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# MOLECULAR MECHANISM OF XY GONADAL DYSGENESIS

Griendy Indig-Weingarten

One of the fundamentals of human sociology is the characterization of the people around us based on gender. We tend to think of gender as a strict binary system where the option is clear: boy or girl. Although society usually honors this dichotomy, biology allows more flexibility to the definition of male versus female. Estimates state that one in every 2000 births is one with a disorder of sex development (The Intersex Society of North America 2006). Some of the disorders are visually obvious while others are only discovered later on in life. Regardless of when the disease first becomes obvious, all of these disorders constitute a variation along the standard development of a male or female. Therefore, to understand disorders of sex development, one must first understand what constitutes normal sex development. As in all of embryology, development is controlled by many different genetic codes and the pathways they encode. Therefore, variation along any gene responsible for any part of the sex differentiation mechanism will result in a deviation from the expected.

## **NORMAL SEX DEVELOPMENT**

The identity of the sperm that successfully penetrates the ovum is the official beginning of sex determination. When meiosis of the spermatocyte is completed, the mature haploid spermatozoa can either have an X or a Y as its twenty-third chromosome. When this particular sperm penetrates the ovum, the identity of its twenty-third chromosome determines the genetic sex. This is the first step of sex determination. Further sex development is divided into two parts: primary sex determination and secondary sex determination. Primary determination involves the development of the fetus's gonads. Once formed, the gonads start producing hormones which initiate secondary sex development. Secondary sex development includes the development of duct systems and external genitalia. Secondary sex development is only complete after puberty (Gilbert 2010).

## **PRIMARY SEX DIFFERENTIATION**

Regardless of their karyotype, initially all embryos present a common set of genital structures. These include the gonads, the internal duct system, and the external genitalia. The development of the genital system begins at the fourth week of embryonic life. The entire genital system is derived from the intermediate mesoderm and urogenital sinus except for the gametes which emigrate from the mesoderm of the yolk sack. The intermediate mesoderm differentiates to form the adrenal cortex and the start of the urogenital ridge originating as the nephrogenic cord. The urogenital ridge is the site of the development of the urinary and reproductive system. The cephalic portion of the nephrogenic cord further matures to form the pronephros, which is mostly an embryonic structure. The more caudal portions of the nephrogenic cord form the mesonephros and the pronephric duct which later forms the mesonephric duct. The genital ridge arises along the middle portion of the mesonephros. This is the central location for the development of the gonadal and genital structures. The caudal (posterior) portion of mesonephric duct fuses with mesonephric tubules and continues to extend and connect to the urogenital sinus.

Then the most caudal area of the nephrogenic ridge further develops into the metanephros which is the permanent kidney (Figure 1) (McMahon et al. 2008).

## GONADS

The gonads are unique embryonic structures because they are the only bipotential organs. They can develop into ovaries or testes. Gonads usually appear at week four but remain undifferentiated until week seven. At about 3-4 weeks, the mesonephros is at its largest size, and it is at this point that the epithelial layer of the mesonephros, also known as the coelomic epithelium, starts to proliferate into the surrounding mesenchyme, or undifferentiated loose connective tissue, and form the primary sex cords. These cords will surround the entering germ cell to form the gonadal cord. At this point, the gonadal tissue will start to differentiate to testes or ovaries.

## MALE GONADAL DEVELOPMENT

An XY fetus, at around the eighth week, will start developing testicular tissue. This begins with the differentiation of the gonadal cord into the Sertoli supporting cells. These cells form processes that surround the primordial germ cells and eventually mature into Sertoli cells. At the same time, cells migrating from the mesonephros, called peritubular myoid cells, form a single layer around the gonadal cords. These peritubular myoid cells contribute to the structural integrity of the testis cord and will support spermatogenesis later in the adult male's life (Buehr et al. 1993). It is at this point (around the eight week) that the gonadal cord is recognizable as the first form of the testis cord. The testis cord then comes in contact with the mesonephric tubules which are connected to the mesonephric duct, causing a capsule called the tunica albuginea to form around the testes. Meanwhile, the testis cords continue to create a dense network called the rete testis. The rete testis functions later on as the carrier of sperm from the seminiferous tubules out of the testicles through the efferent duct which was derived from the mesonephric tubules. At 20 weeks, the testis cord forms the straight and convoluted seminiferous tubules. The straight tubules are connected to the rete testis and do not have any germ cells within them. The convoluted tubules are a continuation of the seminiferous tubules and contain the primordial germ cells. Spermatogenesis takes place in the convoluted tubules (Celio et al. 1999). Once the testis is formed, some primordial germ cells differentiate into a pre-sperm state called T1-prospermatogonia and remain that way until puberty (Gilbert 2010).

