An Update on Genetics of Disorders of Sexual Development Along with Signal Transduction Pathways-Clinical Implications: A Review

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Abstract

Sex determination in mammals is different, as a single bipotential gonad can develop either into a testes or an ovary. If this genetic process which is largely complex gets disrupted during development of a human being it can lead to disorders of sex development. There are two separate processes by which sex development takes place namely i) sex determination whereby the bipotential gonad forms either a testes or ovaries and sex differentiation of internal and external genitalia along with extragonadal tissues like the brain. DSD’s may occur due to different genetic lesion pathways. In this review we have concentrated in details of various genetic pathways causing DSD’s and signal transduction pathways by which various manifestations of gonadal forms like gonadal dysgenesis to ovotestis and genital forms from mild hypospadias or clitoromegaly to ambiguous genitalia phenotype may result. Here we do not touch on sex differentiation of internal genitalia and external genitalia.

Keywords: DSD’s; Genetics; Signal Transduction System; Ambiguous Genitalia; Gonadal Dysgenesis; Ovotestis;

Introduction

In mammals sex development takes place in 2 different and sequential stages namely sex determination and sex differentiation [1]. Sex determination is determined by the complement of sex chromosomes with in an organism and implies that the mechanism which leads to:

- Specification of either a male or female gonad from single undifferentiated gonad [2]. The presence of a Y chromosome makes a bipotential gonad differentiate towards testis specific differentiation and formation of a Y specific gonad, while absence of a Y chromosome leads to.

- Development of an ovary, which is the female specific gonad. During the process of male sex determination, SRY expression located on the Y chromosome initiates a cascade of gene expression within sertoli cells which ultimately drives the morphological differentiation of the testis [3,4]. In sex differentiation secretion of endocrine and local factors like testosterone, dihydrotestosterone and AMH by the testis. And

- Development of male internal and external genitalia (prostate, vasdeferens, penis and scrotum and a reciprocral expression of the mullerian ducts which are the precursors of female internal genital structures (fallopian tubes, uterus and vagina) [5,6]. Disorders of Sex Development (DSD’s) occur when the tightly regulated process is disrupted which occurs primarily due to the result of genetic mutations which interfere with either the development of the testes or ovaries or the action of endocrine and local factors in extragonadal tissues [7,8]. Recent concept of human sex determination and data from mouse models which have added to the understanding of mammalian gonadal development is discussed.

Classification of DSD’s

The Consensus Statement on Management of Intersex disorders was drawn up by world experts and patient advocates under the auspices of the Pediatric Endocrine Society and the European Society for Pediatric Endocrinology in 2006. According to this consensus conference it was decided to review clinical management practices in DSD’s given new advances in diagnostic, introduction of modified and non surgical techniques studies of psychosexual factors which are specific to challenges in clinical practice voiced by patient advocacy organizations. One main recommendation was to not use terms which cause stigma like intersex, pseudohermaphroditism, hermaphroditism, sex reversal [7,8]. Rather this panel aid to label all deviations from sex development to be used under the heading of DSD’s whose definition was congenital condition in which development of chromosomal, gonadal or anatomic sex is atypical*. Within this definition DSD’s can have a wide range of gonadal phenotypes like partial or complete gonadal dysgenesis and ovotestis and external genitalia phenotype like hypospadias clitoroomegaly and ambiguous genitalia or fully masculinized or feminized genitalia which are discordant with karyotype or gonadal phenotypes. With this definition, DSD’s occur in approximately 1 in 100 live births.
birth [8,9]. But the incidence of 46 XY gonadal dysgenesis in which genetic mutations=>testicular disruption is estimated at 1 in 10,000 live births. Neither the distribution of genetic mutation with in these patients, nor the longterm consequences of diagnostic and management challenges associated with 46XY Gonadal dysgenesis are well understood. To understand these issues big multicenter trials, both in usa and Europe are ongoing [10,11,12,13].

