

## Disorders of sex development: a new definition and classification

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A newborn infant with ambiguous genitalia is a complex enough problem to unravel without any further clouding by confusing terms. The nomenclature 'intersex', 'hermaphrodite' and 'pseudohermaphrodite' is anachronistic, unhelpful, and perceived to be pejorative by some affected families. In its place, a consensus statement recommends the term 'disorder of sex development' (DSD), a generic definition encompassing any problem noted at birth where the genitalia are atypical in relation to the chromosomes or gonads. The karyotype is used as a prefix to define the category of DSD, replacing the arcane terminology of male or female pseudohermaphroditism (now known as XY DSD or XX DSD, respectively). The new nomenclature has spawned a simple and logical classification of the causes of DSD. In this chapter new facets of gonadal dysgenesis and novel defects in steroid biosynthesis are reviewed in relation to the DSD classification, and options for early, non-invasive fetal sexing are described. Future research to determine many causes of DSD will benefit from the use of this universal language of scientific communication.

**Key words:** ambiguous genitalia; karyotype; consensus; nomenclature.

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The newborn infant with ambiguous genitalia posing an immediate problem of sex assignment is sometimes described as an endocrine 'emergency'. While this clinical scenario does not meet the traditional criteria for a life-threatening emergency, the problem is certainly immensely distressing to the parents and extended family. They will fail to comprehend why the instantaneous sex assignment that usually occurs at birth is not possible. To compound the problem, health professionals may inadvertently add to the 'ambiguity'. If the anatomy of the external genitalia is not confusing enough, further problems will be engendered by the use of mythological terms such as hermaphroditism and its commoner derivative, pseudohermaphroditism (male or

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female). The word intersex has been in use for some time, but is not favoured by many families with ambiguous genitalia, some of whom regard the term as pejorative. Such a situation cannot be ignored, and there has been a groundswell of opinion that a comprehensive review of the management of intersex is needed. This would recognize the multidisciplinary approach necessary to resolve the problem. Hence a range of health professionals would need to engage in a discourse to produce a consensus document on the management of intersex disorders.

## THE CHICAGO CONSENSUS

The European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society jointly organized a meeting of endocrinologists, surgeons, geneticists, psychologists, and patient advocacy group members, all representing a world community involved with the management of intersex disorders. A consensus document was subsequently published.<sup>1-3</sup> It has become known as the Chicago Consensus by virtue of its generation in the 'windy city'.

The Consensus document is far-ranging and delves into areas of longer-term management and outcome. Already the consequences of proposals contained within the Consensus relating to diagnosis and treatment are being evaluated.<sup>4</sup> A powerful driver to hold a Consensus arose from dissatisfaction with current nomenclature, espoused by both health professionals and patients alike. The generation of a new nomenclature rather serendipitously spawned a radical change in the classification of disorders of sex development; these two components are the subject of this review.

## NOMENCLATURE

The word intersex refers primarily to the clinical scenario of an infant born with external genitalia sufficiently ambiguous that sex assignment is not possible. A prototypic example is a Prader stage III-IV virilized female infant with congenital adrenal hyperplasia (CAH). An example arising from under-virilization of a male infant is partial androgen insensitivity syndrome, manifest as ambiguous genitalia, for which there are scoring systems to quantify the degree of masculinization.<sup>5,6</sup> However, not all cases of CAH manifest as intersex, and the complete form of androgen insensitivity syndrome (CAIS) is patently not an intersex condition. Consequently, the recommended nomenclature to replace intersex is the umbrella terminology 'disorder of sex development' (DSD). This is defined as a congenital condition in which development of chromosomal, gonadal, or anatomical sex is atypical. It can be argued that this embraces such a variety of conditions as to be meaningless in specificity. However, the nomenclature has no diagnostic purpose other than as a starting point towards that goal. The inclusion of the term congenital within the definition excludes all the conditions listed as causes of precocious or delayed puberty. The acronym DSD is not in common usage for any other 'competing' medical disorder.

