YALE JOURNAL OF BIOLOGY AND MEDICINE 94 (2021), pp.657-672.

REVIEW

YALE JOURNAL OF BIOLOGY AND MEDICINE
YJBM
CELEBRATING 90 YEARS 1928–2018

The Embryological Landscape of Mayer-Rokitansky-Kuster-Hauser Syndrome: Genetics and Environmental Factors

Isaac Kyei-Barffour[a], Miranda Margetts[b], Alla Vash-Margita[c,*], and Emanuele Pelosi[d,*]

[a]Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana; [b]Center for American Indian and Rural Health Equity, Montana State University, Bozeman, MT, USA; [c]Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Pediatric and Adolescent Gynecology, Yale University School of Medicine, New Haven, CT, USA; [d]Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a disorder caused by Müllerian ducts dysgenesis affecting 1 in 5000 women with a typical 46,XX karyotype. The etiology of MRKH syndrome is complex and largely unexplained. Familial clustering suggests a genetic component and the spectrum of clinical presentations seems consistent with an inheritance pattern characterized by incomplete penetrance and variable expressivity. Mutations of several candidate genes have been proposed as possible causes based on genetic analyses of human patients and animal models. In addition, studies of monozygotic twins with discordant phenotypes suggest a role for epigenetic changes following potential exposure to environmental compounds. The spectrum of clinical presentations is consistent with intricate disruptions of shared developmental pathways or signals during early organogenesis. However, the lack of functional validation and translational studies have limited our understanding of the molecular mechanisms involved in this condition. The clinical management of affected women, including early diagnosis, genetic testing of MRKH syndrome, and the implementation of counseling strategies, is significantly impeded by these knowledge gaps. Here, we illustrate the embryonic development of tissues and organs affected by MRKH syndrome, highlighting key pathways that could be involved in its pathogenesis. In addition, we will explore the genetics of this condition, as well as the potential role of environmental factors, and discuss their implications to clinical practice.

*To whom all correspondence should be addressed: Alla Vash-Margita, Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Pediatric and Adolescent Gynecology, Yale University School of Medicine, New Haven, CT; Email: alla.vash-margita@yale.edu. Emanuele Pelosi, Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia; Email: p.pelosi@uq.edu.au.

Abbreviations: MRKH, Mayer-Rokitansky-Küster-Hauser; MURCS, Müllerian duct aplasia, renal aplasia, cervicothoracic somite association; MA, Müllerian anomalies; IVF, *in vitro* fertilization; PGT-M, Preimplantation genetic testing, monogenic disorder; PGT-SR, Preimplantation genetic testing, chromosome structural rearrangement; BMP, bone morphogenetic proteins; FGF, fibroblast growth factor; PM, paraxial mesoderm; IM, intermediate mesoderm; LM, lateral mesoderm; PSM, presomitic mesoderm; WDs, Wolffian ducts; UB, ureteric bud; RA, retinoic acid.

Keywords: Müllerian ducts, Müllerian anomalies, MRKH syndrome, Disorders of Sex Development, Wolffian ducts, Genetics, Sex development

Author Contributions: IK-B (ORCID iD: 0000-0003-1890-9821): conception and design, writing, and graphical illustrations; MM: writing, review, and editing; AV-M: writing, review, and editing; EP (ORCID iD: 0000-0003-1890-9821): conception and design, writing, review, and editing.

INTRODUCTION

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome or Müllerian aplasia affects approximately 1 in 5000 women and is characterized by the incomplete development of the female reproductive tract, including uterus, cervix, and upper vagina [1-3]. Herein, we provide an overview of the factors that are known or have been suggested to be associated with MRKH syndrome with the aim to bridge clinical and basic science research. The condition is classified as a rare disease by the National Institutes of Health [4]. Women with MRKH syndrome have a 46,XX karyotype, and typical female development of external genitalia and secondary sexual characteristics. Despite the usually normal development and function of the ovaries, women with MRKH syndrome typically present with primary amenorrhea [5]. As a consequence, diagnosis often occurs around the time of puberty. MRKH syndrome is classified as type I (OMIM 277000) if the female reproductive tract is affected [6] and type II (OMIM 601076) if associated malformations are also present [7]. The most frequent malformations associated with MRKH syndrome are renal anomalies including unilateral agenesis, pelvic kidney, double kidney, and skeletal anomalies including scoliosis, hip dysplasia, and fused vertebrae [8]. Other malformations affect ears and eyes, and less frequently the heart [8]. A severe form of MRKH syndrome type II is Müllerian duct aplasia, renal aplasia, and cervicothoracic somite (MURCS) association, which is characterized by impaired Müllerian, renal, and cervicothoracic development [9]. Other clinical features of MRKH syndrome include shortened vagina, which may lead to dyspareunia if penetrative vaginal intercourse is attempted, cyclical abdominal or pelvic pain, and uterine factor infertility [2,3,10].

