Reproductive System

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Each development stage of human reproductive system is involved with a sophisticated and complex regulatory network. The main organs of the reproductive system (testis/ovary) are originated from the intermediate mesoderm and the subsequent urogenital ridge during embryonic development. The development of external genitalia is closely correlated with the synthesis and function of male hormones. Many congenital disorders related to the differentiation and development of reproductive system are correlated with hereditary factors. This chapter will start from the gonadal development and focus on the hereditary factors contributing to fetal disorders of reproductive system development.

14.1 Normal Embryonic Development of Genitalia and Related Factors

The primordial germ cells migrate from the yolk sac endoderm to the genital ridge from the fourth to fifth week, to form the undifferentiated primordial gonad at about five to six weeks after fertilization. During the early embryonic stage, when male and female reproductive systems are comparable, this period is called the undifferentiated stage of reproductive organs. At the seventh week after fertilization, the primordial gonad of male with XY karyotype is differentiated into the seminiferous tubule and the interstitial cells of the embryo testis (Leydig cell) under *SRY* and *SOX9* genes. In male embryos, the Leydig cells can secrete androgen and interact with its receptors. The Wolffian duct (also called the embryonic duct of the mesonephros) further develops into the epididymis, the seminiferous duct, and seminal vesicles of the embryo. Meanwhile, anti-Müllerian hormone (AMH) secreted by the Sertoli cells will lead to the degeneration of the Müllerian ducts [1]. At 13–16 weeks after fertilization, the primordial gonad of females with XX chromosome karyotype is differentiated into embryonic follicles, thecal cells of embryonic follicles and stromal cells under WNT4, FOXL2, and other factors. Thus, the embryonic ovarian organ is formed. For women, in the absence of androgen and AMH, the Müllerian ducts further develop to form the fallopian tubes, uterus, cervix, and vagina. In the process of gonadal differentiation, multiple genes are involved in temporal and spatial regulations (Fig. 14.1). Any genetic defect involved in the above regulations may lead to errors in gonadal differentiation, resulting in disorders of sexual development or abnormalities in sperms/ova.

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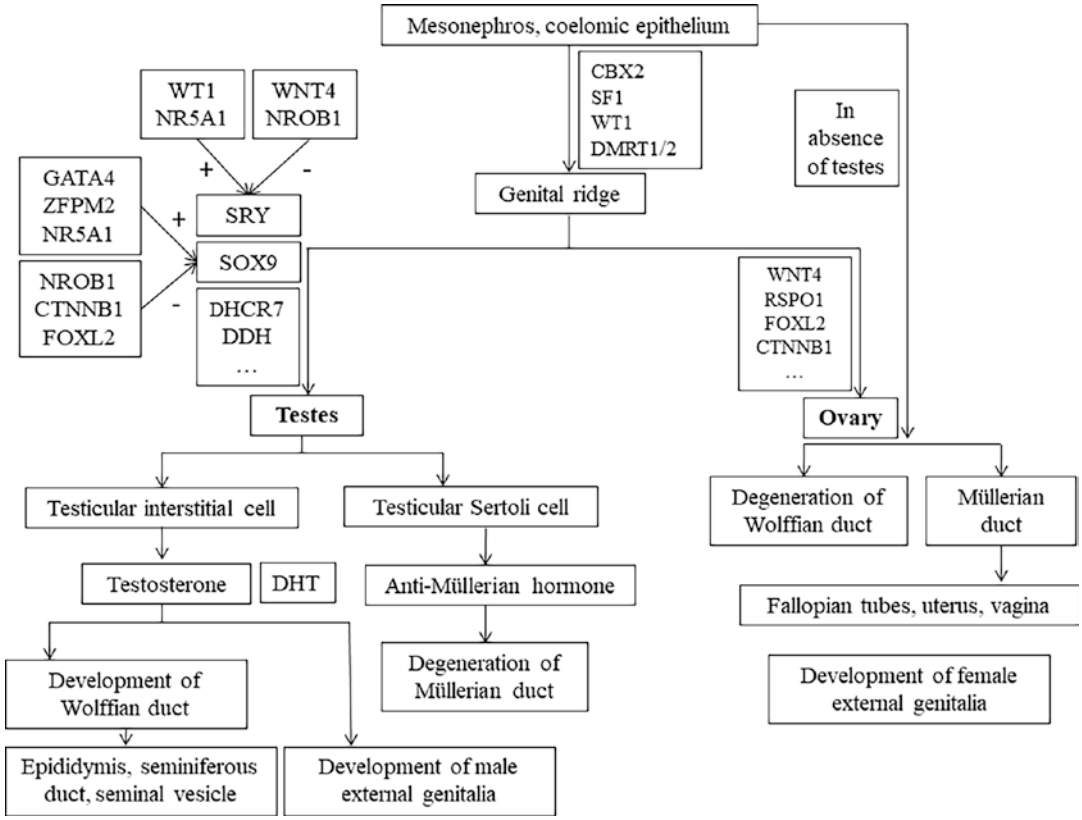

Fig. 14.1 Genetic mechanisms of human sex determination

14.1.1 Main Factors Related to the Development and Differentiation of Testis and External Genitalia

14.1.1.1 SRY

In mammals, sexual development occurs in two distinct and successive stages: sex determination and sex differentiation. In the process of male sex determination, the expression of *SRY* gene located on the Y chromosome initiates the cascade expression of genes in the Sertoli cells, which ultimately drives the morphological differentiation of the testis. *SRY* gene is a regulatory gene that plays a primary role in sex determination. The mRNA levels in the fetus with 46, XY karyotype are upregulated at the urogenital ridge seven weeks post-conception and drive the development of the bipotential gonad into testes [2]. After translation, *SRY* is transferred to the nucleus and binds to the enhancer region of *SOX9*, to drive the differentiation and proliferation of Sertoli cells and the seminiferous tubule tissue of the testis. In the process of sex differentiation, the testes secrete testosterone, dihydrotestosterone, and anti-Müllerian hormone, leading to the development of the male internal and external genitalia (prostate, seminiferous duct, penis, and scrotum) and the degeneration of the Müllerian ducts.

14.1.1.2 SOX9

SOX9 is the second major gene involved in male sex determination, and it encodes an *SRY*-related transcription factor. Expression of SOX9 is essential for testis differentiation, and it acts synergistically with SRY and NR5A1 transcription factors. SOX9 binds to its own promoter, forming a positive feedback loop that maintains high levels of SOX9 expression.

14.1.1.3 NR5A1

NR5A1, also referred as the steroidogenic factor-1 or SF-1, encodes an orphan nuclear receptor that plays an important role in the development of the hypothalamic-pituitary-gonadal-adrenal axis. In the Sertoli cells, NR5A1 acts synergistically with the transcription factor GATA4 at initial formation of the testis. It also binds to the SRY promoter to upregulate the expression of SRY. SF-1 can be detected in the primordial reproductive ridge 32 days after fertilization in human embryos. When the testes can be morphologically recognized, SF-1 is mainly confined to the Sertoli cells of the sex cord and subsequently expressed mainly in the Leydig cells. In addition to the gonad, SF-1 is also expressed in the ventromedial hypothalamic nucleus and pituitary gonadotropin cells.

14.1.1.4 NROB1

NROB1, also known as DAX1, is the nuclear receptor transcription factor that plays an important role in the development of human adrenal cortex and gonad. It is expressed in adrenal cortex, gonad, pituitary, and ventral median nucleus of hypothalamus. In individuals with XY karyotype, there is only a single copy of this gene. The presence of gene duplication mutation will lead to NROB1 overexpression, which may inhibit testicular differentiation. The gene duplication mutation of NR0B1 will result in a dose-dependent XY gonadal dysgenesis and a female phenotype. One of its molecular pathogenic mechanisms found in NROB1 transgenic XY mice is through the direct repression of NR5A1-mediated *SOX9* transcription. In females with the 46XX karyotype, the presence of two fully functioning copies of the *NR0B1* gene are essential to prevent testicular formation. Generally, loss-of-function mutation in NROB1 will lead to hypogonadotropic dysgenesis with primary adrenal insufficiency in males with the 46XY karyotype.

