

## DISORDERS IN HUMAN SEX DEVELOPMENT

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### Summary

Reproduction is one of the most important physiological processes for an organism. It is a process by which organisms will produce more organisms like themselves and therefore perpetuate the species. As individuals, our reproductive system is not essential to stay alive when compared to other critical processes such as having a normal heart or lung function. Nevertheless, it is a fundamental element of who we are. Having a functional reproductive system gives us not only the ability to have children but also defines us as individuals: male or female. For the majority of the human population, assigning themselves as male or female is very simple, but for a very small number of individuals affected by a Disorder of Sex Development (DSD) it is extremely difficult.

These disorders, in which the development of chromosomal, gonadal or anatomical sex is atypical, are very rare but diverse in their presentation. In addition, the clinical presentation of these disorders is complex and is therefore difficult to diagnose without expert clinical advice. Here we compile the description and genetic basis of the most common Disorders of human Sex Development. We also describe in detail the important processes affected by these disorders, such as gonadal development, external and internal genitalia formation and hormonal unbalance. We also discuss extensively the role of several known genes whose function is affected in patients with DSD. While considerable progress has been made in the last 20 years in understanding the genetic basis of human sexual development and DSD, almost 80% of DSD cases still remain unexplained at the genetic level.

## **1. Introduction – Reproduction, Sex and Gonads in Human**

In humans, reproduction is biparental, with two parents required to produce offspring. Human reproduction is sexual, which means two different individuals, male and female with two different reproductive systems are needed to produce offspring. Both male and female produce the germ cells, the gametes, with the male gamete being the sperm and the female gamete being the egg or ovum. These gametes meet in the female reproductive tract where they combine to create a new individual, this is called fertilization. Because the new individual contains a combination of genetic material from both parents, it will not only possess some characteristics traits of his/her parents but also possess his/her own unique characteristics traits.

Each sex has its own unique reproductive system but both have the same purpose: to produce, nurture and transport the sex cells, sperm or ovum, until fertilization. Since fertilization and development of the fetus occurs within the female reproductive system, the female system also contains a unique organ, the uterus, which allows the development of the embryo. Central to the reproductive system are the organs in charge of producing and nourishing the germ cells. In males, the sperm is produced within the seminiferous tubules of the testis. Testes are located in the scrotum, which is an extension of the abdomen. This location, outside of the abdomen, is below the core body temperature and provides the optimal temperature for the development of germ cells. The testes not only produce sperm but also secrete male sex hormones, such as testosterone. Testosterone contributes to the development of the male secondary sexual characteristics, such as growth of the muscle mass, increase in bone density, maturation of the penis, the deepening of the voice and the growth of beard at puberty. The male phenotype, or traits characteristic of a male appearance, are the direct or indirect result of the action of testosterone before and after puberty.

In female, the ovary, located in the abdomen, produces the ovum. Ovulation, the release of the ova into the fallopian tube, is cyclic and under the control of female sex hormones. This cycle of production of ova from the ovary coincides with the cycle of uterine growth, the menstruation cycle. A few days after ovulation occurs, the inner layer of the uterus, called the endometrium, is at its thickest to allow for successful implantation of the potential fertilized egg. The ovaries produce a number of female sex hormones which control both menstruation and development of female secondary sexual characteristics. Female sex hormones include estrogens and progesterone,

powerful hormones that are involved in breast development, vaginal and uterine growth, and reduction of muscle mass. Similar to testosterone action in males, the female sex hormones are responsible for the female phenotype, the traits characteristic of a female appearance.

Human sexual reproduction requires two sexually dimorphic individuals, a male and a female, to produce offspring. In turn, offspring will be either male or female to be able to reproduce and perpetuate the species. The mechanism that determines whether an embryo will be male or female is called sex determination. Conceptually, sex determination is any mechanism in which the sex of the individual is determined and there are a number of different sex determination mechanisms across all living organisms. However the two main mechanisms in place are genotypic sex determination (GSD) and environmental sex determination (ESD). In genotypic sex determination, sex is determined at the time of fertilization by the genetic makeup of the new embryo whereas in environmental sex determination, sex is determined by the environmental conditions, often temperature, surrounding the fertilized ovum. There are many examples of environmental sex determination in fish, turtles, some lizards, and all crocodylians in which the temperature of the egg during development will determine the sex. These animals are poikilotherms, i.e. they are “cold-blooded”, and their body temperature fluctuates with that of the environment. Consequently, the temperature of incubation of the egg will directly affect the temperature of the embryo. However, temperature is not always the trigger of sex determination, and in the case of some coral fish, social interactions determine their sex during juvenile development or even as adults.

