Pathophysiological Bases for the Classification of the Human Sex Development Anomalies

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Abstract

Purpose: To expose a pathophysiological and clinical classification of the anomalies in the sex development more appropriate for the moment and current knowledge after analyzing the processes of sex determination and sex differentiation in the human sex development.

Methods: Revision of the topic "Anomalies in the Sex Development" as presented in books on Obstetrics and Gynecology and search in Medline, Google Scholar and references in articles of all the papers related to Desorders of Sex Development published in recent years.

Synthesis of data: Human sex development can be divided into two different processes:1) sex determination; and 2) sex differentiation, which takes place through hormones produced by gonads information or once formed. In this review, the elements included in both sex determination (chromosomal sex, genetic sex and gonadal sex) and posterior sex differentiation (hormonal sex, gonophoric sex, phenotypic sex and others) are analyzed, also considering all embryological aspects, proposing a new nomenclature and exposing later in detail a pathophysiological and clinical classification of the anomalies in the human sex development (ASDs).

Conclusions: New terms and a pathophysiological and clinical classification for the ASDs are proposed.

Keywords: sex determination, sex differentiation, sex development, disorders, anomalies, classification

1. Introduction

As Eid and Biason-Lauber [1] and Biason-Lauber [2] pointed out, the development of a human embryo as a man or a woman is among the most defining events in a person's life. The phenotypic sex of an individual depends on the type of gonad that develops in the embryo, a process that in itself is determined by the constitution or genetic inheritance of the individual, and different from the development of any other organ since such gonads possess the potential to

differentiate into two functionally distinct organs, testes or ovaries. Sex development can be divided into two distinct processes: 1) sex determination, which is the fate of the undifferentiated gonad to form the testis or ovary; and 2) sex differentiation, which takes place through hormones produced by the gonads information or once formed. Disruption of any of the genes involved in these processes towards testicular or ovarian development could lead to disorders or anomalies of sex development (ASD) [1,2]. Therefore, it is necessary to analyze each of the elements included in the sex determination and sex differentiation, evaluating also the embryological aspects, to later expose a pathophysiological and clinical classification of the disorders of sex development (DSDs) or ASDs according to current knowledge.

2. Method

Revision of the topic as presented in books on Obstetrics and Gynecology [3,4] and search in Medline, Google Scholar and references in articles of all the works related to Disorders of Sex Development published in recent years. Review and analysis of these data.

3. Results and comments

3.1 Sex determination (see Fig 1) includes:

3.1.1. Chromosomal Sex. The primary determination of sex depends on the chromosomes that the individual has in each of the cells: 44 autosomes and 2 sex chromosomes or gonosomes, XX for women and XY for men. Primitive germ cells (oogonia and spermatogonia) contain that even and diploid number of chromosomes, but after meiosis, the fertilizing gametes are left with a haploid number (22+X in the oocyte - "ovum", and 22+X or 22+Y in spermatid- "sperm"), so that when fertilization occurs, the resulting zygote has the specific human diploid endowment. But the determination of the sex of the individual also depends on the formation of undifferentiated gonads (bipotential gonads) in the genital or gonadal ridge (GR) of the embryo, where the germ cells (gonocytes) are going to arrive from the yolk vesicle through the primitive mesentery and up to what later will be ovaries or testes, depending on whether the gonosomes of the zygote are XX or XY. It should also be noted that the chromosomal abnormalities that cause abnormal sexual development can be numerical and/or structural, depending, in turn, on the involvement of determining genes or inducers of organic functions. On the X chromosome, the gonadal determining genes to form the ovary are found in the long arms and proximal part of the short arms; and the extragonadal determinants, that is, genes responsible for height (stature) and other bodily functions, are found throughout the short arm (Xp). Therefore, removal or distal deletion of the short arm will cause changes in height, but not gonadal dysgenesis; whereas a deletion of the long arm will cause pure gonadal dysgenesis, without phenotypic abnormalities. Regarding the Y chromosome: height and other organic functions are found in the long arm of the Yq; while testicular determinant genes are found in the short arm (Yp).
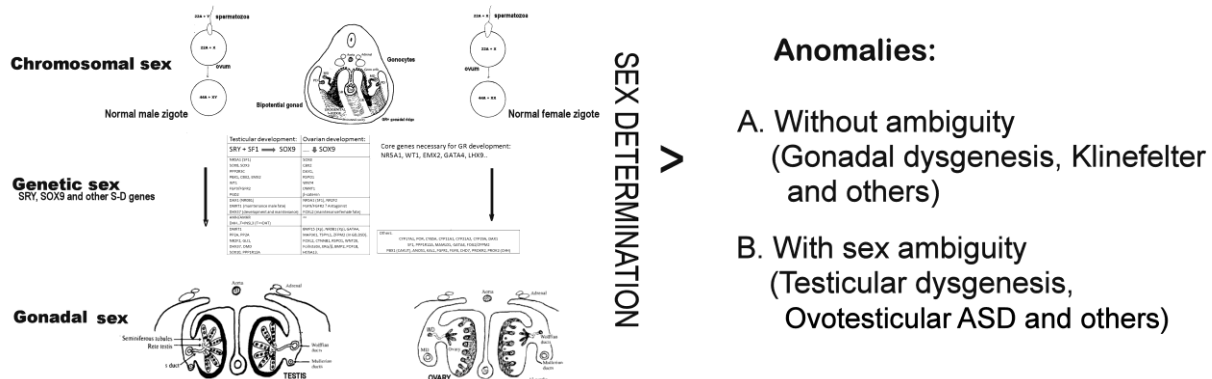
Figure 1. Human sex determination and anomalies.

