seen in a tertiary referral unit over a ten year period with differential diagnosis and suggested management strategies. This table is adapted from those published in Pajkrt et al., 2008 [26] with additional cases added.

Classification	Final diagnosis (number)	Other sonographic findings	Karyotype	Differential diagnosis	Other AIDS to prenatal diagnosis	Management
Abnormal/ambiguous						
Isolated						
	Hypospadias (1)	None	46XY	Inadequate production of testosterone due to Leydig cell hypoplasia or biosynthetic defects — Congenital lipoid adrenal hyperplasia — 17α-hydroxylase deficiency — 3β-hydroxysteroid dehydrogenase deficiency — 17,20-lyase deficiency — 17β-hydroxysteroid dehydrogenase deficiency Partial androgen insensitivity syndrome 5α-reductase deficiency Ovotesticular DSD	• cffDNA for genetic sex • Consider sequencing of the Androgen Receptor gene	Refer to DSD team for investigation and counselling
	Cliteromegaly (1)	None	46XX	Congenital adrenal hyperplasia — 21-OH deficiency — 11-OH deficiency — 3β-hydroxysteroid dehydrogenase deficiency Ovotesticular DSD Maternally derived androgens, eg luteoma of pregnancy Placental aromatase deficiency	• cffDNA for genetic sex • Amniotic steroid levels • Maternal serum androgen levels • Maternal urinary oestrogen levels • Maternal ovarian scan for multicystic change	Refer to DSD team for investigation and counselling. Refer to gynaecology/oncology if luteoma

(*continued on next page*)

Table 1 (*continued*)

Classification	Final diagnosis (number)	Other sonographic findings	Karyotype	Differential diagnosis	Other AIDS to prenatal diagnosis	Management
Luteoma of pregnancy (1)	None	46XX				
With fetal growth restriction						
	Hypospadias (4)	Abnormal maternal and fetal dopplers	46XY	Aneuploidy Confined placental mosaicism	• Fetal biometry • Invasive testing to exclude aneuploidy and determine sex	Serial monitoring in FMU Refer to DSD team for counselling
In combination with urinary tract anomalies						
	Bladder exstrophy (8)	No intra-abdominal bladder with micropenis/no penis/ splayed glans. Low cord insertion	46XY	Cloacal exstrophy	• cffDNA for genetic sex • Detailed anomaly scan	Refer to combined fetal-urology team
	Cloacal exstrophy (3)	Absent intra-abdominal bladder, intra-abdominal cystic mass, dilated bowel, abnormal spine.	46XX	Aneuploidy Other cloacal abnormality	Invasive testing to exclude aneuploidy and determine fetal sex	Refer to combined fetal-urology team
	OEIS (2)	Ompalocoele/ gastroschisis, abnormal spine, no intra-abdominal bladder, hydronephrosis	46XY (1) 46XX (1)	Aneuploidy Cloacal abnormality	Invasive testing to exclude aneuploidy and determine fetal sex	Refer to combined fetal-urology team
	Unknown (2)	Echogenic kidneys	46XY (1) 46XX (1)		Invasive testing to exclude aneuploidy and determine fetal sex	Refer to combined fetal-urology/ nephrology team and clinical geneticist
With other anomalies						
	Mosaic ring chromosome 8	Complex cardiac anomaly, cerebral ventriculomegaly, urachal cyst	46XY mos ring 8	Aneuploidy Bardet Biedel Smith Lemli Opitz syndrome	• Invasive testing to exclude aneuploidy and determine genetic sex	• Autosomal recessive condition so take family history for other affected members and consanguinity.
	Bardet Biedel (1)	Echogeneic kidneys, polydactyly	46XY	Opitz Syndrome Opitz-G or BBB syndrome	• cffDNA for fetal sex only if invasive testing declined	
	Malpeuch syndrome (1)	Cleft lip and palate, IUGR	46XY	VATER association CHARGE association		
	Cranio-cerebellar-	Posterior fossa cyst	46XY	Other genetic syndromes	• Detailed scan	

	cardiac syndrome (1)	with hypoplastic cerebellar vermis, complex cardiac anomaly, talipes		• Maternal urinary steroids to exclude SLO	• Clinical genetics referral • Refer all relevant paediatric specialists for discussion of prognosis
	SLO (2)	Polydactyly, IUFGR, oedema, cleft lip, CNS anomalies.	46XY (2)		
Phenotype discordant with genotype					
Isolated					
	Androgen insensitivity syndrome	None	46XY	Invasive testing and/or cffDNA to confirm discordance between genotype and phenotype. Nb. In situations where there is an abnormality in the SRY gene cffDNA using SRY may give misleading results	Refer specialist DSD team
	Laboratory/clerical error (2)	None	46XY (2)	cffDNA to confirm fetal genetic sex	
With other sonographic abnormalities					
	Campomelic dysplasia (2)	Bowing of femora +/− tibia and fibula. Micrognathia, cardiac anomalies	46XY (2)	cffDNA to confirm fetal genetic sex	Refer clinical geneticist/skeletal dysplasia clinic
	SLO (2)	Polydactyly, IUFR, oedema, cleft lip, CNS anomalies.	46XY (2)		

cffDNA — cell free fetal DNA; DSD — disorders of sex developments; FMU — fetal medicine unit; IUFGR — intra-uterine fetal growth restriction; SLO — Smith Lemli Optitz.

