Disorders of Sex Development with Testicular Differentiation in SRY-Negative 46,XX Individuals: Clinical and Genetic Aspects

Romina P. Grinspon, Rodolfo A. Rey

**Key Words**
Disorders of sex development · SRY · XX maleness

**Abstract**

Virilisation of the XX foetus is the result of androgen excess, resulting most frequently from congenital adrenal hyperplasia in individuals with typical ovarian differentiation. In rare cases, 46,XX gonads may differentiate into testes, a condition known as 46,XX testicular disorders of sex development (DSD), or give rise to the coexistence of ovarian and testicular tissue, a condition known as 46,XX ovotesticular DSD. Testicular tissue differentiation may be due to the translocation of SRY to the X chromosome or an autosome. In the absence of SRY, overexpression of other pro-testis genes, e.g. SOX family genes, or failure of pro-ovarian/anti-testis genes, such as WNT4 and RSPO1, may underlie the development of testicular tissue. Recent experimental and clinical evidence giving insight into SRY-negative 46,XX testicular or ovotesticular DSD is discussed.

Normal Foetal Sex Differentiation

Virilisation of the XX foetus is the result of excessive androgen action during intrauterine development. Androgen excess may be the consequence of a disorder of gonadal (ovarian) development, but it occurs more frequently in individuals with typical ovarian differentiation (table 1). In humans, congenital adrenal hyperplasia, deficiencies in aromatase activity, androgen-secreting tumours and consumption of drugs with androgenic potential belong to the second group and represent the vast majority of cases of 46,XX disorders of sex development (DSD).

Ovarian differentiation is the normal pathway in 46,XX foetuses (fig. 1). However, in rare cases, 46,XX gonads may either completely differentiate into testes, a condition known as 46,XX testicular DSD (previously called XX male) [de la Chapelle et al., 1964], or give rise to the coexistence of ovarian and testicular tissue in the same individual, a condition known as 46,XX ovotesticular DSD (previously true hermaphroditism) [Lee et al., 2006]. In this review, we will address the latest discoveries regarding the causes, underlying genetic mechanisms and clinical aspects of 46,XX DSD with testicular tissue development.
Development into a testis for the male differentiation of the internal and external genitalia, due to the secretion of testosterone and a second testicular factor, subsequently characterised as the anti-Müllerian hormone (AMH) [Josso, 2008]. In their absence, internal and external genitalia follow the female pathway. The degree of virilisation of the internal and external genitalia and of the regression of Müllerian ducts depends on the mass of functional testicular tissue present in the narrow window of foetal dimorphic sex differentiation [Rey and Grinspon, 2011]. A few years after Jost’s work, the determining role of the Y chromosome for testicular development was established [Ferguson-Smith, 2009].

During the indifferent stage, the gonads are bipotential irrespective of their XX or XY karyotype. Gonadal differentiation into a testis or an ovary requires a delicate dosage balance in the timing and levels of expression of several genes [Lin and Capel, 2015]. In most mammalian embryos, the transient expression of SRY, which maps to the Y chromosome, triggers a cascade of gene interactions ultimately leading to the formation of a testis from the indifferent gonadal ridge [Larney et al., 2014]. SRY expression initiates in the middle of the gonad and expands toward the poles [Bullejos and Koopman, 2001]. The timing and level of SRY expression are critical for proper testis differentiation: delayed or decreased expression results in dysgenetic testicular or ovotesticular differentiation in the mouse [Nagamine et al., 1999; Bullejos and Koopman, 2005]. In most mammals, the SRY-box gene

Table 1. 46,XX DSD

<table>
<thead>
<tr>
<th>Abnormal gonadal (ovarian) differentiation</th>
<th>Testicular DSD</th>
<th>Ovotesticular DSD</th>
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<tr>
<td>With no overt gonadal dysgenesis (previously known as XX male)</td>
<td>With overt gonadal dysgenesis: DSD with ambiguous genitalia</td>
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<th>Normal gonadal (ovarian) differentiation</th>
<th>Androgen excess</th>
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<tr>
<td>Foetal</td>
<td>Congenital adrenal hyperplasia: deficiencies of 21-hydroxylase, 11-hydroxylase deficiency, or 3β-hydroxysteroid dehydrogenase</td>
<td>Defective Müllerian duct formation</td>
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<td>Glucocorticoid receptor mutations</td>
<td>Defects of the urogenital sinus and external genitalia: cloacal extrophy, vaginal atresia, etc.</td>
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SOX9 is the earliest upregulated gene in the testis pathway downstream of SRY, followed by CITED4 and other members of the SOX family, including SOX3, SOX10 and SOX13, and many other genes that are critical for testicular differentiation [Quinn and Koopman, 2012; Munger et al., 2013; Lin and Capel, 2015].