3.1.2. Genetic Sex. Traditionally, it was thought that gonadal development involved a primary 'male' pathway that led to testicular development and that depended only on the presence of the Y chromosome and the location of the TDF gene (testis-determining factor) (later known as SRY, Sex-Determining Region Y, at the Yp11.2 locus), while ovarian differentiation was passive, with ovarian development occurring in the absence of SRY expression. However, it is not enough that the gonosomes are XY or XX for the testis or ovary to form in GR. They are also essential: 1) Core or central genes (generally autosomal) that appropriately develop GR. 2) The aforementioned presence or absence of the SRY gene on the Y chromosome; and 3) Various other genes (sex-determining, S-D) present in autosomes (and also on the X chromosome), agonists and antagonists for the development of the gonad in one sense or another. So it is now increasingly recognized that there are several gene networks involved in the development of the bipotential gonad towards a testicular or ovarian destination, and this includes different genes that act antagonistically to regulate gonadal development [5]. Bashamboo et al [6] pointed out that changes in this delicate balance of mutually antagonistic pathways can result in ASD (or DSD) and/or sterility in humans, so that some forms of sterility or infertility can also represent a spectrum of gonadal phenotypes with the same genetic cause [6].

During the bipotential gonad stage, and prior to SRY, several non-specific sex genes are expressed early in the XY and XX GRs, and they include the genes WT1 (11p13), DAX1 (NR0B1, Xp21.2), SF-1 (9q33.3), LHX3 (9q34.3), LIM1, PAX2, GATA4 (8p23.1), EMX2, WNT4 (1p36.12) and DMRT1 (9p24.3), being involved in gonadal differentiation and essential for the development of definitive sex [7]. As we can see, most are autosomal genes that regulate the migration of germ cells and also code for enzymes of steroidogenesis. Some other factors also have to act early in sex development, such as Insulin Receptor-Related (IRR) or "growth factor Y" [8]), and there are also 2 proteins that play an important role in the development of the bipotential gonad that are receptor nuclear gene of the aforementioned SF-1 (NR5A1) and WT1 (Wilms tumor suppressor) genes. Other genes involved in gonadal differentiation include SOX3 (Xp27.1), SOX9 (17q24.3), FGF9, and PGD2, which are more like testicular promoters; whereas DAX1, WNT4 (1p36.12), FOXL2 (3q22.3), RESPO1 (1p34.3) and β-catenin are predominantly ovarian promoters [7]. There are also gene interactions that promote testicular development

(PBX1 and CBX2 -17q25 .3-), and likewise, there is a pro-ovary and anti-testicular gene such as NR2F2 [5]. But CBX2, for example, also promotes the ovarian function [9].

Target genes of the SRY gene are SOX9 (17q24.3), CBLN4 and RT71/ETV2, but perhaps the most important one is SOX9. The activation of SOX9 is produced by the joint action of the SRY and SF-1 (9q33.3) proteins on the TESCO region (testicular enhancer specific of Sox 9 core) [7]. Likewise, it has been established that the SOX9 protein can bind to its own promoter, modulating its own expression [7,10]. And it also belongs to the SOX family the SOX3 gene (Xq27.1), which is similar in structure and function to the SRY gene (67% identity in the nucleotide and absence of introns), which has transactivating activity and works synergistically with SF-1 for testicular differentiation. It has been proposed that SOX3 acts as a repressor of SOX9 expression during female determination; and that in male individuals the expression of the SRY gene eliminates the repression of SOX9 by acting as a repressor of SOX3 [7,11]. Finally, another gene involved in testicular gonadal development is DAX1 (also known as NR0B1), located in Xp21.3 (Xp21.2 according to Eggers et al, [12]) and encoding an orphan nuclear receptor. Doubling the dose of this gene (XX individuals) acts directly by inhibiting SF-1 (SOX9 transcriptional regulator), so that 2 copies of DAX1 prevent testicular formation [7,13].

In the absence of the SRY gene, germ cells (gonocytes) enter meiosis in the GR, and female differentiation (ovarian development) occurs due to the inability of SOX9 expression to reach a critical threshold, together with the expression of signaling factors such as RSPO1 (1p34.3), WNT4 (1p36.12) and FOXL2 that lead to ovary formation by differentiating precursor cells from GR sex-specific somatic cell lines into granulosa cells and antagonizing testicular formation [7,14]. Furthermore, in the XX gonads, the presence of WNT4 antagonizes the male pathway by interfering with the expression of SOX9 [7,15,16]. But although the main role of SF-1 is testicular differentiation, its role in the maintenance of the ovarian formation has also been documented [17]. Genes that are already more specific in the maintenance of sex-specific somatic cell lines in GR are the aforementioned FOXL2 gene (for maintenance of female fate by avoiding trans differentiation of granulosa in Sertoli cells) and the DMRT1 gene (for maintenance of male fate), but there is also a new participant in gonadal development and maintenance which is the DHX37 gene (DEAH-box helicase 37), recently identified as the cause for an important aspect of embryonic testicular regression syndrome [5,18].

