UNIVERSITE SIDI MOHAMMED BEN ABDELLAH
FACULTE DE MEDECINE ET DE PHARMACIE
FES

GENETIC MECHANISMS AND MANAGEMENT OF PATIENTS WITH DISORDERS OF SEX DEVELOPMENT: COMPREHENSIVE OVERVIEW OF THE EXPERIENCE IN HASSAN II UNIVERSITY MEDICAL CENTER- GENETICS/ ONCO-GENETICS UNIT.

THESIS PRESENTED BY:

Dr. KHADIJA BELHASSAN

BORN AT FES, 11 MARCH 1982

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PROFESSOR KARIM OULDIM

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SUMMARY

Human sex-determination (SD) involves complex mutually antagonistic genetic interactions of testis- and ovary-determining pathways. It is clear now that male and female development is achieved through the repression of the alternative state. A gene determining the formation of a testis may function by repressing the female state and vice-versa. Disorders of sex development (DSD) are a heterogeneous group of complex conditions that can affect chromosomal, gonadal, and/or phenotypical sex. In addition to impacts on internal and external genitalia, these conditions can affect fertility potential to various degrees.

Throughout a descriptive retrospective study conducted in the Unit of medical genetics and Onco-Genetics, about 75 cases referred for their DSD management between May 2010 and January 2016 were studied. We were able to highlight the importance of genetic testing tools available in our unit in the establishment of the diagnosis, and management of the received cases of DSD. Nevertheless, the diagnosis of some disorders was difficult through our available means of genetic testing.

Many genes mutated in DSD encode transcription factors characterized by a strictly regulated spatiotemporal expression. Hence, it can be hypothesized that at least part of the missing genetic variation in DSD can be explained by non-coding mutations in regulatory elements that alter gene expression.

Thus, we highlighted here the importance of genetic data generated through, microarray, large scale sequencing, exome and genome sequencing. The impending surge of new genetic data on human sex-determination from sequencing projects will create opportunities for the development of mechanistic models that will clarify how the system operates and importantly provide data to understand how selection and developmental processes interact to direct the evolution of SD.

ABBREVIATION

- **AIS**: ANDROGEN INSENSITIVITY SYNDROME.
- **AMH**: ANTI-MULLERIAN HORMONE.
- **AR**: ANDROGEN RECEPTOR.
- **CAH**: CONGENITAL ADRENAL HYPERPLASIA.
- **CAIS**: COMPLETE ANDROGEN INSENSITIVITY SYNDROME.
- **CGD**: COMPLETE GONADAL DYSGENESIS.
- **CGH**: COMPARATIVE GENOMIC HYBRIDIZATION.
- **CIS**: CARCINOMA IN-SITU.
- **CMA**: CHROMOSOMAL MICROARRAY.
- **DNA**: DESOXYRIBONUCLEIC ACID.
- **DSD**: DISORDER OF SEX DEVELOPMENT.
- **FISH**: FLUORESCENT IN SITU HYBRADIZATION.
- **GCT**: GERM CELL TUMORS.
- **HRM**: HIGH RESOLUTION MELT.
- **LH**: LUTEINIZING HORMONE.
- **OTDSD**: OVO-TESTICULAR DISORDER OF SEX DISORDER.
- **PAIS**: COMPLETE ANDROGEN INSENSITIVITY SYNDROME.
- **PCR**: POLYMERASE CHAIN REACTION.
- **PGD**: PARTIAL GONADAL DYSGENESIS.
- **PMDS**: PERSISTENT MULLERIAN DUCT SYNDROME.
- **SD**: SEX-DETERMINATION.
- **STS**: SEQUENCE TAGGED SITES.
- **TDF**: TESTIS-DETERMINING FACTOR.
- **TDSD**: TESTICULAR DISORDER OF SEX DISORDER.
- **WES**: WHOLE-EXOME SEQUENCING.
- **WGES**: WHOLE-GENOME AND- EXOME SEQUENCING.
- **WGS**: WHOLE-GENOME SEQUENCING.

INTRODUCTION

Disorders of gonadal and sexual development can arise from errors at any of the major steps of normal sex determination. These conditions ranging from gonadal abnormalities to complete incompatibility between chromosomal and phenotypic sex, are now collectively termed disorders of sex development (DSD). [**L. Nussbaum et al, 2016**].

They are among the most common birth defects; worldwide, 1 in 4500 babies are born with significant ambiguous genitalia, and DSDs are estimated to account for over 7% of all birth defects. [**L. Nussbaum et al, 2016**].

The management of such disorders is complex, and one of the most crucial decision is represented by gender assignment. In fact, the primary goal in DSD is to have a gender assignment consistent with the underlying gender identity in order to prevent the distress related to a forthcoming Gender Dysphoria. **[Our team experience].**

Historically, gender assignment was based essentially on surgical outcomes, assuming the neutrality of gender identity at birth. This policy has been challenged in the past decade refocusing on the importance of prenatal and postnatal hormonal and genetic influences on psychosexual development**. [Fisher AD et al 2016].**

The experience in Hassan II University Medical Center Genetics/Onco-Genetics Unit, does not escape these challenges. Thus, and given the complexity of this disorders and their management, DSD individuals and their families need to be supported by a specialized multidisciplinary team, which has been universally recognized as the best practice for intersexual conditions. **[Our team experience].**

It should always be taken into account that every DSD person is unique and has to be treated with individualized care. In this perspective, international registries are crucial to improve the understanding of these challenging conditions and clinical practice, and in providing a better prediction of gender identity. **[Our team experience].**

THEORY PART

I-DEFINITION AND CLASSIFICATION:

1- Definition:

Disorders of sex development (DSD) are rare congenital anomalies with atypical chromosomal, gonadal or anatomical sex organ development. DSD is a generic term introduced in 2005 **[Chicago consensus conference]** that applies to different congenital conditions, many of which are characterized by the unusual appearance of external genitalia and/or atypically developed gonads with potential negative consequences on psychosexual development, fertility and cancer risk. The same consensus meeting tried classifying these complex conditions according to their underlying chromosomal profiles **[Mouriquand P and al 2014].**

2- Classification:

DSD is currently classified into three main groups **[Table 1]**:

The 46,XX DSD patients are individuals who are genetically female, most commonly due to Congenital Adrenal Hyperplasia (CAH), and present with an overdeveloped genital tubercle (clitoris), no vaginal connection to the perineum and enlarged and merged genital folds. The internal genitalia are female and usually normal.

The 46,XY DSD patients are genetically male and constitute a more heterogeneous group, representing a spectrum from normal appearing females to males with hypospadias and infertility. These patients may have underdevelopment of the genital tubercle (hypospadias and/or micropenis) with or without undescended gonads, with or without feminine remnants (mullerian structures). Within this group are patients with dysfunctional gonads (gonadal dysgenesis), impaired steroidogenesis (17 beta hydroxysteroid dehydrogenase deficit), dysfunctional central hormonal control, and dysfunctional target tissues (androgen insensitivity, 5 alpha reductase deficiency)

The chromosomal abnormalities or mosaicisms are mostly represented by the 45,X0/46,XY individuals (mixed gonadal dysgenesis). This group comprises the ovo-testicular DSD. These two last groups of patients bear both female and male genetic and anatomic elements. They typically have asymmetric genitalia with one side more

masculine and the other side more feminine. The gonads can be testis, ovary or both, or dysgenetic gonads with a high risk for gonadal tumor development later in life. **[Andres Calvo et al 2016].**

Table 1: **DSD Classification currently adopted [Andres Calvo et al 2016].**

46,XX DSD

A: Disorders of gonadal (ovarian) development

1. Ovotesticular DSD
2. Testicular DSD (e.g., SRY+)
3. Gonadal dysgenesis

B: Androgen excess

1. Fetal (e.g., 21 hydroxylase deficiency, 11 hydroxylase deficiency)
2. Fetoplacental (aromatase deficiency)
3. Maternal (luteoma, exogenous, etc.)

C: Other (e.g., cloacal extrophy, vaginal atresia, other syndromes)

46,XY DSD

A: Disorders of gonadal (testicular) development

1. Complete gonadal dysgenesis (Swyer syndrome)
2. Partial gonadal dysgenesis
3. Gonadal regression
4. Ovotesticular DSD

B: Disorders in androgen synthesis or action

1. Androgen biosynthesis defect (e.g., 17-hydroxysteroisd dehydrogenase deficiency, 5a reductase deficiency)
2. Defect in androgen action (e.g., CAIS, PAIS)
3. LH receptor defects (e.g., Leydig cell hypoplasia, aplasia)
4. Disorders of AMH and AMH receptor (Persistent Mullerian Duct Syndrome)

C: Other (e.g., severe hypospadias, cloacal extrophy)

Sex chromosome DSD

A: 45,X (Turner Syndrome and variants)

B: 47,XXY (Klinefelter Syndrome and variants)

C: 45,X/46,XY (Mixed Gonadal Dysgenesis, ovotesticular DSD)

D: 46,XX/46,XY (chimeric, ovotesticular DSD)

II-EMBRIOLOGY:

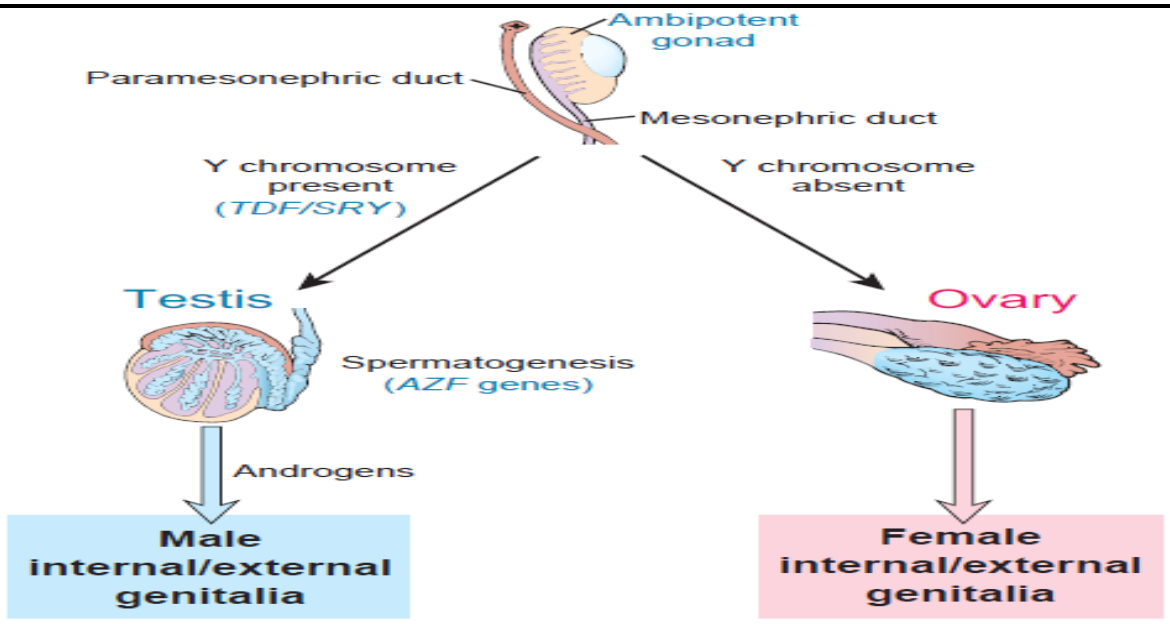
1- Genetic Differenciation:

Development of the genital system is one phase in the overall sexual differentiation of an individual. Sexual determination begins at fertilization, when a Y chromosome or an additional X chromosome is joined to the X chromosome already in the egg. This phase represents the genetic determination of gender. Although the genetic gender of the embryo is fixed at fertilization, the gross phenotypic gender of the embryo is not manifested until the seventh week of development. Before that time, the principal morphological indicator of the embryo's gender is the presence or absence of the sex chromatin (Barr body) in the female. The Barr body is the result of inactivation of one of the X chromosomes. During this morphologically indifferent stage of sexual development, the gametes migrate into the gonadal primordia from the yolk sac. **[Bruce M. Carlson 2014]**

2- Gonadal Differentiation:

By the sixth week of development in both sexes, the primordial germ cells have migrated from their earlier extraembryonic location to the paired genital ridges, where they are surrounded by the sex cords to form a pair of primitive gonads. Up to this time, the developing gonad is ambipotent, regardless of whether it is chromosomally XX or XY. Development into an ovary or a testis is determined by the coordinated action of a sequence of genes in finely balanced pathways that lead to ovarian development when no Y chromosome is present but tip to the side of testicular development when a Y is present. Under normal circumstances, the ovarian pathway is followed unless a particular Y-linked gene, originally designated testis-determining factor (TDF), diverts development into the male pathway. If no Y chromosome is present, the gonad begins to differentiate to form an ovary, beginning as early as the eighth week of gestation and continuing for several weeks; the cortex develops, the medulla regresses, and oogonia begin to develop within follicles. Beginning at approximately the third month, the oogonia enter meiosis I, but this process is arrested at dictyotene until ovulation occurs many years later. In the presence of a normal Y chromosome (with the TDF gene), however, the medullary tissue forms typical testes with seminiferous tubules and Leydig cells that, under the stimulation of chorionic gonadotropin from the placenta, become

capable of androgen secretion. Spermatogonia, derived from the primordial germ cells by successive mitoses, line the walls of the seminiferous tubules, where they reside together with supporting Sertoli cells, awaiting the onset of puberty to begin spermatogenesis **[Fig.1]. [L. Nussbaum et al, 2016].**



[Fig.1]. Scheme of developmental events in sex determination and differentiation of the male and female gonads from the ambipotent gonad.

