Disorders|Differences of Sex Development

An Integrated Approach to Management

John M. Hutson
Sonia R. Grover
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Preface for the Second Edition

This new edition necessitated a change in editors, with Prof. Garry Warne retiring. This provided me with an opportunity to invite Dr. Michele A. O'Connell to supervise the medical chapters and Dr. Aurore Bouty to help me revise the genetics, embryology and surgical chapters. Also, I invited Ms. Chloe A. Hanna, our DSD team coordinator, to join the editorial board, as she has the ear of all the patients and their parents, and her contributions highlight this.

Although the first edition was only published in 2012, there have been significant changes in DSD management and, hence, the need for a new edition. This new edition commences with a new chapter about the language and the background and socio-political changes that engulfed DSD management. Many of these new ways of thinking come from the patients themselves, as the number of adults with DSD increases, and social changes allow this once-forbidden topic to be discussed in the media. The chapter on the genetics of DSD has been completely rewritten to highlight the rapid advances in understanding what genes are involved in genital development. The chapters on normal and abnormal embryology and the hormones regulating sex development have been revised to keep pace with the changing terminology. All the chapters on the main diagnoses have been rewritten by Dr. O'Connell following the retirement of our colleague, Prof. Garry Warne.

We added a small new chapter on 'The Foetus', as prenatal diagnosis is becoming much more common. Chapters on medical, surgical and gynaecological and psychological management have been updated, and some old chapters have been amalgamated to simplify the flow. The previous chapter on 'The Family' has been rewritten to emphasise the importance of psychosocial counselling and support. We have kept the chapter on outcomes from our own centre, as despite the fact that although the follow-up studies are now a bit dated, they represent a potential benchmark and were the best published results worldwide at the time. These can be compared with the new chapter on outcomes elsewhere in the developed as well as in the developing world, courtesy of our colleagues in Chittagong, Bangladesh. Finally, the guide for parents on CAIS has been retained and updated, as it is still sought after by families.

We hope this new edition will continue to educate and inspire the next generation of clinicians to continually strive to improve the lot in life for children with DSD.

Melbourne, Australia
December 2018

John M. Hutson

Acknowledgements for the Second Edition

We thank all our current and previous patients who were born with a DSD for helping us learn how to improve their management. We have not quite achieved yet an adult expert DSD centre to enable DSD patients to transition to the adult world with expert lifelong care, but it is within sight. However, we now have our own paediatric DSD team with monthly meetings across three centres in two countries, which is a major advance.

As the senior member of the editorial team, I would like to thank my coeditors for bringing this new edition to fruition so successfully. Finally, we all thank Ms. Shirley D'Cruz for patiently and extremely competently using her secretarial skill to turn this dream into a reality, yet again!

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About the Editors

John M. Hutson is the Chair of Paediatric Surgery at the University of Melbourne, and one of the consultant Paediatric Urologists at The Royal Children's Hospital and Joint Group Leader for the Surgical Research Group at Murdoch Children's Research Institute.

Professor Hutson has three doctorates (MD, UMelb; MD, Monash; DSc, UMelb) in the area of sexual development and has studied the mechanism of testicular descent for many years. He has developed special expertise and interest in understanding the causes of (and new treatments for) intractable constipation in children. He is the author of numerous papers and books for teaching medical students, as well as monographs on testicular descent, kidney and urogenital malformations and DSD.

Sonia R. Grover is Director of the Department of Gynaecology at RCH. She is a member of the multidisciplinary team at RCH providing care for patients with DSD. Prof. Grover has been involved in research, teaching, supervision of research students and publishing, which has included work on long-term outcomes of women with DSDs, MRKH and CAH and those who have had genitoplasties. Her work also involves supporting the development of adolescent gynaecology services around Australia and overseas, in her role in the International Federation of Paediatric and Adolescent Gynaecology (FIGIJ).

Michele A. O'Connell trained as a Paediatrician in Ireland, before moving to Australia in 2005 to undertake subspecialty training and doctoral research in Paediatric Endocrinology at the Royal Children's Hospital, Melbourne. Since the retirement of Prof Garry Warne, Michele has been the co-ordinating Paediatric Endocrinologist on the RCH multidisciplinary DSD team. She has served as a member and is current co-chair of the Australasian Paediatric Endocrine Group DSD subcommittee. Together with colleagues at RCH and MCRI, she has supervised undergraduate and postgraduate research and has ongoing research collaborations across the fields of DSD, transgender health and paediatric diabetes.

Aurore Bouty is a Paediatric Urologist at the Royal Children's Hospital, Melbourne. She trained as a Paediatric Surgeon in France and subsequently migrated to Australia in 2015 to further specialise in Paediatric Urology. Since then she has benefited from the experience of Professor John Hutson in the management of children born with DSD. Her research interest resides in

the Genetics of DSD, and she is currently a PhD student in Andrew Sinclair's Reproductive Development team at Murdoch Children's Research Institute.

Chloe A. Hanna completed a B.Sc. in Biomedical Science at the RMIT University followed by a Master of Genetic Counselling in 2014 at the University of Melbourne, completing a thesis exploring the psychosocial needs of women with DSD. Chloe previously worked in the disability sector for over 15 years. In 2016, Chloe established the role of Clinical Coordinator for individuals with DSD at the Royal Children's Hospital and currently provides direct support to individuals, families and the broader community throughout Australia. Chloe's key interest is to enhance the multidisciplinary approach to DSD healthcare including psychosocial support pathways.

1 Introduction: Changing Landscapes

Sonia R. Grover, Chloe A. Hanna, and Michele A. O'Connell

What's in a name? *A rose by any other* ***name*** *would smell as sweet.*

Ref Romeo and Juliet. Shakespeare

To open a book with an uncertainty regarding what term and title to use sounds like a poor start. Yet, to put this uncertainty and challenge upfront highlights exactly the issues that this book wishes to tackle.

The cluster of conditions collected under the expression Disorders|Divergences|Differences of Sex Development (DSD), Conditions Associated with Reproductive Development (CARD), Intersex and Variations in Sex Characteristics (VSC) consist of those associated with atypical genetic, phenotypic or hormonal makeup.

Where possible, each individual condition has its own specific or diagnostic term, which attempts to describe the difference or variation in a recognisable, defined way. This serves to enable advancement and achievement of understanding and optimal health care—so that we can share knowledge and experience, and thereby, we are all speaking the same language.

The challenge lies in the 'umbrella' expression or terminology. It is this expression which is fraught with difficulties, as two of the key umbrella expressions (DSD and intersex) are not directly interchangeable, and an individual who may have a condition that falls under a medical DSD classification (e.g. Turner syndrome) may identify with one term but not the other, or indeed neither.

There are also challenges in deciding which conditions or variations should be included and excluded under a given umbrella expression, as well as what care is required to provide optimal outcomes for affected babies, infants, young people and adults.

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The word disorder has been avoided here—yet it does remain in the title of this book. This has been done, partly to reflect the relationship with the first edition, now several years old, and partly because, although we recognise that times have changed and the terminology is changing, to date, there is no consensus on a new term. At the time of writing, this is still the umbrella term most commonly used and recognised in the medical literature. We do acknowledge that it is not a universally accepted term and is perceived by many as having unnecessarily 'pathologising' connotations. For this reason, we have adjusted the title to Disorders|Differences of Sex Development, and we will use the abbreviation 'DSD' throughout the book to allow scope for readers to use or infer their preferred term. As clinicians, we rarely use any umbrella term in our clinical interactions with an individual or family.

This book acknowledges that there are different 'ways of knowing' (Lundberg 2017). Its contents may provide information that not everyone wants or needs to know or understand. For others, this book may only provide an overview and a background to understanding and knowing about DSD.

For families and people with DSD, knowing and understanding their respective conditions as well as about the related challenges and controversies is an important stepping stone to knowing oneself or one's child's condition and subsequently towards gaining optimal health outcomes.

