Molecular genetics of Müllerian duct formation, regression and differentiation

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Abstract

The Müllerian duct forms the female reproductive tract consisting of the oviducts, uterus, cervix and upper vagina. Female reproductive tract function is vital to fertility, providing the site of fertilization, embryo implantation and fetal development. Developmental defects in the formation, and diseases of the female reproductive tract, including cancer and endometriosis, are prevalent in humans and can result in infertility and death. Further, because the Müllerian ducts are initially formed regardless of genotypic sex, mesenchymal-to-epithelial signaling is required in males to mediate Müllerian duct regression and prevents the development of Müllerian-derived organs. In males, defects in Müllerian duct regression result in the retention of female reproductive tract organs and have been described in several human syndromes. Although to date not reported in humans, ectopic activation of Müllerian duct regression signaling components in females can result in aplasia of the female reproduction tract. Clearly, Müllerian duct development is important to human health, however the molecular mechanisms remain largely undetermined. Molecular genetics studies of human disease and mouse models have provided new insights into molecular signaling during Müllerian duct development, regression and differentiation. This review will provide an overview of Müllerian duct development and important genes and signaling mechanisms involved.

Introduction

The Müllerian duct (MD) is the embryonic structure that develops into the female reproductive tract (FRT), including the oviduct, uterus, cervix and upper vagina. The FRT has essential functions in mammals, providing the site of fertilization, embryo implantation and fetal development. Defects in human FRT formation, thought to arise from abnormal embryonic development, are estimated to occur in up to 3% of births and often result in fertility problems. Diseases of the FRT are also prevalent in adult women and include uterine and cervical cancers, and endometriosis. Further, the reproductive tract of males and females initially contain identical pairs of fully formed Wolffian ducts (WDs) and MDs.

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During male sex differentiation, signaling between MD mesenchyme and epithelium mediate MD regression and prevent its development into a FRT. In males, defects in MD regression result in the retention of MD-derived organs and have been described in human persistent Müllerian duct, Urioste and Denys-Drash syndromes. Although to date not reported in humans, activation of the signaling pathways responsible for MD regression in females results in aplasia of the FRT (Kobayashi and Behringer, 2003). While an understanding of MD development is clearly important to human health, the cellular and molecular mechanisms of these processes remain largely unknown. Recent molecular genetics studies of human disease and mouse models have identified multiple genes important for MD development (Table 1). This review will provide an overview of MD formation, regression and differentiation and important genes and signaling mechanisms involved.

Embryology of the Urogenital System

In vertebrates, the urogenital system originates from the intermediate mesoderm and consists of the kidneys, gonads, and urinary and reproductive tracts. Differentiation of the intermediate mesoderm into the urogenital tract begins shortly after gastrulation. First, signaling from the somite and surface ectoderm transduces mesenchymal-to-epithelial conversions in the intermediate mesoderm and the anterior to posterior formation of the nephric ducts, a pair of epithelial tubes joined at the cloaca (Mauch et al., 2000; Obara-Ishihara et al., 1999). Next, the primary kidney or pronephros transiently forms in the posterior region of the nephric ducts and subsequently degenerates (Bouchard et al., 2002; Saxen and Sariola, 1987). Then, posterior to the degenerating pronephros, the mesonephric duct (WD) develops and extends in an anteroposterior direction. The metanephros arises from inductive interactions between the ureteric bud that branches from the caudal WD and mesenchyme (reviewed in (Little et al., 2010)). Soon after formation of the WD, the paramesonephric duct (MD) appears and grows rostral to caudal adjacent to the WD until the duct joins at the urogenital sinus. Initially, the reproductive tracts of males and females are identical, containing two pairs of fully formed WDs and MDs. After sex determination, hormones produced in the fetal testis, anti-Müllerian hormone (AMH), testosterone, and insulin-like 3 (Insl3) trigger regression of the MD, differentiation of the WD into the male genital tract, consisting of the vas deferentia, epididymides, and seminal vesicles, and testicular descent, respectively. In females, lack of AMH, testosterone, and Insl3 in this developmental window permits differentiation of the MD into the female reproductive tract, consisting of the oviducts, uterus and upper vagina, passive degeneration of the WD, and maintenance of the ovaries in an abdominal position, respectively (Fig. 1) (reviewed in (Kobayashi and Behringer, 2003).

The elongating MDs reach and fuse with the urogenital sinus, to form the utero-vaginal duct that will give rise to the caudal uterus, cervix and upper vagina (Orvis and Behringer, 2007). The rostral region of the MD develops into the oviducts and rostral uterus. Uterine morphology between different mammalian species is highly diverse and varies in part because of differences in the extent of rostral MD fusion. For example, fusion in rodents is minimal, resulting in a duplex uterus (consisting of two individual uterine horns connected

at the cervix) while in primates fusion extends more rostrally, resulting in a simplex uterus (consisting of a single uterine cavity) (Kobayashi and Behringer, 2003).

Müllerian Duct Formation

The MD forms in three distinct phases: initiation, invagination and elongation (Fig. 2A). The first phase, initiation, begins with the formation of a placode-like thickening and expression of the LIM (*lin-11, Isl1* and *mec-3*) class homeodomain transcription factor, LHX1, in the rostral mesonephric epithelial cells fated to become MD epithelial cells (Orvis and Behringer, 2007). *Lhx1* has important function in reproductive tract development of both sexes. One *Lhx1* null male neonate had normal testes but lacked WD-derived organs (Kobayashi et al., 2004). *Lhx1* null female mice form normal gonads but lack all MD-derived reproductive tract structures, including the oviducts, uterus and upper vagina (Kobayashi et al., 2004). Transcriptional co-factors, DACH1 and DACH2, function redundantly in and are required for the formation of the MD. The WD forms normally, however double *Dach1/2* mutant mice have severe defects in MD formation and differentiation and reduced MD expression of *Lhx1* and *Wnt7a*. This suggests that DACH proteins act upstream of *Lhx1* and *Wnt7a* and regulate expression, either directly or indirectly, of these and possibly other factors important for MD formation (Davis et al., 2008).

In the second phase of MD formation, invagination, MD-specified cells from the mesonephric epithelium extend caudally towards the WD. Expression of *Wnt4* in the mesonephric mesenchyme is necessary to signal the MD progenitor cells to begin invagination (Kobayashi et al., 2004; Vainio et al., 1999). The MD is absent in male and female *Wnt4* null mice at E11.5 and E12.5 (Vainio et al., 1999). Loss of *Wnt4* does not alter *Lhx1* expression in the MD precursor cells however these cells fail to invaginate, indicating that *Wnt4* is necessary for invagination but not specification (Kobayashi et al., 2004).

The final phase of MD formation, elongation, begins when the invaginating tip of the MD contacts the WD. MD elongation continues in close proximity to the WD until the MD fuses at the urogenital sinus (Masse et al., 2009; Orvis and Behringer, 2007). By E12.5 in the mouse, the MD has reached approximately the halfway point of its elongation path and crosses over the WD to be located medially. Elongation is complete by ~E13.5 with the MD reaching the urogenital sinus (Fig. 2B) (Gruenwald, 1941; Orvis and Behringer, 2007). While controversial in the past it is now believed that the origin of the MD epithelium cells is a cell population of the mesonephric epithelium likely found in the transition area between the pronephros and mesonephros (Guioli et al., 2007; Orvis and Behringer, 2007). There are cellular markers that are distinct between the WD, MD and mesonephric epithelium during MD formation. In mouse at E12.5, the WD expresses the epithelial markers cytokeratin 8 (CK8), pan cytokeratins and E-cadherin (CDH1) and lacks expression of the mesenchymal marker vimentin (Vim). Initially, the newly formed caudal portion of the MD is mesenchymal in nature and expresses Vim but is CDH1 negative. Later, the MD differentiates and expresses the standard epithelial cell markers with expression of CDH1 first evident in the most rostral region of the MD. At mouse E12.5 and E13.5, the

mesonephric epithelium expresses both epithelial and mesenchymal cell markers (Orvis and Behringer, 2007).

Development of the MD is independent of sex genotype and occurs rostral to caudal. Only MD cells at the most caudal tip are in physical contact with WD cells during elongation (Fig. 2B) (Orvis and Behringer, 2007). Mesenchymal cells are present between the MD and WD and mesonephric epithelium in regions rostral to the caudal tip of the MD (Gruenwald, 1941; Orvis and Behringer, 2007). Specification of MD precursor cells and the initial invagination of the mesonephric epithelium occur independently of the WD. However elongation requires signaling and structure from the WD.