In summary, those numerous genes, especially autosomal ones, influence both sex determination and gonadal differentiation as well as subsequent sex differentiation, and therefore, they can produce from complete gonadal dysgenesis to cryptorchidism or infertility due to oligo-azoospermia in men or ovarian failure in women, or even certain minor abnormalities of differentiation such as isolated hypospadias.

3.1.3. Gonadal Sex. Sex determination in mammals is equal to gonadal development [19]; and in the human embryo, the development of the gonads begins already during the fifth week of gestation in the GR itself, forming by interaction between this static part (the somatic precursor cells of the GR) and another dynamic one that are the gonocytes. A protein-like substance called teleferon and produced in the GR, would attract the gonocytes there from the back of the yolk vesicle. But at 6 weeks, the gonads are still not differentiated, being therefore bipotential and

capable of developing in one way or another. The Müllerian and Wolffian ducts are close to each other, and the external genitalia are undifferentiated.

Testicular differentiation begins earlier (7th week) than that of the ovary. The gonocytes that arrive from the posterior part of the yolk vesicle are included in the primary sex cords, which grow towards the gonadal medulla and come into contact with the mesonephric tubules forming the primitive seminiferous tubules. The cells of these cords become support cells (Sertoli cells) in the basement membrane of the tubules; and the included gonocytes are transformed into spermatogonia. From the mesenchyme that surrounds the tubules in the medulla, cells differentiate into interstitial Leydig cells. These, with receptors for chorionic gonadotropin (hCG) induced by the SRY gene (and also the intervention of SOX9 and SF-1), are stimulated to produce testosterone (T). Sertoli cells, on the other hand, produce the anti-Müllerian hormone (AMH) and the androgen transport protein (ABP) responsible for the transport of T through the tubules.

Stimulation by hCG causes hypertrophy of Leydig cells, and at 15 weeks of gestation, fetal T reaches peak levels. Thus, hCG stimulates steroidogenesis in the fetal testes and this contributes to the production of androgens (T) allowing male differentiation. Sertoli cells, however, secrete AMH which, through signaling pathways similar to bone morphogenetic protein (BMP), promotes the regression of Müllerian structures in men [20]. So AMH is responsible for the ipsilateral regression of the Müllerian ducts by the eighth week of gestation, before the onset of T and Wolffian duct stimulation. Although the hormone persists in the serum until puberty, the lack of uterine and tubal regression is the only consistent expression of mutations in the AMH gene.

Similarly, Sertoli cells secrete "desert hedgehog, DHH" which induces the development of steroid-producing fetal Leydig cells to secrete T and INSL3 from approximately 8-9 weeks of development [5,21]. T promotes differentiation of the Wolffian duct in the epididymis, vas deferens and seminal vesicles and, together with INSL3, contributes to testicular descent [38]. And approximately 8 weeks after conception, dihydrotestosterone (DHT), produced mainly by the enzymatic conversion of T, acts on androgen receptors, causing virilization of the external genitalia [5].

AMH also has other extra-Müllerian functions. It exerts an inhibitory effect on the meiosis of the oocyte, intervenes in the descent of the testicles and inhibits the accumulation of surfactant in the lungs. The AMH secreted by the Sertoli cells is detectable in the serum of men in childhood and adolescence, but with a drop to practically undetectable levels after puberty. In contrast, AMH secreted by granulosa cells is not detectable in women until puberty. This difference allows serum determinations to be a sensitive indicator of the presence of testicular tissue in certain ASDs. After puberty, T, aided by meiotic germ cells, suppresses AMH secretion in males, and therefore subjects with androgen insensitivity syndrome (androgen receptor defects) have very high levels of AMH after puberty. The effects of AMH on neural activities in the hippocampus have also been studied, suggesting a potential role in learning and memory, and a possible cause of sex differences in cognitive development and function [23].

In the 46, XX embryo without the active influence of SRY, the bipotential gonad develops in the ovary approximately 2 weeks after that corresponding to the development of the testes, although we have already seen that ovarian development is also an active process that involves networks antagonistic regulators that suppress testicular development. In any case, in the gonad XX, the low levels of AMH and the absence of T cause the Wolff ducts to regress and the Müllerian ducts to develop to form an oviduct and uterus; while the Müllerian tubercle and the mesonephric ducts form the vagina [24,25]. The absence of androgens (DHT) results in the development of female external genitalia.

It is also important to note that the survival and development of germ cells in gonads of both sexes depend on unique interactions with somatic gonad cell populations in GR; so that the inadequate development of somatic cells during the formation of the gonads can also affect germ cells and future fertility [5].