The formation of the gonad into a testis or an ovary and the proper function of the organ are paramount to the correct development of the reproductive system in humans. The disruption of this process, the formation of the gonad and/or its function leads to Disorders of Sex Development (DSD). DSD is an umbrella term that encompasses a wide variety of conditions of different origin. Originally DSD patients were named intersex to describe a person born with characteristics that were not exclusively male or female. In other terms, an intersex person would be a person whose chromosomal, gonadal or anatomical sex is not exclusively male or female. The term DSD alone does not reflect necessarily the diversity of underlying causes of intersex condition. However, the nomenclature of DSD subcategories takes account of this diversity especially by integrating the progresses made in molecular genetics aspect of sex development.

## **2. Several Levels of Sex**

Perhaps one of the lessons we have learned from working with patients with DSD conditions is that defining the sex of an individual is not always easy. Generally, our appearance is primarily used to define us as one sex over another, however for some individuals their external appearance, male or female, may not reflect their internal reproductive system. Furthermore, a person's genetic makeup may be at odds with their physical appearance; for instance XY individuals with ovarian tissue or XX individuals with testicular tissue. Aspects in addition to physical sex must also be considered, such as sexual identity or gender identity. Individuals' perception of their own sex may not

reflect their actual appearance or sexual preferences.

Our current understanding of human sexual development has been greatly influenced by the experimental work performed by Alfred Jost in the middle of 20th century. At a time when many embryologists restricted their work to amphibians and birds, Jost pioneered difficult experimental work on mammalian embryos. Jost castrated rabbit embryos and found that all embryos developed female secondary sexual characteristics, independently of their genetic sex (XX or XY).

He subsequently injected female rabbit embryos with testosterone and all developed male secondary sexual characteristics. From these experiments, Jost concluded that sex differentiates by successive steps; the sex chromosomes (or genetic sex) determine the sex of the gonad (gonadal sex), which then, through the production of sex hormones, determine the physical or external sex of the embryo (phenotypic sex).

Jost's observations further implied that ovarian differentiation, occurring only in the absence of the male Y chromosome, represents the default pathway of sexual differentiation. This paradigm prompted many to search for the male genetic factors that are responsible for testis development and that fail in DSD.

## 2.1. The Genetic Sex

Until the twentieth century, the mechanism of sex determination in humans was unknown. Before that, a number of theories implicating environmental factors such as temperature and nutrition were extremely popular.

The first observation that chromosomal composition could be different between males and females was made in 1891 by the German biologist Hermann Henking. Henking noted the presence of an extra chromosome in some wasp sperm cells (*Pyrrhocoris apterus*) which he named X element (which ultimately led to the term X chromosome). However while Henking hypothesized that the X element could play a role in sex determination, it was the American zoologist Clarence

E. McClung, in 1902, who hypothesized that the number of chromosomes is the cause and not the consequence of sex determination. McClung's observations were the first to indicate a chromosomal mechanism of sex determination not only in insects but in other animals including humans.

At almost the same time, Walter Sutton, a former student of McClung analyzed in detail the chromosomes from germ cells and somatic cells and reached the conclusion that germ cells contained half the number of chromosome of somatic cells (germ cells are haploid and somatic cells are diploid).

Consequently, the fusion of sperm and ovum at the time of fertilization should reconstitute the number of chromosomes observed in somatic cells. We now know that humans have a total of 46 chromosomes which consist of 44 non-sex chromosomes, the autosomes, and 2 sex chromosomes, either XX or XY (Figure 1).

