DEVELOPMENTAL GENETICS OF THE FEMALE REPRODUCTIVE TRACT IN MAMMALS

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The female reproductive tract receives the oocytes for fertilization, supports the development of the fetus and provides the passage for birth. Although abnormalities of this organ system can result in infertility and even death, until recently relatively little was known about the genetic processes that underlie its development. By drawing primarily on mouse mutagenesis studies and the analysis of human mutations we review the emerging genetic pathways that regulate female reproductive-tract formation in mammals and that are implicated in congenital abnormalities of this organ system. We also show that these pathways might be conserved between invertebrates and mammals.

AGENESIS
A condition in which a body part is absent or does not develop completely.

ATRESIA
A condition in which an opening or passage for the tracts of the body is absent or closed.

SEPTATION
Refers to the state of being divided internally by a partition or partitions. In the female reproductive tract, septation is observed longitudinally or transversely.

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The female reproductive tract is essential for the continuation of mammalian species: it provides the site for the fertilization of oocytes by spermatozoa, for implantation and subsequent development of the embryo, and for delivery of the fetus. Abnormalities in female reproductive-tract formation — which are estimated to occur in 0.1–3.0% of live births in humans[1] — can lead to infertility and even death during pregnancy or childbirth. The range of documented defects includes AGENESIS, ATRESIA and SEPTATION of the female reproductive tract[1], which are thought to result from abnormalities that occur during embryonic development.

The female reproductive tract, which in mammals includes the oviducts (fallopian tubes), uterus, cervix and vagina (FIG. 1a), is also a prominent organ site for disease. Malignancies of the cervix and uterus accounted for 14.8% of all cancers among women in the United States in the year 2000 (REF. 2). Surprisingly, despite the importance of this organ system for the fertility and health of women, relatively little is known about the molecular and cellular mechanisms that regulate its development during embryogenesis. Recent findings, predominantly from mouse knockout studies, have identified a set of genes that are essential for the development of this organ system (TABLE 1). By primarily drawing on mouse and human genetic studies, this review examines our knowledge of the genetic pathways that regulate the organogenesis of the female reproductive tract in mammals. Many of these studies show that interactions between the mesenchyme and epithelium of the developing female reproductive tract are important for its formation and differentiation. Interestingly, although mammals and invertebrates differ markedly both in the morphology of their reproductive organs and in their mode of reproduction, some genetic pathways for female reproductive-tract organ development seem to be conserved between them. Other important related issues, such as embryo implantation and postnatal hormone-regulated differentiation, have been reviewed elsewhere[3,4].

Embryology of the female reproductive tract

The development of the vertebrate urogenital system — which comprises the kidneys, gonads, and urinary and reproductive tracts — begins soon after gastrulation, through the differentiation of the INTERMEDIATE MESODERM. This embryonic tissue subsequently proliferates and some cells undergo the transition from the mesenchymal to the epithelial cell type to generate the tubules that compose the male and female reproductive tracts, as well as the kidneys and testes. Before sexual differentiation, mammalian embryos have two pairs of genital ducts: the Wolffian ducts (MESONEPHRIC DUCTS) and the Müllerian

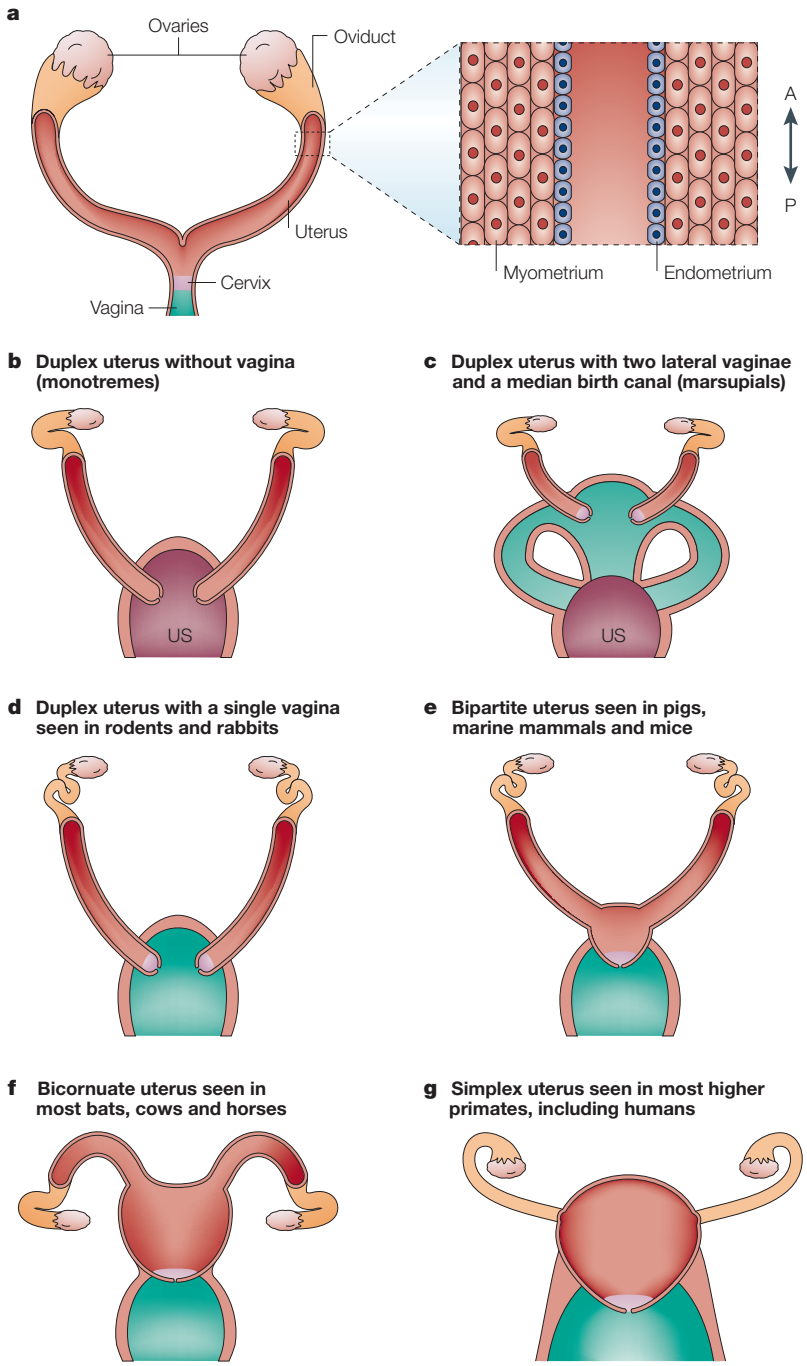


Figure 1 | **Female reproductive-tract variation in mammals. a** | Basic anatomical features of the female reproductive tract. Oocytes leave the ovaries and move into the oviduct, where fertilization occurs. The cervix is the boundary between the uterus and the vagina or urogential sinus. With the exception of in the egg-laying mammals (monotremes), embryos implant in the uterus and are delivered through the vagina. The developing female reproductive tract has two layers, the epithelium and the surrounding mesenchyme, which differentiate into the endometrium and the myometrium, respectively, in the uterus. **b**,**c** | Absent (or limited) fusion of the Müllerian ducts leads to the presence of two uteri. The urogenital sinus (US) is connected to the female reproductive tract (**b**). Müllerian duct fusion is physically blocked by the presence of the ureters, which leads to the formation of three vaginae (**c**). **d** | The duplex uterus shown here has a pair of cervices. **e** | In the duplex bipartite uterus seen in many mammalian species, Müllerian fusion in the uterine region does not occur, or is limited, which leads to the formation of a pair of uterine horns that can support the development of many fetuses. **f** | A larger portion of the uterus forms the uterine body. **g** | Müllerian ducts fuse anteriorly to generate a single uterine body that supports a single fetus or a small number of fetuses per pregnancy. A, anterior (cranial); P, posterior (caudal). Panels **b**–**g** adapted with permission from REF. 5 © (2003) McGraw-Hill.

ducts (PARAMESONEPHRIC DUCTS) (BOX 1). The Wolffian ducts differentiate into structures of the male reproductive tract, such as the epididymides, vas deferentia and seminal vesicles. By contrast, the Müllerian ducts, which subsequently form adjacent to the Wolffian ducts (FIG. 2a), differentiate into the oviducts, uterus, cervix and upper portion of the vagina of the female reproductive tract. The expression of a *lin-11*, *Isl1* and *mec-3* homologue (*Lim1*, also known as *Lhx1*), which encodes a LIM class homeodomain protein, in the epithelium of the Wolffian and Müllerian ducts highlights the initial sexual duality of the forming reproductive systems (see below; FIGS 2b,c, 3).