3- Phenotypic Differentiation:

While the primordial germ cells are migrating to the genital ridges, thickenings in the ridges indicate the developing genital ducts, the mesonephric (also called wolffian) and paramesonephric (also called müllerian) ducts, under the influence of hormones produced by specific cell types in the developing gonad. Duct formation is usually completed by the third month of gestation. In the early embryo, the external genitalia consist of a genital tubercle, paired labioscrotal swellings, and paired urethral folds. From this undifferentiated state, male external genitalia develop under the influence of androgens, beginning at around 12 weeks of gestation. In the absence of a testis (or, more specifically, in the absence of androgens), female external genitalia are formed regardless of whether an ovary is present.

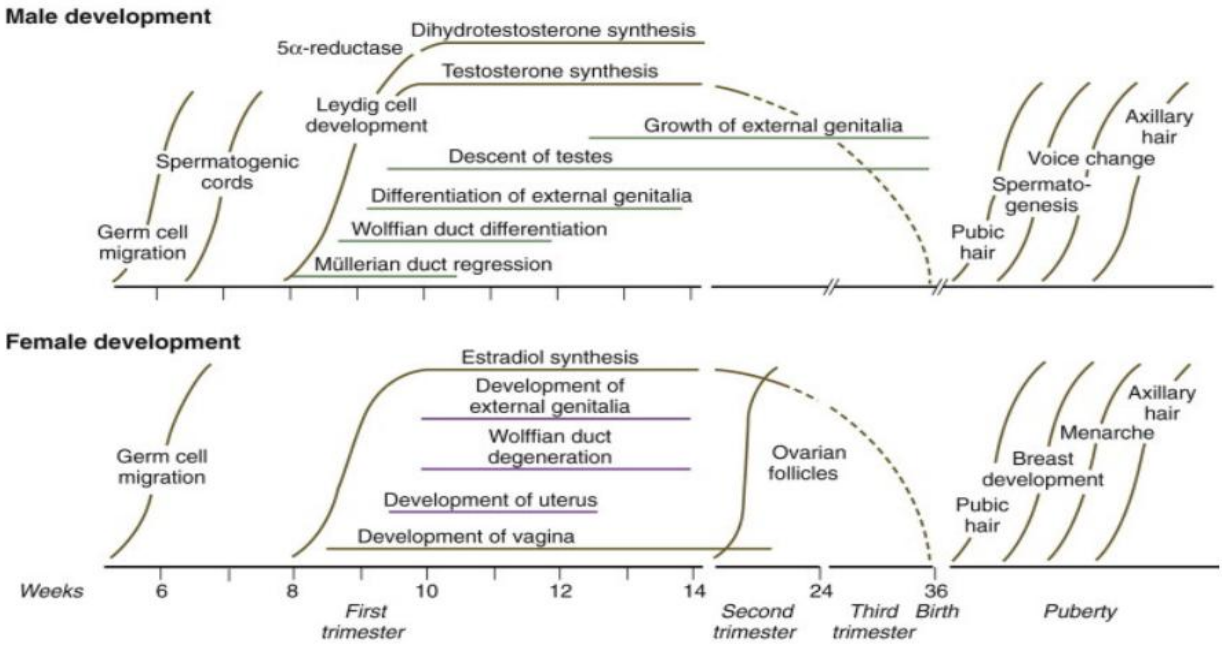
The phenotypic differentiation of gender is traditionally considered to begin with the gonads and progresses with gonadal influences on the sexual duct systems. Similar influences on the differentiation of the external genitalia and finally on the development of the secondary sexual characteristics (e.g., body configuration, breasts, hair patterns) complete the events that constitute the overall process of sexual differentiation. **[L. Nussbaum et al, 2016].**

4- Behavioral Differentiation:

Psychological and behavioral sex difference is related to Sexual differentiation of the brain. Historically, it was believed that such differences were solely due to gonadal hormone secretions. Yet, emerging research is also implicating direct genetic effects of the X and Y chromosomes specifically SRY gene. Under certain circumstances, an individual's genetic gender can be overridden by environmental factors so that the genotypic sex and the phenotypic sex do not correspond. **[Tuck C Ngun et al 2011]**

5- Major events in the sexual differentiation of male and female human embryos:

[Fig.2] resume the major events in the sexual differentiation of male and female human embryos. **[Bruce M. Carlson 2014]**:



[Fig. 2]: Major events in the sexual differentiation of male and female human embryos.

III-Disorders of Sex Development and their Characteristics:

[Table 2] resume the major characteristics of disorders of sex developments **[L. Nussbaum et al, 2016].**

Disorder	Gonadal Sex	Phenotypic Sex	Characteristics
Sex chromosome DSDs			
Klinefelter syndrome	Testes (dysgenetic)	Male	Gonadal dysgenesis; hypogonadism; azoospermia
Turner syndrome	Ovary (streak gonads)	Female	Gonadal dysgenesis; amenorrhea
46,XX testicular DSD	Testes (bilateral)	Normal male (≈80%) or ambiguous (≈20%)	Most present clinically after puberty with small testes, gynecomastia, azoospermia
46,XX ovotesticular DSD	Testicular and ovarian tissue (ovotestis or one of each)	Ambiguous	Uterus may be present; surgery often required to repair external genitalia; raised as male or female
46,XY DSD	Testes (dysgenetic)	Ambiguous	Variable müllerian structures; penoscrotal hypospadias; risk for gonadoblastoma; raised as male or female
46,XY complete gonadal dysgenesis	Undeveloped streak gonads; no sperm production	Female	Normal müllerian structures; risk for gonadoblastoma
46,XY partial gonadal dysgenesis	Regressed testes	Variable (male, female, or ambiguous)	Ambiguous external genitalia with or without müllerian structures; raised as male or female
45,X/46,XY mixed gonadal dysgenesis	Asymmetric (dysgenetic testis and streak gonad)	Variable (male, female, or ambiguous)	Variable phenotype, ranging from a typical (short) male to Turner syndrome female; risk for gonadoblastoma

[Table 2]: DSD, Disorder of sex development. Summarized from Achermann JC, Hughes IA: Disorders of sex development. In Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, editors: *Williams textbook of endocrinology*, ed 12, Philadelphia, 2011, WB Saunders, pp 886-934; and Pagon RA, Adam MP, Bird TD, et al, editors: GeneReviews [Internet]. Seattle, 1993- 2013, University of Washington, Seattle, http://www.ncbi.nlm.nih.gov/books/NBK1116/.

1- Disorders of Gonadal Development:

Gonadal dysgenesis refers to a progressive loss of germ cells, typically leading to underdeveloped and dysfunctional (“streak”) gonads, with consequent failure to develop mature secondary sex characteristics. Gonadal dysgenesis is typically categorized according to the karyotype of a patient. Complete gonadal dysgenesis (CGD)—as in the case of XX males (now formally designated 46,XX testicular DSD) or XY females (now formally designated 46,XY CGD)—is characterized by normal-appearing external genitalia of the opposite chromosomal sex. Cases with ambiguous external genitalia are

said to have partial gonadal dysgenesis. Gonadal dysgenesis can also be associated with sex chromosome DSD; it is a consistent feature of Turner syndrome, and patients with a 45,X/46,XY karyotype have mixed gonadal dysgenesis. **[L. Nussbaum et al, 2016].**

a- Disorders Associated with a 46,XY Karyotype:

The overall incidence of these conditions is approximately 1 in 20,000 live births. Although a number of cytogenetic or single-gene defects have been demonstrated, many such cases remain unexplained. Approximately 15% of patients with 46,XY CGD have deletions or mutations in the SRY gene that interfere with the normal male pathway. However, most females with a 46,XY karyotype have an apparently normal SRY gene. **[L. Nussbaum et al, 2016].**

b- Disorders Associated with a 46,XX Karyotype:

A series of phenotypes known as the 46,XX testicular DSDs (previously termed XX sex reversal) are characterized by the presence of male external genitalia in individuals with an apparently normal 46,XX karyotype. The overall incidence is approximately 1 in 20,000. Most patients have a normal male appearance at birth and are not diagnosed until puberty because of small testes, gynecomastia, and infertility, despite otherwise normal-appearing male genitalia and pubic hair. Most of these patients are found to have a copy of a normal SRY gene translocated to an X chromosome as a result of aberrant recombination. Those 46,XX males who lack an SRY gene, however, are a clinically more heterogeneous group. Approximately 15% to 20% of such patients are identifiable at birth because of ambiguous genitalia, including penoscrotal hypospadias and cryptorchidism (undescended testes); there are no identifiable müllerian structures, and their gender identity is male. A somewhat smaller percentage of patients, however, have both testicular and ovarian tissue, either as an ovotestis or as a separate ovary and testis, a condition known as 46,XX ovotesticular DSD (formerly called true hermaphroditism). Patients with either testicular DSD or ovotesticular DSD who lack a translocated SRY gene have been the subject of intense investigation to identify the responsible genetic defect(s). **[L. Nussbaum et al, 2016].**

2- Ovarian Development & Maintenance:

Ovarian maintenance typically lasts for up to five decades in normal females. Loss of normal ovarian function before the age of 40, as seen in approximately 1% of women, is considered premature ovarian failure (or premature ovarian insufficiency). It has long been thought that two X chromosomes are necessary for ovarian maintenance, because 45,X females, despite normal initiation of ovarian development in utero, are characterized by germ cell loss, oocyte degeneration, and ovarian dysgenesis. Further, patients with 47,XXX or with cytogenetic abnormalities involving Xq, as well as carriers of fragile X syndrome, frequently show premature ovarian failure. Because many non-overlapping deletions on Xq show the same effect, this finding may reflect a need for two structurally normal X chromosomes in oogenesis or simply a requirement for multiple X-linked genes. **[L. Nussbaum et al, 2016].**

3- Disorders of Sex Development Involving Phenotypic Sex:

Patients described earlier illustrate a mismatch between their chromosomal sex and their gonadal sex, frequently leading to gonadal dysgenesis. In contrast, individuals with 46,XX or 46,XY DSD have gonadal tissue that matches their chromosomal sex. However, their mismatch lies in the establishment of phenotypic sex: here, their internal and/or external genitalia show features that are contrary to those expected normally for those of the given chromosomal and gonadal sex. Thus patients with 46,XX DSD have a 46,XX karyotype with normal ovarian tissue but with ambiguous or male genitalia. And those with 46,XY DSD have a 46,XY karyotype and testicular tissue but with incompletely masculinized or female external genitalia. On this basis, patients of both types were thus previously described as having “pseudohermaphroditism,” a term no longer in use. **[L. Nussbaum et al, 2016].**

a- Virilization of 46,XX Infants: Congenital Adrenal Hyperplasia:

These patients include those who have 46,XX karyotypes with a normal uterus and ovaries but with ambiguous or male external genitalia due to excessive virilization. The majority of such patients have congenital adrenal hyperplasia (CAH), an inherited

disorder arising from specific defects in enzymes of the adrenal cortex required for cortisol biosynthesis and resulting in virilization of 46,XX infants. In addition to being a frequent cause of female virilization, CAH accounts for approximately half of all cases presenting with ambiguous external genitalia. Ovarian development is normal, but excessive production of androgens causes masculinization of the external genitalia, with clitoral enlargement and labial fusion to form a scrotum-like structure. Although any one of several enzymatic steps may be defective in CAH, by far the most common defect is deficiency of 21-hydroxylase, which has an incidence of approximately 1 in 12,500 births. Deficiency of 21-hydroxylase blocks the normal biosynthetic pathway of glucocorticoids and mineralocorticoids. This leads to overproduction of the precursors, which are then shunted into the pathway of androgen biosynthesis, causing abnormally high androgen levels in both XX and XY embryos. Whereas 46,XX infants with 21-hydroxylase deficiency are born with ambiguous genitalia, affected 46,XY infants have normal external genitalia and may go unrecognized in early infancy. Of patients with classic 21 hydroxylase deficiency, 25% have the simple virilizing type, and 75% have a salt-losing type due to a mineralocorticoid deficiency that is clinically more severe and may lead to neonatal death. A screening test developed to identify the condition in newborns is now in use in many countries. Prompt medical, surgical, and psychosocial management of 46,XX CAH patients is associated with improved fertility rates and normal female gender identity. **[L. Nussbaum et al, 2016].**

b- Incomplete Masculinization of 46,XY Infants: Androgen Insensitivity Syndrome:

