GENETIC COUNSELING AND THE INTRICACIES OF SEXUAL DIFFERENTIATION

By

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A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors degree
With Honors in

Biology

THE UNIVERSITY OF ARIZONA

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Abstract

The purpose of this thesis was to discuss the biological and clinical components of sexual differentiation. Although it is genetic, mutations leading to disorders of sex development (DSD) can be of great concern to patients and their families. The proposed pathway of sexual determination in combination with the known genes and disorders of sex development help provide necessary information to genetic counselors and medical professionals on how to approach a patient that has been diagnosed with a DSD, while keeping in mind potential psychosexual outcomes. This thesis provides a new look at the proposed pathway as well as information regarding the genes involved in sex differentiation and disorders of sex development. It also provides insight into genetic testing, clinical management, and psychosexual outcomes for patients diagnosed with a DSD.

Introduction

In sex development, there are two different processes: sex determination and sex differentiation. Sex determination is the development that directs the undifferentiated embryo into a sexually dimorphic individual (2). After the sex determination decision has been made, sex differentiation leads to the production of factors by the gonads resulting in the development of the phenotypic sex (2). Mutations occurring during sex differentiation can lead to a disorder of sex development (DSD). DSDs are common but can cause concern within a family. Genetic counselors, in combination with many other medical professionals, can play and important role in handling these complex genetic issues by effectively communicating the disorder of sex development and mutation that may be causing a specific phenotypic sex. The basis of sex differentiation depends on a few different cell types: granulosa cells, Sertoli cells, and Leydig cells. Granulosa cells are somatic cells in the sex cord that are responsible for the development of the female gamete in the ovary. Sertoli cells are somatic cells located in the testes that are essential for the formation of testes and the production and development of mature spermatozoa. Leydig cells are located in the connective tissue of sperm-producing tubules within the testes and are responsible for the production of testosterone. Androgens also have an important role in sex determination of males. After development of the testes, the Leydig cells produce testosterone, thereby promoting Wolffian duct differentiation. The critical period for genitalia virilization depends on the presence of these androgens and the presence of a functioning androgen receptor. Although many of the key genes for sex differentiation and disorders of sex development are known, the pathway and interactions of these genes are still

being studied. Because the pathway is complex and likely incomplete, there are two theories involving sex differentiation. The classical textbook theory states that the presence of a sex-determining region on the Y chromosome will inhibit the 'default' ovarian pathway, leading to the formation of testes. In an XX individual, the sex-determining region is not present, and no inhibition of the 'default' pathway will take place, leading to the formation of ovaries (3). The Z-factor theory was proposed to explain the development of testes without the presence of a sex-determining region. This theory states that the XX gonad expresses a factor with both anti-testes and pro-ovary functions and the sex-determining region acts as an inhibitor of the proposed Z-factor to life the block on the pathway (3). The complicated nature of sexual differentiation requires thoughtful examination and discussion with the patients and families who have been diagnosed with a DSD.

Pathway

The pathway of determination for sexual differentiation is very complex and not well understood. There have been several theories brought forth on how it works, but no evidence has been found to support any of these hypothetical pathways. Figure 1 shows yet another hypothetical pathway of the determination and differentiation of the Müllerian-duct-derived female reproductive pathway and the Wolffian-duct-derived male reproductive pathway. This pathway is derived from the pathways of Biason-Lauber (2) and Ohnesorg, Vilian and Sinclair (7).

