The Evaluation and Management of the Boy with DSD

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Summary:

Atypical genitalia in a boy may have a very wide and diverse aetiology and a definitive diagnosis is often challenging to reach. Detailed clinical evaluation integrated with extensive biochemical and genetic studies play an important role in this process. Such care should be undertaken in highly specialized centres that can also provide access to a multidisciplinary team for optimal long-term care.

Practice points:

1- A boy with suspected DSD should be assessed by an expert clinician who is familiar with the spectrum of conditions, and should be managed an expert multidisciplinary team.
2- A thorough clinical examination is necessary as XY DSD is associated with a wide range of associated conditions
3- Normal biochemical evaluation does not exclude genetic conditions
4- Long term care to people with DSD is an important aspect of management and should include psychological support
5- To improve the care of people with rare conditions such as DSD, knowledge sharing across geographical boundaries is of utmost importance

Research Agenda:

1- Longitudinal studies of physical and psychological outcome in people with XY DSD.
2- Understanding the benefit of reaching a definitive diagnosis in people with XY DSD

Introduction

Although the birth prevalence of cases where there is true genital ambiguity on expert examination may be as low as 1 in 4000 births, atypical genitalia may be as common as 1 in 300 births [1]. Thus, infants with atypical genitalia may have a very variable presentation and rather than treating every affected child in a uniform manner, it is paramount that such a child is first assessed by clinicians with adequate knowledge about the extent of variation in the physical appearance of genitalia, the underlying pathophysiology that may give rise to a disorder of sex development (DSD) and the strengths and weaknesses of the tests that can be performed in early infancy. Unlike 46, XX DSD where the cause is usually clear, identification of a cause of XY DSD is often unclear and may be attributed to a disorder of gonadal development, androgen synthesis or androgen action. Furthermore, many genetic conditions that give rise to XY DSD are associated with a wide range of manifestations. With the rapid advances in diagnostic technology including molecular biology our fundamental understanding of sex and gonadal development has improved. It remains to be seen how these advances are improving the management of people with DSD. In the field of rare conditions, it is imperative that the clinician shares experience and knowledge with others through platforms and forums that facilitate national and international clinical and research collaboration.

Conditions Associated With DSD in Boys

Although XY DSD can be broadly categorised into disorders of gonadal development, androgen synthesis and androgen action (**Table 1**), around 70% of boys who present to a specialist clinic with suspected DSD do not fit into any of these categories and are classed as a non-specific disorder of undermasculinisation [2]. It is possible that environmental factors may also play a role [3,4] and interfere with the normal development of the penis. Infants with XY DSD often have associated anomalies and are often small for gestational age [1,5]. Although several single gene defects and chromosomal rearrangements have been associated with gonadal dysgenesis [6], optimal gonadal development can also be affected

by sex chromosome aneuploidies, including mosaicism. Sex chromosome abnormalities are as common as 1% of pregnancies [7], may affect 1 in 450 newborn infants [8] and may be present in about 1;1,400 of the population [9]. In infants with suspected DSD, sex chromosome abnormalities may be as common as 6% [10]. Androgen synthesis disorders include defects in the testicular steroidogenesis pathway. The defect may interfere only with androgen production, as in cases with 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) deficiency, or involve other steroidogenic pathways in the adrenal gland, as in cases of 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) deficiency. Androgen synthesis would also be suboptimal in cases with Leydig cell defects from Leydig cell aplasia or hypoplasia. Leydig cell defects could also result from hypogonadotropic hypogonadism, leading to micropenis with or without cryptorchidism [11]. The incidence of androgen synthesis disorders such as 17β-HSD3 deficiency is reported at 1:150,000 births [12]. In persistence of Müllerian ducts syndrome (PMDS), boys are born with normal male external genitalia with internal female organs resulting from a defect in anti-Müllerian hormone synthesis or function. Androgen insensitivity syndrome (AIS) has been reported to be the commonest genetic disorder that is associated with DSD [13]. The incidence of AIS has been reported to range from 1 in 20,400 to 1 in 100,000 [14,15]. Recent data from the Danish National Patient Registry has reported that the prevalence of XY women with AIS may be about 4 per 100 000 [16]. Gonadal dysgenesis is reported to be as common as 1 in 10,000 infants [17] but its prevalence in XY females is reported to be lower at 1 per 100,000 [16].