Normal Sexual Differentiation

The gender identity of a person (whether an individual identifies as development of a male or a female) is the result of genetic, chromosomal, and morphological sex as influen ced by the environment of the individual. It includes behavior of any sexual connotation like body gestures and manerisms, habits of speech, recreational preferences and contents of dreams, sexual expression, both homosexual and heterosexual, which can be regarded as the result of all influences on the individuals, both prenatal and postnatal. Specifically gender identity is the result of the following determinants, genetic sex, gonadal sex, the internal genitalia, the external genitalia, the secondary sexual characteristics that appear at puberty and the role assigned by society in response to all the developmental manifestations of sex.

Prenatally sexual differentiation follows a specific sequence of events. 1st is the establishment of genetic sex, 2nd under the control of the genetics the gonads differentiate, determining the hormonal environment of the embryo, the differentiation of the internal duct system and the formation of the external genitalia. It has been apparent that the embryonic brain is also sexually differentiated, similar to that which determines sexual development of the external genitalia. The induction influences of hormones on the CNS can have an effect on the pattern of hormone secretion and sexual behavior in the adult [13,14,15,16,17,18,19,20].

Gonadal Differentiation

Both the X and Y chromosomes evolved from autosomal ancestors in a 300 million year timeline [21]. Most of the ancestral genes on the Y chromosomes deteriorated leaving only a limited number of currently active genes. The male specific area of the Y chromosome encompasses almost all of the active genes (small parts are identical to the corresponding regions of the X chromosome and more importantly the Y chromosome contains the gene that is essential for testicular development.

In human embryos the gonads begin development during the 5th week of gestation as protruabnces overlying the mesonephric ducts. The migration of primordial germ cells into the gonadal ridges occurs between 4-6 weeks of gestation. Although germ cells do not induce gonadal development, if the germ cells fail to arrive, gonads do not develop and only the fibrous streaks of gonadal agenesis will exist. At 6 weeks of gestation age 4 weeks after ovulation the gonads are indifferent but bipotential, possessing both cortical and medullary areas and capable of differentiation into either testes or ovaries. They are composed of germ cells, specially epithelia (potential granulosa/sertoli cells). mesenchyme, potential theca/leydig cells)and the mesonephric duct system, wolffian and mullerian ducts exist side by side, external genitalia are undifferentiated [22]. Subsequent sexual differentiation requires direction to various genes with a single gene determinant on the Y chromosome Testes Determining Factor (TDF) necessary for testicular differentiation beginning at 6-7 weeks of gestation [23]. About 95% of the length of the Y chromosome previously known as the nonrecombining and the male specific region encodes 27 distinct proteins that are male specific involved in testicular function [21]. Deletions in this area consist of material that originated in the X chromosomes over the past few million years.

The distal ends of the short arms of the X and Y chromosome are known as pseudoautosomal regions because during meiosis the homologous distal short arms of the X and Y Chromosomes pair and interchange of genetic material occurs as in autosomal regions. There are actually 2 pseudoautosomal regions in the Y chromosomes, one at the terminal region of short arm and one at the end of the long arm but exchange of genetic material is more common with the short arm [24]. The genes in the pseudoautosomal regions are doubly present in both sexes and therefore escape X inactivation. Gene deletion in this area of the X chromosome (X22.3) are associated with various conditions known as continuous gene syndrome: short stature, mental retardation, X linked ichthysis, Kalmanns syndrome.

Testis

The testes determining gene is located on the distal short arm of the Y, immediately adjacent to the pseudoautosomal region. Loss of the TDF gene causes gonadal dysgenesis. Transfer of the TDF gene to the X results in an XX male. Since the identification of the Y chromosome importance to male differentiation over 4 decades ago 3 proteins have been suggested as the Y encoded, gene-expressed testes determining factor. The 1st was the HY histocompatibility antigen and the 2nd, ZFY (a zinc finger protein). Both were abandoned because of inconsistencies of expression in various cell types (in XX males and XY females) as well as absent expression in indistinguishable males with testes. SRY (sex determining region Y) is almost certainly the true sex determining region in the short arm of the Y chromosome, the only gene on Y Chromosome required for sex determination [25]. SRY is a single exon gene located in the smallest Y chromosome region capable of sex reversal, is expressed in the genital ridge only during the appropriate time of embryonic development. When testicular cord forms. It is deleted or mutated in cases of human XY females; it is present in 46 XY males and it can sex reverse XX mice into males [26,27]. The 204 amino acid protein product contains a 79 aminoacid domain with e motif sharing by recognized family of transcription factors of the high mobility group (HMG) that bind to DNA and regulate gene transcription. The HMG of transcription factors operate by binding and bending DNA, a dynamic conformational mechanism.