The information in this book is the result of knowledge, collected and shared, as well as our different perspectives of knowing based on our different backgrounds as health clinicians. The evidence and knowledge comes from a clinical team that has grown and changed and learnt together and learnt from our patients, as we have provided care to people with DSD for over 30 years. Taking this multidisciplinary skill set, we hope to continue to learn and further develop our knowledge of DSD into the future. While the information provided here is current at the time of writing, it will undoubtedly also change with improvements in our understanding and knowledge in years to come.

1.1 Background

1.1.1 Terminology

In 2005, there was an international consensus meeting that endeavoured to establish some consistency in definitions of various conditions in order to lay the groundwork for international research and guidelines for care in this field (Chicago consensus statement—Hughes et al. 2006). Previously, the management and long-term outcomes of people with this cluster of diagnoses had been poorly studied, as work in this area was challenged by a lack of consistency in terminology to enable accurate comparisons between clinicians and researchers in different cities and countries. Additionally, there were challenges associated with old terminology that were not only confusing but also insensitive to those people with these conditions.

At the Chicago consensus meeting, the 'Disorders of Sex Development' classification system was proposed and thereafter adopted in the medical literature as an umbrella expression to define 'congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical.' There was also a reclassification of the variety of conditions that are encompassed by this label to enable collaboration and more effective comparison internationally with all researchers and clinicians using a common language and classification. Affected individuals and advocates were, and continue to be, vital in the push for more research and long-term follow-up. This classification could be considered a medical or even genetic classification system. This is true as the development of a structured descriptive classification system that allows similar data to be collected, and comparison to improve outcomes was one of the aims of the meeting. Moreover, it was recognised that more accurate data on malignancy risks would be useful for those

trying to contribute to ensuring the best possible health outcomes.

The medical terms 'condition' and 'diagnosis' are not intended as negative expressions or descriptions. One can have hair or eye colour as a description, or a diagnosis of heart or kidney conditions, without there being any intention that the term 'diagnosis' is negative. It is simply a description. One should not be judged for any of these differences. For some people with a 'diagnosis' such as a heart condition, treatment may be recommended, whereas for others, no intervention or specific care is required, or potentially, some monitoring for possible future risks. Likewise, the expression of having a condition or diagnosis related to the field that this book focuses on does not mean that it is a problem; again, it is simply a description.

Since 2006, there has been ongoing debate regarding the most appropriate language with some affected individuals, advocates or clinicians preferring alternative expressions. We acknowledge this, and in using the term DSD, we intend no offence to those who do not identify with this descriptor.

This debate is challenging, as clinicians would rarely use the umbrella expression in the setting of individual patient care. Additionally, it is our experience that affected individuals and their families when asked (e.g. CAH support group meeting Melbourne 2016, MRKH support group Melbourne 2018, (Mortimer 2017)) report that they would prefer the expressions that describe their exact diagnosis and very infrequently would they prefer the expressions intersex or disorder of sex development. Yet for many affected individuals and advocacy groups, 'intersex' is the preferred expression/term (see Darlington statement 2017). A European/UK study specifically undertaken to explore how young people affected by these diagnoses, their parents and focus groups of people with no previous knowledge or experience with these diagnoses or understanding of the different terms found that none preferred DSD; the focus group participants preferred 'intersex' and affected young people and their families preferred descriptive terms (Lundberg et al. 2018). Likewise, in a study undertaken by DSD-life, 45% of participants agreed that DSD applied to their medical condition and 43% considered 'intersex' a bad term; however, almost one-third disagreed that DSD applied to them (Bennecke and De Vries 2016). Recent Australian research also reported broad acceptance of the term 'intersex' amongst affected individuals, with 60% comfortable to use the word in some way to self-describe themselves; of the same cohort, only 3% of respondents reported using the term DSD (Jones et al. 2016). This research also reported that affected individuals preferred words specifically describing their own diagnosis when talking with friends and family and when accessing medical services. In our experience, it is rare for either umbrella term (DSD or intersex) to be used in clinical consultations or in conversations with an affected individual. Thus, although DSD may be used in medical literature, actual clinical practice aligns with the preferences of affected individuals.

In many public and political settings, the expression 'intersex' has become the standard term and has been incorporated into the ambit of the sexuality and gender diverse LGBT grouping, to make LGBTI.

'Disorders', as defined in a medical sense in the Oxford dictionary, refer to a disruption of normal function, a lack of order or regular arrangement. Hence, people may have disorders of metabolism or metabolic disorders, disorders of growth/growth disorders, genetic disorders, bleeding disorders and many others. Importantly, it is not the people who are disordered nor are they less of an individual because of their specific disorder. This issue with language is more pronounced in English than some other languages. In the English language, the expression '*I am cold*' can be confusing, as it could mean *I am cold/freezing* or that I am a cold (distant and potentially unpleasant) person. In other languages, for example, German, this confusion does not exist, as the sentence structure precludes this confusion, so that '*Mir ist kalt*' (I feel cold [e.g. due to weather]) is quite different from '*Ich bin kalt*' (I am a cold person). Yet, it is worth noting that, even in Germany, the debate regarding DSD terminology exists.

Definitions and meanings change over time. Given that this book is about sex development, a simple example relates to colours and dichotomous sex stereotypes. From the mid-1900s, colours have been increasingly used to denote gender, with girls/females being linked to pink and often clothed in pink (with the stereotypical presumption that they have a preference for this colour), whereas boys/males have been linked to the colour blue (Del Giudice 2012). Prior to this, the gender coding of pink and blue was inconsistent and not used in such a gender-dimorphic manner to masculinity and femininity (Paoletti 1997).

The origins of our words are clearly very old, and thus, it is not surprising that the meanings and significance change. English dictionaries tell us that the word 'sex' came from *sexe* (from middle French, 1382), which comes from the Latin *sexus* (gender), derived from *seco, secare* ('divide, cut') in the concept of 'half' of the race. 'Sex' tends to refer to biological differences, and historically, there was male sex and other, or male and a second sex.

In contrast, the expression 'gender' comes from *gendre* (old French), which is derived from Latin *genus* (gender) birth, family, nation. 'Gender' tends to refer to cultural or social aspects of sex.

Historically, the concept of a people who do not fit the dichotomous male/female sex and gender extends well back into Mesopotamian mythology. Inscribed upon a stone tablet from the second millennium BC (Sumer—pre Babylon), there is a myth about the creation of a type of human who is neither man nor woman. The goddess Ninmah fashions a being with 'no male organ and no female organ', for whom there is a position in society—'to stand before the king.' In an Akkadian myth, the goddess of birth establishes a third category of people, which includes demons who steal infants, women who are unable to give birth and priestesses who are prohibited from bearing children (Murray and Roscoe 1997).

In India's three ancient spiritual traditions—Hinduism (Wilhelm 2004), Jainism (Zwilling and Sweet 1996) and Buddhism (Jackson 1996), there is also reference to a third gender. In the Buddhist Vinaya (codified around the second century BC), there were four main categories: male, female, people of dual sexual nature and people of various sexual natures (Jackson 1996; Gyatso 2003).

A 'third sex' known as Hijra (in Hindi हिजड़, in Urdu ہجڑ) exists in India, Pakistan and Bangladesh. These people are mostly men dressed as women, although they are not trying to pass as female. Less than 10% are thought to have a DSD. Although this population has been held up as an example that these societies tolerate non-dichotomous sexuality, it is worth noting that the Hijra hold a very low social status, with a very defined function in society. They appear at weddings and births to bring good luck; refusing their presence will bring bad luck (Khan et al. 2009).

Sexuality is defined as *how* people experience the erotic and express themselves as sexual beings. Again, this has changed over time. In ancient Egypt and Greece, homosexuality was well described and accepted, with evidence from the tomb of Niankhkhnum and Khnumhotep, as well as in Homer's The Iliad. In Persia (1500s–1700s), homosexuality was well accepted in public (with the existence of erotic poems, and male prostitution houses that paid taxes). During the Renaissance, in northern Italy, same-sex love was widespread, although authorities were prosecuting individuals. In the mid-1800s, in European culture, homosexuality was a crime and considered an abnormality that required treatment. Nowadays, in many places, homosexuality is recognised and accepted, including having all the relevant legal rights (many of which were previously denied). The Royal College of Psychiatry considered 'Sexual orientation biological in nature' in 2014, and homosexuality was removed from DSM in 1973 (Bayer 1981). But there are many countries today where sadly homosexuality is still not tolerated or is considered an abnormality. As a general principle, broader societal awareness and acknowledgement of diversity is very important in ensuring its acceptance.