Organ culture studies demonstrated that physical disruption of the WD causes MD truncation, highlighting the link between WD and MD formation (Gruenwald, 1941). The dependence of MD elongation on the WD has also been shown in several mutant mouse models in which the WD either fails to form (*Lhx1* and *Pax2* mutants), degenerates shortly after formation (*Emx2* mutants) or lacks key signaling molecules (*Wnt9b* mutants). In *Lhx1* null mice, the WD is completely absent and the MD fails to form (Kobayashi et al., 2004). Paired-box gene 2 (*Pax2*) is expressed in the WD and MD and is necessary for MD formation. *Pax2* null mice die shortly after birth, failing to form kidneys and reproductive tracts. In *Pax2* homozygous null mice, the rostral portion of the WD forms at E9.5 but does not elongate. By E12.5 the truncated WD has begun to degenerate. MD initiation and invagination occur normally but MD elongation occurs only along the truncated WD. By E16.5, the truncated WD and MD are absent (Torres et al., 1995). Homeobox gene *Emx2* knockout mice lack kidneys, reproductive tracts and gonads in males and females and die shortly after birth due to renal dysfunction. The WD forms normally at E10.5, but by E11.5 the WD is degenerating. The MD fails to form in mutants and is absent at E13.5 (Miyamoto et al., 1997). Although the structure of the WD is unaffected in *Wnt9b* mutant mice, MD elongation is blocked. This suggests that the WD guides elongation through the secreted WNT9B signal. As in studies with a disruption of WD structure, loss of WNT9B signaling did not affect MD specification and initial invagination, only caudal elongation (Carroll et al., 2005).

While the primary cause of MD loss in *Lhx1, Pax2* and *Emx2* null mice is likely a result of WD defects, these homeodomain transcription factors are suggested to have later functions in MD development. Chimera studies suggest that *Lhx1* is required cell-autonomously for the formation of the MD epithelium (Kobayashi et al., 2004). It is also probable *Pax2* functions cell autonomously during MD formation and/or maintenance. PAX2 protein is expressed in both the MD and WD epithelium at E13.5. Further *Pax2* is thought to be required for the mesenchyme-to-epithelium transitions in the intermediate mesoderm necessary for both WD and MD formation (Torres et al., 1995). Similarly, *Emx2* is expressed in both the WD and MD epithelium at E13.5 suggesting additional roles in MD development (Miyamoto et al., 1997).

Caudal growth of the MD epithelium is thought to occur primarily as a result of proliferation of the MD epithelium. Cells from the WD or from the mesonephric epithelium following MD specification do not contribute significantly to the growing MD (Guioli et al., 2007;

Orvis and Behringer, 2007). During elongation, studies in mouse and chick have shown proliferation is occurring along the length of the MD (Guioli et al., 2007; Jacob et al., 1999; Orvis and Behringer, 2007). Organ culture of mouse urogenital ridges in which the rostral MD has been removed leaving the caudal MD tip region showed completion of MD elongation. Therefore, cells contained in the caudal MD tip are capable of completing MD elongation in the absence of the rostral MD (Orvis and Behringer, 2007). In organ culture studies of rat urogenital ridges, migration of MD epithelial cells has been shown to be occurring in the rostral to caudal direction during MD elongation. Further, following extended culture with BrdU, both dividing and non-dividing cells are found in the MD tip suggesting migration may contribute to MD elongation (Fujino et al., 2009).

The PI3K/ AKT pathway also has a role in MD elongation. Treatment with PI3K inhibitors in rat urogenital organ culture blocks MD elongation. PI3K inhibition also deterred lateral migration of the mesenchymal cells that separate the WD and MD. However PI3K inhibition did not affect rostral to caudal migration of the MD epithelial cells in the already formed portions of duct. Slight increases in apoptosis were observed in the MD after PI3K inhibition, but likely do not explain the MD elongation defect. The authors hypothesize that PI3K may be required to activate enzymes that break down the extra-cellular matrix that would otherwise block invasion by the caudal tip (Fujino et al., 2009).

Retinoic acid (RA) signaling is also required for the formation and/or maintenance of the MD. RA, a morphogen derived from vitamin A, has important functions in antero-posterior patterning of the body axis and during limb development (Dreyer and Ellinger-Ziegelbauer, 1996; Robert and Lallemand, 2006). *RA receptor* (*RAR*) genes have redundant function in MD formation. In mouse, single gene mutants of *RARα1, RARα2, RARβ2* or *RAR*γ, have no defects in female reproductive tract development. However the MD is completely absent at E12.5 in *RARα/RARβ2* compound mutants not attributable to any defects in WD formation. Additionally other combinations of *RAR* mutations resulted in partial MD loss caudally. Thus suggesting RA signaling has important functions in MD but not WD formation (Kastner et al., 1997; Mendelsohn et al., 1994). Caudal defects in MD elongation are also observed in *Discs large homolog 1* (*Dlgh1*) null mice that cause MD fusion failure and obstruction which results in aplasia of the cervix and vagina (Iizuka-Kogo et al., 2007).

Sex Differentiation; Müllerian Duct Regression in Males

Anti-Müllerian hormone signaling pathway

During male development mesenchyme-epithelia interactions mediate MD regression to prevent its development into a uterus and oviduct (Fig. 1). MD regression requires binding and signal transduction from the transforming growth factor-β (TGF-β) family member anti-Müllerian hormone (AMH) secreted from the Sertoli cells of the fetal testis and its type 1 and 2 receptors expressed in MD mesenchyme (reviewed in (Josso et al., 1993; Kobayashi and Behringer, 2003). The transcription of the *Amh* gene is directly regulated by multiple factors in the testis-determining pathway including SRY-box containing gene 9 (*Sox9*), steroidogenic factor 1 (*Sf1*), Wilms tumour homologue (*Wt1*), and DSS-AHC critical region on the X-chromosome gene 1 (*Dax1*) (Arango et al., 1999; De Santa Barbara et al., 1998;

Nachtigal et al., 1998; Shen et al., 1994). Females do not express AMH during fetal development thus allowing differentiation of the MD.

The first observable histological change during regression in males is the appearance of the "sworl" pattern of the mesenchymal cells surrounding the MD in the most rostral region (Dyche, 1979; Orvis and Behringer, 2007). At the onset of regression, differences in the MD also appear at the cellular level between the sexes. Initially the forming MD is mesoepithelial in nature with cell markers consistent with a mesenchymal cell tube however the morphology is consistent with a true epithelial cell tube (Dyche, 1979; Orvis and Behringer, 2007). Beginning at E13.5, the female MD begins to express the epithelial cell marker E-cadherin (CDH1) apically and show evidence of apicobasal polarity while the MD in males remains unchanged (Orvis and Behringer, 2007). This has also been observed in several other species including rat (Dohr et al., 1987; Paranko and Virtanen, 1986), human (Magro and Grasso, 1995), chick (Jacob et al., 1999), but not in golden hamster (Viebahn et al., 1987). AMH-induced MD regression occurs in a specific window in time during development and after this time the MD is no longer sensitive to AMH-induced regression (Josso et al., 1976). This window corresponds with the time frame in which the MD is not yet expressing epithelial-specific markers (Orvis and Behringer, 2007). One hypothesis is that the mesoepithial nature of the MD may facilitate regression in males.

Genetic experiments in mouse and naturally occurring mutations in the human have demonstrated that AMH is necessary and sufficient for Müllerian duct regression. *Amh* null male mice have normal development of the testis and male reproductive tract, however Müllerian-derived tissues develop, causing infertility by physically blocking sperm release (Behringer et al., 1994). Further, female transgenic mice ectopically expressing human AMH lack MD-derived tissues (Behringer et al., 1990). Additionally, mutations in the human *AMH* gene are causative of ~45% of Persistent Müllerian Duct Syndrome (PMDS) cases, a rare autosomal recessive disorder. Like *Amh* null mice, male patients with PMDS are normally virilized, but have female reproductive organs including a uterus and fallopian tubes. PMDS is most often diagnosed because of cryptorchidism, a failure of the testis to descend, and/or inguinal hernia (Belville et al., 1999; Belville et al., 2009; di Clemente and Belville, 2006; Salehi et al., 2012).