Following publication of the Consensus statement, a number of responses appeared in the correspondence columns of the journals. Of some concern was use of the word 'disorder' in the DSD definition, although there was almost unanimity of opinion in favour of removing the term 'intersex' from the medical lexicon.<sup>7-9</sup> The fact that individuals with CAIS are normal phenotypic females prompted the suggestion that 'variations of reproductive development' be used as an alternative nomenclature. Furthermore, the acronym (VRD) would avoid the clash with a common congenital cardiac anomaly if 'variation of sex development' (VSD) was

used. A mutation in the CYP21 gene results in a disorder of steroidogenesis and DSD, the pathophysiology as clear as a mutation in the CFTR gene resulting in a disorder of chloride ion transport and chronic lung disease. These examples are clearly not just variations in normal physiology. Adding some diagnostic specificity to the generic DSD definition utilizes knowledge of the karyotype. This is based on recognizing the central role of karyotype analysis in the investigation of most cases of DSD, and knowledge in general about sex chromosomes. The decision to categorize according to karyotype led to the logical step of casting the abstruse and confusing term pseudohermaphroditism into medical antiquity. Table 1 summarizes the components of the revised nomenclature. It is now gaining currency in the scientific literature, whether the study is focused on endocrine, genetic, surgical or psychological issues.<sup>10–14</sup> Furthermore, standard endocrine textbooks are beginning to embrace the nomenclature in the cognate chapters.<sup>15,16</sup> The International Society for Hypospadias and Intersex Disorders (ISHID) entitled its second World Congress: Hypospadias and Disorders of Sex Development. The logic of the revised nomenclature is self-evident; true hermaphroditism is replaced by descriptive terminology which recognizes that the disorder can only be defined by knowledge of gonadal histology, which describes the presence of ovarian follicles as well as testicular tissue. Of course, the karyotypic prefix may be 46,XX (the most frequent), 46,XY or 46,XX/46,XY. The sex reversal nomenclature is one generally favoured by mammalian geneticists but lends itself readily for translocation to the new nomenclature.

CAUSES OF DSD: A NEW CLASSIFICATION

There are numerous modes of classification to bewilder the reader with exhaustive lists of all the possible causes of DSD. No system is perfect, and all will date as advances in molecular genetics result in improved diagnostic insight. Table 2 applies the revised nomenclature in the context of setting out three main diagnostic categories of DSD. One of the categories is retained as ‘sex chromosome anomalies’ as these remain a major component of the causes of DSD. Turner and Klinefelter syndromes are classically described as not being associated with abnormalities of the genitalia at birth, but this is not necessarily the case with Klinefelter syndrome where severe genital anomalies may occur.<sup>17</sup> Advances in molecular techniques such as microarray-based comparative genomic hybridization (CGH), quantitative fluorescent

Table 1. Nomenclature relating to disorders of sex development (DSDs).

Previous	Proposed
Intersex	Disorders of sex development (DSDs)
Male pseudohermaphrodite	46,XY DSD
Undervirilization of an XY male	
Undermasculinization of an XY male	
Female pseudohermaphrodite	46,XX DSD
Overvirilization of an XX female	
Masculinization of an XX female	
True hermaphrodite	Ovotesticular DSD
XX male or XX sex reversal	46,XX testicular DSD
XY sex reversal	46,XY complete gonadal dysgenesis

**Table 2.** A proposed classification of causes of disorders of sex development (DSDs).

Sex chromosome DSD	46,XY DSD	46,XX DSD
A: 47,XXY (Klinefelter syndrome and variants)	A: Disorders of gonadal (testicular) development	A: Disorders of gonadal (ovarian) development
B: 45,X (Turner syndrome and variants)	1. Complete or partial gonadal dysgenesis (e.g. SRY, SOX9, SFI, WT1, DHH etc)	1. Gonadal dysgenesis
C: 45,X/46,XY (mixed gonadal dysgenesis)	2. Ovotesticular DSD	2. Ovotesticular DSD
D: 46,XX/46,XY (chimerism)	3. Testis regression	3. Testicular DSD (e.g. SRY+, <i>dup</i> SOX9, RSP01)
	B: Disorders in androgen synthesis or action	B: Androgen excess
	1. Disorders of androgen synthesis	1. Fetal
	LH receptor mutations	3 $\beta$ -hydroxysteroid dehydrogenase 2
	Smith—Lemli—Opitz syndrome	HSD3B2
	Steroidogenic acute regulatory protein mutations	21-hydroxylase ( <i>CYP21A2</i> )
	Cholesterol side-chain cleavage( <i>CYP11A1</i> )	P450 oxidoreductase ( <i>POR</i> )
	3 $\beta$ -hydroxysteroid dehydrogenase 2 ( <i>HSD3B2</i> )	11 $\beta$ -hydroxylase ( <i>CYP11B1</i> )
	17 $\alpha$ -hydroxylase/17,20-lyase ( <i>CYP17</i> )	Glucocorticoid receptor mutations
	P450 oxidoreductase ( <i>POR</i> )	2. Fetoplacental
	17 $\beta$ -hydroxysteroid dehydrogenase( <i>HSD17B3</i> )	Aromatase ( <i>CYP19</i> ) deficiency
	5 $\alpha$ -reductase 2 ( <i>SRD5A2</i> )	Oxidoreductase ( <i>POR</i> ) deficiency
	2. Disorders of androgen action	3. Maternal
	Androgen Insensitivity Syndrome	Maternal virilizing tumours (e.g. luteomas)
	Drugs and environmental modulators	Androgenic drugs
	C: Other	C: Other
	1. Syndromic associations of male genital development (e.g. cloacal anomalies, Robinow, Aarskog, Hand-Foot-Genital, popliteal pterygium)	1. Syndromic associations (e.g. cloacal anomalies)
	2. Persistent Müllerian duct syndrome	2. Müllerian agenesis/hypoplasia e.g. MURCS)
	3. Vanishing testis syndrome	3. Uterine abnormalities (e.g. MODYS)
	4. Isolated hypospadias ( <i>CXorf6</i> )	4. Vaginal atresia (e.g. KcKusick—Kaufman)
	5. Congenital hypogonadotropic hypogonadism	5. Labial adhesions
	6. Cryptorchidism ( <i>INSL3</i> , <i>GREAT</i> )	
	7. Environmental influences	

