Chapter 2

Review of literature
2.1 Sex-determination and differentiation

Investigations of the molecular events that occur during sex determination, coupled with the analysis of the phenotypes in knockout mice, have increased our understanding of the pathophysiology of some of the clinical defects of sex-determination and differentiation. It is the sex-determining region on Y-chromosome (SRY gene), which dominates the sex-determination pathway (Sinclair et al., 1990). The process of sex determination is identical in both the sexes before SRY expression. The differential gene expression and sex differentiation begins in both the sexes after SRY expression in male gonad starts a series of events leading to the testis development and divergence towards male development (Swain and Lovell-Badge, 2002). The initial changes in genetically male individuals include an increase in proliferation of the coelomic epithelium that surrounds the gonad, formation of a blood vessel at the coelomic surface of the gonad, migration of mesonephric cells into the gonad and formation of testicular cords (Brennan et al., 2002; Buehr et al., 1993; Capel et al., 1999; Martineau et al., 1997; Schmahl et al., 2000). As germ cells are migrating, the urogenital ridge forms from the intermediate mesoderm under the influence of a number of factors, including the transcription factors empty-spericles homeobox gene 2 (Emx2), GATA-4, Lim1, and Lim homeobox 9 (Lhx9) (Fig. 2.1) (MacLaughlin and Donahoe, 2004). Mutations in the genes for these factors produce abnormal gonads in mice, but similar mutations have not yet been implicated in gonadal-dysgenesis syndromes in humans. However, three genes encode interacting proteins that are critical for the formation of the urogenital ridge in humans. The products of the Wilms’ tumor-suppressor gene (WT1) are essential for both gonadal and renal formation. The steroidogenic factor 1 (SF-1) and the duplicated in adrenal hypoplasia congenital on
Figure 2.1: Genes involved in sex-determination and differentiation and the abnormalities associated with their mutations (modified from MacLaughlin and Donahoe, 2004).
the X-chromosome (*DAX1*) proteins are essential for gonadal and adrenal differentiation (Fig. 2.1). Upon testis differentiation, the Leydig cells begin expressing genes involved in steroid (androgen) biosynthesis (MacLaughlin and Donahoe, 2004). The androgen target tissues express 5-α reductase (encoded by SRD5A2), which is essential for the conversion of one metabolic form of androgens (testosterone) to another form (dihydrotestosterone). Both the androgens produced by the testis result in secondary sexual differentiation. The androgen receptor (AR) is critical to the androgen action and hence proper male sexual differentiation (Rajender et al., 2007).

### 2.2 Sex reversal and gonadal dysgenesis

#### 2.2.1 *SRY, SRY* homeobox genes

Patients with pure gonadal dysgenesis have bilateral streak gonads that fail to differentiate. Analysis of these patients and animal models led to the identification of the *SRY* gene located on the distal short arm of the Y-chromosome (Sinclair et al., 1991) and to the detection on autosomes of *SRY* homologues, such as the *SRY* homeobox gene, *SOX9*. The molecular basis for testicular differentiation became clearer when phenotypic males were produced after an *Sry* transgene was introduced into XX mice, confirming the role of *Sry* as a genetic switch that induces testicular differentiation (Koopman et al., 1990). Mutations in the DNA-binding region of the *SRY* gene, which is a member of a large high-mobility-group (HMG) family, were found in a subgroup of 46,XY sex-reversed females with pure gonadal dysgenesis. These patients have characteristic bilateral streak gonads, which are small and fibrotic, without the typical germ-cell or supporting-cell morphology of testes or ovaries (McElreavy et al., 1992). Campomelic
dysplasia, a severe disorder characterized by 46,XY sex reversal, streak gonads, and severe skeletal malformation, occurs in patients with a translocation in the distal arm of chromosome 9p near the SRY box-related SOX9 gene (Wagner et al., 1994) and other genes associated with sex reversal in lower organisms (Ottolenghi et al., 2000). SOX9 and SRY are co-expressed in the male but not in the female urogenital ridge, implicating the two genes in testis determination. The fact that SOX9 activates the transcription of müllerian inhibiting substance (Watanabe et al., 2000; De Santa Barbara et al., 1998) further supports the idea that it has a crucial role in male gonadal development and it is thought to be immediate successor of SRY gene in sex-determination.