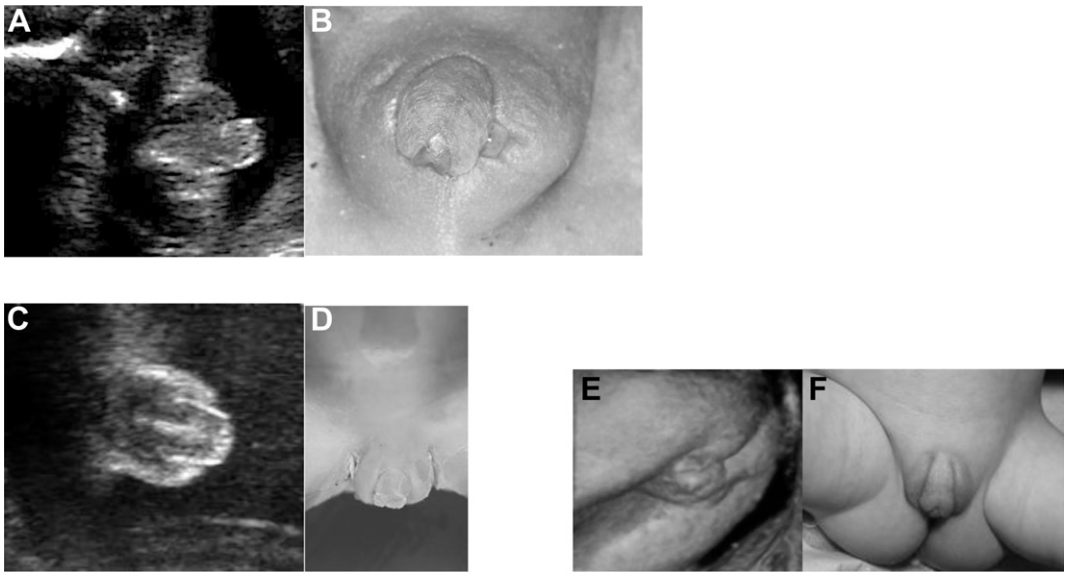

Figure 3 Ultrasound and postnatal images of cliteromegaly (A, B) and hypospadias (C, D) using 2-D ultrasound. A 3-D image taken at 30 weeks gestation and postnatal view is also shown (E, F) (Courtesy T.E. Cohen-Overbeek and I.A.L. Groenenberg, Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam).

Definitive prenatal diagnosis of CYP 21 CAH is limited to families where there is a previously affected child (index case). It requires molecular genetic analysis of both the index case and parents, parental DNA being essential to determine segregation of alleles of the CYP21 gene. Families with affected individuals should be offered genetic counselling, which should be delivered by suitably trained individuals in specialist centres. Molecular genetic analysis is complex [31,32] although less than 12 mutations account for 90—95% of the mutant alleles. The phenotype is not universally correlated to the genotype [32]. Ideally, the prenatal diagnostic pathway should commence with pre-pregnancy parental counselling by an expert team. Parents need to understand that this is an autosomal recessive condition with the consequent 1:4 risk of an affected child in every pregnancy. They need to understand that all affected offspring will require supplementation, but that only affected females are at risk of genital virilisation. Thus the risk of requiring postnatal surgical intervention is only 1:8 overall. Finally, and depending upon the severity, availability of definitive diagnosis, local social policy and parental attitudes, the possibility of pregnancy termination should be discussed. It is worth noting that parental attitudes may sometimes change with time, making it important to reassess the situation in every new pregnancy. In view of the availability of prenatal diagnosis