14.1.1.5 GATA4 and ZFPM2

Heterozygous mutations in the *GATA4* gene may lead to 46, XY disorders of sexual development. GATA4 is also associated with congenital heart disease, suggesting that GATA4 plays a role in gonadal and cardiac development. In the mouse model, *GATA4* mutation disrupted the connection between GATA4 and ZFPM2, resulting in abnormal testicular development. In the porcine model, GATA4 directly activated the SRY promoter, whereas in humans and mice, direct activation of SRY expression was observed only when the WT1 protein was also expressed. In the mouse model, it was found that mutations in *GATA4* or *ZFPM2* resulted in reduced interactions between GATA4 and ZFPM2 proteins, resulting in a decreased ability of any gene (independently or co-expressed) to activate the transcription of target genes such as *AMH*, *SRY*, and *SOX9* [3].

14.1.1.6 DMRT1

In the process of human fetal development, DMRT1 mRNA is detectable in both sexes by 11 gestational weeks, most abundant in Sertoli cell precursors during 10–20 gestational weeks; it is also expressed in oogonia and oocyte by 20 gestational weeks, and it decreases after meiosis [4]. DMRT1 is essential for the maintenance of the fate of Sertoli cells during testis differentiation. The expression of DMRT1 in testicular Sertoli cells of mice after birth maintains the high level of SOX9 expression, thus promoting the expression of testicle-specific genes in Sertoli cells and inhibiting the differentiation of ovarian-specific granulosa cells. Inactivating mutations in *DMRT1* cause these cells to differentiate into granulosa cells instead.

During early embryonic development, CBX2, SF1, WT1, and DMRT1/2 are involved in the regulation of the differentiation from mesonephros and coelom epithelium to genital ridge. During gonadal differentiation, *SRY* gene expression in human embryos with XY karyotype acts as a switch for testicular differentiation, driving the genital ridge to differentiate into testis. SRY is positively regulated by WT1 and NR5A1, while being inhibited by double concentrations of NROB1 and WNT4. SRY protein may activate some downstream genes such as *SOX9*. At the same time, it is also regulated by multiple transcription factors, which in turn initiates a network of testis gene expression and inhibits ovary-specific gene expression (WNT4 and RSPO1).

14.1.2 Major Factors Involved in Differentiation During Normal Development of the Ovary, Fallopian Tube, and Uterus

In response to regulating factors including WNT4, RSPO1, FOXL2, and others, the primordial genital ridge develops into an ovary in females with the 46, XX karyotype. In female embryos with the XX karyotype due to anorchia or loss of function of the testis, the Wolffian duct degenerates without the support of the high concentration testosterone. Meanwhile, the Müllerian duct develops due to the absence of the inhibition of anti-Müllerian hormone, and it is eventually differentiated into the oviduct, uterus, and vagina [5]. The meiosis of fetal germ cells to differentiate into oocyte and ovarian development involves the activation of gene pathways, including RSPO1/WNT4/β-catenin signaling, which is inhibited by SRY. In XX gonads, the Sertoli cell precursors accumulate β-catenin in response to RSPO1/WNT4 signaling transduction and inhibit the activity of SOX9.

14.1.2.1 WNT4

WNT4 plays a key role in the regulation of the Wnt/β-catenin signaling pathway. It also plays an important role in the regulation of mammalian gonadal differentiation and development. Fluorescence in situ hybridization (FISH) analysis reveals that Wnt4 transcripts were predominantly located in the cytoplasm of oocytes. WNT4 is a key regulator of mammalian ovarian development, with the highest levels of Wnt4 expression occurring during the embryonic stage [6, 7]. In mice, knockout of wnt4 may affect ovarian development and steroid synthesis and will lead to sex reversal in females [8].

14.1.2.2 RSPO1

RSPO1 (R-spondin 1) protein is a multipotent signaling ligand, with its key function of enhancing Wnt/β-catenin signaling transduction [9]. RSPO1 is a sex determining factor in female mammals, which plays a key role in reproductive organ development [10]. RSPO1 regulates the expression of gonadal differentiation-related factors via the β-catenin signaling pathway. It can also regulate the division and proliferation of primordial germ cells and the differentiation of somatic cells in gonad to resist the formation of testis, thus determining the female differentiation. Embryonic *RSPO1* mutations may result in impaired development of reproductive organs [11]. The mutations leading to sexual dysplasia identified so far are all involved in the highly conserved N-terminal cysteine-rich domain, which plays a key role in the activation of Wnt/β-catenin signaling pathway [12].

14.1.2.3 FOXL2

FOXL2 is a member of the forkhead box protein-encoding gene family, and its encoded transcription factor is evolutionarily highly conservative [13]. It is one of the genes with its expression first found to be upregulated in the ovarian development of female mice, suggesting that FOXL2 plays an important role in the process of early ovarian differentiation [14]. FOXL2, a nuclear protein expressed in ovarian follicular cells, is the earliest known marker in ovarian differentiation in mammals. It may play a role in ovarian cell differentiation and follicle development and/or maintenance [15, 16].

In addition, there are a variety of other molecules involved in the normal development of the ovary, fallopian tube, and uterus. During early embryonic development, HOX family genes play an important role in the differentiation of reproductive tract. Their transcription factors regulate the structure of the anterior-posterior axis of the Müllerian duct by regulating the corresponding positional information. Homologous expressed genes *HOXa9*, *HOXa10*, *HOXa11,* and *HOXa13* have been found to be expressed along the long axis of the Müllerian duct, and their expressions are overlapped in the mesenchyme of the local genital tract of female mice. The *Wnt* genes also play an important role in regulating the anterior-posterior axis and the radial axis. The expression of transcription factor P63 is the primary marker to distinguish the epithelial cells of the uterine, vagina, and cervix. The *CTNNB1* (catenin beta-1) gene is also involved in the regulation of

endometrial epithelial differentiation and plays an important role in maintaining the characteristics of the uterine epithelium.

14.2 Common Disorders and Pathogenic Genes of Congenital Reproductive System Anomalies

14.2.1 46, XY Gonadal Dysgenesis-Related Disorders

14.2.1.1 Testicular Hypoplasia

14.2.1.1.1 Complete Gonadal Dysgenesis (Swyer Syndrome)

Swyer syndrome is characterized by a female phenotype with ovarian degeneration and dysplasia of secondary sexual characteristics. The gonads are fibrous cords without follicles or normal germ cells, and they are at high risk of developing gonadoblastoma. The internal reproductive organs include bilateral fallopian tubes, uterus, and vagina. Females with Swyer syndrome have normal to tall stature. It is caused primarily by the mutations or deletions of the *SRY* gene on the Y chromosome. Another form of Swyer syndrome is gonadal dysgenesis with intact Y chromosome, which is caused by mutations or deletions/duplications of other genes that regulate gonadal differentiation (such as *SF-1, Dax-1, Wt-1* genes, etc.). It is resulting from testicular insufficiency, such as deficiency in anti-Müllerian hormone and androgens. The genital duct-derived organs are usually the uterus and fallopian tubes, and the external genitalia are under-masculinized [17, 18].

14.2.1.1.2 Partial Gonadal Dysgenesis

Patients with partial gonadal dysgenesis may present with female Turner syndrome signs (short stature, broad chest, cubitus valgus, etc.), ambiguous gender in external genitalia, and hypertrophy of clitoris. There is masculinization presentation in puberty. The gonads are often located in the abdominal cavity, with a cord-like gonad on one side and a malformed testis on the other. It may be induced by *DHH* gene mutation [18].

14.2.1.1.3 Common Disorders Leading to Male Gonadal Dysgenesis

Dax-1 Duplication Mutation

Dax-1 gene duplication mutation may induce gonadal dysgenesis in 46, XY males. The patients present with female external genitalia, with rare cases of reproductive duct-derived organs being the uterus and fallopian tube. The gonad is hypoplastic testis or ovary, the blood sex hormone level is decreased, and the gonadotropin level is increased. If the ovarian function is relatively normal, the sex hormone and gonadotropin levels are close to the normal female level, and the patient may combine with mental retardation, growth retardation, and facial-cranial malformation [18, 19].