The morphology of the female reproductive tract can differ markedly among mammalian species (FIG. 1b–g). Müllerian duct formation is similar between species and the morphological diversity mainly results from differences in the extent of fusion of the two Müllerian ducts anteriorly[5]. At one extreme are monotremes and marsupials in which Müllerian fusion is absent or limited, which leads to the formation of two uteri ('duplex' uteri in FIG. 1b,c). At the other extreme, the Müllerian ducts of higher primates (including humans) fuse more anteriorly, which results in the formation of a single ('simplex') uterus with a single cervix and vagina (FIG. 1g). Anatomical variation of the female reproductive tract can even be observed within a species; for example, subspecies of bats can have different types of uterus[6].

Molecular genetics in the mouse

Targeted mutagenesis in the mouse has identified several genes that are essential for female reproductive-tract development. On the basis of their mutant phenotypes, the genes that are knocked out in these mice can be categorized into those that are required for initial Müllerian duct formation in both sexes, for its regression in males or for its differentiation in females. Each of these three main developmental stages are discussed below. Molecular expression analyses in wild type and knockout mice have also contributed to understanding the relationships between genes and have been used to build a molecular genetic pathway for female reproductive-tract development.

Müllerian duct formation. A small set of homeodomain-containing transcription factors and signalling molecules are required for female reproductive-tract formation in mice. One of these, paired-box gene 2 (*Pax2*), encodes a homeodomain transcription factor that is homologous to the *Drosophila* PAIR-RULE GENE *paired* (*prd*) (REF. 7). *Pax2*-null mutant mice die soon after birth, have no kidneys and lack a reproductive tract owing to the degeneration of the Wolffian and Müllerian ducts during embryogenesis — a phenotype that is consistent with the expression of this gene in the kidney and in the epithelium of the Wolffian and Müllerian ducts. However, the anterior portion of both tracts initially forms in *Pax2*-null mutants[8]. A closely related gene, *Pax8*, is co-expressed with *Pax2* in the developing Wolffian and Müllerian ducts and kidney, although

Table 1 | **Mouse genes that are required for female reproductive tract development**

Gene name	Genetic map position	Molecule encoded	Tissue of expression	Female reproductive-tract phenotype abnormality (mode of inheritance)	References
Formation					
Pax2	Ch19 (43.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	8
Lim1 (*Lhx1*)	Ch11 (48.0 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	11
Emx2	Ch19 (53.5 cM)	Homeodomain transcription factor	ME,WE	Absence of FRT (R)	12
Wnt4	Ch4	Wnt family secreted protein	MM	Absence of FRT (R)	17
Ltap	Ch1 (93.4 cM)	Transmembrane protein with PDZ domain	ND	Imperforate vagina (D)	22,23
Hoxa13	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Delay or arrested formation (R)	51
Regression					
Mis (*Amh*)	Ch10 (43.0 cM)	TGFβ superfamily secreted protein	Sertoli cells	Ectopic FRT in males (R)	27,28
Misr2 (*Amhr2*)	Ch15 (57.4 cM)	TGFβ superfamily type 2 Ser/Thr transmembrane receptor	MM	Ectopic FRT in males (R)	35
Wnt7a	Ch6 (39.5 cM)	Wnt family secreted protein	ME	Ectopic FRT in males (R)	42
Differentiation					
Wnt7a	Ch6 (39.5 cM)	Wnt family secreted protein	ME	Homeotic transformation of oviduct to uterus and uterus to vagina, no uterine glands, abnormal mesenchyme differentiation (SD)	53
Hoxa10	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of anterior uterus to oviduct (R)	49,52
Hoxa11	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Partial homeotic transformation of uterus to oviduct (SD)	49,99
Hd (*Hoxa13*)*	Ch6 (26.33 cM)	Homeodomain transcription factor	MM,WM	Homeotic transformation of cervix to uterus (SD)	100
Ovo1 (*Ovol1*)	Ch19	C2H2-type zinc-finger protein	ND	Subfertility with dilated uterus and cervix, constricted or imperforate vagina (R)	101

This table lists all of the mouse genes that are known to be involved in female reproductive tract (FRT) development. *The *Hoxa13* mutation in the *Hypodactyly* (*Hd*) mutant is not a null allele, but is thought to be a dominant-negative allele[73,74]. *Amh*, anti-Müllerian hormone; *Amhr2*, anti-Müllerian hormone type 2 receptor; C2H2, two cysteine two histidine; Ch, chromosome; cM, centimorgan; D, dominant; *Emx*, empty spiracles homologue; *Hoxa*, homeobox A; *Lim1*, *lin-11*, *Isl1* and *mec-3* transcription factor homologue; *Lhx1*, LIM homeobox protein; *Ltap*, Loop-tail-associated protein; ME, Müllerian duct epithelium; *Mis*, Müllerian-inhibiting substance; *Misr2*, Müllerian-inhibiting substance type 2 receptor; MM, Müllerian duct mesenchyme; ND, not determined; *Ovol*, Ovo homologue-like; *Pax*, paired box gene; R, recessive; SD, semidominant; TGF, transforming growth factor; WE, Wolffian duct epithelium; WM, Wolffian duct mesenchyme; *Wnt*, wingless-related MMTV integration site.

INTERMEDIATE MESODERM
A region of the embryonic mesoderm that forms the urogenital system, including the kidneys, gonads and their tracts.

MESONEPHRIC DUCT
A tubule that forms by posterior extension of the pronephric duct and differentiates into the urinary and male reproductive tract: the Wolffian duct.

PARAMESONEPHRIC DUCT
A tubule that forms parallel to the mesonephric duct and differentiates into the female reproductive tract: the Müllerian duct.

PAIR-RULE GENE
A class of segmentation gene that determines segments along the anterior–posterior axis. The expression of pair-rule genes in a pattern of seven stripes that are perpendicular to the axis is regulated by another class of segmentation genes: the gap genes.

PRONEPHROS
The first kidney that appears in the embryo at the anterior end of the nephric duct. This is a transitional organ that subsequently degenerates during embryogenesis and is thought to be non-functional in mammals.

CHIMAERA ASSAY
A technique that assesses the mode of action of gene products by generating animals from a mixture of cells that are derived from two or more genetically distinct animals.

CELL-AUTONOMOUSLY
A mode of gene effect that is restricted to the cell in which the gene is expressed.

Pax8-mutant mice have normal reproductive tracts and kidneys[9]. *Pax2*;*Pax8* double mutants lack Wolffian duct and PRONEPHROS formation[10], which indicates that their combined function might be required for both the formation and maintenance of the male reproductive tract. It is possible that *Pax2*/*8* genes also have redundant roles in the Müllerian duct epithelium, but Müllerian duct development in *Pax2*/*8* double mutants has not been reported[10].

Another homeodomain-containing protein with a role in female and male reproductive tract development is Lim1, which was mentioned above[11]: *Lim1*-null mutant mice lack oviducts, a uterus and the upper portion of the vagina in females, and lack Wolffian duct derivatives in males. In females, a new CHIMAERA ASSAY for female organs showed that *Lim1* is required CELL-AUTONOMOUSLY in the developing epithelium of the oviduct and uterus[11]. *Lim1* is probably required for the formation of the Müllerian duct epithelium, because *Lim1*-mutant cells were not present in the Müllerian ducts of chimaeras, even at E12.5, which is when the Müllerian duct begins to form.