In addition to disorders of testis formation during embryological development, causes of DSD in 46,XY individuals include abnormalities of gonadotropins, inherited disorders of testosterone biosynthesis and metabolism, and abnormalities of androgen target cells. These disorders are heterogeneous both genetically and clinically, and in some cases they may correspond to milder manifestations of the same cause underlying ovotesticular DSD. Whereas the gonads are exclusively testes in 46,XY DSD, the genital ducts or external genitalia are incompletely masculinized. There are several forms of androgen insensitivity that result in incomplete masculinization of 46,XY individuals. Here we illustrate the essential principles by considering the X-linked

syndrome known as androgen insensitivity syndrome (once known as testicular feminization). As the original name indicates, testes are present either within the abdomen or in the inguinal canal, where they are sometimes mistaken for hernias in infants who otherwise appear to be normal females. Although the testes in these patients secrete androgen normally, end-organ unresponsiveness to androgens results from absence of androgen receptors in the appropriate target cells. The receptor protein, specified by the normal allele at the X-linked androgen receptor (AR) locus, has the role of forming a complex with testosterone and dihydrotestosterone. If the complex fails to form, the hormone fails to stimulate the transcription of target genes required for differentiation in the male direction. The molecular defect has been determined in many hundreds of cases and ranges from a complete deletion of the AR gene to point mutations in the androgen-binding or DNA-binding domains of the androgen receptor protein. Affected individuals are chromosomal males (karyotype 46,XY) who have apparently normal female external genitalia but have a blind vagina and no uterus or uterine tubes. The incidence of androgen insensitivity is approximately 1 in 10,000 to 20,000 live births, and both complete and partial forms are known, depending on the severity of the genetic defect. In the complete form, axillary and pubic hair are sparse or absent, and breast development occurs at the appropriate age, but without menses; primary amenorrhoea is frequently the presenting clinical finding that leads to a diagnosis. Gender assignment is typically not an issue, and psychosexual development and sexual function (except for fertility) are that of a typical 46,XX female. **[L. Nussbaum et al, 2016].**

IV- Mechanism of Sex Determination in Humans: Insights from Disorders of Sex Development:

This Chapter will consider the gene mutations responsible for the non-syndromic forms of disorders of sex development (DSD) and how recent genetic findings are providing insights into the mechanism of sex determination. High throughput sequencing technologies are having a major impact on our understanding of the genetic basis of rare human disorders, including DSD. **[Anu Bashamboo et al 2016]**

A- Disorders of Gonadal Dysgenesis:

1- Gene Mutations and 46,XY Gonadal Dysgenesis (GD):

a- SRY:

Approximately 15% of 46,XY Gonadal Dysgenesis patients carry mutations in the testis-determining gene SRY **[McElreavey et al, 1999].** The human SRY protein is expressed in Sertoli cells and germ cells from the moment of testis determination until adulthood. SRY mutations are usually de novo, but some are inherited from a fertile father suggesting that these inherited mutant proteins retain partial biological activity, and the incomplete penetrance could be caused by stochastic effects around a threshold level of biological activity required for testis formation **[Phillips et al., 2011].** Although SRY mutations are usually associated with gonadal dysgenesis, a 46,XY woman with premature menopause was reported to carry a de novo p.Gln2Ter mutation **[Brown et al., 1998].**

b- SOX9:

A key master gene in gonadal development and the target of SRY signaling is the SOX9 gene on chromosome 17. SOX9 is normally expressed early in development in the genital ridge and is required for normal testis formation. Mutations in one copy of the SOX9 gene, typically associated with a skeletal malformation disorder called camptomelic dysplasia, lead to complete gonadal dysgenesis in approximately 75% of 46,XY cases. In the absence of one copy of the SOX9 gene, testes fail to form, and the ovarian pathway is followed instead. The phenotype of these patients suggests that the critical step for the male pathway is sufficient SOX9 expression to drive the formation of testes, normally after up-regulation by the SRY gene. In 46,XY CGD, with either a mutation in SRY or a mutation in SOX9, the levels of SOX9 expression remain too low for testis differentiation, allowing ovarian differentiation to ensue. **[L. Nussbaum et al, 2016].**

c- NR5A1:

As many as 10% to 15% of patients with a range of 46,XY DSD phenotypes carry mutations in the NR5A1 gene, which encodes a transcriptional regulator of a number of genes, including SOX9 and DAX1. These mutations are associated with inadequate

androgenization of external genitalia, leading to ambiguous genitalia, partial gonadal dysgenesis, and absent or rudimentary müllerian structures. **[L. Nussbaum et al, 2016].**

NR5A1 belongs to the subfamily of transcription factors known as nuclear receptor subfamily 5 (group A, member 1), which is highly conserved in vertebrates **[Morohashi et al., 1992].**

d- GATA4 & the Cofactor FOG2:

GATA4 belong to a class of evolutionarily conserved lineage-limited zinc finger transcription factors that participate in cell fate determination, proliferation, and maturation **[Zaytouni et al., 2011].** Both GATA4 and GATA6 are expressed in the somatic tissues of the embryonic testis **[Ketola et al., 1999].** GATA4 cooperatively interacts with NR5A1 to regulate the expression of genes critical for testis determination and differentiation **[Viger et al., 2008].** The abnormal physical interaction of GATA4 with the cofactor FOG2, present with severe testicular dysgenesis. FOG2 is a zinc finger cofactor that modulates the activity of GATA4 **[Zaytouni et al., 2011].** It is suggested that FOG2 is involved in the early stages of testis determination. Using exome sequencing, 2 independent cases, of 46,XY gonadal dysgenesis each with missense mutations in the FOG2 gene were identified **[Bashamboo et al., 2014].** There was no history of cardiac anomalies in either the patients or their families. Functional studies indicated that the failure of testis development in these cases could be explained by the impaired ability of the mutant FOG2 proteins to interact with GATA4. These studies established GATA4 and FOG2 mutations as causes of 46,XY DSD.

e- CBX2:

The Polycomb group (PcG) of proteins defines a subset of factors that physically associate and function to maintain the positional identity of cells from the embryo to adult stages. PcG has long been considered a paradigmatic model for epigenetic maintenance of gene transcription programs. Its complexes catalyze mono-ubiquitination of histone H2A on lysine 119 and tri-methylation of histone H3 on lysine 27.

A single patient with 46,XY gonadal dysgenesis and mutations in the human CBX2 gene has been reported. This was a 46,XY girl who carried 2 independent mutations in

CBX2; a paternally inherited and a maternally inherited mutation **[Biason-Lauber et al., 2009].** Histology of the gonads at 4.5 years revealed apparently normal ovaries. Although polycomb group proteins are traditionally regarded as transcriptional repressors, there is evidence that at least in some cellular or promoter contexts CBX2 acts as a transcriptional activator of NR5A1 and SRY expression **[Biason-Lauber et al., 2009]**. In the patient described above, the presence of apparently normal ovaries suggests that CBX2 actively represses fetal ovarian development in an XY individual in the early stage of gonad formation.

f- MAP3K1:

The mitogen-activated protein kinases (MAPKs) an evolutionarily conserved gene, historically, these were considered to be cellular housekeeping factors, and it was a surprise to find that mutations in at least some of its factors could generate gonad-specific phenotypes. In humans, mutations in MAP3K1 have been identified in cases of 46,XY DSD **[Loke et al., 2014].** The mechanism whereby MAP3K1 mutations cause a failure of testis determination is unclear. The phenotype associated with MAP3K1 mutations is 46,XY CGD, a phenotype that is also associated with mutations in the SRY gene. This suggests that MAP3K1 signaling is required for the early stages of testis determination in humans.**[Anu Bashamboo et al. 2016]**

g- DMRT1, an Evolutionary Conserved Sex-Determining Gene:

Deletions of terminal 9p are associated with monosomy 9p syndrome, which is characterized by intellectual disability together with a distinctive series of somatic anomalies, and in approximately 70% of 46,XY individuals anomalies of testis development are seen that range from a completely female phenotype to a male phenotype with hypospadias and/or cryptorchidism **[Ottolenghi and al, 2000].** Evidence to indicate that the key player in human testis determination is DMRT1 came through the identification of a de novo missense mutation in a patient with 46,XY CGD **[Murphy et al., 2015].** There were no other somatic anomalies in this healthy girl. The histology of the gonad was similar to that of an *SRY* mutation and showed no evidence of testicular material, suggesting that the mutation was indeed impacting on primary testis determination. It is suggested that the lack of testis determination seen in this patient is

due to a combination of haplo-insufficiency and dominant negative activity. It may be required for *DMRT1* mutation to be pathogenic (or penetrant) to show either dominant negative activity on the wild-type allele or alter the normal interactions of the protein. Which results in a failure of testis determination**. [Anu Bashamboo et al. 2016]**

h- DAX1:

The DAX1 gene in Xp21.3 encodes a transcription factor that plays a dosage-sensitive role in determination of gonadal sex, implying a tightly regulated interaction between DAX1 and SRY. Although production of SRY at a critical point in early development normally leads to testis formation, an excess of DAX1 resulting from duplication of the gene can apparently suppress the normal male-determining function of SRY, leading to ovarian development. **[L. Nussbaum et al, 2016].**

2- 46,XX Testicular & Ovotesticular DSD:

a- SOX Gene Mutations:

In recent years it has become evident that the ectopic expression of HMG-box containing proteins in the urogenital ridge at the moment of sex determination may result in testicular development in a chromosomal XX female. Although most cases of 46,XX TDSD/OTDSD are caused by the presence of the SRY gene, usually on the X chromosome, the remaining cases stay unexplained. As compared to 46,XY DSD, there are relatively fewer known causes of 46,XX DSD. In human, SOX3 loss-of-function mutations are associated with mental retardation and growth hormone deficiency **[Laumonnier et al., 2002].** However, some 46,XX SRY negative testicular DSD patients have been reported who carry rearrangements at the SOX3 locus on the X chromosome. **[Sutton et al., 2011].** Complete or partial duplications of chromosome 22 in 46,XX SRY negative individuals are associated with various degrees of masculinization **[Seeherunvong et al., 2004].** Further delimitation of the minimal region was demonstrated by a de novo duplication of 22q11.2q13 in a 46,XX SRY negative male with mild hypospadias, dysmorphic features, and hypotonia **[Polanco et al., 2010].** Human SOX10 maps to 22q13.1 and may be responsible for the phenotype**. [Anu Bashamboo et al. 2016]**

b- RSPO1/WNT4/β-Catenin Signaling:

Little is known about the genetic pathway(s) involved in human ovary development. In XX individuals, activation of the β-catenin signaling pathway by the proteins RSPO1 and WNT4 is necessary for granulosa cell differentiation leading to ovarian development. Stabilization of β-catenin by the RSPO1/WNT4 pathway results in transcription of its target genes. Mutations involving RSPO1 and WNT4 are associated with exceptionally rare syndromic forms of 46,XX testicular/ovotesticular DSD.**[Parma et al., 2006].** Mutations involving RSPO1 have not been reported in non-syndromic cases of testicular and ovotesticular DSD **[Anu Bashamboo et al 2016].**

c- NR5A1and 46,XX DSD:

NR5A1 is expressed in the granulosa cells of the early developing ovary**. [Anu Bashamboo et al 2016]**. Primary ovarian insufficiency, also termed premature ovarian failure, is defined by the arrest of normal ovarian function before the age of 40 years and includes premature menopause, primary and secondary amenorrhea as well as ovarian dysgenesis. Analyzing cases of 46,XX primary ovarian insufficiency for mutations in the NR5A1 gene leaded to the discovery of the NR5A1 p.Arg92Trp mutation. p.Arg92Trp mutation specifically results in testis formation in a chromosomal female background. The mutation involves a highly conserved amino acid residue. The p.Arg92Trp mutation is predicted to disrupt DNA binding. The p.Arg92Trp mutation could be associated either with inappropriate activation of testis-specific pathways in the ovary or with disruption of pathways that oppose testis development and maintain ovarian integrity. Specifically, the NR5A1 and β-catenin proteins physically interact to upregulate the expression of the NR0B1 (Dax-1) gene on the X chromosome **[Mizusaki et al., 2003].**

B- Ovarian Development & Maintenance:

Duplications of at least two genes SOX9 and SOX3 have been described, suggesting that increased levels of transcriptional regulators can overcome the absence of SRY and initiate the testis-specific pathway. Both gene duplications and regulatory mutations can increase the level of SOX9 expression to bypass the requirement for SRY. Similarly, duplications of the X-linked SOX3 gene, which is very closely related in

sequence to the SRY gene, can stimulate increased SOX9 expression, replacing the usual need for SRY. Nearly a dozen specific genes have been implicated in familial cases of premature ovarian failure and in various forms of 46,XX gonadal dysgenesis. **[L. Nussbaum et al, 2016].**

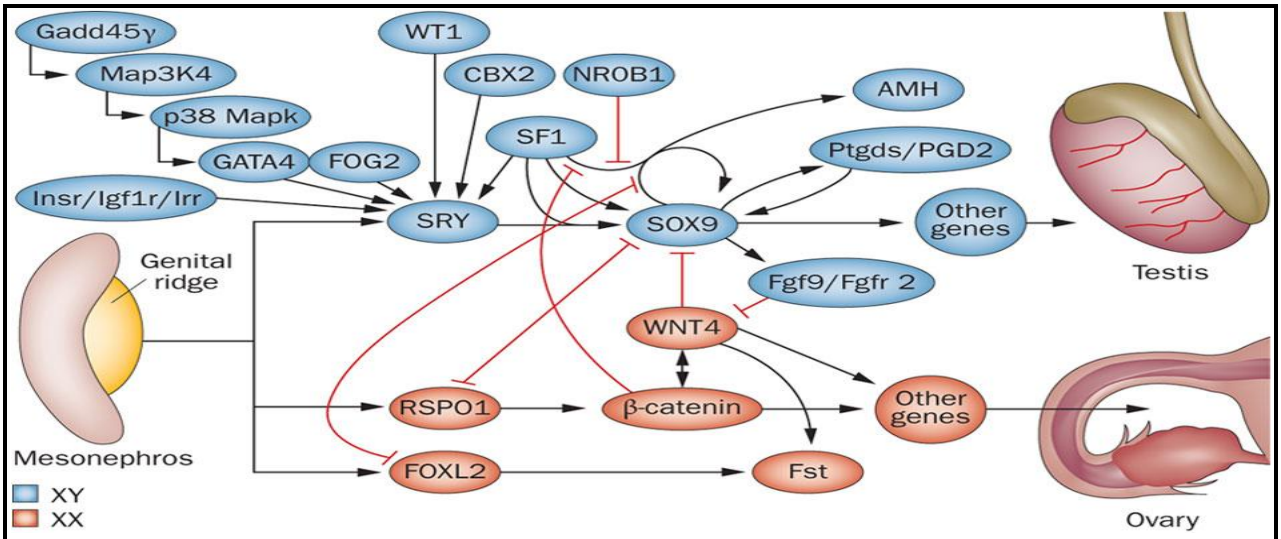
C- Non-coding Variation in Disorders of Sex Development:

Genetic studies in Disorders of Sex Development (DSD), representing a wide spectrum of developmental or functional conditions of the gonad, have mainly been oriented towards the coding genome. Application of genomic technologies, such as whole exome sequencing, result in a molecular genetic diagnosis in ~50% of cases with DSD. Many of the genes mutated in DSD encode transcription factors such as SRY, SOX9, NR5A1, and FOXL2, characterized by a strictly regulated spatiotemporal expression. Hence, it can be hypothesized that at least part of the missing genetic variation in DSD can be explained by non-coding mutations in regulatory elements that alter gene expression, either by reduced expression, misexpression or overexpression of their target genes. In addition, structural variations such as translocations, deletions, duplications or inversions can affect the normal chromatin conformation by different mechanisms, stressing in the importance of epigenetics as mechanism of DSD. **[Baetens D, et al. 2016].**

D- Gene Regulatory Networks in Embryonic Gonadal Development:

Genes shown in **[Fig.3];** are known to have a role in sex development on the basis of studies in humans and mice. Testis-related genes (blue) and ovary-related genes (red) are depicted in regulatory pathways leading to Sertoli-cell and granulosa-cell specification, respectively. Arrows do not necessarily imply direct actions. Sexual dimorphism is triggered in the XY genital ridge by the expression of SRY in the somatic cells at 6 weeks gestation in humans. In XY gonads, SOX9 expression is subsequently upregulated by SRY and SF-1 binding to TESCO. SOX9 is a key hub gene for testis development. SOX9 maintains its own expression through binding to TESCO with SF-1. SOX9 also regulates expression of genes required for testis formation such as AMH, FGF9, PTGDS and probably other genes. SOX9 also suppresses the expression of ovarian genes such as RSPO1 and FOXL2. Sexual dimorphism in the XX

genital ridge is triggered by R-spondin-1 (encoded by RSPO1) and FOXL2. WNT4, β-catenin and FST are also expressed in a female-specific manner, promoting ovarian development. Additionally, R-spondin-1, FOXL2, WNT-4 and β-catenin have roles in preventing differentiation of testis by repressing SOX9 expression. **[Ono, M et al, 2012]**



[FIG.3]. Gene regulatory networks in embryonic gonadal development

PRACTICAL PART

I- INTRODUCTION:

DSD has always been deriving a true drama for the families as well as for the patients .In most cases, the classical examinations that are clinical, biological or radiological, lead always only to the detection of the anomaly without being able to decide the etiology nor the definitive diagnosis, thing that only the medical genetics can detect thanks to the progress of the molecular biology which offered more explicit examinations. DSD can be detected in antenatal stage during systematic consultation of gynecologist-obstetrician via ultrasound or often noted clearly at birth, such as sexual ambiguities. Others are unperceived during childhood and appear only in adult stage, it is the case of the patients with primary amenorrhea, puberty delay, sterility, or unmatched sexual characters with the assigned civil sex. As soon as the disorder is recognized, it is necessary to determine the final sex as soon as possible in order to eliminate any psychological and social trauma. These individuals as their families must be assumed by multidisciplinary team which will decide, according to several considerations, tests to be performed, diagnostic to retain and consequently, the therapeutic approaches to follow or even surgical decisions to take. Throughout the study of 75 cases, we could highlight the role of each genetic diagnostic method in the establishment of the diagnosis, emphasize some DSD etiologies and the therapeutic approach of some others.

1- Material & Methods:

a- Patients Inclusion Criteria:

It is a retrospective descriptive study conducted at the medical genetics and Onco-Genetics unit of Hassan II University Medical Center, this work concerns 75 cases. We included all the patients who required the realization of a karyotype for clinical presentation suggestive of DSD. The work is founded on the exploitation of the clinical records of the cases referred between May 2010 and January 2016. We classified our patients, according to the new classification of the DSD, as follows:

- ❖ Chromosomal abnormalities: 55 patients
- ❖ Abnormalities 46, XY DSD: 9 patients
- ❖ Abnormalities 46, XX DSD: 11 patients

b- **Genetic analysis tools used**:

The karyotype was performed in all patients by culturing the blood cells for 72 hours on a complete culture medium made up by bovine fetal serum, gentamicin sulfate, L-glutamine and phyto-hemagglutinin. The cells are then blocked in metaphase by the colchicine. After a hypotonic shock with the KCI, they are fixed by an acetic acid methanol mixture. The chromosomal preparations obtained are analyzed in a semi-automatic way thanks to specialized software coupled to a camera.

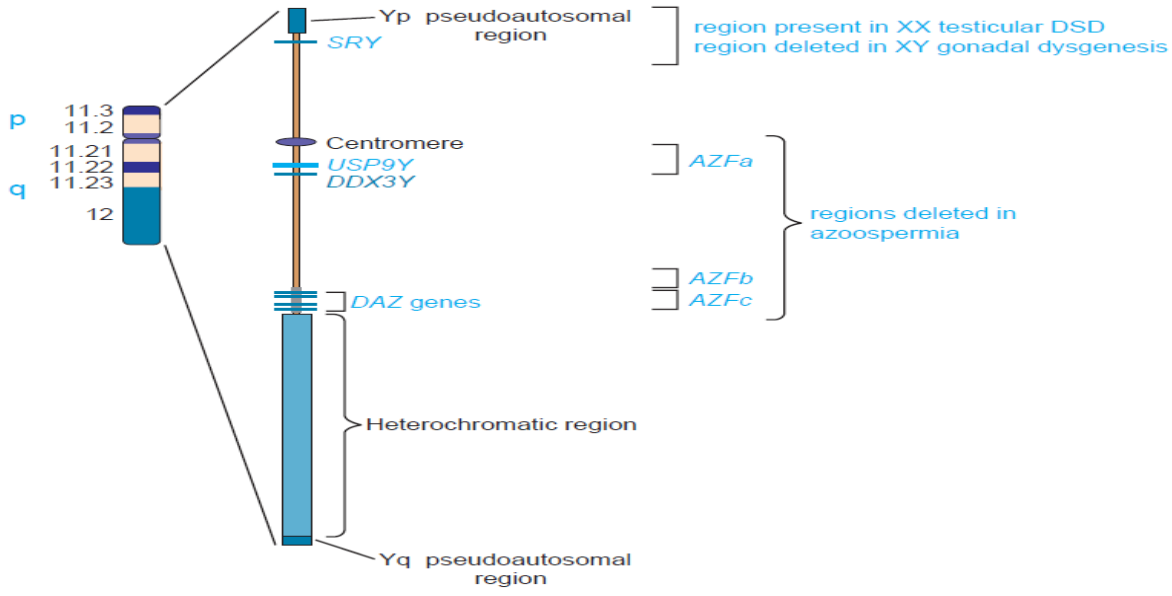
The technique of FISH using the probe Vysis CEP X (DXZ1) Spectrum Green / Vysis LSI SRY Probe Spectrum Orange has been applied in some cases according to the clinical indication: This technique was used to detect:

- Homogeneous monosomy and mosaïsm in Turner syndrome,
- Mixed gonadal digenesis,
- Presence of a triple X syndrome,
- Chimerism and finally,
- Double Y syndrome.

For the SRY gene, we carried out two types of PCR:

- ❖ The multiplex PCR in which we used in the same mix two pairs of primers, amplifying SRY and DXS1684 locus. This first PCR was to detect the presence of the SRY gene.
- ❖ PCR sequencing, HRM box for a more targeted molecular analysis.

We also analyze the micro deletions of the Yq area in men presenting azoospermia or severe oligo-asthenospermia with normal chromosomal formula. Most of these deletions are found in three distinct areas, designated under the terms of AZFa, AZFb and AZFc. AZFc is the zone being the most frequently object of deletions. **[Fig.4].** These deletions are detected by PCR technique using specific anonymous sequences of the Y chromosome (Sequence Tagged Sites, STS) or specific starter's candidate's genes. We used the Kit Y chromosome AZF Analysis System from Promega.



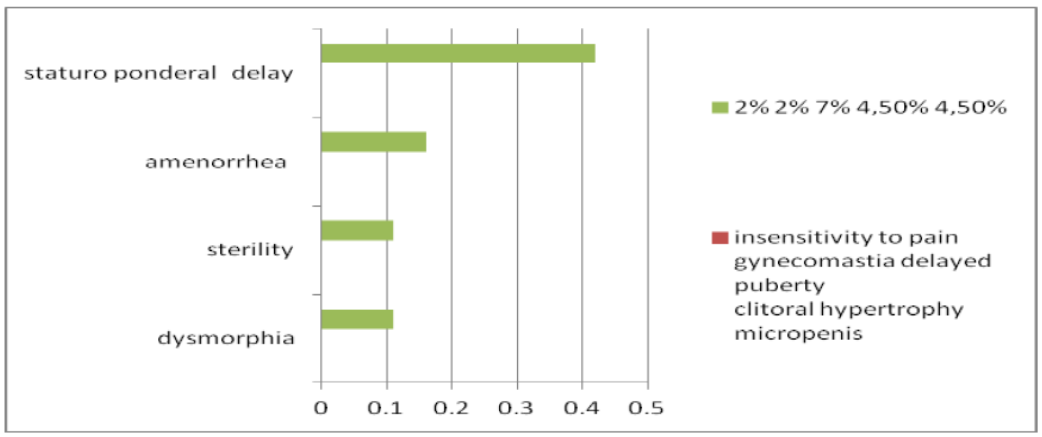
[Fig.4]. The Y chromosome in sex determination and in disorders of sex development (DSDs). Individual genes and regions implicated in sex determination, DSDs, and defects of spermatogenesis are indicated. **[L. Nussbaum et al, 2016].**

2- Results:

75 patients suspected of DSD were included in the study. This number, in no case represents the actual frequency of this category of disorders in our population as the tendency of patients is to hide their diseases to be protected from the stigma. Parents of children with DSD exhibit overprotective parenting and perceive their child as being vulnerable. These emotions and behaviors exhibited by parents may limit the medical consultation rate in our population.

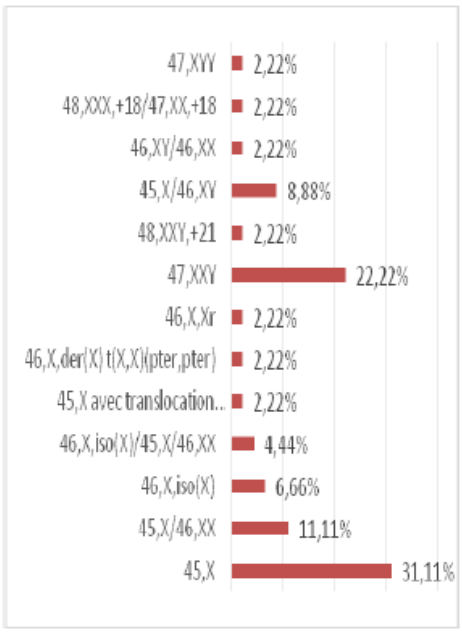
a- First Group: Chromosomal Abnormalities: 55 Patients:

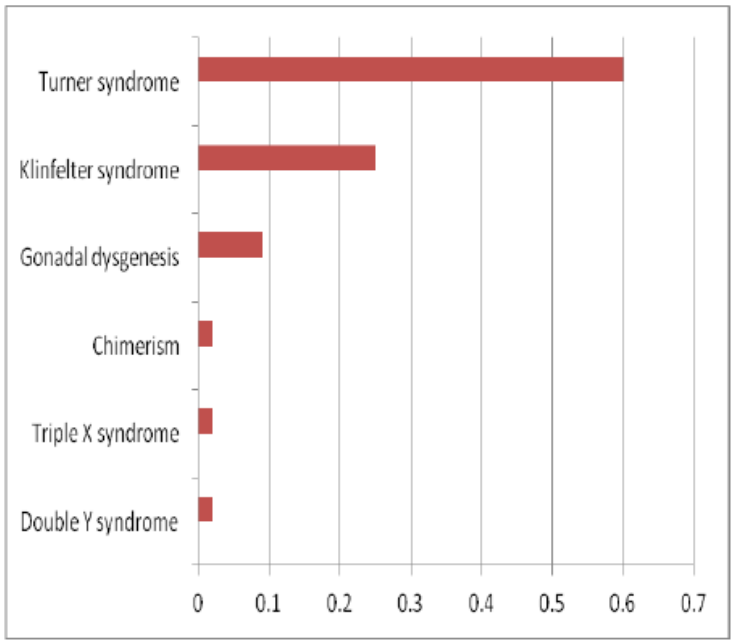
The median age of diagnosis in this group is 18.18 years; it is relatively high compared to the age of diagnosis of the all cases in our study (14.3 years). This can be explained by the delayed manifestations of these disorders. We note a female prevalence (69% of the cases), against 29% for the male civil sex and 2% for the unspecified sex. 82.2% of the cases result from a non-consanguineous marriage. The most common presenting sign in female sex cases is the staturo-ponderal delay. It is found in 55%. In the second place comes the amenorrhea (23%) and the dysmorphic features (13%). Among patients of male sex, sterility is present in 38%, then comes the stature ponderal delay (15%), and the micropenis (15%). **[Fig.5]**



[Fig.5]. Distribution of the patients of group 1 according to the reason of consultation.