Wt-1 Defect Syndrome

Wt-1 (Wilms' tumor suppressor 1) gene is expressed in the kidney, gonad, and primordial genital ridge. The missense mutation in exon 9 of *Wt-1* may lead to Denys-Drash syndrome, which is characterized by under-masculinization of external genitalia, cord-like gonad or hypoplastic testis, genital duct-derived organs being uterus and fallopian tubes, and with complicated renal lesions. Plasma gonadotropin level is increased, and gonadal hormone level is decreased. As the gonad is at high risk of developing blastoma, the probability of developing Wilms' tumor is about 4%. The splice site mutation in exon 9 of *Wt-1* gene will result in Frasier syndrome, with clinical manifestations including a cord-like gonad, genital duct-derived organs being dysplastic uterus and fallopian tube, and female external genitalia and may be concurrent with gonadoblastoma and renal lesions. *Wt-1* gene heterozygous deletion will lead to WAGR syndrome, which is characterized by Wilms' tumor; aniridia or iris malformation; urogenital dysplasia, including renal agenesis, horseshoe kidney, urethral atresia, hypospadias and cryptorchidism; and mental retardation [20].

SOX9 Defect

The main clinical manifestations include limb flexion and gonadal dysgenesis, which are

inherited as autosomal dominant inheritance disorders. Limb flexion includes long bone flexion, hypoplasia of scapula, pelvic deformity, small thorax, 11 pairs of ribs, cleft palate, macrocephaly, micromandible, orbital hypertelorism, and various degrees of cardiovascular and renal malformations. In three-fourth of patients, the gonads may develop to hypoplastic testes or ovaries, with the presence of the uterus and fallopian tubes, and the epididymis and seminiferous duct are absent or hypoplastic. Approximately 70% of patients have ambiguous external genitalia, and the remainder have female or male phenotypes [21].

SF-1 Defect

Mutations in the *SF-1* gene may lead to gonadal dysgenesis and adrenocortical insufficiency, which are manifested as female external genitalia, presence of uterus and fallopian tubes, gonadal dysgenesis, or absence of secondary sexual characteristic development during puberty, which are combined with manifestations of adrenocortical insufficiency [22].

14.2.1.2 Disorders in Androgen Synthesis or Androgen Dysfunction

14.2.1.2.1 Androgen Synthesis Disorder

5α-reductase Deficiency

It is an autosomal recessive disorder of sex differentiation due to deficiency of 5α-reductase resulting in insufficient androgen effect. 5α-reductase is a membrane protein located on the microsomes of target cells, which involves in the catalytic conversion of testosterone to the more potent dihydrotestosterone. In the process of the differentiation of external genital in a male fetus, dihydrotestosterone guides the bidirectional differentiation potentials of the genitalia primordium to differentiate toward the male direction, which is closely correlated with the development of scrotum and prostate. Male patients with 5α-reductase deficiency tend to develop feminization of external genitalia, which is clinically manifested as different degrees of sexual differentiation disorders, persistent urogenital sinus (blind-ended vagina), with clitoritis-like penis that can erect, with normal testes, seminiferous duct, and epididymides, but the prostate may appear as a form of residue. The testes may be located in the scrotum or groin, and the seminiferous duct and epididymis may open into the blind-ended vagina. At birth, the external genitalia present as a female phenotype, typically presenting with a pseudo-vaginal perineoscrotal hypospadias. During puberty, increased testicular secretion of testosterone drives male puberty development, testicular descent and enlargement, penis becoming longer and thicker, voice becoming rough, and muscularity. However, they may have sparse beard and pubic hair, armpit hair and body hair, a small prostate, and oligozoospermia. The patients have male personality and sexual awareness [20].

StAR Deficiency

Steroidogenic acute regulatory protein (StAR) transports cholesterol into the mitochondrial membrane, which is the first and rate-limiting step in the synthesis of steroid hormones. It is expressed in the adrenal gland and the gonads. Mutations in the *StAR* gene will result in cholesterol accumulation in adrenocortical cells and Leydig cells, and the resulting disruption of cell function may lead to congenital lipoid adrenal hyperplasia, which is inherited as an autosomal recessive disorder. The 46, XY male patients have female external genitalia, with a blind-ended vagina and absence of uterus and fallopian tubes. The gonads are presented as testes, which are located in the abdominal cavity, inguinal canal, or labia majora. After birth, due to the complete blockade of adrenocortical hormone synthesis, the newborn may have the manifestation of severe adrenocortical insufficiency [18, 23].

3β-hydroxysteroid Dehydrogenase Deficiency

3β-hydroxysteroid dehydrogenase (HSD3B2) is involved in the catalytic conversion of Δ5-steroids to Δ4-steroids. HSD3B2 deficiency will lead to the disorders in the synthesis of aldosterone, cortisol, and testosterone, resulting in the accumula-

tion of Δ5-steroids in the body. Among them, dehydroepiandrosterone (DHEA), a weak androgen, may lead to masculinization in female patients and varying degrees of feminization in male patients. There are two isozyme types of 3β-hydroxysteroid dehydrogenase, HSD3B1 and HSD3B2. HSD3B1 is distributed in peripheral tissues such as placenta, skin, and mammary gland, while HSD3B2 is distributed in adrenal gland and gonad. And the two enzymes are 93% homologous. The enzymatic activity of HSD3Bl is five times stronger compared to HSD3B2. Mutations in *HSD3B1* may lead to death of the fetus because of the inability to synthesize progesterone. Mutations in *HSD3B2* gene will result in adrenal and gonadal dysfunction, which is inherited as an autosomal recessive disorder. Male patients usually have micropenis with moderate to severe hypospadias in their external genitals, and adrenocortical crisis may occur after birth. Gynecomastia may occur during puberty, which is presumably attributed to the compensatory effect of HSD3B1 in peripheral tissues. In some patients, sufficient amount of testosterone can be generated to support penile development and spermatogenesis after glucocorticoid replacement therapy, due to the compensatory effect of HSD3B1 [20, 23].

17α-hydroxylase/17,20-lyase Deficiency

CYP17A1 has both of the functions of 17α-hydroxylase and 17,20-lyase. It exists in adrenal gland and gonad and is involved in the catalytic conversion of pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively. Mutations in *CYP17A1* may result in blockade of synthesis of cortisol and androgen, and the elevation of ACTH as feedback stimulates the increase in synthesis of deoxycorticosterone and corticosterone, further leading to hypernatremia and hypokalemia. The external genitalia of male patients often show micropenis with hypospadias. And a severe patient may have completely female external genital and blind-ended vagina, presence of epididymides and seminiferous duct, and absence of uterus and oviducts, and the gonads are testes locating anywhere in the descending process [20, 23].

17β-hydroxysteroid Dehydrogenase (HSD17B3) Deficiency

HSD17B3 is a testicular mitochondrial enzyme involved in the catalytic conversion of androstenedione to testosterone. This gene mutation may induce testosterone synthesis disorders. Most such patients have female external genitals. In a few patients, external genitals are ambiguous, with blind-ended vagina, presence of epididymis and seminiferous duct, and absence of uterus and fallopian tube, and the gonad is testis, which is often located in the groin. During puberty, with the increase in androstenedione, testosterone, estrone, and gonadotropin levels, patients may have masculinization, hairiness, voice change, and increased muscle mass. Some patients have different degrees of gynecomastia [20, 23].

14.2.1.2.2 Androgen Dysfunction (Complete/Partial Androgen Insensitivity Syndrome)

Androgen insensitivity syndrome is an X-linked recessive genetic disorder due to androgen receptor deficiency. Androgen receptors are the macromolecules that mediate the critical role of androgens in target cells. Defect in the genes encoding the androgen receptors is the main pathogenic cause. The development of the male embryo into a normal male phenotype requires, in addition to adequate testosterone secretion from the embryonic testes, the presence of androgen receptors on the primordium of the external genitalia and the mesonephric ductal structures such as the prostate in the embryo in order for these structures to develop into the tissues of normal male reproductive organs. Androgen insensitivity syndromes are resulting from defects of the androgen receptor itself or post-receptor defects. The patients may have different degrees of clinical manifestations, either completely female phenotype or subfertile males with normal male genitalia [20, 23], which can be divided into complete and incomplete types. Patients with complete androgenic insensitivity syndrome is born with a completely female phenotype, with a normal sized clitoris and scarce pubic hair with feminine distribution but with scant or absent axillary hair. The vagina is blind-ended, without uterus and adnexa.

