Genetic Counselling

21

David Amor

21.1 Introduction

DSD have a range of aetiologies, but for most DSD, genetic factors are prominent. As understanding of the genetic contributions increases, clinicians are increasingly faced with questions about why the DSD occurred and whether it will happen again. This chapter provides an overview of the process of genetic counselling, emphasizing the elements most relevant to genetic counselling for DSD, and then individual DSD, summarizing the specific aspects of genetic counselling for each condition.

21.2 Overview of Genetic Counselling

Genetic counselling is an important component of the management of people with DSD and their families and may take place in a variety of clinical settings. Genetic counselling for DSD frequently takes place within a multidisciplinary team, and it is typically carried out by clinical geneticists, genetic counsellors, or by other health professionals with specific expertise in genetic counselling.

Genetic counselling for DSD can take place at any stage of life but most commonly occurs in one of four clinical contexts:

1. a couple with personal or family history of DSD planning a pregnancy;
2. prenatal diagnosis of DSD;
3. following diagnosis of DSD in a neonate or young child; or,
4. an adolescent or young adult affected by DSD and seeking information about their own diagnosis.

Genetic counselling is a complex process that is difficult to encapsulate in a single definition; however, the content and process of genetic counselling typically includes, to varying degrees, six main elements (Harper 2010), which are summarized below.

21.2.1 Diagnostic and Clinical Aspects

Genetic counselling depends on accurate diagnosis, and it is essential that the diagnosis be made

D. Amor (✉)
Department of Neurodisability and Rehabilitation, The Royal Children's Hospital, Melbourne, VIC, Australia

Murdoch Children's Research Institute, Melbourne, VIC, Australia

Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia

Victorian Clinical Genetics Services, Melbourne, VIC, Australia
e-mail: david.amor@mcri.edu.au

J. M. Hutson et al. (eds.), *Disorders|Differences of Sex Development*,
https://doi.org/10.1007/978-981-13-7864-5_21

as firm as possible before risk[1] estimates are given. In addition to careful history and examination, additional data may be obtained from a variety of sources, such as interviewing multiple family members, seeking archived medical records, pathology reports and death certificates. In DSD, a careful endocrine, chromosome and molecular assessment is necessary before accurate genetic counselling can be given. Once a diagnosis is confirmed, it is important that the counsellor understands the natural history of the DSD and is able to communicate this to the individual and their family in a meaningful way.

21.2.2 Documentation of Family and Pedigree Information

The collection of family information is best achieved by drawing a family tree or pedigree. A clearly drawn pedigree provides a permanent record of genetic information in a particular family and conveys genetic information more clearly than other forms of family history documentation. Pedigrees drawn during the consultation also serve as a psychosocial tool, providing the opportunity to explore and better understand family dynamics and relationships. Careful inquiry may be required to elicit a family history of genital variation, information about which may not have been widely communicated within the family. Information about family history of stillbirth, neonatal death and consanguinity should also be sought.

Special care is required when drawing pedigrees in DSD families. Symbols used in constructing pedigrees include male (□), female (○) and sex unknown (◇); in DSD families, the symbols for male and female should be used to denote sexual identity rather than chromosomal sex. Where known, chromosome sex can be included as an annotation.

Although many DSD follow classical Mendelian inheritance, two points warrant emphasis. First, for X-linked recessive diagnoses that cause sex reversal (e.g. Androgen insensitivity syndrome—AIS), the pedigree at first glance may not suggest X-linked inheritance because affected individuals are phenotypically female rather than male. Second, there exist Mendelian DSD that are penetrant for only one chromosomal sex; for these diagnoses, the phenotype may skip one or more generations, and the risk of having an affected child is half of the genetic risk.

21.2.3 Recognition of Inheritance Patterns and Risk Estimation

Once all available information has been collated, it should be possible to make a risk estimation. In the setting of DSD, request for risk estimation usually takes one of three forms:

1. risk of having a second affected child;
2. risk of transmission from an affected parent to a child; or,
3. risk of having an affected child when a more distant relative is affected.

Risk figures in genetic counselling can be given either as odds (e.g. ‘one in four’) or as percentages (e.g. 25%), with the method of explanation tailored to the needs of the client. For Mendelian diagnoses, an exact risk can be given on the basis of the known inheritance pattern, typically ranging from ‘negligible risk’ to 50% risk. For chromosome variations and non-Mendelian diagnoses, risk estimation is usually empirical, based on observed data rather than theoretical predictions. The accuracy of empirical risk estimation depends on the quality of the available observed data and the degree to which it matches the client’s personal situation. For very rare diagnoses, the only source of risk estimation might be from case reports of recurrence (or lack thereof).

[1] In this chapter, the term ‘risk’ is used in the context of the likelihood of a child of having a disorder/difference of sex development (DSD), but this term may not be appropriate in all clinical situations. In particular, when a particular DSD is viewed as a variation rather than a condition, terms such as ‘chance’ or ‘likelihood’ may be more appropriate. It is important to check in with parents to ensure they are comfortable with the language.

21.2.4 Communication

Genetic concepts are typically complex and need to be coupled with effective communication if genetic counselling is to be successful. Information about the psychological, psychosocial and practical impact of the diagnosis; genetic test results; natural history of the DSD; and available treatments should be conveyed in a way that is easily comprehended, taking into account the scientific literacy of the family. Genetic counselling must also be sensitive to the sexual identity of those affected, and care must be taken to avoid assuming that chromosomal sex will necessarily determine gender identity.

Most genetic counselling sessions are followed up by a letter in plain language to the client, summarizing the main points discussed and documenting any risk estimate. Finally, although generally distinct from psychotherapeutic counselling, genetic counselling frequently has a therapeutic element, arising from the willingness of the counsellor to listen to and acknowledge the client's experiences, the focus on the agenda of the client and the empathy shown during the consultation. Where possible sharing resources about these variations, including appropriate peer support contacts, should be included in this information.