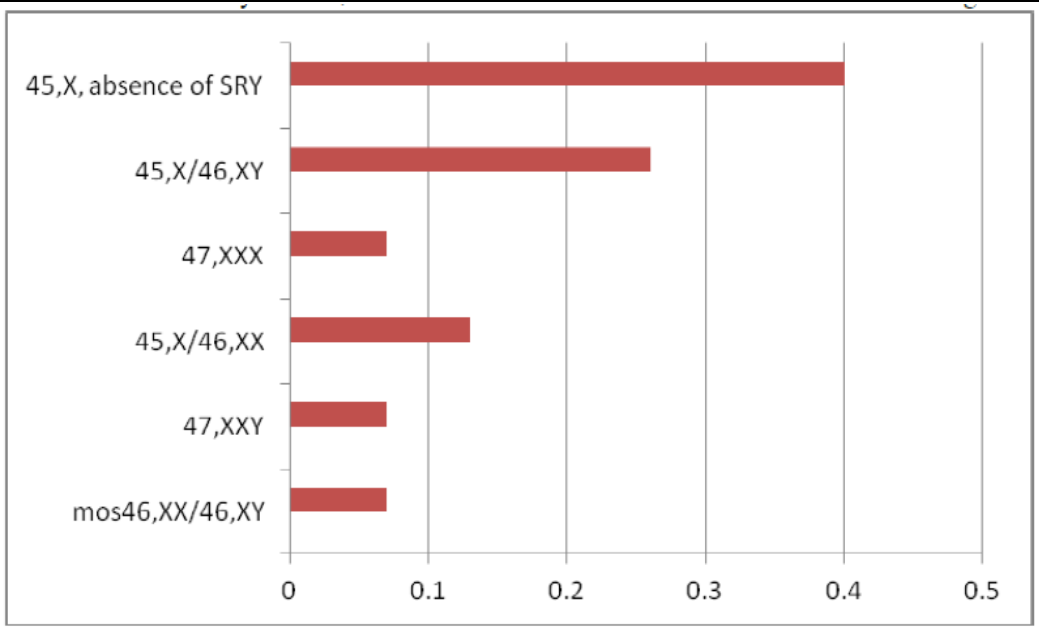
The cytogenetic analysis demonstrates well the clear prevalence of monosomy X (31.11%), followed by the chromosomal formula 47, XXY. **[Fig.6].** Thus, Turner syndrome is retained at 60%, followed by Klinefelter syndrome (25%). The percentage of the mixed gonadal disgenesis is considerable (9%). The chimerism, the triple X and the double Y syndromes are minority in our sample, they are represented by a single case for each one, and represent 2% each. **[Fig.7].**





[Fig.6]. [Fig.7]. Karyotyping results in Group 1, and distribution of Group 1 patients according to their final diagnosis.

FISH analysis was performed for 33.33% of the cases particularly those with Turner's syndrome, the results obtained are listed in **[Fig.8]**:

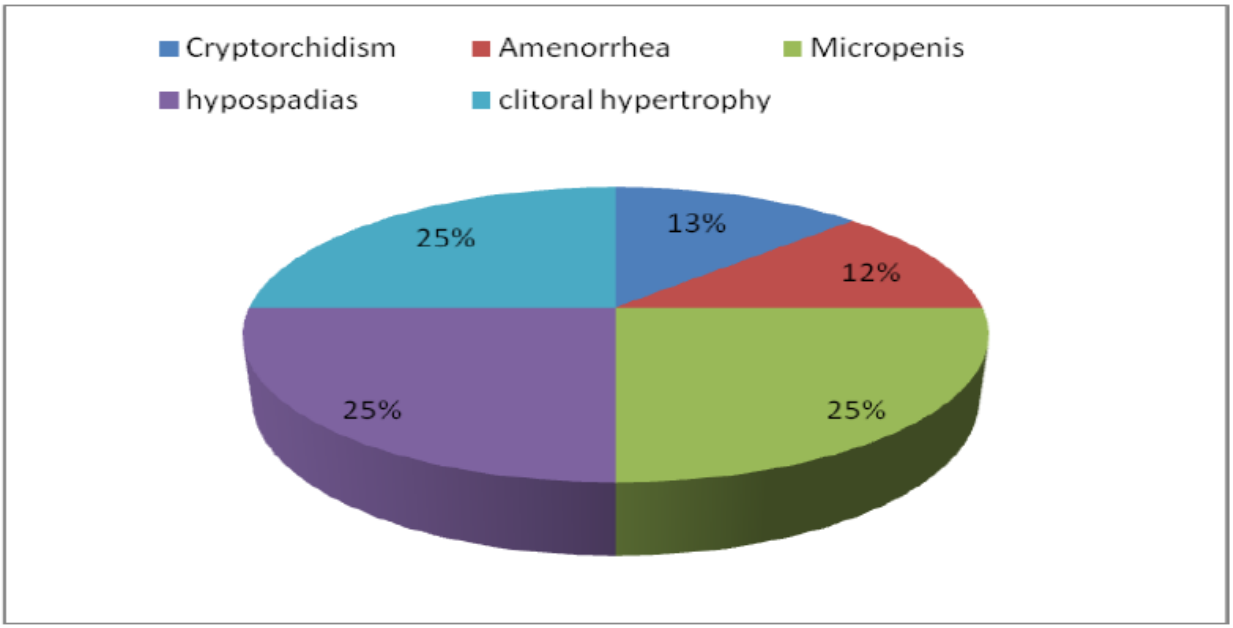


[Fig.8]. Group 1 Patients distribution based on the results of FISH.

We note a diversity of results according to the indication of the test. The absence of the Y chromosome is noted at 40% of the cases. The exploration of AZF area was carried out among patients presenting azoospermy with normal chromosomal formula (7% of the cases). No microdeletion was found.

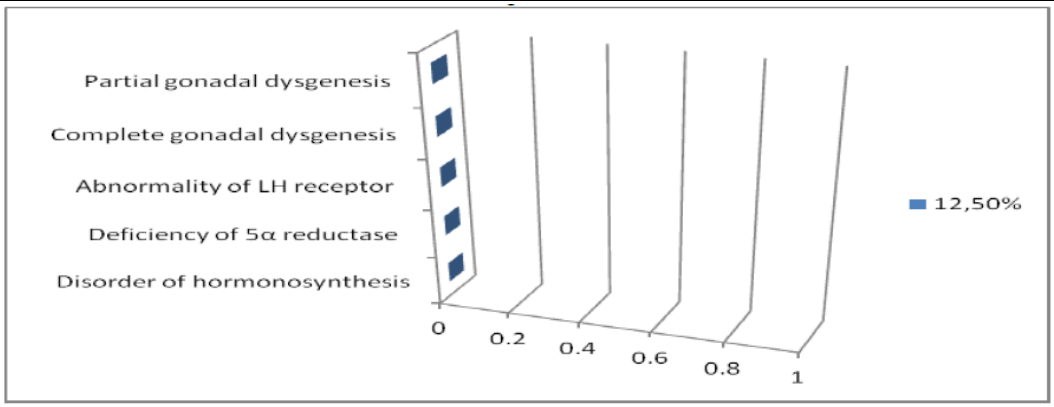
b- <u>Group 2: DSD 46, XY (9 cases):</u>

The average age of diagnosis in this group is 5.25 years. It largely exceeds the 3 years limit, which could involve significant psychological impact and poor social integration. 87.5% are from unspecified sex, 12.5% of female sex and no patient of male sex. Consanguinity is reported at 75% of the cases. The hypospadias, the micropenis and clitoral hypertrophy are the most frequent reasons for consultation, secondly comes amenorrhea and clitoral hypertrophy. They can be isolated or associated with other symptoms. **[Fig.9]**



[Fig.9]. Group 2 Patients distribution according to the reason of consultation.

The Karyotype shows the chromosomal formula 46, XY in all cases .The molecular analysis confirms the presence of SRY gene in 6 cases. The 5 α reductase deficiency is retained in 37.5% of cases. **[Fig.10].**

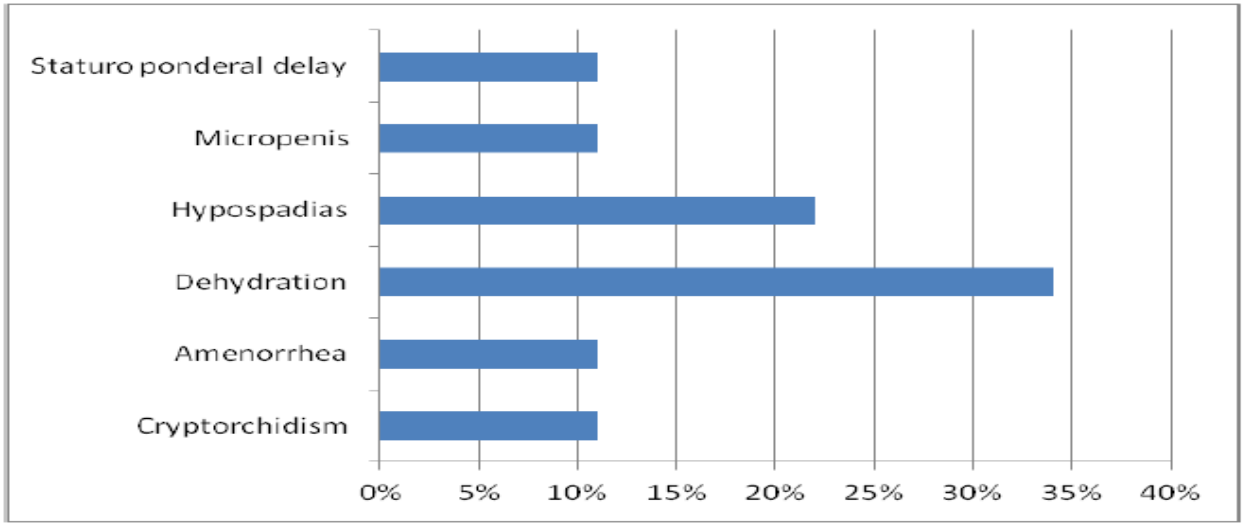


[Fig.10]. Group 2 Patients distribution according to the final diagnosis.

c- Group 3: DSD 46, XX. (11 Cases):

In this last group, the diagnostic average age is 3.22 years. 89% of the cases have an unspecified sex and 11% a female civil sex. 67% of the patients are from a non-consanguineous marriage. The most common presenting sign is acute dehydration

(34%), and then comes the hypospadias (22%). These warning signs are most often associated with other signs. **[Fig.11]**



[Fig.11]. Group 3 patients distribution according to the reason of consultation.

The karyotype was carried out for all the cases; the common result was 46, XX. The molecular analysis of gene SRY shows its presence at 11% of cases (male XX with positive SRY). The congenital adrenal hyperplasia accounts for 89% of the diagnoses retained in this group.