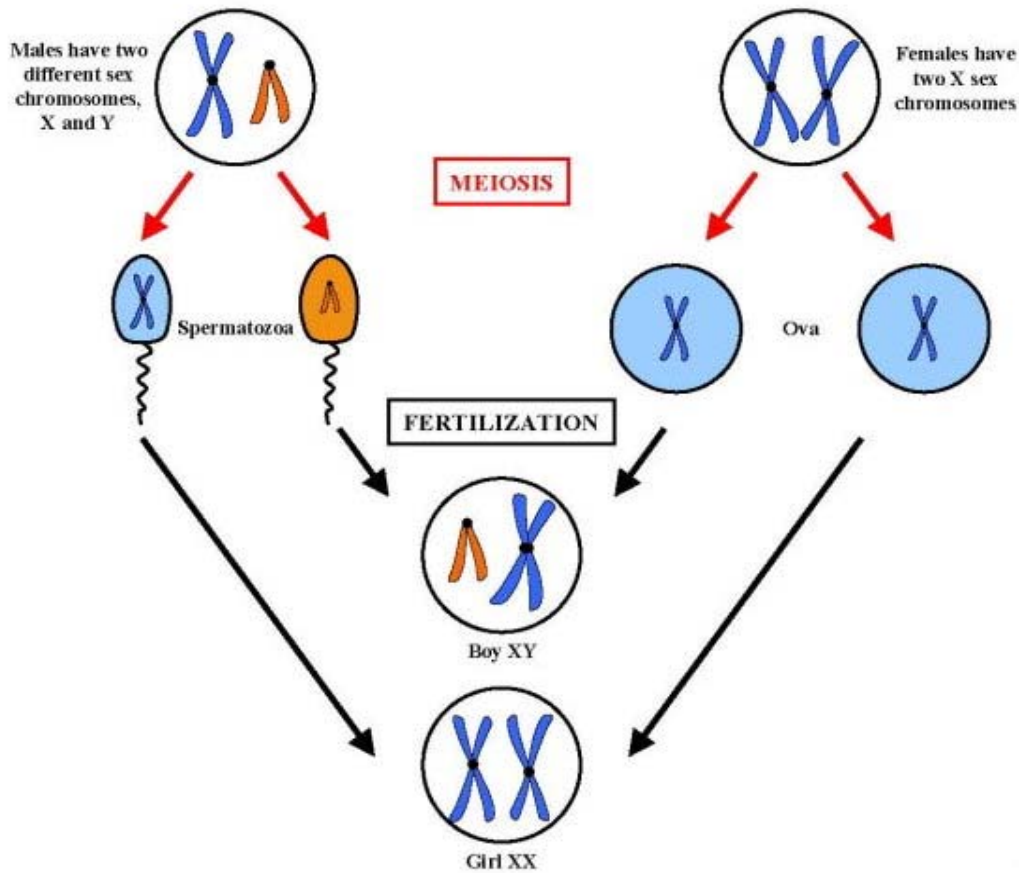


Figure 1. Genotypic sex determination in humans.

In 1959, more than 50 years after Sutton's observations about chromosome numbers in germ cells, it was discovered that the Y chromosome is the male-determinant for both mouse and human. By studying Turner syndrome patients who have a karyotype 45,X and develop as female and Klinefelter syndrome patient who have a karyotype 47, XXY and develop as male, scientists reached the conclusion that, to develop a male phenotype, one must have a Y chromosome. It took until 1990, more than 30 years, to pinpoint the minimal sex determining region within the Y chromosome and identify the SRY gene (Sex determining Region Y). Numerous molecular and genetic studies followed in both humans and mice showing that SRY is undisputedly a master gene for sex determination.

Although our chromosomal sex is determined at the time of fertilization, 46,XY males or 46,XX females, studies of DSD patients has revealed that chromosomal sex does not always determine phenotypic sex. 46,XY patients who are presenting as female and 46,XX patients who are presenting as male are examples of discrepancies between phenotypic sex and the genotypic sex. Although a majority of 46,XX males are found to have a small portion of the Y chromosome including SRY, some do not and the etiology of their condition remains unexplained.

Male somatic cells are diploid and contain 44 autosomes and two sex chromosomes, the X chromosome (Blue) and the Y chromosome (Orange). Female somatic cells are

diploid and contain 44 autosomes and two sex chromosomes, both X chromosomes (Blue).