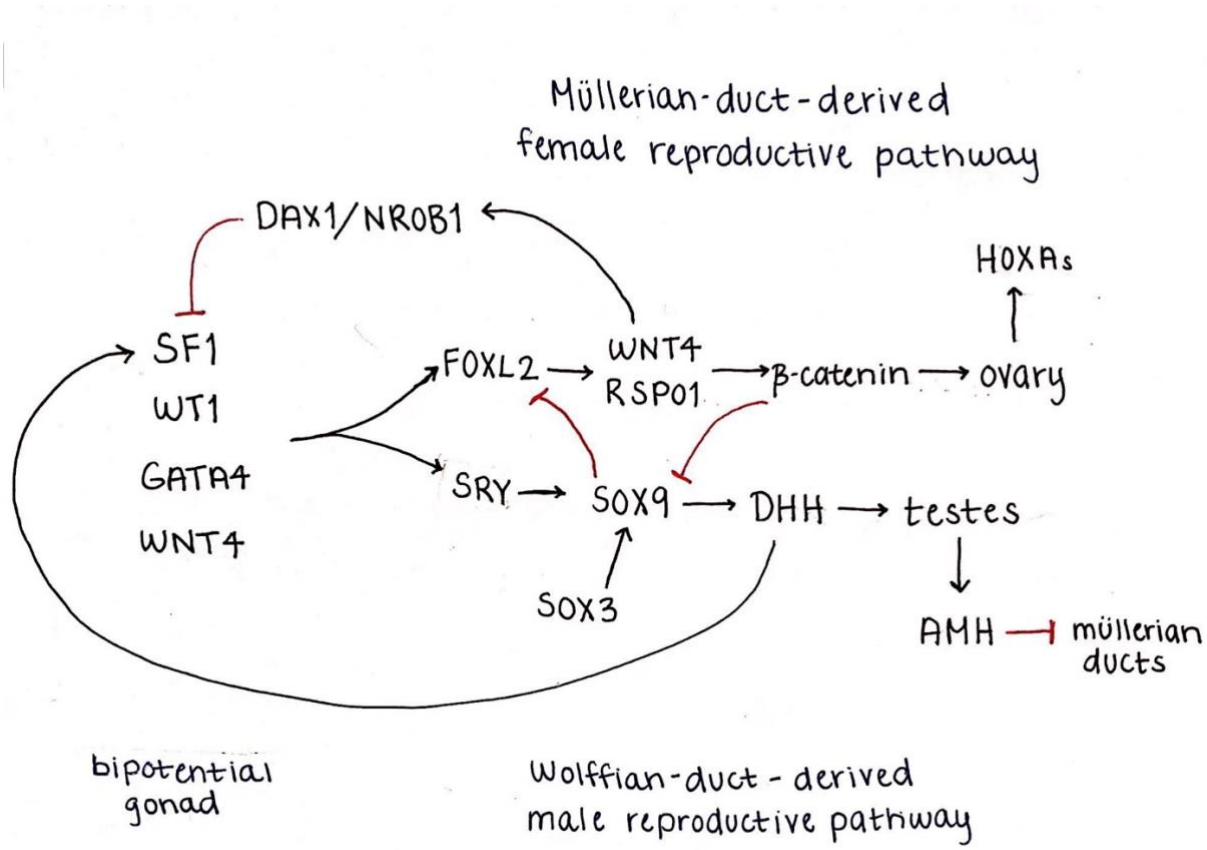

Figure 1: Theoretical Pathway of Sex Determination. The GATA4 transcription factor in combination with the WT1 and SF1 transcription factors are expressed in the bipotential or undifferentiated gonad. The pathway can then go one of two ways, through the Müllerian-duct-derived female reproductive pathway or through the Wolffian-duct-derived male reproductive pathway. In the female reproductive pathway, the FOXL2 transcription factor will promote transcription of the RSPO1 and WNT4 signaling molecules. The WNT4 signaling molecule will upregulate DAX1/NR0B1 which will then inhibit expression of SF1 and therefore stop the progression of the Wolffian-duct pathway. RSPO1 will stabilize the production of beta-catenin. Beta-catenin will inhibit SOX9 expression and will allow for continuation of the Müllerian-duct pathway and development of the ovaries. The HOXA genes are present in the developing ovaries and are important throughout the development of the ovarian system. In the male reproductive pathway, WT1 and SF1 transcription factors work closely together and positively regulate SRY expression. Either the SRY transcription factor or SOX3 transcription factor will lead to the production of SOX9. SOX 9 then inhibits FOXL2 expression and will allow for continuation of the Wolffian-duct pathway as well as promote transcription of the DHH signaling molecule. DHH upregulates SF1 expression and leads to the development of the testes. The presence of AMH will inhibit the Müllerian-duct pathway and promote the Wolffian-duct pathway. The presence of MAPK (specifically MAP3K1) upregulates FOXL2 and inhibits SOX9 expression, therefore forcing the Müllerian-duct pathway. Pathway was derived from pathways shown by Biason-Lauber (2) and Ohnesorg, Vilian and Sinclair (7).

Genes

Many genes have been described that affect the development and differentiation of the gonads. Mutations in these genes can lead to a defect in the pathway and therefore a disorder of sexual development. The function, interactions, and potential mutations in the genes shown in Figure 1 have been described below and in Table 1.

