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46, XY Gonadal dysgenesis; clinical characteristics and genetic aspects

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Abstract

Background: Disorders of sex development (DSD) have varied phenotypic presentation and multiple genetic etiologies. 46, XY gonadal dysgenesis is also a form of DSD which comprises a spectrum of clinical conditions in which the development of the fetal gonad is abnormal. 46, XY gonadal dysgenesis can have varied genetic etiology and can be the consequence of defects of any gene involved in the process of gonad formation.

Methods: A retrospective study was conducted and patients with 46, XY with gonadal dysgenesis and female phenotype were studied. Thorough elicitation of history and detailed clinical examination was done. Genetic analysis in the form of karyotype was done in all the cases along with mutation study. We identified 4 individuals with this phenotype from a large cohort of 46, XY DSD patients. Karyotyping was done in all cases. DNA samples from subjects were studied by

either whole exome sequencing or target gene panel approach.

Results: This study investigated the genetic etiology of 46, XY gonadal dysgenesis patients using various genetic testing like karyotyping and exome sequencing including several DSD-associated genes in humans. This study included 4 patients with 46, XY DSD. We identified mutations in 2 (50%) patients in the WT1 gene. Among them, one patient harbored pathogenic variant, while the other patient had variant of uncertain significance.

Conclusion: Targeted exome sequencing is an efficient tool to improve the diagnostic yield of DSD, despite its phenotypic and genetic heterogeneity. Knowing the exact cause in the terms of mutation can help in better understanding of the disease mechanism, phenotype and genetic correlation; patient counselling and management.

Keywords: Gonadal dysgenesis, Swyer syndrome, Frasier syndrome, streak gonads

Introduction

46, XY gonadal dysgenesis is a form of DSD which comprises a spectrum of clinical conditions in which the development of the fetal gonad is abnormal. Gonadal dysgenesis can be classified as either complete (CGD) or partial (PGD) depending on the gonadal morphology. [1,2] The complete form of gonadal dysgenesis was first described by Swyer et al. and is characterized by female type external and internal genitalia, lack of secondary sexual characteristics, and the presence of bilateral dysgenetic gonads.[3] In PGD in which a Y chromosome is present, there is incomplete testis determination and the external phenotype depends on the degree of testicular function.

46, XY gonadal dysgenesis can have varied genetic etiology and can be the consequence of defects of any gene involved in the process of gonadal formation. Mutations and deletions in the SRY (sex-determining gene on the Y chromosome) have been reported in the literature to account for 10-20% of the cases of 46, XY CGD. [4] Mutations in NR5A1, MAP3K1 and SRY are commonly observed as the molecular cause of 46, XY gonadal dysgenesis. [5] Other mutations or copy number variations identified have included DHH, NROB1 (DAX 1), WNT4, WT1, GATA4, DMRT1 (9p24.3) deletion and CBX2 (17q25) deletion. [6-14] In many cases, the cause of XY CGD remains unknown. Our study will focus on the main genes causing gonadal dysgenesis in humans, presenting as an isolated or syndromic phenotype.

Objective

The aim of the present study was to investigate the different clinical characteristics in individuals with 46, XY gonadal dysgenesis; the genetic aspects and to discuss the relationships with variable clinical phenotypes and genes involved.

Methods and Materials

This is a retrospective study from March 2017 to Feb 2020. The study was approved by our ethics committee. Individuals reared as female with male genotype and gonadal dysgenesis were included in the study. Four patients were studied, out of which two of them were referred to us mainly for primary amenorrhea and the other two with renal failure. Systematic investigations were done to give a proper diagnosis. Genetic analysis in the form of karyotype was done in all the cases along with mutation study.

In all cases, a detailed history was taken and examination was done. Informed consent was obtained from the patient before performing the investigation. Hormonal profile was advised. Anatomical factors of the

uterus and ovaries were evaluated with the aid of USG and MRI. They were referred to our cytogenetic laboratory for chromosomal analysis. Chromosome analysis was performed using standard G-bands by trypsin using Giemsa (GTG)-banding techniques on cultured lymphocytes. Metaphase spreads were obtained from blood lymphocytes using standard procedures. Chromosome analysis was carried out using Applied Spectral Imaging (ASI), Israel software.