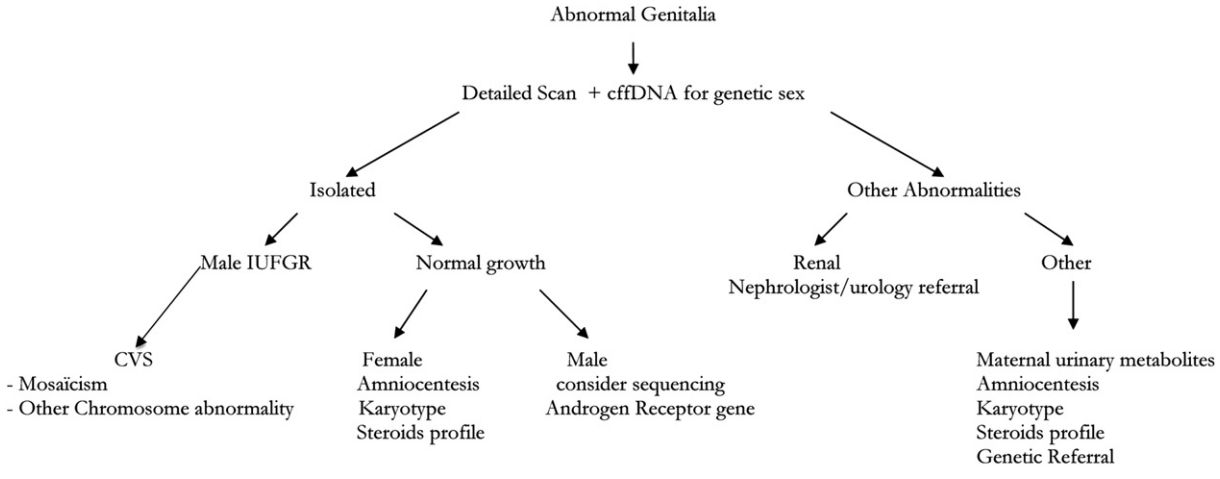

Figure 4 Suggested management algorithm for unexpected presentation of genital abnormalities on prenatal ultrasound. (Adapted from Pajkrt and Chitty 2008 [26]).

and treatment, experts managing these families should strive to emphasise the need for early referral in pregnancy, an ideal difficult to achieve.

When a family request prenatal diagnosis for CYP 21 CAH, the first step is to confirm the pregnancy and perform an ultrasound scan for accurate dating and exclusion of multiple fetuses or an empty gestational sac [23]. Genetic fetal sex determination can be performed reliably using NIPT and analysis of cffDNA obtained from maternal plasma from 7 weeks' gestation. Definitive molecular genetic diagnosis of CYP21 still requires analysis of chorionic villi following CVS from 11 weeks' gestation. Readers should be aware that the progress in NIPT is rapid and technological advances have recently allowed definitive diagnosis of other autosomal recessive and X-linked conditions [33,34] and so definitive molecular diagnosis of CYP21 using NIPT may not be that far distant.

In rare cases, the unexpected identification of a fetus with abnormal external genitalia at the time of a routine ultrasound scan, subsequently found to have a 46XX karyotype, may lead to analysis of the CYP 21 gene both in the fetus, using cultured amniocytes, chorionic villi or (rarely) fetal blood, and parental DNA for definitive molecular diagnosis (Table 1). Realistically this situation only arises at or after 20 weeks' gestation when routine anomaly scanning is performed. As this is beyond the potential prenatal treatment window (see below), parents should be carefully counselled regarding the risks and benefits of invasive prenatal diagnosis versus the risk of miscarriage versus diagnosis at birth.

Issues arising in CYP 21 CAH prenatal treatment

Prenatal treatment aimed at preventing masculinisation of affected female fetuses may be effective for fetuses at risk for classic CYP 21 CAH but is not appropriate for non-classic types (13—16). Although today's early diagnosis of fetal sex allows restriction of steroid treatment to mothers carrying female fetuses, as summarised in the consensus statement on CAH management, *"the appropriateness, ethics, and outcomes of the prenatal treatment of CAH with dexamethasone remain controversial"* [28]. The report concludes that provided treatment with maternal dexamethasone is commenced early in pregnancy (prior to 9 weeks after the last menstrual period) genital virilisation in affected female fetuses is ameliorated, an effect that by itself is considered as positive [35—38]. However, the statement that, *"it completely eliminates virilisation in more than 85%,"* should be viewed with caution as there has been no systematic evaluation of patients undergoing prenatal treatment by urethroscopy performed by a pediatric urological surgeon and the level of confluency between the vagina and the urethra is not known [30,31,38]. Furthermore, significant variations in outcome have been observed. These can have several aetiological factors including late onset of prenatal treatment, unreliable dating of the pregnancy, inappropriate dexamethasone administration (either dose and/or frequency of administration), poor maternal compliance and possible differences in androgen sensitivity.

Although no clinically significant adverse effects of long-term prenatal exposure to dexamethasone has been reported, data on long-term follow-up is limited [37,39,40] and questions have been raised as to the possible adverse effects on cognitive function in a small series [39—41]. In terms of maternal side-effects, treated mothers experience greater weight gain, oedema, and striae than untreated mothers but no evidence of an increased incidence of either hypertension or gestational diabetes has been observed [36,38,40]. Further concerns have been raised recently, following reports in both humans and animals, regarding the potential effects of prenatal dexamethasone exposure on gene expression during the early and critical developmental period. These observations have raised further questions as to the safety of such treatment [39—44]. Taking all these factors into consideration, there is a widely held view that prenatal dexamethasone treatment of a mother carrying a female fetus at risk of CYP 21 CAH should only be performed by a multidisciplinary experienced team in the context of a clinical trial with commitment to long-term follow-up of both mother and child.

Conclusions

Prenatal presentation of DSD is rare but demands sensitive and timely management by an experienced and expert team, which should include expertise in fetal medicine, genetics, paediatric endocrinology and paediatric urology. Rapid developments in molecular genetics and other technology will influence diagnosis and management, possibly rendering some of the conclusions of this paper outdated. Existing and any future prenatal treatment requires co-ordinated and long-term follow-up studies to determine the true benefits and costs. Since these conditions are rare, this will require multicentre, probably international, studies.

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