Amhr2 positive cells in the MD mesenchyme transduce the AMH hormone signal secreted from the fetal testis (Mishina et al., 1999). AMH signaling occurs in a paracrine manner and begins with AMH binding to its type 2 receptor (AMHR2). AMHR2 then forms a heteromeric complex with and then phosphorylates and activates a type 1 receptor. This activation results in the phosphorylation of an R-Smad and supposedly formation of an R-SMAD/SMAD-4 complex that translocates into the nucleus to transcriptionally activate AMH signaling pathway target genes. AMH type 1 receptors ALK2 (AVCR2) and ALK3 (BMPR1A), and AMH R-Smad effectors (SMAD1, SMAD5 and SMAD8) function redundantly in MD regression and are shared with the bone morphogenetic protein (BMP) pathway. ALK3 is considered the primary type 1 receptor required for regression however ALK2 is capable of transducing the AMH signal in the absence of ALK3. Conditional knockout of *Alk2* in the MD mesenchyme does not block MD regression. However approximately half of all *Alk3* conditional mutant males and 100% of mutants with

conditional knockout of both *Alk2* and *Alk3* failed to regress the MD (Jamin et al., 2002; Orvis et al., 2008).

ALK2 and ALK3 are believed to function in a distinct temporal and spatial manner during regression. Initially, *Amhr2* is expressed in the mesonephric epithelium. In the mesonephric epithelium, ALK2 appears to mediate AMH signaling which directs *Amhr2* positive cells to undergo an epithelial to mesenchymal transition and migrate to surround the MD epithelium forming the distinct "sworl" pattern observed at E15.5 (Zhan et al., 2006). This is followed by the breakdown of the basement membrane and subsequent loss of the epithelium (Dyche, 1979; Orvis and Behringer, 2007; Trelstad et al., 1982). In females, *Amhr2* expressing cells are found in the mesonephric epithelium on the antimesometrial side of the MD, but without AMH signaling these cells do not undergo migration to surround the MD epithelium or cellular changes up to at least E15.5 (Fig. 3) (Arango et al., 2008; Orvis et al., 2008; Zhan et al., 2006).

Mechanisms known to be involved in regression include epithelial cell migration, epithelial to mesenchymal transformations and apoptosis (Allard et al., 2000; Austin, 1995; Hutson et al., 1984). The pattern of regression of the MD is hypothesized to occur in a rostral to caudal wave corresponding to *Amhr2* expression. A statically imaged time course of *ex vivo* cultured rat urogenital ridges (males and AMH-treated females) during regression showed rostrally a reduction in MD diameter (Picon, 1969; Tsuji et al., 1992). Further Allard et al. showed a positive correlation between the rostral-caudal wave of *Amhr2* expression and the pattern of increased apoptosis of the MD epithelium during regression in rat (Allard et al., 2000). In contrast, studies from the *Amhr2-lacZ* knock-in mouse model indicate that although *Amhr2* expression initiates in a rostral to caudal wave, it is expressed along the entire length of the MD at E13.5 well before any overt changes in the male MD (Fig. 3) (Arango et al., 2008). Further studies will be needed to clarify the spatial-temporal patterning and cell behaviors, including migration and apoptosis, during MD regression.

Genetic studies in mice and human indicate that AMHR2 is the sole type 2 receptor required for AMH signaling and is likely dedicated to the AMH-signaling pathway. Male *Amhr2* and *Amh* mutant mice have identical phenotypes; normally virilized with persistent MD-derived organs (Behringer et al., 1994; Mishina et al., 1996; Mishina et al., 1999). Additionally, the phenotype of hAMH expressing transgenic female mice is rescued by *Amhr2* mutation (Behringer et al., 1990; Mishina et al., 1999). Further, mutation of the *AMHR2* gene accounts for about half of the cases of PMDS with known molecular etiology (Belville et al., 2009; di Clemente and Belville, 2006; Salehi et al., 2012).

The *Wilms' tumor 1* (*Wt1*) transcription factor gene is a direct activator of *Amhr2* transcription. *Amhr2* transcript levels are reduced in *Wt1* null mice and both transcripts are co-expressed in the developing MD. Additionally, *Wt1* expression mirrors the sexually dimorphic pattern observed for *Amhr2* expression during MD regression in the urogenital ridge. Further, *in vitro* assays show WT1 activates transcription of *Amhr2* and binds to elements in the *Amhr2* proximal promoter (Klattig et al., 2007). Two alternate splice variants of *Amhr2, Amhr2-* exon 2 and *Amhr2-* exon 9 and 10, are found in adult rat and mouse. The *Amhr2* splice variants act in a dominant negative manner when co-transfected

with full length AMHR2 and AMH in *in vitro* luciferase reporter assays. The dominant negative effect of the splice variants was reduced at higher levels of AMH concentration corresponding to local levels of AMH in the gonads. The authors hypothesize that splice variants may be expressed at high levels only in a particular subset of gonadal cells and regulate AMH signaling in these cells. Alternatively, splice variants may have site specific effects independent of the presence of AMH ligand or may have a role in the transport of ligand into the cell and might be important in trafficking across the blood brain barrier (Imhoff et al., 2013). Expression of these splice variants during MD regression has not been determined and it is currently unknown what if any role they have during reproductive tract development.

Wnt signaling plays multiple roles in MD development and is needed for formation, regression and differentiation. Prior to the onset of MD regression in males, WNT7A signaling from the epithelium to mesenchyme of the MD activates *Amhr2* expression in both sexes and is also required for appropriate differentiation of the MD. *Wnt7a* mutant males retain MD-derived organs because *Amhr2* expression is lost in the MD mesenchyme, thus blocking the AMH-signaling pathway. Consistent with the differentiation defects observed in the *Wnt7a* mutant female reproductive tract, in mutant male, the ectopic female reproductive tract shows no evidence of oviduct coiling and is a simple epithelial tube (Parr and McMahon, 1998). This is in contrast to *Amh* and *Amhr2* null mouse models where the differentiation of the mutant female and male MD-derived organs is relatively normal (Behringer, 1994; Behringer et al., 1994; Mishina et al., 1996). The *Frizzled* (*Fzd*) genes encode the seven trans-membrane protein receptors for the WNT ligand which are required for both canonical and non-canonical WNT signaling pathways. A dedicated FZD receptor has not been identified for WNT7A in the MD. Previous studies showed interactions between WNT7A and FZD10 activated the WNT pathway (Kawakami et al., 2000). Additionally, in mouse, *Wnt7a* and *Fzd10* have overlapping expression patterns in the MD (Nunnally and Parr, 2004). Although this identified FZD10 as a potential candidate receptor for WNT7A, *Fzd10* knockout mice have no reproductive tract phenotype (Fzd 10: MGI Direct Data Submission MGI:3604450). Similar to *Fzd10, Fzd1* expression is found in the MD mesenchyme and epithelium at E14.5, but *Fzd1* knockout males have no MD regression defects (Deutscher and Hung-Chang Yao, 2007; Lapointe et al., 2012). This suggests multiple FZD receptors are capable of interaction with WNT7A and function redundantly during MD regression.

AMH signaling; downstream molecular mechanisms

Several studies suggest WNT signaling is also important to the downstream molecular signaling cascade required for MD regression during male reproductive tract differentiation. However, the exact role of WNT signaling following activation of the AMH signaling pathway remains unclear. Either inactivation or constitutive activation of β-CATENIN (CTNNB1) in the MD mesenchyme causes retention of MD-derived tissues in mutant males independent of AMH expression suggesting tight control of CTNNB1 activation is necessary for MD regression (Kobayashi et al., 2011; Tanwar et al., 2010). In the canonical WNT signaling pathway, nuclear localized CTNNB1 in a complex with T-cell factor/ lymphoid enhancer factor (TCF/LEF) transcription factors regulate expression of target

genes. CTNNB1 is also known to have roles in cell tight junction formation and adhesion and may therefore be functioning independent of WNT signaling during regression (Brembeck et al., 2006). CTNNB1 activates *Lef1* transcription and upregulates *Lef1* promoter activity *in vitro* (Filali et al., 2002; Vadlamudi et al., 2005). Additionally, *Ctnnb1* inactivation results in the loss of LEF1 up-regulation normally observed in the MD mesenchyme of males during regression (Kobayashi et al., 2011). This suggested LEF1 may be required downstream of WNT/β-CATENIN signaling to induce MD regression during male reproductive tract differentiation. However, *Lef1* null male mice have normal MD regression (Mullen and Behringer, unpublished observations) (van Genderen et al., 1994). Several additional WNT pathway factors have also been identified that are expressed in a sex-specific pattern in the mesenchyme during AMH-induced MD regression including *Wnt4, Wnt5a*, and Frizzled-related Wnt pathway genes *Sfrp1, Sfrp2, and Sfrp5*. Knockout of *Wnt4* in the MD mesenchyme does not interfere with MD regression (Kobayashi et al., 2011). No defects in MD regression have been reported in *Wnt5a*$^{-/-}$ mice (Mericskay et al., 2004). Likewise, loss of function of *Sfrp2* and *Sfrp5* caused no defects in MD regression (Cox et al., 2006). Double knockout *Sfrp1*$^{-/-}$/*Sfrp2*$^{-/-}$ mice appear to have a slight delay, but MD regression is complete at later embryonic stages (Warr et al., 2009). These results suggest that WNT pathway factors have redundant function during MD regression or alternatively are not required for regression. Further studies will be needed to clarify the roles of WNT signaling during later stages of MD regression.