2.3 Gonadal and adrenal abnormalities

2.3.1 DAX1

The DAX1 gene codes for a member of the nuclear receptor family of proteins. Since this protein lacks a DNA-binding domain but has a ligand-binding domain, it presumably regulates gene expression through protein–protein interaction (Parker et al., 1999). DAX1 mutations are associated with adrenal hypoplasia congenital (AHC) (Ito et al., 1997), a syndrome of adrenal insufficiency due to impaired development of the adrenal cortex, and hypogonadotropin hypogonadism (HH) as a result of impaired development of the pituitary and the gonads (Fig. 2.1). Dax1 antagonizes the synergy between Sf-1 and Wt1 (Nachtigal et al., 1998) in mice, thereby inhibiting the transcription of Sf-1 downstream genes, most likely by recruiting corepressors (Crawford et al., 1998) or by blocking binding of Sf-1 to DNA (Zazopoulos et al., 1997).
An intersex disorder resulting in dysgenetic and often asymmetric gonads is the enigmatic syndrome of mixed gonadal dysgenesis, which is most often associated with a mosaic 45,X/46,XY karyotype (Donahoe et al., 1979), although a 46,XY karyotype is found in 40 percent of patients (Robboy et al., 1982). The mosaicism is characterized by the presence of at least two gonadal germ-cell lines with different chromosomal complements (Telvi et al., 1999). The percentage of cells with an intact XY genotype dictates the degree of testicular differentiation. In the classic form, there is a streak gonad on one side and a dysgenic fibrotic testis with disordered tubular architecture on the other side, retained müllerian ducts caused by a deficiency of müllerian inhibiting substance, and incomplete genital masculinization as a result of a deficiency of testosterone. An X-linked molecule like DAX1 may have a role in mixed gonadal dysgenesis, since DAX1 suppresses testicular differentiation (Parker et al., 1999). The presence of two X chromosomes, albeit in different cells, one from 45,XO and the other from 46,XY, may be sufficient to prevent sustained testicular growth and differentiation by providing excessive DAX1 (or another inhibiting molecule), which suppresses testicular development. This concept is supported by the observation that the presence of a second X chromosome in XXY humans with Klinefelter’s syndrome and in XXY mice leads to abnormalities of germ-cell development with early entry into meiosis (Mroz et al., 1999).
2.4 Gonadal and renal abnormalities

2.4.1 The Frasier Syndrome and WT1

The Frasier syndrome is characterized by both, gonadal dysgenesis and renal abnormalities that result in streak gonads coupled with the nephrotic syndrome (Fig. 2.1). If it occurs in the XY genotype then it is coupled with sex reversal. Study of the phenotype of Wt1-knockout mice revealed that the gene is involved in the early steps of the differentiation of both gonads and kidneys, helping to explain the association of gonad and kidney malfunction in the Frasier syndrome. Alternative splicing of the Wt1 gene in mice can result in up to 24 protein isoforms. Mutations of two of these isoforms lead to striking clinical manifestations, thereby demonstrating their importance in human sex determination. They are the iKTS and the +KTS variants, in which there is deletion (i) or maintenance (+), respectively, of three amino acids, lysine (K), threonine (T), and serine (S) between the third and fourth zinc fingers of the DNA-binding domain of this transcription factor. Hammes et al. (2001) found that altering the expression of KTS in mice influences both kidney and testicular function. In the Frasier syndrome, the splice site of WT1 that normally preserves the KTS triplet is mutated; therefore, patients with the syndrome produce only WT1 protein without KTS. Gonads lacking KTS have decreased production of the sex-determining region of the Y chromosome (SRY), a urogenital ridge protein that is critical for testicular differentiation. In these iKTS gonads there is also a decrease in müllerian inhibiting substance (MIS), a glycoprotein hormone derived from Sertoli cells that causes regression of the male müllerian ducts, and whose presence is an early marker of testicular differentiation (Teixeira et al., 2001). The findings in the Frasier syndrome indicate that the +KTS WT1 isoform must be produced.
either at the same time or before the urogenital ridge produces the SRY that will induce gonadal differentiation. Individuals with 46,XY karyotype usually have a female phenotype with retained müllerian ducts as well as nephropathy. The severity of the nephropathy varies, however, with the position of the mutation that disrupts the KTS region; some genotypes lead to renal failure in infancy, whereas others cause milder forms of nephritic syndrome compatible with increased longevity. Patients with the Frasier syndrome who have a mutation that inactivates KTS, however, are not susceptible to Wilms’ tumor.