Associated Malformations Including SGA in DSD

Associated anomalies in infants with atypical genitalia have been reported to occur in over 30% of cases [13,18- 20] with several cases having multiple malformations [18]. Although congenital malformations were reported primarily in cases with a mosaic karyotype or with syndromic DSD [21,22], they have also been reported with increased frequency in monogenic cases [18]. In addition to a high rate of associated congenital malformations, XY DSD infants may also be small for gestational age (SGA) [1,5,13,18,19). Infants with no clear cause of

undervirilisation are more likely to be SGA rather than those who have a molecular genetically proven diagnosis of partial androgen insensitivity syndrome [5]. On the other hand, associated malformations were more prevalent in cases with gonadal developmental disorders and amongst these, the commonest malformations include those that affect the cardiac, central nervous system (CNS) and renal system [18]. Many transcription factors are involved in the development of gonads and other organs and this could explain the high frequency of associated malformation in gonadal development disorders [6]. A mosaic karyotype may also influence the type of associated malformation, as boys with 45,X/46,XY DSD tend to have malformations that resemble Turner Syndrome [21]. Smith-Lemli-Opitz syndrome and P450 oxidoreductase (POR) deficiency are well known to be associated with other anomalies [22,23]. Other androgen synthetic disorders have been reported to be associated with other conditions [24]. Associated malformations have also been reported in cases of 17β-HSD3 and 5α-reductase 2 (5ARD2) mutations [18]. Given that renal development may also be androgen-dependent [25] it is notable that renal malformations have also been reported in cases of AIS, including those with a confirmed AR mutation [18,26]. The high rate of associated anomalies in XY DSD, especially in cases with no definite diagnosis, should direct the clinician to a meticulous clinical evaluation, trying to gather all possible information that might facilitate the genetic diagnosis.

History & Physical Evaluation

Clinical evaluation should begin with a complete medical history and a thorough general examination. The medical history should focus on antenatal course and results of the antenatal tests, specifically any structural abnormalities seen in the prenatal ultrasound, and whether karyotyping has been determined. Any maternal history of exposure to medication or chemical disruptors should be reported. Family history should be explored thoroughly, especially consanguinity, as many conditions that are associated with XY DSD are inherited. It should also record any neonatal or early infantile fatality and family members with any of the following conditions: DSD, hypoglycaemia, salt-losing crisis, genital surgery, hormonal replacement and

infertility or amenorrhea. The physical examination should include a detailed general evaluation to look for evidence that may suggest associated malformations or a dysmorphic syndrome. Vital signs, particularly blood pressure, should be determined. The genital exam should include careful inspection and palpation. The labioscrotal fold should be inspected for texture, shape, skin pigmentation and the degree of fusion, which could indicate the extent of the androgen effects. Additionally, the labioscrotal fold and inguinal area should be inspected and palpated for any evidence of gonads, which may include the need to milk it down for proper evaluation. The stretched phallus length should be measured and compared to the normative data. The number and the location of phallus openings should be noted. Lastly, use of a scoring system such as the external masculinisation score (EMS) will allow an objective record of the external genitalia [27].

Biochemical Evaluation

Current consensus guidelines for the evaluation of boys with DSD recommend biochemical assessment as an essential initial diagnostic step [27]. Basic biochemistry is available in most paediatric centres and steroid hormone profiling offers the opportunity for fast and comprehensive analysis for both diagnosis, as well as for monitoring of treatment progress [28]. Amongst specialist centres there is increasing availability of these investigations [29]. However, recent European guidelines advise that more complex biochemical endocrine investigations for DSD should be performed as part of a standardised quality framework in certified laboratories and clinicians managing boys with DSD should therefore be aware of this prior to initiating these tests [28]. Table 2 summarises the typical first tier of biochemical investigations recommended at the likely different ages of presentation with DSD. In the case of the newborn, biochemistry is often unreliable before the age of 36 hours and should be deferred until thereafter to ensure accuracy [27]. Future investigations will then be dictated by the clinical phenotype and the initial results. In terms of diagnostic utility, however it should be noted that a recent study reviewing the biochemical and genetic investigations performed in 122 boys with 46, XY DSD in a single specialist centre demonstrated that biochemical