The testis is not in fixed site, which may be located in the abdominal cavity or in the groin, even in the labia majora in a few cases. The clinical manifestations of incomplete androgen insensitivity syndrome are diverse, most of which are male or showing a male tendency appearance. The most common is the male phenotype at birth but with hypospadias, mostly with cryptorchidism, and without spermatogenic function. The Müllerian structures are absent, and mesonephric duct-derived organs can be present but with dysplasia [23, 24].

14.2.1.2.3 Leydig Cell Anergy Syndrome

The HCG/LH receptor on the testis is located on the membrane surface of the interstitial membrane of the testis. When HCG/LH binds to the receptor, it triggers the G protein to be allosteric, activates the cAMP-dependent protein kinase, and initiates the synthesis of testosterone through a cascade reaction. Leydig cell anergy syndrome is induced by mutation in the gene encoding the HCG/LH receptor, which prevents the testis from responding to HCG/LH. At the same time, the differentiation and development of Leydig cells depend on the stimulation of HCG/LH. The absence of HCG/LH will lead to the hypoplasia or absence of Leydig cells. Such patients do not have complete masculinization. The most severe cases have completely female appearance at birth, with urethral meatus and vaginal meatus, clitoris hypertrophy, or scroto-labial fusion, and incomplete testicular descent may be found in the groin, labia majora, or scroto-labial fusion. A hypoplastic epididymis and seminiferous duct may be present. Small penis with hypospadias can be seen in mild cases. Serum gonadotropin level is increased, and testosterone and estrogen levls are decreased. The testosterone does not respond to hCG stimulation test. The testes biopsies reveal lack of Leydig cells, but Sertoli cells are normal and have nearly normal seminiferous tubules and incomplete spermatogenesis [20, 23, 25].

14.2.1.2.4 Persistent Müllerian Duct Syndrome (PMDS)

It is mainly induced by mutations in the genes encoding anti-Müllerian hormone or the genes encoding anti-Müllerian hormone receptor and is named as persistent Müllerian duct syndrome type I and persistent Müllerian duct syndrome type II, respectively. The clinical manifestations are the same for these two types. Anti-Müllerian hormone (AMH) is secreted by Sertoli cells of the testis and induces the degeneration of the Müllerian ducts. If this hormone and its receptor are deficient, the Müllerian duct will not be degenerated and differentiated into the uterus and fallopian tubes. Patients with 46, XY generally have normal testicular development but with fallopian tubes and a uterus, cryptorchidism, and testes in the groin. Testes and fallopian tubes can also co-exist in the pelvic cavity [26].

14.2.1.2.5 Hypogonadotropic Hypogonadism

Hypothalamic or pituitary dysfunction due to various congenital or acquired factors may lead to decreased secretion of gonadotropins (FSH, LH), thus resulting in hypoplasia of the testis and decreased secretion of androgens. Clinical manifestations include testicular dysplasia, micropenis, and low levels of plasma gonadotropin and testosterone [17].

14.2.2 46, XX Gonadal Dysgenesis Disorders

The etiologies of 46, XX DSD include disorders associated with gonadal (ovarian) dysfunction, hyperandrogenism, and other structural abnormalities or syndromes [17].

14.2.2.1 Ovarian Dysgenesis

14.2.2.1.1 Ovotesticular Development Disorders

Ovotesticular disorder of sexual development is the co-existence of ovarian follicles and seminiferous tubules in the same patient. Specific phenotypes depend on relative gene expression patterns and the function of the gonad. Gonadal histological type can include ovary, testes, ovotestis, and dysgenesis types [18]. The underlying mechanism leading to ovotesticular disorders in 46, XX (SRY-) individuals may involve activation of

genes involved in testicular development in the absence of *SRY* and/or under-expression of ovarian/anti-testicular genes. Genes associated with ovotesticular developmental disorders include *NR5A1, SOX3, SOX10, WNT4, RSPO1*, etc. [27].

14.2.2.1.2 Testicular Sexual Development Disorder

There is a pseudo-autosomal region at the end of the short arm of X and Y chromosomes, which contains homologous genetic information, and exchange of genetic material occurs during meiosis pairing. *SRY* gene is located near the proximal end of the pseudo-autosomal region. Hence, if the short arm ends of X and Y chromosomes have unbalanced exchange including *SRY* gene, the 46, XX sex chromosome karyotype containing *SRY* may be generated, or the 46, XY karyotype without *SRY* may be formed [24]. In *SRY*-positive 46, XX patients, the dose of Y to X translocation is heterogeneous, from only encompassing the *SRY* region to possibly occupying 40% of the short arm of the Y chromosome. About one-third of patients have a cut point in the protein kinase gene region, and the more the short arms are translocated, the greater extent of masculinization the phenotype is. Approximately 80% of XX males are induced by Y to X translocations. The remaining 20% may involve mutations in other autosomal or X-linked genes, such as duplications of the *SOX9* locus or potential *SOX9* regulatory elements, or the presence of an underlying Y chromosome that is difficult to detect [26]. The patient's chromosome karyotype was 46, XX, with the clinical manifestations of male phenotype with small testis and (or) micropenis, cryptorchidism, and gynecomastia in some cases [18].

14.2.2.1.3 Gonadal Dysgenesis

Simple 46, XX gonadal dysgenesis syndrome is partly induced by defect of the FSH receptor (FSHR) located on 2p. Other etiologies are unknown and are presumed to be related to mutations in genes or receptor genes associated with ovarian organogenesis, such as mutations in primordial germ cell migration genes [24, 25]. Patients with this disease have a karyotype of 46, XX. The external genitalia are female, and other clinical manifestation include primary amenorrhea, no secondary sex characteristics developed by pubertal age, and normal height. Their gonads may be bilateral striated tissue, one side striated, contralateral underdeveloped ovaries, or bilateral underdeveloped ovaries. Sex hormone levels are reduced with elevated gonadotropin levels.

14.2.2.2 Clitoral Hypertrophy Due to Hyperandrogenism

14.2.2.2.1 Fetal Source Androgen Overload (21-hydroxylase Deficiency)

21-hydroxylase (*CYP21A2*) deficiency is the most common cause of the abnormal genital development in individuals with 46, XX gonadal dysgenesis. Approximately 95% of CAH are 21-hydroxylase deficiency caused by *CYP21A2* mutation, which will convert 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone substrates for the synthesis of cortisol and aldosterone, respectively. The classical type, with an incidence of about 1:15,000, can be divided to salt-wasting type and simple virilizing type. There is another more modest nontypical type. Female external genital abnormalities in classical salt-wasting 21-hydroxylase deficiency usually occur during the fetal or neonatal period, with external genital ranging from clitoral hypertrophy to perineal hypospadias to labial fusion. The extent of masculinization of the external genital can be so extensive that the appearance of external genitals of affected female infants resembles that of males with undescended testes on both sides. Unless determined by neonatal screening, infants with congenital adrenal hyperplasia typically presented with loss of weight gain, feeding difficulties, somnolence, dehydration, hypotension, hyponatremia, hyperkalemia, and masculinization of the external genitalia with vulvar pigmentation during the first two to three weeks after birth. It can be fatal when the diagnosis is delayed or missed. Current newborn screening has reduced mortality from acute adrenal insufficiency in this disease [28].

14.2.2.2.2 Fetal Placental-Derived Androgen Excess (Aromatase Deficiency, P450 Oxidoreductase)

Aromatase Deficiency

An autosomal recessive disease is caused by mutations in the *CYP19A1*, which encodes an aromatase. This enzyme is converted into estrogen in gonadal and extragonadal tissues, including the placenta. The placental tissue of aromatase deficient fetuses is unable to convert dehydroepiandrosterone sulfate from fetal adrenal glands to estrogen, with subsequent accumulation of its precursor androstenedione and testosterone. Affected female patients were born with an ambiguous phenotype of external genitalia, and their mothers developed masculine signs after the first trimester of pregnancy. Affected female patients may develop ovarian cysts in childhood, may not develop secondary sexual characteristics at puberty, and may develop primary amenorrhea and hyperandrogenemia [29]. If untreated, men and women with this disease will likely develop osteoporosis and tall stature.