Exome sequencing was done in all the cases covering all the genes involved in gonadal dysgenesis. For case 2, nephrotic gene panel was advised to rule out the genetic cause of nephrotic syndrome, whereas clinical exome was done in case 4. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80-100X coverage on the Illumina sequencing platform. Selective capture and sequencing of the protein coding regions of the genome/genes was performed. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases. [15-18]

Case Description

Patient 1

A 14-year-old girl was referred to us with complaints of primary amenorrhea. There was absence of breast development with the hypopigmented areola. She had no axillary or pubic hair. External genitalia was of female type. On investigation, hormone assay; serum follicle stimulating hormone (FSH) and serum luteinizing hormone (LH) were found to be very high, 93.7 and 28.19 mIU/ml, respectively. Ultrasound showed a rudimentary uterus and MRI revealed a hypoplastic uterus with absent fallopian tubes. Karyotype was 46, XY. Diagnosis of Swyer syndrome was made based on the investigations. Clinical exome report showed no pathogenic mutation. Diagnostic laparoscopy revealed a small uterus with normally present fallopian tubes [Figure 1]. Ovaries could not be visualized; fibrous bands were seen on either side which appeared to be streak gonads [Figure 2]. Given streak gonads and genotype of XY, bilateral removal of whitish structures was done and sent for histopathology [Figure 3]. The report showed dysgenetic streak gonads with ovarian differentiation. After proper counselling, she was started on hormonal replacement therapy (HRT) with conjugated estrogen for 3 months. Six months after the initiation of HRT, her secondary sexual characteristics have shown improvement. Breast development occurred (Tanner stage II).

Patient 2

A four-year-old female child born out of consanguineous marriage was referred to us who presented with hypertension gradually deranging renal function that progressed to chronic kidney disease since 1 year of age. On USG pelvis, small uterus was noted and gonads could not be detected. Renal biopsy revealed focal segmental glomerulosclerosis (FSGS). Provisionally diagnosed as a steroid-resistant nephrotic syndrome (SRNS). Genetic analysis revealed two variants in two different genes; WT1 in Intron 9; c.1432+5G>A (5'Splice site), classified as pathogenic and responsible for Frasier syndrome/ Denys-Drash syndrome; and second in COL4A5 gene at Exon 28; c.2215C>G (p. Pro739Ala) classified as likely benign [Figure 4]. The molecular diagnosis of Frasier syndrome was in correlation with patient phenotype and genotype. Karyotype was 46, XY and external genitalia was completely female. On follow up, the patient is on dialysis but not doing well and might succumb to renal failure.

Patient 3

A 14-year-old female, not attained menarche. On physical examination, it was seen that the patient was of short stature (<5th percentile) and increased arm span. She had a broad chest with widely spaced nipples and low posterior hairline. Her secondary sex characters not developed; absent axillary and pubic hair; no breast development (Tanner I). Her hormone profile was of hypergonadotropic hypogonadism; S. FSH-127.08 ng/ml; S. LH-15.03 ng/ ml; S. Prolactin-18.57 ng/ml; S. Testosterone-0.15 ng/ ml. USG revealed hypoplastic uterus and absent or streak gonads. Her karyotype showed 45, X [40]/ 46, XY [60]. Clinical exome revealed no pathogenic variants and showed the presence of SRY gene. On laparoscopy streak gonads were noted along with small uterus and fallopian tube [Figure 5,6 &7]. Bilateral gonadectomy was done. On histopathology, the left gonad showed undifferentiated gonadal tissue whereas the right gonad showed ovarian stroma with few atrophic follicles and areas of undifferentiated gonadal tissue with Leydig-like cells [Figure 8]. Basically, both the gonads showed dysgenetic features. Diagnosis of Partial XY gonadal dysgenesis was made.