GATA4

The GATA4 transcription factor consists of two zinc fingers which are necessary for DNA recognition, DNA binding, and stabilization of protein-DNA interaction as well as being critical for protein-protein interactions of GATA family transcription factors with other non-familial transcription factors (2,7). GATA4 is the only transcription factor within the family that is expressed within somatic cells in the bipotential gonad without being found in the germ cells (2). According to Biason-Lauber (2), GATA4 is expressed in the somatic cells of the bipotential gonad of both sexes; however, it becomes sexually dimorphic. Expression is upregulated in the XY Sertoli cells and is downregulated in the Leydig cells (2,3), but is downregulated in all cell types in XX gonads (2). Abundant GATA4 expression is maintained in Sertoli cells throughout embryonic development but is significantly downregulated after histological differentiation of the ovary (3). This suggests that GATA4 may be involved in early gonadal development and potentially sexual dimorphism (3). The GATA4 transcription factor is known to regulate the expression of many sex-determining genes, such as SRY, SOX9 and AMH (2). A defect within the GATA4 transcription factor is often associated with congenital heart defects and/or genital ambiguity within 46,XY individuals (2,3).

WT1

Wilms' tumor suppressor 1, or WT1, encodes a zinc-finger DNA-binding protein (2, 3, 7), has a role in development and tumor suppression (3), and can act as either a transcriptional repressor or activator (7). There are two different isoforms of WT1, +KTS and -KTS, which have been shown to play critical roles during embryogenesis (2). The -KTS isoform binds to the SRY promoter region, leading to transactivation of SRY; however, the +KTS isoform plays a role in the regulation of the SRY transcript (2). The expression of WT1 beings simultaneously with SF1 and are thought to be dependent (3). To ensure proper formation of the bipotential gonad, it is thought that WT1 activates an SF1-independent pathway and also ensures gonad formation by controlling SRY expression (3). WT1 may also target WNT4, DAX1, and AMH, which are thought to be activated by WT1 (-KTS) variants (3). A mutation in WT1 can cause Denys-Drash syndrome and Frasier syndrome. Frasier patients carry a mutation resulting in the loss of the WT1 +KTS isoform (2). By eliminating the isoform, SRY levels reduce and may result in the increase of AMH expression, which inhibits the development of Müllerian structures (2).

SF1

Steroidogenic factor 1, also known as SF1 or NR5A1, is a key transcription factor involved in the regulation of several steroidogenic enzymes (7). SF1 is downregulated during ovarian development but is expressed continuously through testicular development, strictly in Sertoli and Leydig cells (7). Therefore, we can assume that SF1

plays a role in the formation of undifferentiated gonads and sex determination (7). A mutation within SF1 is shown to cause 46,XY gonadal dysgenesis as well as premature ovarian failure and adrenal failure (7).

SRY

The SRY transcription factor is found on the Y chromosome—making it vulnerable to degradation; therefore, it is minimally conserved and has some functional flaws (2, 3). The high mobility group (HMG) domain is the only region conserved between mammalian species and serves as the site of almost all clinical mutations causing XY gonadal dysgenesis (2, 7). SRY engages a male-specific cascade of molecular events, however, the continued expression of SRY is not necessary for the rest of the pathway (2). SRY acts on a single gene, SOX 9, and the expression of SOX9 is then reinforced by positive regulatory feedback loops (2). SRY is positively regulated by many transcription factors such as WT1, SF1/NR5A1, and GATA4 (7). A mutation in SRY can result in SOX9 being silenced and therefore the development of the ovarian structures occurs, with beta-catenin (a protein that, in combination with WNT4 and RSPO1, promotes the ovarian pathway) playing a crucial role in driving the process, leading to 46,XY DSD due to pure gonadal dysgenesis (2).

SOX3

SOX3 is a member of the SOX family of transcription factors (2). It is an X-linked gene that is thought to be the ancestral gene of the Y-linked SRY transcription factor because they are functionally interchangeable (2, 7). SOX3 induces differentiation of the testes

by upregulation of SOX9 expression through a mechanism similar to that of SRY (2). SOX3 is not expressed during embryonic development in either sex; therefore, loss of function mutations that occur within SOX3 do not affect sex determination (2, 7).

SOX9

SOX9 expression is upregulated shortly after the expression of SRY begins (2). Together, SRY and SOX9 create a positive feedback loop responsible for maintaining expression and promoting the development of Sertoli cells (5). The expression of SOX9, though, is not entirely dependent on SRY (2, 3). According to Biason-Lauber (2, 3), normal SOX9 transcriptional regulation in the gonads consists of an SRY-independent initiation, an SRY-dependent upregulation, and SRY-independent maintenance. Within the male, SF1 activates SRY expression which together upregulate SOX9 expression; however, in the female, it is downregulated (2, 3). The downregulation in the female is likely to be passive, which implies the presence of currently unknown repressors (2, 3). After the expression of SRY has stopped, SOX9 is maintained at high levels through direct autoregulation (2, 3). Mutations in SOX9 can result in campomelic dysplasia, gonadal dysgenesis, testicular or ovotesticular DSD and male-to-female sex reversal (2, 3).