P450 Oxidoreductase Deficiency

Cytochrome P450 oxidoreductases are electron donors for all microsomal P450 enzymes and other non-P450 enzymes. Mutations in this enzyme may affect the activity of enzymes involved in the synthesis of glucocorticoids, mineralocorticoids, and estrogens. The signs and symptoms range from mild to severe, depending on the mutation. Newborns with 46, XY may present with ambiguous external genitalia. Patients with severe P450 oxidoreductase deficiency may have skeletal deformities such as craniosynostosis, midface retrusion, forehead protrusion, arachnoid protrusion, bowing of femora, and radiohumeral synostosis, which is known as Antley-Bixler syndrome. Patients with the disease may also manifest with nostril atresia, mental retardation, and developmental retardation. The mothers of some of these newborns will have virilizing manifestations during pregnancy due to lack of aromatase activity [30].

14.2.2.2.3 Excess Maternal Androgens (Luteoma, Intake of Androgen Drugs)

Maternal Luteoma

Gestational luteoma is a benign, non-neoplastic lesion of the ovary. It is induced by the increased activity of luteinizing cells stimulated by the androgen that produces chorionic gonadotropin (HCG). Approximately two-third of female newborns born to masculinized mothers show some degree of masculinization [31].

Krukenberg's Tumor of Ovary

Krukenberg's tumor of ovary arises from the metastatic adenocarcinomas of gastrointestinal tissue or the breast. These tumors may produce androgens due to luteinization of the tumor stroma. During pregnancy, high-level androgen production due to elevated HCG levels may lead to masculinization of the pregnant woman and the female fetus [32].

Exogenous Androgen

In addition to gestational luteoma and androgen secretory tumor, maternal androgen hyperplasia during pregnancy may be induced by exposure to exogenous androgens. Some female fetuses may show masculinization of external genitalia at different degrees when the mothers are treated with progesterone for habitual abortion or other reasons in the first trimester of pregnancy. In addition, maternal intake of androgens is also a possible cause of fetal masculinization [33].

14.2.2.3 Others (Cloacal Exstrophy, Vaginal Atresia, MURCS Association, and Other Syndromes)

Other disorders due to the factors of nonchromosomal/nonhormonal abnormalities that lead to 46, XX gonadal dysgenesis include cloacal exstrophy, vaginal atresia, MURCS association, etc. Since the differentiation process in embryonic development is affected, the affected patients have reproductive tract developmental malformations, which are often accompanied by renal and

urethral malformations. And the routine treatment is surgical correction [20, 21].

14.3 Clinical Practice

14.3.1 Intrauterine Sex Determination and Management of a Fetus with Sex Chromosome Abnormality Found Prenatally by Noninvasive Method

14.3.1.1 Current Medical History

This is a 26-year-old woman with no family history of genetic disorders or other risk factors. During the second trimester of pregnancy, she requested the noninvasive prenatal testing (NIPT) as a primary screening test for fetal Down syndrome. The NIPT result showed a low risk for trisomy 21, trisomy 18, and trisomy 13, and additional analysis revealed a high risk of sex chromosome aneuploidy. Prenatal genetic counseling at 17 gestational weeks was recommended.

14.3.1.2 Prenatal Genetic Counseling

NIPT showed sex chromosomes aneuploidy. However, NIPT is a primary screening method, rather than diagnostic method. According to the previous data, the positive predictive value of sex chromosomes aneuploidy is 20–30%. Therefore, it is suggested that invasive prenatal diagnosis can be considered to confirm whether there are abnormalities in sex chromosomes.

14.3.1.3 Laboratory Tests and Results

14.3.1.3.1 Results of Chromosome Microarray Analysis of Amniotic Fluid Cells

The amniotic fluid cells were analyzed with chromosomal microarray-Affymetrix CytoScan 750k. The microarray results revealed there is an about 155 kb deletion involving Xp22.33q28 region (Fig. 14.2).

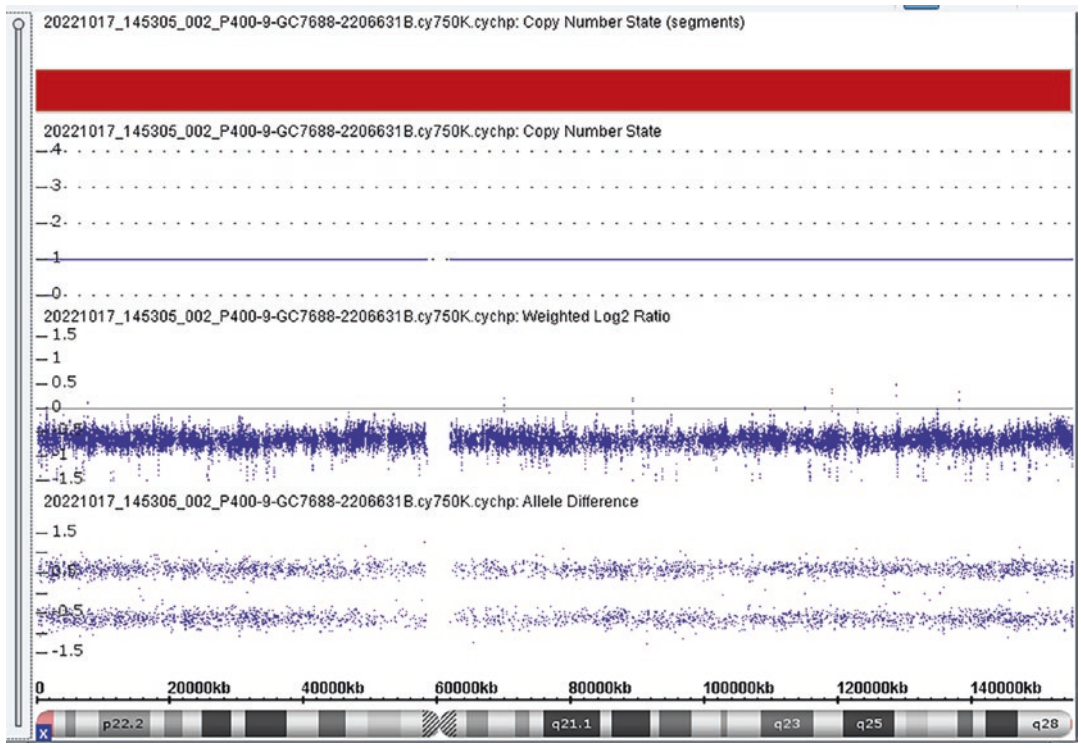

Fig. 14.2 Results of microarray study of DNA from amniocentesis

14.3.1.3.2 Results of Karyotype Analysis of Cultured Amniotic Fluid Cells

G-banded karyotyping on cultured amniotic fluid cells was conducted, in which a total of 50 metaphase cells were analyzed, showing a karyotype of 45, X (monosomy X) (Fig. 14.3).

14.3.1.3.3 Genetic Test of Sex Determining Gene (*SRY*)

The *SRY* gene was amplified by PCR using specific primers and analyzed by electrophoresis. The *SRY* gene, which is located at Yp, encodes the testis determining factor protein required for the development of male genitalia. If *SRY* genes are detected in the sample, a band with the size of 279 bp will be amplified by PCR. Therefore, PCR of amniotic fluid cell's DNA using primers for *SRY* produced a product of the expected size, confirming the presence of the *SRY* gene in this sample (Fig. 14.4).

14.3.1.3.4 Fluorescence In Situ Hybridization (FISH) Results

FISH was performed on interphase and metaphase cells in cultured amniotic fluid, using a probe specific for the centromere of the X chromosome (CEPX-green fluorescent labeling) and a probe specific for the sex-determining gene *SRY* in the short arm of the Y chromosome (red fluorescent label), with 500 cells counted. The results showed that 490 cells presented only one green, fluorescent signal marking the X chromosome (Fig. 14.5a), and ten cells presented *SRY* double-positive signals, with a proportion of 10/500 (2%) (Fig. 14.5b). The results showed monosomy of X in 98% cells, and 2% cells were *SRY* double positive. The FISH results showed a chimeric karyotype, with most of the cells missing one sex chromosome, and 2% of the cells were *SRY* double-positive, suggesting the presence of iso (Y) or idic (Y).