Patient 4

A one-year-old girl presented with anasarca for 3-4 days. There was abdominal distension with breathing difficulty. Swelling was noted all over the face and legs. She was having proteinuria in the range of nephrotic syndrome. Patient was being treated for Nephrotic syndrome with acute kidney injury. There was no improvement in renal function following therapy; hence, the patient received regular peritoneal dialysis.

Renal biopsy was done which showed focal segmental glomerulosclerosis (FSGS). Karyotyping was advised to rule out Turner syndrome as hydrops fetalis was noted earlier in antenatal sonogram. The patient was reared as female but later proved to be a genetic male owing to the 46, XY karyotype; however, normally developed female external genitalia was observed. Abdominal computed tomography did not reveal cryptorchidism or abnormal renal mass. Further exome sequencing was advised which revealed mutation in WT1 gene. Heterozygous mutation was noted in exon 9 of WT1 gene c.721T>C (p. Cys241Arg) and was categorised as variants of uncertain significance. It appeared to be a novel mutation causing Denys Drash syndrome.

Discussion

We had these four patients with different clinical findings and different etiology for gonadal dysgenesis, all having 46, XY genotype. Here, we would discuss the type of gonadal dysgenesis we encountered in terms of its developmental aspect, molecular and genetic etiology as well as their recommended testing and clinical significance.

Developmental and molecular aspect of 46, XY gonadal dysgenesis

Gonadal dysgenesis is a genetic condition as a consequence of errors in cell division and or modifications in genetic material which leads to complete or partial loss of gonadal development. The gonadal dysgenesis may happen either at fertilization or shortly in early developmental stages of the embryo and fetus. Complete gonadal dysgenesis genotypes are known as either 46, XX or 46, XY. Here, we would discuss only 46, XY gonadal dysgenesis, which can be either complete or partial based on its characteristic phenotype and genetic aetiology. These patients with full gonadal dysgenesis can have streak gonads, female external genitalia and hypergonadotropic hypogonadism

with associated amenorrhea and lack of secondary sexual characteristics. [19] It has been estimated that 15-20% of all cases of 46 XY gonadal dysgenesis are due to mutations in the SRY gene. [20] The SRY gene on the Y chromosome plays a crucial role in testicular development. Sertoli cells found in testis express the SRY gene which assists in various events leading to sexual differentiation. Sertoli cells also help in the development of Leydig cells which then produce androgens and insulin-like factor3 causing the regression of Mullerian duct structures and differentiation of Wolffian duct structures. [21] Mutations in SRY lead to completely dysgenetic gonad or a streak gonad whereas, in the presence of normal SRY when other genes are the cause of dysgenesis, some immature seminiferous tubules have still been noted. [22] Studies have shown the association of the genes SOX9, GATA4, FOG2, NR5A1, and WT1, many of which code for transcription factors. [21]

Additionally, MAP3K1 is also a common gene associated with 46, XY gonadal dysgenesis and has been shown to be involved in 13 to 18% of patients. MAP3K1 expression downregulates SOX9 leading to abnormal testicular development. This down regulation of the testicular development pathway would ultimately lead to impaired gonadal development. [23] SOX9 is crucial for testes development and is associated with β-catenin, a factor playing a role in sexual development. [23] NR5A1 receptor can have a role in activating beta-catenin, which is a regulator of sex development in different mammals and has to be in adequate amount to carry out ovarian development, along with inhibition of proper testicular development. [21]

Although many genes have links with gonadal development and sexual differentiation, as well as many possible mutations, deletions, and translocations associated with 46 XY, and mosaic forms the exact mechanisms and genetic causes leading to such disorders cannot be explained thoroughly.

Investigating 46, XY gonadal dysgenesis

A patient with delayed puberty or amenorrhea with elevated serum LH and FSH levels, should be considered for a karyotyping test. Pelvic ultrasound or MRI may show streak gonads which would again indicate a karyotype. After initial analysis and karyotype, further testing for specific gene mutations should be advised to figure out the cause of the patient's gonadal dysgenesis as well as to have an understanding of what other symptoms they may demonstrate based on specific genetic alterations. [24] Diagnostic laparoscopy is advised to look for streak gonads and its timely removal to prevent its transformation to gonad blastoma.