Investigations of the DNA binding properties of testosterone to estradiol that is down regulated in the male embryo and AMH.
Steroidogenic Factor1 (SF1) and DAX1 are nuclear receptors for which specific ligands have not been identified (orphan receptors). SF1 influences the expression of genes which encode steroidogenic enzymes and AMH and when genetic expression of SF1 is disrupted in mice gonads and adrenals fail to develop [39,40,41]. SF1 (in the mouse), the gene encoding SF1 has been designated FtzF1 expresses a direct gonadal differentiation. Later its expression in the leydig cells is important for testosterone production and in the adrenal hypoplasia and DAX 1 is believed to work with SF1 in regulating development and function of steroid producing tissues [42]. SF1 and DAX1 interactions are complicated and necessary for the transcription of steroid producing tissues [33]. SF1 and DAX1 interactions are complicated and necessary for the transcription of steroid producing tissues [42]. SF1 and DAX1 interactions are complicated and necessary for the transcription of multiple genes involved in normal sex determination and normal adrenal development and function.

The WT1 gene is named after wilm’s tumor nephroblastoma, because it is one of the genes on chromosome 11 deleted in patients of the tumor. Mutant mice lacking WT1 fail to develop kidneys and gonads. WT1 mutations however could not be detected in 25 patients with a congenital absence of uterus and vagina, indicating that WT1 is necessary for normal renal and gonadal development but not early Mullerian duct development [43]. Phenotypic girls with renal disease may have a WT deficiency and an XY genotype.

Testicular differentiation begins at 6-7 weeks. 1st testes controls subsequent sexual development, therefore SRY presumably controls autosomal genes. Thus testicular hormones activate or repress genes to direct development away from an otherwise predetermined cause of female differentiation.

The SOX (SRY like box) genes are similar to the sequence of SRY but are not located on the Y chromosomes. DAX1 is involved in both early sex differentiation and normal gonadogenesis [39,40,41]. SF1 (in the mouse), the gene encoding SF1 has been designated FtzF1 expresses a direct gonadal differentiation. Later its expression in the leydig cells is important for testosterone production and in the adrenal hypoplasia and DAX 1 is believed to work with SF1 in regulating development and function of steroid producing tissues [42]. SF1 and DAX1 interactions are complicated and necessary for the transcription of steroid producing tissues [33]. SF1 and DAX1 interactions are complicated and necessary for the transcription of multiple genes involved in normal sex determination and normal adrenal development and function.

HCG stimulation produces leydig cell hypertrophy and peak fetal pH levels are seen after 15-18 weeks of pregnancy [44]. It is suggested that HCG stimulates steroidogenesis in the early fetal tests, so that androgens production will ensue and masculine differentiation can be accomplished [44]. However normal masculine differentiation in the mouse models lacking LH receptors and molecular evidence indicates that fetal Leydig cells (but not adult cells) respond to ACTH as well as HCG [45]. A primary role for ACTH is supported by the report of a male with inactivating mutation of gene for HCG/LH receptors who develop other sexual development in time and a functionally active testis controls subsequent sexual development, therefore SRY presumably controls autosomal genes. Thus testicular hormones activate or repress genes to direct development away from an otherwise predetermined cause of female differentiation.

Genes other than SRY are also required for proper gonadal development. These autosomal genes regulate migration of the germ cells and coding for the steroidogenic enzymes. The formation of testis precedes
female genitalia (lack of AMH) along with a vas deferens and epididymis (testosterone stimulation) [46].