1.1.2 Recent History of DSD

From a more recent historical perspective, a number of important developments that have impacted significantly on the clinical management of individuals with DSD occurred in the 1980s and 1990s.

Patient advocacy groups were first established in the late 1980s and early 1990s and became vocal, establishing a 'contested collaboration' (Kessler 1998; Davis 2015). Some patient advocacy groups took a high-profile approach (Intersex Society of North America (now named Accord Alliance), Organisation Intersex International), appearing at conferences and challenging the attending clinicians. Important limitations in clinical care such as a lack of robust outcome studies and insufficient psychological support were thus increasingly brought to the attention of the clinicians. Unfortunately, in many countries, the ongoing lack of funding for comprehensive prospective patient databases to support high-quality research means that longitudinally acquired data on medium- and long-term outcomes are still relatively limited.

In the late 1990s, John Colapinto publicised the long-term outcome of the life of David Reimer following a disastrous surgical accident, whereby he suffered irreparable damage to his penis when cauterising equipment malfunctioned during a circumcision at the age of 8 months in Canada in 1966 (Colapinto 1997, 2000, 2001). At the age of 22 months, David Reimer's parents were advised to raise 'John' (one of twin boys) as 'Joan' following the recommendations of Dr. John Money, a renowned psychologist at the time, based at John Hopkins University. Money's theory of gender identity development, which was then increasingly popular, claimed that gender was a societal construct that was malleable; hence, nurture rather than nature determined gender identity (Money 1985). Thus, appropriate nurturing and 'corrective'/feminising surgery (orchidectomy and feminising genitoplasty) were recommended to reinforce the gender role. As children, David (Joan) and his twin brother were not told that they were both born boys; nor did they know the story of David's surgeries. Money regularly presented this 'John/Joan' case as a success story in academic settings, which led to both dissemination of and increased support for the theory, hence potentially influencing medical/surgical decisions in the management of children with DSD where sex of rearing was uncertain in infancy. Sadly, this was despite there reportedly being evidence when David (Joan) was as young as 6 years old that he was increasingly unhappy with his female sex assignment (Diamond and Sigmundson 1997). He went on to have pubertal induction with oestrogen, but on learning his personal history as an adolescent, he transitioned to living as a male with the name David. In allowing his story to be told publically in the late 1990s, a number of issues relating to his care were highlighted, not only in relation to apparent inaccuracies in Dr. Money's theory and reporting of the case, but also in relation to the importance of open disclosure and optimal information sharing in clinical settings and decision-making. While David's path arose from an acquired injury rather than a congenital DSD, there were many similarities and implications for care pathways in DSD, which is why it is included here.

Furthermore, in the 1990s, there were several publications regarding gender change from female to male in classical CAH (Warne 1992; Meyer-Bahlburg et al. 1996). These cases additionally highlighted that future gender identity was beyond the control of managing clinicians and emphasised the need for awareness amongst all parties (family and clinicians) that, while sex assignment can occur at birth, gender cannot be known by anyone other than a given individual.

From the early 2000s, along with the increasing recognition of the role of patient advocacy and a desire to review management strategies and long-term outcomes, these events culminated in the 2005 Chicago meeting, the changed definition and terminology, as well as recommendations in relation to management of various conditions (Hughes et al. 2006). Management guidelines continue to be revised and evolve as knowledge also improves (Ahmed et al. 2016; Lee et al. 2016; Cools et al. 2018). While the terminology remains

contentious, since the introduction of the medical DSD classification system, there has been a significant increase in DSD-related scientific publications and DSD-specific international meetings and conferences, all of which will serve to further advance knowledge in the field. In tandem with this, our understanding of the underlying genetic variants that may be associated with different DSD has also increased exponentially. These will be discussed further in Chap. 2.

1.1.3 Clinical Definition of DSD

If the challenge regarding definitions is now extended to the clinical definition of DSD, further problems arise. There are a number of diagnoses for which there is ongoing debate as to whether they belong under the DSD umbrella. In particular, this applies to structural anomalies such as bladder exstrophy and cloacal anomalies. Hypospadias, in its more severe forms, is increasingly recognised as a DSD, with specific genetic testing allowing recognition of variations in hormone production and androgen receptor sensitivity. But should the less severe forms of hypospadias be considered a DSD? In line with the decision to stay with the terminology of 'DSD' that arose at the Chicago meeting, this book will encompass those conditions that were accepted at that consensus meeting (Table 1.1).

Table 1.1 Summary of new terminology

Title	Previous terminology
Disorders of sex development	Intersex
46,XY DSD	Male pseudohermaphrodite Under-virilised male Under-masculinised male
46,XX DSD	Female pseudohermaphrodite Virilised female Masculinised female
Ovo-testicular DSD	True hermaphrodite
46,XY complete gonadal dysgenesis	XY female XY sex reversal
46,XX testicular DSD	XX male XX sex reversal

Adapted from Hughes et al. (2007)

1.1.4 Incidence of DSD

The incidence of DSD is clearly influenced by which definition is used. A few incidences of the common forms of DSD are shown in Table 1.2. Many estimates of the relative proportion of the different diagnoses have used the selection criteria as ambiguous genitalia. Few papers have used the Chicago definition. A study at The Royal Children's Hospital in Melbourne attempted to utilise the Chicago definition but limited the cohort to children aged up to 10 years. This means that adolescents presenting with lack of pubertal development and girls with primary amenorrhoea were not identified in this cohort (Table 1.2).

Beyond acknowledging the challenges in terminology, definitions and incidence of these conditions, this discussion is not the primary purpose of this book. This book aims to explore the challenges of providing optimal care in situations where outcomes are often uncertain.

1.1.5 Clinical Care: Historical Perspective and Changes Over Time

History has a place to play here, as what is known in medical and psychological spheres has changed over time, and thus, what care is possible has changed rapidly in recent decades. This is important, as a comparison of care provided 30 or 40 years ago will reflect significantly different knowledge.

The first report of a female with congenital adrenal hyperplasia (CAH) is thought to be from 1865 (De Crecchio 1865). Although the association between altered function of the adrenal gland and excessive sex steroid production ('adrenogenital syndrome') was understood in the early twentieth century, it was not until 1950 that the successful use of cortisone as a therapeutic intervention to alleviate excess ACTH stimulation was first reported. The endocrine basis of congenital adrenal hyperplasia (CAH) was only discovered in 1953 by Lawson Wilkins, an endocrinologist in Baltimore (Wilkins 1965). Further advances in

Table 1.2 Diagnostic breakdown of reported DSD cohorts

Authors	Country	Cohort size	Study population	Common diagnoses
Parisi et al. (2007)	United States	250	Children with DSD assessed by hospital gender team (excluding Klinefelter, Turner and multiple congenital abnormality patients)	CAH 14% AIS 10% MGD 8%
Thyen et al. (2006)	Germany	80	Infants with ambiguous genitalia	CAH 18% AIS 16% MGD 9%
Al Agha et al. (2001)	Australia	51	Infants with ambiguous genitalia	CAH 31% AIS 10% MGD 6%
Bhullar et al. (2011)	Melbourne	199	All children aged 0–10 years identified with DSD using consensus statement	Perineal hypospadias 34% CAH 22% Exstrophy 14%
Joshi et al. (2006)	India	109	Infants with ambiguous genitalia	CAH 28% AIS 15% 5ARD 12%

CAH congenital adrenal hyperplasia, *AIS* androgen insensitivity syndrome, *MGD* mixed gonadal dysgenesis, *5ARD* 5α-reductase deficiency

genetics and classification of different enzyme deficiencies followed in the 1960s. Synthetic glucocorticoid and mineralocorticoid medications have been available since the 1950s; however, these medications are still not readily accessible in some parts of the world. Thus, even survival for a child with salt-losing 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) is a relatively recent expectation, and even today in some parts of the world, the mortality of affected children, particularly boys with severe salt-wasting CAH, remains high (see Chap. 22). In some parts of the world, the essential hormonal treatment is available only on the black market, thus compromising health and increasing mortality and morbidity.