21.2.5 Information on Available Options

Many genetic counselling sessions result in the client being presented with choices. In DSD genetic counselling, choices frequently centre on reproductive options, but they may also include decisions about whether to pursue further genetic testing, and whether to pass on information to other family members.

21.2.6 Support in Decision-Making and for Decisions Made

A key element underlying genetic counselling is non-directiveness; in practice, this means that a goal of genetic counselling is to provide clients with the appropriate information to enable them to make their own informed decisions, taking into account their attitudes, perceptions and beliefs. The genetic counsellor may facilitate and support the client's decision-making but should avoid advising the client on which choices to make. Finally, the genetic counsellor should assess the need for further counselling and follow-up.

21.3 Reproductive Options

For many DSD, there is a significant risk of recurrence for siblings or offspring; following diagnosis of these DSD, families are faced with difficult choices about future pregnancies. Reproductive choices are very personal, and not all options will be considered by all families. Where future pregnancies may have a DSD, there are six main reproductive options that may be available:

1. possibility of having another affected child;
2. deciding not to have further children;
3. prenatal diagnosis—some families may wish to know—to prepare themselves and their family or consider termination of pregnancy;
4. preimplantation genetic testing (PGT);
5. use of donor gametes or donor embryos; and,
6. adoption.

For some individuals with a DSD, reproduction using their own gametes will not be possible. For these people, conception using donor gametes may be possible, and provided the donor is not a genetic relative, the possibility of recurrence will be avoided.

21.4 Genetic Testing

Genetic testing is now a routine part of clinical practice and plays a key role in management of DSD, for which genetic testing falls into two categories: cytogenetic analysis and for single-gene disorders.

21.4.1 Cytogenetics

Cytogenetic analysis, and particularly information about the sex chromosomes, is a key part of the diagnostic process of DSD, and this is the only way to accurately diagnose chromosome DSD. Standard microscope karyotype should be performed in all cases, with at least 30 cells examined in order to exclude mosaicism. The presence of SRY is routinely tested using fluorescein *in situ* hybridization (FISH). Phenotypic sex determination involves a cascade of genes located on the autosomes and sex chromosome, and DSD have been associated with a number of autosomal deletions and duplications. Some of these include known DSD loci, such as SOX9, whereas others presumably harbour DSD genes that are yet to be discovered.

In recent years, traditional chromosome analysis has been replaced by chromosome microarray analysis, also known as molecular karyotyping. Chromosome microarray allows the determination of copy number at more than a million different loci across the genome, offering detection of small chromosome deletions and duplications at a resolution that is more than 100 times greater than microscopic karyotyping. Molecular karyotyping is a valuable diagnostic tool for detecting gene copy-number changes affecting known DSD genes and is also used for research to identify new DSD loci (Ledig et al. 2010). The diagnostic yield of molecular karyotyping is greatest in individuals with a DSD who have additional syndromal features, such as intellectual disability or associated birth defects. Despite the clear benefits of molecular karyotyping, microscopic karyotyping should still be performed in DSD cases due to its ability to detect balanced translocations and low-level mosaicism.

21.4.2 Gene Testing

Genetic testing for a number of DSD genes is now available on a clinical basis, including testing of SRY, androgen receptor (AR), DHH, NR5A1 (SF-1), SOX9, WT1 and CYP21A2. This number will undoubtedly increase as new genes are identified and linked to DSD. Detection of a specific gene variant can assist by confirming and refining a clinical diagnosis, clarifying the risk to other family members, and providing the opportunity for molecular prenatal diagnosis in future pregnancies. Families should be aware, however, that genetic testing does not provide clear answers in all cases. In some cases, a sequence variation of unknown significance may be identified. These are variations in DNA for which there is insufficient evidence to clearly classify the variant as being disease-causing. In other cases, no variant is identified, and there are several explanations for this finding. These include that a disease-causing variant is present in a gene that was not tested, or a gene that is yet to be discovered, or that there is a variant in the tested gene, but that it was not detected by the particular testing method used. In addition, failure to detect a causative pathogenic variant may be because the DSD is due to a non-genetic cause.

Testing for single genes is now mostly limited to cases where there is a strong suspicion of a pathogenic variant in a particular gene, based on clinical or biochemical phenotype. When multiple genetic aetiologies are being considered, a preferable approach is to use next-generation DNA sequencing (NGS), which enables large numbers of DSD genes to be simultaneously tested at relatively low cost, providing a new and valuable tool for the diagnosis of DSD. In the setting of 46,XY DSD, testing a 'panel' of 64 DSD genes has been shown to make a molecular diagnosis in about one-third of individuals tested (Baxter et al. 2015).

21.5 Genetic Counselling for Specific DSD

21.5.1 Sex Chromosome DSD

21.5.1.1 47,XXY

Klinefelter syndrome (47,XXY) is caused by the presence of an additional X chromosome in an otherwise male karyotype. Sibling recurrence risk for 47,XXY is low and probably not signifi-

cantly increased compared to the background population risk, with only one documented occurrence of brothers with Klinefelter syndrome (Woods et al. 1997). There is a modest maternal age effect, resulting from approximately 30% of Klinefelter syndrome resulting from a maternal meiosis I error (Gardner and Amor 2018).

In the absence of medical intervention, men with Klinefelter syndrome are infertile. Instances of documented natural fertility are extremely rare (Laron et al. 1982; Terzoli et al. 1992; Juul et al. 2007) and may be accounted for by undetected XY/XXY mosaicism. In fact, testicular XY/XXY mosaicism appears to be relatively common, even in males with non-mosaic XXY on blood karyotype. Evidence from testicular biopsies of male with non-mosaic Klinefelter syndrome indicates that spermatogenesis, where present, originates not from XXY cells, but from foci of testicular tubules where the spermatogonia have a 46,XY karyotype, most likely representing clones of spermatogonia that have randomly lost one X chromosome.