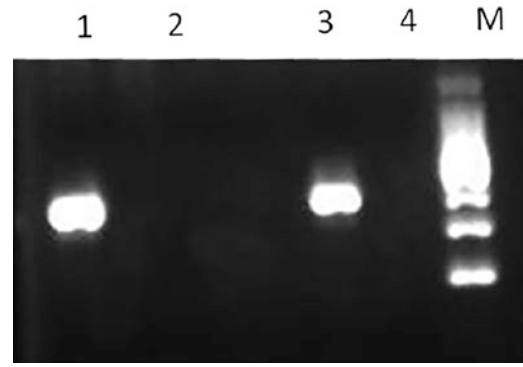

Fig. 14.4 PCR amplification electrophoresis of *SRY* in amniotic fluid samples. M marker; (1) amniotic fluid cells, (2) normal female, (3) normal male, (4) blank

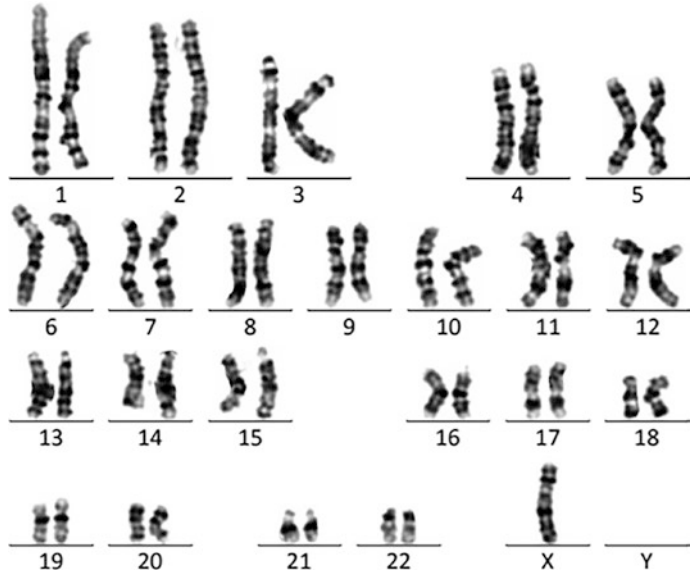

Fig. 14.3 G-banded karyotyping of cultured amniotic fluid cells

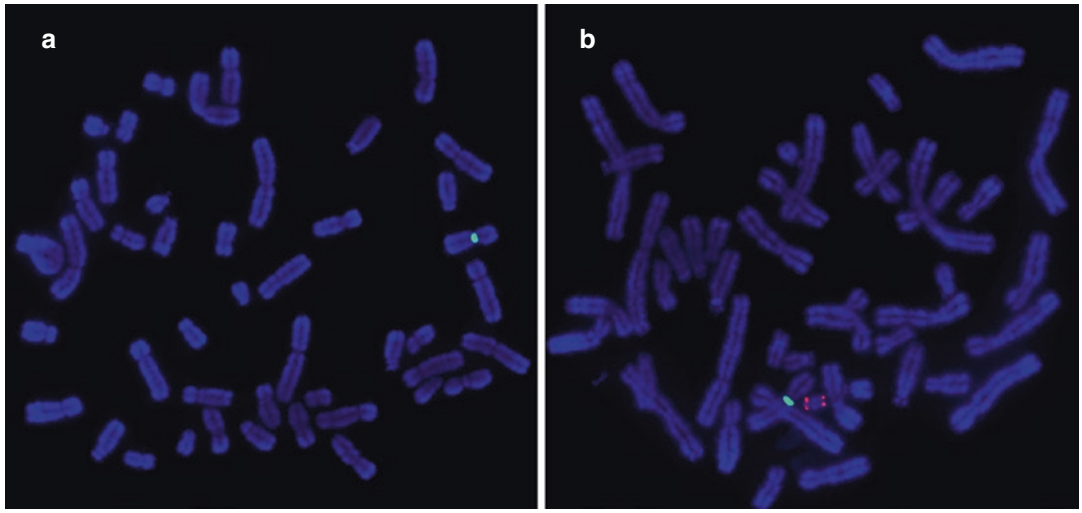

Fig. 14.5 Metaphase FISH results: FISH was performed using a probe specific for the centromere of the X chromosome (green) and a probe specific for the *SRY* site (red). (**a**) One Green signal in metaphase nuclei represents the 45, X cell line. (**b**) One green signal and two red signals in metaphase nuclei represent 46, X, iso (Y) or idic (Y)

14.3.1.4 Obstetrical Ultrasound Examination

The ultrasound examination reveals male external genitalia.

14.3.1.5 Post-Testing Consultation and Discussion

The chromosomal microarray analysis and karyotyping of the fetus showed the karyotype of 45, X, which was related to Turner syndrome. The main features of this syndrome include female appearance, short stature, webbed neck, cubitus valgus, underdevelopment of secondary sexual characteristics, congenital ovarian dysplasia, and primary amenorrhea. Most of the patients being infertile, some of the patients having mild intellectual disability, and some of the patients had congenital malformations in organs and tissues such as heart, kidney, and skeleton. Thus, the 45, X karyotype usually presents as a female appearance. But in rare cases, monosomy X patients are males, usually resulting from an unbalanced Y-autosomal translocation resulting in the retention of a short arm of the Y chromosome, which contains the sex-determining gene *SRY*, or the presence of a low proportion of the Y chromosome, which contains the *SRY* gene. The *SRY* gene, normally located at Yp, encodes the testis-determining protein and is essential for the development of the male genitalia. The fetus in this case showed male external genitalia determined by the ultrasound. The gender determined by karyotyping was inconsistent with the result of the ultrasound. Therefore, the presence of *SRY* and the presence of structural abnormalities involving the region where *SRY* is located should be further confirmed. Subsequent PCR confirmed the existence of *SRY* gene, and the FISH test with *SRY* site-specific probe further confirmed the existence of the region of *SRY* gene. Meanwhile, it was also confirmed that some of the cells were *SRY* double-positive, suggesting a chimeric karyotype. Taken together the results of chromosome microarray analysis and the FISH test, the karyotype was presumed to be 45, X/46, X, iso (Y), or idic (Y). This chimeric karyotype is commonly found in patients with gonadal dysgenesis, with high phenotypic heterogeneity. It may be manifested as Turner syndrome in women, as ambiguous external genitalia due to mixed gonadal dysgenesis, or as partial gonadal dysgenesis with the appearance of male external

genitalia. Meanwhile, the results of this case indicated that the prenatal routine test indicated 45, X, which was non-chimeric karyotype. However, due to the limitation of prenatal routine technology for low percentage chimerism, there was still the possibility of low percentage chimerism of Y chromosome or *SRY*. Hence, such cases should be comprehensively determined by combining the results of karyotyping, molecular test, FISH, and chromosome microarray analysis. Moreover, as the chimerism proportion is not directly related to the phenotype, it is challenging to predict the phenotype based on the abnormal proportion in the karyotype before the delivery. It is necessary to combine with ultrasound evaluation to estimate the possible phenotypic characteristics, so as to help evaluate the fetal prognosis and help the family decide whether to continue the pregnancy.

Combined with various results, the ultrasound result of the fetus suggested male external genitalia. Taken together with the results of genetic testing and literature retrieval, the fetus may be a male with partial gonadal dysgenesis or a normal male after birth. But the gonad has a risk of developing gonadal tumor, with an average probability of 15%. However, due to the structural abnormality of Y chromosome, the spermatogenic function will be affected, and the patient will not have childbearing potential in the adulthood. In addition, most cases will present with a short stature.

14.3.1.6 Follow-Up

The parents selected to continue the pregnancy and postnatal follow-up revealed male external genitalia.

14.3.2 Intrauterine Diagnosis and Management of Twin Pregnancy in Patients with Congenital Adrenal Hyperplasia

14.3.2.1 Current Medical History

A 37-year-old pregnant woman with 16 gestational weeks was diagnosed with twin pregnancy by ultrasound during pregnancy. The patient came for prenatal genetic counseling because her husband and daughter had 21-hydroxylase deficiency.