polymerase chain reaction (QF-PCR) and pyrophosphorolysis-activated polymerization (PAP) for analysis of free fetal DNA from maternal blood and amniotic fluid samples are already being applied in clinical practice for detecting chromosome aneuploidy and for prenatal sexing.<sup>18–24</sup>

One benefit of the ability to detect fetal DNA in maternal plasma is the avoidance of dexamethasone treatment for a pregnancy at risk for CAH, when a male fetus can be identified as early as 7 weeks of gestation by analysis of the Y chromosome with SRY-specific probe (personal observation). Cell-free fetal DNA has a very short half-life (a matter of minutes) as compared to that of fetal cells that can persist for decades postpartum.<sup>25</sup> However, it is important to process the maternal blood sample using more than one centrifugation step, otherwise remnants of intact fetal cells from a previous pregnancy may be a contaminant.<sup>26</sup> A new technique requires careful validation before being applied to deriving information as critical as fetal sexing which is subsequently used in deciding treatment plan. It is estimated that fetal DNA can be detected as early as 5 weeks of gestation, and this is 100% accurate by 8 weeks, based on a Y-specific DAZ repetitive probe.<sup>27</sup> The combination of reverse transcriptase polymerase chain reaction (RT-PCR) detection and the PAP assay provides both sensitivity and specificity of 100%, with 95% confidence for the risk of an erroneous result being <8.1%.<sup>23</sup> When the fetal DNA analysis predicts female sex, a maternal discrimination test needs to be performed with several polymorphic markers, or a universal fetal DNA marker used in maternal blood which is methylated in maternal DNA but hypomethylated in the placenta.<sup>28</sup> It is our practice to perform the free fetal DNA analysis starting at 7–8 weeks of gestation and re-check the result in a sample collected 1 week later. There is a 21% rise in fetal DNA levels per week of gestation during the first trimester of pregnancy.<sup>29</sup> Home testing kits are now advertised, with names such as 'Baby Gender Mentor', whereby capillary blood spots can be collected onto filter paper for dispatch to the laboratory, with a fetal sexing result promised within 24–48 hours. Although the website provides precise details of when in pregnancy the sample should be collected and what complicating factors may render the test unreliable, it does not provide data on validation and quality control. It is also of some concern that unregulated public access to such important tests has not been formally considered with respect to ethical issues.<sup>30</sup>

It is logical that the karyotype should underpin the other two categories shown in Table 2 for the new DSD classification system. The advances in cytogenetics and molecular genetics are now applied to sex chromosome analysis, with impressive rapidity in the provision of results. The XX DSD and XY DSD categories are both subdivided according to a primary disorder of gonad development versus a disorder of sex-steroid biosynthesis arising from an otherwise morphologically normal gonad. In the case of XX DSD, this is generally extraneous to the gonad, as exemplified by 21-hydroxylase deficiency.