Some men with Klinefelter syndrome are now able to become fathers with the assistance of testicular sperm extraction (TESE), which is able to obtain sperm in approximately 50% of XXY males (Fullerton et al. 2010). The chances of obtaining sperm might be improved if sampling and storage occur immediately after puberty; however, the benefits of this approach are yet to be proven (Gies et al. 2016). The few single sperms obtained by TESE are injected into the egg using intracytoplasmic sperm injection (ICSI). The first such child from a father with Klinefelter syndrome conceived using this technique was born in 1997 (Bourne et al. 1997), and since then more than 100 genetic children have been born to people with non-mosaic Klinefelter Syndrome (Fullerton et al. 2010).

These successes raise the question of whether there are genetic risks to the offspring. In terms of clinical outcome, results are reassuring, with only one documented instance of foetal XXY (Ron-El et al. 2000). Nonetheless, there is some evidence that sperm from XXY males have a higher incidence of aneuploidy compared to XY males, and that this aneuploidy affects autosomes as well as sex chromosomes (Levron et al. 2000; Rives et al. 2000). These aneuploidies are most likely the result of a compromised testicular environment rather than the presence of XXY cells per se, and the risk of variation is similar to that for azoospermic men with a 46,XY karyotype (Levron et al. 2000; Palermo et al. 2002). On the basis of this information, there may be a small increased risk for both sex chromosome and autosome aneuploidy in the offspring of XXY males, and preimplantation genetic diagnosis (PGD) or prenatal genetic diagnosis could be offered to these couples (Staessen et al. 2003).

For males with mosaicism XY/XXY in the blood, natural fertility may be possible, and a semen analysis in late adolescence can help predict the likelihood of natural pregnancy. In mosaic males with oligospermia or azoospermia, treatment with ICSI ± TESE may be beneficial, and XY/XXY mosaicism appears to be associated with a higher rate of sperm retrieval (Seo et al. 2004) and a lower rate of sperm aneuploidy (Ferlin et al. 2005) compared to non-mosaic XXY.

21.5.1.2 45,X

Turner syndrome (TS) is associated with partial or complete loss of one X chromosome (Nielsen and Wohlert 1991; Stochholm et al. 2006) and is characterized by ovarian insufficiency which occurs before puberty in most cases. Whilst infrequent at birth, 45,X karyotype is common at conception and is identified in approximately 10% of products of conception from spontaneous abortion (Kajii et al. 1980). There is no evidence of an increased risk of sibling recurrence.

Approximately 30% of women with TS have a mosaic karyotype in the peripheral blood, with a 45,X cell line detected in conjunction with one or more other cell line, for example, 45,X/46,XX, 45,X/47,XXX, and 45,X/46,XX/47,XXX, accounting for variability in phenotype and ovarian function (Hanson et al. 2001).

Spontaneous puberty occurs in 15–30% of girls with TS and 2–5% experience menarche (Pasquino et al. 1997). Oocytes are present in the

ovaries of approximately a quarter of adolescents with TS, but spontaneous pregnancy is rare (Bernard et al. 2016), and for many women with TS, *in vitro* fertilization (IVF) with a donor egg is the most viable option. In a French study of 480 women with Turner syndrome, only 2/181 women (1.1%) with a non-mosaic 45,X karyotype achieved a spontaneous pregnancy (Bernard et al. 2016). In a woman with non-mosaic 45,X, any period of fertility is likely to be short-lived; in some centres, sampling and storage of oocytes in early adolescence, or ovarian cryopreservation in prepubertal girls, might offer a future possibility (Oktay et al. 2016).

Current evidence suggests that 45,X germ cells are unable to complete meiosis (Modi et al. 2003), suggesting that spontaneous pregnancy in women with 45,X TS relies on the presence of 46,XX germ cells in the ovaries, with the occasionally observed follicles originating from small numbers of 46,XX germ cells (Hall et al. 2006). Fertility is more likely to be retained in women with 45,X/46,XX mosaicism than standard monosomy (45,X), although premature ovarian insufficiency is common (Blair et al. 2001), and the risk of chromosomally atypical offspring in women with mosaic TS appears to be increased compared to the general population (Uehara et al. 1999; Sybert 2002; Bernard et al. 2016). Of note, a non-mosaic 45,X peripheral karyotype does not preclude the presence of 45,X/46,XX mosaicism in the ovary, and a peripheral blood karyotype is not a completely reliable predictor of ovarian status (Mortensen et al. 2010). In the study of Bernard et al. a pregnancy was achieved by 19/130 women with 45,X/46,XX mosaic Turner syndrome (Bernard et al. 2016).

A significant proportion of women with TS have a structural difference of the second X chromosome, resulting in partial X monosomy. Examples are deletion Xp, ring X and isochromosome Xq, and for these categories, mosaicism with 45,X or 46,XX cell lines is common. Many structural differences of the X chromosome are compatible with spontaneous menarche and pregnancy, and there are many examples of mother-daughter transmission (Lachlan et al. 2006). Risk to offspring is increased, and genetic counselling is recommended: male embryos that inherit the structurally atypical X will usually be non-viable, and female offspring are at risk of a more severe phenotype than the mother if X-inactivation does not completely favour the intact X.

Regardless of whether conception is spontaneous or assisted in TS, increased rates of miscarriage and foetal variation have been described (Bernard et al. 2016). In a review of the outcomes of 160 pregnancies in 74 women with TS, 29% resulted in miscarriage; 20% were associated with foetal anomalies, such as TS and Down syndrome; and 7% resulted in perinatal foetal death (Tarani et al. 1998). Intrauterine growth restriction and prematurity occur in approximately 50% (Bodri et al. 2006). In view of the high risk of foetal anomalies, antenatal diagnostic testing should be offered to all pregnant TS women, with the pregnancy managed as high risk from a foetal perspective. Miscarriages may be due to karyotypic variants such as TS or Trisomy 21, altered uterine environment related to developmental variation or poor endometrial receptivity due to hypo-oestrogenism (Abir et al. 2001; Doger et al. 2015; Bernard et al. 2016).