DHH

Desert hedgehog, DHH, is a member of the hedgehog family of signaling molecules that is necessary for the upregulation of SF1 in Leydig cells (2). DHH is the only one of its family that plays a role in testicular development and fertility (7) and is expressed in

both somatic cell population of the developing XY gonads and later in Sertoli cells (2). There is no expression of DHH found in the ovaries (2). A mutation in DHH can cause 46,XY complete or partial gonadal dysgeneses (2, 7).

AMH

Anti-Müllerian hormone (AMH) is part of the transforming growth factor-bets family and is one of the first known hormones produces by the developing testes (7). AMH is synthesized in fetal Sertoli cells and is responsible for the regression of the müllerian ducts, including the oviducts, uterus, and upper vagina (2, 7), therefore making it required for sex-specific differentiation of the reproductive tract (7). Mutations in AMH result in persistence of the fallopian tubes and uterus in males (2), but a lack of AMH does not affect testicular differentiation (7).

FOXL2

FOXL2 is a single-exon gene encoding a transcription factor and nuclear protein (2). It is one of the earliest known markers for ovarian differentiation (2) and plays a role in the early stage of ovarian somatic development (3). FOXL2 plays an important role in granulosa cell homeostasis because it upregulates the expression of aromatase, the enzyme responsible for androgen transformation into estrogen within granulosa cells (2). FOXL2 is expressed in both the postnatal and adult follicular cells (2) and the continued expression within the ovary is critical to maintaining the ovarian phenotype (7). A mutation in FOXL2 can lead to premature ovarian failure (POF) and a failure of FOXL2 continued expression causes ovarian aging and tumorigenesis (2).

WNT4

WNT4, along with RSPO1, is expressed both before sex determination and within the gonads of both sexes (2). Together, these genes are necessary for XX and XY gonadal development (2). WNT4 is upregulated in the ovary and WNT signaling prevents abnormal testosterone production in XX embryonic gonads (2); however, it alone cannot override the male development pathway (7). The function of WNT4 is critical for the organization of the ovarian structure because female germ cells play central part in maintenance of the ovary, which can be displayed by the fact that when oocytes are not present, ovarian follicles never for or they eventually degenerate (3). When the germ cell shave reached the final destination within the gonad, the WNT4 signaling molecule appears to maintain oocyte viability (3). WNT4 also upregulates DAX1, which then antagonizes SF1, and inhibits steroidogenic enzymes (3). A duplication event within WNT4 results in a patient with ambiguous genitalia, dysgenic gonads, and what appears to be remnants of both the Müllerian and Wolffian ducts (2). SERKAL syndrome results because of an inactivation of both copies of WNT4 (2). Other signs of WNT4 mutations are absence of the uterus but no other Müllerian abnormalities, and excess of androgen (2). Too much activity of WNT4 can cause 46,XY DSD, otherwise known as feminization of the male, and too little activity of WNT4 can cause 46,XX DSD, otherwise known as masculinization of the female (2).

RSPO1

R-spondin 1 (RSPO1) is a signaling molecule that stabilizes beta-catenin and is highly expressed at the critical time of gonad development (2). It is believed to activate then

synergize with WNT4 in embryonic ovaries to stabilize beta-catenin and allow for the continuation of the female reproductive pathway (7). RSPO1 is not required for determination or function of the testes (2). A mutation in RSPO1 has a range of results including developmental delay and sex reversal (2).

HOXA

The HOX gene family are regulatory molecules that encode conserved transcription factors which control aspects of cell differentiation (3). These genes are both present and essential for development of the müllerian tract in the embryonic stage and function of the adult uterus (3).

DAX1/NR0B1

Rather than having a characteristic nuclear receptor zinc finger DNA-binding domain, DAX1 encodes an orphan nuclear hormone receptor with a ligand-binding domain (2, 7). It has a transcriptional silencing domain which is through to interact directly with corepressors to mediate repression (2). Most notably, DAX1 acts as a repression of SF1 transactivation in both steroidal and gonadal tissues (2, 3). A mutation inactivating DAX1/NR0B1 can lead to adrenal insufficiency with glucocorticoid and mineralocorticoid deficiency or 46,XY DSD (2).