## CAUSES OF XX DSD

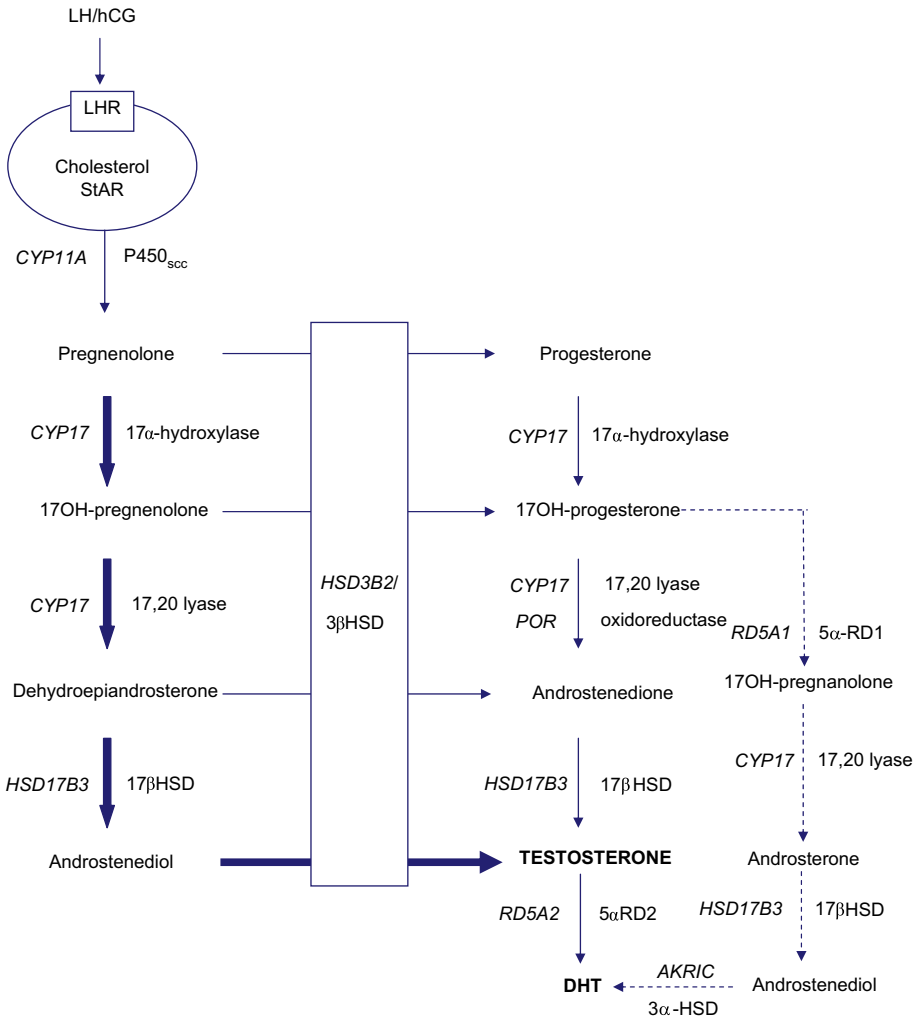
CAH remains the commonest cause of ambiguous genitalia of the newborn, whatever classification system is used in DSDs. The new classification system takes cognizance of a few additions to the limited list of causes of XX DSDs. Aromatase deficiency is now a well-characterized disorder of steroidogenesis, one of whose manifestations is the notable double virilizing effect on the mother and her female newborn from excess fetal androgen substrates.<sup>31,32</sup> Different tissue-specific promoters regulate the expression of aromatase in oestrogen-producing locations such as the placenta,

ovary, breast, and adipose tissue. In the case of XX DSD, there is exposure of the mother and a female fetus to fetal adrenal androgens that are normally aromatized by the fetally derived placenta. Only a total of seven virilized female infants with aromatase deficiency who fail to feminize at puberty have been reported.<sup>33</sup> Affected males are normal at birth, but present in early adulthood as excessively tall with delayed bone maturation, decreased bone mineralization, and features of the metabolic syndrome.

As with most disorders of steroidogenesis, there can be variability in phenotypic expression of this enzyme deficiency. Levels of residual aromatase activity of mutant enzymes as measured *in vitro* are generally associated with some breast development at puberty.<sup>34</sup> Indeed, it is possible to predict Tanner staging of breast development at puberty on the basis of quantitative aromatase activity, with up to 20% activity realizing stage 4 as well as normal uterine growth. A similar predictability with functional analysis of mutant 21-hydroxylase enzymes together with molecular modelling has been shown to distinguish classical from non-classical forms of CAH.<sup>35</sup> The key to considering aromatase deficiency as a cause of XX DSD includes the history of recurrent maternal virilization in subsequent pregnancies with resolution in the non-pregnant state. Other sources that are maternal in origin need to be excluded, such as polycystic ovarian syndrome, luteoma and hyperreactio luteinalis, although the latter two conditions rarely recur. Sometimes there can be severe recurrent virilization during pregnancy without a known cause.<sup>36</sup>