3.2 Sex differentiation (post gonadal) (see Fig 2). The second process known as sex differentiation takes place once the sex determination decision has been made through factors produced by the gonads and that differentiated phenotypic sex. However, many of the genes responsible for sex determination are also responsible for gonadal steroidogenesis and therefore for subsequent sex differentiation. This includes:
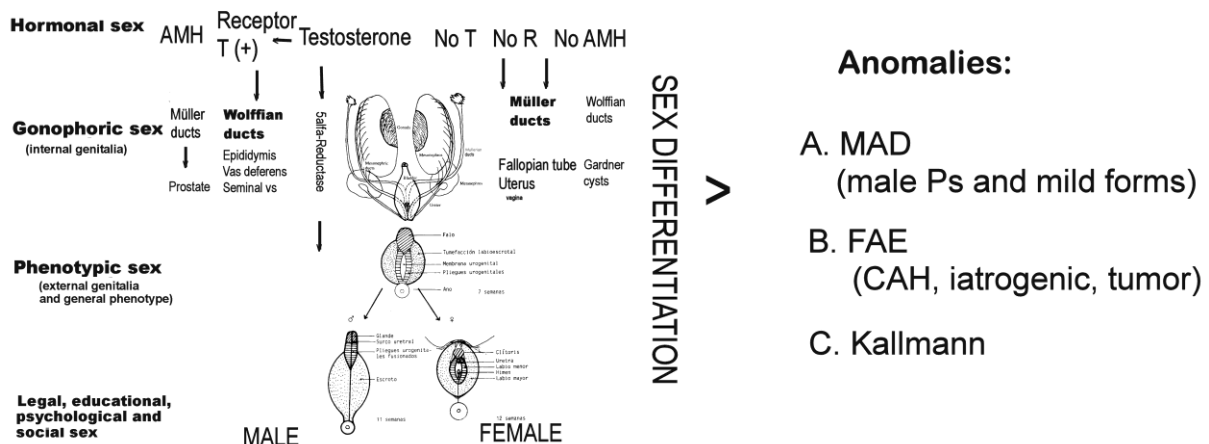

Figure 2. Human sex differentiation and anomalies.

3.2.1. Hormonal Sex. Sex differentiation towards a male or female phenotype (despite the fact that the determination has been to the testicle, ovary, or nothing) will already depend on the gonadal secretions, that is, on whether the gonad that has been formed (testicle) produces or not determined steroid hormones (T), that the appropriate enzymes exist for those steroids to follow the steroidogenetic pathways in their metabolism, and then that the appropriate receptors for such steroids exist. It is therefore advisable to remember steroidogenesis and its general scheme with the main enzymes involved, their arrangement in the gonads or in the adrenal gland as well as the mechanisms of steroid action in order to properly understand the alterations in sex differentiation or pseudo hermaphroditisms (Ps). Fig 3 shows a general scheme of steroidogenesis and the enzymes involved [3].
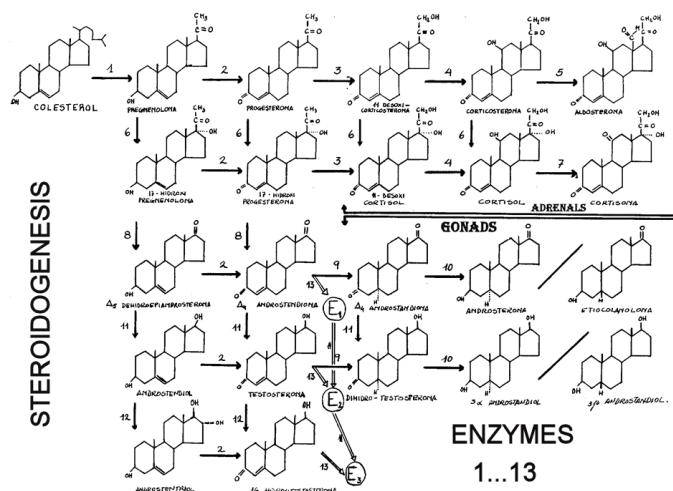
Figure 3. General diagram of steroidogenesis in gonads and adrenals (taken from … with permission). Legends in figure.- Enzymes: 1= Cholesterol 20-22 desmolase; 2= 3-β-ol-dehydrogenase and/or delta4-5-isomerase; 3= 21-hydroxylase; 4= 11-β-hydroxylase; 5= 18 keto oxidase; 6= 17-α-hydroxylase; 7= 11-dehydrogenase; 8= 17-20 desmolase or lyase and/or 17-α-oxireductase; 9= 5α-reductase; 10= 3-β-ol oxireductase; 11= 17-β-hydroxysteroid dehydrogenase or oxireductase; 12= 16-α-hydroxilase; 13= 3-hydroxylase, aromatase.

Furthermore, as we have already seen, AMH is produced early in the Sertoli cells in the fetal testicle, while T, as well as INSL3, are produced in Leydig cells at 7-8 weeks of development. Ovarian development is, however, posterior and does not produce these substances.

The mechanism of action of steroid hormones is exerted by binding to specific receptors located in the target tissues or organs (also controlled by genes). In the case of T, it is also a bit more complex because the cells have to transform this T into DHT, which is the active hormone. Such transformation takes place in the cytoplasm, binds to specific receptors, and passes into the nucleus, where the receptor-DHT complex stimulates RNA synthesis by binding to the nuclear receptor [3].