14.3.2.2 Prenatal Genetic Counseling

This is a 37-year-old woman for genetic consulting, who previously gave birth to a female child. Her daughter presented with vomiting, diarrhea, skin pigmentation, and clitoral hypertrophy after birth. Neonatal screening test showed increased 17- hydroxyprogesterone (17-OHP) (120 nmol/L), and *CYP21A2* gene analysis showed that she harbored homozygous c.518T > A (p.I173N) mutation and was diagnosed as 21-hydroxylase deficiency (21-OHD). In the following pedigree analysis, this woman carried a heterozygous mutation of c.518T > A (p.I173N), and her husband carried a homozygous mutation of c.518T > A (p.I173N). She requested for prenatal diagnosis. The amniotic fluid samples were taken from two fetuses (F1 fetus and F2 fetus) at 16 weeks of pregnancy, and genetic testing was performed.

14.3.2.3 Laboratory Tests and Results

14.3.2.3.1 Results of Genetic Test of *CYP21A2* and *SRY* Genes in Amniotic Fluid Cells

The amniotic fluid cells of F1 fetus and F2 fetus were analyzed using the STR polymorphism linkage analysis to rule out the maternal contamination. There was no maternal contamination discovered in the amniotic fluid samples. Then, F1 fetus and F2 fetus were tested for *CYP21A2* and *SRY* gene. The F1 fetus exhibited homozygous mutation c.518T>A (p.I173N) in *CYP21A2* gene and *SRY* gene (−). The F2 fetus had heterozygous mutation of c.518T > A (p.I173N) in *CYP21A2* gene with *SRY* gene (+).

14.3.2.3.2 Findings of Fetal Ultrasound (Fig. 14.6)

The F1 placenta was located in the posterior wall of the uterus, and the clitoris of the external genitalia was slightly hyperechoic. The F2 placenta was localized in the posterior wall of the uterine fundus, and the external genitalia was the male type.

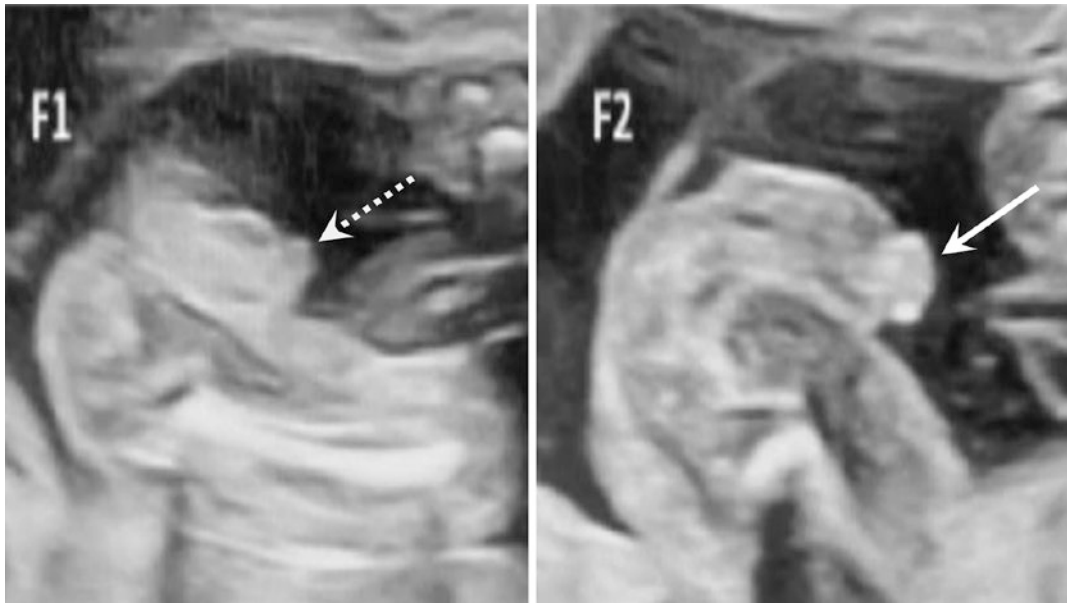

Fig. 14.6 Fetal ultrasound showed that the F1 placenta was located in the posterior wall of the uterus, and the clitoris of the external genitalia was slightly hyperechoic (dashed arrow). The F2 placenta was located in the posterior wall of the uterus fundus, and the external genitalia was a male type (solid arrow)

14.3.2.4 Post-Testing Consultation and Discussion

Congenital adrenal hyperplasia is a group of autosomal recessive hereditary disorders, due to the deficiency of various catalytic enzymes in the adrenal corticosteroid synthesis pathway, and the negative feedback of corticosteroid synthesis may lead to the hypersecretion of adrenocorticotropic hormone (ACTH); among them, 21-hydroxylase deficiency is the most common one. The daughter of the consulting patient had symptoms of vomiting and diarrhea after birth. Physical examination showed skin pigmentation and clitoral hypertrophy. The newborn screening results showed that 17OHP was obviously elevated. The *CYP21A2* genetic test showed that she carried homozygous mutation of c.518T > A (p.I173N). Therefore, the daughter was diagnosed with 21-hydroxylase deficiency and treated with hydrocortisone and 9 α-fludrocortisone, and her condition was stable at present.

The consulting patient's husband was 36 years old, with the height of 160 cm and weight of 82 kg. He complained that he had strong motor ability since childhood and had a history of precocious puberty. The consulting patient said that she became pregnant after she has been married for 3 months. Since then, she did not take any contraceptive measures but was not pregnant for 4 years. Laboratory tests performed at initial visit at his age of 36 years showed increased 17-OHP (213 nmol/L), ACTH (160 pg/mL), and androstenedione (>10 ng/mL). B ultrasound of the adrenal showed he had bilateral adrenal rest tumor. Due to harboring *CYP21A2* gene homozygous mutation of c.518T > A (p.I173N), the husband was diagnosed as nonclassical 21-OHD. Most adult males with nonclassical 21-OHD may no longer require medication. However, excessive testosterone can negatively inhibit FSH and LH, which may affect reproductive function in partial patients, and medication is required. The husband of the consulting patient was given oral hydrocortisone 20 mg/day after the diagnosis was confirmed. The consulting patient became pregnant 3 months later after her husband took the oral hydrocortisone. Combined with intrauterine ultrasound and prenatal genetic diagnosis of the amniotic fluid samples, F1 female fetus was identified as a 21-OHD patient,

and the F2 male fetus was identified as a 21-OHD carrier with the normal phenotype.

14.3.2.5 Follow-Up

The family chose fetal reduction of the fetus F1 at 20 weeks of gestation. Four weeks after the surgery, the fetus F2 developed normally, and the reduced fetus F1 was atrophied. F2 fetus was diagnosed as a carrier of 21OHD confirmed by gene diagnosis after birth, with normal clinical phenotype.

14.4 Research Progress

14.4.1 Common Environmental Risk Factors for Reproductive System Disorders of Intrauterine Origin

In the process of fetal development, critical molecular and cellular processes must respond to various hormones and other growth factors in a complete manner to enable the normal function of the fetus after birth. Chemicals or environmental disturbances in environmental exposure may affect these processes and may change the development and differentiation of gonads and external genitals as well as the postnatal endocrine function and other vital processes for reproduction, and some chemicals with different structures and effects have been shown to affect the synthesis and function of the hormones.

In recent years, the total birth rate has remained below the replacement levels (2.1 children per woman) in many countries worldwide. Decreases in the total birth rate may be correlated with male reproductive disorders, including testicular cancer, disorders of sexual development, cryptorchidism, hypospadias, low testosterone levels, poor semen quality, childlessness, altered sex ratios, and increased demand for assisted reproductive technologies. Reproductive disorders in adult males may occur in the uterus during the fetal period. Although they may be induced by genetic mutations, recent evidence suggests that these issues are often associated with environmental exposure (chemicals and other interfering endocrine substances) of the fetal testicles. Environmental factors can also adversely affect the adult endocrine system. These environmental factors may act directly or through epigenetic mechanisms as a result of increased environmental exposures due to modern lifestyles, and the effects of environmental exposure may persist for generations after exposure [33].