The genetics of MRKH syndrome is complex, and key mechanisms regulating reproductive tract development are still poorly understood [11]. Familial clustering indicates a genetic component in the pathogenesis of MRKH syndrome. However, discordant Müllerian anomalies (MA) phenotypes in monozygotic twins suggest a role for environmental factors [12]. Several genes have been suggested as candidates for MRKH syndrome. However, functional validation is still lacking, and the etiology of MRKH syndrome remains poorly defined. As with most rare conditions, the absence of data regarding etiology, heritability and associated malformations continue to pose challenges for patients with the diagnoses, their families, and their health care team.

Major challenges in clinical care of these patients involve addressing reproduction as well as the ability to have penetrative vaginal intercourse. Creation of a vaginal canal includes patient-controlled dilation and various surgical techniques of vaginoplasty that include Abbe-McIndoe procedure with use of dermal graft as well as modifications using amniotic membranes, inert materials, oral mucosa, and autologous *in vitro* grown vaginal tissue [2,13,14]. Another surgical approach for vaginoplasty is a laparoscopic Vecchietti procedure that has been successfully modified from the original approach via laparotomy and demonstrated comparable outcomes [15]. Advances in reproductive technologies have provided opportunities for biological children to women with MRKH syndrome through *in vitro* fertilization (IVF) of gestational carriers and uterine transplant. However, limited data exist on transgenerational inheritance patterns of the MRKH syndrome related to assisted reproduction, and these opportunities pose unanswered questions regarding genetic transmission of the condition to female biological offspring of women with MRKH syndrome. A recent systematic literature review reported on 125 women with MRKH syndrome undergoing 369 cycles of IVF with gestational surrogacy and delivering 71 newborns [16]. This review did not provide information on the genetic outcomes in the offspring [16]. Uterine transplant, albeit still experimental, gives women with MRKH syndrome an option to carry a pregnancy with a biological child [17]. Johannesson et al. (2021) reported on the success of 55% live birth rate per attempted transplant, and 79% live birth rate per technically successful transplant [18]. The authors described that all female neonates were born without congenital anomalies but did not specify whether uterine or renal anomalies were evaluated in seven female neonates [18]. That notwithstanding, increasing accessibility to fertility treatment options may lead to utilization of prenatal diagnostics such as preimplantation genetic testing for single gene / monogenic disorders (PGT-M) or PGT for chromosome structural rearrangements (PGT-SR) in the future for women with MRKH syndrome desiring to have biological children [19,20]. Limited evidence is available regarding the inheritance of urogenital anomalies in the biological children of women with MRKH syndrome who underwent surrogacy or uterine transplant. A survey of IVF programs performing surrogate procedures for women with congenital absence of the uterus and vagina failed to find genetic transmission of MRKH syndrome in 17 female children [21]. Nonetheless, clinicians caring for women with MRKH syndrome may have an obligation to inform their patients of factors affecting transmission of the condition as technology advances and options of having a biological child become more accessible. The impacts of environmental factors should be ascertained and considered as well.

In this review, we explore the embryogenesis of organ systems affected by MRKH syndrome and discuss the roles that candidate genes and environmental factors may play during their development.