Emx2 is a mammalian homologue of the *Drosophila* head-gap gene *empty spiracles (ems)*, which is thought to be required for the formation of both Müllerian and Wolffian ducts in the mouse. *Emx2*-null mutant mice lack reproductive tracts, gonads and kidneys. During development, the entire Wolffian duct starts

to degenerate at E11.5 and no Müllerian ducts are observed in *Emx2*-null mutants at E13.0 (REF. 12).

Retinoic-acid signalling also seems to be important for the formation and/or maintenance of the Müllerian ducts. Although female mice that are mutant for single retinoic-acid receptor genes (including *RARα1*, *RXRα1*, *RARβ2* and *RARγ*) have normal reproductive tracts, females with compound mutations completely lack this organ, and mice with other mutant combinations partially lack the caudal portion of the female reproductive tract[13,14]. These studies show a redundant requirement of retinoic-acid receptors for female reproductive-tract development.

Wnt gene family members encode secreted glycoproteins that are homologous to the *Drosophila* SEGMENT-POLARITY GENE *wingless* (*wg*) and a subset (*Wnt4*, *Wnt5a* and *Wnt7a*) is involved in the development of several female reproductive organs[15]. *Wnt4*-mutant female mice lack a female reproductive tract but, surprisingly, differentiate a normal male reproductive tract; this is thought to be because *Wnt4*-mutant females have ectopic LEYDIG CELLS in their ovaries[16], which leads to Wolffian duct differentiation. No Müllerian duct forms in both *Wnt4*-mutant males and females from E11.5, before normal Müllerian duct regression takes place in males[17]; this indicates that *Wnt4* might be required for the initial step of Müllerian duct formation before sexual differentiation occurs. Analysis of *Lim1* expression in *Wnt4* mutants uncovered the presence of presumptive Müllerian duct precursor cells, which indicates that *Wnt4* is required for tubule formation of the Müllerian duct but not to specify the Müllerian duct precursor cells[11].

It is noteworthy that many of the genes described above that are essential for Müllerian duct formation are expressed in the developing kidney and are required for proper kidney organogenesis[18,19]. Similar mechanisms might therefore operate in the development of the kidney and the Müllerian duct. From this point of view, *Wnt4* expression in the Müllerian duct mesenchyme, rather than in the COELOMIC EPITHELIUM of the mesonephros, is probably required for female reproductive-tract development; this is because *Wnt4* is expressed in the metanephric mesenchyme-derived tissues but not in the epithelial ureteric bud-derived component of the kidney[20]. *Pax2* and *Pax8* are thought to be required for mesenchyme-to-epithelium transitions, including Wolffian duct formation from the

SEGMENT-POLARITY GENES
Segmentation genes that are required for patterning the body along the anterior–posterior axis. They are expressed in a pattern of 14 stripes at the onset of gastrulation and following the expression of pair-rule genes.

LEYDIG CELL
Interstitial mesenchymal cells of the mammalian testis that are involved in the synthesis of testosterone.

COELOMIC EPITHELIUM
An epithelial tissue that lines the surface of the body wall and abdominal organs.

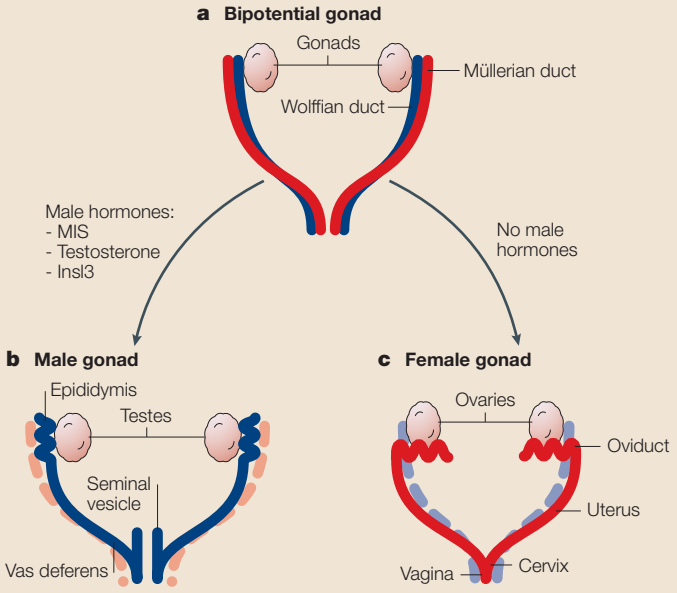
EPIDIDYMIS
(Plural epididymides). The distal portion of the male reproductive tract that receives the sperm from the testis.

VAS DEFERENS
(Plural vas deferentia). The proximal portion of the male reproductive tract through which the sperm travels from the epididymis to the urethra.

Box 1 | Sexual differentiation of the reproductive system

Before sexual differentiation, both male and female embryos have bipotential gonads, as they possess both Wolffian and Müllerian ducts (a). These ducts can differentiate into male or female reproductive organs according to the hormonal status of the fetus. Owing to the expression of the testis-determining gene on the Y chromosome, *Sry*, the bipotential gonad of males becomes the testis, which secretes several hormones including testosterone, Müllerian inhibiting substance (MIS; also known as anti-Müllerian hormone, AMH) and insulin-like growth factor 3 (Insl3)[93] (b). Testosterone promotes Wolffian duct differentiation into the male reproductive tract through the formation of the EPIDIDYMIDES, VAS DEFERENTIA and seminal vesicles, and MIS eliminates the Müllerian ducts (pink dashed line). In mice, the elimination of the Müllerian duct system in male fetuses is essentially complete by embryonic day (E) 16.5 (REF. 11). All three hormones are involved in testicular descent. In females, the bipotential gonad becomes the ovary (c). In the absence of male hormones, the Wolffian ducts degenerate (blue dashed line), whereas the Müllerian ducts persist and differentiate into the female reproductive tract, including the oviduct (fallopian tube), uterus, cervix and upper portion of the vagina.

Two Müllerian ducts fuse to form a single vagina at the posterior region. The derivation of the vaginal epithelium is controversial. It is widely accepted that the upper two-thirds of the vagina derives from the Müllerian duct and the lower one-third derives from the urogenital sinus[94,95]. This idea largely depends on the fact that *testicular feminization* (*Tfm*) male mice retain a shortened vagina, called the 'sinus vagina'. *Tfm* male mice have a female phenotype that is caused by a mutation in the androgen receptor (*Ar*) gene, which results in androgen insensitivity, but they are still responsive to MIS signalling to regress the Müllerian ducts. The residual vaginal tissue in *Tfm* mice was considered to be derived from the urogenital sinus, not from the Müllerian duct. However, recent analysis of androgen-treated female mice indicates that the entire vagina might derive from the Müllerian duct[96]. Cell-lineage analysis is needed to clarify this question. A, anterior (cranial); P, posterior (caudal).