Sexually dimorphic expression patterns during regression have also been identified for *Matrix metalloproteinase 2* (*Mmp2*). *Mmp2* is upregulated in the male MD mesenchyme during regression and this up regulation is lost in *Amh* null males. Morpholino knockdown of *Mmp2* in organ culture blocks regression and decreases MD epithelium apoptosis (Roberts et al., 2002). Null *Mmp2*-mutant mice however have no defects in MD regression. This may suggest redundant function with other genes (Itoh et al., 1997; Roberts et al., 2002). The PI3K/ AKT pathway may also have a role in AMH signal transduction during regression. Activated phospho-AKT (p-AKT) is present in equal amounts in the WD and MD in both sexes prior to regression. In rats, synchronous with the initiation of MD regression, p-AKT is decreased in males at E15.5 and is undetectable at E16.5. Females maintain p-AKT expression. This pattern is also observed in mice (Fujino et al., 2009). Although PI3K signaling has been shown to prevent apoptosis and the epithelial to mesenchymal transitions that take place during MD regression, it is not clear if this reduction in p-AKT is a cause or effect of regression (Allard et al., 2000; Dyche, 1979; Fujino et al., 2009; Trelstad et al., 1982; Zhan et al., 2006). AMH signaling has been shown to inactivate the PI3K pathway by blocking autophosphorylation of the EGF receptor in the MD epithelium by inhibiting tyrosine kinase (Hurst et al., 2002; Hutson et al., 1984). Although multiple genes have been identified using candidate approaches, the role of many of these signaling pathways and molecules remains unclear due to the possibility of functional redundancy.

Müllerian duct differentiation in females

Once the MD is formed it differentiates into a functional oviduct, uterus, cervix and upper vagina. Correct patterning and differentiation of the MD is dependent on a complex network

of *Hox* and *Wnt* genes. Further it is known that steroid hormones also regulate many of the genes necessary for proper MD differentiation during organogenesis and adulthood (Masse et al., 2009). Abdominal B (*AbdB*) homeobox genes (*Hoxa9, Hoxa10, Hoxa11* and *Hoxa13*) of the mammalian *Hoxa* cluster are required for differentiation and segmental patterning of the MD. *AbdB* genes are expressed along the anterior-posterior axis of the MD according to their 3' to 5' order in the *Hoxa* cluster. *Hoxa9* is expressed in the oviduct. *Hoxa10* is expressed in the mesenchyme of the uterus. *Hoxa11* is expressed in the posterior uterus and cervix. *Hoxa13* is expressed in the cervix and upper vagina (Taylor et al., 1997; Warot et al., 1997). *Hox10* expression is necessary for correct specification of tissue boundaries in the male and female reproductive tract. In *Hoxa10* null mice, male and female reproductive tracts display posterior to anterior homeotic transformation. At E17.5, *Hoxa10* is present only in the portion of the MD that will differentiate into the uterus. Mutations in *Hoxa10* result in homeotic transformation of 25% of the proximal uterus into oviduct (Benson et al., 1996). Three heterozygous mutations in the *HOXA10* gene with predicted loss of function have been associated with uterine malformations. Patients with *HOXA10* mutations had uterine defects ranging from septate uterus and vagina with a duplex cervix to a didelphic uterus indicative of MD fusion defects (Cheng et al., 2011; Ekici et al., 2013). In adult uterus, *Hoxa10* represses *Emx2* and is found in an inverse expression pattern suggesting a further role for *Emx2* in MD patterning and differentiation (Troy et al., 2003). Overlapping expression of patterns of *Hoxa10* and *Hoxa11* suggests they have partially redundant function during MD differentiation. Further, exposure to the non-steroidal estrogen diethylstilbestrol (DES) in mice caused a posterior shift in *Hoxa9* expression likely a result of down regulation of *Hoxa10* and *Hoxa11* (Block et al., 2000). Sex steroids mediate *Hoxa10* and *Hoxa11* expression levels. Women exposed to DES during development have malformations of the reproductive tract consistent with anterior transformation (Cermik et al., 2003; Taylor et al., 1999). *Hoxa11* null mice have a thinner and shorter uterus lacking glands consistent with a partial homeotic transformation (Gendron et al., 1997). Mutant mice in which the *Hoxa11* homeodomain was replaced with the *Hoxa13* homeodomain displayed posterior homeotic transformation of the female reproductive tract with the posterior uterus becoming cervix/vagina. This demonstrates that *Hoxa11* and *Hoxa13* have unique functions in MD differentiation and that *Hoxa13* is upstream of factors required for differentiation of the MD into cervix and vagina (Zhao and Potter, 2001). Although *Hoxa13* null mutants die between E13.5 to E14.5, mutant female embryos are missing the caudal portion of the MD suggesting *Hoxa13* has function during MD formation in addition to MD differentiation. Defects in caudal MD formation were also observed in a *Hoxa13* paralogue, *Hoxd13*, mutant females at birth (Warot et al., 1997).

The WNT pathway is required for MD patterning and differentiation. *Wnt7a* has important function in both MD regression in males and MD differentiation in females. *Wnt7a* is expressed throughout the MD epithelium prior to birth. After birth expression is maintained in the oviductal and uterine epithelium but is down-regulated in the vaginal epithelium (Miller et al., 1998). In adult and neonate *Wnt7a* mutant females, the uterus is smaller in length and diameter and the uterine wall is thinner with less smooth muscle. Oviduct differentiation occurs but coiling and elongation are absent and uterine glands are not present. Posteriorly, a homeotic transformation occurs of oviduct to uterus and uterus to

vagina in the *Wnt7a* null females (Miller and Sassoon, 1998; Parr and McMahon, 1998). *Wnt7a* also appears to be necessary for the maintenance of *Hoxa10* and *Hoxa11* expression with the *Wnt7a* null females showing reduced expression of the two genes. Additionally, the similarity between the *Hoxa11* null and *Wnt7a* null mutant phenotype (thin, small uterus lacking glands) is consistent with upstream regulation of *Hoxa11* by *Wnt7a* (Miller and Sassoon, 1998; Parr and McMahon, 1998).

Recent studies using conditional knockout and tissues explanted under the kidney capsule have also clarified the role for WNT signaling at later stages of MD differentiation. These WNT signaling molecules include *Wnt4, Wnt5a* and *CTNNB1*, which were not previously described because of early embryonic lethality (*Wnt5a and CTNNB1*) or early roles in MD formation (*Wnt4*). The role of *Wnt4* in MD formation is well established. A recent conditional knockout study also demonstrates *Wnt4* is important for MD differentiation. Conditional inactivation of *Wnt4* in the uterine luminal and glandular epithelium, stroma and myometrium using *Progesterone receptor* (*PR*)-*Cre* resulted in reduced uterine gland number and a stratified luminal epithelial layer instead of a simple columnar epithelial cell layer (Franco et al., 2011). The receptor for *Wnt4* during MD development has not been identified. However, *Frizzled1* (*Fzd1*) expression has been found in the developing mesenophros in both the MD mesenchyme and epithelium (Deutscher and Hung-Chang Yao, 2007). Further, 3 of 17 *Fzd1*−/− null females had a uterine phenotype similar to the *Wnt4* conditional mutant females suggesting FZD1 may be acting as the WNT4 receptor (Lapointe et al., 2012). However because of the low penetrance of uterine defects in the *Fzd1*−/− mutant females it is likely other FZD receptors are also able to transduce the WNT4 signal.