Genes involved in ovarian differentiation of the bipotential gonad increase their expression somewhat later. WNT4 and RSPO1 stabilise β-catenin, encoded by CTNNB1, which promotes the expression of ovarian genes, such as FST (follistatin) and FOXL2. The latter also counteracts SOX9 and other pathways involved in early testis development [Carré and Greenfield, 2014; Biason-Lauber and Chaboissier, 2015; Lin and Capel, 2015; Suzuki et al., 2015] (fig. 1).

**46,XX Ovotesticular and Testicular DSD**

Ovotesticular DSD is a rare form of DSD, with variable prevalence and karyotypes in different regions of the world. However, all studies agree that 46,XX is the most common karyotype observed in blood samples, ranging from ~65 to 90% [Verkauskas et al., 2007; Wiersma and Ramdial, 2009; Matsui et al., 2011]. The remaining cases carry a Y chromosome (46,XY, 46,XX/46,XY or other mosaicisms), which explains the development of testicular tissue. In 46,XX patients with testicular or ovotesticular DSD, the SRY gene may be present, owing to an abnormal translocation to the X chromosome or more rarely to an autosome. A precise frequency of SRY-positive 46,XX DSD is difficult to estimate due to inclusion and/or ascertainment biases of the various studies. In unselected samples of limited numbers (between 30 and 33 patients), the reported prevalence of SRY-positive 46,XX ovotesticular DSD cases ranges from 10% [McElreavey et al., 1992] to 33% [Verkauskas et al., 2007]. Yet, frequencies may range from 0% of cases when SRY was studied in blood cells of 46,XX ovotesticular DSD patients with ambiguous genitalia [Matsui et al., 2011] to 100% of cases when SRY was determined in blood cells of 46,XX testicular DSD with complete virilisation [Vorona et al., 2007] or in gonadal tissue of 46,XX ovotesticular DSD cases with ambiguous genitalia [Ortenberg et al., 2002]. Despite imprecision, evidence that SRY could not explain all cases of 46,XX maleness became progressively stronger.

When testicular tissue differentiates in an SRY-negative XX gonad, 2 different mechanisms can be envisaged: the increased expression of pro-testis genes or the insufficient expression of pro-ovarian/anti-testis genes (fig. 1). An effort to make an accurate diagnosis is important for the provision of proper genetic counselling and long-term management. In the next sections, we will review recent evidence on the genetic mechanisms underlying XX maleness in SRY-negative individuals and on the clinical management of these patients.

**Mechanisms Underlying Testis Differentiation in SRY-Negative Individuals**

**Increased Expression of Pro-Testis Genes**

SOX9

The SOX (SRY-related HMG box) protein family includes a group of transcriptional regulators containing a highly conserved high-mobility-group domain [Lefebvre et al., 2007]. This domain was first identified in SRY. SOX9, mapping to 17q24.3 in the human, is expressed in several tissues including chondrocytes and testes but also in bile duct, the central nervous system, hair follicles, heart, lung, pancreas, and retina. In the XY mouse embryo, an increase in SOX9 expression is induced by SRY in the gonadal ridge. SOX9 then upregulates FGF9 and PGD2, and a positive feedback loop is established to further upregulate SOX9, which progressively becomes independent of SRY (fig. 1). SOX9 is responsible for Sertoli cell specification, thus initiating testis differentiation [Eggers et al., 2014], and for triggering AMH production [de Santa Barbara et al., 1998; Arango et al., 1999]. SOX9 haploinsufficiency results in dysgenetic 46,XY DSD associated with campomelic dysplasia [Foster et al., 1994; Wagner et al., 1994].