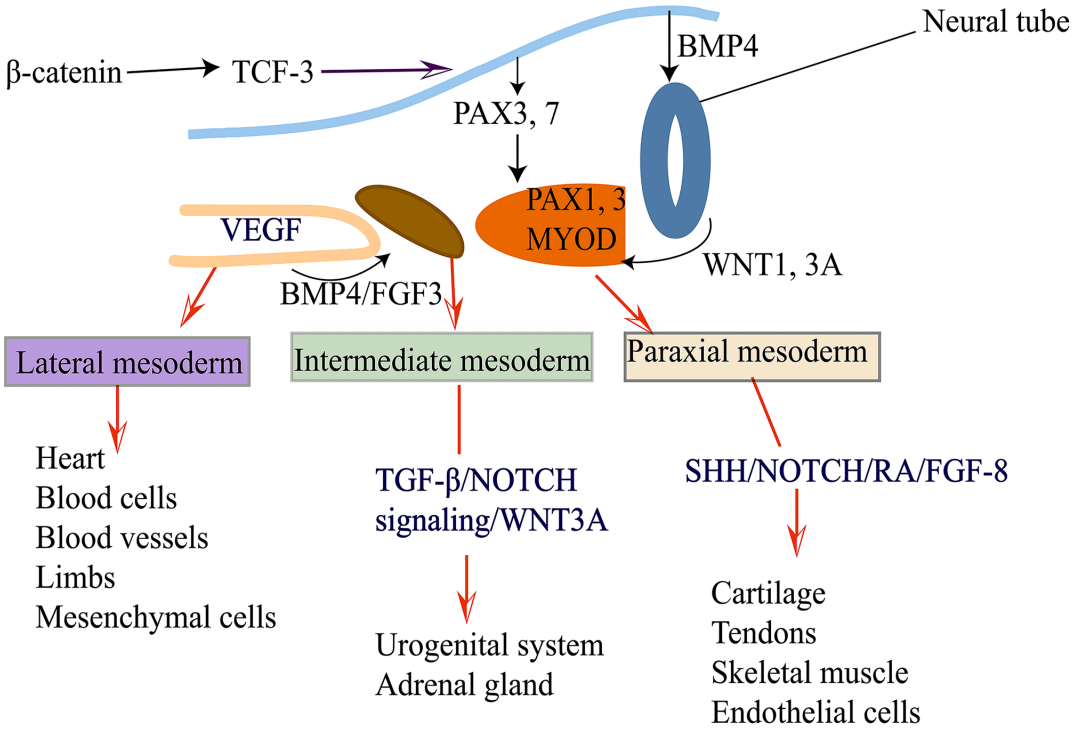

Figure 1. **Molecular regulation of mesodermal patterning**. β-catenin induces TCF-3, which regulates the expression of transcription factors and regulatory proteins including *Pax3*,*7*, *Bmp4*, and *Wnt1*,*3a*). These signals lead to the differentiation of the three main components, lateral mesoderm, intermediate mesoderm, and paraxial mesoderm. Expression of VEGF in the lateral mesoderm (LM) initiates progenitor cell specification for the development of the heart, blood vessels, limbs and mesenchymal cells. BMP4/FGF3 signaling from the LM stimulates TGF-β/Notch signaling, which activates *Wnt3a* expression in the intermediate mesoderm driving urogenital system and adrenal gland development. Expression of *Wnt1,3a* from the neural tube upregulates *Pax1,3* and *MyoD* in the paraxial mesoderm. *Pax1,3* and *MyoD* stimulate SHH/NOTCH and RA/FGF-8 signaling to differentiate the paraxial mesoderm into cartilage, tendons, skeletal muscles, and endothelial cells.

EARLY DEVELOPMENT OF MESODERMAL TISSUES

Tissues affected by MRKH syndrome share common embryonic origin and genetic programs. Proper development of the mesoderm is critical as the reproductive tract, kidneys, skeleton, and heart – the organs most commonly affected by MRKH – all originate from this germ layer. Additionally, early differentiation of these organs are mostly regulated by the same main pathways, including WNT [22], bone morphogenetic proteins (BMP) [23], and fibroblast growth factor (FGF) [24]. Therefore, perturbations disrupting early events during mesodermal development could involve multiple tissue primordia or include signaling factors that are necessary for the correct formation of more than one organ. Genetic variations involving these pathways need particular attention and could explain, at least partially, complex presentations like MRKH syndrome type II.

During gastrulation, activation of *Tcf-3* by β-catenin is a key event that activates specific pathways driving mesoderm differentiation into paraxial, intermediate, and lateral mesoderm [25] (Figure 1). The paraxial mesoderm (PM) gives rise to muscles and most of the skeleton. The entire urogenital system, including the reproductive tract and the kidneys, derives from the intermediate mesoderm (IM). Finally, the lateral mesoderm (LM) differentiates into the heart, vascular system, smooth muscles, and skeleton of the limbs [26]. Mesoderm differentiation is a complex process requiring the coordinated and balanced expression of several genes. In addition to WNT/β-catenin signaling, several other factors play fundamental roles in regulating the specification and development of the mesodermal germ layer into its main components. Low levels of BMP drive IM development and inhibit the expression of PM genes. Conversely, BMP is expressed at high levels in the LM, ensuring its development while repressing IM-specific gene expression [27]. It is believed that the gradient of BMP signaling causes the differential expression of specific *Fox* factors acting as effectors of mesodermal patterning [28]. LM is specified by *Foxf1* expression, whereas high and low levels of *Foxc1* and