Another important part of testicular development is the differentiation of the Leydig cells. These cells originate from mesenchyme, or the undifferentiated loose connective tissue in between the testis cords. These Leydig cells eventually produce testosterone which will be crucial to further male differentiation. Because of its steroid

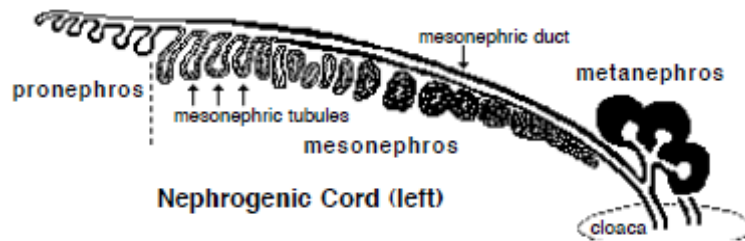


Figure 1: Nephrogenic Cord.

Source: <http://vanat.cvm.umn.edu/TFFlectPDFs/LectEmbUrinarySys.pdf>.

producing nature, Leydig cells tend to cluster near the testicular vasculature (Wilhelm 2007).

## FEMALE GONADAL DEVELOPMENT

In the case of an XX embryo, the gonads usually develop into ovaries. The ovaries start developing during the eight week. In the absence of testis determining genes, the genital ridge will differentiate into ovaries. However, unlike testis, in which the testis cord forms early on in embryogenesis, the functional unit of the female gonads—the ovarian follicles—completely mature only at puberty. During fetal development, primordial follicles are formed. These follicles are usually formed around 16-24 weeks after fertilization (Celio et al. 1999). The ovarian development begins with the migration of the primordial germ cells into the genital ridge and the gonadal cord partially atrophies. Later on, the primordial germ cells proliferate and form the primordial oocyte, or the oogonium. At around the fifth month, the oogonia are arrested at prophase of the first meiosis and are subsequently surrounded by pregranulosa cells which differentiated from the gonadal cord. These granulosa cells are connected with cellular bridges and high levels of vasculature. One of the key differences between the male and female primordial germ cells is that while all the male cells will continue to proliferate and mature to sperm from the age of puberty throughout a lengthy adult life, only the female cells that have matured and were arrested at prophase of the first meiotic division in utero will mature into oocytes.

## MOLECULAR GENETICS OF GONAD DEVELOPMENT

### TESTIS PATHWAY

Genetic sex is defined as the presence of an XX or XY karyotype. In either case, one X will be inactivated, allowing the X or the Y to be the defining chromosome in sex determination. The Y chromosome carries a dominant testis-determining gene which actively promotes testicular development. Absence of the gene, regardless of the identity of the rest of the chromosome, will cause the embryo to go into “default” development and continue to develop as a female. It is for this reason that sex determination is sometimes equated with testis determination. This was proven by Alfred Jost when he castrated rabbits in utero and the rabbits’ development continued according to the female pattern (Jost 1972). Later research showed the testis-determining gene to be the *SRY* gene on the short arm of the Y chromosome. In fact, the research that discovered the connection between the *SRY* gene and testis determination was carried out on XY females and Turner Syndrome patients (Berta et al. 1990).

### *SRY*: SEX- DETERMINING REGION Y

The *SRY* gene is located on the upper short arm of the Y chromosome. In studies using XX reversed males and XY reversed females, results showed that a fetus with the Y short arm, even when missing the long Y arm, would develop testis. Accordingly, any individual missing the Y short arm would not develop testis. Additionally, if the *SRY* gene is inserted in the genome of a normal XX mouse, the XX mouse would develop testis (Koopman et al. 1991). The *SRY* gene is expressed in the bipotential gonads immediately before differentiation of the Sertoli cells begins, at around eight weeks, and is deactivated a few days later (Cotinot et al. 2002). Although *SRY* is deactivated after a short window, while active, it synthesizes the *SRY*

transcription factor whose primary role is to activate the *SOX9* gene, allowing differentiation to continue even after *SRY* is deactivated.