Conversely, ectopic SOX9 expression in undifferentiated gonads of transgenic XX mice results in sex reversal with testis development and male phenotype, demonstrating that high SOX9 expression suffices to trigger testis differentiation in the absence of Sry [Vidal et al., 2001]. Adult gonads contain seminiferous tubules with Sertoli cells but no spermatogenesis, as expected in a male with 2 X chromosomes. Similarly, XX dogs with an interstitial duplication of chromosome 9 which carry the SOX9 locus develop through the male pathway [Rossi et al., 2014].

Testicular and ovotesticular DSD in 46,XX SRY-negative patients have been described in association with duplications of chromosome 17 carrying the SOX9 locus. The first infant with ambiguous genitalia and scrotal gonads, who carried a de novo mosaic 46,XX,dup(17) (q23.1q24.3) containing the SOX9 gene, as revealed by FISH, was reported in 1999 [Huang et al., 1999]. The
study suggested that the extra dose of SOX9 is sufficient to initiate testis differentiation in the absence of SRY; however, the gonadal histology was not described. A recent case of a boy with normal genitalia has also been reported with a SOX9 duplication [Lee et al., 2014].

In line with the previous observation and with data in mice identifying relevant cis-activating elements within a SOX9 gonad-specific enhancer [Sekido and Lovell-Badge, 2008], duplications or triplications of potential SOX9 regulatory sequences have subsequently been identified as associated with XX maleness. In fact, a 178-kb duplication 600 kb upstream [Cox et al., 2011] and a 96-kb triplication 500 kb upstream [Vetro et al., 2011] of the SOX9 coding sequence were initially identified as the cause of familial 46,XX testicular DSD in SRY-negative patients with small testes and azoospermia (table 2). Also, a 148-kb tandem duplication of the region ∼500 kb upstream of SOX9 was found in an SRY-negative 46,XX patient with ovotesticular DSD, who presented with ambiguous genitalia. Two further cases with partially overlapping 17q24.3 duplications ∼500 kb upstream of SOX9 presented with dissimilar phenotypes: one was an infertile male with testes, while the other was an ovotesticular DSD with ambiguous genitalia [Vetro et al., 2015]. Finally, 3 recent reports [Xiao et al., 2013; Hyon et al., 2015; Kim et al., 2015] have identified 7 further SRY-negative patients with 46,XX testicular or ovotesticular DSD, who carried duplications ranging from 68 to 83.8 kb, located ∼510–600 kb upstream of SOX9, which overlapped with previously reported rearrangements and allowed refining the minimal region to a 40.7–41.9-kb element.

SOX3

SOX3 is a single exon gene located in Xq27.1, which encodes a protein that is most similar to SRY and that is required for normal brain, pituitary and craniofacial development in mice and humans. Although SOX3 is not required for normal testicular differentiation, as revealed