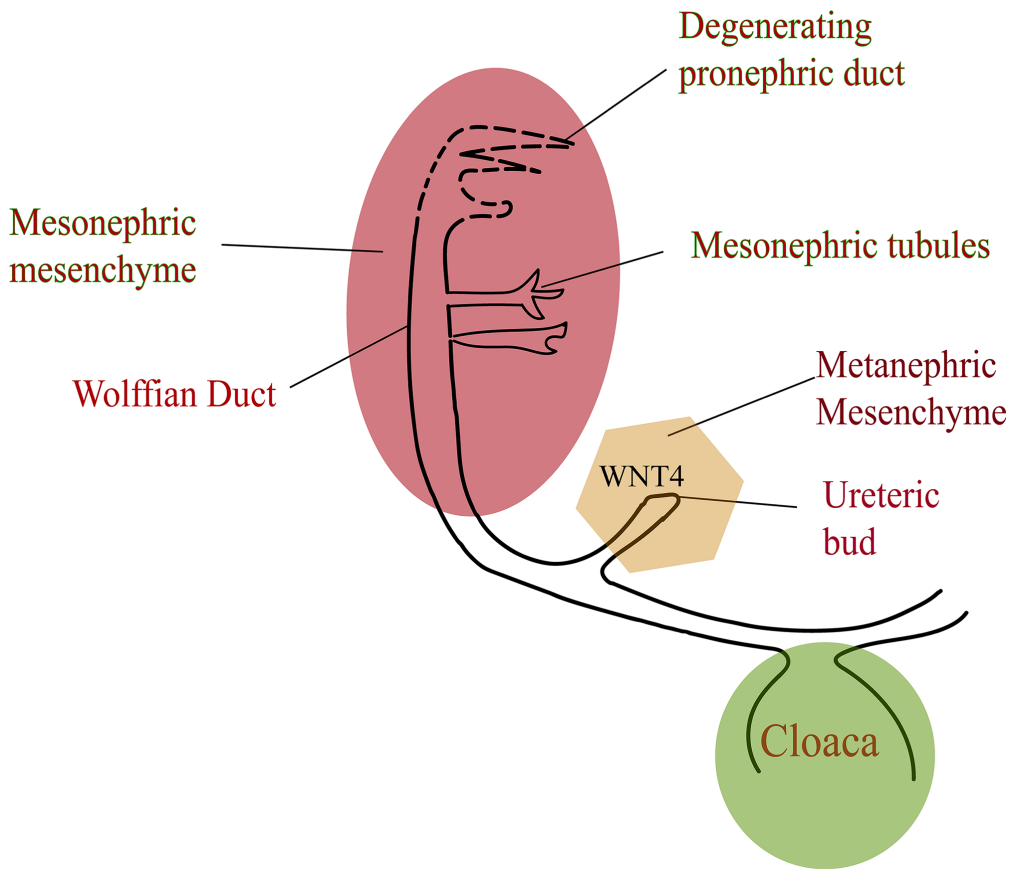

Figure 2. **Development of the embryonic kidneys**. The pronephric ducts are primordial ducts forming from the intermediate mesoderm. They extend caudally forming the Wolffian ducts, which invade the mesonephric mesenchyme and give origin to the mesonephric tubules. In some species, these tubules transiently assume excretory functions until the ureteric buds branch out into the metanephric mesenchyme and develop into the metanephros, or permanent kidneys.

Foxc2 determine the development of PM and IM, respectively. In addition, the FGF pathway is a critical regulator of embryonic segmentation in vertebrates [29]. FGF factors establish a posterior-to-anterior concentration gradient, which induces cell fate and provides positional information in the presomitic mesoderm (PSM) [30]. In the PM, FGF controls the maturation of paraxial cells into segmented tissue [31].