### *SOX9*

The *SOX9* gene is located on the seventeenth chromosome. SOX genes are called transcription factors due to their nature of transcribing proteins that can bind to specific locations on DNA to control gene activity. *SOX9* is involved in many developmental processes, particularly skeletal formation. In fact, the role of *SOX9* in testis formation was discovered when a link between *SOX9* and a condition called campomelic dysplasia was discovered. Campomelic dysplasia patients exhibit multiple skeletal abnormalities. Interestingly enough, however, 75% of the 46 XY patients with campomelic dysplasia were sex-reversed females (Cooke et al. 1985). This led researchers to discover that when *SOX9* is activated in the genital ridge, it induces testis formation. Studies have shown that mice missing the *SRY* gene but possessing an extra copy of the *SOX9* will develop as male. Accordingly, mammals possessing the *SRY* gene but lacking the *SOX9* gene will not develop male (Huang et al. 1999).

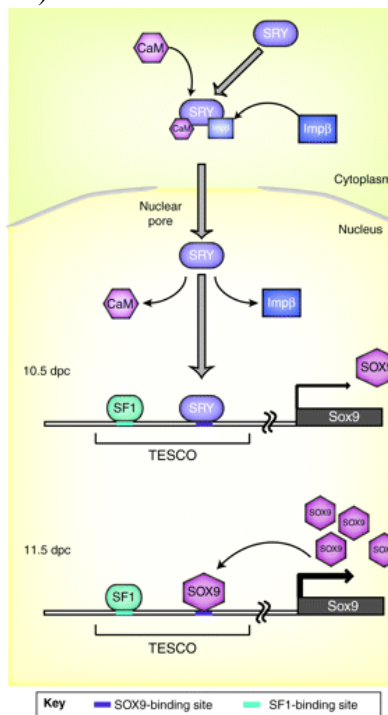
*SOX9* works in several different manners:

- It activates its own promoter, creating a positive feedback loop which allows it to be active for a longer period of time (Sekido and Lovell-Badge 2008).
- It blocks genes that are involved in ovary formation (de Santa Barbara et al. 2000).
- It can bind and activate different genes necessary for testicular development.
- It promotes the gene expression necessary to produce fibroblast growth factors that are critical for Sertoli Cells precursors.
- It binds to the gene that promotes the production of anti-mullerian factor (de Santa Barbara et al. 2000).

*SOX9* is detected in the somatic cells in the human fetal gonads as early as the sixth week. When the Sertoli cells start differentiating, *SOX9* is expressed in the nuclei of the cells (de Santa Barbara et al. 2000). Studies done on *SOX9* have found that it very often works together with a nuclear receptor called steroidogenic factor-1 (SF-1) which is activated by the *NR5A1* gene. The relationship between the *SRY*, *SOX9*, and SF1 is explained in Figure 2.

### *NR5A1*

*NR5A1* is sometimes referred to as the SF-1 gene



In the cytoplasm, SRY is bound by calmodulin (CaM) and importin  $\beta$  (Imp $\beta$ ), which recognize the N- and C-terminal nuclear localization signals (NLSs) on SRY, respectively, and recruit it to enter the nucleus. At 10.5 dpc, SRY and steroidogenic factor 1 (SF1) bind directly to specific sites ('TESCO', testis-specific enhancer of *Sox9* core) that lie within the gonadal specific enhancer of *Sox9* (indicated by the coloured regions on the DNA) and upregulate *Sox9* expression cooperatively. At 11.5 dpc, after initiation of *Sox9* expression, an auto-regulation system operates in which SOX9 also binds directly to TESCO with SF1 to prolong and amplify *Sox9* expression. Abbreviations: SOX9, SRY box containing gene 9; SRY, sex-determining region on the chromosome Y.

**Figure 2:** Cellular mechanism of SRY function. Source: <http://dev.biologists.org/content/137/23/3921.full>

because it encoded the SF-1 receptor. The *NR5A1* gene spans seven exons and is located on the ninth chromosome (Johns Hopkins University School of Medicine 2011). It functions in gonadal differentiation and in steroidogenesis. Knockout mice (mice that were genetically altered) missing the *NR5A1* gene developed with complete adrenal and gonadal agenesis. Studies show that SF-1 is involved early in embryogenesis during development of the bipotential gonad. Although it is not completely understood how SF-1 functions in the embryogenetic stage, mice missing the part of the *NR5A1* gene that encodes for SF-1 never developed a genital ridge. SF-1 is already active in humans at four weeks when the gonadal ridge starts to form, and its levels remain high in the testis even once the *SRY* shuts off. SF-1 aids in activating *SOX9* and continues to work with *SOX9* to elevate the anti-mullerian hormone transcription. As the name steroidogenic indicates, SF-1 is involved in hormone production. It is involved in multiple steroid-producing functions, such as collaborating with *SOX9* to produce AMH (anti-mullerian hormone), and activating genes involved with testosterone production in Leydig cells (Shen et al. 1994). Mice lacking the SF-1 gene lack both adrenal glands and gonads, proving that the SF-1 gene is involved in early embryogenesis of the urinary and reproductive system (Luo et al. 1994).