### Table 2. Genetics and phenotypes of SRY-negative 46,XX DSD with testicular tissue

<table>
<thead>
<tr>
<th>Proposed pathogenesis</th>
<th>Genetic findings</th>
<th>Genitalia</th>
<th>Gonadal histology</th>
<th>Hormone analysis</th>
<th>References</th>
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<tr>
<td>Increased SOX9 expression</td>
<td>duplication of SOX9 gene</td>
<td>ambiguous genitalia (n = 1)</td>
<td>not reported</td>
<td>not reported normal FSH, LH, AMH and post-hCG T</td>
<td>Huang et al., 1999</td>
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<td></td>
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<td>male genitalia (n = 1)</td>
<td>testes</td>
<td>high FSH and LH low to normal T</td>
<td>Lee et al., 2014</td>
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<td>infertile male ± gynaecomastia (n = 8)</td>
<td>testes with germ cell depletion</td>
<td>low FSH and LH post-hCG T</td>
<td>Cox et al., 2011</td>
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<td></td>
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<td>ambiguous genitalia (n = 4)</td>
<td>ovotestes/testes</td>
<td>low T post-hCG</td>
<td>Vetro et al., 2015</td>
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<tr>
<td>Increased SOX3 expression</td>
<td>duplication of SOX3 gene</td>
<td>infertile male (n = 2)</td>
<td>not reported</td>
<td>high FSH and LH low to normal T</td>
<td>Sutton et al., 2011</td>
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<td>male genitalia (n = 2)</td>
<td>not reported</td>
<td>not reported</td>
<td>Sutton et al., 2011</td>
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<td></td>
<td>rearrangement of SOX3 regulatory regions</td>
<td>ambiguous genitalia (n = 4)</td>
<td>testes</td>
<td>not reported</td>
<td>Mizuno et al., 2014</td>
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<td>ambiguous genitalia (n = 1)</td>
<td>one testis/one ovary</td>
<td>normal FSH, LH and T for male</td>
<td>Haines et al., 2015</td>
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<td>ambiguous genitalia (n = 1)</td>
<td>ovotestes</td>
<td>normal FSH and LH, low AMH and T for male</td>
<td>Grinspon et al., 2015</td>
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<td>Increased SOX10 expression</td>
<td>duplication of chromosome 22q</td>
<td>normal male (n = 1)</td>
<td>not reported</td>
<td>not reported low T for male</td>
<td>Sezherunvong et al., 2004</td>
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<td></td>
<td>trisomy 22</td>
<td>ambiguous genitalia (n = 1)</td>
<td>one testis/one ovary</td>
<td>not reported</td>
<td>Aleck et al., 1999</td>
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<td>Decreased WNT4 expression</td>
<td>WNT4 point mutation</td>
<td>ambiguous genitalia in SERKAL syndrome (n = 2)</td>
<td>dygentic testes</td>
<td>low T for male post-hCG</td>
<td>Nicholl et al., 1994</td>
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<td>ambiguous genitalia (n = 1)</td>
<td>dygentic testes (n = 1)</td>
<td>not reported</td>
<td>Mandel et al., 2008</td>
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<td>Decreased RSPO1 expression</td>
<td>RSPO1 2.7-kb deletion</td>
<td>ambiguous genitalia, palmoplantar keratoderma (n = 4)</td>
<td>dygentic testes (n = 1)</td>
<td>not reported</td>
<td>Parma et al., 2006</td>
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<td></td>
<td>RSPO1 point mutation</td>
<td>ambiguous genitalia, palmoplantar keratoderma, onychodystrophy, hearing impairment (n = 1)</td>
<td>ovotestes</td>
<td>normal FSH, LH and oestradiol for female</td>
<td>Tomaselli et al., 2008</td>
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hCG = Human chorionic gonadotropin; T = testosterone.
by mutations in humans and mice [Weiss et al., 2003], its ectopic overexpression in the developing XX gonads provokes testicular development in a transgenic mouse model. Elegant experimental studies have shown that, just as SRY, SOX3 can act synergistically with SF1 to upregulate SOX9 expression and trigger Sertoli cell differentiation in the bipotential gonad [Sutton et al., 2011].

Concordantly, different rearrangements of the SOX3 locus have been associated with 46,XX testicular DSD, suggesting that overexpression of SOX3 can cause XX male sex reversal in humans. Duplications encompassing SOX3 have been reported in 5 SRY-negative 46,XX patients with male or ambiguous genitalia and functional evidence of the existence of testicular tissue [Sutton et al., 2011; Moalem et al., 2012; Vetro et al., 2015], although no histological study of the gonads was reported in most of them. In 5 other patients, the condition was associated with rearrangements involving the regulatory region of SOX3. A duplication of a common 206-kb region, located 566 kb upstream of the SOX3 coding sequence, was found in four 46,XX patients with testicular DSD [Mizuno et al., 2014], while a 773-kb duplication of chromosome 1 integrated ~82 kb distal to SOX3 was described in a patient with testicular differentiation on one side and an ovary on the other side [Haines et al., 2015]. We have recently reported the first case of an SRY-negative 46,XX patient with ambiguous genitalia associated to a SOX3 duplication presenting with bilateral ovotestes [Grinspon et al., 2015]. It should, however, be mentioned that a SOX3 duplication may not be sufficient to induce testicular differentiation when normal regulatory sequences are lacking in the duplicated DNA fragment [Igarashi et al., 2015].