Polycystic ovary syndrome (PCOS) is one of the major endocrine disorders affecting women of childbearing age, but its etiology is still unclear. Some studies suggest that environmental factors, especially the intrauterine environment during the fetal period, play a key role in the development of PCOS. Androgens and endocrine-disrupting substances in the mother, such as bisphenol A, may contribute to the development of polycystic ovary syndrome in the fetus. Alterations in the uterine environment, including hormonal imbalances, may affect fetal gonadal development [34].

14.4.2 Progress in Prevention and Control Strategies for Hereditary Reproductive Disorders

In the primary prevention of hereditary reproductive disorders, it is of great significance to provide comprehensive guidance such as health education, pre-pregnancy care, and genetic counseling to couples preparing to give birth to children, especially conducting carrier screening among the high-risk groups. For couples who were found to carry pathogenic mutations in hereditary reproductive system diseases, genetic counseling and marriage and childbearing guidance should be conducted to evaluate the reproductive risks, select the best mode of reproductive and pregnancy management, and so as to prevent the birth of children with severe disorders and effectively reduce the risk.

The commonly used prenatal screening techniques include noninvasive DNA prenatal screening, etc. Carrier screening based on next-generation sequencing (NGS) technology is a new method for primary prevention of genetic

reproductive problems. The secondary prevention strategy for hereditary reproductive system disorders includes prenatal screening and prenatal diagnosis, especially on the basis of the clinical and genetic diagnosis of the proband. Genetic analysis for high-risk fetuses in families with genetic disorders through prenatal genetic diagnosis provides a practical and effective way for the prevention of these disorders. Prenatal diagnosis techniques include fetal ultrasound, karyotype analysis, fluorescence in situ hybridization, chromosome microarray analysis, Sanger sequencing, NGS, multiplex ligation-dependent probe amplification, etc. Currently, chromosome microarray analysis, NGS, and their derived technologies are rapidly developing and gradually become the mainstream technologies for prenatal diagnosis and genetic etiological diagnosis of birth defects [35]. The third level strategy in the three-level prevention and control plan for hereditary reproductive system disorders is neonatal disease screening. Considering the limitations in the screening techniques such as enzymology and metabolites through tandem mass spectrometry, gene sequencing especially the NGS will become an important method for screening genetic reproductive disorders.

References

1. Nistal M, Paniagua R, Gonzalez-Peramato P, Reyes-Mugica M. Perspectives in pediatric pathology, chapter 6. Male undermasculinization. Pediatr Dev Pathol. 2015;18:279–96.
2. Rey R, Picard JY. Embryology and endocrinology of genital development. Bailliere Clin Endocrinol Metab. 1998;12:17–33.
3. Arboleda VA, Sandberg DE, Vilain E. DSDs: genetics, underlying pathologies and psychosexual differentiation. Nat Rev Endocrinol. 2014;10:603–15.
4. Zarkower D, Murphy MW. DMRT1: an ancient sexual regulator required for human gonadogenesis. Sex Dev. 2022;16:112–25.
5. Rey RA, Grinspon RP. Normal male sexual differentiation and aetiology of disorders of sex development. Best Pract Res Clin Endocrinol Metab. 2011;25:221–38.
6. Farhadi A, Fang S, Zhang Y, et al. The significant sex-biased expression pattern of Sp-Wnt4 provides novel insights into the ovarian development of mud crab (Scylla Paramamosain). Int J Biol Macromol. 2021;183:490–501.
7. Pellegrino M, Maiorino R, Schonauer S. WNT4 signaling in female gonadal development. Endocr Metab Immune Disord Drug Targets. 2010;10:168–74.
8. Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. Nature. 1999;397:405–9.
9. Park S, Cui J, Yu W, Wu L, Carmon KS, Liu QJ. Differential activities and mechanisms of the four R-spondins in potentiating Wnt/beta-catenin signaling. J Biol Chem. 2018;293:9759–69.
10. Dellambra E, Cordisco S, Delle Monache F, et al. RSPO1-mutated keratinocytes from palmoplantar keratoderma display impaired differentiation, alteration of cell-cell adhesion, EMT-like phenotype and invasiveness properties: implications for squamous cell carcinoma susceptibility in patients with 46XX disorder of sexual development. Orphanet J Rare Dis. 2022;17:275.
11. Tallapaka K, Venugopal V, Dalal A, Aggarwal S. Novel RSPO1 mutation causing 46,XX testicular disorder of sex development with palmoplantar keratoderma: a review of literature and expansion of clinical phenotype. Am J Med Genet A. 2018;176:1006–10.
12. Parma P, Radi O, Vidal V, et al. R-spondin1 is essential in sex determination, skin differentiation and malignancy. Nat Genet. 2006;38:1304–9.
13. Kim SY, Weiss J, Tong M, Laronda MM, Lee EJ, Jameson JL. Foxl2, a forkhead transcription factor, modulates nonclassical activity of the estrogen receptor-alpha. Endocrinology. 2009;150:5085–93.
14. Benayoun BA, Caburet S, Dipietromaria A, et al. The identification and characterization of a FOXL2 response element provides insights into the pathogenesis of mutant alleles. Hum Mol Genet. 2008;17:3118–27.
15. Nicol B, Estermann MA, Yao HH, Mellouk N. Becoming female: ovarian differentiation from an evolutionary perspective. Front Cell Dev Biol. 2022;10:944776.
16. Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ. Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature. 2006;441:1109–14.
17. Lee PA, Houk CP, Ahmed SF, Hughes IA, International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine S, the European Society for Paediatric E. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. Pediatrics. 2006;118:e488–500.
18. Witchel SF. Disorders of sex development. Best Pract Res Clin Obstet Gynaecol. 2018;48:90–102.
19. Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. Best Pract Res Clin Endocrinol Metab. 2015;29:607–19.
20. Diaz A, Lipman Diaz EG. Disorders of sex development. Pediatr Rev. 2021;42:414–26.
21. Sandberg DE, Gardner M. Differences/disorders of sex development: medical conditions at the inter-

section of sex and gender. Annu Rev Clin Psychol. 2022;18:201–31.
22. Domenice S, Machado AZ, Ferreira FM, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. Birth Defects Res C Embryo Today. 2016;108:309–20.
23. Mohnach L, Fechner PY, Keegan CE. Nonsyndromic disorders of testicular development overview. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews((R)). Seattle: University of Washington; 1993.
24. Kutney K, Konczal L, Kaminski B, Uli N. Challenges in the diagnosis and management of disorders of sex development. Birth Defects Res C Embryo Today. 2016;108:293–308.
25. Ostrer H. Disorders of sex development (DSDs): an update. J Clin Endocrinol Metab. 2014;99:1503–9.
26. Biason-Lauber A. Control of sex development. Best Pract Res Clin Endocrinol Metab. 2010;24:163–86.
27. Grinspon RP, Rey RA. Disorders of sex development with testicular differentiation in SRY-negative 46,XX individuals: clinical and genetic aspects. Sex Dev. 2016;10:1–11.
28. Merke DP, Auchus RJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med. 2020;383:1248–61.
29. Bulun SE. Aromatase and estrogen receptor alpha deficiency. Fertil Steril. 2014;101:323–9.
30. Miller WL. P450 oxidoreductase deficiency: a disorder of steroidogenesis with multiple clinical manifestations. Sci Signal. 2012;5:11.
31. Wang YC, Su HY, Liu JY, Chang FW, Chen CH. Maternal and female fetal virilization caused by pregnancy luteomas. Fertil Steril. 2005;84:509.
32. Zulfiqar M, Koen J, Nougaret S, et al. Krukenberg tumors: update on imaging and clinical features. AJR Am J Roentgenol. 2020;215:1020–9.
33. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev. 2016;96:55–97.
34. Abruzzese GA, Silva AF, Velazquez ME, Ferrer MJ, Motta AB. Hyperandrogenism and polycystic ovary syndrome: effects in pregnancy and offspring development. WIREs Mech Dis. 2022;14:e1558.
35. Hayward J, Chitty LS. Beyond screening for chromosomal abnormalities: advances in non-invasive diagnosis of single gene disorders and fetal exome sequencing. Semin Fetal Neonatal Med. 2018;23:94–101.