Vaginal agenesis was recognised in ancient Greek times with endeavours to create a vagina dating back to 460 BC by Hippocrates (Goldwyn 1977). Today, there are a number of different techniques of creating a vagina, including approaches that require no surgery, yet we still do not know which approach results in the best long-term outcomes (McQuillan and Grover 2014a, b).

The capacity to provide hormone replacement therapy for people with non-functioning ovaries or testes to allow the development of secondary sexual characteristics and, importantly, provide the necessary protection for bone and cardiovascular health became available only in the last few decades.

Children with bladder and cloacal exstrophy still die in many parts of the world due to lack of access to the necessary complex surgery.

The first surgery for CAH was undertaken in the late 1950s. Open disclosure to patients and their families began in the Royal Children's Hospital and some other places in the 1980s, although in many places, this may not be still happening. Previously, this full disclosure and explanation regarding the underlying diagnosis and previous interventions (if relevant) was not given, in the misguided belief that knowing might be more painful or difficult. Clearly, evidence and experience have shown that this approach was not appropriate.

Over the last decade, psychosocial support for DSD has been recognised to be an essential aspect of medical care. Testimony from cohorts of adults who have been treated as infants and children without these supports and resources has highlighted their importance.

Therefore, over time, evolution in medical understanding and interventions have allowed

gradual improvements in the understanding of, and care across, a number of diagnoses. However, there have been many areas where proposed therapeutic interventions have been subsequently shown over time not to result in the desired outcome. Surgical interventions in particular have been the subject of increased debate and with increased recognition of the potential for adverse outcomes in this regard, approaches have changed in recent years and continue to evolve (Hrabovszky and Hutson 2002). The practice of early genital surgery and sex assignment has shifted in recent decades. For example, in the late twentieth and early twenty-first centuries, in boys with partial androgen insensitivity syndrome (PAIS), where a female sex of rearing was assigned based on genital appearance at birth and predictions of little further virilisation of appearance in puberty, interventions such as removal of testes in childhood were commonly undertaken. It is now recognised that this intervention can lead to harm and many affected individuals who had such surgery in childhood are deeply unhappy that this occurred. Prior to 1990, 35% of those with 46,XY DSD diagnosed as PAIS, variations of gonadal development or androgen biosynthesis were assigned male, compared to 68% after 1999 (Kolesinska et al. 2014). Various factors contribute to this trend, including shifting cultural and societal views, improved surgical reconstruction techniques and better understanding about the potential fertility, oncogenic risk and adult gender identity in this cohort. Gender dysphoria is not uncommon in individuals with PAIS, but this is regardless of sex assigned and not convincingly influenced by such surgery. While malignancy risk is higher in intra-abdominal gonads in those with PAIS, this risk is low pre-puberty; hence, removal of gonads is now recognised to be more appropriately considered at an age when the individual can be involved in this decision for themselves (see Chap. 7). Deferring such surgery also allows for potential effects of endogenous hormone production in puberty and an individual's own gender identity to be established.

In contrast, those conditions where there are gonads with Y genetic material but the gonads are non-functioning (i.e. with no fertility potential and no hormone production), there can be a malignancy risk of up to 30%, even in childhood. Hence, removal of these gonads is considered important to prevent cancer.

Other surgeries performed in individuals with DSD are also the subject of much debate. Feminising genitoplasty for girls with CAH is a case in point, with opinions on this intervention ranging from support in those with significant virilisation (Prader stage 3+) due to the high incidence of satisfaction with outcomes (e.g. RCH follow-up studies (Lean et al. 2005, 2007; Crawford et al. 2009)) to opinions that it should not be performed in infancy (Creighton et al. 2001) but rather deferred until the girl herself is old enough to be involved in the decision.

1.1.6 Human Rights and DSD: Where to from Here?

Internationally, in recent years, human rights agencies and UN treaty bodies, agencies and special rapporteurs have increasingly called on Member States to strengthen protections for the human rights of people born with variations in sex characteristics (e.g. San Francisco 2005, Germany 2012, Switzerland 2012). In 2013, the UN Human Rights Council called upon all states to repeal laws allowing 'intrusive and irreversible treatments for children with intersex variations'. Such interventions included genital surgery and involuntary sterilisation without the free and informed consent of the person concerned (UNHRC 2013). In 2016, a group of UN and international human rights experts published a statement on Intersex Awareness Day (26 October) that sought for governments to prohibit medical procedures on intersex infants, children and adolescents without 'the full, free and informed consent of the person concerned (http://www.ohchr.org/EN/NewsEvents/Pages/DisplayNews.aspx?NewsID=20739&LangID=E).

It is clear therefore that there is growing impetus for change in this space in recent years. Rulings such as those from the UN where people with intersex variations who have experienced so-called 'normalising' surgery or treatment have

been recognised as 'victims of abuses and mistreatment', and where medical interventions have been labelled 'harmful practices,' may understandably be confronting for clinicians and surgeons who have offered such interventions with best intentions for optimal outcomes. Nonetheless, although not universal, suboptimal historical outcomes cannot be ignored and the lived experience of affected individuals has both changed practices and greatly increased awareness of the need to continually scrutinise all interventions undertaken. However, it remains the case today that the life-course and outcomes of a given individual with some DSD (such as PAIS or androgen biosynthetic variations) can be very difficult to predict in infancy or childhood, and decisions that may result in future regret are, and will likely remain, difficult to fully eliminate. It should be noted that this may indeed be the case whether a decision to intervene or to defer intervention is made. Deferring intervention, particularly where outcomes in relation to this are unknown (and should not automatically be presumed to be better), is as much a decision as opting to intervene.

How best to progress to ensure ongoing improvement to maximising optimal outcomes for affected individuals not surprisingly remains the subject of much debate. Clinically, there has been a significant shift over the last decade towards optimising care through management in specialised multidisciplinary teams, using a clinical ethics framework (see Chap. 15 for detailed discussion). A clinical ethics framework incorporates key concerns from the human rights field, but framed in a way that gives room for nuanced considerations of the circumstances of each individual child. Human rights discourse has a tendency to be black-and-white, implying that one approach is always the right thing for every individual. It works best for civil and political rights, which can reasonably be seen to transcend individual difference. In contrast, the principle-based approach of clinical ethics aims to better acknowledge the complexity of seeking to promote each individual's well-being in their specific circumstances. There are multiple aspects of a person's well-being, and the clinical ethics framework allows for structured ethical consideration of these in decision-making for each individual.

Many individual DSD or variations are very uncommon, hence infrequently encountered even in large tertiary clinical centres. This, along with the many uncertainties in outcomes, means that decisions in relation to care are often highly complex and need to be taken in the context of current knowledge, with open discussion of the uncertainties and controversies in approaches, while being individualised for a given child and family's unique circumstances.

Approaches to these difficult issues vary internationally. In 2015, Malta became the first country to institute a legal ban to prohibit deferrable interventions or surgery. Groups in other countries are also seeking legal frameworks. For example, in March 2017, Australian and Aotearoa/New Zealand intersex organisations and independent advocates issued a joint consensus statement (Darlington Statement 2017) calling for the criminalisation of deferrable medical interventions and the development of human rights-based lifetime standards of care. This statement also declared, however, that the Family Court system (in Australia) has 'failed to adequately consider the human rights and autonomy of children born with variations of sex characteristics'; hence, oversight in this setting is not thought to be optimal (nor likely feasible). An alternative put forward in place of the Court was an 'independent effective human rights-based oversight mechanism/s consisting of human rights experts, clinicians and intersex-led community organisations'.