In an XX individual with/without the active influence of a Y chromosome, the bipotential gonad develops into an ovary about 2 weeks later than testicular development. The cortical zone develops and contains germ cells, whereas the medullary portion regresses with its remnants being the rete ovarii, a compressed nest of tubules and leydig cells into the hilus of the ovary. Normal ovarian differentiation requires the presence of germ cells indicating some forms of communication between germ cells and the somatic cells. The germ cells proliferate by mitosis, reaching a peak of 5-7 million by 20 weeks of pregnancy. By 20 weeks the fetal ovary achieves mature compartmentalization with primordial follicles containing oocytes, initial evidence of follicular and an incipient stroma. Degeneration (atresia) begins even earlier by birth, only 1-2 million germ cells remain. These have become surrounded by a layer of follicular cells forming primordial follicles with oocytes that have entered the 1st meiotic division. Meiosis is arrested in the prophase of the 1st meiotic division until reactivation of follicular growth that may not occur until years later. Excessively rapid atresia (germ cell attrition) in gonadal dysgenesis (45X) accounts for the streak gonads seen in these cases [47]. A complete 46XX complement is necessary for ovarian development [48]. The 2nd X chromosome, therefore contains elements essential for ovarian development and maintenance.

Genetic Pathways of sex determination

Testis

In humans levels of SRY mRNA are upregulated in the urogenital ridge at 7 weeks after conception and drive the bipotential gonad towards testis formation in 46XY individuals [49,50]. Following translation the SRY protein translocates to the nucleus and binds to the enhancer region of SOX 9 to drive the differentiation and proliferation of sertoli cells and testis tubule organization [51,52,53]. Definitive evidence that SRY is the initiating factor in human male sex determination was provided by the discovery of deletions in the S’ or 3’ regulatory regions of SRY which alter the timing or levels of SRY expression and cause 46XY gonadal dysgenesis [3,54,55,56,57].

SRY related transcription factor SOX 9 is the 2nd major gene involved in male sex determination. In humans autosomal dominant mutations in SOX 9 causes campomelic dysplasia with a 46XY DSD and external genitalia ranging from ambiguous genitals to a female phenotype [58,59]. SOX9 protein expression is required for testis determination and in conjunction with SRY and the transcription factor NR5A1, the SOX9 protein binds to its own promoter to perpetuate a positive feedback loop, which maintains high levels of SOX9 expression. In mouse models, SOX 9 mRNA expression is also maintained by activation of the Fgf9-fgfr2 and prostaglandin D2(PGD2) signaling pathway [60,61,62,63]. Clinical syndromes associated with skeletal dysplasias have been described for mutations in FGFR2 [64]. However mutations which result in gonadal dysgenesis and DSD’s in humans have not been identified as yet in components of the FGF9-FGFR2 or PGD2 signaling pathways which suggests that phenotypes associated with such mutations might result in embryonic lethality or have redundant functions in the genetic network which drive sex determination.

A number of mutations in the genes encoding additional transcription factors which are involved in sex determination have been involved in human DSD’s. NR5A1 (also called steroidogenic factor, or SF1) encodes an orphan nuclear receptor, which is a major contributor to the development of the hypothalamo-pituitary-gonadal-adrenal axis [65,66,67]. High expression of Nr5a1 in the mouse bipotential gonad suggested a role for this gene in cell proliferation before the onset of testis determination and in the upregulation of both Sry and Sox9 gene expression [68]. Initial reports brought into light the involvement of Nr5a1 mutations in gonadal and adrenal dysgenesis in 46XY individuals having a female phenotype [67]. In presertoli cells NR5A1 synergizes the transcription factor GATA4 at the onset of testis determination and binds to the SRY promoter to upregulate SRY expression [69]. Further analysis of NR5A1 in individuals with DSD’s or with infertility issues has shown that mutations in this gene are associated with a variety of phenotypes or mild hypospadias to ambiguous genitalia along with infertility in adulthood [70,71,72,73]. Further studies have also shown that 46XY patients with mutations in SF1 who are phenotypically female may present with clitoromegaly which is secondary to increased testosterone levels at the onset of puberty despite their dysgenetic gonads [74].