#### OVARY PATHWAY

In order to provide a complete picture of development, the molecular pathway of the ovaries is briefly discussed, although these genes are not involved in XY Gonadal Dysgenesis.

##### WNT4

Early on, *WNT4* is expressed in the genital ridge in both male and female embryos. However, its expression, while still maintained in the fetal ovaries, is undetectable in the fetal testes. Mice that lack the gene will fail to develop ovaries (Cotinot et al. 2002).

##### R-SPONDIN-1

Another critical gene is R-spondin-1, a protein expressed by *RSPO1*. An XX fetus with gene mutation in *RSPO1* will be XX male. *RSPO1* acts together with *WNT4* to produce beta-catenin, which activates further ovarian development, and block *SOX9* production. An XY fetus with a duplication of the *WNT4* and *RSPO1* on its chromosome will develop ovaries instead of testis (Gilbert 2010).

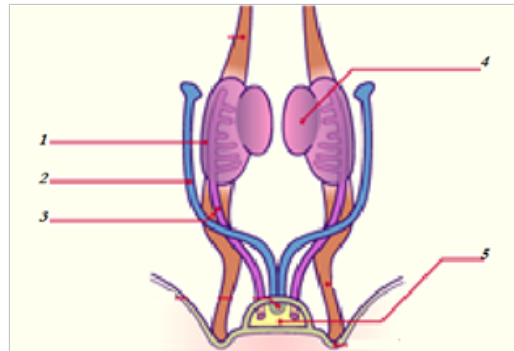
(There are many other genes involved in both testis development and ovarian development that are not discussed in this paper.)

#### SECONDARY SEX DEVELOPMENT

Once the gonads are formed, they start to produce hormones. These hormones are initiators of secondary sex development. Once these hormones begin to be secreted, secondary sex development can start. It is important to note that primary and secondary developments do overlap, as the testes and ovaries continue to develop once steroids are produced.

## INTERNAL DUCT SYSTEM

The gonads are just one part of the sex differentiation process. The ducts system, like the gonads, start out in an undifferentiated state. In the undifferentiated stage, the embryo presents with mesonephric ducts, also known as the Wolffian ducts. Paramesonephric ducts, which appear in the sixth week, form part of the urogenital cord. The Wolffian ducts form from the nephrogenic cord and develop toward the cloaca. Mesonephric tubules, another part of the mesonephros involved in the development of the internal ducts, arise from the mesenchyme of the mesonephros and eventually fuse with the mesonephric duct to create a passage to the cloaca (Schoenwolf et al. 2009). The Mullerian Duct is formed when the coelomic epithelium, near the top of the mesonephros, invaginates. Both the Mullerian and the Wolffian ducts continue to grow caudally towards the urogenital sinus (Figure 3) (Sweeney 1998).



**Figure 3:** Internal Duct System

- 1-Mesonephros
  - 2-Paramesonephric Duct
  - 3-Mesonephric Duct
  - 4-Indifferent Gonads
  - 5-Urogenital Sinus
- Source: Celio et al. 1999.

## MALE DUCT SYSTEM DEVELOPMENT

During early embryogenesis, the Wolffian duct serves as an excreting duct for the mesonephros. Once the metanephros becomes functional, most of the mesonephric duct atrophies, and the part that remains is dependent on the testosterone released from the Leydig cells in the fetal testis. In the eighth week of gestation, the Leydig cells start releasing testosterone and thus begin two important aspects of the internal male duct system development. One is the atrophy of the paramesonephric duct, and the other is the further development of the mesonephric duct (Celio et al. 1999). The undifferentiated state includes both sets of ducts, and with the start of duct differentiation, the paramesonephric duct starts to atrophy due to the effect of anti-mullerian hormone released by the Sertoli cells within the seminiferous tubules. It is interesting to note that a small part of the paramesonephric duct does remain and differentiates into the appendix testis (Jacob and Barteczko 2005). Meanwhile, the Leydig cells begin releasing testosterone to support the development of the mesonephric duct. Most of the top portion of the Wolffian duct atrophies, leaving only the portion which differentiates into the efferent duct, epididymis, vas deferens, and seminal vesicles.