After meiotic division in the gonads, males produce two different types of haploid sex cells bearing either X or Y chromosomes whereas females produce only X-bearing haploid sex cells. As a result of fertilization, the combination of haploid genetic information from the spermatozoa and ovum, an embryo will be either XY, male or XX, female.

## 2.2. The Gonadal Sex

The gonad is unique because it is structurally, biochemically and functionally different between males and females. Despite their numerous differences, the male and female brains are structurally very similar; however, the testis and the ovary are two totally different organs.

Historically, the gonad pathology was the primary site of investigation in patients with DSD because clinicians and scientists lacked sufficient knowledge or tools, such as chromosomal composition, gene mutations or hormonal levels to explain these disorders.

This “gonad centered” view of sex determination and of DSD conditions has impacted upon the patient’s perception of themselves and also on the scientific research directions, which focus mainly on the development of the gonad above all other systems.

However, in mammals including humans, the formation of the gonad is in fact the critical turning point of the formation of the male or female internal and external genitalia.

While terminally differentiated gonads are different organs in adult males and females, during early human development they initially form an undifferentiated gonadal ridge, which is indistinguishable between the sexes.

The undifferentiated gonad arises from the intermediate mesoderm. Intermediate mesoderm is the tissue that will form the entire urogenital system, including the kidneys and the gonads, and their respective ductal systems. Firstly, a set of ducts differentiate from the intermediate mesoderm into a structure named the mesonephros.

The formation of the undifferentiated gonad then begins with the thickening of the epithelium (or ridge) of the mesonephros around 33 days post-ovulation, which is around 5 weeks into human gestation.

At this stage, the urogenital ridge is indistinguishable between male and female (Figure 2). At 41 days post-ovulation in XY embryos, male sex determination is switched on by the expression of the Y chromosome gene SRY. This expression induces dramatic changes in the morphology of the gonad, and by 44 days post-ovulation, around 6-7 weeks into gestation, sex cords (seminiferous tubules or testis cords) typical of the testis

can be observed. In the female, in the absence of the SRY gene, the ovary does not form sex cords. At the same stage, in both sexes, a ductal structure develops, the paramesonephric duct also known as the Müllerian duct.