2.4.2 The Denys–Drash Syndrome and WT1
Mutations outside the KTS region result in a WT1 protein that affects gonads later in development, leading to the Denys–Drash syndrome, in which gonads differentiate more completely than the gonads of patients with the Frasier syndrome. Thus, affected patients have a less severe functional deficiency. For example, male gonads are sufficiently developed to produce müllerian inhibiting substance, which ensures that regression of the müllerian ducts is normal, but the synthesis of testosterone is impaired. Although individuals with 46,XY karyotype have a predominantly male phenotype, low testosterone levels can cause male pseudohermaphroditism with various degrees of hypospadias and undescended testes (Little et al., 1995). Patients with the Denys–Drash syndrome also have a high incidence of Wilms’ tumors and a nephropathy characterized by focal glomerular and mesangial sclerosis, which often results in end-stage renal disease and ultimately renal transplantation in the second or third decade of life. These multiple molecular WT1 variants resulting from alternative splicing of the KTS amino
acid triplet have different clinical implications. Study of patients with the iKTS mutation has alerted clinicians to the fact that phenotypic girls with focal glomerular sclerosis or the nephrotic syndrome should be screened for XY sex reversal. Also, phenotypic girls with XY sex reversal who retain müllerian structures, because the gonadal dysgenesis occurs before the production of müllerian inhibiting substance, should be screened for the nephrotic syndrome. In addition, boys with mild undervirilization characterized by hypospadias and undescended testes who also have proteinuria may have the Denys–Drash (+KTS) variant and should be monitored carefully for focal glomerular nephropathy and Wilms’ tumor. Wilms’ tumor can be associated with aniridia, genitourinary anomalies, and mental retardation—the WAGR syndrome (Miller et al., 1964). These complex phenotypic associations are thought to occur because of the proximity of WTI on chromosome 11p13 to the paired box homeotic (PAX6) gene and two other genes in that region that are expressed in the embryonic brain. Patients with the Beckwith–Weidemann syndrome of hemihypertrophy (Koufos et al., 1989), caused by mutations of a gene on chromosome 11p15, are also prone to Wilms’ tumor.

2.5 Steroidogenic Factor 1 (SF-1)

Another important gene in early gonadal development is SF-1 (Parker et al., 1999), which encodes a transcription factor homologous to steroid hormone receptors, but whose ligand is unknown, placing the receptor in a class of orphan nuclear hormone receptors. SF-1 binds DNA and regulates the expression of a number of genes that participate in sexual development. These include müllerian inhibiting substance (Shen et al., 1994; Arango et al., 1999; Watanabe et al., 2000), all the cytochrome P-450 steroid
hydroxylase enzymes (Parker et al., 1995) and 3 B-hydroxysteroid dehydrogenase (Leers-Sucheta et al., 1997), which are required for the synthesis of sex steroid hormones. Sf-1−knockout mice fail to develop adrenal glands and gonads, and die at birth (Lala et al., 1992). A human with adrenal insufficiency and 46,XY sex reversal was found to have a mutation in SF-1 (Achermann et al., 1999; Taketo et al., 1995). Wt1 and Sf-1 have been shown to interact in mice, with Wt1 enhancing the effect of Sf-1 on downstream genes (Nachtigal et al., 1998).

2.6 True hermaphroditism

An unusual cause of ambiguous genitalia is true hermaphroditism, in which both ovarian and testicular tissues are present either in the same or in a contralateral gonad. This disorder is rare in North and South America but quite common in Africa and the Middle East. Asymmetry of gonads and subsequently of reproductive ducts and external genitalia is common, with testes, ovaries, and ovotestes present in various combinations in patients with a predominantly 46,XX karyotype (Donahoe et al., 1978). The sex of rearing is dictated by the phenotype, which is directed by the predominant gonad. In true hermaphroditism, the gonads have less severe dysgenesis (Nihoul-Fekete et al., 1984) than do the gonads of patients with mixed gonadal dysgenesis. The molecular events leading to this unique disorder have not been elucidated, but a few cases have been attributed to translocation of a fragment containing the SRY gene to a cryptic site on the X chromosome (Berkovitz et al., 1992).
2.7 Male pseudohermaphroditism

An important cause of male pseudohermaphroditism with sexual ambiguity is failure of androgen production or an inadequate response to androgen, both of which can cause incomplete masculinization of individuals with the 46,XY karyotype. The clinical spectrum varies from mild failure of masculinization, with hypospadias and undescended testes, to complete sex reversal with a female phenotype.