Cytochrome P450 oxidoreductase (POR) deficiency is the latest addition to the list of causes of XX DSD. This condition only emerged as a distinct entity through the realization that apparent combined deficiencies of 17-hydroxylase and 21-hydroxylase enzymes is a single disorder due to lack of POR, a membrane-bound flavoprotein that plays a central role in electron transfer from NADPH to P450 enzymes.<sup>37</sup> It was initially characterized in patients with Antley-Bixler syndrome (a skeletal dysplasia syndrome comprising craniosynostosis, brachycephaly, radio-ulnar/humeral synostosis and bowed femora) in whom some had ambiguous genitalia and abnormal steroidogenesis.<sup>38,39</sup> Most patients with Antley-Bixler syndrome have an autosomal dominant activating mutation in the *FGFR2* (fibroblast growth factor receptor 2) gene but do not have genital anomalies. In contrast, mutations in POR were only found when the syndrome was associated with genital anomalies and an abnormal pattern of steroids. Subsequently, it has been established that POR deficiency can cause DSD unassociated with a skeletal dysplasia characteristic of the Antley-Bixler syndrome. Reference to Table 2 shows that POR deficiency uniquely falls in both camps of disorders relating to XX DSD and XY DSD. How this occurs is illustrated in Figure 1.

POR is a cofactor to all microsomal cytochrome P450 enzymes, which include 17-hydroxylase and 17,20-lyase combined activities, 21-hydroxylase and aromatase. 17,20-Lyase activity is impaired more than 17-hydroxylase activity by POR deficiency, resulting in a disproportionate accumulation of 17OH-progesterone compared with androgens. This increased substrate may be converted efficiently to 5 $\alpha$ -reduced androgens by a newly proposed 'back-door pathway' to potent androgens such as DHT.<sup>40</sup> The primary evidence for such a pathway is found in the Tamar wallaby, but there are indirect data to suggest that such a pathway may operate specifically in human fetal steroidogenesis.<sup>41,42</sup> Aromatase activity would also be affected by POR deficiency, hence increasing fetal androgens and contributing to virilization in an affected female fetus. Paediatric endocrinologists have been intrigued by the observation in some infants with the severe form of CAH of a mismatch between markedly elevated serum 17OH-progesterone levels yet only a modest increase in testosterone



**Figure 1.** Pathway of testicular steroidogenesis. Bold arrows indicate a predominant  $\Delta^5$  pathway of steroid production. The dotted lines denote a 'back-door' pathway to dihydrotestosterone (DHT) synthesis postulated to be specific to the fetus. The steroidogenic enzymes and their cognate genes are shown. LH, luteinizing hormone; hCG, human chorionic gonadotrophin; LHR, LH receptor; StAR, steroidogenic acute regulatory protein; HSD, hydroxysteroid dehydrogenase deficiency; RD5A1, 5 $\alpha$ -reductase type I; RD5A2, 5 $\alpha$ -reductase type 2; AKR1C, aldo-keto reductase.

and mild virilization. This may be partly explained by the finding of a heterozygous POR mutation in a severe salt-loser with a proven CYP21 mutation causing CAH but only mild virilization.<sup>43</sup> The observation is a unique example of an autosomal recessive disorder manifesting in the heterozygous state. POR as a cause of XY DSD has a relatively simple explanation based on POR deficiency having a more profound effect on 17,20-lyase activity, a key regulator of androgen biosynthesis.<sup>44</sup>

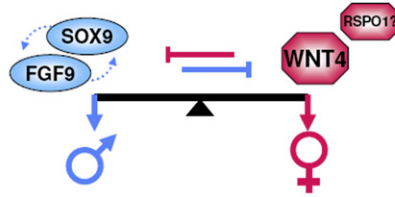
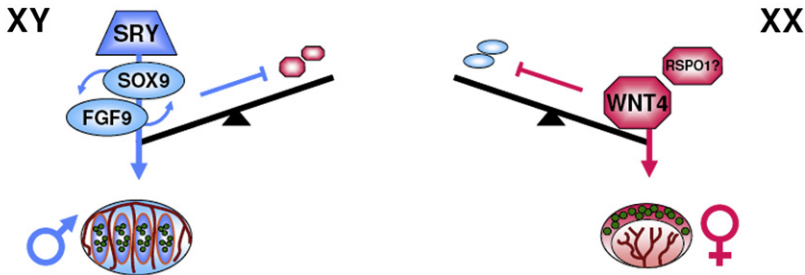
The pathways of steroidogenesis depicted in [Figure 1](#) suggest that the dichotomy in the effects of POR deficiency on XX DSD and XY DSD can be rationally explained. However, the reality is a more complex melange of interacting pathways that are variously affected according to the nature of individual POR mutations.<sup>45,46</sup> It has been proposed that POR deficiency may represent the commonest form of CAH. That may be the case, bearing in mind the pleiotropic function of POR as a cofactor in steroidogenesis. It is clear that specific steroid analysis has a role to play in the investigation of DSD of no apparent cause, particularly in idiopathic under-masculinized states. Analysis of urinary steroid profiles by gas chromatography/mass spectrometry detects the biochemical signature of the 'back-door pathway' characterized by increased pregnanediol, pregnanetriolone, and pregnanetriol, and an increased androsterone/aetiocholanolone ratio.<sup>47</sup> Applying this test routinely in the investigation of unestablished causes of DSD should in time enable a reliable estimate of the prevalence of non-syndromal POR deficiency to be established.