SOX10

SOX10, a gene closely related to SOX9, maps to chromosome 22q13.1 in humans and is involved in neural crest and glial development. As for SOX3, a functional role for SOX10 has not been established in normal gonadal development [Pingault et al., 2013], presumably because loss-of-function during gonadal development is likely to be masked by the action of other members of the SOX family. However, overexpression of Sox10 in the gonads of transgenic XX mice results in the development of testes and a male phenotype [Polanco et al., 2010]. Interestingly, complete or partial duplications of chromosome 22 have been reported in a number of SRY-negative XX cases with ovarian dysgenesis and/or testicular tissue differentiation [Polanco et al., 2010]. Relevant to the present review are a patient with masculinised external genitalia, dysgenetic testes and Müllerian remnants with a 47,XX,+22 karyotype [Nicholl et al., 1994], a patient with ambiguous genitalia (ovotesticular DSD) associated to an inverted duplication of 22q13.122qter [Aleck et al., 1999], and a patient showing only mild hypospadias and bilaterally palpable testes in whom a duplication of 22q11.22q13 was reported [Seeherunvong et al., 2004].

DMRT1

The DMRT gene cluster is located in human chromosome 9p24.3 and comprises DMRT2, DMRT3 and DMRT1 [Brunner et al., 2001]. DMRT genes have a conserved DNA-binding motif, the DM domain, and play critical roles in gonadal differentiation and gametogenesis. DMRT1 encodes a male-specific transcription factor and has the most prominent role – amongst DMRT genes – in regulating testicular differentiation in all vertebrates. Distal 9p deletions encompassing the DMRT cluster are associated with dysgenetic and ovotesticular DSD in 46,XY patients [Matson and Zarkower, 2012].

Although no SRY-negative 46,XX DSD patients with testicular tissue and DMRT1 overexpression have as yet been identified, increased expression of Dmr at1 in the fetal gonads of transgenic XX mice is sufficient to drive testicular differentiation and male sex development [Zhao et al., 2015].

Insufficient Expression of Pro-Ovarian Genes

WNT4

WNT4 belongs to the WNT family, a large group of secreted glycoproteins encoded by 19 distinct genes that are expressed in a tissue-specific fashion. WNT4 maps to 1p36.12, and there is clinical and experimental evidence of its role in sex differentiation. WNT4 cooperates with RSPO1, leading to a reduction in the phosphorylation and degradation of β-catenin. The consequent increase in β-catenin induces WNT-responsive genes and antagonises SOX9 [Carré and Greenfield, 2014; Biason-Lauber and Chaboissier, 2015]. WNT4 upregulates DAX1 expression, which antagonises SF1, and thereby inhibits steroidogenic enzymes. WNT4 overexpression has been associated to sex reversal in a XY patient [Jordan et al., 2001]. Apart from its role in ovarian differentiation, WNT4 is also directly involved in Müllerian duct formation [Biason-Lauber and Chaboissier, 2015] and plays a critical role in the formation of the kidneys, adrenals, pituitary gland, and mammary tissue.

The first experimental evidence of the role of WNT4 in sex development derives from the inactivation of the Wnt4 gene in mice [Vainio et al., 1999]. Wnt4 knockout XX mice are virilised, the Müllerian duct is absent, and
the development of the Wolffian ducts resembles that of the male. The gonads are also masculinised, as revealed by the enhanced expression of the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, required for testosterone synthesis and normally suppressed in the foetal ovary.

Heterozygous loss-of-function mutations of WNT4 have been described in in three 46,XX patients with mild virilisation. The main complaint was primary amenorrhoea due to Müllerian defects characteristic of the Mayer-Rokitansky-Kuster-Hauser syndrome and clinical signs of androgen excess [Biason-Lauber et al., 2004, 2007], but no testicular tissue could be identified [Philibert et al., 2008]. Conversely, the inactivation of both copies of WNT4 results in a severe clinical condition, known as the SERKAL syndrome, characterised by 46,XX DSD with ambiguous genitalia, renal agenesis, adrenal hypoplasia, and pulmonary and cardiac abnormalities. The presence of testicular DSD and ovotesticular DSD were reported in the same family [Mandel et al., 2008].