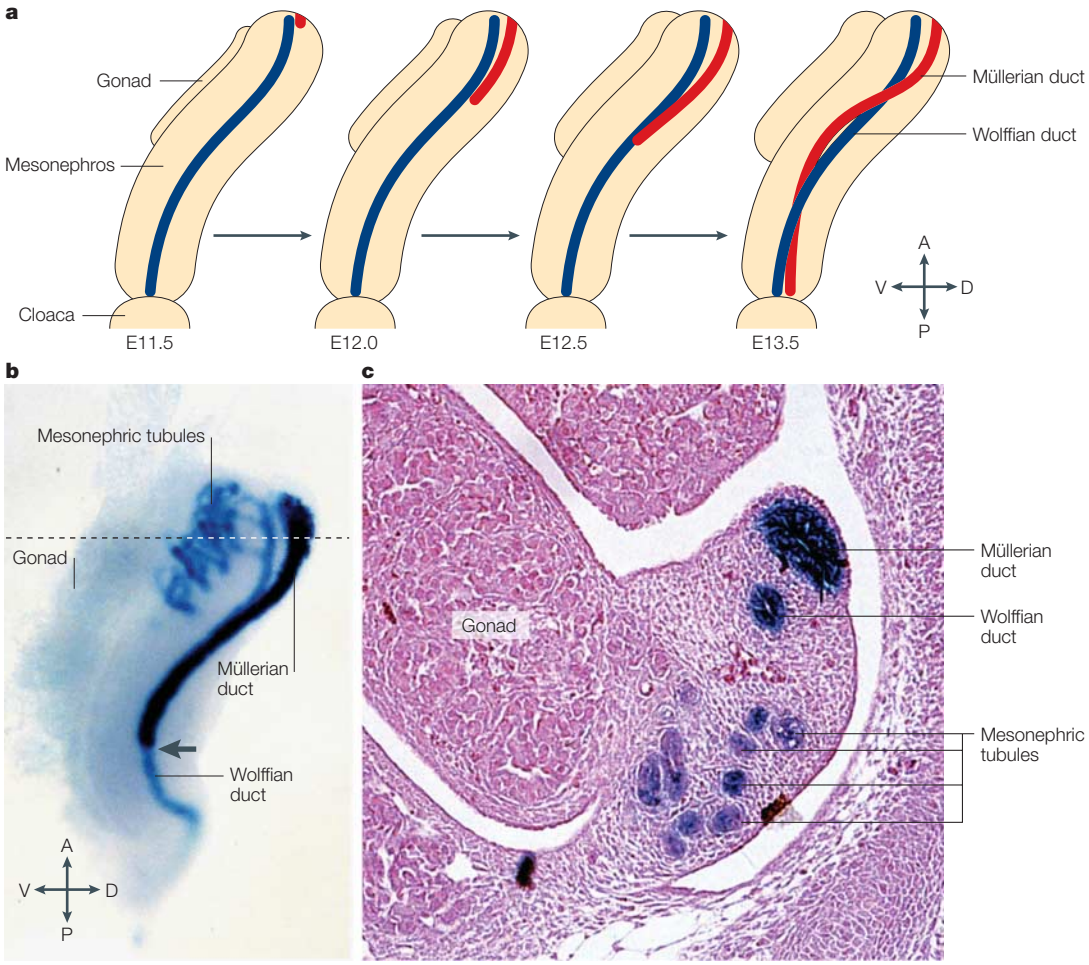


Figure 2 | **Formation of the Müllerian ducts. a** | Schematic diagram of Müllerian duct formation in mammals. The Müllerian duct forms as an invagination of the surface epithelium of the MESONEPHROS at around embryonic day (E) 11.5 in mice and this epithelial invagination extends posteriorly until it reaches the CLOACA at ~E13.5. **b** | The extending epithelium of the Müllerian duct is visualized at E12.5 by *Lim1* (*Lhx1*)–*lacZ* expression[11]. Note that the Wolffian duct (blue) has reached the cloaca posteriorly, but the Müllerian duct is still in the process of extending posteriorly. The grey arrow points to the posterior tip of the extending Müllerian duct. **c** | Cross section of the gonadal/mesonephric region (dashed line in **b**). Blue staining by *Lim1–lacZ* expression is observed in the epithelium of the Wolffian and Müllerian ducts and the mesonephric tubules. A, anterior (cranial); D, dorsal; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; P, posterior (caudal); V, ventral. Panel **c** adapted from REF. 11 © (2003) The Company of Biologists Ltd.

MESONEPHROS
The second kidney that forms next to the pronephros posteriorly during embryogenesis. In mammals, this is a transient embryonic organ that subsequently degenerates but is thought to be functional. The urinary function is postnatally taken over by the metanephros.

CLOACA
The terminal end of the hindgut before division into the rectum and urogenital sinus. The dorsal part of the cloaca differentiates into the rectum and anal canal, and the ventral part differentiates into the urogenital sinus.

PLANAR-CELL POLARITY
The polarity of epithelial cells in the plane of the epithelium, which is orthogonal to their apical–basal axis.

SERTOLI CELLS
Tall columnar epithelial cells of the mammalian testis that are involved in the synthesis of Müllerian-inhibiting substance.

mesenchyme of the intermediate mesoderm[10] and formation of the nephron from the metanephric mesenchyme[21]. The same mechanism might also be involved in epithelium invagination during Müllerian duct formation.

Modulation of Wnt signalling is also involved in female reproductive-tract development. *Loop-tail* (*Lp*) was identified as a semidominant spontaneous mutation in the mouse[22]. The *Lp* (*Ltap*) gene encodes a four-transmembrane protein with a PDZ domain (loop-tail-associated protein, Ltap; also known as Vangl2 or Lpp1) that is homologous to *Drosophila* Strabismus/Van Gogh (Stbm/Vang)[23], which is a component of the Frizzled–Dishevelled tissue-polarity pathway in invertebrates and vertebrates[24]. As well as having tail loops, *Loop-tail* heterozygous mutant females have an imperforate vagina[22]. Because Stbm/Vang modulates canonical and non-canonical Wnt signalling pathways to establish epithelial PLANAR-CELL POLARITY (PCP) (reviewed in REF. 25), the establishment of PCP might be an essential step in Müllerian duct morphogenesis and Ltap could modulate the Wnt signalling pathway during this process.

Müllerian duct regression. In males, the Müllerian duct system forms initially but subsequently regresses (BOX 1). Mutations that cause Müllerian duct persistence in males have provided insights into the genetic and molecular pathways that regulate the regression process. The elimination of the Müllerian ducts in male fetuses is caused by Müllerian-inhibiting substance (MIS; also known as anti-Müllerian hormone, AMH), which is a transforming growth factor-β (TGF-β) superfamily member that is secreted by the SERTOLI CELLS of the fetal testis (reviewed in REF. 26). Fetal ovaries do not produce MIS and so the Müllerian duct system can persist and

VIRILIZE
(Masculinize). To produce or cause male sexual characteristics.

PARACRINE
A form of cell–cell communication that depends on a secreted substance that acts over a short distance and does not enter the circulation.

AUTOCRINE
A mode of action of a secreted substance by which it affects the cell that secretes it.

ANIMAL-CAP ASSAY
An experimental system to study inductive interactions in the early embryogenesis of urodele amphibians and, subsequently, *Xenopus*. The animal cap of the blastula can respond to the appropriate inductive signal or transgene expression to produce a range of differentiated tissues.

MATRIX METALLOPROTEINASES
A family of proteinases that modify the extracellular matrix and require a metal in the catalytic process.

differentiate. Two pieces of evidence indicate that MIS is both necessary and sufficient for regression of the Müllerian duct system. *Mis*-mutant male mice have testes and are normally VIRILIZED but they also have a uterus and oviducts[27] (FIG. 3a,b). Also, when *Mis* is overexpressed in female transgenic mice, Müllerian duct-derived organs are eliminated[28] (FIG. 3c,d). The expression of *Mis* in the fetal testis is directly regulated by SRY-box containing gene 9 (*Sox9*), steroidogenic factor 1 (*Sf1*, also known as *Nr5a1*), Wilms tumour homologue (*Wt1*) and DSS-AHC critical region on the X chromosome gene 1 (*Dax1*, also known as *Nr0b1*), which link MIS to the testis-determination pathway[29–32].

MIS signalling is mediated by its type II receptor (Misr2, also known as Amhr2), which is expressed in the mesenchyme of the Müllerian duct by E13.5 in mice[33]. This stage of *Misr2* expression is consistent with the crucial period for Müllerian duct regression that occurs between E13 and E14 in mice, as determined by the removal of the testis from the urogenital ridge at different time points in organ culture[34]. Misr2 is probably dedicated specifically to MIS signal transduction because *Misr2*-mutant males have the same phenotype as *Mis*-mutant males[35]. Further evidence is provided by the fact that mutations in *Misr2* block the elimination of the Müllerian duct system and the ovary degeneration that is observed in transgenic female mice that overexpress human MIS[36].