NroB (also called DAX1) is present on the X chromosome (at p 21.3) and the NR5A1 encodes an orphan nuclear receptor which has a function in mammalian sex determination [75]. Duplications encompassing NROB1 in humans or transgenic overexpression of NroB1 in mice [75,76,77].

• Dose dependent XY gonadal dysgenesis and a female phenotype. As XY individuals have only one copy of this gene a duplication which result in DAX1 overexpression is enough to block testis determination. One of the molecular mechanisms identified in XY mice transgenic for NroB1 is through direct inhibition of Nr5a1 mediated transcription of SOX9 [77]. In 46XY female individuals, NroB1 gene are crucial for preventing testis formation. Loss of function mutations or deletions of NroB1.

• Congenital adrenal hyperplasia and life threatening adrenal failure which is associated with abnormalities of male genital development secondary to decreased steroidogenesis [78].

The GATA4 and ZFPM2 (also called FOG2) genes encode transcription factors which are critical for testis development. The discovery of a familial heterozygous missense mutations in GATA4 which resuted in a Gly 221 Arg mutation and development of a 46XY DSD which was associated with congenital heart disease, underscores the fundamental role of GATA4 in both gonad and cardiac development [79]. Genetic associations between 2 unrelated patients with rare mutations in ZFPM2 who also had 46XY gonadal dysgenesis is described recently which further amplifies the important role of GATA4 –ZFPM2 interactions in testis determination [80]. In mouse models mutations which...
disrupt association between Gata4 and Zfpm2 give rise to abnormal testis development [81]. In a porcine model GATA4 directly activated the SRY promoter, however in humans and mice, direct activation of SRY expression has only been observed when the WTI protein is coexpressed [81]. The studies in mice support the finding in patients with mutations in these genes and offer additional mechanistic insights into sex determination. Mutations in Gata4 and Zfpm2 led to reduced interaction between Gata4 and Zfpm2 protein which resulted in decreased ability of either gene (independently or when coexpressed) to activate transcription of target genes such as Amh, Sry and Sox9 [79,80,81].

Deletions which encompass the region surrounding the DMRT1 and DMRT2 genes which are located on chromosome 9p have been found in multiple cases of 46XY gonadal dysgenesis with ambiguous genitalia [82,83]. Fine mapping of this region has narrowed the minimal region associated with 46XY gonadal dysgenesis to a small 260kb region upstream of the DMRT1 and DMRT2 genes [82]. In addition to its role in somatic cell determination with in the gonads, a highly significant locus on chromosome 9p near DMRT1 was identified in genomic wide association studies performed in patients with gonadal germ cell tumours [84]. The finding that DMRT1 is associated with a propensity towards germ cell tumors is consistent with the phenotype of Dmrt1 knock out mice, which have profound failure in postnatal maintenance of the germline and on a specific genetic background also have increased susceptibility to gonadal germ cell tumor formation [85,86,87].

Within testis differentiation DMRT1 is critical for the maintenance of sertoli cell fate. Once the testis fate has been established the phenotype of the heterogenous cells within testis must be actively maintained. In mice postnatal expression of Dmr1 simultaneously promotes testis specific expression in sertoli cells through maintenance of high levels of Sox9 expression and repress ovary specific granulosa cells differentiation. Loss of Dmr1 from sertoli cells in the postnatal mouse testis leads to transdifferentiation of these cells into granulosa cells.

A person having multieoxic deletions in WWOX gene present on chromosome 16 reported with 46XY gonadal dysgenesis which suggested that the gene was involved in testis development [88]. Pathological analysis of gonads showed immature testis and the presence of premalignant gonadal germ cells [88]. With this phenotype there is a possibility that WWOX may function in the same development pathway as DMRT1 to promote somatic cell differentiation of sertoli cells and maintain germ cells. Additional studies in mice support the hypothesis as XY mice homzygous presence of some of testicular tissue within a 46XX gonad.