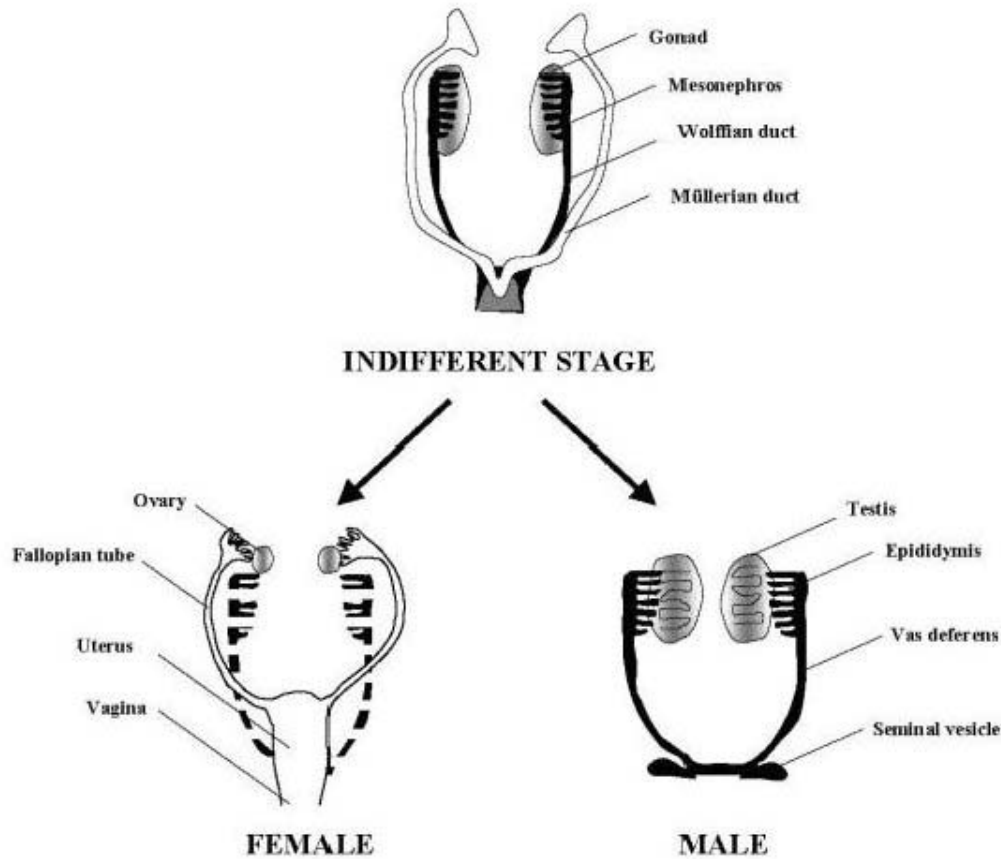


Figure 2. Differentiation of the reproductive system.

The undifferentiated reproductive system before SRY expression is depicted at the top. Male and female differentiated reproductive systems at birth are depicted at the bottom. (Adapted from Wilhelm D., Palmer S., et al. (2007))

In males, a week later, as the testes grow and differentiate, they start to secrete testosterone which will then trigger the differentiation of the mesonephric ducts into the Wolffian duct. The Wolffian duct will in turn differentiate into the epididymis, vas deferens and prostate. At the same time, the testis secretes another hormone known as anti-Müllerian hormone (AMH) or Müllerian inhibiting substance (MIS) which will specifically induce the regression of the Müllerian duct.

In females, as testosterone is not produced, the mesonephric ducts does not grow and does not differentiate into the Wolffian duct. These ducts will eventually degenerate. On the other hand, as AMH is not produced by the ovary, the Müllerian duct remains intact and will eventually differentiate into the fallopian tubes, uterus and the upper third part of the vagina.

Although male and female gonads are fundamentally two different organs, they do share some striking similarities. Both organs are composed of two major cell populations, the somatic cells that give the gonad its organization, produce hormones and provide support for the second cell population, the germ cells.

In the testis, the somatic cells consist of the Sertoli cells which support and nurture the germ cells, the Leydig cells which produce testosterone and the peritubular myoid cells which provide architectural support for the formation and the maintenance of the seminiferous tubules. In the ovary, the granulosa cells form a multilayer of cells surrounding the germ cell (the oocyte) and provide support and nutrients throughout germ cell development.

Granulosa cells are also steroidogenic cells as they produce sex hormones such as estradiol. The outer layer of this structure, the follicle, is composed of theca cells. They could be interstitial or stromal cells that produce androstenedione, a precursor of estradiol. Androstenedione is metabolized by the granulosa cells to form estradiol.

### **2.3. The Phenotypic Sex**

The phenotypic sex is primarily our physical appearance. In many animals, including humans, males and females are dimorphic and generally exhibit a sex-specific physical appearance. For instance, we are familiar with the fact that men usually have beard and women have breasts. While there are numerous examples of physical dimorphism between males and females, dimorphism is also observed in internal organs unrelated to the reproductive system.

Males typically have higher bone density and larger lung volume per body mass than females. Among all internal organs showing sexual dimorphism, the brain has probably been the most controversial subject of many studies. While male and female brains are similar, they show striking differences at the level of the anatomy, biochemical function and behavior. The anatomy of the brain is well documented in humans and male and female brains display differences in size of particular regions.

A number of brain regions are enlarged in the male brain (hypothalamus, stria terminalis, and amygdala) when compared with the female brain and a number of regions in the female brain (caudate nucleus, hippocampus, Broca's area, anterior commissure, and right parietal lobe) are enlarged when compared to the male brain.

The size difference in these regions could have a direct influence in the cognitive function and behavior of the brain, and a well known example of such a behavioral sex difference is the aggressive trait. Aggression, whether physical, or simply the use of foul language, is more common in males than females.

Although the male and female hormones secreted by the gonads contribute to the sexual dimorphism between male and female brains, there seems to be a direct genetic influence too. Studies from mouse models have shown that sex-specific brain differentiation may be under the influence of both hormones and an individual's genetic make-up.