Deferring decisions about surgery until an age when an individual may develop the capacity to be involved in decisions relating to their own care fits in with rights-based considerations such as autonomy and bodily integrity. However, the effects of deferring such decisions on the overall well-being of the child and future adult are not known. There are currently no data to support the hypothesis that such a management plan will invariably have preferable outcomes to interventions performed earlier, with informed parental consent on the child's behalf. As surgical prac-

tices and techniques have evolved (albeit to varying extents internationally), regulating to put a blanket ban on all surgery in infancy on the basis of sub-optimal outcomes using historical evidence from outdated surgical practices, is argued to not be a sound approach.

There is, however, some agreed ethical ground. Perhaps one of the most notable advances in recent decades is much greater involvement of parents of young children and older children themselves in decision-making. As part of best practice, clinicians discuss with parents and older children both what is known and not known about the child's particular condition and introduce some of the existing controversies in relation to potential management. Awareness of and openness to change over time (e.g., in future, gender identity relative to assigned sex in infancy) are increasingly promoted. Parents' and adolescents' decision-making and consent to any intervention is arguably much better informed than it has been in the past.

We do not presume to give final answers to these difficult issues in this book, but rather raise them to highlight the many changes that are occurring in the current context in which children and adolescents with DSD are managed. Like all ethical issues, good ethics depends on good facts. Recent and ongoing scientific advances such as the generation of international/multicentre registries (e.g. iDSD, iCAH and the DSD Translational Research Network) and ongoing strides in our understanding of the genetics relating to DSD will increasingly allow the collection of higher quality prospectively acquired data in very specific conditions, to optimally inform progress in this regard. Good ethics also depends on sustained reflection and deliberation on the values underpinning decision-making, recognition of pluralism about values in our communities and awareness of the limits of one's own perspective. So we can expect good ethics to lead to further debate and further change over time.

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2 The Molecular Basis of Sex Determination and Differentiation: Implications for Understanding DSD

Aurore Bouty, Katie Ayers, and Andrew Sinclair

Sexual reproduction in mammals requires two sexes characterised by specific genetic and anatomical features. The phenotypic sex of an individual is largely driven by the type of gonad that develops in the embryo, a process itself determined by sex chromosome complement. Human males and females both have 22 pairs of autosomes and differ only in their sex chromosome complement. Typically, females have two X chromosomes (46,XX), while males have one X and one Y (46,XY). From the initial bi-potential gonad, a cascade of genes allows differentiation into a testis or an ovary, a process known as sex determination. Once gonads have developed and differentiated, they start producing sex-specific hormones, which, in turn, determine the development of secondary sexual characteristics, including the differentiation of the external genitalia (Eggers et al. 2014).

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Disruption to the genetic network underlying these pathways can lead to DSD, a group of congenital conditions where chromosomal, gonadal or anatomical sex is atypical (Hughes et al. 2006). Studies in both humans and mice have identified a number of genes that play critical roles in internal and external genitalia development (Eggers and Sinclair 2012). In this chapter, we will review some of the key genes and genetic pathways involved in gonad and genital development and demonstrate how defects in these genes result in DSD. For a summary of genes involved in human DSD, see Table 2.1. Disruption of the sex chromosome complement can also lead to DSD but is out of the scope of this review.

2.1 Formation of the Bi-potential Gonad

In mammals, testes and ovaries arise from bilateral bi-potential gonads, which derive from the genital ridge. A number of genes have been shown to be critical for the development of these undifferentiated gonads.

In mice, *Gata4* (GATA-binding factor 4) is the earliest expressed gene specific for the genital ridge development (Piprek et al. 2016). In mice, loss of *Gata4* expression leads to the absence of genital ridges (Hu et al. 2013). The Homeobox protein *Emx2* and Lim/homeobox protein *Lhx9* are

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Table 2.1 Genes known to be involved in DSD

		Gene	Locus	OMIM	Associated DSD	Inheritance
46,XX DSD						
	Disorders of ovarian development					
		BMP15	Xp11.22	300247	Ovarian dysgenesis	AD
		FOXL2	3q22.3	608996	POI alone or with blepharophimosis, ptosis and epicanthus inversus syndrome	AD
		NR5A1	9q33.3	184757	POI	AD
		RSPO1	1p34.3	609595	46,XX OT DSD with palmoplantar hyperkeratosis	AR
		SOX3	Xq27.1	313430	46,XX T or OT DSD—gain of function	XL: dup
		SOX9	17q24.3	608106	46,XX T DSD—duplication	AD: dup
		SRY	Yp11.2	480000	46,XX T DSD—gain of function	Translocation
		WNT4	1p36.12	603490	46,XX T DSD	AR
		WT1	11p13	607102	Frasier and Denys-Drash syndrome	AD
		HSD17B4	5q23.1	233400	Perrault syndrome (with ovarian dysgenesis in 46,XX)	AR
		FSHR	2p16.3	136435	Ovarian dysgenesis	AR
	Androgen excess					
		NR0B1	Xp21.2	300473	CAH	XLR
		ARX	Xp21.3	300215	X-linked lissencephaly with ambiguous genitalia	XL
		CYP11A1	15q24.1	118485	CAH	AR
		CYP11B1	8q24.3	610613	CAH (11-beta-hydroxylase deficiency)	AR
		CYP17A1	10q24.32	609300	17, 20-lyase deficiency	AR
		CYP19A1	15q21.2	107910	Aromatase deficiency	AR
		CYP21A2	6p21.33	613815	CAH (21-hydroxylase deficiency)	AR
		HSD3B2	1p12	613890	CAH (3-beta-hydroxysteroid dehydrogenase deficiency)	AR
		NR3C1	5q31.3	138040	46,XX hyperandrogenism	AD
		POR	7q11.23	124015	Cytochrome P450 oxydoreductase deficiency	AR
		STAR	8p11.23	600617	CAH (cholesterol desmolase deficiency)	AR
	Other (Müllerian agenesis)					
		WNT4	1p36.12	603490	MRKH	AD
		HOXA13	7p15.2	142959	Hand-foot-uterus syndrome, MRKH	AD
46, XY DSD						
	Disorders of testicular development					
		CBX2	17q25.3	602770	CGD	AR
		DHH	12q13.12	605423	PGD or CGD	AR, AD
		DMRT1	9p24.3	602424	CGD	AD: deletion
		DMRT2	9p24.3	604935	CGD	AD: deletion
		GATA4	8p23.1	600576	GD	AD
		NR0B1	Xp21.2	300473	GD—gain of function	XL: dup
		NR5A1	9q33.3	184757	Various forms of 46,XY DSD	AD
		MAP3K1	5q11.2	600982	GD	AD
		SOX9	17q24.3	608106	GD and campomelic dysplasia	AD
		SRY	Yp11.2	480000	46,XY ovarian DSD	AD
		TSPYL1	6q22.1	604714	Sudden infant death syndrome with dysgenesis of the testes syndrome	AR
		WNT4	1p36.12	603490	46,XY OT DSD or CGD—duplication	AD: dup
		WT1	11p13	607102	Frasier and Denys-Drash syndrome	AD
		ZFPM2	8q23.1	603693	GD	AD
		FGFR2	10q26.13	176943	GD with cranioynostosis. Apert syndrome	AD
	Disorders of androgen synthesis or action					
		AKR1C2	10p15.1	600450	Various forms of 46,XY DSD	AR
		AKR1C4	10p15.1	600451	Various forms of 46,XY DSD	AR

Table 2.1 (continued)