Ovary

Till the development of molecular and genetic tools in the last decade it was thought that ovarian sex determination was a passive default pathway which occurred in the absence of SRY expression has not been found for ovarian differentiation. Also very few morphological changes which suggest ovarian development have been found to occur at the developmental time point when a XY biotential gonad begins to develop the organizational development of the testis.

Though there is no visible morphological changes, gene expression in XX somatic cells in the biotential gonad have been shown to drive differentiation of granulosa cells steroid producing theca cells [90]. The main signals for initiation of granulosa cells differentiation are not clear. However high mRNA levels of the signaling factors WNT4 and RSPO1 upregulate expression of and stabilize the transcription factor CTNNB1 (also called β-catenin) which suppresses male specific Sox9 expression, maintains Wnt4 gene expression and promote germ cell proliferation [91,92,93,94].

Morphological differentiation of human ovaries take place at 7 week gestation age, the development stage when female germ cells enters 1st steps of meiosis. Much the knowledge of this developmental process has been gained by animal model studies. In mice female germ cells meiosis gets triggered by cell extrinsic factors like retinoic acid and expression of Stra 8 [95,96]. During the point of high levels of Wnt4 signalling through any of Frizzled or Lrp5-Lrp6 receptors drive both germ cells meiosis and differentiation of theca cells [97]. Just like the developing testis following the point of fetal ovarian determination, the ovarian phenotype and granulosa cell differentiation are actively maintained by the expression of FoxL2 protein and the expression of the estrogen receptors α and β [98,99,100]. Loss of FoxL2 expression in mouse adult granulosa cells.

- Upregulation of Sox2 along with trans differentiation however with male sex determination, initial ovarian determination independent on Wnt4and Rsop1 signalling the ovarian phenotype needs other proteins like Fox L2 and the estrogen receptors.

Failure of correct ovarian development in individuals with the 46XX gonadal dysgenesis

- One of the 2 distinct states, gonadal dysgenesis or the presence of some of testicular tissue within a 46XX gonad. Gonadal dysgenesis has been suggested to have a distinct aetiology from that of a DSD, however it is believed that failure of ovarian development, especially during the early stages is similar to 46XY gonadal dysgenesis. The primary difference between 46 XX and 46 XY gonadal dysgenesis is that, in the former case, the phenotype of the internal and external genitalia is congruent. In contrast in persons having 46 XY karyotype there is some degree of phenotypic variability of the external genitals which can range from hypospadias to a female phenotype and can include ambiguous genitalia [7].

Gonadal dysgenesis associated with the 46 XY karyotype manifests clinically as primary ovarian insufficiency defined as premature depletion of ovarian follicles and onset of menopause before 40yrs of age [101]. This condition is represented by a range of phenotypes from individuals who have not entered puberty before 40yrs of age [102]. This condition has varying phenotypes from cases who have not entered puberty till 15yrs of age to those who experience early cessation of ovulation called secondary
amenorhea [102,103]. Though primary ovarian insufficiency can result due to different nongenetic causes like autoimmunity, chemotherapy and environment factors a genetic basis is thought to be the main cause in 10-15 % of all cases [104,105]. Female sex determination is closely linked to the starting of meiosis in germ cells [106]. The commonest genetic cause of gonadal dysgenesis in a phenotypic female is 45 X Turner Syndrome, which occurs in 1 in 2000 live births [107]. The follicle depletion seen among patients with 45 X Turner Syndrome can occur both from haploinsufficiency of critical genes on the X chromosomes which escape X inactivation and from incorrect pairing of the X chromosomes during meiosis. Deletions/mutations affecting expression of the autosomal gene FOXL2 can cause 46 XX gonadal dysgenesis with blepharophimosis, ptosis and epicanthus inversus syndrome [BPES] in humans and goats [108,109]. There are 2 types of BPES in humans i. e. BPES type I which is a sex limited autosomal dominant form with full spectrum of disease. This severe phenotype occurs from truncating mutations which:

- Haploinsufficiency of FOXL2 protein [108]. BPES type II is limited to the blepharophimosis phenotype, occurs in both male and female persons and has no gonadal phenotype. Subsets of patients having this limited form of the disease have small duplications within the FOXL2 gene [110]. Rarely both BPES type I and II can be present with in a single family [110]. Autosomal dominant mutations in the nuclear orphan receptors NR5A1.