Pregnant women with TS require coordinated multidisciplinary tertiary medical and obstetric care, as they are at very high risk for complications during pregnancy, such as thyroid dysfunction, obesity, diabetes, hypertension, pre-eclampsia, deterioration of congenital heart disease, heart failure, aortic dissection and sudden death (Bernard et al. 2016).

21.5.1.3 45,X/46,XY

The karyotype 45,X/46,XY is associated with a broad range of clinical phenotypes from Turner syndrome to typical male. Presumably, these differences reflect the distribution of each cell line in different parts of the body, and particularly the presence of a Y-containing cell line in the gonad. Frequently, the Y chromosome is structurally atypical, with the structural variation presumably predisposing to loss during mitosis.

There is substantial difference in phenotype according to whether cases are ascertained prenatally or postnatally. Prenatally diagnosed

cases are phenotypic male in 90% of cases but may be at later risk of infertility. The other 10% of prenatally ascertained cases exhibit phenotypic features of TS and/or genital variations (Telvi et al. 1999). Postnatally ascertained cases present with a broad range of phenotypes, including Turner syndrome, infertility in otherwise phenotypic males and genital variation. Although in theory Y chromosome instability might be familial, in practice, sporadic occurrence appears to be the rule. There is a risk of gonadoblastoma in dysgenetic gonads for individuals with a karyotype of 45,X/46,XY due to the Y chromosome component.

21.5.1.4 46,XX/46,XY

The 46,XX/46,XY karyotype usually results from the fusion of dizygotic twin XX and XY embryos (XX/XY chimerism), although several other mechanisms have been proposed. Associated phenotypes include ovotesticular DSD, genital ambiguity, phenotypic male and phenotypic female. Occurrence is always sporadic. 46,XX/46,XY is occasionally encountered at prenatal diagnosis; in this circumstance, both cell lines may be present in the foetus, or alternatively, the second cell line results from contamination of the sample by maternal cells, or from an undiagnosed 'vanished' twin (Amor et al. 1999).

21.5.2 46,XY DSD

21.5.2.1 46,XY Complete Gonadal Dysgenesis (Swyer Syndrome) and 46,XY Partial Gonadal Dysgenesis DSD

The category of 46,XY gonadal dysgenesis comprises two conditions that are phenotypically distinct but genitally overlapping.

1. 46,XY complete gonadal dysgenesis (CGD) is associated with a 46,XY karyotype, typical female external genitalia with typical Müllerian structures and underdeveloped gonads with no sperm production.
2. 46,XY DSD (partial gonadal dysgenesis) is characterized by ambiguous/atypical genitalia, dysgenic testes, reduced or absent sperm production and variable presence of Müllerian structures.

46,XY gonadal dysgenesis can be familial and is a unique example of a Mendelian condition that can be inherited as an X-linked recessive, Y-linked, autosomal dominant or autosomal recessive trait. When inheritance is autosomal, penetrance is typically limited to individuals with an XY karyotype.

About 15% of individuals have deletions or loss of function variants in the SRY gene that are detectable by FISH or gene sequencing. The contribution of other genes is unknown; however, duplication of the gene NR0B1 (DAX1) accounts for some X-linked 46,XY gonadal dysgenesis (Barbaro et al. 2007), homozygous (or compound heterozygous) variants in desert hedgehog (DHH) account from some autosomal recessive 46,XY gonadal dysgenesis (Canto et al. 2004), and autosomal dominant 46,XY gonadal dysgenesis can result from heterozygous variants in DHH, NR5A1 (SF1) (Philibert et al. 2010) and MAP3K1 (Pearlman et al. 2010).

Genetic testing is available and can be used to inform genetic counselling in familial and sporadic cases of non-syndromic 46,XY gonadal dysgenesis. Analysis of SRY and chromosome microarray analysis are the first-line genetic investigations, followed by genetic analysis of multiple genes via an NGS panel.

In familial cases of 46,XY gonadal dysgenesis and cases where a causative gene variant is identified, this information will inform genetic counselling. The identification of a gene variant allows carrier testing of family members and prenatal diagnosis in at-risk pregnancies.

Men with SRY variants are usually infertile; therefore, most variants arise *de novo* in the proband rather than being present in the father. Nonetheless, siblings of the proband might still be at low risk because of gonadal or somatic mosaicism in the father. Variants in SRY that result in partial loss of function can cause 46,XY DSD, and penetrance may be incomplete, complicating genetic counselling.

In cases where there is no family history and no identified genetic variant, the situation is less

straightforward. No empiric sibling recurrence risk data exist, but risk is likely to be low. In the absence of molecular confirmation, prenatal diagnosis can be potentially offered by looking for the combination of an XY karyotype on chronic villus sampling (CVS), amniocentesis or non-invasive prenatal testing (NIPT) and female external genitalia on ultrasound scan.

Some individuals with 46,XY DSD may be able to reproduce with the aid of assisted reproduction technologies. For 46,XY women, assisted conception is possible if a uterus is present (Michala et al. 2008). Individuals with 46,XY may be able to reproduce using their own gametes and assisted reproduction technology. For variants affecting the SRY gene, the variants will be passed to all sons but not to daughters of the proband.

21.5.2.2 XY Ovarian DSD

The development of typical female anatomy and ovarian tissue (usually dysgenetic) in the presence of a 46,XY karyotype is very rare. There is a single case report of this presentation as an autosomal recessive entity caused by compound heterozygous variants in the gene CBX2 (Biason-Lauber et al. 2009).