The *Wnt5a* gene is required for development of the posterior (caudal) region of the MD and glandular genesis. *Wnt5a* null mice have short, coiled uterine horns, but lack a cervix and vagina. In kidney capsule explant studies of mutant uterine horns, both *Wnt7a* in the luminal epithelium and *Wnt5a* in the uterine stroma were required for gland formation independent of canonical pathway member *Lef1* (Mericskay et al., 2004). Conditional deletion of *Ctnnb1* in the MD mesenchyme using *Amhr2-Cre* causes a hypoplastic uterus with uterine hypotrophy, reduced uterine glands and uncoiled oviducts. In *Ctnnb1* conditional mutants, reduced proliferation but not apoptosis contributes to the hypoplasia of the uterus. No differences were found in the expression patterns of *Wnt4* and *Wnt5a* in the mesenchyme or *Wnt7a* in the epithelium (Deutscher and Hung-Chang Yao, 2007). The mutant phenotype including lack of coiling mimics the *Wnt7a* null phenotype (Parr and McMahon 1998). Distinct from the *Wnt7a* null mice, deletion of *Ctnnb1* in the MD mesenchyme resulted in the differentiation of smooth muscle cells into adipose tissue postnatally causing the uterus to become fat filled. This suggests other WNTs may be needed for uterine differentiation or alternatively the role of *Ctnnb1* in the mesenchyme is independent of WNTs and is functioning instead to control cell adhesion or the formation of cell tight junctions. These studies suggest that the initial differentiation of the myometrium does not require *Ctnnb1* but in its absence there is a progressive shift from smooth muscle tissue to adipose tissue (Arango et al., 2005; Deutscher and Hung-Chang Yao, 2007). *Ctnnb1* conditional ablation using *PR-Cre* resulted in a thinner uterus of normal length with reduced gland numbers at

sexual maturity. Constitutive activation of CTNNB1 using *PR-Cre* reduced uterus length and caused hyperplasia of uterine glands. Reductions in the size of the uterus as a result of ablation or activation of *Ctnnb1* suggest tight control of WNT signaling is required for proper MD differentiation and development (Jeong et al., 2009).

The *Wnt 4* and β-*catenin* (*Ctnnb1*) conditional mutant females and *Wnt7a−/−* and *Wnt5a−/−* null females have similar defects in MD differentiation. These are also similar to the uterine phenotype caused by DES exposure suggesting a link between WNT signaling and estrogen signaling. WNT signaling ligands in the luminal epithelium may be required to prevent the formation of a stratified epithelial layer in the uterus in response to estrogen signaling (Franco et al., 2011).

Human Müllerian duct formation and differentiation defects

Aberrant development of the MD is a relatively frequent cause of human birth defects. Defects include MD aplasia, MD persistence, and MD fusion and patterning defects. Multiple medical syndromes are associated with female reproductive tract abnormalities. Molecular genetic studies of these patients have identified candidate factors involved in some cases. However the molecular genetic cause of these syndromes remains unknown in the majority of cases (Kobayashi and Behringer, 2003). Several well-characterized syndromes are described; Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (OMIM 277000), maturity-onset diabetes of the young type 5 (MODY5) (OMIM 604284), persistent Müllerian duct (PMDS) syndrome (OMIM 261550), Urioste syndrome (OMIM 235255), Denys-Drash syndrome (DDS) (OMIM 194080) and Hand-foot-genital (HFG) syndrome (OMIM 140000).

Müllerian duct aplasia syndromes

MRKH occurs in about 1 in 4500 female births and is characterized by the absence of the uterus and upper vagina in genetic females (46XX) (Folch et al., 2000). In the majority of affected patients, ovarian development is normal and the lower third of the vagina is present. The molecular etiology of most cases of MRKH is unknown and occurs most often due to sporadic mutations but familial cases have been described with autosomal dominant inheritance with incomplete penetrance and variable expressivity. MRKH syndrome is further classified as type I (typical; isolated to the reproductive tract) or type II (atypical; associated with additional developmental defects) (Ledig et al., 2011). Müllerian duct aplasia, unilateral renal agenesis, and cervicothoracic somite anomalies (MURCS) association is a severe form of MRKH type II. Patients with MURCS association have renal and skeletal defects in addition to uterine and vagina aplasia or agenesis (Oppelt et al., 2006). Plausible causes of MRKH are mutations increasing either AMH or AMHR2 activity and/or expression that could result in MD regression in females. However, no defects in either the *AMH* or *AMHR2* genes have been discovered to date (Oppelt et al., 2005).

Multiple genes have been associated with MRKH including *WNT4, TCF2* (also known as *HNF1β* or *v-HNF1*), *LHX1* and *short stature homeobox* (*SHOX*). Heterozygous loss of function mutations in the human *WNT4* gene causes a complete absence of a uterus and upper vagina. In addition, female patients with *WNT4* mutations also have excess androgens

and symptoms of virilization (Biason-Lauber et al., 2007; Biason-Lauber et al., 2004). This is consistent with the phenotype of *Wnt4* null mice in which the MD does not development and testosterone biosynthesis is ectopically activated in the ovary (Vainio et al., 1999). The most common mutations associated with MRKH are deletions of chromosomal region 17q12 which contains both *TCF2* and *LHX1* genes with approximately 6% of examined cases of MRKH carrying this deletion. TCF2 is a POU domain containing transcription factor widely expressed during development with function in epithelial differentiation (Coffinier et al., 1999a; Kolatsi-Joannou et al., 2001). Heterozygote mutations of *TCF2* were first associated with MODY5 with additional malformations in renal development and function. A subset of female patients with heterozygote mutations in *TCF2* have malformations of the reproductive tract including bicornuate uterus, uterus didelphys, and Müllerian aplasia and renal defects in the absence of diabetes (Bingham et al., 2002; Lindner et al., 1999). This suggests an important role for *TCF2* in urogenital tract formation and maintenance. In mouse, *Tcf2* is expressed in the reproductive tract epithelium during development and persists in the adult (Coffinier et al., 1999a; Reber and Cereghini, 2001). However the function of *Tcf2* in mouse urogenital development remains undetermined due to the early embryonic lethality of *Tcf2* null mutant mice and the normal phenotype of the heterozygous mutants (Barbacci et al., 1999; Coffinier et al., 1999b). To date, MKRH syndrome has also been associated with five heterozygous mutations in the human *LHX1* gene; four missense mutations and a frame shift mutation leading to a stop codon (Ledig et al., 2012; Ledig et al., 2011; Sandbacka et al., 2013). Additionally, *Lhx1* has been shown in mice to be essential for MD formation (Kobayashi et al., 2004). Partial duplication of the *SHOX* gene was found in two daughters with MRKH type I and their unaffected father (Gervasini et al., 2010).

Müllerian duct persistence syndromes

The development of a uterus and oviduct in human males has been noted in three syndromes; PMDS, Urioste syndrome and DDS. PMDS patients have normal testis development and the presence of MD-derived female reproductive organs. The syndrome is usually diagnosed while correcting undescended testes in pediatric patients. Reduced fertility is common in PMDS patients and potential causes include structural abnormalities caused by MD remnants, cryptorchidism past the age of 2 years, and damage to the vas deferens during orchidopexy. There is also an increased risk of malignancy in the ectopic MD-derived organs if not surgically removed and in the testes due to cryptorchidism. Eleven cases of malignancy in the retained MD organs have been reported and laparoscopic removal of the MD structures in PMDS patients is recommended (Farikullah et al., 2012). PMDS is further classified with type I males having undetectable levels of AMH and type II males with normal AMH levels. The majority of PMDS cases are caused by mutations in the *AMH* (type I) or *AMHR2* (type II) genes with each representing about half of the cases with known molecular etiology (Belville et al., 2009; di Clemente and Belville, 2006; Salehi et al., 2012).

Urioste syndrome is an autosomal recessive disorder associated with the retention of MD-derived tissues in males. In addition to a persistent MD phenotype patients also have lymphangiectasia and postaxial polydactyly. The molecular basis of this syndrome is currently unknown (Urioste et al., 1993).

Denys-Drash syndrome (DDS) is characterized by partial gonad dysgeneis, congenital or infantile nephropathy, and Wilms' tumor. The molecular cause of DDS in almost all cases is dominant loss of function mutations in the zinc finger DNA binding domain of WT1. In multiple cases of DDS, patients have MD-derived uterus and vagina remnants in addition to developed vas deferens and epididymis (Barakat et al., 1974; Denys et al., 1967; Manivel et al., 1987). *Amh* and *Amhr2* are regulated by WT1 therefore reductions in AMH ligand and its receptor are postulated to cause the defects in MD regression seen in DDS patients (Hossain and Saunders, 2003; Klattig et al., 2007; Nachtigal et al., 1998). The most common *WT1* gene mutation in DDS is a missense mutation in exon 9, 1180C>T (R394W). The presence of retained MD structures is found in some but not all patients including those from the same family (Coppes et al., 1992; Zhu et al., 2013). Mouse models heterozygous either for the *Wt1* null allele or the R394W mutation have no evidence of MD regression defects (Gao et al., 2004). Together this suggests that genetic background and/or environmental factors may play an important role in determining the penetrance of MD regression defects in DDS patients.