2.7.1 Inadequate androgen production or metabolism

Another cause of undervirilization arises from defects in the synthesis of testosterone in patients with mutations in the steroidogenic enzymes responsible for the conversion of cholesterol to dihydrotestosterone, namely; steroidogenic acute regulatory protein (Stocco et al., 2001), cytochrome P-450 17-hydroxylase (Di Cerbo et al., 2002), 3b-hydroxysteroid dehydrogenase (Morissette et al., 1995), and 17-ketosteroid reductase (Peltoketo et al., 1999). These defects cause low levels of androgen. Mutations in the 5a-reductase type 2 gene (Andersson et al., 1991) result in low levels of dihydrotestosterone, which cause penoscrotal hypospadias, prepenile scrotas, and an enlarged prostatic utricle (Andersson et al., 1991; Jenkins et al., 1991), often requiring surgical reconstruction (Donahoe et al., 1977). Regression of the Müllerian duct occurs because the normal Sertoli cells produce normal or even elevated (Lee et al., 1996; Lee et al., 1997; Rey et al., 1994) levels of Müllerian inhibiting substance. Many genetic males with a deficiency of 5a-reductase type 2 are born with female external genitalia and are raised as females. The curious virilization that occurs in these patients at puberty often leads to a change in sexual identity (Imperato-McGinley et al., 2002). This paradox
Figure 2.2. The genomic organization of androgen receptor gene and protein. The color-coding of the gene and the protein shows the corresponding regions of the gene encoding various regions of the protein. The names of the protein domains are followed by the description of their functions.
is explained by a normal increase at puberty in the activity of the 5-a reductase type 1 isoform, which results in sufficient dihydrotestosterone to complete the virilization of these genetic males.

2.7.2 Inadequate response to androgens

The inadequate response to androgens results in the end organ resistance to the male hormones, resulting in various degrees of abnormal male sexual differentiation, named as androgen insensitivity syndrome (AIS).

2.7.2.1 AR gene, AR protein and sexual differentiation

AR gene, the most commonly mutated gene in AIS, has been mapped on Xq11.2 and consists of 8 exons separated by intronic sequences, encoding a protein with 919 amino acid residues (Figure 2.2). The gene was initially cloned by Matias et al. (2000), later many laboratories have cloned the gene independently. Exon 1 of the gene is the largest in size and harbours CAG and GGN repeat sequences, encoding variable number of polyglutamine and polyglycine amino acid residues in N-terminal domain of the protein (Fig 2.2).

AR protein is a member of ligand activated super-family of steroid receptors having domain organization. AR protein has domain organization with N-terminal domain (NTD), DNA-binding domain (DBD) and C-terminal ligand binding domain (LBD). NTD is involved in transactivation function of AR protein, DBD binding DNA in complex form with ligand, LBD is involved in ligand binding. LBD, in addition to the
Figure 2.3. Various functional domains/key residues of androgen receptor protein.
ligand binding, is also involved in dimerization of the receptors and binding of specific ligands. The NTD has at least two functional domains essential for transactivation i.e. activation function 1 (AF-1) and activation function 5 (AF-5) (Fig 2.3). LBD consists of activation function 2 (AF-2), which interacts with the activation function domains in NTD. This interaction named as N-C interaction is critical for downstream activation of the genes controlled by AR. The DBD consists of two zinc fingers which help in DNA-binding.

The crystal structure of the LBD has now been determined, which has defined the critical role of certain residue for ligand binding (fig 2.4). Elucidation of 3-D crystallographic structure of AR-LBD has established 12 α helices and four β strands arranged in two β sheets, which make a typical helical sandwich to form ligand-binding pocket (Matias et al., 2000) (fig 2.4). The 12 helices of AR are folded into a three-layered sandwich. Helices H1/2, H3, H7 and H10/11 form two outer layers while inner layers consist of a ligand binding pocket and a non-ligand-binding hydrophobic core (helices H4/5, H8 and H9). The determination of the crystal structure has helped to correlate the mutations with the phenotype in various studies.