21.5.2.3 Complete (CAIS) or Partial Androgen Insensitivity Syndrome (PAIS)

Androgen insensitivity syndrome is inherited as an X-linked recessive trait and is caused by variants in the androgen receptor (AR) at Xq11–12. The androgen receptor is the only gene known to be associated with AIS, and sequencing of the AR gene detects variants in >95% of individuals with CAIS. PAIS appears to be genetically heterogeneous and variants in the AR gene are found in <50% of individuals (Gottlieb and Trifiro 2017). Nearly 1000 different AR variants have been shown to cause AIS (http://androgendb.mcgill.ca/). The phenotype of CAIS is relatively consistent within families; however, the phenotypes of PAIS show intrafamilial variability (Deeb et al. 2005). *De novo* variants are relatively common, being observed in 27% of families with only one affected individual (Hiort et al. 1998); therefore, genetic testing is necessary to provide accurate genetic counselling. Gonadal mosaicism has also been reported for AIS (Boehmer et al. 1997); therefore, there is a small risk of recurrence even following an apparently *de novo* variant.

When an XX female is known to be heterozygous for an AR variant, for each offspring, there is a one in four chance of the offspring having a 46,XY karyotype and being affected by AIS, and a one in four chance of the offspring having a 46,XX karyotype and being a carrier of AIS. Prenatal diagnosis and preimplantation genetic testing (PGT) are available for AIS. Fertility may be preserved in some males with partial AIS, in which case all XX offspring will be carriers of AIS and all XY offspring will be unaffected males.

Note that AIS is allelic to Kennedy disease (Spinobulbar muscular atrophy), which is caused by expansion of a polyglutamine tract within the AR gene. People with Kennedy disease have mild AIS.

21.5.2.4 Hormone Biosynthetic Defects

Defects in androgen biosynthesis can lead to typical testis development but incomplete androgenization of male genitalia. Some conditions are accompanied by deficiencies of adrenal hormones. Examples are 17β-hydroxysteroid dehydrogenase deficiency, 3-beta-hydroxysteroid dehydrogenase deficiency, cholesterol desmolase deficiency and 17α-hydroxylase deficiency. Inheritance is autosomal recessive; therefore, there is a one in four risk of recurrence in each pregnancy; however, the genital phenotype is only expressed in individuals with a 46,XY karyotype.

Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive multiple congenital anomaly syndrome caused by deficiency of the enzyme 7-dehydrocholesterol reductase. In XY individuals, there is incomplete androgenization of the male genitalia.

21.5.3 Rare Syndromes

21.5.3.1 Campomelic Dysplasia and SOX9

Campomelic dysplasia is a rare skeletal dysplasia characterized by long bone bowing (campomelia), cleft palate, club feet and distinctive facies. In XX individuals, typical female genital development occurs; however, most XY campomelic dysplasia individuals have either female or ambiguous/atypical external genitalia and variable internal genitalia. SOX9 is the only gene associated with campomelic dysplasia, and inheritance is autosomal dominant, although most cases result from *de novo* variants. About 5% of affected individuals have a chromosome translocation, visible in standard karyotype, disrupting the SOX9 locus at 17q24.3–25.1. Translocations are usually *de novo*, but parental karyotypes should, nonetheless, be checked, as translocations with breakpoints a long distance from SOX9 may have incomplete penetrance. The remaining 95% of individuals have a sequence change, or less commonly a large deletion, affecting SOX9. Germ line (Cameron et al. 1996) and somatic (Smyk et al. 2007) mosaicism for SOX9 variants has been reported. Notably, some individuals with SOX9 variants and ambiguous/atypical genitalia have typical skeletal development.

21.5.3.2 WT-1

Denys-Drash syndrome and Frasier syndromes are both caused by autosomal dominant variants in the gene WT-1 located at 11p13, with the majority of variants being *de novo*. Denys-Drash syndrome is characterized by early-onset nephropathy, increased risk of Wilms tumour and 46,XY DSD. In Frasier syndrome, nephropathy is later in onset, there is 46,XY complete gonadal dysgenesis, and tumour risk is primarily for gonadoblastoma. For both conditions, XX individuals usually have typical genital development.

21.5.4 Isolated Anomalies

21.5.4.1 Hypospadias

Hypospadias is a common variation where the urethra opens on the ventral side of the penis and is associated with a range of Mendelian syndromes, chromosome variations and DSD. For isolated hypospadias, the risk of recurrence in male siblings is around 1 in 20, and a similar risk applies for sons of an affected male. X-linked hypospadias has been reported in association with variants in the gene MAMLD1 (Fukami et al. 2006) and with variants in the androgen receptor (Allera et al. 1995).

21.5.4.2 Cryptorchidism

Cryptorchidism is a common presentation in males and is thought to result from a combination of genetic and environmental factors. The recurrence risk in male siblings of isolated cases is around 1 in 20 (Czeizel et al. 1981). Cryptorchidism is also associated with a range of Mendelian syndromes and chromosome variations.

21.5.5 46,XX DSD

21.5.5.1 XX Testicular DSD

Most males with XX testicular DSD arise as a result of the presence of SRY in an otherwise typical XX karyotype. The phenotype is similar to that of Klinefelter syndrome, with male external genitalia, small testes, azoospermia and, if untreated, signs of testosterone deficiency. In contrast to Klinefelter syndrome, males with XX testicular DSD do not have increased stature or learning difficulties (Ferguson-Smith et al. 1990). In most males with SRY+ XX testicular DSD, Yp material (including SRY) is present on the X chromosome as a result of atypical exchange during meiosis I during gametogenesis in the father. This is nearly always a sporadic event (Weil et al. 1994; Wang et al. 1995). Far less commonly, SRY has been translocated onto a terminal arm of an autosome (Dauwerse et al. 2006; Queralt et al. 2008), and in this circumstance, sex-limited autosomal dominant inheritance is observed. There is also on record a typically fertile XY individual in whom SRY was translocated onto the X chromosome (Abbas et al. 1993); in this circumstance, all XX offspring would have XX testicular DSD. Because of these rare familial occurrences, in order to prove sporadic occurrence, it is necessary to perform FISH to search for SRY in the father and confirm

that SRY is located only on the Y chromosome. Men with SRY+ XX testicular DSD are infertile.