Table 1: Genes Involved in Sex Development

Gene	Chromosome Location	Protein Function	Molecular Defect	Human syndrome/phenotype
DAX1/NR0B1	Xp21.3	Nuclear receptor	Duplication	XY gonadal dysgenesis with disorganized testes cords and hypogonadotropic hypogonadism
DHH	12q13.1	Signaling molecule	LOF	XY partial or complete gonadal dysgenesis
FOXL2	3q23	Transcription factor	Normal	Premature ovarian failure (POF)
GATA4	8p23.1-p22	Transcription factor	LOF	XY ambiguous genitalia or reduced phallic length
			Deletion downstream	XY complete gonadal dysgenesis
RSPO1	1p34.2	Signaling molecule	LOF	XX testicular and ovotesticular DSD
			GOF/ Duplication	XY gonadal dysgenesis
SF1/ NR5A1	9q33	Nuclear receptor/ transcription factor	LOF	XY gonadal dysgenesis, premature ovarian failure (POF), adrenal insufficiency
SOX3	Xp27.1	Transcription factor	LOF/ Deletion, Duplication	XX testicular DSD
SOX9	17q24.3-q25.1	Transcription factor	LOF	XY gonadal dysgenesis combined with campomelic dysplasia (CD)
			GOF	XX female-to-male sex reversal
			Duplication	XX testicular DSD
			Translocation upstream	XY ovotesticular DSD
			Duplication/ triplication upstream	XX testicular DSD

			Deletion upstream	XY ovarian DSD with acampomelic campomelic dysplasia (ACD), gonadal dysgenesis, female, or ambiguous external genitalia
SRY	Yp11.3	Transcription factor	LOF	XY ovarian DSD
			GOF/ translocation	XX testicular DSD
WNT4	1p35	Signaling molecule	LOF	XX Mullerian duct agenesis, testosterone synthesis, and coelomic vessel formation
			Duplication	XY gonadal dysgenesis (male-to-female sex reversal)
WT1	11p3	Transcription factor	LOF	Wilms tumor
				Denys-Drash syndrome
				Frasier syndrome

Table 1 Derived from tables shown by Biason-Lauber (2, 3). LOF—loss of function mutation. GOF—gain of function mutation.

Disorders of Sex Development

Mutations in these sex determining genes lead to a defect in the pathway, resulting in a particular disorder of sex development. These disorders have extremely variable phenotypes and overall incidences within the population. The DSD, gene mutation, characteristics, and overall incidence have been described below and in Table 2.

46,XX Testicular DSD

46,XX testicular DSD is characterized by the appearance of male external genitalia in an individual with a 46,XX karyotype (6). Most of these individuals have a copy of the normal SRY gene that has been translocated to an X chromosome as a result of abnormal recombination (6). They have a normal male appearance and are usually not diagnosed until puberty because of small testes, gynecomastia, and infertility, although they have normal-appearing male genitalia and pubic hair (6). Some 46,XX male individuals lack a SRY gene. About 15-20% of these individuals can be identified at birth because of ambiguous genitalia, including undescended tests, but there is no identifiable müllerian structures and they identify as male (6). The overall incidence of 46,XX testicular DSD is approximately 1 in 20,000 births (6).

46,XX Ovotesticular DSD

46,XX ovotesticular DSD is characterized by the presence of both ovarian and testicular tissue as either ovotestes or as separate ovary and testes (3, 6). Ambiguous genitalia with or without the presence of a uterus can be observed in individuals with 46,XX

ovotesticular DSD (6). Surgery is often necessary to repair the external genitalia and the individual can be raised as male or female (6). This form of DSD is very rare and only a small percentage of patients have this condition (3, 6).

Complete Gonadal Dysgenesis

Complete gonadal dysgenesis (CGD) can refer to XX males or XY females and is characterized as normal appearing external genitalia of the opposite chromosomal sex (6). 46,XY CDG individuals (XY females) often have dysfunctioning gonads composed mainly of fibrous tissue (streak gonads), no sperm production, and normal müllerian structures (6). They appear phenotypically female and have a risk of gonadoblastoma (6). These individuals have either a mutation in SRY or SOX9, therefore the levels of SOX9 expression are too low for testes differentiation and thus ovarian differentiation takes place (6). This form of DSD is prevalent in about 1.5 per 100,000 live born females (5).

Partial Gonadal Dysgenesis

Partial gonadal dysgenesis is a rare disorder characterized by ambiguous external genitalia with or without müllerian structures (6). These individuals can be raised as male or female (6).

Mixed Gonadal Dysgenesis

Mixed gonadal dysgenesis is one of the more common forms of DSD. It can be characterized in one of two ways: an individual with a 45,X/ 46,XY karyotype with

dysgenetic testes and streak gonad, or any individual with a varying degree of asymmetrical gonadal dysgenesis (3, 6). The phenotype can range from a typical male to a Turner syndrome female and a risk for gonadoblastoma (6).