2.7.2.2 Androgen receptor mutations

Androgen-receptor mutations (Lubahn et al., 1988; Trapman et al., 1988) result in the androgen insensitivity syndrome (AIS) in which testes can be intra-abdominal or in the inguinal canals, but Wolffian structures and external genitalia fail to respond to high levels of testosterone and its target-tissue metabolite, dihydrotestosterone. Adequate
Figure 2.4. Crystal structure of androgen receptor in complex with methyltrienolone (R1881) (androgen analogue). Panel A and B show the ribbon model at two different angles in complex with the white colored ligand. Panel C shows the narrow ribbon Jmol view of the molecule in complex with the ligand. Panel D shows the WebMol view of the molecule in complex with the ligand. The ligand is a ringed molecule in a different color than the protein molecule.
Müllerian inhibiting substance produced by the otherwise normal testes, however, results in complete regression of müllerian ducts. AIS represents an array of phenotypes, from mild androgen insensitivity (MAIS) to complete androgen insensitivity (CAIS) with the several intermediate forms of PAIS (Fig 1.2).

AR gene has been extensively studied in AIS patients and a large number of mutations have been identified worldwide, and are available at AR gene mutation database (Gottlieb et al., 2004) (web: http://www.mcgill.ca/androgendb/). Of these, approximately 90% have been reported in CAIS and PAIS. A significant number of these mutations have been supported by in vitro functional assays to result in loss of ligand-binding or transactivation potential of the mutant receptor molecule. Most of the mutations resulting in androgen insensitivity are the substitutions along with a low frequency of deletions/insertions. Structural–functional correlation of these mutations is now possible because of the availability of crystal structure for AR-LBD (Matias et al., 2000).

Almost every kind of mutation has been reported in the AR gene; however, certain generalizations have been made (McPhaul 2002). One of the first mutations reported was a group in which single nucleotide substitutions resulted in the insertion of premature termination codon within the open reading frame of the AR gene. In different pedigrees, similar mutations have been localized to each of the eight exons of the AR gene and associated uniformly with CAIS (McPhaul 2002). Mutations associated with normal androgen-binding represent another relatively homogenous class of defects. Nucleotide sequencing in such patients localized the mutations to DBD, which associated with a
broad range of androgen resistant phenotypes, including CAIS and PAIS (McPhaul 2002). Mutations that disrupt N and C-terminal interactions of AR protein form another category of AR mutations, which retain normal ligand binding (Langley et al., 1998). The final category of mutations is associated with qualitative abnormalities of ligand binding such as alterations of ligand affinity, thermal instability of ligand-binding and rapid dissociation of ligand from the receptor protein. Uniformly, these defects have been traced to amino acid substitution mutations within the LBD of the receptor protein and associate with the entire range of androgen resistant phenotypes (McPhaul 2002; Grino et al., 1988).

2.8 Müllerian agenesis

The undifferentiated gonad coexists with both male and female reproductive ducts. The paramesonephric or müllerian duct forms the uterus, fallopian tubes and the upper vagina, and under the influence of testosterone, the mesonephric or wolffian duct forms the vas deferens, seminal vesicles, and epididymides. A transcription factor gene common to the development of both müllerian and wolffian systems is PAX2. This gene is required for normal intermediate development of the mesoderm in both sexes; mutations in mice lead to müllerian duct, wolffian-duct, and renal agenesis (Torres et al., 1995). A mutation in the PAX2 gene has been reported in a family with the renal-coloboma syndrome (Sanyanusin et al., 1995), which partially reproduces the results seen in mice. Failure of müllerian development occurs in 46,XX female patients with Mayer–Rokitansky–Küster–Hauser syndrome, which is characterized by vaginal or complete müllerian agenesis and kidney abnormalities, including a pelvic kidney or the