endocrine abnormalities were detected in only around one quarter of affected boys. Interestingly, a genetic abnormality was detected in 20% of the boys with normal endocrine biochemistry [2]. Increasingly, it is therefore likely that biochemical investigations will in future be paired with molecular genetic analysis to provide a more comprehensive opportunity to offer boys a definitive diagnosis.

Genetic Evaluation

With improvements in technology, genetic evaluation is more readily accessible for boys with DSD than ever before. According to a recent international survey of centres caring for people with DSD, genetic testing may even be considered prior to biochemistry, for example for establishing a diagnosis in conditions such as 5ARD2 deficiency or 17β-HSD3 deficiency [29]. The importance of providing affected individuals and families with a definitive genetic diagnosis lies in the fact that subsequent management may be guided by the possibility of long term fertility or by future gonadal tumour risk. In addition, genetic counselling can be offered to families regarding recurrence risk and the possible long-term outcomes of the condition can be discussed. With advances in next generation sequencing (NGS), it is now possible to investigate large numbers of genes quickly and more cheaply [30]. The initial step in genetic evaluation in boys with DSD remains in undertaking karyotype analysis either by polymerase chain reaction (PCR) or fluorescent in situ hybridization (FISH) to confirm whether the boy is XX or XY [27]. Thereafter depending on availability, multiplex ligation dependent probe amplification (MPLA) or comparative genomic hybridisation (CGH) may be appropriate. Approximately 30% of DSD cases have been reported as having copy number variants (CNVs) on array-CGH [2,31] and this technology may be particularly useful in cases with additional malformations [32]. Furthermore, CNVs in the non-coding region of gene associated with DSD have been reported [33]. Some centres now offer targeted panels for key genes involved in DSD pathogenesis [2], with the likelihood of detecting an abnormality ranging from 10% [2] to 43% [34] in boys with XY DSD and depending on the number of genes included in the panel.

Additionally, the targeted panel increases the chance of getting a genetic diagnosis compared with a single gene test [35].

Functional Assessment

Historically, AIS was diagnosed based on the analysis of androgen binding in genital skin fibroblast [36,37]. This approach has become less popular over time, especially when investigating PAIS as the yield of molecular genetic diagnosis still remained low despite abnormal binding studies [36]. The effects of exogenous testosterone or human Chorionic Gonadotropin (hCG) stimulation on penile growth has been used as an indirect method to assess androgen sensitivity [38]. A number of regimens have been described in the literature [39-41]. The largest study to date which employed a randomized controlled design with over 90 cases in each arm showed that the administration of parenteral testosterone enanthate 2mg/kg monthly for 2 months was associated with an increase in penile length of 35% [42]. A concern with parenteral use of testosterone is the systemic effect of testosterone. Thus, some clinicians have used topical dihydrotestosterone (DHT) and percutaneous administration of DHT in a dose of 0.2-0.3 mg/kg once daily for a period of 3-4 months has been reported to increase phallic length by 0.5 to 2.0 cm in five boys with a penile length less than 2.5cm [43]. However, topical use may also be associated with raised systemic levels of androgens [44]. Given the variable response, it is unclear whether the penile response to androgens can be reliably used as a test of androgen sensitivity. Androgen sensitivity has also been evaluated biochemically by measuring the sex hormone binding globulin (SHBG) following androgen exposure as part of hCG stimulation [45] or following stanozolol therapy [46]. Although this response may sometimes be able to identify cases of androgen insensitivity [47], the variability of the SHBG response has prevented it from being used regularly. The expression of apolipoprotein D (APOD), an androgen-responsive protein, in the genital fibroblast following DHT application has been studied in a relatively small cohort of cases with XY DSD. APOD expression was strikingly reduced in AIS compared with the normal population and in comparison with other forms of XY DSD [48]. In a relatively large cohort of cases with 46, XY