Müllerian duct fusion and patterning defect syndrome

HFG is an autosomal dominant syndrome that results in shortened thumbs and great toes and genital defects including hypospadias in males and a range of female reproductive tract defects from a longitudinal vagina or double vagina to double uterus and cervix. Incomplete MD fusion during embryogenesis gives rise to these defects in females with HFG (Goodman and Scambler, 2001). The similarity of the *Hypodactly* (*Hd*) mutant mouse phenotype with a spontaneous dominant negative mutation in the first exon of the *Hoxa13* gene to the limb and genital defects in humans first identified *Hoxa13* as a potential candidate gene (Post et al., 2000). Although, MD fusion defects are not present in *Hd* and *Hoxa13*–/– mice, mild hypospadias of the vagina is observed in a portion of the mutant females (Post et al., 2000; Warot et al., 1997). Additionally, one in six female compound mutants of *Hoxa13* and its paralogue, *Hoxd13*, had MD fusion defects (Warot et al., 1997). Further, six heterozygous mutations in the human *HOXA13* gene have been reported in families with HFG to date (Goodman et al., 2000; Mortlock and Innis, 1997).

Conclusions

Much progress has been made in understanding the molecular genetics of MD development and several important signaling pathways have been identified (Fig. 4.). Study of knockout and conditional knockout mouse model phenotypes and molecular genetic studies of human diseases of FRT development have provided key insights into the complex signaling cascade involved. However potential functional redundancy of many of these factors including WNT signaling pathway members, MMPS and HOX genes has made it difficult to assess their *in vivo* function. For example, MD regression requires tight regulation of CTNNB1 activation but thus far a single WNT required for MD regression has not been identified. Further it is likely other factors yet to be identified are involved in MD development. Expression profiling using next generation sequencing technologies should identify genes that are differentially expressed during MD formation and differentiation. By using *Cre* recombinase lines expressing in the MD epithelium (*Wnt7a–Cre*) and mesenchyme (*Amhr2-Cre*) it will

be possible to globally uncover the transcriptome of these juxtaposed tissue types. Mesenchyme-epithelia interactions are important regulators of development and many defects in FRT development are the result of aberrant cell-cell communication and signaling. Understanding the molecular and cellular mechanisms of MD formation and differentiation will give key insights into FRT development and disease.

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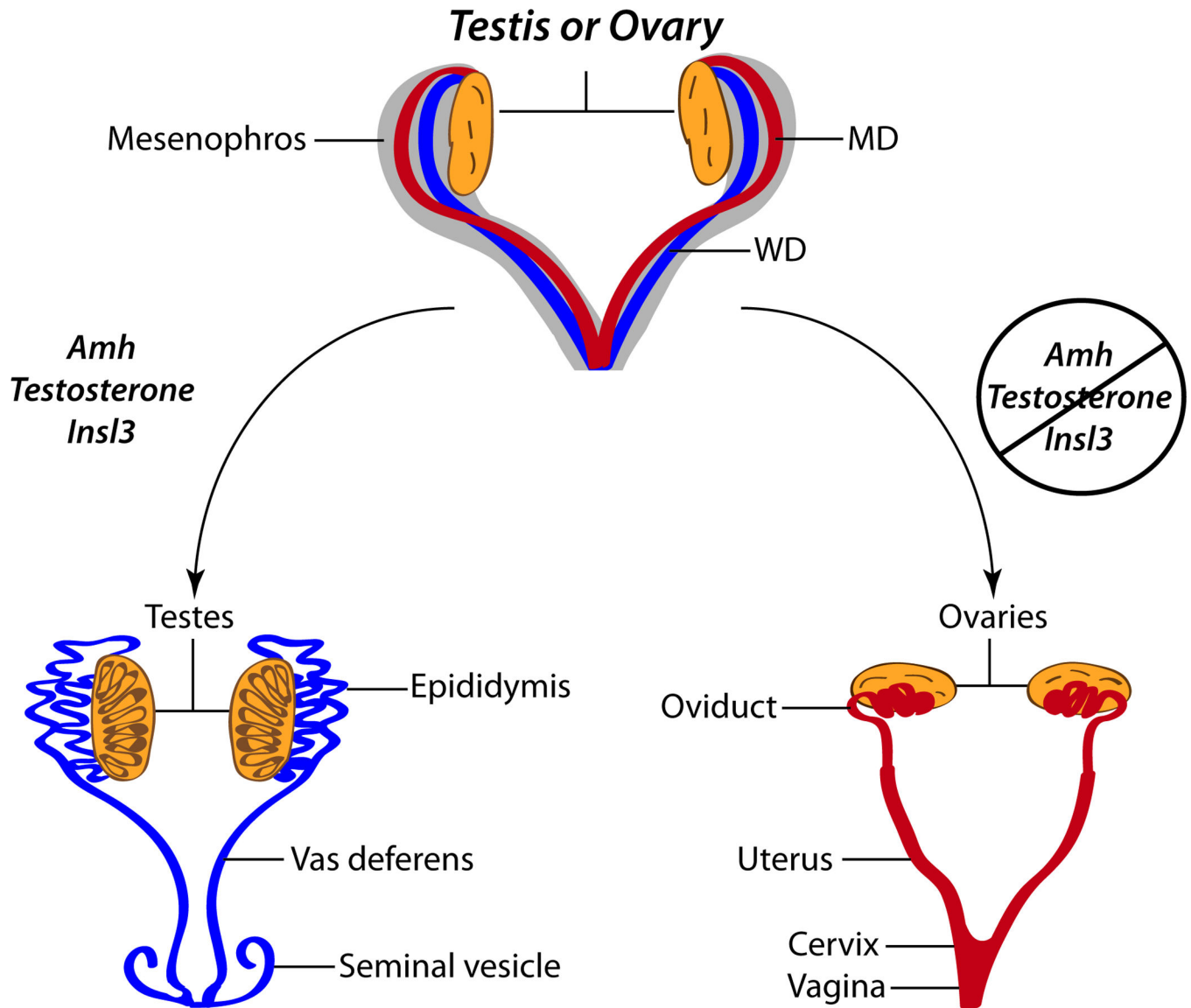
FIG. 1. Sexual differentiation of the reproductive tracts
The reproductive tracts prior to sexual differentiation are equivalent and contain a fully formed Wolffian duct (WD; represented in blue) and Müllerian duct (MD; represented in red). Hormones produced in the fetal testis, anti-Müllerian hormone (Amh), testosterone, and insulin-like 3 (Insl3), enable regression of the MD, differentiation of the WD into the male genital tract, and testicular descent, respectively. In females, lack of AMH, testosterone, and Insl3 at this developmental time permits differentiation of the MD into the female reproductive tract, passive degeneration of the WD, and maintenance of the ovaries in an abdominal position, respectively. The WD differentiates into the male reproductive tract consisting of the vas deferentia, epididymides, and seminal vesicles. The MD develops into the female reproductive tract consisting of the oviducts, uterus and upper vagina. Adapted from (Kobayashi and Behringer, 2003).
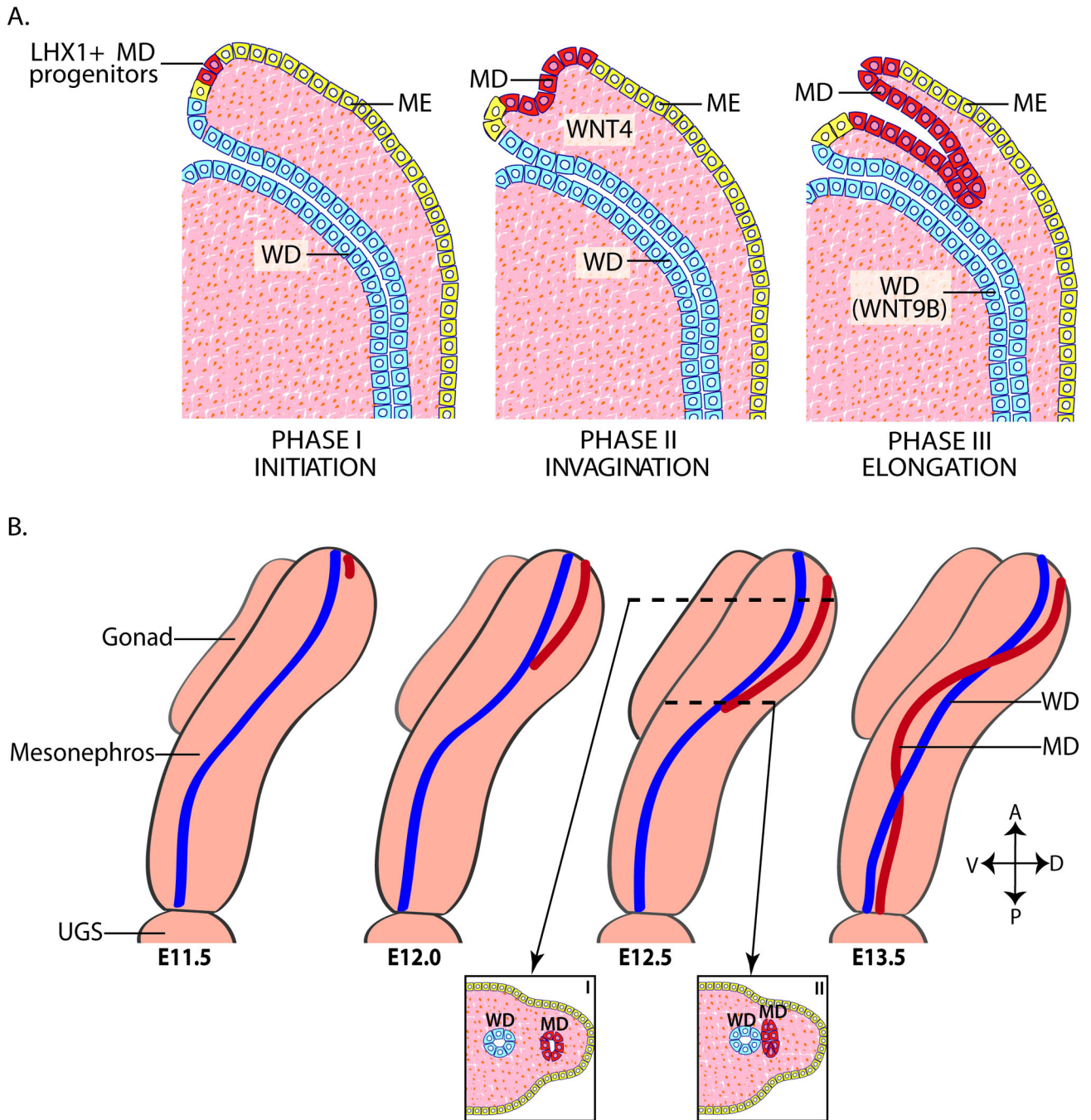
FIG. 2. Müllerian duct formation