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more severe agenesis of the kidney (Donahoe et al., 1980). Inactivation of Wnt-4, the
gene encoding a member of the Wingless family of proteins, may be implicated in this
disorder. Wnt is an acronym for a drosophila homologue of the Wingless family of proteins that is found in the mouse genome at a site where the mouse mammary tumor virus growth factor often integrates. The Wnt-4 protein is secreted by the müllerian-duct epithelium and induces the development of the müllerian mesenchyme. Early inactivation of Wnt-4 causes failure of the formation of müllerian-duct derivatives in both sexes; however, a functional effect is manifested only in females, since in normal males, the müllerian duct regresses under the influence of müllerian inhibiting substance. Coincident kidney defects are lethal at birth in mice (Stark et al., 1994; Vainio et al., 1999), but humans with less severe phenotypes can survive. Homeobox (Hox) transcription factors 9, 10, 11, and 13 are necessary for normal uterine and vaginal development; abnormalities in the expression of the genes for these factors account for some uterine and vaginal atresias (Ma et al., 1998). Mutations in a Hoxa13 allele are the cause of the hand–foot–genital syndrome, in which there are deformities of the hands and feet, vaginal abnormalities in females or hypospadias in males, spinal abnormalities, and extrophy of the bladder and cloaca (Goodman et al., 2001). Transfection of constructs with this mutation into the chicken-hindgut region reproduced these abnormalities (de Santa Barbara et al., 2002). Diethylstilbestrol has been known since 1971 (Herbst et al., 1971) to alter müllerian development. The fact that diethylstilbestrol suppresses another Wnt gene - Wnt-7a, and alters Hox gene expression in müllerian ducts in mice (Miller et al., 1998) provides a plausible molecular mechanism for the uterine
abnormalities, vaginal adenosis, and rarely carcinoma observed in patients who were exposed to diethylstilbestrol in utero (Herbst et al., 1971).

2.9 Persistent Müllerian duct syndrome

Persistent müllerian duct syndrome occurs in 46,XY males as a rare form of male pseudohermaphroditism that is caused by a defect in either the gene for the müllerian inhibiting substance, (Teixeira et al., 2001; Belville et al., 1999; MacLaughlin et al., 2001; Haqq et al., 1998) located on chromosome 19p13 (Cohen-Haguenauer et al., 1987), or its type II receptor, located on chromosome 12q13 (Visser et al., 1995). Patients with this syndrome (Belville et al., 1999) have retained müllerian ducts and unilateral or bilateral undescended testes, and they may also have crossed testicular ectopia caused by herniated uterine structures, which drag the contralateral gonad into one scrotum (Hutson et al., 1987) with its ipsilateral gonad.

2.10 Congenital adrenal hyperplasia

Congenital adrenal hyperplasia is caused by the inability of the adrenal to synthesize (Speiser et al., 2001) sufficient cortisol, leading to excess testosterone and resulting in severe masculinization in 46,XX females. More severe forms involve decreased aldosterone production and salt wasting (Speiser et al., 2001). The most common mutation occurs in the cytochrome P-450 21-hydroxylase enzyme (Pucholt et al., 1980; White et al., 1985; Merke et al., 2002); a less common form (5 percent of cases) results from a loss-of-function mutation in 3-β hydroxysteroid dehydrogenase (Morissette et al., 1995). Still rare is 11-β hydroxylase deficiency, which can also result in prenatal or
postnatal virilization (Chua et al., 1987; Mornet et al., 1989). Insufficient production of cortisol and the resultant failure of negative feedback in the hypothalamic–pituitary–adrenal axis causes excess corticotropin production, leading to adrenocortical hyperplasia. In addition, cortisol precursors are shuttled to other steroid pathways, causing high levels of adrenal androgenic steroids, which masculinize the female external genitalia to form a glans penis, rather than a clitoris, and scrotum, rather than labia majora (Fig. 4). Under the influence of the excess androgens, the vagina fails to complete its descent to the perineum, causing a common urogenital canal or sinus with incomplete separation of the vagina and urethra. Ovaries and müllerian structures are otherwise normal, because their development is independent of sex steroids at this stage. The diagnosis can be made in utero, and early maternal dexamethasone therapy can ameliorate the masculinized phenotypes (Speiser et al., 2001; MErke et al., 2002; New, 2001). Surgical reconstruction can be performed in infancy to restore the female phenotype (Schnitzer et al., 2001).