3- Discussion:

a- Discussion Of Statistical Results:

❖ **Incidence/ Prevalence Of DSD According To The International Statistics:**

There is very limited data available on the precise incidence of DSD worldwide. This reflects both the rarity of some of these conditions as well as the challenge of achieving a definitive clinical diagnosis. In the newborn, truly ambiguous genitalia that may pose a problem for binary gender assignment has an estimated incidence of 1: 4,500– 5,500 births. Overall, around 50% of all cases of DSD with truly ambiguous genitalia are due to either CAH or 46,XY mixed gonadal dysgenesis caused by a 45,X/46,XY mosaicism. The incidence of 46,XY DSD is estimated to be 1: 20,000 births and of 46,XY gonadal dysgenesis around 1: 100,000 births. TDSD/OTDSD are estimated to occur in 1: 100,000 births. More commonly, developmental anomalies of the external genitalia may exist in 1 in 300 newborn infants. These include undescended testis or anomalies of the

opening of the urethra on the penis (hypospadias). However, most of this published data on the incidence of DSD is available only from Western countries; therefore, the worldwide prevalence of DSD is unclear. A German study indicated that the incidence of ambiguous genitalia in infants of non-German background was 4 times higher compared to the general population, which they attributed to an increase in autosomal recessive forms of DSD due to higher rates of consanguinity in the migrant populations. There is some evidence to support the hypothesis that there is a higher rate of DSD in societies with a higher rate of consanguinity. The incidence of ambiguous genitalia in Saudi Arabia has been estimated at 1: 2,500 live births, whilst in Egypt it has been estimated at 1: 3,000 live births, which is higher than the reported frequency of 1: 4,500–1: 5,500 in European countries. Another hindrance in defining the prevalence of DSD is the lack of an accurate or even any diagnosis in many cases. In the German study almost half of the children did not have a definitive diagnosis by the age of 6 months. Excluding cases where the biochemical profile indicates a specific error in steroidogenesis, it has been estimated that a specific molecular diagnosis is obtained in about 20% of cases of DSD and that only 50% of 46,XY children with DSD will receive a definitive clinical diagnosis. The detailed genetic analyses of individuals with DSD have been a powerful tool in the identification of genes involved in sex determination and therefore DSD. **[Anu Bashamboo et al 2016]. [Table.3]**

Sex	Disorder	Karyotype	Approximate Incidence
Male	Klinefelter syndrome	47,XXY	1/600 males
		48,XXXY	1/25,000 males
		Others (48,XXYY; 49,XXXYY; mosaics)	1/10,000 males
	47,XYY syndrome	47,XYY	1/1000 males
	Other X or Y chromosome abnormalities		1/1500 males
	XX testicular DSD	46,XX	1/20,000 males
		Overall incidence: 1/300 males	
Female	Turner syndrome	45,X	1/4000 females
		46,X,i(Xq)	1/50,000 females
		Others (deletions, mosaics)	1/15,000 females
	Trisomy X	47,XXX	1/1000 females
	Other X chromosome abnormalities		1/3000 females
	XY gonadal dysgenesis	46,XY	1/20,000 females
	Androgen insensitivity syndrome	46,XY	1/20,000 females
		Overall incidence: 1/650 females	

[Table.3]. Incidence of Sex Chromosome Abnormalities. **[L. Nussbaum et al, 2016].**

- **Our cohort: Group 1: chromosomal abnormalities:**

Patients with chromosomal differentiation disorders represent 72.58% of all the cases in our study, this percentage is higher than that found in Mr. Rabah Shawky cohort (41.29%) **[R. Shawky, et al 2012]** and even higher than the Johannes Nielsen cohort (23.4%) **[J. Nielsen, et al 1991]**. The predominant breeding sex in this group is female (69%) in contrast to the Rabah M. Shawky cohort where male sex is predominant (60.35%). this is due to the importance of Turner syndrome compared to other disorders in our sample. In this group, the diagnostic average age is 18.18 years. It is 12.46 years According to Rabah M. Shawky and one year in MacLean cohort **[MacLean. N, et al 1961]**. Thus, the age of diagnosis is relatively late in our group. The rate of consanguinity is 13%. This rate is lower than Rabah M. Shawky cohort (55.5%). The stature- ponderal delay constitutes the most frequent call sign. Rabah M. Shawky reports that infertility is the most frequent reason for consultation (48.55%). as for the Sidibe cohort, the first call sign was sexual ambiguity **[Sidibe. AT, et al 2005]**. Chromosomal differentiation disorders include several pathological entities: Turner syndrome, Klinefelter syndrome, gonadal digenesis and mixed chimerism. **[Hughes IA et al 2006].** Turner syndrome, which diagnosis is made by karyotype, affects 60% of patients in our study, while Rabah M. Shawky brings it back to 26.98% of the cases. Johannes Nielsen, in his turn speaks, about 5.3% of cases. This syndrome is caused by number or structural abnormalities affecting the X chromosome. Theoretically, the number abnormality constitutes 70% of the cases; 50%homogeneous and 20% in mosaic. The percentage of the structural abnormalites is estimated to be 30% including: the isochromosome X, translocations X, ring X (5%) and deletions (5%). In our group, monosomy X constitutes 51.85% of Turner cases, the monosomy in mosaic (18.51%), the isochromosome (11.11%), ring X (3,71%) and translocations (7,42%). So these results are consistent with accepted data. The differences in the chromosomal formula between our group and the other data are shown in **[Table.4]**

	Monosomy X	Monosomy in mosaic	Isochromosome X	Ring X	translocations	deletions
Rabah M. Shawky	33,46%	64,89%	0,81%	_	_	0,81%
Johannes Nielsen	11%	22%	11%	11%	_	11%
Groupe1	51,85%	18,5%	11,11%	3,71%	7,42%	_

[Table.4] Comparative between group 1 and other data concerning the various forms of Turner syndrome.

Klinefelter syndrome constitutes 25% of chromosomal differentiation disorders, of which 91% are homogeneous and 9% in mosaic, this is in conformity with the allowed data. The differences of the chromosomal formula between our group and the other data are illustrated in **[Table5]:**

	homogeneous	mosaic
Rabah M. Shawky	83,84%	11,35%
Johannes Nielsen	90%	10%
Groupe1	91%	9%

[Table5] Comparative between group 1 and the other data concerning the various forms of klinefelter syndrome.

The mixed gonadal disgenesis constitutes 8.88% of the chromosomal disorders in our study. According to Johannes Nielsen the DGM represents 0.3%. The Malaysian data of Kannan shows on the other hand a percentage of 7.1% **[Kannan. TP, et al. 2008].** Chimerism 46, XX/46, XY account for 2.22% of the disorders in our series. Johannes Nielsen reported it in 0.3% of cases, and Rabah M. Shawky in 8.25%. The analysis of the results of this first group shows that the karyotype is the pillar of the diagnosis. The use of new methods of genetic exploration, such as the FISH and the PCR, allows further diagnostic approach. Indeed, FISH analysis makes it possible to confirm the result of the karyotype and specify the proportions of the mosaic on a broader population in a shorter time. PCR SRY/DXS1684, in turn, allows us to establish a fairly comprehensive care for turner patients by removing the risk of gonadoblastoma. In this group, the molecular study of SRY gene was applied to 20% of cases especially turner

patients of which theoretically 12% have SRY gene in their genome (which indicates the presence of risk to develop gonadoblastoma). It was noted that the 11.11% of tested cases have a positive SRY gene with a neoplasic risk. Thus, a gonadectomy was indicated.

- **Our Cohort: Group 2: DSD 46, XY:**

The 46, XY DSD constitute 12,90% of the whole of our cases. This rate is higher than that of the Rabah M and Shawky series (7.26%). Sallahi and Borer bring back 28% and 50% respectively of 46, XY DSD **[Sallahi, et al. 2003] [Borer, et al. 1995].** The average age of diagnosis is 5.25 years in this group. In 62% of the cases the diagnosis is made before 3 years. Whereas in Sallahi cohort, the average age of diagnosis is 4 years going from 4 months to 9 years, the diagnosis is carried at the first year of life in 50% of the cases. In Aguilar Martinez cohort **[Aguilar Martinez et al. 1990]** the diagnosis at birth is made in 100% of the cases. In our group, the diagnosis is carried tardily compared to the other series. Consanguinity is found in 25% of the patients. This rate is significantly lower than that reported by Ftouhi **[Ftouhi.B, et al. 1990]** (62% of the cases) and Sallahi (50% of the cases). Sexual ambiguity constitutes the principal reason for consultation (75%), as in Ftouhi and Sallahi cohorts. According to DSD classification, based on Chicago consensus, the 46, XY DSD gather several pathologies: disorders of gonadal development (testicular), synthesis or action disorders of the androgens, abnormality of the AMH and its receptor, and LH receptor abnormalities. The most retained diagnosis in our study is the 5 α reductase deficiency, whereas according to Sallah, the testosterone deficiency is diagnosed in 3 cases (50%), the androgens insensitivity in 1 case (16.6%). Abbadi found 8 cases of androgens insensitivity in 29.6% **[Abbadi, Thesis Project].** In this second group, all the patients profited from karyotype which could direct the rest of the paraclinic investigations in order to pose the diagnosis. The molecular analysis of SRY gene was carried out for 3 subjects (37.5% of the patients of the group). It was absent among 2 patients (66% of the cases). The diagnoses retained for these cases were the partial and complete gonadal disgenesis. The theory says that 15% of the patients suffering from these disorders present changes or deletions of SRY gene. It is necessary to remove the gonads which present, in these 2 cases, a possible hazard of malignity.

❖ **Our cohort: Group 3: sexual differentiation disorders 46, XX:**

46, XX DSD constitute 14.52% of all studied cases. The average age of diagnosis is 5.25 which is relatively late compared to the other series. The rate of consanguinity is about 33%. This rate is lower than that of Sallahi (80%). Sexual ambiguity is present at 44% of cases, acute dehydration at 34%, amenorrhea at 11%, and stature-ponderal delay at 11%. According to Amri **[F. Amri, et al. 1997]**, in 36 cases; 10 patients (27%) had sexual ambiguity (ranging from stage I to stage V of Prader); 6 patients had dehydration (17%) and neonatal screening in 2 other children (6%). The karyotype was realized for all the patients. In Sallahi cohort, the karyotype was 46 XX in all cases, except one, which presented an abnormality: 46XX, - 11, - 19. 46,XX DSD include: gonadal development disorders (ovarian), congenital adrenal hyperplasia (CAH), placental aromatase deficiency and finally the maternal hyper-androgenism. The diagnosis of CAH was retained in 89% of our patients and 1 case was male 46, XX SRY positive, which is consistent with literature data. According to the Mohamed El-Sherbiny cohort **[F. Amri, et al. 1997]**, the congenital adrenal hyperplasia concerns 70% of the patients. According to Amri, it constitutes 100% of the cases. The clinical data and the result of the karyotype allow directing the etiologic diagnosis. Thus several paraclinic explorations are necessary in particular hormonal. FISH analysis was applied to 44% of the cases in this group, the objective was to confirm the results of the karyotype affirming the absence of chromosome Y. The molecular analysis of SRY gene was carried out at 56% of the cases. It was present in one patient only (20%), the theory thus says that 90% of the patients with testicular DSD possessed fragments comprising SRY gene on their X chromosome, the diagnosis of male 46, XX SRY-positive testicular DSD was retained.

b- <u>Discussion Of The Role Of Medical Geneticist:</u>

Establishing a specific molecular diagnosis is helpful in the clinical management of cases and in offering accurate genetic counselling for the family with a member presenting DSD. In those cases, where a clear steroidogenic defect has been identified biochemically, targeted single-gene analysis will confirm the diagnosis in most cases. However, in 46,XY DSD with no clear abnormality of steroidogenesis, the yield from diagnostic genetic testing has often been poor, costly and limited, with less than 50% of

affected cases having an identified genetic alteration. With the advent of genomic medicine, the ability to better diagnose, predict and treat disease is anticipated to transform many aspects of care for individuals with a DSD, its utility may reside in ending diagnostic uncertainty.

Vast improvements in genetic sequencing technology combined with a huge reduction in costs has provided a stimulus for change, with next-generation sequencing and whole-genome and- exome sequencing (WGES) becoming available in clinical practice. Diagnostic DNA laboratories are moving from single-gene sequencing (sequential analysis) to next-generation sequencing assays (parallel testing), designed to sequence multiple DSDs genes on a targeted panel in one analysis or whole-exome sequencing with predetermined filters that target DSD genes. A targeted panel is advantageous as it yields high-quality coverage of the genes of interest, whilst minimizing the risk of incidental findings. The clinical geneticist at the specialist DSD center can evaluate complex genetic syndromes and advise which genetic testing technique is appropriate and cost-effective for each clinical situation, once urgent karyotype testing and copy number variant analysis have been completed. With the advent of targeted panels and WGES, more extensive biochemical and radiological investigations might be reserved until answers from analysis are obtained, with the potential to avoid further costly investigations. However, there are challenges in bringing WGES into routine practice, which is expected to become mainstream in the next years. Pretest counselling needs to be broader, to cover all potential test outcomes, whilst explaining the limitations of this approach. In addition, in line with international recommendations, patients and parents should be informed about the possibility of unsolicited findings of medically relevant disease variants. It is, therefore, recommended that procedures such as WGES should only currently be considered following informed consent and as part of an ethics approved study. **[S. Faisal Ahmed et al 2016].**

Close involvement of the clinical genetics service can ensure that the multidisciplinary team covers all aspects of genetic counselling including provision of information to the family, the mode of inheritance of the disorder and the choices or options available for dealing with this risk. Established links with the clinical genetics service are also useful

when considering prenatal testing or interventions such as steroid therapy in CAH. As the scope for prenatal noninvasive diagnosis using free-floating foetal DNA becomes more realistic, the close involvement of the clinical geneticist at a very early stage in at-risk pregnancies will become even more important.

c- Discussion Of Clinical & Molecular Management:

- **Clinical Diagnosis and main Genetic Testing:**

Most genetic causes of non-syndromic disorders of testicular development are not known. Approximately half of affected individuals will have an underlying genetic etiology identified through molecular genetic testing. **[Barseghyan H, et al 2015].** Non-syndromic 46,XY DSD and 46,XY CGD must be distinguished from syndromic forms, in which additional organ systems, growth, and cognitive development may also be affected. Pathogenic variants in DHH, MAP3K1, NR5A1, SRY, NR0B1, DMRT1 are most known causative of non-syndromic 46,XY disorders of testicular development. Determination of the child's karyotype, frequently accompanied by chromosomal microarray, and performance of FISH, are an essential part of the investigation of such patients and can help guide clinical, surgical and psychosocial management, as well as genetic counseling. Direct mutation analysis or exome sequencing are the second part needed for diagnosis. Our Genetic Unit still lacking competencies in diagnosis of syndromic form of DSD, and genetic tools in exploring other genes rather than only SRY gene in non-syndromic DSD.