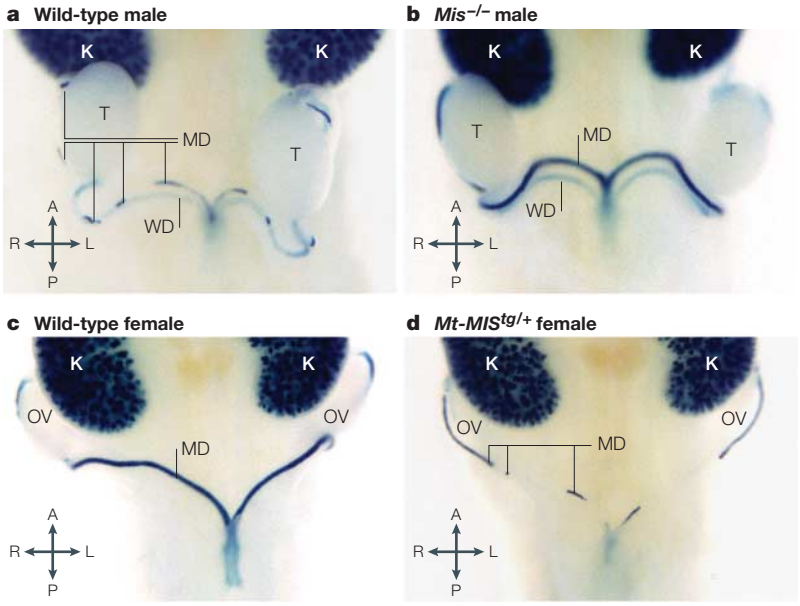


Figure 3 | **Müllerian duct regression.** The developing Müllerian ducts are visualized by *Lim1–lacZ* expression[11] in the mouse embryo at embryonic day (E) 15. **a** | In XY male mice, Müllerian-inhibiting substance (MIS) is produced by the testes and eliminates the Müllerian ducts. The regressing Müllerian ducts (MD) have a fragmented pattern at this stage. **b** | When *Mis* is mutated by gene targeting in XY mice, there is no Müllerian duct regression. **c** | There is no Müllerian duct regression in the absence of MIS in XX female mice. **d** | When human *MIS* (*AMH*) is overexpressed using a metallothionein (*Mt*) promoter in XX mice, ectopic regression of the Müllerian duct is observed. A, anterior (cranial); K, kidney; L, left; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; OV, ovary; P, posterior (caudal); R, right; T, testis; WD, Wolffian duct. Panels **c** and **d** adapted from REF. 11 © (2003) The Company of Biologists Ltd.

The identity of the type I receptor for MIS remains controversial, but both biochemical and antisense knockdown phenotypic data indicate that Alk2 (also known as Acvr1) and Alk6 (also known as Bmpr1b) can mediate MIS signals[37–39] — although *Alk6* is not essential for Müllerian duct regression. Another MIS type I receptor candidate is Alk3 (also known as Bmpr1a). *Alk3* (*Bmpr1a*)-mutant mice die during gastrulation[40], but conditional inactivation of *Alk3* in the Müllerian duct mesenchyme induces males to have a female reproductive tract that is identical to *Mis* and *Misr2*-mutant males[41]. This indicates that *Alk3* is required for Müllerian duct regression. It is possible that several type I receptors can mediate MIS signals in Müllerian duct regression or that different type I receptors mediate MIS signals in different tissues. The involvement of the bone morphogenetic protein (BMP) receptors Alk2/3/6 in MIS signalling indicates that the Smad1/5/8 proteins, which are typically downstream of BMP receptors, might mediate Müllerian duct regression.

Wnt genes have a function not only in Müllerian duct formation, as discussed in the previous section, but also in its regression in males. In *Wnt7a*-mutant male mice the Müllerian ducts do not regress, which leads to the formation of female reproductive-tract organs[42]. *Wnt7a* expression in the Müllerian duct epithelium (which begins at E11.5; REF. 17) is essential for the expression of *Misr2* in the surrounding mesenchyme in both sexes. It is not clear whether Wnt7a acts as a PARACRINE signal from the epithelium to the mesenchyme or as an AUTOCRINE signal in the epithelium of the female reproductive tract. Frizzled genes encode seven transmembrane proteins that serve as Wnt receptors. It will be important to identify which Frizzled protein is the receptor for Wnt7a, and in which tissue the receptor is expressed in the developing Müllerian duct. One candidate Wnt7a receptor is Frizzled10 (Fzd10). This protein interacts with Wnt7a to induce Wnt-downstream genes in a *Xenopus* ANIMAL-CAP ASSAY. Fzd10 might also be a Wnt7a receptor in the developing chicken limb[43], in which Wnt7a is involved in patterning[44].

MIS binds Misr2 in the Müllerian duct mesenchyme, and induces the morphological changes that eventually cause the degeneration of the Müllerian duct system[34]. However, the molecular mechanisms that regulate these cellular changes are unclear. Apoptosis of the epithelial cells of the Müllerian duct is associated with disruption of the basement membrane, which is mainly composed of type IV collagen and laminin[45]. One candidate for a molecular signal from the mesenchyme to the epithelium is MATRIX METALLOPROTEINASE 2 (Mmp2)[33], although *Mmp2*-mutant male mice are normal except for a subtle growth delay and are fertile without Müllerian duct-derived tissues[46]. The molecular nature of the signals between the Müllerian duct mesenchyme and the epithelium during regression remains a relatively unexplored area of research.

Müllerian duct differentiation and pattern formation. After the Müllerian ducts form in the female fetus, they differentiate into the oviducts, uterus, cervix and a

portion of the vagina along the anterior–posterior axis, (FIG. 1a). These different structures have distinct morphologies and cytoarchitecture. Tissue-recombination assays indicate that tissue identity is initially specified in the mesenchyme, which subsequently instructs the differentiation of the associated epithelium[47]. Some genes are expressed in region-specific patterns in the developing female reproductive tract (described below). A study using AFFYMETRIX GENE-CHIP ANALYSIS also identified genes that are differentially expressed in the uterus and cervix of the female reproductive tract[48].

Abdominal B (*AbdB*) homeobox genes reside at the 5′ end of the four mammalian HOX CLUSTERS and are expressed in the posterior compartments of the body axis. The number of *AbdB* genes has grown considerably with organismal complexity: mammals have 16 *AbdB* genes whereas *Drosophila* has only 1 (REF. 49). In mice, *AbdB* genes have partially overlapping expression patterns in the mesenchyme of the reproductive tract. Along the anterior–posterior axis of the Müllerian duct, *AbdB* genes are expressed according to their 3′–5′ order in the *Hox* clusters; for example, *Hoxa9* is expressed in the oviduct, *Hoxa10* in the uterus, *Hoxa11* in the uterus and cervix, and *Hoxa13* in the cervix and upper vagina[50,51].

Mutations in *Hoxa10* cause an anterior HOMEOTIC TRANSFORMATION of the reproductive tract in males and females: the anterior part of the vas deferens becomes the more anterior epididymis in the male and the anterior part of the uterus transforms into the more anterior oviduct in the female[52]. These data indicate that *Hoxa10* is required for defining tissue boundaries in the reproductive tract, and they are consistent with the expression of *Hoxa10* in the mesenchyme of the presumptive uterus, and also with its absence from the presumptive oviduct at E17.5 (REF. 52). The expression patterns of *Hoxa10* and *Hoxa11* overlap in the uterus during embryogenesis; genetic data indicate both that *Hoxa11* specifies regional identity along the anterior–posterior axis of the female reproductive tract and that genes in a *Hox* cluster might have partially redundant functions[49].

When the homeobox of the *Hoxa11* gene is replaced by the homeobox of *Hoxa13*, posterior homeotic transformation occurs in the female reproductive tract: the uterus, in which *Hoxa11* but not *Hoxa13* is normally expressed, becomes similar to the more posterior cervix and vagina, in which *Hoxa13* is normally expressed[48]. This indicates that the homeodomains in Hoxa11 and Hoxa13 are not functionally equivalent for female reproductive-tract development and that Hoxa13 regulates distinct downstream targets that are required for differentiation of the cervix and vagina.