		Gene	Locus	OMIM	Associated DSD	Inheritance
		AMH	19p13.3	600957	PMDS	AR
		AMHR2	12q13.13	600956	PMDS	AR
		AR	Xq12	313700	CAIS, PAIS	XL
		ARX	Xp21.3	300215	X-linked lissencephaly with ambiguous genitalia	XL
		ATRX	Xq21.1	300032	46,XY DSD associated with alpha-thalassaemia X-linked intellectual disability syndrome	XL
		CDKN1C	11p15.4	600856	Genital anomalies associated with Beckwith-Wiedemann and IMAGE syndrome	AD
		CYB5A	18q22.3	613218	various forms of 46,XY DSD	AR
		CYP11A1	15q24.1	118485	46,XY DSD with adrenal insufficiency	AR
		CYP17A1	10q24.32	609300	17, 20-lyase deficiency	AR
		HSD17B3	9q22.32	605573	17-beta-hydroxysteroid dehydrogenase III deficiency	AR
		HSD3B2	1p12	613890	3-beta-hydroxysteroid dehydrogenase deficiency	AR
		LHCGR	2p16.3	152790	Leydig cell hypoplasia, precocious puberty	AR
		POR	7q11.23	124015	Cytochrome P450 oxidoreductase deficiency	AR
		SRD5A2	2p23.1	607306	Steroid 5-alpha-reductase deficiency	AR
		STAR	8p11.23	600617	CAH (cholesterol desmolase deficiency)	AR
		BBS9	7p14.3	615986	Bardet-Biedl syndrome	AR
		CHD7	8q12.2	608892	CHH or KS, CHARGE syndrome	AD
		FGF8	10q24.32	612702	CHH or KS	AD
		FGFR1	8p11.23	147950	CHH or KS	AD
		FSHB	11p14.1	136530	CHH	AD
		GNRH1	8p21.2	152760	CHH	AR
		GNRHR	4q13.2	138850	CHH	AR
		HESX1	3p14.3	601802	KS or CPHD	AD
		KAL1	Xp22.31	300836	CHH or KS	XL
		KISS1R	19p13.3	604161	CHH or KS	AD
		LEP	7q32.1	164160	CHH with obesity	AR
		LHX3	9q34.3	600577	CPHD	AR
		PROK2	3p13	607002	CHH or KS	AD
		PROKR2	20p12.3	607123	CHH or KS	AD
		PROP1	5q35.3	601538	CPHD	AR
		TAC3	12q13.3	162330	CHH	AR
		WDR11	10q26.12	606417	CHH or KS	AD
	Other (isolated hypospadias, cryptorchidism)					
		AR	Xq12	313700	Isolated hypospadias	XL
		CYP11A1	15q24.1	118485	Isolated hypospadias	AD
		HSD3B2	1p12	613890	Isolated hypospadias	AD
		SRD5A2	2p23.1	607306	Isolated hypospadias	AD
		ATF3	1q32.3	603148	Isolated hypospadias	AD
		HOXA13	7p15.2	142959	Guttmacher syndrome	AD
		INSL3	19p13.11	146738	Cryptorchidism	AD
		MAMLD1	Xq28	300120	Hypospadias	XL
		RXFP2	13q13.1	606655	Cryptorchidism	AD

also involved in the early development of the bipotential gonad. Mice deficient in *Emx2* lack gonads, kidneys and genital tracts (Pellegrini et al. 1997). In humans, a microdeletion encompassing *EMX2* has been found in a patient with 46,XY DSD (Piard et al. 2014). *Lhx9*-null mice also fail to develop gonads, and deficient XY mice develop as female (Birk et al. 2000). However, variants in this gene have yet to be associated with DSD in humans. Lhx9 regulates the expression of the

orphan nuclear receptor *Nr5a1*, also known as steroidogenic factor 1 (SF1), a transcription factor that is expressed in both gonads and adrenal glands. *Nr5a1*-null mice lack both gonads and adrenal glands (Luo et al. 1994; Val et al. 2003). Another player, the Wilms tumour protein homolog (WT1) protein, in particular, the –KTS isoform, is necessary for early bi-potential gonad development and *Wt1*-null mice fail to develop gonads and kidneys, which is lethal (Kreidberg et al. 1993). In mice, *Cbx2* is also involved in early gonad formation, and mice deficient in *Cbx2* display delayed gonad development and male-to-female sex reversal (Eggers et al. 2014). Finally Insulin-like growth factors (IGFs) are involved in the regulation of genital ridge formation (Pitetti et al. 2013). All these genes are evolutionarily conserved among vertebrates. In mice null for *Gata-4*, the genital ridges do not form at all, suggesting that *Gata-4* is required for initiation of bi-potential gonad formation. However, in mice null for *Sf1*, *Wt1*, *Emx2* and *Lhx9,* the bi-potential gonads form but quickly degenerate, suggesting that these genes are involved in maintenance and further development (Piprek et al. 2016). Homeodomain proteins such as HOXA9, HOXA10, HOXA11 and HOXA13 are thought to be responsible for the precise determination of the site of genital ridge formation. Other homeobox genes such as Sine Oculis Homeobox Homologs (*Six1* and *Six4*), pre-B-cell leukemia Homeobox 1 (*Pbx1*) or Podocyte Expressed 1 (*Pod1*) are implicated in the regulation of genital ridge formation, and development in mice is reviewed in Piprek et al. (2016). Finally, it is interesting to note that many of these bi-potential gonad genes continue to play a later role in the gonads, in particular, during testis sex determination.

2.2 Testis Determination and Development

2.2.1 SRY

Attempts to identify the exact sequence responsible for male development, referred to as testis-determining factor (TDF), focussed on males with a 46,XX karyotype. In 90% of these males, translocation of a small piece of the Y chromosome is observed, and molecular studies finally identified the translocated gene responsible, denoting it the *SRY* (sex-determining region of the Y) gene (Sinclair et al. 1990). Further evidence of the key sex-determining role of *SRY* came from the identification of causative variants in this gene in 46,XY phenotypic females (Berta et al. 1990). Final proof that *SRY* is critical for male development was demonstrated using transgenic mouse studies, which showed that expression of *Sry* alone is sufficient for XX mice to develop testis and become males (Koopman et al. 1991). Therefore, in both mice and humans, *Sry* is necessary and sufficient to induce testis development (Eggers et al. 2014). More recently, *SRY* has been demonstrated to determine Sertoli cell fate in mice by repressing the ovarian pathway and activating testicular differentiation genes (Li et al. 2014).

2.2.2 SOX (SRY Box) Transcription Factors

SOX9 is a member of the SRY-related HMG box protein family of transcription factors. In mice, *Sox9* is the immediate downstream target of *Sry,* and its expression is activated when *Sry* levels reach a critical threshold. Although *Sox9* is expressed in the genital ridge of both male and female mouse embryos, it becomes sexually dimorphic after *Sry* expression peaks, with a considerable increase in XY compared to XX gonads. Conditional gonad-specific knockout of Sox9 in XY mouse embryos leads to the development of ovaries, while ectopic overexpression in XX embryonic mouse gonads results in testicular development (Eggers et al. 2014). An upstream region of *Sox9*, known as the testis-specific enhancer core element (TESCO), is required for upregulation of mouse *Sox9* expression (Sekido and Lovell-Badge 2008). Another region upstream of SOX9 in humans, known as RevSex, has been involved in various forms of DSD (Kim et al. 2015; Sreenivasan et al. 2017). However, this region remains to be defined in detail.

Many of the downstream targets of SOX9 have been characterised. In mice, *Sox9* initiates expression of anti-Müllerian hormone (*Amh*) and

cerebellin 4 (*Cbln4)*. Another downstream target of Sox9 in mice is fibroblast growth factor 9 (*Fgf9*), which acts through its receptor FGFR2 for the development of Sertoli cells and through a feedback loop to upregulate Sox9 (Bagheri-Fam et al. 2008, 2017). In humans, patients with deletion of chromosome 10q26.13, which includes the *FGFR2* locus, demonstrate atypical sexual differentiation (Bagheri-Fam et al. 2008).

Sox9 seems to be regulated by nuclear receptor subfamily 0 group B member 1 (Nr0b1), also known as *Dax1* (Ludbrook et al. 2012*)*. However, both the contribution and the precise function of *DAX1* in testis development remain unclear, as it is considered both a pro-testis (Meeks et al. 2003) and an anti-testis gene (Swain et al. 1998). Several studies in both mice and human suggest that *DAX1* acts within a narrow window and that both the activity and concentration of NR0B1 are critical (Ludbrook and Harley 2004).