Disorders of gonadal development under the umbrella of XX DSD are relatively infrequent if Turner syndrome is excluded as a mainstream DSD. Until recently, there has also been little evidence of the presence of ovary-specific gonad-determining factors whose inactivation would result in a form of gonadal dysgenesis. The concept that there may be 'anti-testis' genes to enable ovarian determination to occur arose as a result of observing the effects of over-expression of genes such as DAX-1 and WNT4 from duplications in chromosomal regions Xp21 and 1p35, respectively.<sup>48,49</sup> However, DAX-1 cannot be considered an ovarian-determining gene as its targeted disruption in XX mice does not abrogate normal ovary development.<sup>50</sup> It is more appropriate to consider ovarian development in the context of a balance between the opposing forces of male- versus female-promoting factors. Tipping the balance in one direction by inactivating one of these factors may be sufficient to realize sex reversal.<sup>51</sup> This idea recapitulates a concept espoused some time ago which stated that a putative gene, named 'Z', is expressed in the XX gonad and represses the testis-determining pathway, thus allowing an ovary to develop.<sup>52</sup> The corollary is that SRY is a testis inducer by its action in repressing a repressor, alias 'Z'. The 'Z' hypothesis has underpinned one plausible explanation as to how a testis can form in the 10% of XX males who lack an SRY gene.<sup>53</sup> The balancing act to determine the fate of gonad development probably involves an interplay between the FGF and WNT4 signalling pathways. Male-to-female sex reversal results from mutations in *Fgf9*, whereas female-to-male sex reversal, albeit partial, occurs when *Wnt4* is mutated.<sup>54</sup> There are now case reports of WNT4 mutations in humans demonstrating partial female-to-male sex reversal, thus implying a role for this signalling molecule in the ovary.<sup>55–57</sup> More persuasive evidence of a specific ovarian inducer is based on complete female-to-male sex reversal associated with an inactivating mutation in the human R-spondin1 (*RSPO1*) gene.<sup>58</sup> This growth factor activates the WNT4–catenin signalling pathway, suggesting that *RSPO1* and WNT4 act cooperatively to antagonize testis determination in XX gonads. [Figure 2](#) proposes how a delicate balance between *SOX9/FGF9* and *WNT4/RSPO1* in the indifferent gonad may be played out in determining the fate of the gonad.<sup>59</sup>

## CAUSES OF XY DSD

Disorders which come under the XY umbrella of the DSD classification are far more numerous, but paradoxically the success rate in establishing a precise diagnosis is far lower than in XX DSD. The observation that only 10–15% of cases of complete



**A** Bipotential Gonad**B** Mammalian SDM Switch

**Figure 2.** Model of opposed signals in mammalian sex determination (SDM). (A) In the bipotential gonad, male-promoting (SOX9 and FGF9) and female-promoting (WNT4 and possibly RSPO1) hold each other in check. (B) The presence of SRY (XY) reinforces the positive feedback between SOX9 and FGF9, which then out-competes the female signals and drives testis differentiation. In the absence of SRY (XX), the female-promoting signals shut down the male loop and drive ovarian differentiation. Reproduced from DiNapoli and Capel (2007, *Molecular Endocrinology* Sept 20 Epub ahead of print) with permission.