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### Bibliography

Antal, Z. and Zhou P. (2009). Congenital adrenal hyperplasia: diagnosis, evaluation, and management. *Pediatrics in Review* 30(7): e49-57. [Good general review on the management of CAH]

Australian Human Rights Commission (2009). Sex Files: the legal recognition of sex in documents and government records. [http://www.humanrights.gov.au/genderdiversity/sex\\_files2009.html](http://www.humanrights.gov.au/genderdiversity/sex_files2009.html). [A comprehensive list of recommendations to the Australian government on the legal recognition of sex in documents and government records]

Bernard, P. and Harley V. R. (2007). Wnt4 action in gonadal development and sex determination. *International Journal of Biochemistry and Cell Biology* 39(1): 31-43. [Introduction to the molecular aspects of WNT4 function]

Berta, P., Hawkins J. R., et al. (1990). Genetic evidence equating SRY and the testis-determining factor. *Nature* 348(6300): 448-50. [Landmark paper identifying SRY as the key testis-determining gene in human]

Biason-Lauber, Konrad A. D., et al. (2009). Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene. *American Journal of Human Genetics* 84(5): 658-63. [Identification of CBX2 mutation in a 46,XY phenotypic female patient with normal ovaries. CBX2 is the most recent gene identified in DSD]

Bocklandt, S. and Vilain E. (2007). Sex differences in brain and behavior: hormones versus genes. *Advances in Genetics* 59: 245-66. [Discusses the influence of hormones and genetic in brain sexual dimorphism]

Fukami, M., Wada Y., et al. (2006). CXorf6 is a causative gene for hypospadias. *Nature Genetics* 38(12): 1369-71. [The discovery of mutations in MAMLD1 gene causing hypospadias]

Ivell, R. and Hartung S. (2003). The molecular basis of cryptorchidism. *Molecular Human Reproduction* 9(4): 175-81. [A good review on the etiology of undescended testes]

Jost, A., Vigier B., Prepin J. and Perchelet J. P. (1973). Studies on sex differentiation in mammals. *Recent Progress in Hormone Research* 29:1-41. [A review on Jost's ground-breaking experiments]

Lee, P. A., Houk C. P., et al. (2006). Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. *Pediatrics* 118(2): e488-500. [The most recent document on the clinical management of DSD, including a revision of the nomenclature of DSD reflecting patient's concern and advances in molecular genetics]

Lin, L. and Achermann J. C. (2008). Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. *Sexual Development* 2(4-5): 200-9. [A comprehensive review on SF1 molecular function]

Parma, P., Radi O., et al. (2006). R-spondin1 is essential in sex determination, skin differentiation and malignancy. *Nature Genetics* 38(11): 1304-9. [A landmark paper reporting the first mutation in a gene leading to 46,XX DSD]

Sekido, R., Bar I., et al. (2004). SOX9 is up-regulated by the transient expression of SRY specifically in Sertoli cell precursors. *Developmental Biology* 274(2): 271-9. [The molecular link between three genes already known to be involved in sex determination]

Sim, H., Argentaro A., et al. (2008). Boys, girls and shuttling of SRY and SOX9. *Trends in Endocrinology and Metabolism* 19(6): 213-22. [A comprehensive review on some of the mechanisms involving SRY and SOX9 in sex determination]

Uller, T., Pen I., et al. (2007). The evolution of sex ratios and sex-determining systems. *Trends in Ecology and Evolution* 22(6): 292-7. [A good review on the different modes of sex determination in animals]

Wilhelm, D., Palmer S., et al. (2007). Sex determination and gonadal development in mammals. *Physiological Reviews* 87(1): 1-28. [A comprehensive review covering many aspects of the sex determination in mammals with insight from mouse models]

### **Biographical sketch**

**Pascal Bernard** is a senior research officer at Prince Henry's Institute of Medical research in Clayton, Australia. He received his PhD from Lausanne University, Lausanne, Switzerland. He uses mouse models of gene loss of function and gain of function to study the molecular genetic mechanisms of sex determination. His research is aimed at understanding the molecular mechanisms of action of the WNT proteins during ovarian and testicular development.