Turner Syndrome

Turner syndrome is characterized by the 45,X karyotype. It is a chromosomal disorder in which either the entirety of the X chromosome is absent, or the missing chromosome is present in some cells but not in others (3). Individuals with Turner syndrome can be diagnosed in the neonatal period due to low birth weight, short neck, and lymphedema of the hands and feet (5). Other characteristic physical abnormalities include short stature, broad chest, low hairline, low set ears, webbed necks, epicanthal fold, down-slanting palpebral fissures, micrognathia, and delayed puberty (3, 5). Turner syndrome can be associated with congenital hear disease, hypothyroidism, diabetes, vision problems, hearing difficulties among many other autoimmune diseases (3). It is estimated that 1 in 2,500 live-born female births are diagnosed with Turner syndrome (5).

Klinefelter Syndrome

Klinefelter syndrome is characterized by the 46,XXY karyotype. Individuals with Klinefelter syndrome are male, not sex-reversed females, likely due to chromosomal X inactivation of DAX1 (7). Typical characteristics include small testes, infertility, gynecomastia, and delayed puberty (3, 5). A vast majority of individuals with Klinefelter syndrome are infertile due to the failure of germ cell development and patients are often

clinically identified for the first time because of infertility (6). An estimated 1 in 500 males has Klinefelter syndrome (5); however, because of the extremely mild and variable phenotype, many cases are thought to go undetected (6).

Denys-Drash Syndrome

Denys-Drash syndrome is caused by a mutation in the zinc-finger domain of the WT1 gene (5, 7) and is characterized by renal failure and genital abnormalities (7). Individuals with Denys-Drash syndrome have ambiguous genitalia or a female phenotype, an XY karyotype, dysgenetic gonads and often develop Wilms' tumor (2).

Frasier Syndrome

Frasier syndrome is a rare disorder caused by a mutation in the splice donor site of the WT1 gene resulting in a loss of the +KTS isoform (2, 5, 7) which leads to 46,XY DSD gonadal dysgenesis (2, 7). Individuals often have normal female external genitalia, streak gonads, an XY karyotype, and often develop gonadoblastoma (2).

SERKAL Syndrome

SERKAL syndrome is caused by a homozygous recessive missense mutation in WNT4 (5) and is characterized by 46,XX DSD, or female-to-male sex reversal (2, 5). Individuals often have ambiguous genitalia, gonadal morphology ranging from ovotestes to normal testes, renal agenesis, adrenal hypoplasia, as well as lung and heart anomalies (2).

Persistent Müllerian Duct Syndrome

Persistent Müllerian Duct syndrome is a rare autosomal recessive disorder caused by a mutation in AMH and is characterized by the presence of müllerian structures in a genotypic male (5). Individuals often have normal phallic development and testicular function, both testes on the same side (possibly embedded in the broad ligament), and abnormal development of male excretory ducts (5). Fertility is possible in individuals with Persistent Müllerian Duct syndrome if at least one testis is scrotal with intact excretory ducts; however, most individuals are infertile (5).

Camptomelic Dysplasia

Camptomelic dysplasia is caused by a mutation in one copy of the SOX9 gene and is generally associated with skeletal malformation (6). In the absence of one copy of SOX9, the testes fail to form, and the ovarian pathway is followed instead (6).

Androgen Insensitivity Syndrome

Androgen insensitivity syndrome (AIS) is the most common molecular diagnosis of 46,XY DSD newborns (1). It is an X-linked genetic disease caused by a nonsynonymous point mutation in the androgen receptor gene (1). An unaltered androgen receptor is necessary for male reproduction (1). AIS can be found in three forms: complete androgen insensitivity syndrome (CAIS), partial androgen sensitivity syndrome (PAIS), and mold form androgen sensitivity syndrome (MAIS) (1). AIS individuals are chromosomal males due to the 46,XY karyotype, but phenotypic females with normal female external genitalia, a blind vagina, no uterus or uterine tubes (6).

Testes can be present within the abdomen or in the inguinal canal and are often mistaken for hernias in infants who otherwise appear to be normal phenotypic females (6). The testes in these patients secrete androgen normally, however, end-organ unresponsiveness to androgens results from absence of the androgen receptors in the target cells (6). Androgen insensitivity syndrome occurs in approximately 1 in 10,000-20,000 live births (6).

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is an inherited disorder resulting in virilization of 46,XXY infants due to defects found in specific enzymes of the adrenal cortex that are required for cortisol biosynthesis (6). CAH individuals generally have normal ovarian development but have excessive androgen production leading to masculinization of external genitalia, clitoral enlargement, and formation of a scrotal-like structure due to labial fusion (6). CAH accounts for around half of all ambiguous genitalia cases with an incidence of 1 in 12,500 births (6).