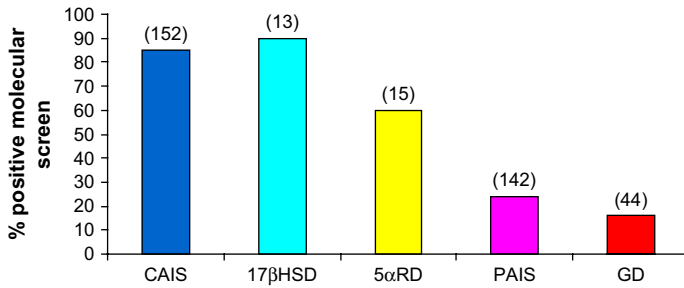
gonadal dysgenesis (Swyer syndrome) are caused by a mutation in SRY has spawned the idea that a multitude of other genes must be involved in testis determination. Candidate genes explored on the basis of knowledge of the phenotype of mouse knockout models include DAX-1, SF-1, WNT4, SOX3, LHX9 and FOG-2.<sup>60–63</sup> Almost never is a mutation identified which explains the sex reversal. However, there is now increasing evidence from single case reports and analysis of cohorts of patients with gonadal dysgenesis that haplo-insufficiency of SF-1 as a result of heterozygous mutations of this gene are a relatively frequent cause of XY DSD without adrenal insufficiency.<sup>64–68</sup> One mutation located uniquely in the ligand-binding domain (Leu437Gln) was identified in a male with penoscrotal hypospadias.<sup>66</sup> This widens the phenotype considerably for de novo/germ-line or X-limited dominant mutations of SF-1. Bilateral congenital anorchia (vanishing testis syndrome), when associated with micropenis, may also result from a heterozygous mutation in SF-1.<sup>69</sup> There are several syndromic causes of gonadal dysgenesis associated with known genes – such as campomelic dysplasia (SOX9),  $\alpha$ -thalassaemia/mental retardation (ATRX), Denys–Drash syndrome (WT-1), and X-linked lissencephaly (ARX) – but mutations have rarely been identified in association with gonadal dysgenesis alone.

The new classification embraces the numerous and previously well-documented defects in androgen biosynthesis. It includes the aforementioned syndrome of P450 oxidoreductase deficiency as it applies to XY DSD, a defect in the fetus-specific ‘back-door’ pathway to DHT synthesis. If androgen (including DHT) production is

normal, then a defect in androgen signalling must be considered. In the context of causes of XY DSD, resistance to the action of androgens is the commonest cause, akin to CAH in the causation of XX DSD. The paradigm for resistance to androgens is CAIS, formerly known as the testicular feminization syndrome. This is a further example of a changing nomenclature brought about by expressions of disquiet about the former terminology from individuals who have CAIS. The clinical description has changed little from that originally given in detail by Morris and sometimes eponymously attributed to his name.<sup>70</sup> In contrast, advances in molecular genetics have provided an explanation for the pathogenesis of the phenotype in the majority of subjects with CAIS. Missense and nonsense mutations, common to many single-gene disorders, also feature to cause inactivation of the AR. Androgen insensitivity may also result from mutations which disrupt the intramolecular interaction between the N-terminal and C-terminal regions of the AR, a requirement of transcriptional activation that is unique to the AR.<sup>71–73</sup> Another functional defect that can (rarely) cause androgen insensitivity is failure of trafficking of the AR to the nucleus.<sup>74</sup> When no mutation is found in the coding region or intron/exon boundaries of the AR gene, some defect in the interaction of the AR with one of a myriad of co-regulator proteins may be invoked.<sup>75</sup> The absence of a putative AR-specific co-activator in genital skin fibroblasts from a patient with CAIS and a normal AR gene has been reported, but there has been no subsequent report as to the identity of the protein.<sup>76,77</sup>

Partial androgen insensitivity syndrome (PAIS) is defined by a phenotype comprising variable degrees of under-masculinization despite normal age-related androgen production and AMH function (as shown by absence of Mullerian structures) and normal testis histology. The syndrome is proven to be a defect in androgen signalling in only a minority of cases.<sup>78,79</sup> What causes partial resistance to androgens in the majority of subjects with the phenotype of PAIS remains a biological mystery. The phenotypes of AR-mutant-positive and AR-mutant-negative cases of PAIS are almost indistinguishable, although a positive family history is found more commonly in the former category.<sup>80</sup> Furthermore, there is a strong association between severe hypospadias (a common feature of PAIS) and low birth weight.<sup>81,82</sup> Intrauterine growth restriction (IUGR) is characteristically the result of a decrement of growth rate occurring in early fetal life, a period which coincides with completion of urethral development.<sup>83</sup> It is plausible that epigenetic effects may modulate early fetal development through the methylation status of fetal growth genes such as H19 and IGF2.<sup>84</sup> This hypothesis would need to be tested in monozygotic twins discordant for IUGR and hypospadias. Overall, the diagnostic yield when molecular analyses are applied to XY DSDs is coupled with variable success, with figures of 60–90% for CAIS and androgen biosynthetic disorders, whereas the rate is considerably lower in gonadal dysgenesis and PAIS (see Figure 3). There is considerable scope for rectifying this unsatisfactory situation through more detailed defining of DSD phenotypes and increased collaboration between clinicians and molecular geneticists.