## FEMALE DUCT SYSTEM DEVELOPMENTS

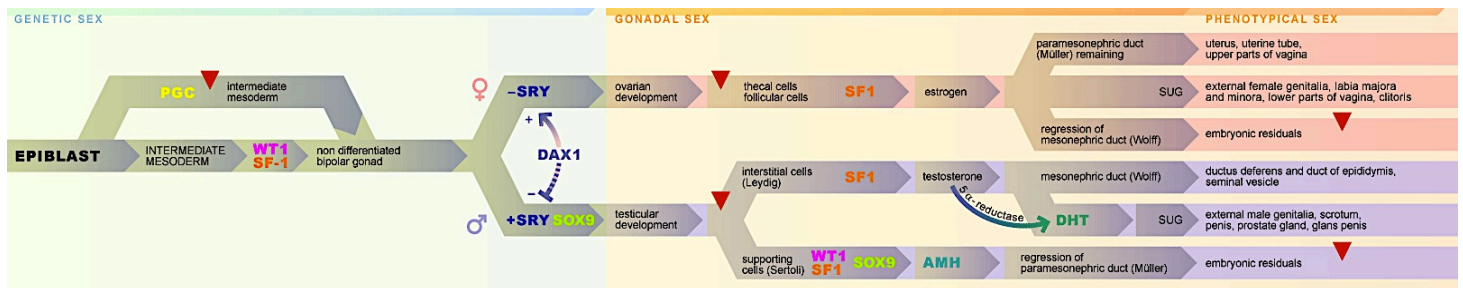
In a human embryo, the absence of the anti-mullerian hormone causes the undifferentiated ducts to develop into the female duct system. The mesonephric ducts and tubules atrophy, and the paramesonephric duct further develops. In the eighth week, the two lower portions of the mullerian duct fuse. This fused portion is the site of the uterus. The upper portion of the paramesonephric duct proliferates outwards towards the gonad and develops as the fallopian tubes. The lowest portion forms the utero-vaginal canal (Celio et al. 1999).

## EXTERNAL GENITALIA DEVELOPMENT

The external genitalia arise from the genital tubercle, the urogenital groove and sinus, and the labioscrotal fold.

As opposed to male development where testosterone plays a key role in differentiation, estrogen is not involved in female sexual differentiation. Instead, it is produced by the ovaries to function as support for follicular maturation. In fact, both male and female fetuses are exposed to high amount of estrogen from their mother. The embryo has a predisposition to develop internal female duct systems (fallopian tubes and uterus), so unless there is an active intervention by anti-mullerian hormone (secreted by Sertoli cells) and testosterone (secreted by the steroidogenic Leydig cells) to promote development of the epididymis, vas deferens, efferent ducts, and other male organs, a female system will develop. Furthermore, male external genitalia are dependent on specific hormonal activity. The undifferentiated embryo is very rich in androgen receptors. Once the Leydig cells start producing androgen, particularly dihydro testosterone, the male external genitalia develop, first into a phallus, and then further into the mature penis, phallic urethra, and scrotum. The absence of androgen causes the development of the clitoris, urethra, vagina, and labia. This further demonstrates that male external genitalia formation requires active intervention (Schoenwolf et al. 2009). This is why it is said that an embryo, if left to its own devices, will go to “default development” and develop female.

This concludes a summary of sex differentiation (Figure 4). Most discoveries of the mechanisms involved in this development were discovered when studying patients with an abnormal sex development or disorder of sex development.



**Figure 4:** Timeline of the primary and secondary sex development. Source: [http://www.embryology.ch/images/imagegraphe/u1e\\_schemdifferentEn.gif](http://www.embryology.ch/images/imagegraphe/u1e_schemdifferentEn.gif).

## DISORDERS OF SEX DEVELOPMENT

Disorders of sex development are defined as any congenital condition in which development of chromosomal, gonadal, or anatomical sex is atypical (The Intersex Society of North America 2006). As seen earlier, sex differentiation is a complex and lengthy process, involving many different genomic pathways. As a result, there are many different variations along the developmental process which can cause atypical presentation. If a mutation exists which impedes the development of one of the necessary functions, it is expected that an abnormal phenotype will occur. Disorders of sex development is a very broad term which spans a spectrum of various different phenotypical presentations. The defining factor in determining the outcome of a variation depends on which point of development it affects. The first step of sex differentiation is genetic sex. Examples of disturbances in the proper genetic sex



development are Turners Syndrome (45 XO female), Klinefelters Syndrome (47 XXY male), XYY syndrome, and a few others. The next step in sexual determination is gonadal sex. Disorders of gonadal sex include XX Gonadal Dysgenesis and XY Gonadal Dysgenesis. The final part of sex development is anatomical sex. Variations in anatomical sex are the broadest of the three steps of sex development. Conditions range from phenotypical female, ambiguous genitalia, and phenotypical male. These conditions include congenital adrenal hyperplasia, androgen insensitivity syndrome, hypospadias, 5-alpha-reductase deficiencies, and many others (The Intersex Society of North America 2006).