Table 2: Disorders of Sex Development and their Characteristics

Disorder	Gonadal Sex	Phenotypic Sex	Characteristics	Prevalence
46, XX Testicular DSD	Testes (bilateral)	Normal male (80%) or ambiguous (20%)	Present clinically after puberty with small testes	1/2000 births
46, XX Ovotesticular DSD	Testicular and Ovarian Tissue	Ambiguous	Uterus may be present; surgery often required to repair external genitalia; can be raised male or female	Rare (3-10% of DSD)
Complete Gonadal Dysgenesis	Streak gonads (no sperm production)	Female	Normal Mullerian structures	1.5/100,000 births
Partial Gonadal Dysgenesis	Regressed Testes	Variable (male, female or ambiguous)	Ambiguous external genitalia with or without Mullerian structures; can be raised male or female	Rare
Mixed Gonadal Dysgenesis	Testes (dysgenetic) and streak gonad	Variable (male, female or ambiguous)	Variable phenotype, ranging from typical male to Turner syndrome female	Common
Turner Syndrome	Ovary (streak gonads)	Female	Gonadal dysgenesis	1/2500 females
Klinefelter Syndrome	Testes (dysgenetic)	Male	Gonadal dysgenesis	1/600 males
Denys-Drash Syndrome	Testes (dysgenetic)	Variable (female or ambiguous)	Renal failure, genital abnormalities	Rare
Frasier Syndrome	Streak gonads	Female	Gonadoblastoma	Rare
SERKAL Syndrome	Testes or ovotestes	Ambiguous	Renal, adrenal, lung and heart anomalies	Rare

Persistent Müllerian Duct Syndrome	Testes (uterus and fallopian tubes)	Male	Infertile, but fertility is possible; normal phallic development and testicular function; both testes on same side; abnormal development of male excretory ducts	Rare
Camptomelic Dysplasia	Ovary	Female	Male-to-female sex reversal; skeletal malformation	Rare
Androgen Insensitivity Syndrome	Testicular and Ovarian Tissue	Female	Normal female external genitalia, blind vagina, no uterus or uterine tubes; testes present in abdomen	1/ 10-20,000 births
Congenital Adrenal Hyperplasia	Testicular and Ovarian Tissue	Variable (female or ambiguous)	Normal ovarian development, excessive androgen production, clitoral enlargement, labial fusion	1/ 12,500 births

Table 1 Derived from tables shown by Nussbaum, McInnes, and Willard (6).

Hypothetical Z-gene

The Z-factor theory is the alternatively proposed model that accounts for the XX individuals develop testes in the absence of the SRY transcription factor (2, 4). This theory differs for the classical textbook theory in that the XX gonad expresses some sort of ‘Z-factor’ molecule that plays a role in both pro-ovary and anti-testes and in the XY gonad, SRY inhibits this ‘Z-factor’ molecule to lift the block on the male pathway (2, 3) rather than the ovarian pathway being some sort of default pathway when there is no presence of the sex-determining region on the Y chromosome (2). Some have proposed that this one particular gene has two separate function; to suppress SRY, and to initiate the female pathway (4). Others, however, proposed that rather than one gene it was two genes, one for each function (4). The Z-factor molecule is not known but there are a few proposed candidates. It is thought that this Z-gene in on Xp21 because it is the location of duplications in some of the 46,XY sex reversal cases (4). One possible candidate for the Z-like gene in SOX3 because it is located on this specific region of the X chromosome (4). Another proposed candidate is RSPO1, which acts as a repressor of SRY and when it is de-repressed activated SOX9 (3). This is still largely under study.

Genetic Testing

Genetic testing is an extremely important part of understanding, evaluating, and making decisions concerning patients with DSD. Testing can be done using a number of methods, each specialized for specific scenarios that are encountered in the determination and differentiation of DSD. Peripheral blood karyotype analyses are useful in the detection of X and Y chromosomes, balanced chromosome

rearrangements, and large structural rearrangements (5). The fluorescence in situ hybridization (FISH) analyses using X and Y centromere-specific probes is useful in assessing sex chromosome mosaicism, unknown marker chromosomes, and chromosomal rearrangements (5). Chromosomal microarray analyses, specifically single nucleotide polymorphism (SNP) microarrays and comparative genomic hybridization (CGH), can detect gene variations at the submicroscopic level (5). CGH can identify genes associated with DSD as well as interrogate multiple genes simultaneously, which accelerates the diagnostic process and limits the financial burden that accompanies multiple individual gene tests (5). CGH cannot detect balanced chromosomal translocations or low-level mosaicism (5). Genome-wide association studies (GWAS) detects novel loci associated with DSD that are located outside of the coding region and interprets the phenotypic function impact of the variant; however, it's usefulness is limited due to phenotypic and genetic heterogeneity as well as low incidences for specific DSD disorders (5). Next-generation sequencing (NGS) techniques, such as whole-exome sequencing and whole-genome sequencing, identify the molecular basis of many diseases (5). Whole-exome sequencing (WES) targets the coding regions of thousands of genes simultaneously while whole-genome sequencing (WGS) targets the entire genome, but neither of these techniques detect larch copy number variants, repetitive sequences, or aneuploidy (5). This type of testing detects variants outside of sexual differentiation, which leads to a gray area regarding the ethics of genetic testing. As a genetic counselor, or any medical professional, it is important to have informed consent from a patient before conducting NGS. The possibility of an incidental finding, unrelated to DSD, has the potential for significant medical impact (5).