- Can also cause POF in 46XX patients and account for <3% of all genetic cases of this condition [48,111,112]. Besides FOXL2, ovarian determination is dependent on the presence of functional WNT4 or functional RSPO1 [113]. Genetic mutations in the WNT4gene can cause a wide range of functional effects including impaired lipid modification, defects in receptor signaling and aggregate formation [114,115].

Testicular or ovotesticular DSD’s which are associated with the 46 XX genotype are rare and arise due to ectopic expression of SOX family genes which are related to the major testis determining gene SRY within the fetal bipotential gonad. Upto 90% of isolated 46XX testicular DSD involve a translocation of SRY to the X chromosome or an autosome [116]. Part of remaining cases can be explained by rare duplications or deletions in the promoter and enhancer region of SOX genes including SOX9, SOX3P and SOX10 [117,118,119,120,121,122,123,124]. In these cases testicular tissue develops and causes some bit of masculinization of both internal and external genital structures. Individuals with rare syndrome 46 XX testicular or ovotesticular DSD’s have been shown to harbor mutations in RSPO1 [71].

Main points of Sex Determination

I. Migration of primordial germ cells to the urogenital ridge.

II. Differentiation of the bipotential gonadal tissue under the direction of WT1 and SF1

III. SRY activation of male specific genes, especially, SOX9, to produce the testes by cell proliferation, migration and vascularization

IV. Ovarian differentiation by suppression of SOX9 through the activity of DAX1 and Wnt4

V. Gonadal streaks without germ cells in XX or XY individuals (female phenotype): Deficiencies in WT1 or SF1

VI. Lack of testicular development in XY individuals, pure gonadal dysgenesis (female phenotype): Deficiencies in SRY or SOX9

VII. Male phenotype in a 46XX individual: Presence of SRY

VIII. Mixed gonadal dysgenesis in mosaics (varying phenotype): Excess DAX1

Sex Determining Pathways

Although transcription factors have a key role in male sex determination, other factors and pathways have recently been recognized an essential part during this process.

Signal Transduction Pathways

Different studies have been done regarding cellular proliferation in bipotential gonads and during time before sexually dimorphic changes occur in sex determination. At the onset of male sex determination, the WNT4 and RSPO1 signalling pathway become sexually dimorphic with downregulation of WNT4 and RSPO1 expression and upregulation of SOX9 expression in the developing testis [70,71,73].

Mitogen Activated Protein Kinase (MAPK) signaling is the dominant pathway in testis determination. Heterozygous missense mutations in MAP3K1 have been shown in 6 cases of 46 XY DSD’s [125,126]. Also functional studies of human cell lines point that gain of function mutations in MAP3K1 shift the balance of sex determination from testis development (driven by SOX9-FGF9 signalling) toward ovarian development (mediated by WNT4 and β-catenin signaling) [127]. Studies on mice have demonstrated that phosphorylation of the transcription factor Gata4 is regulated through MAP3K1 signalling. This pathway might also regulate Sry expression during fetal gonadal development via modulation of chromatin structures [128].

Also mutation in the signaling pathway Desert Hedgehog(DHH) pathway have also been described in a small subset of patients with mini fasicular neuropathy [129,130]. Also autosomal recessive mutations in the Hedgehog Acetyl Transferase (HHAT) have been found in a patient with 46 XY DSD with gonadal dysgenesis and chondroplasia [131]. Gonad specific deletions in Hhat mice occur in abnormal testistubule formation, decreased gonadal size and testicular dysgenesis. Increased expression of S0X9 and Cyp11a1, which encodes a steroidogenic enzyme marker of Leydig cell in the mice showed that differentiation of both sertoli and Leydig cells was affected, however Sry expression remained normal, which indicated that these disruptions happen downstream of Sry expression [131].