A minority of males with XX testicular DSD are SRY negative and presumably arise as a result from inappropriate activity of the gene cascade that is typically switched on only in response to SRY. Not surprisingly, atypical genitalia occur more commonly in these individuals. The cause of testicular development in these individuals is not well understood, but atypical dosage of genes in the sex-determining pathway is likely to play a part. One family has been reported with autosomal dominant sex-limited transmission of SRY-testicular DSD, resulting from a 178 kb duplication that is 600 kb upstream of SOX9 (Cox et al. 2011). In the absence of family history, risk of recurrence is likely to be low. XX testicular DSD has been diagnosed prenatally, following the detection of discordance between chromosomal and ultrasonographic sex (Trujillo-Tiebas et al. 2006).

21.5.5.2 46,XX Ovotesticular DSD

Individuals with 46,XX ovotesticular DSD have both testicular and ovarian tissue (containing spermatogonia or oocytes respectively either within the one gonad or in separate gonads). The karyotype on peripheral blood is 46,XX and SRY is absent. The likely explanation is localized activation of testicular development, for example, by cryptic mosaicism within the gonad for cells containing the SRY gene (Ortenberg et al. 2002; Queipo et al. 2002) or other mosaic variants that lead to testicular development. Most cases are sporadic, but the existence of rare familial cases (Ramos et al. 1996; Slaney et al. 1998) indicates the existence of X-linked or autosomal predisposition genes. Genomic rearrangements that increase the expression of SOX9 or SOX3 have been implicated in some cases (Grinspon and Rey 2016).

21.5.5.3 Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is a family of disorders characterized by impaired synthesis of cortisol from cholesterol in the adrenal cortex. At least eight forms exist, and all follow autosomal recessive inheritance. The most important type is 21-hydroxylase deficiency, which causes virilization in XX individuals and variable salt wasting in XX and XY individuals. Molecular testing of the gene *CYP21A2* detects variants in most cases and can be used for prenatal diagnosis. For families with familial CAH, genetic counselling is recommended to discuss future pregnancies and referral to an endocrinologist during a pregnancy to discuss the best management for the baby postnatally.

21.5.5.4 Rare Syndromes

Several rare syndromes exist in which male genital development occurs in the setting of an XX karyotype and other variations.

Variants in the R-Spondin1 gene cause a syndrome of XX testicular DSD, palmoplantar keratosis and squamous cell carcinoma of the skin (Parma et al. 2006). Inheritance is autosomal recessive, and in XY individuals, only PPK and SSC are present.

Microphthalmia with linear skin defects syndrome (MLS) is an X-linked male-lethal disorder associated with X-chromosome rearrangements that result in monosomy from Xpter to Xp22, including the gene HCCS. In some individuals, the chromosome rearrangement is an X;Y translocation, resulting in sex reversal due to translocation of SRY onto the X chromosome (Mucke et al. 1995).

Multiple congenital anomalies in association with 46,XX sex reversal have also been reported in an individual with a chromosome 22 duplication, 46,XX,dup(22)(q11.2q13). The person had male external genitalia and intrascrotal gonads (Seeherunvong et al. 2004).

21.5.5.5 Isolated Anomalies

Müllerian anomalies including uterine and vaginal agenesis, and duplication of the uterus and vagina, are relatively common, and undoubtedly underdiagnosed. Usually, individuals have typical ovarian function, and presentation is commonly at puberty with atypical menstruation or amenorrhea, or later with infertility or pregnancy complications. Although genetic factors are likely

to contribute, most isolated Müllerian anomalies are sporadic, and risk of recurrence is low. There is a known association with renal and skeletal changes, which in its most severe form is known as Müllerian duct aplasia-unilateral renal aplasia-cervicothoracic somite dysplasia (MURCS) association (Duncan et al. 1979). MURCS association is usually sporadic, but familial forms exist, and autosomal dominant inheritance with incomplete penetrance has been suggested (Guerrier et al. 2006). Variations of female internal and external genitalia are also associated with a large number of rare genetic syndromes and a variety of chromosome anomalies.

References

Abbas N, McElreavey K, Leconiat M, et al. Familial case of 46,XX male and 46,XX true hermaphrodite associated with a paternal-derived SRY-bearing X chromosome. C R Acad Sci III. 1993;316(4):375–83.

Abir R, Fisch B, Nahum R, et al. Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update. 2001;7(6):603–10.

Allera A, Herbst MA, Griffin JE, et al. Mutations of the androgen receptor coding sequence are infrequent in patients with isolated hypospadias. J Clin Endocrinol Metab. 1995;80(9):2697–9.

Amor D, Delatycki MB, Susman M, et al. 46,XX/46,XY at amniocentesis in a fetus with true hermaphroditism. J Med Genet. 1999;36(11):866–9.

Barbaro M, Oscarson M, Schoumans J, et al. Isolated 46,XY gonadal dysgenesis in two sisters caused by a Xp21.2 interstitial duplication containing the DAX1 gene. J Clin Endocrinol Metab. 2007;92(8):3305–13.

Baxter RM, Arboleda VA, Lee H, et al. Exome sequencing for the diagnosis of 46,XY disorders of sex development. J Clin Endocrinol Metab. 2015;100(2):E333–44.

Bernard V, Donadille B, Zenaty D, et al. Spontaneous fertility and pregnancy outcomes amongst 480 women with Turner syndrome. Hum Reprod. 2016;31(4):782–8.

Biason-Lauber A, Konrad D, Meyer M, et al. Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene. Am J Hum Genet. 2009;84(5):658–63.

Blair J, Tolmie J, Hollman AS, et al. Phenotype, ovarian function, and growth in patients with 45,X/47,XXX Turner mosaicism: implications for prenatal counseling and estrogen therapy at puberty. J Pediatr. 2001;139(5):724–8.