DEVELOPMENT OF THE RENAL SYSTEM

The first event during the development of the genitourinary tract is the formation of a ductal system forming the primordium of the future urinary system. This starts with the emergence of the pronephric duct at the end of the third week of gestation in humans and embryonic day 8 (E8.0) in the mouse. These ducts migrate caudally to form the Wolffian ducts (WDs) around gestational week 4 in humans and E8.5 in the mouse [32]. Development of the WD is a necessary event for the differentiation of the female reproductive tract as it will be discussed in the following section. The WDs form mesonephric tubules in the adjacent mesonephric mesenchyme. In several mammalian species, these tubules perform the functions of an embryonic kidney. In humans, this occurs only for a few weeks before the caudal portion of the WDs gives rise to the ureteric bud (UB), which invades the surrounding mesenchyme and forms the metanephros, the future permanent kidneys [33]. The WNT/β-catenin pathway plays a critical role in WD development and is necessary to maintain the WDs epithelium in a precursor state [34]. *Wnt4* is expressed in the metanephric mesenchyme and acts as an inducer of mesenchymal-to-epithelial transition required for kidney development [35] (Figure 2). Both deletion and overexpression of a stabilized form of β-catenin result in urogenital anomalies ranging from kidney hypoplasia to agenesis [36]. Downstream of WNT/β-catenin, a network of effector factors play critical roles in urogenital system development [7]. *Pax2* and *Pax8* expression ensure renal lineage specification and survival [37]. PAX2 induces the expression of critical transcription factors including *Lxh1*, which is required for

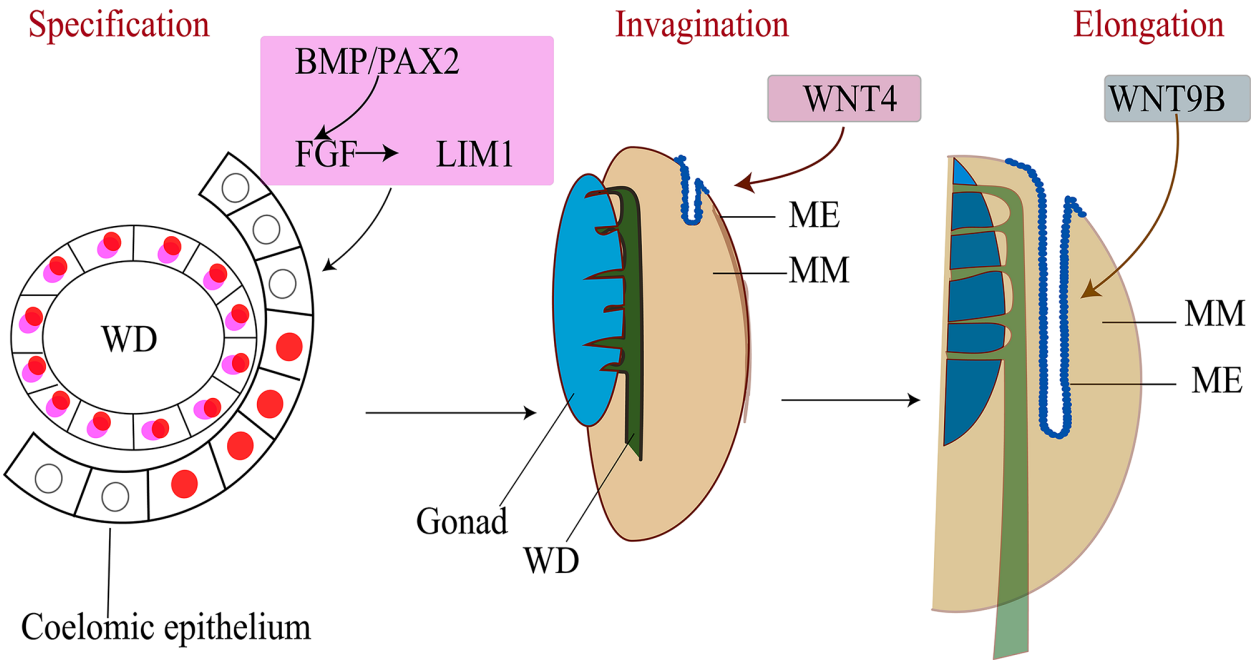

Figure 3. **Phases of early development of the Müllerian ducts**. During specification, BMP signaling stimulates the expression of *Pax2* in coelomic epithelial cells (precursors of MD epithelial cells, (red)). WD-derived inductive signaling stimulates fibroblast growth factor (FGF) signaling in the *Pax2*-positive cells to activate the expression of *LXH1* and commit their fate to Müllerian duct development. During invagination, *Pax2/LXH1* positive Müllerian epithelium (ME) invaginates into the mesonephric mesenchyme (MM) by WNT4 signaling from the MM. In the elongation phase, WD-derived WNT9B signaling guides posterior elongation of the nascent MD to the urogenital sinus.