As well as its involvement in the differentiation of the Müllerian ducts, *Hoxa13* might also be required for the formation of the Müllerian duct in both sexes. Targeted *Hoxa13*-null mouse mutants die between E11.5 and E15.5, probably as a result of STENOSIS of the umbilical artery[51]. At E13.5 or E14.5, *Hoxa13*-mutant female embryos lack the caudal portion of the Müllerian duct, probably owing to a delay in or arrest of the invagination of the Müllerian duct along the anterior–posterior axis. Moreover, this function that might be shared by *Hoxd13*, which is a *Hoxa13* PARALOGUE. *Hoxd13* is expressed in the terminal region of the urogenital and digestive tracts, and partially overlaps with *Hoxa13* expression. Unlike *Hoxa13* mutants, *Hoxd13*-homozygous mutant mice are viable, and males are subfertile with subtle abnormalities in their accessory sex glands[51]; however, Müllerian agenesis in the caudal portion was observed in some compound *Hoxa13*$^{+/-}$*; Hoxd13*$^{-/-}$-mutant females at birth[51].

Interestingly, *Wnt7a* is not only required for Müllerian duct regression in males (see the previous section) but also for differentiation of the female reproductive tract. Initially, *Wnt7a* is expressed throughout the entire Müllerian duct in embryos, whereas after birth it becomes restricted to the oviductal and uterine epithelium[15]. There is no oviductal coiling and uterine-gland formation in *Wnt7a*-mutant adult females[53], and *Wnt7a*-mutant females have shallow VAGINAL FORNICES[54]. Also, the reproductive tract of *Wnt7a*-mutant female adults is posteriorized. The posterior oviduct of *Wnt7a* mutants becomes more similar to the uterus and the mutant uterus also has characteristics of the vagina[53].

From 1938 until 1971, a synthetic oestrogen, diethylstilbestrol (DES), was used by millions of pregnant women to prevent miscarriage. Prenatal or perinatal exposure to DES disturbs the development of the reproductive tract in both humans (male and female) and mice[55]. Interestingly, the uterine phenotypes of *Wnt7a*-mutant female mice resemble those of wild-type female mice that are prenatally treated with DES[54]. Subsequent studies have shown that perinatal downregulation of *Wnt7a* expression might account for the uterine defects that are observed in DES-treated females[54]. DES treatment also alters *AbdB Hox* gene-expression patterns in the female reproductive tract[56,57]. These animal studies provide a molecular explanation for the reproductive defects that are observed in the children of women that used DES during pregnancy.

Molecular genetics in humans

Defects of the female reproductive tract are sometimes found in both newborn girls and boys, and are therefore thought to result from abnormalities of the Müllerian ducts during embryogenesis. These defects include Müllerian aplasia, Müllerian persistence (in males) and incomplete Müllerian fusion. TABLE 2 shows some autosomal genes that, when mutant, are responsible for dominant and recessive syndromes in female reproductive-tract formation, regression and differentiation. Although most of our understanding of how the female reproductive tract develops derives from the description of human genetic syndromes, the loci that underlie many of these syndromes have not yet been mapped or molecularly characterized.

Müllerian duct formation. Relatively few genes have been identified that regulate Müllerian duct formation in humans. Humans with heterozygous mutations in hepatic nuclear factor 1β (*HNF1β*, also known as *vHNF* or *TCF2*), which encodes a homeodomain transcription

AFFYMETRIX GENE-CHIP ANALYSIS
The examination of gene-expression profiles by the high-density array of single-stranded DNA nucleotides.

HOX CLUSTERS
A group of linked regulatory homeobox genes that are involved in patterning the animal body axis during development. Homeobox genes are defined as those that contain an 180-base-pair sequence that encodes a DNA-binding helix–turn–helix motif (a homeodomain).

HOMEOTIC TRANSFORMATION
When one embryonic axial segment alters its identity to that of another.

STENOSIS
A narrowing or obstruction of the opening or channel of a tract, which prevents the normal flow through it.

PARALOGUE
A homologous gene that originates by gene duplication.

VAGINAL FORNIX
(Plural vaginal fornices). An anatomical recess that is formed by the projection of the cervix into the upper part of the vagina. There are four fornices in a female: the anterior fornix, the posterior fornix and two lateral fornices.

Table 2 | **A selection of human syndromes that affect female reproductive tract development**

Syndrome name	OMIM*	FRT abnormalities in patients	Mode of inheritance	Genomic location	Gene mutated	Molecule encoded	References
Formation							
Maturity-onset diabetes of the young type V (MODY5)	604284	Vaginal aplasia and rudimentary uterus	AD	17cen–q21.3	*TCF2* (*HNF1β*)	Homeodomain transcription factor	58
McKusick–Kaufman syndrome (MKKS)	236700	Hydrometrocolpos by vaginal atresia	AR	20p12	*MKKS* (*BBS6*)	Chaperonin	68,102, 103
Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome	277000	Absence of the vagina and uterus	AR	ND	ND	ND	104
MURCS association	601076	Müllerian duct aplasia	SP	ND	ND	ND	105
Regression							
Persistent Müllerian duct syndrome (PMDS) type I	261550	Persistence of Müllerian derivatives	AR	19p13.3 –p13.2	*MIS* (*AMH*)	TGFβ superfamily secreted molecule	69
Persistent Müllerian duct syndrome (PMDS) type II	261550	Persistence of Müllerian derivatives	AR	12q13	*MISR2* (*AMHR2*)	TGFβ superfamily type 2 Ser/Thr transmembrane receptor	69
Urioste syndrome	235255	Persistence of Müllerian derivatives	AR	ND	ND	ND	70,106, 107
Differentiation							
Hand–foot–genital (HFG) syndrome	140000	Longitudinal vaginal septum	AD	7p15–p14.2	*HOXA13*	Homeodomain transcription factor	75
Cat eye syndrome (CES)	115470	Hypoplastic uterus, vaginal atresia	SP	21q11	ND	ND	108
Fryns syndrome (FRNS)	229850	Uterus bicornis or hypoplasia	AR	ND	ND	ND	109

*Reference number for the entry in the online Mendelian inheritance in man (OMIM) database of genetic disorders (see online links box). AD, autosomal dominant; *AMH*, anti-Müllerian hormone; *AMHR2*, anti-Müllerian hormone type 2 receptor; AR, autosomal recessive; *BBS*, Bardet–Biedl syndrome; cen, centromere; FRT, female reproductive tract; *HNF*, hepatocyte nuclear factor; *HOXA*, homeobox A; *MIS*, Müllerian-inhibiting substance; *MISR2*, Müllerian-inhibiting substance type 2 receptor; MURCS, Müllerian duct aplasia, unilateral renal aplasia and cervicothoracic somite dysplasia; ND, not determined; SP, sporadic; *TCF*, transcription factor; *TGF*, transforming growth factor.

factor, develop maturity-onset diabetes of the young type 5 (MODY5)[58]. This syndrome can include renal dysfunction and genital malformation; a subset of female carriers also has Müllerian aplasia, which includes vaginal aplasia and a rudimentary uterus[58]. The fact that *HNF1β* mutations are also found in patients with renal and Müllerian anomalies in the absence of diabetes[59] indicates that *HNF1β* is essential for the formation and/or maintenance of the Müllerian ducts in humans. In mouse embryos, *Hnf1β* is expressed in the epithelium of the reproductive tract during embryogenesis and after birth[60,61], and it was proposed that *Hnf1β* directly regulates cadherin 16 (Cdh16, also known as Ksp-cadherin) in urogenital organs[62,63]. *Hnf1β* function in the urogenital system of the mouse is still unclear, because homozygous mutants die ~E7 and heterozygous mutant mice are phenotypically normal[64,65]. A recently developed conditional allele of *Hnf1β* will prove useful in clarifying the function of *Hnf1β* in female reproductive-tract development in the future[66].