RSPO1

RSPO1 is one of the 4 members of the R-spondin family, expressed during foetal development in the central nervous system, the limb buds and the body trunk, and the proximal posterior part of the anterior limb bud [Chassot et al., 2008]. The human RSPO1 gene is located in chromosome 1p34.3. As already mentioned, several lines of evidence have demonstrated that RSPO1 synergises with WNT4 in XX gonads to stabilise β-catenin, thus acting as an activator of the canonical signalling pathway in ovarian development [Biason-Lauber and Chaboissier, 2015].

In mice, the ovarian phenotype of the XX Rspo1 knockout is strikingly similar to that of Wnt4 knockout [Chassot et al., 2008]. Penetration of the phenotype is slightly variable, and gonad morphology may range from dysgenetic gonads to ovotestes. Interestingly, β-catenin overexpression is able to rescue the abnormal masculinisation of the gonads of XX Rspo1 knockout mice, thus confirming that RSPO1 acts through the β-catenin signalling pathway.

In humans, the first reported cases with loss-of-function mutations of the RSPO1 gene were associated with SRY-negative 46,XX DSD, palmoplantar hyperkeratosis and squamous cell carcinoma of the skin [Parma et al., 2006]. The presence of ‘functional’ testes was assumed based on the absence of Müllerian derivatives and the masculinisation of the internal and external genitalia; however, no histological study of the gonads is reported. A second case with 46,XX virilisation associated with palmoplantar keratoderma, congenital bilateral corneal opacities, onychodystrophy, and hearing impairment was reported later. Laparoscopy showed an apparently normal uterus and left fallopian tube. Histology of the gonad biopsy showed the presence of ovotestis and a seminoma [Tomaselli et al., 2008]. Notably, the normal reproductive phenotype of 46,XY individuals suggests that RSPO1 is not required for testis differentiation and function.

FOXHL2

FOXHL2 is a single exon gene encoding a forkhead/winged helix nuclear protein acting as a transcription factor, and mapping to 3q22.3 in humans. In most vertebrates, FOXL2 is one of the earliest markers of foetal differentiation of the ovary. In mice, Foxl2 does not seem essential for ovarian development in the XX foetus, but forced expression impairs testis differentiation in the XY foetus [Ottolenghi et al., 2007]. FOXL2 continues to be expressed in the postnatal ovary, and ablation of FOXL2 expression in the XX adult ovary results in its transdifferentiation to testis [Uhlenhaut et al., 2009]. In 46,XX patients, loss-of-function of FOXL2 is associated with the blepharophimosis/ptosis/epicanthus inversus (BPES) syndrome with or without ovarian dysgenesis [Crisponi et al., 2001], but no development of testicular tissue or signs of virilisation have been reported.

Clinical Features

In the previous passages of this review, we analysed 46,XX DSD with testicular tissue differentiation from a pathogenic standpoint and referred to them as ‘testicular’ or ‘ovotesticular’ DSD according to whether ovarian tissue was present. In all cases, the testicular component could be more or less dysgenetic. While the existence of testicular tissue can be easily demonstrated, since it can be inferred from the assessment of genital virilisation and of the levels of circulating testosterone and AMH, the ascertainment of the existence of ovarian tissue is not always possible. In fact, the presence of the ovaries do not have any impact on foetal development of the genitalia [Jost, 1953], and there are no reliable markers of ovarian function in infants; therefore, the diagnosis of ovotesticular DSD may be missed.

From a clinical point of view, virilised 46,XX patients with testicular tissue can present with an apparently normal male phenotype or with variable degrees of genital
ambiguity (table 1). Evidence exists that testicular and ovotesticular DSD are different manifestations of the same disorder of gonadal development, especially by the existence of different phenotypes in the same family and even in monozygotic twins [Maciel-Guerra et al., 2008]. Notwithstanding, we will separately address the clinical features of completely virilised patients and those with ambiguous genitalia.