(A) MD (represented in red) formation occurs in three phases: initiation, invagination, and elongation. Phase I (Initiation); MD progenitor cells in the mesonephric epithelium (represented in yellow) are specified and begin to express LHX1. Phase II (invagination): In response to Wnt4 signaling from the mesenchyme, LHX1+ MD progenitor cells invaginate caudally into the mesonephros towards the WD (represented in blue). Phase III (Elongation): The tip of the MD contacts the WD and elongates caudally in close proximity to the WD requiring structure and WNT9B signaling from the WD. (B) Beginning at ~ E11.5 in mice,

the MD invaginates and extends posteriorly guided by the WD. During elongation, mesenchymal cells separate the WD and MD anterior to the growing tip (I). However at the MD tip, the MD and WD are in contact (II). At ~E12.5, the MD crosses over the WD to be located medially. Elongation is complete by ~E13.5 with the MDs reaching the UGS. Adapted from (Kobayashi and Behringer, 2003). A, anterior (dorsal); E, embryonic day in mouse; D, dorsal; ME, mesonephric epithelium; MD, Müllerian duct; P, posterior (caudal); urogenital sinus, UGS; V, ventral; WD, Wolffian duct.
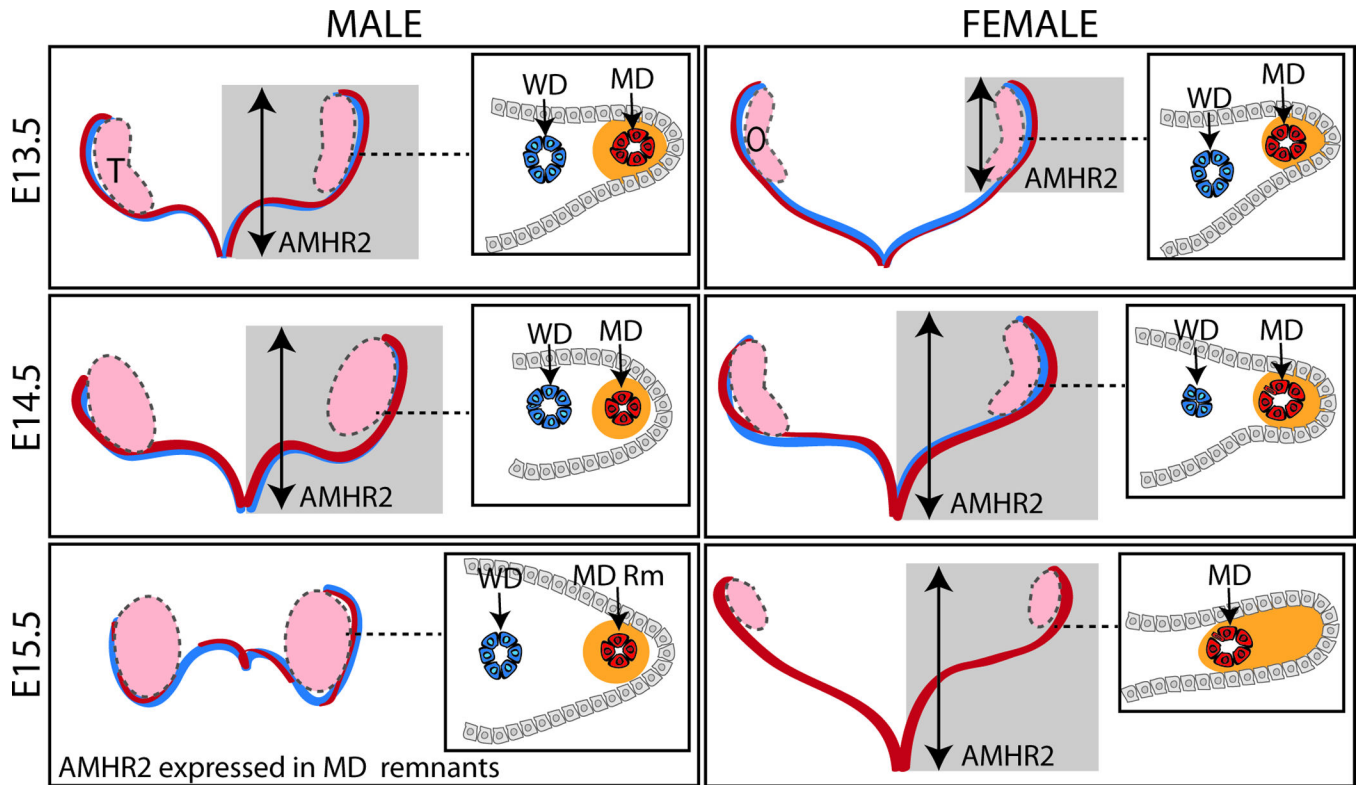
FIG. 3. Müllerian duct regression