McKusick–Kaufman syndrome (MKKS) includes several developmental anomalies, including HYDROMETROCOLPOS (HMC), postaxial polydactyly (PAP) and congenital heart disease (CHD). Female MKKS patients have vaginal atresia with hydrometrocolpos[67]. The *MKKS* gene, which is ubiquitously expressed in fetuses and adults, seems to encode a chaperonin-related protein and therefore might be involved in protein folding[68].

HYDROMETROCOLPOS
The distension of the uterus and vagina by the accumulation of secreted fluid; this usually reflects a mechanical obstruction.

Several human syndromes with Müllerian duct aplasia are frequently observed. Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome is an autosomal recessive disorder. Patients are genetically female (46XX) with normal ovaries and external genitalia; however, they lack a vagina and frequently have uterine agenesis or dysgenesis. Another human syndrome with Müllerian agenesis is MURCS association (Müllerian duct aplasia, unilateral renal aplasia and cervicothoracic somite dysplasia). The molecules that are responsible for these syndromes have not yet been identified.

Müllerian duct regression. There are human syndromes in which males retain Müllerian duct-derived tissues. Persistent Müllerian duct syndrome (PMDS) is a rare form of autosomal recessive male pseudohermaphroditism: male patients have testes and are normally virilized, but also retain ectopic female reproductive-tract organs, including uterine and fallopian duct tissue. PMDS individuals are often identified because of an associated cryptorchidism (undescended testis). There are two different types of PMDS: MIS is not detected in PMDS type I patients, whereas MIS levels are normal in type II patients. The genes that encode the ligand (*MIS*) and its type II receptor (*MISR2*) are mutated in PMDS type I and type II patients, respectively[69].

Another autosomal recessive syndrome that is associated with the persistence of Müllerian duct derivatives in males is Urioste syndrome[70]. This syndrome includes

not only PMDS, but also LYMPHANGIECTASIA and postaxial polydactyly. The molecular basis of this syndrome and its relationship to MIS signalling are not yet understood. Of course, any mutation that causes testicular dysfunction or degeneration before MIS production during embryogenesis will indirectly lead to the persistence of Müllerian duct derivatives in genetic males.

Müllerian duct differentiation and pattern formation. Hand–foot–genital syndrome (HFG) is an autosomal dominant disorder. HFG patients have shortened thumbs and shortened great toes, a bicornuate or duplex uterus in females and HYPOSPADIAS in males. The bicornuate or didelphic uterus is thought to result from incomplete Müllerian duct fusion during embryogenesis. The hand and foot defects that are observed in HFG syndrome patients are similar to those of a spontaneous mouse mutant, *Hypodactyly* (*Hd*). *Hd* is a semidominant mutation[71] and *Hd*-heterozygous mice have a shorter digit I on all limbs and are fertile. Most *Hd*-homozygous mutant mice are embryonic lethal, although rare escaper mutants have a single digit on all limbs and both males and females are infertile. *Hd*-homozygous mutant escaper females have mild hypoplasia of the vagina and clitoris[71,72]. Positional cloning of the *Hd* locus identified a 50 base pair (bp) deletion in the first exon of the *Hoxa13* gene[72], which is thought to be a DOMINANT-NEGATIVE mutation[73,74]. Subsequently, several mutations have also been found in the *HOXA13* gene of HFG patients[75]. Because one characteristic of HFG syndrome is incomplete Müllerian duct fusion, *HOXA13* might be required for defining the anterior position of Müllerian duct fusion, and the duplicated uterus in HFG patients might result from anterior homeotic transformation with a posterior shift of the Müllerian duct-fusion boundary.

Although female HFG patients have incomplete Müllerian duct fusion, the same abnormality is not found in *Hd* or *Hoxa13*-mutant female mice[51,72]. However, when the *Hoxa13* mutation was combined with a *Hoxd13* mutation, one of six *Hoxa13*$^{+/-}$*; Hoxd13*$^{-/-}$ compound-mutant females had improper Müllerian duct fusion in the vagina, which was not observed in *Hoxd13*$^{-/-}$ mutant females[51]. This indicates that *Hoxa13* is also required for correct Müllerian duct fusion in mice, and that *Hoxa13* and *Hoxd13* function redundantly during this process. It is thought that the morphological diversity in the female reproductive tract mainly results from different extents of Müllerian duct fusion (FIG. 1). Different spatial and/or temporal expression patterns of *AbdB* genes, including *Hoxa13* and *Hoxd13*, during embryogenesis might explain the diversity that is seen in the female reproductive tracts of different mammalian species.

Molecular conservation during evolution

The anatomy of the female reproductive tract differs markedly among mammalian species (FIG. 1). However, the fundamental genetic pathways that control the development of the female reproductive tract have been conserved. This is true even between vertebrates and invertebrates, thereby reinforcing a pattern that has been documented for other organs[76–78].

One of the best studied systems of organogenesis is uterine–vulval development in hermaphrodites of the nematode *Caenorhabditis elegans*[79,80]. The *C. elegans abnormal cell lineage-11* (*lin-11*) gene is orthologous to mouse *Lim1*, which, as noted above, is essential for female reproductive-tract development[11]. Interestingly, *lin-11* is expressed in the ventral uterine-intermediate precursor cells and their progeny, and its function is required for uterine and vulval development during nematode embryogenesis[81]. *Pax2*-null mutant female mice lack a uterus and oviducts[8]. In *C. elegans* hermaphrodites that are mutant for *egg-laying defective-38* (*egl-38*) — a *PAX* homeobox gene that is homologous to vertebrate *PAX* group II (*Pax2*, *Pax5* and *Pax8*) genes — four uterine cells are abnormally transformed into neighbour cells, which results in egg-laying defects[82].

A *Hox* gene, *lin-39*, is required for generating the vulval precursor cells (VPCs) at the first larval stage and subsequently specifies the vulval fate at the third larval stage in developing *C. elegans* hermaphrodites. When *lin-39* is replaced with the posterior *Hox* gene *mab-5*, the vulval fate is homeotically transformed into the posterior fate[83]. Some components of Wnt signalling are also involved in vulval development, including the β-catenin homologue *β-catenin/armadillo related*-1 (*bar-1*) and the adenomatosis polyposis coli (APC) homologue *adenomatosis polyposis coli related-1* (*apr-1*)[84]. Interestingly, the components of Wnt signalling and the homeobox gene *lin-39* interact genetically to regulate vulval development[84]. Moreover, *bar-1* and *apr-1* are required for the maintenance of *lin-39* expression in the developing vulva[85,86]. These data indicate that Wnt signalling regulates *Hox* gene expression for proper vulval development in *C. elegans*. In mice, *Wnt7a* is required for proper differentiation of the oviduct and uterus, and some *AbdB Hox* genes, including *Hoxa10* and *Hoxa11*, are required for proper regional specification along the anterior–posterior axis of the female reproductive tract, as described above. Intriguingly, in *Wnt7a*-mutant female mice, expression of *Hoxa10* and *Hoxa11* in the uterine mesenchyme is lost ~5–12 weeks after birth, although these genes are expressed normally at postnatal day 10 (REF. 53). Therefore, *Wnt7a* is required for maintenance of *Hoxa10* and *Hoxa11* expression in the uterus in mice.

These findings indicate that molecules that regulate female reproductive-system development have been conserved between vertebrates and invertebrates. So, the definition of the genetic pathways that regulate the formation and differentiation of the female reproductive-tract organs should benefit from genetic studies in organisms from both of these classes. This, in turn, should provide clues to help understand and diagnose abnormalities in the female reproductive tract of humans.

Conclusions

Recently, the genetic cascade for Müllerian duct development has started to become defined (BOX 2). The examination of mouse mutant phenotypes, molecular expression analysis in mutants and promoter analysis have all contributed to an understanding of

LYMPHANGIECTASIA
Dilation of the lymphatic vessels that is caused by lymphatic damage, which leads to the blockage of local lymphatic drainage.