3.2.2. Gonophoric sex (internal genitalia). If the testis has formed in the GR, the T produced in it and transported along the tubules by the ABP develops the mesonephric or Wolffian duct to form the epididymis, vas deferens, and seminal vesicles. Mesonephric tubules near the testicular gonad persist and transform into efferent ductules, which open into the mesonephric duct that constitutes the epididymis in this region. At the same time, AMH produced in Sertoli cells inhibits the development of the paramesonephric or Müllerian ducts. Then T and INSL3 contribute to testicular descent.

If an ovary has formed in the GR (and also when the gonad is absent or dysgenetic), then the absence of T stabilizes or prevents the development of the mesonephric ducts (Wolffian ducts), and their atretic remnants are the paraophorus, paraovarian and Gartner´s duct. However, the lack of AMH allows the development of the paramesonephric ducts (Müller ducts) to become the internal female genitalia. The cranial portions of the Müllerian ducts form the Fallopian tubes and the fused caudal portions form the uterus. However, the adequate fusion, resorption of the

separating septum and the correct formation of the uterus by the Müllerian ducts is induced at all times by the mesonephric or Wolffian ducts located laterally on both sides. This is how the uterus is formed up to the cervix; but lower down, the Müllerian tubercle and the caudal portions of the mesonephric ducts form the vagina [24-26]. The development of the urinary system is closely related to that of the genital system [24-30].

In summary, in the presence of the XY gonad, T promotes the differentiation of the Wolffian duct into the epididymis, the vas deferens and seminal vesicles and, together with INSL3, contributes to testicular descent. In the XX gonad, low AMH levels and the absence of T cause the Wolffian ducts to regress and the Müllerian ducts to develop to form the oviduct and uterus; and form the vagina below, between the Müller's tubercle and the caudal portions of the Wolffian ducts.

3.2.3. Phenotypic sex (external genitalia and general phenotype). The development of the external genitalia also depends on hormonal action, not directly but by effect of DHT, formed from T and thanks to the enzyme 5α-reductase. The external genitalia are very similar in both sexes (genital tubercle -phalo- in ventral position to the cloacal membrane, labioscrotal swellings and urogenital folds, and a continuous urethral groove with the urogenital opening) until the end of the 9th week, although its shape end is not established until the end of week 12. If the testis is present and T $\gg$ DHT (by 5α-reductase) is produced, the phallus lengthens to form the penis, and the urogenital folds fuse together along the length from the lower surface of the penis to form the penile urethra; as a result, the external urethral orifice is brought up to the glans penis. The folds themselves from the corpus spongiosum of the penis; and the genital or labioscrotal prominences form the bags into which the testicles descend at about 18 weeks, pulled by the gubernaculum and under the effect of T and INSL3. If an ovary is formed, or in the absence of male inducers, the tubercle becomes a clitoris, the folds (labia minora) do not fuse, and the urethral sulcus of the urogenital sinus remains open, thus creating the introitus into which they lead directly the logically shorter female urethra and also the vagina after opening the urogenital membrane whose remains form the hymen. The labioscrotal eminences form the labia majora [3, 24].

Therefore, from eight weeks after conception, DHT, produced mainly by enzymatic conversion of T, acts on the androgen receptors, causing virilization of the external genitalia. In the presence of the XX gonad, the absence of androgens (and the influence of the several determining genes analyzed in the previous section) results in the development of female external genitalia.

3.3 Anomalies of the sex development (ASDs). Classification.

Indeed, the origin of abnormal sex development situations can be: 1) Well in the chromosomes and SRY genes (and other SD genes located in autosomes) that such chromosomes can carry, and therefore, in the formation of the gonad; Or, 2) In the glands that produce hormones that are involved in sex development (gonads, adrenal, pituitary), as well as in the metabolism of these hormones, in their action on the target organs and in the content of receptors. Therefore, the first classification that we could make of the ASDs would be:

• Due to anomalies in the determination: of chromosomes, S-D genes and/or gonadal formation.

• Due to anomalies in the differentiation of the genitalia and phenotype, which will be due to abnormalities in hormonal secretions or their action on the target organs, without anomalies in the chromosomes or in the gonads.

Table 1. New nomenclature for Anomalies or Disorders of Sex Development

Previous	Chicago consensus	Proposed
Intersex status	DSDs	Anomalies in the sex development (ASDs) or DSDs
True hermaphroditism	Ovotesticular DSD	Ovotesticular ASD or OT-DSD
Male pseudohermaphroditism (Undervirilization or Undermasculinization of a XY male)	46, XY DSD	Male (XY) with Androgen Deficiency (**MAD**) or male Ps
Female pseudohermaphroditism (Overvirilization or Masculinization of a XX female)	46, XX DSD	Female (XX) with Androgen Excess (**FAE**) or female Ps
XX male or XX sex reversal	46, XX testicular DSD	Male XX
XY sex reversal?	46, XY complete gonadal dysgenesis	Pure gonadal dysgenesis (Swyer syndrome)

Table 1 shows the terms previously used in the ASDs, their correspondence with the Chicago consensus and classification, and the new nomenclature that we propose for them. In accordance with it and with what was previously stated on sex determination and differentiation, our classification would include:

3.3.1. Anomalies in sex determination (chromosomal and/or gonadal), with or without sex ambiguity (generally):

A. Unambiguous sex abnormalities would (usually) include: 1) Gonadal dysgenesis presenting: a) Ovarian agenesis (gonadal agenesis); b) Gonadal dysgenesis with chromosomal and/or phenotypic alteration: Turner syndrome; or c) Ovarian dysgenesis or hypoplasia (Greenblatt syndrome). 2) Variants of gonadal dysgenesis: a) Mosaic variants; b) Structural abnormalities of the second sex chromosome; and c) Pure gonadal dysgenesis (Swyer syndrome). 3) Triple X constitution and other polysomies. 4) Sex reversal (XX males). 5) Klinefelter syndrome (males). And 6) Other infertility conditions associated with male oligo-azoospermia or female primary ovarian failure (dysgenetic infertility).

B. The anomalies with sex ambiguity should (usually) include: 1) Testicular dysgenesis (XY), which in turn should include to the dysgenetic male Ps (or partial gonadal dysgenesis) and the

mixed gonadal dysgenesis; 2) true hermaphroditism (or ovotesticular disorder, OT-ASD); and 3) some mutations in the NR5A1 gene/SF-1.

3.3.2. The anomalies in sex differentiation (posterior or postgonadal), that is, without chromosomal or gonadal abnormalities (histological [31]), frequently present sex ambiguity or Ps to different degrees, and they should include:

A. Male Ps or Male (XY) with Androgen Deficiency (MAD), if the karyotype is XY and there are testes, even if the phenotype is female. This can occur due to enzyme deficiencies or defects in androgenic action. So male Ps or MAD should include: 1.- Gonadotropin-resistant testes and fetal gonadotropic deficiency (Leydig cell hypoplasia). 2.- Deficiencies in the testicle itself or its secretions, which include: 1) Embryonic testicular regression or testicular regression syndrome (anorquia); and 2) Disorders of androgen production: Male Ps or MAD due to blockage in steroidogenesis. 3.- Defects in androgenic action or androgenic insensitivity syndromes (AIS) due to: 1) 5α-reductase deficiency. 2) Disorders in androgen receptor function including: a) The complete form of testicular feminization or Morris syndrome (CAIS); b) Incomplete forms of testicular feminization (PAIS); c) Reifenstein syndrome. And 3) Receptor-positive resistance. 4.- Other mild forms of male Ps or without Ps as: a) The "infertile male" syndrome; b) Congenital Cryptorchidism; c) Hypospadias; and d) Lack of AMH and therefore regression of the Müllerian ducts (uterine hernia syndrome).

B. Female Ps or Female (XX) with Androgen Excess (FAE), if the karyotype is XX and there are ovaries, even if the phenotype is male. It mainly includes the Congenital Adrenal Hyperplasia (CAH) due to enzymatic deficiencies (especially 21-hydroxylase deficiency (P450c21). But they should also include the iatrogenic causes (hormone therapy), the maternal virilizing tumor, and the aromatase deficiency (P450arom). And finally,

C. Other anomalies of sex differentiation without Ps or ambiguity may be due to idiopathic or congenital hypogonadotropic hypogonadism (CHH) in both males and females, and here we include Kallmann syndrome.

4. Discussion

Anomalies or Disorders of Sex Development (ASD or DSD) were defined as "congenital conditions within which the development of chromosomal, gonadal and anatomical sex is atypical" at the 2005 Chicago Consensus Meeting and later published as a Consensus Statement in 2006 [32-34]. However, there have been and there are many controversies, both due to the negative connotations perceived by organizations and professionals when terms such as "disorders" are used (perhaps due to the perceived implications that "sex" involves sexual behavior), and because some health professionals have considered inaccurate, not very descriptive and confusing certain terms such as "intersex", "pseudohermaphroditism", "hermaphroditism", "sex reversal", etc., or they were felt pejorative among families and patient support groups. Other authors [35,36] prefer the term "differences" and use the same acronym DSD (differences of sex development). However, many authors [37,38] do not consider the nomenclature proposed in the Chicago consensus as the most appropriate. Aaronson and Aaronson [39] proposed a comprehensive classification of DSD based on gonadal histology

(ovarian, ovotesticular, testicular and dysgenetic DSD), but we believe it is preferable, as we have done in this review, to analyze normal or physiological sex determination, the embryological aspects, and the postgonadal sex differentiation, exposing then their anomalies or disorders as they would be included in a pathophysiological and clinical classification that we understand more accurate and appropriate at the present time [38]. Additionally, the abnormalities in the sex differentiation caused by alterations in gonadal hormonal secretions (including those due to hypothalamic-pituitary abnormality), or their effects on the target organs and phenotype, are those that should be included among the ASDs, and are generally also due to abnormalities of genes responsible for enzymes in steroidogenesis. We have already seen that the NR5A1 gene is a key transcriptional regulatory gene in the HHG axis and that central hypothalamic-pituitary abnormalities must also be considered for proper sex differentiation. However, ASDs (or DSDs) should not include genitourinary malformations as a consequence of abnormalities in the structural anatomical formation during embryonic development of the mesonephric (Wolffian ducts) and paramesonephric (Müllerian ducts) genital ducts in both sexes, including the system urinary [26-30,38].