Patients with 46, XY CGD, or Swyer syndrome, most commonly present in adolescence with delayed puberty or primary amenorrhea due to their non-functional gonads. [25] The endocrine evaluation usually shows elevated levels LH and FSH. Imaging studies, including pelvic ultrasound or MRI, demonstrate the presence of a uterus and may show bilateral streak gonads. If gonadectomy or gonadal biopsy is performed, gonadal histology reveals the presence of bilateral dysgenetic streak gonads. Mutations and deletions in the *SRY* (sex-determining gene on the Y chromosome) have been reported in the literature to account for 10-20% of the cases of 46, XY CGD. [3, 26] Other mutations identified have included NR5A1 (9q33), DHH (12q13.1), NROB1 (DAX 1), WNT4, DMRT1 (9p24.3) deletion, CBX2 (17q25) deletion, and a heterozygous mutation in MAP3K1 (5q11.2). [3,11,12,13,14,24,27,28] In many cases, the cause of XY CGD remains unknown. In our study, we

could not find any mutation on clinical exome for case 1 (Swyer syndrome) but could know that the SRY gene was present. Though array-CGH could not be done so we don't know if any microdeletion or duplication might be responsible for the syndrome.

For patients with suspected XY CGD, the following testing was recommended by McCann et al to establish the diagnosis. [24] [Figure 9] The investigation is mainly to be carried on three modalities; hormonal evaluation, imaging and genetic testing. Regarding genetic testing, they suggest that if karyotype and array CGH are normal, sequencing for SRY, NR5A1, and DHH should be considered. So, it is a candidate-gene approach. However, we did exome sequencing instead of following a candidate-gene approach, the advantage of which was that almost 25-30% of the genetic cause can be ruled out as most of them are due to mutations. Only three genes with copy number variation are attributed to causing 46, XY GD and accounts for very few cases. Also, we could know the status of the SRY gene with this test, therefore a separate test to look for SRY deletion was not required. Performing many single-gene sequencing is not that cost-effective and also there is a possibility of missing the responsible gene. Moreover, if the differential diagnosis is too wide then it can rule out the other causes of 46, XY DSD as well. Therefore, exome sequencing is a preferred choice in today's scenario as it has become an affordable and comprehensive test.

In case of Partial XY gonadal dysgenesis, both the clinical phenotypes and karyotypes are variable. The most common karyotype of patients with XY PGD is 45, X/46, XY, which was observed in our case 3. Imaging shows the absence of fully developed Müllerian structures, depending on the degree of testicular dysgenesis. Gonadal histology may reveal either bilateral dysgenetic testes or one streak gonad and a contralateral dysgenetic or normal-appearing testis. They often have elevated basal LH and FSH levels. The genetic testing and rest of the investigations can be done in similar ways as for 46, XY CGD [Figure 9]. The differential diagnosis should include 46, XY partial gonadal dysgenesis and syndromic 46, XY gonadal dysgenesis (such as Frasier syndrome, campomelic dysplasia and 46, XY DSD with adrenal insufficiency).

Frasier syndrome and Denys-Drash syndrome

Frasier syndrome is characterized by gonadal dysgenesis and nephropathy. It is caused by very specific mutations in the WT1 gene located in 11p23. Patients with the 46, XY karyotype have normal female genitalia with streak gonads and are at high risk for developing gonad blastoma. The type of nephropathy observed in this syndrome is mainly focal segmental glomerulosclerosis. These patients present with kidney problems in 2nd and 3rd decades; most cases at puberty. Rarely, the renal symptoms might manifest at a younger age; youngest example at 6 months of age. In our case, the patient presented with renal problems at 1 year of age.