- **Additional Non-syndromic DSD Conditions to Consider in our daily practice:**

There are many other genes involved in non-syndromic DSD to be considered to diagnose in our Genetic Unit, the majority of that genes are related to autosomal chromosomes, and their pathway is related to hormones biosynthesis. The underdeveloped infrastructure of biomedical analysis in our institution concerning Hormones, is one of the causes of defects in diagnosis in this kind of conditions. **[Table.6]**

Disease Mechanism	Disease Name	Gene	Distinguishing Features
Hormone biosynthetic defects	Lipoid adrenal hyperplasia	*STAR*	Severe deficiency of adrenal & gonadal steroids; all XY individuals are phenotypically female; severe salt wasting
	P450scc (formerly cholesterol desmolase) deficiency	*CYP11A1*	Acute adrenal insufficiency; elevated ACTH & plasma renin, low or absent adrenal steroids; XY individuals are phenotypically female
	3-beta-hydroxysteroid dehydrogenase deficiency	*HSD3B2*	Acute adrenal insufficiency w/elevated pregnenolone, 17-hydroxypregnenolone & DHEA; XY individuals have severe hypospadias with micropenis
	17-alpha-hydroxylase deficiency/17,20-lyase deficiency	*CYP17A1*	Hypertension, hypokalemic alkalosis, elevated ACTH, LH & FSH; XY individuals have absent or incomplete virilization of external genitalia
	Cytochrome P450 Oxidoreductase Deficiency	*POR*	Combined deficiency of p450c17 & p450c21 causing accumulation of steroid metabolites; ambiguous genitalia in XX individuals & incomplete virilization in XY individuals
	17-beta-hydroxysteroid dehydrogenase deficiency	*HSD17β3*	Interferes w/conversion of androstenedione to testosterone; XY individuals have absent or incomplete virilization of external genitalia but may virilize at puberty
	5-alpha-reductase deficiency	*SRD5A2*	Interferes w/conversion of testosterone to dihydrotestosterone; possible virilization at puberty; XY individuals may appear phenotypically female or have ambiguous genitalia w/hypospadias & blind vaginal pouch
	Aldo-keto reductase deficiency	*AKR1C2, AKR1C4*	Alternative pathway for DHT synthesis in fetal testis; XY individuals may appear phenotypically female or have ambiguous genitalia
LH receptor defects	Leydig cell hypoplasia	*LHCGR*	Leydig cell hypoplasia or agenesis; T levels low; LH/FSH elevated; decreased response to hCG stimulation testing
LH deficiency	Kallmann syndrome	*KAL1*	See Isolated Gonadotropin-Releasing Hormone Deficiency; XY individuals typically have micropenis w/a normally formed scrotum
Androgen receptor defects	Androgen Insensitivity Syndrome	*AR*	Lack of virilization due to impaired androgen binding or transactivation; includes complete & partial defects; T levels normal or high
	CBX2-related complete gonadal dysgenesis	*CBX2*	One case reported ; phenotypic female w/a 46,XY karyotype, uterus & histologically normal ovarian tissue

[Table.6] Other Non-syndromic DSD Conditions with their corresponding genes and clinical manifestations**. [Lauren Mohnach, et al 2016]**

Our Genetic Unit started implementing the genetic testing for the androgen receptor defect, in patients with susceptible androgen insensitivity syndrome.

- **Additional Syndromic DSD Conditions to Consider in our daily practice:**

The Syndromic forms of DSD as differential diagnosis of the non-syndromic forms of DSD have to be considered in our daily practice in our Genetic Unit, the majority of that genes are related to autosomal chromosomes with very well described syndromic features. **[Table.7]**

Disease Name	Gene	Clinical Features
Alpha-thalassemia X-linked mental retardation syndrome	*ATRX*	Distinctive craniofacial features, genital anomalies, hypotonia, severe intellectual disability, mild-to-moderate anemia secondary to alpha-thalassemia
Antley-Bixler syndrome with disordered steroidogenesis	*POR*	Craniosynostosis, hydrocephalus, distinctive facies, choanal stenosis or atresia, low-set dysplastic ears w/stenotic external auditory canals, skeletal anomalies, renal anomalies, reduction of cognitive function, developmental delay
Campomelic dysplasia	*SOX9*	Distinctive facies, Pierre Robin sequence w/cleft palate, shortening & bowing of long bones, club feet, laryngotracheomalacia w/respiratory compromise
GATA4-related disorders	*GATA4*	Testicular anomalies and congenital heart defects
Smith-Lemli-Opitz syndrome	*DHCR7*	Pre- & postnatal growth retardation, microcephaly, moderate to severe intellectual disability, distinctive facial features, cleft palate, cardiac defects, underdeveloped external genitalia in males, postaxial polydactyly, 2-3 syndactyly of the toes. Caused by deficiency of the enzyme 7-dehydrocholesterol.
X-linked lissencephaly with ambiguous genitalia	*ARX*	Lissencephaly w/severe intellectual disability; genitalia of XY individuals can range from ambiguous to phenotypically female.
WT1-related disorders (see Wilms Tumor Overview)	*WT1*	**Fraiser syndrome.** Focal & segmental glomerulosclerosis of the kidney & 46,XY CGD **Denys-Drash syndrome.** Mesangial sclerosis of the kidney, Wilms tumor, & 46,XY DSD
9p24 deletions	*DMRT1*	Trigonocephaly, dysmorphic features (widely spaced eyes, arched eyebrows, low-set ears, long philtrum, thin vermilion of the upper lip), congenital heart defects, underdeveloped external genitalia in males, intellectual disability
11p13 microdeletion	*WT1*	*W*ilms tumor, *a*niridia, *g*enitourinary anomalies, & mental *r*etardation (i.e., intellectual disability) syndrome (WAGR)

[Table.7]. Syndromic DSD Conditions with their corresponding genes and clinical manifestations**. [Lauren Mohnach, et al 2016].**

d- Discussion Of Long Term Management:

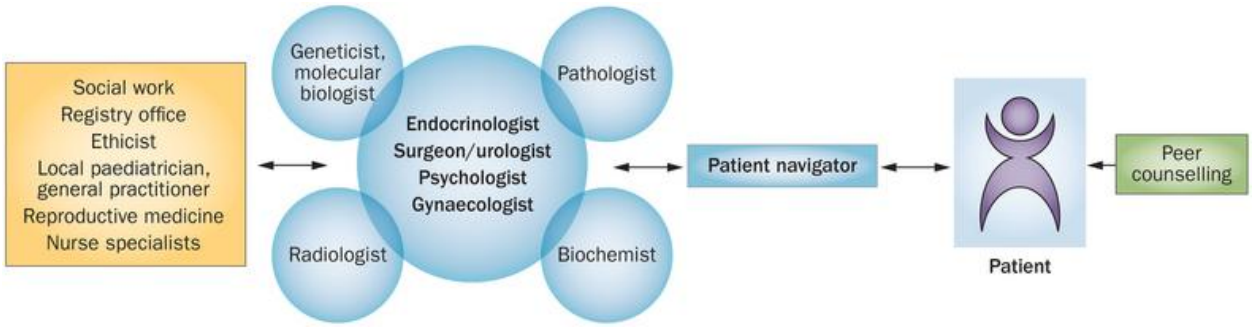
❖ Treatment Of Manifestations:

It is paramount that any child or adolescent with a suspected disorder of sex development (DSD) is assessed by an experienced clinician with adequate knowledge about the range of conditions associated with DSD. If there is any doubt, the case should be discussed with the regional DSD team. In most cases, particularly in the case of the newborn, the pediatric endocrinologist within the regional team acts commonly as the first point of contact. This clinician should be part of a multidisciplinary team experienced in management of DSD and should ensure that the affected person and parents have access to specialist psychological support and that their information needs are comprehensively addressed. The underlying pathophysiology of DSD and the strengths and weaknesses of the tests that can be performed should be discussed with the parents and affected young person and tests undertaken in a timely fashion. Finally, in the field of rare conditions, it is imperative that the clinician shares the experience with others through national and international clinical and research collaboration. Evaluation and long-term management is best performed at a center with an

interdisciplinary care team (including clinical geneticists, endocrinologists, surgeons, and mental health professionals) experienced in the diagnosis and management of DSD; all individuals should receive a sex of rearing; surgical decisions should be made after detailed discussion with the family regarding risks, benefits, and limitations of any proposed surgery; surgical intervention (hypospadias repair, orchiopexy, scrotoplasty, and phalloplasty in males and clitoroplasty, vaginoplasty, and urogenital sinus mobilization in females) should focus on functionality; whenever possible, removal of tissue and irreversible procedures should be avoided; streak gonads and nonfunctional dysgenetic gonads should be removed to decrease the risk for gonadoblastoma; dysgenetic gonads with residual function that are not removed require tumor surveillance; if gonads are retained, surveillance for the development of contra-sexual puberty is warranted if sex of rearing is discordant with gonadal sex; sex steroid therapy (testosterone in males and estrogen or estrogen/progesterone in females) is important for the development of secondary sexual characteristics and for normal adolescent bone mass accrual; 46,XY individuals with a pathogenic variant in NR5A1 should be monitored for adrenal insufficiency; most affected individuals are infertile, although assisted reproductive technologies may help achieve pregnancy in some cases. In our facility the interdisciplinary team is still not working in a perfect harmony for the well management of DSD patients.

- **Surveillance:**

Regular follow up with an interdisciplinary DSD team including endocrinology, genetics, obstetrics/gynecology, psychology, Genetic Counseling, and urology is primordial in managing patient with DSD, but still difficult in our institution. **[Fig.12].**



[Fig.12]. Composition of a team managing patients with disorders of sex development. **[Olaf Hiort, et al 2014]**

e- General Strategy Evaluation Of DSD Management In Our Genetic Unit:

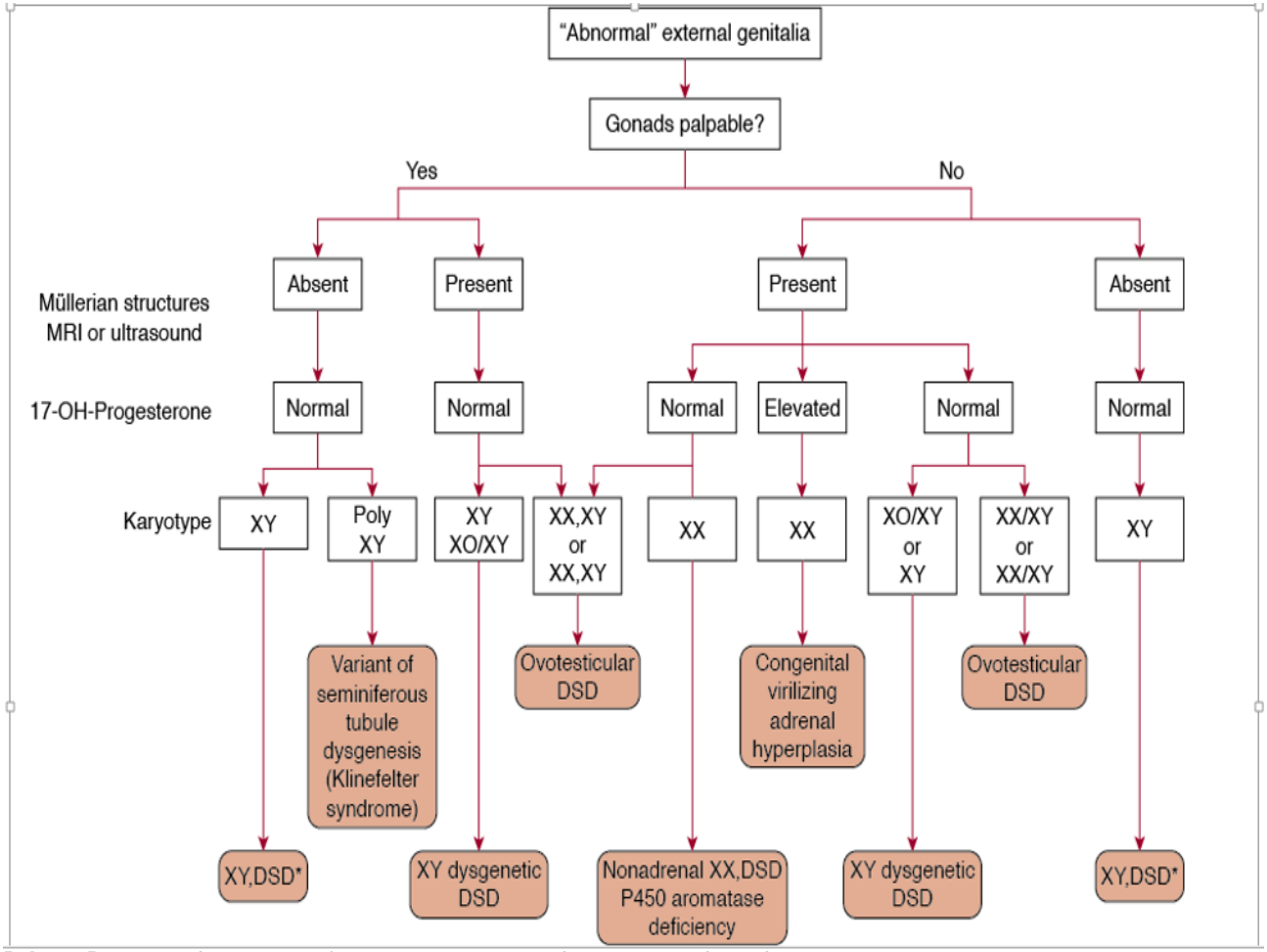
The initial evaluation of an individual suspected of having a non-syndromic DSD is to determine the chromosome complement.