Acknowledgments

References

[1] Eid, W., Biason-Lauber, A. (2016). *Why boys will be boys and girls will be girls: Human sex development and its defects*. Embryo Today, 108:365-379. doi: 10.1002/bdrc.21143.

[2] Biason-Lauber, A. (2016). *The Battle of the Sexes: Human Sex Development and Its Disorders*. Results Probl Cell Differ, 58:337-82. doi: 10.1007/978-3-319-31973-5_13.

[3] Acién, P. Tratado de Obstetricia y Ginecología: Obstetricia. (1998). Cap 2, p28. Ed Molloy, Alicante. ISBN 84-605-8341-4.

[4] Acién, P. Tratado de Obstetricia y Ginecología: Ginecología. (2004). Cap 11:371-428. Ed Molloy, Alicante. ISBN 84-609-0564-0.

[5] Gomes, N.L., Chetty, T., Jorgensen, A., Mitchell, R.T. (2020). *Disorders of Sex Development-Novel Regulators, Impacts on Fertility, and Options for Fertility Preservation*. Int J Mol Sci, 21(7). pii: E2282. doi: 10.3390/ijms21072282.

[6] Bashamboo, A., Eozenou, C., Rojo, S., McElreavey, K. (2017). *Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development*. Clin Genet, 91:143–156. doi: 10.1111/cge.12932.

[7] García-Acero, M., Moreno, O., Suárez, F., Rojas, A. (2019). *Disorders of Sexual Development: Current Status and Progress in the Diagnostic Approach*. Curr Urol, 13:169–178. DOI: 10.1159/000499274

[8] Erickson, R.P., Strnatka, D. (2011). *Insulin Receptor-Related (IRR) Is Expressed in Pre-Implantation Embryos: A Possible Relationship to "Growth Factor Y" and Sex Determination*. Mol Reprod Dev, 78:552. doi: 10.1002/mrd.21334.

[9] Bouazzi, L., Sproll, P., Eid, W., Biason-Lauber, A. (2019). *The transcriptional regulator CBX2 and ovarian function: A whole genome and whole transcriptome approach*. Sci Rep, 9:17033. doi: 10.1038/s41598-019-53370-4.

[10] Kashimada, K., Koopman, P. (2010). *SRY: the master switch in mammalian sex determination*. Development, 137:3921–3930. doi: 10.1242/dev.048983.

[11] Graves, J.A. (1998). *Interactions between SRY and SOX genes in mammalian sex determination*. Bioessays, 20:264–269. doi: 10.1002/(SICI)1521-1878(199803)20:3<264::AID-BIES10>3.0.CO;2-1.

[12] Eggers, S., Sadedin, S., van den Bergen, J.A., Robevska, G., Ohnesorg, T., Hewitt, J, et al. (2016). *Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort*. Genome Biol, 17:243. doi: 10.1186/s13059-016-1105-y.

[13] Ludbrook, L.M., Bernard, P., Bagheri-Fam, S., Ryan, J., Sekido, R., Wilhelm, D., Lovell-Badge, R., Harley, V.R. (2012). *Excess DAX1 leads to XY ovotesticular disorder of sex development (DSD) in mice by inhibiting steroidogenic factor-1 (SF1) activation of the testis enhancer of SRY- box-9 (Sox9)*. Endocrinology, 153:1948–1958. doi: 10.1210/en.2011-1428.

[14] Arboleda, V.A., Lee, H., Sánchez, F.J., Délot, E.C., Sandberg, D.E., Grody, W.W., Nelson, S.F., Vilain, E. (2013). *Targeted massively parallel sequencing provides comprehensive genetic diagnosis for patients with disorders of sex development*. Clin Genet, 83:35–43. doi: 10.11 11/j.1399-0004.2012.01879.x.

[15] Cederroth, C.R., Pitetti, J.L., Papaioannou, M.D., Nef, S. (2007). *Genetic programs that regulate testicular and ovarian development*. Mol Cell Endocrinol, 265-266:3–9. doi: 10.1016/j.mce.2006.12.029.

[16] Schuijers, J., Clevers, H. (2012). *Adult mammalian stem cells: the role of Wnt, Lgr5 and R-spondins*. EMBO J, 31:2685–2696. doi: 10.1038/emboj.2012.149.

[17] Lourenco, D., Brauner, R., Lin, L., De Perdigo, A., Weryha, G., Muresan, M., et al. (2009). *Mutations in NR5A1 associated with ovarian insufficiency*. N Engl J Med, 360:1200–1210. doi: 10.1056/NEJMoa0806228.

[18] Ostrer, H. (2014). *Disorders of sex development (DSDs): an update*. J Clin Endocrinol Metab, 99:1503-9. doi: 10.1210/jc.2013-3690.

[19] Biason-Lauber, A.. (2017). *Human Sex Development: from Basic Science to Clinical Practice and Back*. Pediatr Endocrinol Rev, 15:8-20. doi: 10.17458/per.vol15.2017.l.humansexdevelopment.