A new phenomenon which now needs to be included in DSD classification is the observation that some disorders – such as undescended testis and hypospadias – may be linked to the effects of environmental chemicals on the developing male reproductive tract.<sup>85,86</sup> There are plenty of potential culprits contained within the environmental soup of 100,000 chemicals to which humans are exposed.<sup>87</sup> It appears that the mechanism of the effect is an imbalance in the androgen/oestrogen equilibrium prevailing during fetal life, although some compounds are specifically oestrogenic or anti-androgenic in their mode of action. Others may interfere with the pathway of sex-steroid biosynthesis.<sup>88</sup> A concomitant change in testis cancer prevalence



**Figure 3.** Mutational analysis of cases of XY DSD from the Cambridge DSD database. Numbers in each diagnostic category are shown in parenthesis. CAIS, complete androgen insensitivity syndrome; 17βHSD, 17β-hydroxysteroid dehydrogenase deficiency; 5αRD, 5α-reductase deficiency; PAIS, partial androgen insensitivity syndrome; GD, gonadal dysgenesis.

and sperm counts has given rise to the proposal that this quartet of male reproductive tract disorders constitutes a testis dysgenesis syndrome (TDS) which has its origins in fetal life.<sup>89</sup> It is an attractive unifying hypothesis to invoke an environmental cause. Chemicals such as phthalates and pesticides may cause endocrine-disrupting effects. Most of the reproductive toxicological effects of such compounds have hitherto been demonstrated only in animal experiments. A reliable and reproducible marker of anti-androgenic effect in rodent studies is the reduction in ano-genital distance (AGD) in male offspring following administration of a compound such as the antifungal agent vinclozolin to pregnant dams.<sup>90</sup> There are now data for this anthropometric measurement in human newborns demonstrating that the AGD is larger in males than in females, and that the length is inversely related to the level of pesticide exposure during pregnancy, as documented in maternal urine samples.<sup>91</sup> Such a relatively simple marker of exposure now allows further epidemiological studies to determine whether there are changing patterns of urogenital disorders in humans which can be linked to environmental effects.

## IMPACT OF THE NEW DEFINITION AND CLASSIFICATION

There is already evidence that health professionals involved in the management of families with DSD are moving towards a universal language of communication. This is seen in scientific publications, in conference programming, and in standard textbooks of endocrinology.<sup>15</sup> The Consensus firmly places the need for psychosocial support at the heart of team management for DSD, recognizing that medical and surgical issues are not the sole components of care. What is conveyed to the parents of a newborn infant with ambiguous genitalia in the first hours after birth will imprint on their minds for years to come. Is it not too fanciful to think that a clinical psychologist should not, *de facto*, be part of the multidisciplinary team imparting knowledge at this early critical stage? The changes in terminology have largely gained favour from patient advocacy groups. Supportive arguments include clarity of terms which are inclusive of numerous conditions affecting the urogenital tract, use of terms that refer only to the clinical manifestations of a condition and not to the psychosexual characteristics of an individual, and enablement of improved chances of optimal care when heterogeneous conditions are classified under one generic umbrella.<sup>4</sup>

The impact on DSD management in terms of tumour risk is directly the result of an evidence base generated in recent years from a number of centres which have data on gonadal histology across a range of conditions which cause DSD.<sup>13,92,93</sup> The choice of timing of gonadectomy in CAIS, for example, can now be made on the basis of an option to allow spontaneous onset of puberty from endogenous oestrogen production coupled with a low tumour risk into adulthood. There has been a sea change in surgical practice with the recognition that sex assignment is not inextricably linked to surgical intervention, and there is often an opportunity to allow the affected child to reach an age of sufficient cognitive development to become involved in management decisions that will have lifelong implications. Above all, health professionals and patient advocacy groups are beginning to work in close harmony rather than in discord, and recognize that together they must address the enormous uncertainties that pervade the management of DSD.

## THE FUTURE

It is clear that the Consensus statement raised more questions than it answered. There is recognition that more research is needed to improve diagnosis, refine medical, psychological and surgical management, and above all gather evidence on outcome. Information on outcome is particularly sparse in XY DSD. Such research is best conducted in a multicentre, multidisciplinary manner to enable sufficient numbers of cases to be studied. Progress on this front is under way in Europe through the auspices of the European Society for Paediatric Endocrinology supporting the establishment of a register of DSD patients investigated following agreed protocols. Allied to this activity is funding from the European Community for a multinational study of the molecular pathogenesis of DSD and its longer-term outcome. All participants in the research programme are operating on the basis of implementing the new DSD nomenclature and classification that brings with it a clarity of purpose to the research.

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