DSD, the expression of APOD in the genital skin fibroblast was reduced in patients with AIS [49] and its study has also allowed the identification of a group of patients who did not have a mutation in the *AR* coding sequence but did have reduced AR expression in genital skin fibroblast. Recently, gene expression in peripheral blood mononuclear cell following a formal hCG stimulation test has also been studied as a method of assessing androgen sensitivity [50]. PIWI-interacting RNAs (piRNAs) were upregulated in the cases that showed a testosterone response to hCG compared to those in the non-responder group. This finding provides a helpful insight into the short-term effects of androgens whilst assessing functional sufficiency or sensitivity of androgens from readily available samples. By combining molecular genetics with routinely performed endocrine investigations, this approach also allows for a less invasive procedure than other methods.

Management

With the exception of disorders that affect glucocorticoid or mineralocorticoid synthesis, no specific medical therapy is usually required in infancy for most cases of XY DSD. Although the effect of infantile testosterone therapy on subsequent penile length has been reported [51,52], sufficient data on the different types of DSD is lacking. There is also some evidence to support the use of early gonadotrophin therapy in cases of hypogonadotrophic hypogonadism but long-term studies are lacking [53]. Generally, the most important aspect of XY DSD management is communication with the parents and resolution of any immediate issues regarding sex assignment. The management of XY DSD requires a well-trained multidisciplinary medical team that will provide optimal medical, surgical and psychological care for the patient and their family [27]. Psychological evaluation is an important aspect of XY DSD management. The parents of a child with atypical genitalia should be assessed, and should also be counselled by an experienced psychologist. Parents of new children have a number of queries and often find it difficult to talk to others [54]. They often find senior clinical staff a useful source of support but may rely on the premise that the condition can be treated.

Sex assignment is influenced by many factors. These factors include etiological causes, external genital features, internal reproductive anatomy, possibility of spontaneous pubertal development, the capacity for sexual activity and fertility potential, and the ethnic or cultural background of the parents [55]. In particular, prenatal androgen exposure is a factor that should also be considered when making a decision regarding gender assignment, as it has been theorized that gender identity in patients with 5 α reductase deficiency could be influenced by the extent of prenatal androgen exposure [56].

For sex assignment, each patient should be evaluated and managed individually, and the available outcome studies should be reviewed regularly. It has been generally accepted that all patients with complete androgen insensitivity syndrome (CAIS) and complete gonadal dysgenesis are raised as female, as studies have indicated that all identify themselves as female [57,58]. The decision of the assigning the sex of other XY DSDs is still controversial. Recently, it has been reported that the trend in sex assignment has changed, and now favours male sex assignment [59]. This change may have been influenced by the current outcome studies available. Patients diagnosed with either 17β-HSD3 or 5ARD2 deficiencies during the neonatal period could virilise during puberty, and 60 % of patients diagnosed with either of these two disorders have been reassigned from the female to male gender during pubertal period [60]. Additionally, with advances in vitro fertilization technologies, fertility has been documented in some cases with 5ARD2 deficiency [61]. This is an important development and should be considered during the decision of sex assignment. Among patients with partial androgen insensitivity syndrome (PAIS), androgen biosynthetic defects, and partial gonadal dysgenesis, gender identity corresponds with the sex of rearing, whether they are raised male or female [58]. In patients with gonadal dysgenesis, hCG stimulated testosterone secretion and the clinical response to exogenous testosterone should facilitate the decision of gender assignment, as these tests may indicate the possibility of spontaneous pubertal development.

Long term care, monitoring, education and provision of support to the patient and family are fundamental aspects of DSD management. During childhood, intermittent visits with the multidisciplinary medical team allows for the opportunity of the child and parents to express any concerns they may have, and to provide them the needed medical and psychological support. Additionally, regular visits reinforce a positive relationship between child and physician. Given that it is increasingly being realised that some causes of XY DSD such as NR5A1 gene abnormalities may be associated with a gradual deterioration in testes function [62], PAIS with a confirmed mutation in *AR* may be associated with early gynaecomastia [63], sex chromosome variations may be associated with learning or behaviour concerns, regular visits provide the forum for monitoring of these concerns. Around the time of puberty, the need for hormonal therapy should be considered and evaluated. Hormonal therapy should be initiated with low doses of the appropriate sex steroid hormone, which should be gradually increased to mimic spontaneous pubertal development [64]. However, unlike the typical case of constitutional delayed growth and puberty, testosterone therapy can be considered at an earlier age in boys with confirmed organic hypogonadism.