Clinical Management and Psychosexual Outcomes

When a child is born with a DSD, it may be alarming and confusing for the parent. It is important to review the pregnancy, family histories, and perform a physical examination of the child. Instead of guessing whether the child is a boy or girl, a team of pediatric endocrinologists, urologists, surgeons, radiologists, nurse educators, obstetrics and gynecology specialists, geneticists, neonatologists, and behavior health professionals will care of the infant and analyze the child, making sure to include the parents in the discussion (5). Several things should be examined, including the symmetry of the external genitalia, presence of gonads, genital pigmentation, extent of labioscrotal fusion, length and diameter of phallus, location of urethral meatus, number of perineal openings, presence of posterior labial fusion, and estimation of ano-genital ratio (5). Karyotype and genetic testing are essential in the determination of a DSD. Ultrasound studies are also useful to detect presence of the uterus and any renal anomalies (5). Certain phenotypic expressions can also guide the genetic and laboratory testing. Virilization of the external genitalia with bilateral non-palpable gonads is often associated with congenital adrenal hyperplasia, normal symmetric female external genitalia with labial masses if often associated with androgen insensitivity syndrome, and asymmetric external genitalia is often associated with gonadal dysgenesis or ovotesticular DSD (5). Other important factors to discuss with the parent and medical team include sex of rearing, medical management plans, gonadal development, genetic testing results, recurrence risks, and follow-up plans (5). It is also important to consider non-medical factors including healthy psychological development,

quality of life, and the ability of the parents to cope with uncertainties, as well as medical factors including surgical possibilities, fertility potential, and need for hormone replacement (8). The psychosexual outcomes of children with DSD is a very important aspect to keep in mind when diagnosing a child; however, the evidence of these outcomes have only been studied with respect to a few different disorders, specifically CAH, CAIS, and PAIS. Studies regarding the psychosexual outcomes of CAH indicate that the majority of individuals raised as females develop and maintain female gender identity throughout their life, although they tend to have a less strong female identification, gender discomfort, and gender dysmorphia when compared to females without CAH (8). However, another study reports that only 5% of female-reared women with CAH experienced gender dysmorphia and made a complete social transition into the male role (8). Individuals with CAIS who had been reared as females, with little exception, develop and maintain a female gender identity, although there is more variability in gender behavior (8). A study showed that women with CAIS recalled more male-typical preferences in adolescence, including toys and activities; however, none of the 98 women with CAIS interviewed reported gender dysmorphia or complete social transition into the male role (8). Greater gender fluidity is seen in individuals with PAIS, with 11% of female-reared individuals identifying as gender dysmorphic or socially transitioning into the male role and 14% of male-reared individuals identifying as gender dysmorphic or socially transitioning into the female role (8).

Conclusion

The known interactions between different genes affecting sex development and differentiation give valuable insight into the pathway of sex determination. Of the two proposed theories of sex differentiation, I believe the Z-factor theory to be more accurate. I believe it is likely that one particular gene has the two separate functions of suppressing the sex-determining region of the Y chromosome (SRY) and initiating the ovarian pathway. I also believe that it is possible that this proposed Z-factor molecule is either SOX3 or RSPO1, but I think RSPO1 is a better candidate. The pathway and gene functions can also give valuable insight into the cause of many disorders of sex development. It is shown that mutations in a single gene, such as SOX9 or GATA4, can have profound effects on the phenotype of an individual and even lead to other health concerns. As a genetic counselor or medical professional, it is imperative to know which types of genetic testing should be done for each DSD to give the most accurate results and also to be able to communicate these results effectively. Although diagnosing a DSD is not easy, the best way to go about it is to have an open line of communication with many other medical professionals and keep in mind the future well-being and psychological effects of the patient. Being diagnosed with a DSD or having a family member with a DSD diagnosis can be unsettling and scary if you don't know what to expect. Being well aware of the clinical management and psychosexual outcomes of a DSD will make the patient feel more comfortable with the diagnosis and help them to lead a happy and healthy life.

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