### **XY GONADAL DYSGENESIS**

One of the defining moments in male or female sex differentiation is the identity of the mature undifferentiated gonad. Once the gonad has differentiated to either testis or ovary, it initiates steroidogenic activity which will define the rest of the anatomical development. If gonadal development were to halt at the stage of indifference, such an occurrence would be called XY gonadal dysgenesis. The term gonadal dysgenesis involves the broader group of conditions that would cause the dysgenesis, or failure to develop, of the gonads. In Turner Syndrome, gonadal development fails due to a monosomy of the sex chromosome; the gonads are missing crucial genes for gonadal development. In a condition known as XX gonadal dysgenesis, gonads which should develop into ovaries are missing ovarian defining genes or receptors to complete ovarian development. Perhaps the most fascinating is that of an XY fetus developing phenotypically as a female due to gonadal dysgenesis in a condition called XY gonadal dysgenesis (Sinclair and Cameron 2004).

### **CLINICAL PRESENTATION**

XY Gonadal Dysgenesis is usually diagnosed when a patient who failed to develop secondary female sex characteristic undergoes testing to determine the cause. The patient usually presents a female phenotype but no or sparse pubic and underarm hair, no or little breast tissue development, and amenorrhea. Examinations and ultrasound images reveal usual female external genitalia, fallopian tubes, and uterus but no visible ovaries. The incidence of occurrence is hard to estimate, with some papers stating numbers as frequent as 1:30,000 and others quoting numbers as low as 1:150,000 (Wilhelm 2010).

### **TREATMENT**

Although XY Gonadal Dysgenesis is not a diagnosis that comes along with serious medical conditions (except in gene specific abnormalities that will be discussed later), it does come along with some medical aspects that need to be addressed.

#### **HORMONE REPLACEMENT THERAPY (HRT)**

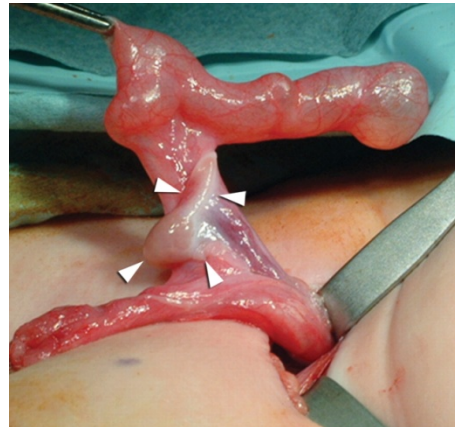
One option is to substitute the hormones that the patient is missing due to the dysfunctional gonads. Patients with XY Gonadal Dysgenesis need to start hormone replacement therapy to initiate pubertal development, and they need to remain on the therapy to maintain optimal health. The doctor will usually start off the patient on a low dosage of estrogen, most often in the form of a 0.3 mg tablet of premarin, a form of estrogen. The dose will increase over a span of 2-3 years. The incremental increase is intended to mimic the hormonal activity that normally would take place at the start of

puberty. During this time, all the expected secondary sex characteristic should develop.

Because the uterus is also maturing during this time, it is important to initiate menstruation with progesterone to reduce the risk of uterine cancer. A doctor will usually recommend bringing on a menses a minimum of four times a year for the upkeep of optimal uterine health. There are a number of different forms of HRT: tablets, patches, gels, injections, and implantable pellets. Some health professionals recommend taking a combined form of estrogen and progesterone, and some add a low dose of testosterone to more accurately mimic the natural hormone production in healthy females. There are conflicting reports as to what form and dose is optimal, and most patients find that the process to find the right regimen depends on what works best for them personally (WebMD 2009).