WD elongation and the formation of tubular structures in the developing kidney [38] and *Emx2*, which regulates successful kidney morphogenesis [39]. Expression of *Eya1, Wt1*, and *Six2* is also critical for the maintenance of the ureteric bud [40]. In addition, GATA3 and the retinoic acid (RA) regulate ureter budding by inducing the expression of the *Ret* receptor, resulting in the fusion of the nephric ducts at the cloaca [41].

DEVELOPMENT OF THE FEMALE REPRODUCTIVE TRACT

Following WD development, the Mullerian ducts (MDs) form in a process characterized by three main but poorly understood phases [42] (Figure 3). The first is initiation and begins at E11.5 through activation of BMP signaling and the induction of *Pax2* and *Pax8* expression in the cranial coelomic epithelium adjacent to the WDs [43]. The BMP/PAX2 axis together with FGF signaling activates the expression of the transcription factor *Lxh1* in the coelomic epithelium stimulating the specification of MD epithelial cells [44]. In the second phase, invagination, LXH1-positive cells invade the mesonephric mesenchyme to form the nascent Müllerian duct [45]. Elongation is the third phase and is regulated by inductive factors coming from the WD including WNT4 and WNT9B [45]. Initiation and invagination of MDs seem to be independent of the WDs. However, the presence of the WDs is necessary for MD elongation [46]. Although it was initially believed that WDs donated cells to the MDs during development [47], it has been established that the WDs mainly act as a guide during this process [48]. By E13.5, the MD development is completed, and the two ducts meet at the urogenital sinus.

Further differentiation of WDs and MDs into sex-specific reproductive tracts depends on gonadal development (Figure 4). In the male, the *Sry* gene on the Y chromosome triggers a signaling cascade leading to the development of testes (reviewed by [49]). These produce testosterone, stimulating WD differentiation into the male reproductive tract, and anti-Müllerian hormone (AMH), which causes MD degeneration [50]. In the female, the absence of *Sry* results in the development of the ovaries by the action of specific genes including *Foxl2* and *Wnt4* [51]. The lack of testosterone and AMH causes regression of the WDs and further differentiation of the MDs into the female reproductive tract. The anterior regions of the MDs develop into the oviducts and the uterus, whereas the caudal portions fuse at the urogenital sinus to form the uterovaginal duct, giving rise to the cervix and the upper vagina [45].

The genetic program regulating the development of the female reproductive tract is still poorly characterized. The WNT pathway through the stabilization of β-catenin