Males with Frasier syndrome have the typical male genotype (46, XY), but have gonadal dysgenesis, therefore they are phenotypically female presenting with amenorrhea. External genitalia could be either ambiguous or completely female. They can have small uterus and fallopian tubes. The underdeveloped gonads referred to as streak gonads are non-functional and often become cancerous, so they are usually removed surgically early in life. In our case parents refused laparoscopic intervention so the gonadal state could not be known. The nephrotic syndrome with slowly progressing renal disease finally resulted in end-stage renal failure. On renal biopsy, focal segmental

glomerulosclerosis was noted. Our case had typical findings on renal biopsy but the renal disease manifested early i.e. at 1 year of age. At four, she progressed to end-stage renal failure.

The other group having XX karyotype have the less severe phenotype, frequently not clinically identified as FS. They have normal and functioning female genitalia. Clinically, they present only with renal disease. However, these females can transmit the disease to their offspring.

Denys-Drash syndrome is a rare genetic disorder characterized by early-onset nephrotic syndrome that rapidly progresses to renal failure during the first few years of life, pseudo-hermaphroditism, and a high risk of developing Wilm's tumor. [29] The initial manifestations of DDS can be vague and indistinguishable. Here, the child initially presented with anasarca and was eventually diagnosed with DDS with WT1 gene heterozygous variant.

In Denys-Drash syndrome, the characteristic glomerular damage causing the nephrotic syndrome manifests as diffuse mesangial sclerosis and this may be the presenting feature, either at birth or in the 1st few years of life. In our case patient had focal segmental glomerulosclerosis instead of diffuse mesangial sclerosis which is not very common. In addition to the nephrotic syndrome, there could be Wilms's tumour and /or intersex. Wilms's tumour was not detected yet in our patient. A wide variety of WT1 mutations can cause Denys-Drash syndrome, therefore genotype-phenotype correlation becomes difficult.

In contrast to Denys-Drash syndrome, Frasier syndrome is a consequence of very specific WT1 mutations that hinders splicing at the second alternative splice donor site. [30, 31, 32] Mutations are detected in intron 9 of the WT1 gene and cause deficiency of the normally much abundant KTS positive isoforms and thus a reversal of the normal KTS positive/negative ratio from 2:1 to 1:2. [32] As the WT1 protein produced in Frasier syndrome is normal, with normal binding capabilities, the impact of these mutations points out the importance of precisely balanced expression of WT1 isoforms for normal function. This has a great impact on tumour risk because patients with Frasier syndrome have one normal copy of WT1 and one which can only produce the KTS negative isoform. Loss of allele will prevent the cells to produce the KTS positive isoform of WT1 but still have high KTS negative isoform.

The molecular basis for the intersex state in patients with Frasier syndrome and 46 XY is more difficult to explain than in Denys-Drash syndrome because KTS positive isoforms only have a limited ability to activate SF1.[33] Interactions between WT1 isoforms, SF1, MIS, and DAX1 in the Frasier syndrome is depicted in Figure 10.

Those having 46 XX karyotype and Frasier syndrome have normal gonadal development, indicating the lower significance of WT1 in normal female gonadal development. [31] Also, the molecular basis of nephropathy in Frasier syndrome is not properly understood.

In conclusion, identifying Frasier syndrome and DDS is crucial for early diagnosis and proper follow-up. All children presenting with nephrotic syndrome during the first year of life and those with SRNS with either Wilms' tumour or genital abnormalities should be screened for mutations in WT1. Therapeutic approaches and the monitoring of Wilms' tumour and gonad blastoma need to be considered on an individual basis.

Conclusion

Gonadal dysgenesis is a complex condition with associated symptoms spanning across many organ systems. Clinicians must be familiar with gonadal dysgenesis and its investigation protocol so that such patients do not get unnoticed or misdiagnosed and could be managed properly to improve their development and decrease their mortality. DNA sequencing technologies have enhanced investigations into specific genetic causes of these disorders and has become crucial for diagnosis, management and prognosis of such disorders caused by gene mutation. However, it's difficult to understand the particular pathway the genetic alterations take to develop the phenotype. As genetic technologies advances we believe that we will be able to acquire an even better perspective into the causes of gonadal dysgenesis, as well as better insight into human sexual development.