Studies in mice have demonstrated a certain level of redundancy among members of the *SOX* gene family. In humans, *SOX8* has recently been identified in patients with 46,XY DSD (Portnoi et al. 2018). In mice, *Sox10* is thought to be sufficient to drive testis development when overexpressed/duplicated but is not required for testis determination when other members of the Sox family are present (Polanco et al. 2010). Likewise, *Sox3* is not normally expressed in the developing gonads in mice or humans. However, a transgenic mouse line with ectopic overexpression of *Sox3* showed XX female-to-male sex reversal. Sex reversal is also seen in 46,XX patients with a *SOX3* duplication (Sutton et al. 2011). This supports the notion that other *Sox* genes can functionally replace *SRY* or *SOX9* and drive testis differentiation.

2.3 Regulators of Sry Expression

2.3.1 GATA Genes and ZFPM2

GATA4 and *GATA6* belong to the *GATA* family of zinc finger transcription factors that recognise a GATA factor present in the promoter of many genes. Padua et al. demonstrated that these genes are necessary for normal testis development, as conditional double-mutant mice lacked normal steroidogenic function in the testes (Padua et al. 2015). In mice, expression of *Gata4* becomes sexually dimorphic with high expression in the Sertoli cells, where it acts with zinc finger protein, FOG Family Member 2 (*Zfpm2*), as a dimer that is required for *Sry* regulation (Manuylov et al. 2011). *Zfpm2* expression is directly controlled by *Six1* and *Six4*, which may be functionally redundant (Fujimoto et al. 2013).

2.3.2 NR5A1 Gene encodes Steroidogenic Factor 1 (SF1)

In mice, the *Nr5a1* gene has been identified as another target of *Six1* and *Six4*, which seem responsible for the control of gonad precursor cell formation and determination of gonadal size (Kawakami et al. 2000). SF1 together with SRY is also required for proper expression of SOX9 in the developing testis (Park et al. 2005).

2.3.3 WT1

The WT1 protein exhibits alternative splicing, and a specific isoform, Wt1 +KTS, has an important function in very early testis development, as mice lacking *Wt1 +KTS* undergo male-to-female sex reversal as a result of failure to upregulate *Sry* expression (Hammes et al. 2001). In humans, causative variants in the *WT1* gene have been associated with numerous cases of syndromic or isolated DSD (Hastie 2017). In particular, variants in *WT1* are responsible for Frasier syndrome, which includes a male-to-female sex reversal (Barbaux et al. 1997), Denys-Drash syndrome, where patients present with atypical genitalia (Pelletier et al. 1991) and WAGR syndrome (Wilms tumour, aniridia, genitourinary malformations and mental retardation) (Le Caignec et al. 2007).

2.3.4 Mitogen-Activated Protein Kinase Pathway

Using a forward genetic screen in mice, Bogani et al. demonstrated that mitogen-activated protein kinase kinase kinase 4 (MAP3K4, also known as

MEKK4) was necessary for normal expression of *Sry* during testis development (Bogani et al. 2009). They subsequently showed that *Gadd45γ* was required to promote *Map3k4*-mediated activation of p38 MAPK signalling for testis determination in mice (Warr et al. 2012). More recently, the same team has found that *Map2k6* and more moderately *Map2k3* had functions in mouse sex determination through positive effects on *Sry* (Warr et al. 2016). Interestingly, while *MAP3K1* does not appear to be required for normal testis determination in mice (Warr et al. 2011), numerous variants in this gene have been reported in 46,XY DSD with gonadal dysgenesis (Pearlman et al. 2010) (Loke et al. 2014).

2.4 The Hedgehog Signalling Pathway

Hedgehog is an important morphogen that controls patterning and differentiation of numerous organs during embryogenesis. Desert Hedgehog (*Dhh*) is the sole HH member expressed in the developing XY mouse gonad. Differentiated Sertoli cells secrete DHH, which subsequently binds to its receptor protein patched homologue 1 (*Ptch1*) on pre-Leydig cells and activates the hedgehog signalling pathway in these cells, resulting in their differentiation. Null mice for *Dhh* have disrupted testis cords and lack mature Leydig cells (Clark et al. 2000). Hedgehog acyl transferase (HHAT) also plays a role in proper testis cord formation and the differentiation of foetal Leydig cells in both humans and mice (Callier et al. 2014).

2.5 DMRT1

Although the primary sex-determining gene in several non-mammalian vertebrates such as the chicken is present on the sex chromosomes (Lambeth et al. 2014), Doublesex and Mab-3-related Transcription Factor 1 (*Dmrt1*) have evolved to become dispensable in mammals (Raymond et al. 2000). It plays an important role, however, in maintaining Sertoli cells in postnatal mouse testis by blocking testicular retinoic acid signalling from activating genes involved in female sex determination (Matson et al. 2011; Minkina et al. 2014). It is one of the downstream targets of GATA4 (Zaytouni et al. 2011), and disruptions to this gene have been implicated in human DSD (Marsudi et al. 2018). Loss of *DMRT1* gene was seen in a Mos 45,XY,-9[8]/46,XY,r(9)[29]/47,XY,+idic r(9)×2[1]/46,XY,idic r(9)[1]/46,XY[1] female presenting with short stature (11, p.28). Partial deletion of *DMRT1* causes 46,XY ovotesticular disorder of sexual development (Ledig et al. 2012).

2.6 Epigenetic Regulation of Testis Determination

Epigenetic modifications including modification of histones such as methylation can alter gene expression. Studies in mice have shown that the H3K9 demethylase Jmjd1a positively controls *Sry* expression by regulating H3K9me2 marks (Kuroki et al. 2013). In humans, this gene is increasingly reported in various cancers but not DSD.

2.7 Ovarian Differentiation

While numerous genes and pathways have been implicated in testis differentiation and development, much less is known about ovarian differentiation. Initially, It was thought to be a 'default' pathway in the absence of SRY. However, a number of specific genes and genetic pathways have now been implicated in ovarian development, and several of these cause DSD in humans when disrupted.

2.7.1 Forkhead Box L2 (Foxl2)

Foxl2 is a member of the forkhead box gene family of evolutionarily conserved transcription factors. It is one of the earliest upregulated genes in the developing ovary, suggesting a role in early ovary differentiation, the importance of which may differ between species (Baron et al. 2005). For instance, sex reversal is observed in goats with homozygous loss of FOXL2 (Gustin et al. 2016) but not in mice (Schmidt et al. 2004). In mice, it appears to play a major role in postnatal maintenance of the ovary (Uhlenhaut et al. 2009).

In mice, genes regulating testis development are upregulated shortly before birth and follicle activation is impaired in postnatal stages (Garcia-Ortiz et al. 2009). In humans, heterozygous loss-of-function mutations in *FOXL2* cause autosomal-dominant blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, OMIM #110100) (Crisponi et al. 2001). There are two forms of the syndrome; type 1, with associated premature ovarian insufficiency and type 2, without it (Meduri et al. 2010). Variants in *FOXL2* have also been associated with isolated premature ovarian insufficiency without BPES.

A recent study in 79 patients with 46,XX *SRY*-negative testicular or ovotesticular DSD with virilisation found two new heterozygous frameshift variants in the orphan nuclear receptor *NR2F2*, encoding the transcription factor chicken ovalbumin upstream promoter transcription factor 2 (COUP-TF2) (Bashamboo et al. 2018). Although, in mice, *Coup-Tf2* negatively regulates the expression of the pro-testis *Sox9* gene, virilisation and testis development were not reported in *Coup-Tf2*$^{+/-}$ XX female mice (Rastetter et al. 2014). This indicates that nuclear receptors might have divergent functions in mouse and human biology (Bashamboo et al. 2018).