In female sex determination WNT4 and RSPO1 signalling via β-catenin is critical for normal ovarian development. In human dominant missense mutations in WNT4 were shown to be associated with mullerian aplasia and hyperandrogenism, a
finding which is supported by the phenotypes of female Wnt4 deficient mice[91,113,114]. Rare recessive mutations in WNT4 cause SERKAL syndrome which is associated with multiple development anomalies in the kidneys, adrenal gland lungs as well as testicular DSD’s with ambiguous genitalia in persons having 46 XX Karyotype [132]. In Wnt4 knockout mice findings seen before human discoveries showed early loss of germ cells and increased expression of male steroidogenic enzymes, which:

- Elevated androgens levels during wolffian duct formation [91]. Studies have shown that Wnt4 also functions to upregulate retinoic acid signals that both initiate the onset of meiosis in germ cells and maintain germ cells throughout adult life [94,95,133].

Along with WNT4, RSPO1 is upregulated in the developing human ovary and missense mutations in this gene causes syndromic forms of 46 XX testicular and ovotesticular DSD’s which are associated with palmo-plantar hyperkeratosis and a predisposition for development of squamous cell carcinoma in the skin [92,94]. Findings in mouse model suggest that Rspo1 is needed for ovarian determination in both normal XX individuals and in people with XY sex reversal [37,92]. Therefore the interaction between signaling pathways and transcription factors in ovarian determination is without clear hierarchy unlike that which exists with SRY signaling in testis determination is that multiple pathways are needed to program the heterogeneous group of cells with in the differentiating gonads.

Conclusions

Classification of patients with DSD’S has become largely changed with the use of multidisciplinary steps as well as huge research networks which make us understand the pathophysiology of DSD’S much better. By Giving a constant framework for diagnosis, clinicians can start to find patients having similar phenotype and get at bigger understanding of medical, social as well as psychological factors, which lead to the total well being of patients having DSD’s with the rarity of humans having DSD’s, different multicentre studies are needed for finding methods which will lead to consistent diagnosis and optional medical care for these people. Even though the focus on identification of geneic mutations which lead to altering the process of sex determinion and sex differentiation gender by itself may not fully explain the vast range of health or psychological issues which might be experienced by a person having a DSD. Yet mutations found in critical developmental genes have given important insight into pathophysiological mechanisms of DSD’s along with means of classification which can be used to follow the long term effects of genetic mutations and treatment outcomes in these patients. Multiple lines of evidence suggest that newly identified mutations would have deleterious effect on protein function. Examples of this are given here. Borges et al investigated the effects of IMAGE(Intrauterine Growth Restriction Metaphysial dysplasia, adrenal hypoplasia and genital anomalies)associated mutations on protein stability, cell cycle progression and cell proliferation. Mutations in the PCNA binding site of CDKN1C significantly increased CDKN1C protein stability and prevent cell cyle progression in the S Phase. IMAGE mutant CDKN1C protein decreased cell growth significantly, which:
more than both the wild type or BWS protein. [134]. Bramble et al. used whole exome sequencing to study mutant protein function in case of FSHR mutation, being a rare cause of 46XX gonadal dysgenesis with primary amenorrhea due to hypogonadotropic ovarian failure. They found cellular localization of FSHR protein as well as FSH stimulated cAMP production by using flow cytometry which demonstrated an average of 48% reduction in cell surface signalization. The mean fluorescent signal of cAMP stimulated by FSH was decreased by 50 % in the mutant transfected cells.[135]. To study this altered protein function.

Davidson showed that application of synthetic small interfering RNA Linked to the vector peptide Penetratin 1= a rapid, very effective uptake of siRNA by populations of cultured primary mammalian hippocampal and sympathetic neural networks[136]. Thus we have focused just on the genetics of sex determination without trying to go into abnormalities of sex differentiation e.g defects in androgen biosynthesis which are also known causes of DSD’s [137,138,139].

References

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64. Wong M, Ramayya MS, Chrousos GP, Driggers PH, Parker KL. Cloning and


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