Bodri D, Vernaeve V, Figueras F, et al. Oocyte donation in patients with Turner's syndrome: a successful technique but with an accompanying high risk of hypertensive disorders during pregnancy. Hum Reprod. 2006;21(3):829–32.

Boehmer AL, Brinkmann AO, Niermeijer MF, et al. Germ-line and somatic mosaicism in the androgen insensitivity syndrome: implications for genetic counseling. Am J Hum Genet. 1997;60(4):1003–6.

Bourne H, Stern K, Clarke G, et al. Delivery of normal twins following the intracytoplasmic injection of spermatozoa from a patient with 47,XXY Klinefelter's syndrome. Hum Reprod. 1997;12(11):2447–50.

Cameron FJ, Hageman RM, Cooke-Yarborough C, et al. A novel germ line mutation in SOX9 causes familial campomelic dysplasia and sex reversal. Hum Mol Genet. 1996;5(10):1625–30.

Canto P, Soderlund D, Reyes E, et al. Mutations in the desert hedgehog (DHH) gene in patients with 46,XY complete pure gonadal dysgenesis. J Clin Endocrinol Metab. 2004;89(9):4480–3.

Cox JJ, Willatt L, Homfray T, et al. A SOX9 duplication and familial 46,XX developmental testicular disorder. N Engl J Med. 2011;364(1):91–3.

Czeizel A, Erodi E, Toth J. Genetics of undescended testis. J Urol. 1981;126(4):528–9.

Dauwerse JG, Hansson KB, Brouwers AA, et al. An XX male with the sex-determining region Y gene inserted in the long arm of chromosome 16. Fertil Steril. 2006;86(2):463.e461–5.

Deeb A, Mason C, Lee YS, et al. Correlation between genotype, phenotype and sex of rearing in 111 patients with partial androgen insensitivity syndrome. Clin Endocrinol. 2005;63(1):56–62.

Doger E, Cakiroglu Y, Ceylan Y, et al. Reproductive and obstetric outcomes in mosaic Turner's syndrome: a cross-sectional study and review of the literature. Reprod Biol Endocrinol. 2015;13:59.

Duncan PA, Shapiro LR, Stangel JJ, et al. The MURCS association: Müllerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia. J Pediatr. 1979;95(3):399–402.

Ferguson-Smith MA, Cooke A, Affara NA, et al. Genotype-phenotype correlations in XX males and their bearing on current theories of sex determination. Hum Genet. 1990;84(2):198–202.

Ferlin A, Garolla A, Foresta C. Chromosome abnormalities in sperm of individuals with constitutional sex chromosomal abnormalities. Cytogenet Genome Res. 2005;111(3–4):310–6.

Fukami M, Wada Y, Miyabayashi K, et al. CXorf6 is a causative gene for hypospadias. Nat Genet. 2006;38(12):1369–71.

Fullerton G, Hamilton M, Maheshwari A. Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009? Hum Reprod. 2010;25:588–97.

Gardner RJM, Amor DJ. Gardner and Sutherland's chromosome abnormalities and genetic counseling. New York: Oxford University Press; 2018.

Gies I, Oates R, De Schepper J, et al. Testicular biopsy and cryopreservation for fertility preservation of pre-