Long term studies that look at the outcome of boys with DSD are limited, and most of the literature is comprised of retrospective studies that are subject to selection bias [65]. The transition of care from paediatric to adult medicine should be planned early on in the patient's life. For optimum care, patients should be seen in a transition clinic where care is joined between paediatric and adult medicine. There are limited data regarding the transition among patients with DSD, and a proposed strategy has been published [66]. The concept of transition in general should be focused on the factors that optimize and ensure the continuity of medical and psychosocial care, as well as to provide comprehensive care in the adult clinic setting. Patients with DSD often receive medical and surgical intervention, which could, themselves lead to complications. In a retrospective study, 24% of patients who have had previous hypospadias repair experienced complications that required additional corrective surgery and long-term follow up was recommended [67]. Patients who have retained their testes are at risk

for developing a gonadal tumour. The risk rate of developing a gonadal tumour depends on different factors, and is determined by the type of disorders the patient is diagnosed with [68]. There are several other long-term outcomes such as cardiovascular health, metabolic health and bone health where there is little information available. Linkage studies have revealed that some of these outcomes may be affected in people with DSD [69] and it is believed that this may be related to suboptimal hormone replacement in adulthood. Quality of life (QoL), and in particular sexual QoL, in DSD should be assessed regularly and managed accordingly throughout their life. A comprehensive assessment of the patient's QoL should include an examination of their friendships, education, work life relations and their sexual life and activities. While some available studies show an impairment of the sexual QoL among patients with XY DSDs, the data remains inconclusive [70]. Incorporation of standardized clinician reported and patient reported outcome measurement as part of routine clinical assessment is becoming increasingly routine in clinical practice [71]. It is anticipated that collection of such measures will become an essential component of the delivery of care for boys and men with DSD.

Table 1 Classification of 46, XY DSD

Disorders of gonadal development

- **Complete gonadal dysgenesis (CGD)**
- **Partial gonadal dysgenesis(PGD)**

Disorder of androgen synthesis

- **Smith-Lemli- Opitz syndrome**
- **Steroidogenic acute regulatory (StAR) protein deficiency**
- **P450 side chain cleavage (scc) deficiency**
- **17-α-hydroxylase/17,20-lyase deficiency**
- **3-β- hydroxysteroid dehydrogenase type 2 (3β-HSD2) deficiency**
- **P450 oxidoreductase (POR) deficiency**
- **17-β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) deficiency**
- **5α- reductase 2 (5ARD2) deficiency**

Disorder of androgen action

- **Complete androgen insensitivity syndrome (CAIS)**
- **Partial androgen insensitivity syndrome (PAIS)**

Leydig cell defect

- **Leydig cell hypoplasia**
- **LH deficiency**

Persistence of Müllerian ducts syndrome (PMDS)

- **Defect in AMH synthesis**
- **Defect in AMH receptor**

Other

323

Age at first presentation	Biochemical evaluation	Genetic evaluation
Newborn	17 OH-progesterone	Urgent:
	Electrolytes	PCR or FISH analysis using Y
	Glucose	and X-specific markers
	Testosterone	Non-urgent:
	Androstenedione	CGH array
	Renin	Targeted NGS.
	AMH	
	Urine steroid profile	
Adolescent	LH	
	FSH	
	testosterone	
	Prolactin	

Table 2. Investigations for initial presentation of a boy with DSD. Abbreviations: 17OHP: 17 OH-progesterone; AMH: anti-Müllerian hormone; LH: luteinising hormone; FSH: follicle stimulating hormone; PCR: polymerase chain reaction; FISH: fluorescence in situ hybridisation; CGH: comparative genomic hybridisation; NGS: next generation sequencing.

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