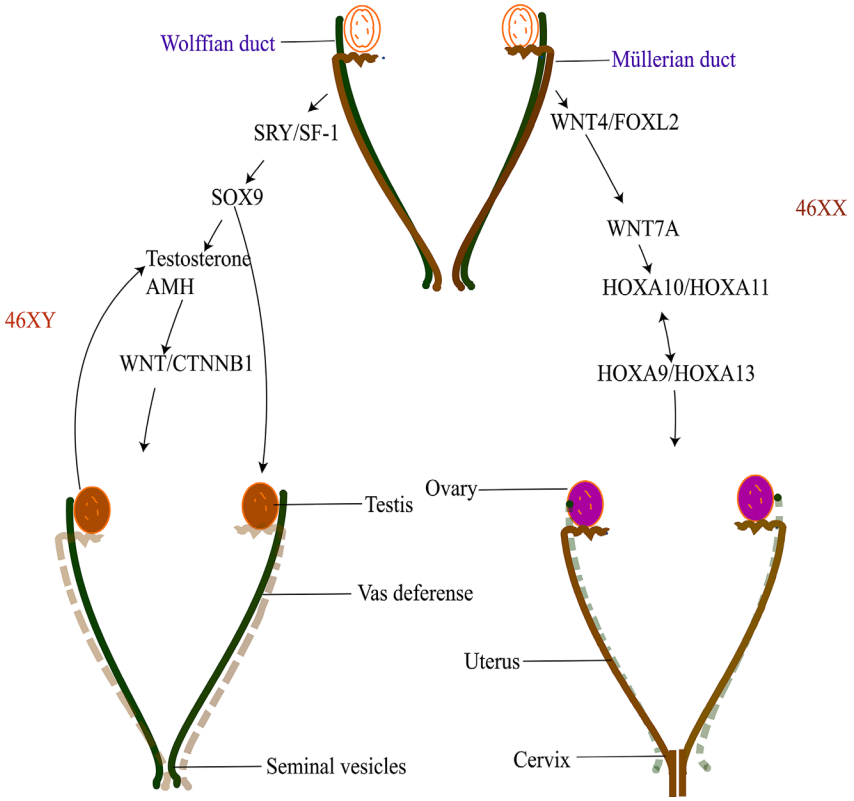

Figure 4. **Molecular mechanism of sex differentiation**. Before sex determination, embryos have undifferentiated, or bipotential gonads and both MDs and WDs. In the male, the Y-linked SRY protein interacts with steroidogenic factor-1 (SF-1) to increase the expression levels of *Sox9*. This drives the differentiation of Sertoli cells and Leydig cells within the testes. Sertoli cells produce anti-Müllerian hormone (AMH) to stimulate MD regression, whereas Leydig cells produce testosterone stabilizing WD development through signaling including WNT/β-catenin. In the female, expression of *Foxl2* inhibits the expression of *Sox9*. As the gonads develop into ovaries and male factors are not produced, the WDs degenerates and the MDs develop into the female reproductive tract under the action of several factors including WNT7A, and members of the HOX family.

plays a central role during the initial formation of both ducts as well as their development into definitive tracts [52]. The WNT/β-catenin pathway is expressed in both the epithelium and the mesenchyme of the MDs [53], and several members have been shown to be critical for MD development [54,55]. *Wnt4*-null female mice develop normal WDs, but they do not develop MDs, suggesting a specific role for MD initiation [54]. Conversely, ablation of *Wnt5* results in defective elongation of the developing MDs and lack of endometrial glands [56]. In addition, the incomplete demarcation between the vagina, uterus, and oviduct as well as lack of uterine glands and myometrial aberrations have been reported in *Wnt7a* knockout female mice [55]. Another group of factors critically important for the differentiation of the MDs is the family of *Hox* genes, which show a characteristic expression pattern along the female reproductive tract. *Hoxa9* is expressed in the oviduct, *Hoxa10* in the mesenchyme of the uterus, *Hoxa11* in the posterior uterus and cervix, and *Hoxa13* is the most caudal with expression in the cervix and upper vagina [57]. Ablation of either *Hoxa10* or *Hoxa11* leads to homeotic transformation of the uterus to oviducts, whereas knockout of *Hoxa13* results in agenesis of the caudal portion of the MD. In addition, $Hoxa13^{+/-}$;$Hoxd13^{-/-}$ compound mutation leads to homeotic transformation of the cervix into uterus [58].

DEVELOPMENT OF THE SKELETAL SYSTEM

Several pathways involved in the development of the urogenital system also regulate skeletogenesis. The PM undergoes a series of events leading to the conversion of a seemingly uniform population of mesenchymal cells into distinct clusters, or somites that will later differentiate into muscles, connective tissues, and bones [59]. Somitogenesis is a cell-autonomous process regulated by a network of finely synchronized factors. NOTCH, FGF, and WNT pathways induce an oscillating wave of signaling activity that triggers cells in the posterior end of

each presumptive somite to undergo mesenchymal-to-epithelial transition [60]. As a result, somite boundaries are established and somites bud off [61].

The fate of each developing somite is determined by its position along the anterior-posterior axis [62], a process mainly controlled by the Hox genes. Despite this rigid specification, cells within each somite retain a high degree of plasticity until late somitogenesis [63], and full commitment to a particular cell lineage is only achieved after segmentation when somites are surrounded by a layer of epithelial cells [64].

The differentiation of sclerotomes, the somites that will become skeletal tissue, is regulated by a network of interacting factors including WNT and BMP proteins, PAX1/9, RA, and HOX members [65,66]. Mesenchymal organization involved in intervertebral cartilage maturation is regulated by FOXL2 and SOX9 through epithelial-to-mesenchymal (EMT) transition [67]. In the lateral mesoderm, EMT processes induce *Wnt7a* and *Sox9* expression, which activate *Runx2* and drive mesenchymal condensation to form limb buds [68].

Finally, specification, migration, and differentiation of neural crest cells (NCC), which form cranial bones and cardiac structures, are also regulated by WNT, BMP, and FGF factors [69]. These proteins induce the expression of *Pax3/7*, *Dlx5*, and *Msx1/2*, which in turn fine tune *Wnt*, *Bmp*, and *Fgf* gene expression through a feedback mechanism [69].