At E13.5 in mice, male and female WD (represented in blue) and MD (represented in red) are fully formed. In males, AMHR2 is expressed throughout the length (represented by gray box and arrow) and in the MD mesenchyme (represented in orange) in a tight ring around the MD epithelium (cross-section inset). In females, AMHR2 expression is caudally expressed and found in the MD mesenchyme on the antimesometrial side of the MD epithelium. By E14.5, expression of AMHR2 is found along the length of the MD in both sexes. At E14.5 in females, the WD is beginning to degenerate and is absent by E15.5. Portions of the MD have regressed in males by E15.5 in an apparently random pattern and AMHR2 expression is limited to the remaining MD remnants. E, embryonic day in mouse; MD, Müllerian duct; O, ovary; Rm, remnant; T, testis; WD, Wolffian duct.
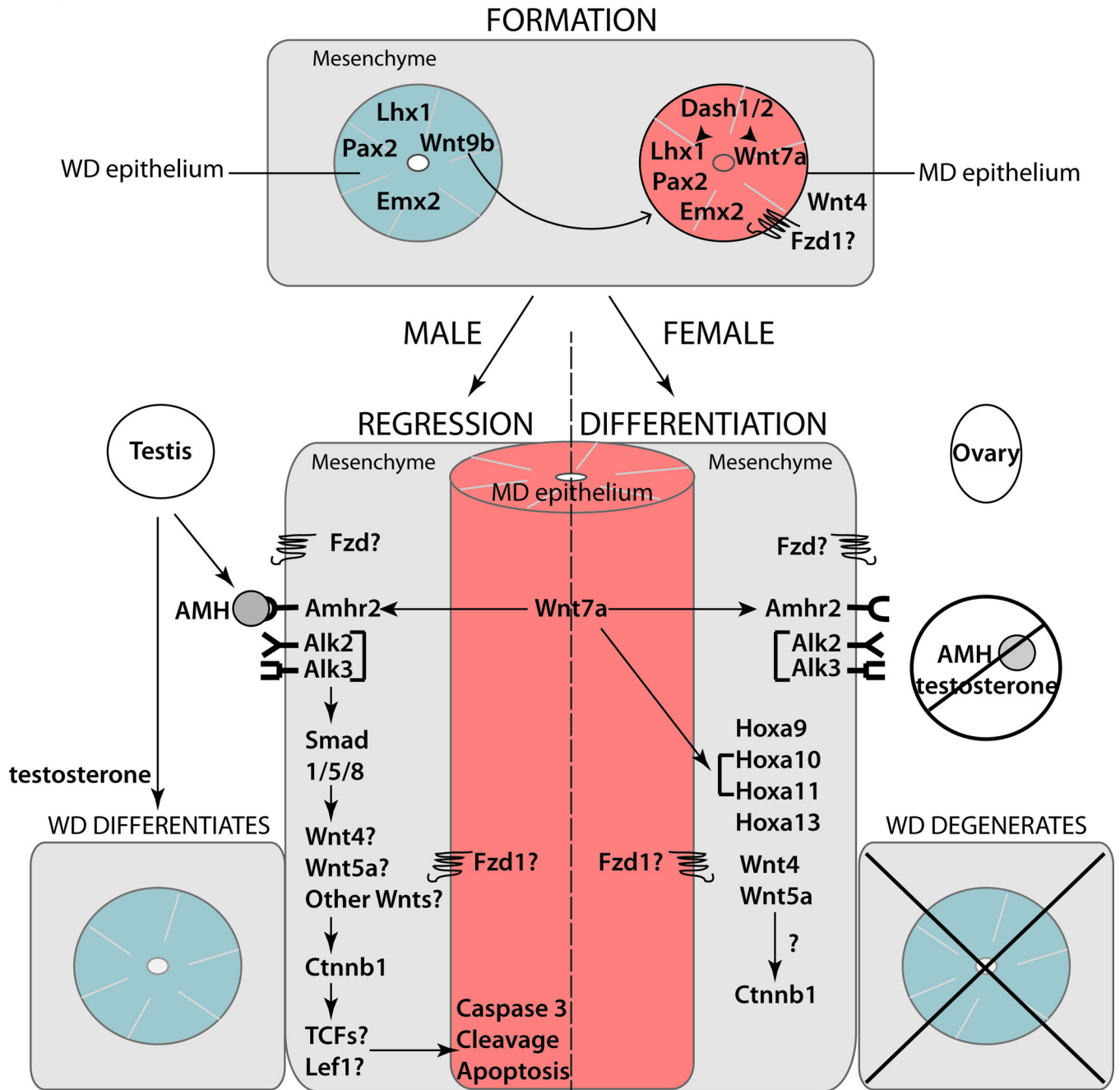
FIG. 4. Genes involved in Müllerian duct development

Multiple factors and signaling pathways in the mesenchyme (represented in gray) and epithelium of both ducts (WD epithelium represented in blue; MD epithelium represented in red) act in concert to direct MD development. Hormones and key genes required for MD formation, regression in males and differentiation in females are indicated in this figure. MD, Müllerian duct; WD, Wolffian duct.

TABLE 1

Mouse and human genes involved in Müllerian duct development

This table lists known genes involved in the development and differentiation of the Müllerian duct as demonstrated by mouse models and disease causing gene mutations in humans.

Gene	Molecule Encoded	Expression (mouse)	Reproductive Tract Phenotype (*References*)	
			Mouse	**Human**
Amh	TGFβ Superfamily secreted protein	Sertoli Cells	Ectopic FRT in males (N) (Behringer et al., 1994)	PMDS type I, Ectopic FRT in males (AR) (Belville et al., 1999; Belville et al., 2009; di Clemente and Belville, 2006; Salehi et al., 2012)
Amhr2	TGFβ Superfamily type II Ser/Thr Transmembrane receptor	MM	Ectopic FRT in males (N) (Mishina et al., 1996)	PMDS type I, Ectopic FRT in males (AR) (Belville et al., 1999; Belville et al., 2009; di Clemente and Belville, 2006; Salehi et al., 2012)
beta catenin (*Ctnnb1*)	Signaling Protein Adhesion	MM, ME WE, WM	Males - Ectopic FRT (C) (Kobayashi et al., 2011) Females- Hypotrophic uterine horns & defective oviduct coiling Myogenesis to adipogenesis switch (C) (Arango et al., 2005; Deutscher and Hung-Chang Yao, 2007; Jeong et al., 2009)	NA
Digh1	Scaffolding protein	WE	Cervix and vagina aplasia (Iizuka-Kogo et al., 2007)	NA
Emx2	Homeodomain Transcription Factor	ME, WE	No FRT (N) (Miyamoto et al., 1997)	NA
Hoxa10	Homeodomain Transcription Factor	MM, WM	Homeotic transformation of anterior uterus to oviduct (N) (Benson et al., 1996)	Defects in MD fusion (AD) (Cheng et al., 2011; Ekici et al., 2013)
Hoxa11	Homeodomain Transcription Factor	MM, WM	Partial homeotic transformation of uterus to oviduct (N) (Gendron et al., 1997)	NA
Hoxa13	Homeodomain Transcription Factor	MM, WM	Agenesis of the caudal MD (N) (Warot et al., 1997) Homeotic transformation of cervix to uterus (*Hd*-Dominant negative allele of *Hoxa13*) (Post et al., 2000) Homeotic transformation of anterior cervix to uterus (compound *Hoxa13$^{+/-}$; Hoxd13$^{-/-}$*) (Warot et al., 1997)	HFG syndrome (AD) (Goodman et al., 2000; Mortlock and Innis, 1997)
Lhx1	Homeodomain Transcription Factor	ME, WE	No FRT (N) (Kobayashi et al., 2004)	MRKH syndrome (AD) (Ledig et al., 2012)
Pax2	Homeodomain Transcription Factor	ME, WE	No FRT (N) (Torres et al., 1995)	NA
RAR α,β,γ	Retinoic acid receptors	NA	Compound mutants; varying degrees of MD defect (malformation -> absence) (Mendelsohn et al., 1994)	NA
Tcf2	Homeodomain Transcription Factor	NA	NA	MODY5 with vaginal aplasia and rudimentary uterus (AD) (Bingham et al., 2002; Lindner et al., 1999).
Wnt4	Wnt secreted protein	MM	No FRT (N) (Vainio et al., 1999)	MRKH syndrome (AD) (Biason-Lauber et al., 2007)

Gene	Molecule Encoded	Expression (mouse)	Reproductive Tract Phenotype (*References*)	
			Mouse	Human
			Stratified luminal epithelial layer and reduced uterine gland numbers (C) (Franco et al., 2011)	
Wnt5a	Wnt secreted protein	MM, ME (<E13.5) MM (>E13.5)	Posterior MD growth defects; absence of uterine glands (N) (Mericskay et al., 2004)	NA
Wnt7a	Wnt secreted protein	ME	Males - Ectopic FRT (N) (Parr and McMahon, 1998) Female - Homeotic transformation of oviduct to uterus and uterus to vagina no uterine glands abnormal mesenchyme differentiation (N) (Miller and Sassoon, 1998; Parr and McMahon, 1998)	NA
Wnt9B	Wnt secreted protein	WE	Absence of uterus and upper vagina (N) (Carroll et al., 2005)	NA
Wt1	Zinc Finger Transcription Factor	MM	NA	DDS, Ectopic FRT in males in some cases (Barakat et al., 1974; Denys et al., 1967; Manivel et al., 1987)

AD, autosomal dominant; AR, autosomal recessive; C, conditional; DDS, Denys-Drash syndrome; FRT, female reproductive tract; HFG, hand-foot-genital; ME, Müllerian epithelium; MM, Müllerian mesenchyme; MODY5, maturity-onset diabetes of the young type 5; MRKH, Mayer-Rokitansky-Küster-Hauser; N, null; NA, not available; PMDS, persistent Müllerian duct syndrome; WE, Wolffian epithelium; WM, Wolffian mesenchyme.