2.7.2 Wnt4, Rspo1 and β-catenin

Two components of the Wnt signalling pathway, Wnt4 and Rspo1, play major roles in ovarian development. Both function through the activation of β-catenin, which, in turn, regulates a variety of genes important for ovarian development. β-Catenin and Wnt4 antagonise the pro-testis genes *Sox9* and *Fgf9* (Chassot et al. 2014). Foxl2 expression is also partially dependent on RSPO1, β-catenin and Wnt4, as deletion of both Foxl2 and Wnt4 or Rspo1 and Foxl2 results in a more severe phenotype than the single knockout models. Gonads in these mice develop as ovotestes, indicating a partial sex-reversal (Chassot et al. 2014). Additionally, while Rspo1 null mice exhibit a decreased number of germ cells, null Wnt4 mice have an initial normal number of germ cells that suffer from massive apoptosis. Therefore, it seems that Rspo1 stimulates germ cell proliferation, while Wnt4 is required for germ cell survival (Chassot et al. 2014). In humans, overexpression of *WNT4* has been associated with 46,XY sex reversal (Jordan et al. 2001). Additionally, heterozygous loss-of-function variants in *WNT4* have been reported in 46,XX patients with virilisation (Biason-Lauber et al. 2004; Philibert et al. 2008). Loss-of-function variants in *RSPO1* have been associated with a recessive syndrome including 46,XX testicular or ovotesticular DSD, palmoplantar hyperkeratosis and predisposition to squamous cell carcinoma of the skin (Parma et al. 2006; Tomaselli et al. 2008; Naasse et al. 2017).

2.8 Internal Genital Tract Development

As further detailed in the embryology chapter, mammalian male and female internal genital tracts derive from the paramesonephric and mesonephric ducts. Differentiation into these tracts is triggered by the differentiation of the bi-potential gonad into ovary or testis, according to the genetic sex of the individual. Numerous genes have been involved in the development of the female reproductive tract, in both mice and humans. The Mayer–Rokitansky–Küster–Hauser syndrome (MRKH) is a syndrome where the Müllerian tract development is incomplete, resulting in aplasia of the upper third of the vagina and uterus. Most studies of this condition have demonstrated recurrent changes in chromosomal regions 1q21.1 (Cheroki et al. 2008; Ledig et al. 2011), 16p11.2 (Nik-Zainal et al. 2011; Sandbacka et al. 2013), 17q12 (Cheroki et al. 2008; Bernardini et al. 2009; Ledig et al. 2011; Nik-Zainal et al. 2011; Sandbacka et al. 2013) and 22q11.21 (Cheroki et al. 2006, 2008; Ledig et al. 2011; Nik-Zainal et al. 2011). Most likely involved genes in these regions are *RBM8A* for 1q21.1, *TBX6* for 16q11.2 and *LHX1* and *HNF1B* for 17q12. Patients presenting with MRKH combined with hyperandrogenism have exhibited variants in WNT4 (Biason-Lauber et al. 2004, 2007; Philibert et al. 2008, 2010). For a complete description of candidate genes, refer to the review from Ledig et al. (Ledig and Wieacker 2018).

Table 2.2 Hypospadias candidate genes (48)

Humans		Animal models	Expression studies
SHH	HOXA4	DHH	PTCH1
GLI1	HOXB6	Wnt5a	Frizzled
GLI2	MAP3K1	Ctnnb1	
GLI3	CHD7	Hoxa13	
FGF8	NR5A1	Hoxd13	
FGF10	MAMLD1	EfnB2	
FGFR2	BMP4	EphB2	BMP2
ESR1	BMP7	EphB3	
ESR2	WT1	FKBP52/FKBP4	
WTAP	AKR1C3		
DGKK	HSD3B2		
HSD3B1	CYP11A1		CTGF
HSD17B3	CYP19A1		CYR61
ATF3	SRD5A2		GADD45B
BNC2	AR		ZEB1
MID1	VAMP7		
32		9	7

2.9 External Genitalia Development

As further detailed in the embryology chapter, after gonad differentiation and hormone production, the external genitalia will usually differentiate into female or male structures. From a genetic standpoint, differentiation of the genital tubercle and closure of the urethral plate to form the male penis and penile urethra are of particular interest. Several studies in both humans and mice have been carried out, and a recent review of the literature determined 48 candidate genes involved in the development of the penis and potentially responsible for hypospadias (Bouty et al. 2015). For a summary of these findings, see Table 2.2.

2.10 Genetics and DSD

Genetic variants are thought to underlie most DSD, and a wide variety of genetic changes have been implicated in DSD. As described above, 46,XY DSD can be caused by variants in a number of genes involved in both early gonad development and testis differentiation. Failure in gonadal development can cause complete gonadal dysgenesis or streak gonads, whereas failure in those genes involved in testis differentiation can cause the development of ovarian tissue or ovo-testis, as the ovarian pathway is no longer repressed. Some genes cause DSD in a dominant manner (i.e. SOX9 and MAP3K1), whereas others require both alleles to be affected (i.e. DHH). Large chromosomal deletions or duplications can also cause DSD. In particular, in 46,XX DSD, duplication of SOX3 or SOX9 or its regulatory regions can cause testicular development in the absence of a Y chromosome and *SRY*. Another well-studied phenotype of 46,XX DSD is premature ovarian insufficiency (POI, OMIM #311360). Apart from chromosomal abnormalities, such as 45,X Turner syndrome, this heterogeneous condition can result from variants in genes affecting the development of the ovary, DNA division and repair, follicle development and hormonal signalling, metabolism and immune regulation. For a thorough description of genes involved in this diagnosis, see review from Tucker et al. (Tucker et al. 2016). An up-to-date list of causative genes for POI is presented in Table 2.3. POI can occur prior to pubertal changes, part way through the development of secondary sexual characteristics or may present as an early menopause.

Table 2.3 Candidate genes for premature ovarian insufficiency (POI)

Gene	Inheritance	Phenotype
AARS2	AR	Leukodystrophy + POI
AFF2	XLD (susceptiblity)	POI
AIRE	AR	Autoimmune polyglandular syndrome, type 1 + POI
ATM	AR	Ataxia telangiecstasia + POI
BLM	AR	Bloom syndrome + POI
BMP15	XLD	POI
BMPR1B	AR	Acromesomelic chondrodysplasia + POI
C10ORF2	AR	Perrault syndrome + POI
CLPP	AR	Perrault syndrome + POI
CSB-PGBD3	AD	POI
CYP17A1	AR	POI
CYP19A1	AR	POI, foetal masculinization
DMC1	AR	POI
EIF2B2	AR	Ovarioleukodystrophy + POI
EIF2B4	AR	Ovarioleukodystrophy + POI
EIF2B5	AR	Ovarioleukodystrophy + POI
EIF4ENIF1	AD	POI
FANCA	AR	Fanconi anaemia + POI
FANCC	AR	Fanconi anaemia + POI
FANCG	AR	Fanconi anaemia + POI
FANCM	AR	POI
FIGLA	AR	POI
FOXL2	AD	BPES type 1 + POI
FMR1	XLD (premutation)	POI
FSHR	AR	POI
GALT	AR	Galactosaemia + POI
GDF9	AR	POI
HARS2	AR	Perrault syndrome + POI
HAX1	AR	POI
HFM1	AR	POI
HSD17B4	AR	Perrault syndrome + POI
LARS2	AR	Perrault syndrome + POI
LMNA	AD	Cardiomyopathy + POI
MCM8	AR	POI
MCM9	AR	POI
MSH4	AR	POI
NANOS3	AR	POI
NBN	AR	Nijmegen breakage syndrome + POI, infertility
NOBOX	AD	POI
NOG	AD	Proximal symphalangism + POI
NR5A1	AR	POI (DSD in males)
NUP107	AR	POI (XX gonadal dysgenesis)
PGRMC1	AD	POI
PMM2	AR	Congenital disorder of glycosylation + POI
POF1B	XLR	POI
POLG	AR, AD	Progressive external opthalmoplegia and parkinonism + POI
POLR2C	AD	POI
PSMC3IP	AR	POI, XX ovarian dysgenesis
RCBTB1	AR	POI, retinal dystrophy, intellectual disability

(continued)