- **Chromosome Analysis:**

One genetic testing strategy is to perform a karyotype using conventional staining methods of a sufficient number of cells to detect mosaicism for sex chromosome aneuploidy (i.e., 45,X/46,XY) and fluorescence in situ hybridization (FISH) for the presence of SRY. Another genetic testing strategy not available in our Genetic Unit is to perform a chromosomal microarray (CMA), as this will determine the sex chromosome complement, evaluate for the presence or absence of SRY, and screen for deletion/duplication syndromes in which individuals may have genital anomalies within the DSD spectrum . If the karyotype is already known, CMA may still be pursued, particularly for individuals in whom a syndromic diagnosis is being considered, in such cases our Unit try to find a foreign institution to make the microarray testing for our patients in a research collaboration basis. If the individual has a 46,XY chromosome complement but is SRY negative, the cause of the individual's nonsyndromic disorder of testicular development has been determined. If FISH or CMA detects a deletion of SRY, a limited karyotype can be considered to determine if the deletion was caused by a translocation or a complex rearrangement of genetic material.

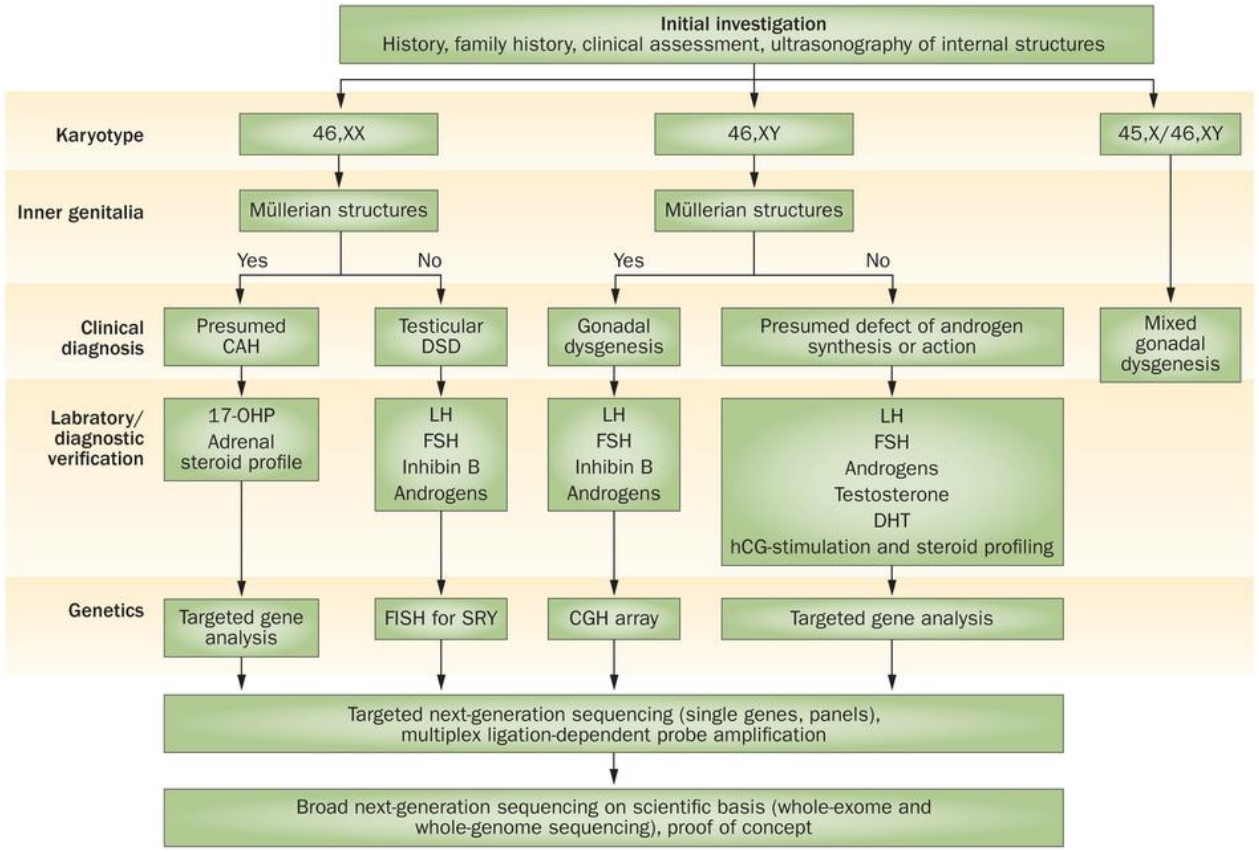
❖ Molecular Genetic Testing:

Molecular testing approaches can include single-gene testing, use of a multi-gene panel, and more comprehensive genomic testing. The only molecular genetic testing available in our genetic Unit is SRY, AZF, and the Androgen receptor which is under development. In the aim to develop the Molecular Genetic Testing in our Genetic Unit we should develop more single-gene testing and perform serial single-gene testing of the three most important genes in DSD: DHH, MAP3K1, NR5A1 based on the individual's clinical findings and/or the order in which pathogenic variants most commonly occur. As perspectives in developing our Molecular Genetic Testing and Molecular Cytogenetics, we should consider: targeted deletion/duplication analysis of NR0B1, and chromosomal microarray to screen for small deletions of 9p24 including DMRT1. Moreover, and with the emergence of New Generation Sequencing, A multi-gene panel that includes DHH, MAP3K1, NR5A1, SRY and other genes of interest may also be considered as future perspective. More comprehensive genomic testing including whole-exome sequencing (WES) or whole-genome sequencing (WGS) may be considered as an ultimate goal. **[Fig.13]. [Fig.14]**



[Fig.13]. General strategy of DSD management in our Genetic Unit:

History: family history, pregnancy(hormones, virilisation, inspection).
Clinical examination: Papation of inguinal region and labioscrotal folds; rectal examination.
Karyotype analysis/fish.
Initial studies of blood analysis: plasma 17-hydroxyprogesterone and 17 hydroxypregnenelone, androstendione, dehydroepiandrosterone, testosterone, and dihydrotestosterone.
Serum electrolytes.
Sonogram or MRI of kidneys, ureters, and pelvic contents.
Provisional diagnosis.
Vaginogram(urogenital sonogram): selected cases.
Endoscopy, laparoscopy, gonadal biopsy: restricted to XY,DSD, ovotesticular DSD, and selected instances of nonadrenal XX,DSD.
Molecular genetic studies.



[Fig.14]. Evaluation of disorders of sex development by clinical, laboratory and genetic investigations. **[Olaf Hiort, et al 2014]**

f- Management Of Malignancy Risk:

The diagnosis of DSD raises concerns of tumor risk and treatment as well as future fertility preservation. Thus, their management is complex and evaluation of tumor risk is aided by advances in genotyping for Y-chromosomal material not evident in traditional karyotyping. More complete genetic screening for DSD patients (even in those with only hypospadias and unilateral undescended testes) should increasingly become the standard of care.

DSD patients are at increased risk for the development of testicular carcinoma in-situ (CIS) and germ cell tumors (GCT), including seminoma, non-seminoma, juvenile granulosa cell, gonadoblastoma, and dysgerminoma. Cancer risk factors include Y-chromosomal material and gonadal position, especially for streak gonads. The 46 XX DSD patients [congenital adrenal hyperplasia (CAH)] with no genetic Y-chromosomal

material are not at higher risk of cancer. Post-pubertal complete androgen insensitivity syndrome (AIS) patients remain prone to tumor development if the testes remain in the abdomen. Estimates of the risk of GCT in partial AIS for untreated undescended testes may be as high as 50%. The cancer risk of scrotal testes in partial AIS is unknown. CIS occurs almost exclusively in patients with hypovirilization, most notably in AIS. Persistent Mullerian Duct Syndrome (PMDS) confers the usual cancer risk associated with cryptorchidism, but also a possible tumor risk of the Mullerian remnant. Several markers are under investigation for tumor evaluation in the DSD population beyond hCG and AFP markers; (Oct3/4, TSPY, WT-1). Developments in pathologic and histologic diagnosis will further challenge the traditional understanding of the oncologic management and surveillance of these patients. **[Martin Kathrins et al. 2016]**

g- Genetic Counseling:

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. Genetic risk assessment can be established by the use of family history and genetic testing to clarify genetic status for family members. Genetic Counseling have to address all personal, cultural, or ethical issues that individuals may face. Non-syndromic disorders of testicular development can be inherited in a mode of sex-limited autosomal recessive (like DHH gene), sex-limited autosomal dominant (like MAP3K1 gene, NR5A1 gene, and heterozygous deletion of DMRT1 gene), Y-linked (SRY gene), or X-linked manner (hemizygous duplication of NR0B1 gene). Genetic counseling and risk assessment depend on determination of the specific cause and the sex chromosome complement of the individual who harbors the pathogenic variant.

h- Related Genetic Counseling Issues:

- **Gender identity, gender assignment and reassignment:**

The management of such disorders is complex, and one of the most crucial decision is represented by gender assignment. In fact, the primary goal in DSD is to have a gender

assignment consistent with the underlying gender identity in order to prevent the distress related to a forthcoming Gender Dysphoria. Historically, gender assignment was based essentially on surgical outcomes, assuming the neutrality of gender identity at birth. This policy has been challenged in the past decade refocusing on the importance of prenatal and postnatal hormonal and genetic influences on psychosexual development.

- **Family planning:**

The optimal time for determination of genetic risk and discussion of the availability of prenatal testing is before pregnancy.

- **Medical Assisted Reproduction:**

While patients with some DSDs may have functioning gonads with viable germ cells but an inability to achieve natural fertility secondary to incongruent internal or external genitalia, other patients may have phenotypically normal genitalia but infertility due to abnormal gonad development. Discussion of fertility issues with the patient and family is essential to the optimal treatment of each patient and an important part of the multi-disciplinary approach to evaluating and counseling these families.

i- <u>Prenatal Testing & Preimplantation Genetic Diagnosis:</u>

Once a genetic cause of DSD has been identified in an affected family member, prenatal testing and preimplantation genetic diagnosis for a pregnancy at increased risk are possible options, but still unavailable in our country genetics facilities.

CONCLUSION

Sex determination and differentiation require the balanced and sequential activation of transcription factors, signaling molecules, hormones and their receptors. Disorders of sex development (DSD) have heterogeneous groups of etiologies caused by mutations or deletions of genes involved in sex development. The DSD is categorized into 46, XX DSD, 46,XY DSD, sex chromosome DSD, ovotesticular DSD, and 46,XX testicular DSD. Precise diagnosis is essential for sex assignment, surgical correction of external genitalia, prevention of gonadal tumors, psychiatric support, and genetic counseling. The increased genetic knowledge in the field has opened up new diagnostic possibilities. The first line genetic testing for DSD is the assessment of the karyotype and the *SRY* gene. The follow-up genetic tests are performed for confirmatory diagnosis; the evaluation of copy number variants by array comparative genomic hybridization (CGH), direct sequencing of a specific gene, and functional analyses of mutations. A lot of genes can be analyzed by molecular laboratories and the number of available genes is growing. DNA analyses should be done under clinical assessment on the basis of family history, prenatal history, physical findings focused on external genitalia, endocrinologic data, and radiologic findings. Genetic counseling is essential to help patients and their families understand the disease status and the risk for recurrence in future pregnancies, and participate in the process of sex assignment. Children with DSD should be managed with a multidisciplinary team, including pediatric endocrinology, molecular genetics, cytogenetics, neonatology, urology, and psychiatry.

In our Genetics Unit and throughout the study of 75 cases, we were able to highlight the role of each genetic test in the establishment of the diagnosis, the etiology and the therapeutic approach of some DSD.

Initially we could classify the patients into categories according to their genotypic formula, then, thanks to the karyotype we could diagnose some chromosomal

abnormalities such as Turner and Klinefelter syndromes. Other disorders were diagnosed thanks to the combination with the biological, clinical and radiological results. Thus, we were able to deal with the reached patients and prescribe the possible treatments to them. On the other hand, the diagnosis of some disorders was difficult through cytogenetic only. FISH, meanwhile, confirmed the result of the karyotype and specified the proportions of the Mosaic on a larger population in a short time. PCR SRY/DXS1684, in turn, allowed us to establish complete assumption for Turner patients by removing the risk of gonadoblastoma. For XY women, the PCR contributed to the understanding of the etiology by elimination of certain assumptions (such as the deletion of *SRY* gene). We could deduce starting from the *SRY* gene sequencing that the result of molecular biology cannot be useful in the absence of a well-illustrated clinical, biological and radiological observations, and that the etiology research requires exploration of other genes also important as the gene of the sexual determinism. We finally concluded that genetics and molecular biology, does not allow dealing with DSD without contribution of other disciplines. In order to present the best assumption to the suffering patients of DSD, we should explore other genes, and implement the new genomics technologies.

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