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Legend Figures

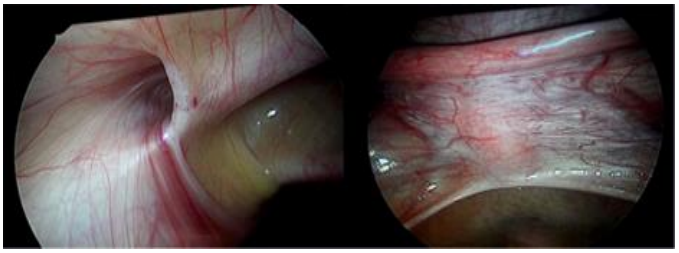

Figure 1: Showing small uterus with normally present fallopian tubes and inguinal canal was seen on both sides which were blind and empty in case 1 (Swyer syndrome).

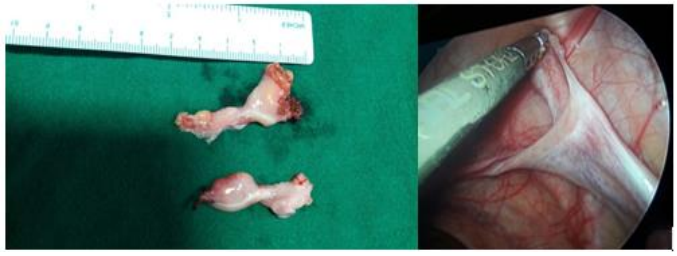

Figure 2&3: Showing streak gonads in case 1.

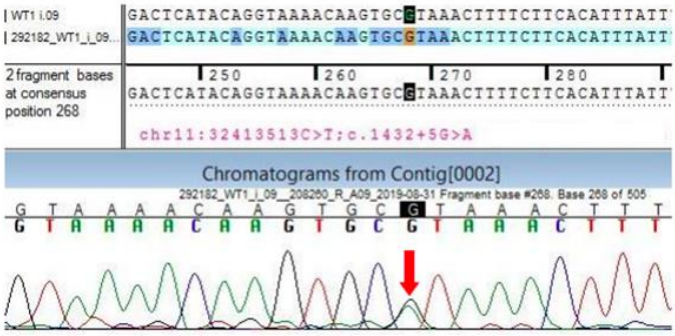

Figure 4: Sequence chromatogram and alignment to the reference sequence showing the variation in intron 9 of the WT1 gene [chr 11:32413513C>T; c.1432+5G>A (5'Splice site proximal)] detected in heterozygous condition (case 2).