[20] Clarke, T.R., Hoshiya, Y., Yi, S.E., Liu, X., Lyons, K.M., Donahoe, P.K. (2001). *Mullerian inhibiting substance signaling uses a bone morphogenetic protein (BMP)-like pathway mediated by ALK2 and induces SMAD6 expression*. Mol Endocrinol, 15:946–959. doi: 10.1210/mend.15.6.0664.

[21] Barsoum, I.B., Bingham, N.C., Parker, K.L., Jorgensen, J.S., Yao, H.H. (2009). *Activation of the Hedgehog pathway in the mouse fetal ovary leads to ectopic appearance of fetal Leydig cells and female pseudohermaphroditism*. Dev Biol, 329:96-103. doi: 10.1016/j.ydbio.2009.02.025.

[22] Ahmed, S.F., Bashamboo, A., Lucas-Herald, A., McElreavey, K. (2013). *Understanding the genetic aetiology in patients with XY DSD*. Br Med Bull, 106:67-89. doi: 10.1093/bmb/ldt008.

[23] Wang, K., Xu, F., Campbell, S.P., Hart, K.D., Durham, T., Maylie, J., Xu, J. (2020). *Rapid actions of anti-Müllerian hormone in regulating synaptic transmission and long-term synaptic plasticity in the hippocampus*. FASEB J, 34:706-719. doi: 10.1096/fj.201902217R.

[24] Acién, P. (1992). *Embryological observations on the female genital tract*. Hum Reprod, 7:437-445. doi: 10.1093/oxford journals. humrep.a137666.

[25] Sánchez-Ferrer, M.L., Acién, M.I., Sánchez del Campo, F., Mayol-Belda, M.J., Acién, P. (2006). *Experimental Contributions to the Study of the Embryology of the Vagina*. Hum Reprod, 21:1623-8. doi: 10.1093/humrep/del031.

[26] Acién, P., Acién, M. (2007). *Malformations of the female genital tract and embryological bases*. Current Women´s Health Reviews, 3:248-288.

[27] Acién, M., Acién, P. (2013). *Classification of the Female Genital Tract Malformations and its Embryological Origin. Diagnostic and Therapeutical Considerations*. Current Women's Health Reviews, 9:1-29.

[28] Acién, P., Acién, M. (2013). *Embryological considerations and update classification of female genital malformations*. Giorn It Ost Gin, 35/4:507-511.

[29] Acién, P,, Aciénm M,, Sánchez-Ferrer, M. (2004). *Complex malformations of the female genital tract. New types and revision of classification*. Hum Reprod, 19:2377-84. doi: 10.1093/humrep/deh423.

[30] Acién, P., Acién, M. (2016). *The presentation and management of complex female genital malformations*. Hum Reprod Update, 22:48-69. doi: 10.1093/humupd/dmv048.

[31] Lepais, L., Morel, Y., Mouriquand, P., Gorduza, D., Plotton, I., Collardeau-Frachon, S., Dijoud, F. (2016). *A novel morphological approach to gonads in disorders of sex development*. Mod Pathol, 29:1399-1414. doi: 10.1038/modpathol.2016.123.

[32] Lee, P.A., Houk, C.P., Ahmed, S.F., Hughes, I.A., in collaboration with the participants in the International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. (2006). *Consensus Statement on Management of Intersex Disorders*. Pediatrics, 118:e488-e500. DOI: https://doi.org/10.1542/peds.2006-0738.

[33] Houk, C.P., Hughes, I.A., Ahmed, S.F., Lee, P.A., and Writing Committee for the International Intersex Consensus Conference Participants. (2006). *Summary of Consensus Statement on Intersex Disorders and Their Management*. Pediatrics, 118:753-757. DOI: https://doi.org/10.1542/peds.2006-0737.

[34] Hughes, I.A., Houk, C., Ahmed, S.F., Lee, P.A. (2006). *Consensus statement on management of intersex disorders*. J Pediatr Urol, 2:148-162. doi: 10.1016/j.jpurol.2006.03.004.

[35] Cools, M., Nordenström, A., Robeva, R., Hall, J., Westerveld, P., Flück, C., Köhler, B., Berra, M., Alexander, S., Schweizer, K., Pasterski, V. (2018). *COST Action BM1303 working group 1 Caring for Individuals With a Difference of Sex Development (DSD): A Consensus Statement.* Nat Rev Endocrinol, 14:415-429. doi: 10.1038/s41574-018-0010-8.

[36] Camats, N., Flück, C.E., Audí, L. (2020). *Oligogenic Origin of Differences of Sex Development in Humans*. Int J Mol Sci, 21(5). pii: E1809. doi: 10.3390/ijms21051809.

[37] Acién, P. (2020). *Disorders of Sex Development*. Open Acc J Repro & Sexual Disord, 2(5). OAJRSD.MS.ID.000150. doi:10.32474/OAJRSD.2020.02.000150.

[38] Acién, P., Acién, M. (2020). *Disorders of Sex Development: Classification, Review, and Impact on Fertility*. J Clin Med, 9:3555. Doi:10.3390/jcm9113555.

[39] Aaronson, I.A., Aaronson, A.J. (2010). *How should we classify intersex disorders?*. J Pediat Urol, 6:443e446. doi:10.1016/j.jpurol.2010.04.008