#### GONADECTOMY

Patients with XY gonadal dysgenesis must undergo a gonadectomy, surgery to remove the streak gonads so that they do not develop into dysgerminomas (germ cell tumors). The risk of an XY gonadal dysgenesis patient developing cancer is estimated at around 30% occurrence rate, which is why doctors recommend that gonadectomy be preformed soon after diagnosis. Dysgerminomas can develop in adults with streak gonads due to their indifferent embryonic structures which are not intended to be mature organs. The gonadal tissue of the streak gonads contains germ cells and many pre-Sertoli/granulose cells scattered throughout the stroma. These cells can develop into gonadoblastomas, benign neoplasms, exclusive to these types of cells. These neoplasms can become malignant and form dysgerminomas and other malignant growths (Michala et al. 2008). Cases have been reported in which patients as young as nine months have developed dysgerminomas (Dumic et al. 1993); therefore doctors recommend removing the gonads as early as possible. With the advances of modern surgical techniques, most gonadectomies are done laparoscopically and involve minimal recovery with little scarring (Figure 5).



**Figure 5:** Laparoscopic removal of streak gonads (indicated by the white arrows) in patient with XY Gonadal Dysgenesis.

Source:

<http://radiographics.rsna.org/content/28/7/1891/F14.large.jpg>

#### GENETIC CAUSES OF XY GONADAL DYSGENESIS

Five genes have been identified in relation to XY gonadal dysgenesis. Because of the rarity of the condition and the constantly developing field of molecular genetics, many of their mechanisms are poorly understood.

##### *SRY*

Mutations in the *SRY* gene (the testis-determining gene) have been found in 10-15% of all XY Gonadal Dysgenesis. Most mutations are in the HMG (High Mobility Group) box on the *SRY* gene. The HMG box is capable of sequencing specific DNA binding and bending. This is crucial in the *SRY* gene, because it is the specific bending pattern which activates the *SRY* in the gonads. The bends act by changing the

chromatin structure in the regulatory region of the target gene that starts the assembly of the regulatory complex. The identification of HMG as the critical region was formed when researchers noticed that there are variations in the non-HMG portions of the *SRY* nucleotide sequences which do not have any effect on testicular development. This finding demonstrated that these regions are not involved in testis development. As previously mentioned, *SRY* is expressed in the genital ridge for a brief time, stimulating Sertoli cell and testis determination (Sinclair and Cameron 2004). A few factors have been identified as regulators for *SRY*. The WT1 may activate *SRY* through an *SRY* promoter region which it expresses. It is also hypothesized that the *SRY* gene itself might have a transcription site on it for auto regulation (Vilain et al. 1992). The roles identified in *SRY* include the induction of the mesonephric cells into the ridge to form the testis cord, proliferation of the cells, and inducement of Sertoli cell differentiation. The lack of *SRY* also causes the failure of the *SOX9* and any other gene relying on *SRY* as its regulatory “switch.”

*SRY* related XY gonadal dysgenesis has been observed in familial lines, but most cases are de novo mutations which cause the loss of binding and its subsequent failure to initiate testis differentiation. Although *SRY* is expressed in somatic cells in different areas in the body, no other effects have been observed as a result of the *SRY* mutation (Simpson 2008).

#### *DHH*

*DHH*, the desert hedgehog gene, is located on the twelfth chromosome and is composed of three exons. *DHH* expression was observed in the testis of fetal mice. In the ovaries of fetal mice, however, little *DHH* expression was observed. This led to the initial belief that *DHH* is involved in testis development. The product of the *DHH* is found in Sertoli cells and in Schwann cells. Therefore, patients with *DHH* mutation usually have polyneuropathy (many nerves acting simultaneously). Unlike *SRY* which directly affects Sertoli cell development, *DHH* affects the development of different cells called peritubular myoid cells, which are cells that make a thin lining around the testis cord. Interruption of peritubular cell proliferation will cause a failure in the development of the testis cord, which will cause a failure in gonadal development. In fact, *DHH*-deficient knockout mice showed male sterility, peritubular defects, and testis-development failure. In addition to its role in testis cord development, studies suggest that the *DHH* is a regulator signal for the differentiation of Leydig cells in the fetal testis and is also involved in upregulating the SF-1 factor (Canto et al. 2004).

Patients with *DHH* mutation may have complete or partial Gonadal Dysgenesis. Partial gonadal dysgenesis usually presents itself with slightly masculinized external genitalia and some internal male ductal development. Some development does occur, a result of some androgen production, usually due to a mosaic presentation, meaning that not all cells have the mutation of the *DHH* gene. In complete gonadal dysgenesis, most patients possess homozygous mutations which do not allow any *DHH* expression to reach the threshold for testicular development.