pubertal boys with Klinefelter syndrome: a pro/con debate. Fertil Steril. 2016;105(2):249–55.
Gottlieb B, Trifiro MA. Androgen insensitivity syndrome. GeneReviews®. 2017. doi: NBK1429 [book accession].
Grinspon RP, Rey RA. Disorders of sex development with testicular differentiation in SRY-negative 46,XX individuals: clinical and genetic aspects. Sex Dev. 2016;10(1):1–11.
Guerrier D, Mouchel T, Pasquier L, et al. The Mayer-Rokitansky-Kuster-Hauser syndrome (congenital absence of uterus and vagina)—phenotypic manifestations and genetic approaches. J Negat Results Biomed. 2006;5:1.
Hall H, Hunt P, Hassold T. Meiosis and sex chromosome aneuploidy: how meiotic errors cause aneuploidy; how aneuploidy causes meiotic errors. Curr Opin Genet Dev. 2006;16(3):323–9.
Hanson L, Bryman I, Barrenas ML, et al. Genetic analysis of mosaicism in 53 women with Turner syndrome. Hereditas. 2001;134(2):153–9.
Harper P. Practical genetic counselling. Oxford: Oxford University Press; 2010.
Hiort O, Sinnecker GH, Holterhus PM, et al. Inherited and de novo androgen receptor gene mutations: investigation of single-case families. J Pediatr. 1998;132(6):939–43.
Juul A, Aksglaede L, Lund AM, et al. Preserved fertility in a non-mosaic Klinefelter patient with a mutation in the fibroblast growth factor receptor 3 gene: case report. Hum Reprod. 2007;22(7):1907–11.
Kajii T, Ferrier A, Niikawa N, et al. Anatomic and chromosomal anomalies in 639 spontaneous abortuses. Hum Genet. 1980;55(1):87–98.
Lachlan KL, Youings S, Costa T, et al. A clinical and molecular study of 26 females with Xp deletions with special emphasis on inherited deletions. Hum Genet. 2006;118(5):640–51.
Laron Z, Dickerman Z, Zamir R, et al. Paternity in Klinefelter's syndrome—a case report. Arch Androl. 1982;8(2):149–51.
Ledig S, Hiort O, Scherer G, et al. Array-CGH analysis in patients with syndromic and non-syndromic XY gonadal dysgenesis: evaluation of array CGH as diagnostic tool and search for new candidate loci. Hum Reprod. 2010;25(10):2637–46.
Levron J, Aviram-Goldring A, Madgar I, et al. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's syndrome. Fertil Steril. 2000;74(5):925–9.
Michala L, Goswami D, Creighton SM, et al. Swyer syndrome: presentation and outcomes. BJOG. 2008;115(6):737–41.
Modi DN, Sane S, Bhartiya D. Accelerated germ cell apoptosis in sex chromosome aneuploid fetal human gonads. Mol Hum Reprod. 2003;9(4):219–25.
Mortensen KH, Rohde MD, Uldbjerg N, et al. Repeated spontaneous pregnancies in 45,X Turner syndrome. Obstet Gynecol. 2010;115(2 Pt 2):446–9.
Mucke J, Happle R, Theile H. MIDAS syndrome respectively MLS syndrome: a separate entity rather than a particular lyonization pattern of the gene causing Goltz syndrome. Am J Med Genet. 1995;57(1):117–8.
Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet. 1991;87(1):81–3.
Oktay K, Bedoschi G, Berkowitz K, et al. Fertility preservation in women with Turner syndrome: a comprehensive review and practical guidelines. J Pediatr Adolesc Gynecol. 2016;29(5):409–16.
Ortenberg J, Oddoux C, Craver R, et al. SRY gene expression in the ovotestes of XX true hermaphrodites. J Urol. 2002;167(4):1828–31.
Palermo GD, Colombero LT, Hariprashad JJ, et al. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod. 2002;17(3):570–5.
Parma P, Radi O, Vidal V, et al. R-spondin1 is essential in sex determination, skin differentiation and malignancy. Nat Genet. 2006;38(11):1304–9.
Pasquino AM, Passeri F, Pucarelli I, et al. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. J Clin Endocrinol Metab. 1997;82(6):1810–3.
Pearlman A, Loke J, Le Caignec C, et al. Mutations in MAP3K1 cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination. Am J Hum Genet. 2010;87(6):898–904.
Philibert P, Leprieur E, Zenaty D, et al. Steroidogenic factor-1 (SF-1) gene mutation as a frequent cause of primary amenorrhea in 46,XY female adolescents with low testosterone concentration. Reprod Biol Endocrinol. 2010;8:28.
Queipo G, Zenteno JC, Pena R, et al. Molecular analysis in true hermaphroditism: demonstration of low-level hidden mosaicism for Y-derived sequences in 46,XX cases. Hum Genet. 2002;111(3):278–83.
Queralt R, Madrigal I, Vallecillos MA, et al. Atypical XX male with the SRY gene located at the long arm of chromosome 1 and a 1qter microdeletion. Am J Med Genet A. 2008;146A(10):1335–40.
Ramos ES, Moreira-Filho CA, Vicente YA, et al. SRY-negative true hermaphrodites and an XX male in two generations of the same family. Hum Genet. 1996;97(5):596–8.
Rives N, Joly G, Machy A, et al. Assessment of sex chromosome aneuploidy in sperm nuclei from 47,XXY and 46,XY/47,XXY males: comparison with fertile and infertile males with normal karyotype. Mol Hum Reprod. 2000;6(2):107–12.
Ron-El R, Strassburger D, Gelman-Kohan S, et al. A 47,XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome: case report. Hum Reprod. 2000;15(8):1804–6.
Seeherunvong T, Perera EM, Bao Y, et al. 46,XX sex reversal with partial duplication of chromosome arm 22q. Am J Med Genet A. 2004;127A(2):149–51.
Seo JT, Park YS, Lee JS. Successful testicular sperm extraction in Korean Klinefelter syndrome. Urology. 2004;64(6):1208–11.

Slaney SF, Chalmers IJ, Affara NA, et al. An autosomal or X linked mutation results in true hermaphrodites and 46,XX males in the same family. J Med Genet. 1998;35(1):17–22.

Smyk M, Obersztyn E, Nowakowska B, et al. Recurrent SOX9 deletion campomelic dysplasia due to somatic mosaicism in the father. Am J Med Genet A. 2007;143A(8):866–70.

Staessen C, Tournaye H, Van Assche E, et al. PGD in 47,XXY Klinefelter's syndrome patients. Hum Reprod Update. 2003;9(4):319–30.

Stochholm K, Juul S, Juel K, et al. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. J Clin Endocrinol Metab. 2006;91(10):3897–902.

Sybert VP. Phenotypic effects of mosaicism for a 47,XXX cell line in Turner syndrome. J Med Genet. 2002;39(3):217–20.

Tarani L, Lampariello S, Raguso G, et al. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. Gynecol Endocrinol. 1998;12(2):83–7.

Telvi L, Lebbar A, Del Pino O, et al. 45,X/46,XY mosaicism: report of 27 cases. Pediatrics. 1999;104(2 Pt 1): 304–8.

Terzoli G, Lalatta F, Lobbiani A, et al. Fertility in a 47,XXY patient: assessment of biological paternity by deoxyribonucleic acid fingerprinting. Fertil Steril. 1992;58(4):821–2.

Trujillo-Tiebas MJ, Gonzalez-Gonzalez C, Lorda-Sanchez I, et al. Prenatal diagnosis of 46,XX male fetus. J Assist Reprod Genet. 2006;23(5):253–4.

Uehara S, Yaegashi N, Maeda T, et al. Risk of recurrence of fetal chromosomal aberrations: analysis of trisomy 21, trisomy 18, trisomy 13, and 45,X in 1,076 Japanese mothers. J Obstet Gynaecol Res. 1999;25(6):373–9.

Wang I, Weil D, Levilliers J, et al. Prevalence and molecular analysis of two hot spots for ectopic recombination leading to XX maleness. Genomics. 1995;28(1): 52–8.

Weil D, Wang I, Dietrich A, et al. Highly homologous loci on the X and Y chromosomes are hot-spots for ectopic recombinations leading to XX maleness. Nat Genet. 1994;7(3):414–9.

Woods CG, Noble J, Falconer AR. A study of brothers with Klinefelter syndrome. J Med Genet. 1997;34(8): 702.