**46,XX DSD with Testicular Tissue and Complete Virilisation**

According to the nomenclature suggested by the Chicago consensus [Lee et al., 2006], this includes 46,XX testicular DSD and, very rarely, 46,XX ovotesticular DSD. The former corresponds to the original concept of ‘XX male’, as defined by Albert de la Chapelle [1972], i.e. ‘a male phenotype, male psychosexual identification, testes or gonads of testicular type without macroscopic or microscopic evidence of ovarian tissue, and absence of female genital organs’. It is estimated that 90% of XX males are SRY-positive [Vorona et al., 2007]; since no distinction has been made in most of early clinical descriptions of XX males regarding the existence or not of Y chromosomal sequences including SRY, we are not able to make a detailed, specific characterisation of SRY-negative XX males. When testes have differentiated normally during foetal development, irrespective of the presence or absence of SRY, clinical features of XX males are expected to be the same.

In these typical cases, diagnosis is usually delayed until adult life when the male patient is examined because of infertility. Less frequently, boys may be referred to a paediatric endocrinologist for short stature or small testes at the age of puberty. In fact, the stature of XX boys is lower than that of age-matched XY pairs, and similar to that of age-matched XX girls, in spite of similar circulating levels of hormone of the growth axis, including IGF1 and IGFBP3 [Aksglaede et al., 2008]. The existence of Y-specific growth genes may be the explanation. Testes are smaller in these patients, as in patients with Klinefelter syndrome, in adulthood [Vorona et al., 2007]. Testicular volume does not differ from that of control boys during childhood. The underlying reason is that testicular volume depends mainly on Sertoli cells before puberty. Also at pubertal onset, testicular volume increases progressively to 4 ml, and even to 6–8 ml, mainly due to Sertoli...
Sertoli and Leydig cell function is normal in childhood and at the onset of puberty in 46,XX males, as revealed by normal testis hormone and gonadotropin levels [Boucekkine et al., 1994; Rey et al., 1999]. Testicular dysfunction occurs during pubertal development, when the normal increase in testicular volume is led by germ cell proliferation. The existence of 2 X chromosomes and the absence of a Y chromosome results in meiotic failure, leading to dramatic germ cell loss, regression of testicular volume, azoospermia and low semen volume (fig. 2) [Huang and Yen, 2008]. Seminiferous tubule dysfunction is reflected in low inhibin B and increasing FSH, whereas a mild Leydig cell dysfunction may exist in some cases leading to low-normal testosterone and increased LH. A typical feature is the elevation of oestrogen levels responsible for gynaecomastia [Vorona et al., 2007; Aksglaede et al., 2009].

**46,XX DSD with Testicular Tissue and Ambiguous Genitalia**

When the amount or functional capacity of the testicular tissue is insufficient, various degrees of genital ambiguity may occur. This applies to both testicular DSD and ovotesticular DSD. As already mentioned, the differential diagnosis between ovotesticular and testicular DSD is based on histological analysis. Ovotesticular DSD requires the existence of seminiferous cords and ovarian follicles with oocytes (fig. 3). The clinical presentation does not differ from that of other types of DSD, with a range going from a rather male phenotype with mild hy-}

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**Fig. 3.** Histology of ovotesticular DSD. Histological aspect of an ovotestis, whose diagnosis requires the presence of seminiferous cords (or tubules) and ovarian follicles with oocytes. The mere existence of a fibrous ovarian-like stroma, without follicles, is insufficient for the diagnosis. Modified with permission from Chemes et al. [2003].
Concluding Remarks

Evolving knowledge triggers changes in nomenclature. The old definitions of XX males and true hermaphroditism have left their place to the most recent 46,XX testicular DSD and ovotesticular DSD. However, this homology is not straightforward. Whereas the concept of XX males did not include genital ambiguity, XX testicular DSD includes all 46,XX patients with testes, irrespective of whether they have a normal capacity of producing androgens and AMH or whether they are dysgenetic. Clearly, a male phenotype results in the former case, and ambiguous genitalia in the latter. Also, with the availability of new technologies, we have gained insight into the mechanisms underlying testicular tissue development in 46,XX SRY-negative individuals. Thus, inadequate expression of SOX3, SOX9, SOX10, WNT4, and RPSPO1 have already been associated with XX testicular or ovotesticular DSD in humans, while other candidate genes are being considered on the basis of knowledge derived from experimental models. The impact on clinical practice of the complete unveiling of the genetic aetiologies of XX male-ness is as yet only partially appraised.

Disclosure Statement

The authors have no conflicts of interest to declare.

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