*DHH* is also expressed in Schwann cells along peripheral nerves. Therefore, mutations in *DHH* can cause a condition called polyneuropathy. Polyneuropathy is a neurological disorder which causes many nerves to malfunction simultaneously. Impaired nerve transmission occurs due to reduced insulation of nerves by the affected Schwann cells (Canto et al. 2004). *DHH*-related gonadal dysgenesis is estimated to

account for 20% of all complete gonadal dysgenesis cases and 50% of all partial gonadal dysgenesis cases.

#### *NR5A1* (SF-1)

*NR5A1* encodes the SF-1 (steroidogenic factor-1) and is sometimes referred to as the SF-1 gene. It is a nuclear receptor that binds to and regulates the transcription of many target genes involved in gonadal and adrenal development. SF-1 was first studied in XY gonadal dysgenesis patients displaying complete adrenal failure. This was a direct indication of the role SF-1 plays in the formation of both gonadal and adrenal glands. These patients had homozygous mutations and did not have any SF-1 expression. In most human studies, it is rare to find a patient with complete adrenogonadal failure due to *NR5A1*. Later studies dealt with patients with heterozygous missense and frameshift mutation affecting only parts of the gene. These patients presented little or no adrenal failure. Many of these patients displayed androgen biosynthesis failure and impaired leydig cell development. Some even presented testicular tissue development (Achermann et al. 2002). This highlighted the fact that SF-1 acts within a dose-dependent function as opposed to acting as an on and off switch.

The prevalence of *NR5A1* XY gonadal dysgenesis is low.

#### *CBX2*

A fourth gene whose mutation can cause XY Gonadal Dysgenesis is the Chromobox homolog protein 2 (*CBX2*). The *CBX2* gene is part of Polycomb Recessive Group 1 on the seventeenth chromosome, which encodes polycomb group proteins. These proteins form large protein complexes capable of chromatin remodeling on primordial germ cells. Chromatin remodeling initiates the transition from mitotic division early in embryogenesis to meiosis once they are in a specific gonad. Research also indicates that the *CBX2* might have a role as a promoter of the SF-1 (Baumann and De La Fuente 2011). Knockout mice missing the *CBX2* gene expressed skeletal abnormalities and sex reversal (Lauber et al. 2009).

#### *DMRT1*/ 9p DELETION

Deletion of the tip of the 9p chromosome is associated with gonadal dysgenesis. This association, however, was discovered quite recently, so most research is still based on presumption. Because the deletion of the tip of 9p affects a number of chromosomes, patients present with complex phenotypic features including ambiguous genitalia and craniofacial abnormality. Deletion of the *DMRT1* gene, located on the ninth chromosome, has been identified as a cause of XY gonadal dysgenesis. *DMRT1* is unregulated in the genital ridge and is, therefore, linked to testis development. Research shows that *DMRT1* is critical for maintaining the activity of other male differentiation genes like *SOX9*. It is a sequence-specific transcriptional regulator, meaning that it will regulate the transcription of other genes. In this case, it is likely that it binds to *NR5A1* and promotes SF-1 production to upkeep the continued gene expression of *SOX9*. Loss of the *DMRT1* gene in mice, even after undergoing complete male development, can cause reversal of the Sertoli cells into granulosa cells by promoting the production of female promoting genes. In humans, this deletion does not result in female development as it does in mice, but it does halt male development, causing XY gonadal dysgenesis (Matson et al. 2011).

## CONCLUSION

With the discovery of sex chromosomes in 1921, the scientific world anticipated that the genetics of male and female differentiation would become clearer. However, it was not until the 1990s that the *SRY* gene was discovered when XY females were studied. The study of XY females also revealed other genes involved in the different aspects of sex differentiation. The connection between *DHH* and the development of the peritubular myoid cells is evident in patients with XY gonadal dysgenesis due to a mutation of the *DHH* gene. *NR5A1* plays a crucial role in encoding the steroidogenic factor-1 which plays multiple roles in gonadal cord development and further differentiation of the testis. Another gene mutation in XY gonadal dysgenesis patients, a mutation in *CBX2*, provides insight into its role in regulation of primordial germ cell development. Studying the role of *DMRT1* in regulating continued male differentiation helps scientists gain a deeper understanding of the mechanisms involved in testicular development.

Science is constantly evolving, and research conducted on patients with XY gonadal dysgenesis and other disorders of sex development will further the understanding of the different components of male differentiation and development.

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