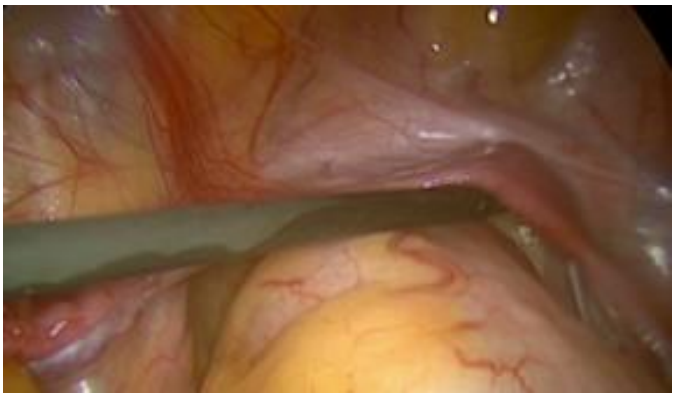

Figure 5: showing hypoplastic uterus in case 3

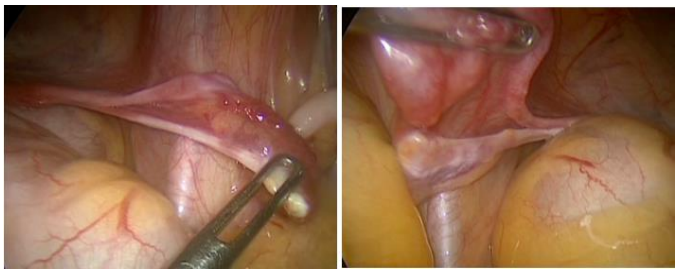

Figure 6&7: Showing streak atrophic gonads in case 3

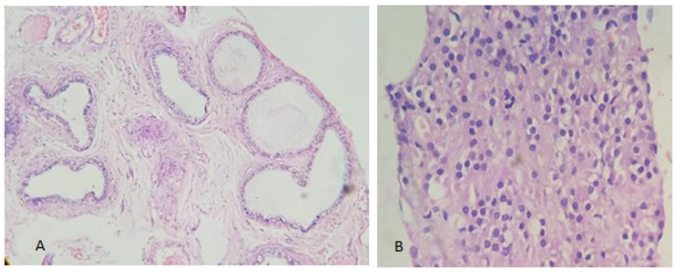

Figure 8: Histopathology of gonad (A) showing ovarian stroma and atrophied follicle along with (B) un differentiated gonadal tissue in case 3 (H & E x200).

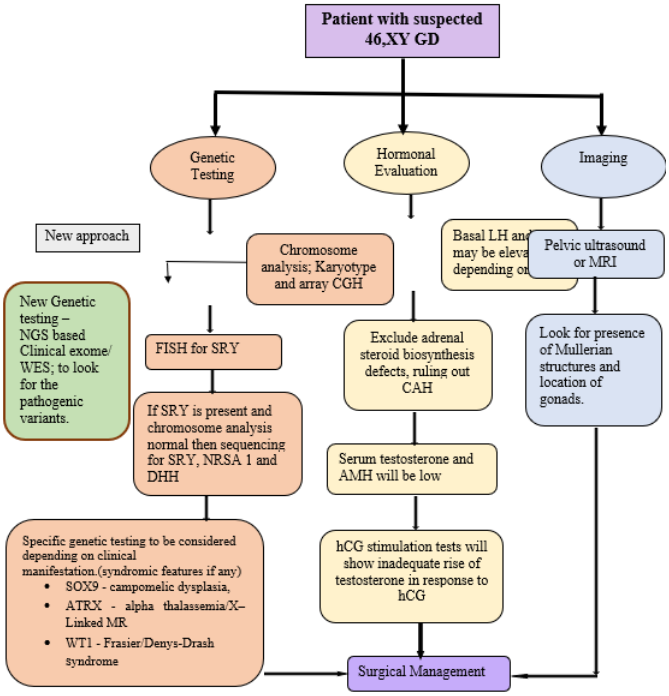

Figure 9: Diagnostic algorithms showing investigation for diagnosis of 46, XY DSD. The "new" scheme suggests that updated genetic testing may be performed as second line of investigation just after karyotype, leading to a molecular diagnosis rather than a lengthy step wise single gene approach.

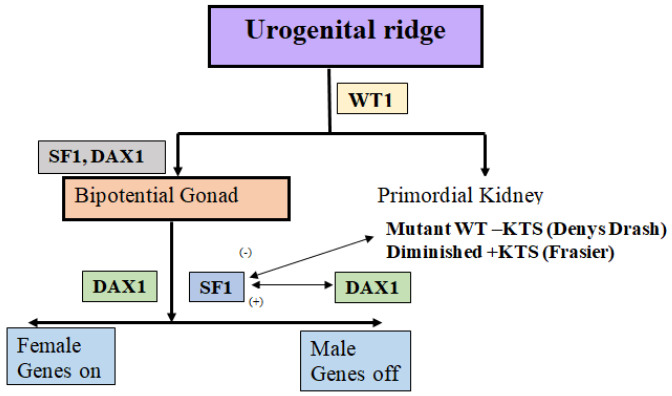

Figure 10: The interaction between the Wilms's tumour suppressor (WT1) protein isoforms (KTS negative (−KTS) and positive (+KTS)), steroidogenic factor 1 (SF1), Mullerian inhibiting substance (MIS), and DAX1 in Denys-Drash and Frasier syndromes.