HYPOSPADIAS
A congenital defect in which the urethra opens abnormally on the ventral side of the penis, rather than at the distal tip of the glans.

DOMINANT-NEGATIVE
A form of mutation that interferes with the function of its wild-type gene product.

the developmental genetics of female reproductive-tract organogenesis. However, several questions remain to be answered. For example, further factors must be involved in female reproductive-tract development. Expression profiling using microarray gene-chip technology should identify genes that are differentially expressed during female reproductive-tract development. Subsequent functional analysis, especially using knockout technologies, will help to place more factors in this genetic cascade. We anticipate that large-scale mouse-mutagenesis projects might also yield mutations in new genes that affect the female reproductive tract.

It also remains to be determined how Müllerian duct invagination is initiated at the anterior end of the mesonephros and how its elongation is guided along the anterior–posterior axis. Recently, important cytological changes and several key genes that regulate invagination during tubulogenesis have been found[87,88]. The invaginating Müllerian duct is a simple long epithelial tubule, without branching. Perhaps these processes and molecules are also involved in Müllerian duct formation.

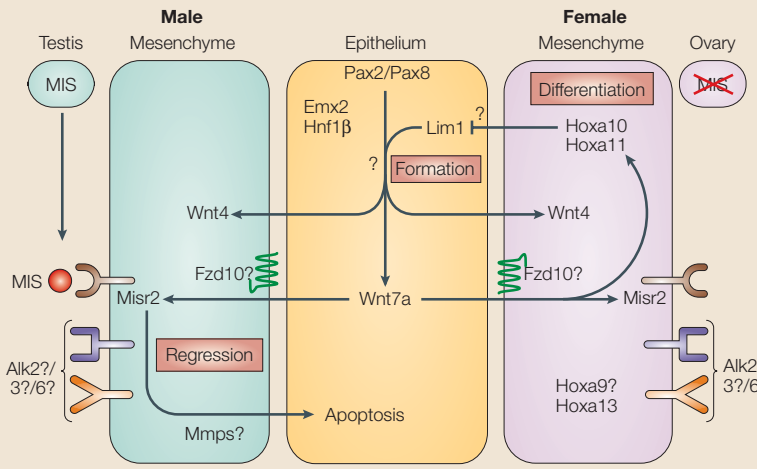
It is noteworthy that many mutations in mice, including those in *Pax2*, *Lim1*, *Emx2* and *RARα/RARγ*, cause both Wolffian and Müllerian duct aplasia[8,11–13], but none lacks the Wolffian duct alone; this indicates that Müllerian duct formation might require the presence of the Wolffian duct[89]. Unidentified inductive and/or guidance molecules might be secreted by or expressed on the cell surface of the Wolffian duct for Müllerian duct development. However, the genes mentioned above are also expressed in the Müllerian duct[8,11,12], where they are thought to function specifically[11]. The specific functions of these genes in Müllerian duct development might have to be re-examined by conditional gene inactivation in the Müllerian or Wolffian ducts alone. It is also possible that Müllerian duct development affects Wolffian duct development. *Wnt7a*-null mutants fail to regress the Wolffian duct, although *Wnt7a* is expressed only in the Müllerian duct epithelium[54]. The molecular mechanisms that regulate Wolffian duct regression are largely unknown.

Box 2 | **Genetic model for female reproductive-tract development**

The female reproductive tract forms from the Müllerian ducts and is composed of an epithelial tube and adjacent mesenchyme. During Müllerian duct formation, before sexual differentiation, empty spiracles homologue 2 (*Emx2*), hepatocyte nuclear factor 1β (*Hnf1β*), *lin-11*, *Isl1* and *mec-3* homologue (*Lim1*), paired-box gene (*Pax2*), *Pax8* and wingless-related MMTV integration-site family member 7a (*Wnt7a*) are expressed in the epithelium and *Wnt4* is expressed in the mesenchyme. All of these genes, except *Pax8*, are essential for Müllerian duct formation. Genetic interactions between these genes are largely unknown but expression analysis, mutant phenotyping and epistasis studies in the mouse point to the model illustrated here. *Wnt4*-null mutants form Müllerian duct-precursor cells, which express *Lim1*, but these cells fail to form an invaginating tubule[11]. This indicates that *Lim1* might be required to specify Müllerian duct-precursor cells and/or convert these cells into the epithelial tissue of the Müllerian duct. It is possible that Lim1 and Pax2/8 cooperatively regulate some factors, including the *Wnt* genes (such as *Wnt4* and *Wnt7a*, as shown here)[97] during Müllerian duct formation. Wnt7a that is secreted by the epithelium and, perhaps, acts through Frizzled homologue 10 (Fzd10), induces expression of the Müllerian-inhibiting substance type II receptor gene (*Misr2*) in the mesenchyme of both sexes, which makes the Müllerian ducts of males and females competent for MIS-induced regression.

In males, MIS is expressed and secreted by the testis. MIS binds to Misr2 on the Müllerian duct mesenchyme (possibly with the receptors that are encoded by activin receptor-like kinase (Alk)2/*Alk3*/*Alk6*), which leads to elimination of the epithelium by transformation to mesenchymal cells or by apoptosis[45,98] This signal induces the expression of matrix metalloproteinase 2 (*Mmp2*) and possibly of other *Mmp* genes in the Müllerian duct mesenchyme, which leads to apoptosis in the Müllerian duct epithelium; *Mmp2* alone, however, is dispensable for Müllerian duct regression.

In females, there is no production of MIS, which allows the persistence and differentiation of the Müllerian ducts. Homeobox A (*Hoxa*) genes are expressed along the anterior–posterior axis of the Müllerian ducts and specify the identities of tissues such as the oviduct, uterus, cervix and vagina. *Wnt7a* is also required for the postnatal maintenance of *Hoxa10* and *Hoxa11* expression. It is possible that *Hoxa10* in the mesenchyme represses *Lim1* expression in the epithelium of the developing oviduct[11].



CRE/LOXP
A site-specific recombination system that is derived from the *Escherichia coli* bacteriophage P1. Two short DNA sequences (*loxP* sites) are engineered to flank the target DNA. Activation of the Cre recombinase enzyme catalyses recombination between the *loxP* sites, which can lead to the excision of the intervening sequence when two *loxP* sites have the same orientation on the same DNA strand.

In the Müllerian duct mesenchyme, conditional gene inactivation has been successfully carried out using *Misr2*-CRE mice[41]. So, *Misr2* provides a molecular entry point to genetically manipulate the Müllerian duct mesenchyme. However, *Misr2* is expressed in the Müllerian duct relatively late and it cannot be used to study the function of genes that are expressed in the mesenchyme during Müllerian duct formation. Transcriptional enhancers from genes that are expressed earlier in the Müllerian duct mesenchyme (such as *Wnt4*) will provide useful molecular tools. By contrast, there is, at present, no mouse model for conditional gene inactivation in the Müllerian duct epithelium. Although the activation of heterologous genes by *Lim1*, *Hnf1β* and *Cdh16* enhancers has been accomplished in the Müllerian duct epithelium, expression was also seen in the Wolffian duct epithelium[11,60,61,90–92]. Further promoter analysis will be required to identify Müllerian duct epithelium-specific enhancers to generate *Cre*-expressing mice in the Müllerian duct epithelium. Interestingly, *Wnt7a* is specifically expressed in the Müllerian duct epithelium but not the Wolffian duct, so the *Wnt7a* promoter might be a useful molecular tool for this purpose. Finally, it is essential to identify the *cis*-acting regulatory sequences that direct Müllerian duct-specific expression to define the genetic cascade for Müllerian duct development. It is notable that many of these developmental genes are also expressed in the female reproductive tract of the adult, have roles in uterine tissue remodelling and implantation, and might be linked with cancer. Ultimately, a molecular and cellular understanding of Müllerian duct formation and differentiation should lead to insights into female reproductive-tract development and disease.

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Competing interests statement

The authors declare that they have no competing financial interests.

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