2.11 DMRT (9p) deletions and sex reversal

Deletions of chromosomes 9p have been associated with primary sex reversal of 46,XY individuals, indicating that this region harbors genes essential for testicular development (Bennett et al., 1993). Relatively large deletions on 9p have been associated with a syndrome called 9p deletion syndrome, in which patients have phenotypes including mental retardation and craniofacial abnormalities (Alfi et al., 1976). XY individuals with 9p deletion syndrome have a high frequency of partial or complete sex reversal, with female or ambiguous external and internal genitalia and partial or complete gonadal

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dysgenesis (Bennett et al., 1993; Jotterand et al., 1976; Fryns et al., 1986; Crocker et al., 1988; Hoo et al., 1989). Smaller 9p deletions can cause the same extent of sex reversal and gonadal dysgenesis without the other symptoms of 9p deletion syndrome (Veitia et al., 1997; Flejter et al., 1998; Veitia et al., 1998; Guioli et al., 1998). This suggests that gonadal dysgenesis and the other symptoms of 9p deletion syndrome are caused by the loss of different genes. Molecular and cytogenetic analysis of 9p deletions in sex-reversed patients has identified a range of deletion breakpoints from 9p21 to 9p24, with no clear correlation between the extent of the deletion and the severity of sex reversal. The wide range of deletion breakpoints suggests that the deletions are removing, rather than interrupting, a critical gene. The smallest sex-reversing 9p deletions described delete only a portion of the most distal region, 9p24.3 (Flejter et al., 1998; Guioli et al., 1998). This suggests that one or more genes within the 9p24.3-9pter interval is required for testis development.

It was found that sex-determination was conserved between nematodes and insects (Raymond et al., 1998). Shen et al. (1988) found that the mab-3 gene of C.elegans (required for male sexual differentiation) (Shen et al., 1988), encodes a protein with a novel DNA-binding motif that is also found in the Drosophila sexual regulator doublesex (dsx) (Baker et al., 1980; Burtis et al., 1989; Erdman et al., 1993; Erdman et al., 1996). This motif was named as DM domain after dsx and mab-3. Raymond et al. (1998) the same authors previously identified DMRT1 (Doublesex and MAB-3 related transcription factor 1; formerly called DMT1) and considered it a strong candidate for sex-determination, given its sequence similarity with other sex-determining genes (Raymond et al., 1998). Later two genes namely DMRT1 and DMRT2 were identified from this
region (Raymond et al., 1999). This suggests that sex reversal due to 9p deletion might result from loss of both genes. Consistent with this possibility, sequencing of the entire coding region of *DMRT1* and the DNA-binding domain of *DMRT2* from a large number of XY females revealed only a single possible point mutation in *DMRT1*.

Despite the lack of point mutations in *DMRT1* and *DMRT2*, FISH analysis of the smallest reported sex-reversing 9p deletion showed the absence of both genes, implying that all sex-reversing 9p deletions reported thus far remove both genes. Therefore, the authors suggested that sex reversal in 9p-deleted individuals might be due to hemizygosity of both *DMRT1* and *DMRT2*. Several possible mechanisms have been proposed for XY sex reversal associated with 9p deletions, including haploinsufficiency of the deleted gene(s) or the uncovering of a recessive mutation on the remaining chromosome. The relatively high frequency of XY sex reversal observed in 9p deletion syndrome suggests haploinsufficiency, because otherwise the frequency of pre-existing mutant alleles would have to be correspondingly high.

### 2.12 WNT4 gene and sex reversal

Most of the genes discussed above play role in male differentiation, and result in male to female sex reversal, when mutated. Till date, no gene has been shown to play a role in ovarian development equivalent to the role played by SRY and SOX9 genes in testicular development. WNT4 is one gene encoding the signaling molecule, which has been implicated in ovary development. Wnt4 is expressed in the embryonic gonad of both
sexes at early stages of development and after the action of Sry in the XY gonad its expression becomes ovary specific (Vainio et al., 1999). Vainio et al. (1999) studied a mouse model in which Wnt4 is ablated (Stark et al., 1994) and observed that, whereas both male and female Wnt4-knockout mice have similar defects in kidney development and adrenal function but gonadal development and steroidogenic function are affected exclusively in female Wnt4-knockout mice. Later it was shown that the lack of Wnt4 leads to masculinization of XX embryos, and also that Wnt4 inhibits the migration of adrenal precursors and endothelial cells into the ovary (Jeays-Ward et al., 2003; Vainio et al., 1999). In addition, overexpression of Wnt4 in the testis interferes with proper testicular vascular development and androgen production (Jeays-Ward et al., 2003; Jordan et al., 2003). Wnt4 and Dax1 have been proposed to act in the same molecular pathway with WNT4 regulating Dax1 expression (Jordan et al., 2001; Mizusaki et al., 2003). Consistent with this, these genes have a similar expression pattern during gonad development and Wnt4 mutant animals show decreased levels of Dax1 expression.