PROPOSED ETIOLOGIES OF MRKH SYNDROME

MRKH syndrome is a complex and multifactorial condition, and the study of its etiology has been hindered by small cohort sizes, poor standardization, and lack of functional validation. Familial cases are usually explained by an autosomal dominant pattern of inheritance, characterized by incomplete penetrance and variable expressivity [70]. One issue limiting our understanding of the genetics of MRKH syndrome is the poor investigation of family members alongside affected women, limiting the power of genetic analysis. A second challenge is the possibility of mosaicisms [71], which could account for, at least partially, discrepancies between mouse and human variants in affecting MD development [72]. Thirdly, the candidate gene approach used so far provides limited information without functional genomic analysis, which is currently severely lacking [73]. Finally, monozygotic twins with discordant MRKH syndrome phenotypes suggest environmental contributions that may play a role, either alone, or in combination with genetic predisposition. However, research in this space is limited [74]. As a result, the etiology of MRKH syndrome remains unexplained. More advanced research strategies are required to improve timely and accurate diagnosis and optimize clinical management.

GENETIC ETIOLOGIES OF MRKH SYNDROME

Several candidate genes have been proposed as result of genetic analyses in women affected by MRKH syndrome or developmental studies in animal models [73]. Here we focus on a selected few whose fundamental role in the development of the urogenital tract has been established – mostly in mouse models or that have been recently identified representing potential promising candidates [7,55,75,76] (Table 1).

WNT Genes

Located on *1p36.12, WNT4 encodes* for a secreted protein regulating TCF-dependent signaling [77]. During embryonic development, WNT4 has important morphogenic roles regulating cell fate and patterning processes [78]. Loss-of-function mutations of *WNT4* are associated with 46,XX sex reversal, kidneys dysgenesis, and Müllerian aplasia [79]. In addition, variations within exon 1 of *WNT4* have been reported in MRKH syndrome [80]. However, some investigators have proposed *WNT4* deficiency as a presentation distinct from the classic MRKH syndrome due to the feature of hyperandrogenism [80].

An additional factor, *Wnt5a* is involved in several developmental processes through the activation or inhibition of WNT/β-catenin signaling pathways [81]. *Wnt5a* plays critical roles in the paraxial mesoderm during somitogenesis regulating proliferation and patterning [82]. During MD development, *Wnt5a* is necessary for posterior elongation of the developing reproductive tract and its ablation results in vaginal agenesis [56]. To date, no *WNT5A* mutations have been found in patients with MRKH syndrome [83]. However, specific deletion of *Wnt5a* in the MD mesenchyme caused partial Müllerian agenesis in a mouse model [84].

Wnt7a participates in several developmental processes mainly through the canonical WNT/β-catenin signaling pathway [85]. *Wnt7a* is specifically expressed in the epithelial cells of the MD and plays key roles during its development [86]. This factor is involved in the induction of cell polarity during the differentiation of the female reproductive tract and plays critical roles in uterine smooth muscle patterning and the maintenance of the uterine function [87]. However, a molecular analysis of 11 MRKH syndrome patients did not reveal pathogenetic variations of *WNT7A*, suggesting a lack of association [88]. Although the sample size in this study was small, mutations of *WNT7A* have not been reported in MRKH syndrome to date.

Further, *Wnt9b* is expressed in the inductive epithe-

Table 1. Genes Involved in the Development of the Female Reproductive Tract from Studies in Mouse Models, and Candidate Gene Variations Found in MRKH Syndrome

Gene	Murine FRT phenotype	Variants in MRKH	References
WNT4	Kidney dysgenesis, FRT agenesis	p.L12P;p.R83C	[80]
WNT5A	Vaginal agenesis, absence of uterine glands	-	[56,83]
WNT7A	Homeotic transformation of oviduct to uterus and uterus to vagina	-	[86,88]
WNT9B	FRT dysgenesis	p.Q326Ter	[75]
CTNNB1	Uterine hypoplasia	-	[91,92]
LRP10	-	dup 14q11.2;p.D419N	[73,94]
LHX1	Uterine hypoplasia	del 17q12	[10,95]
HNF1B	-	del 17q12;p.C1027T	[98,99]
HOXA10	Homeotic transformation of uterus to oviduct	p.Y57C	[101,103,109]
HOXA11	Partial homeotic transformation of uterus to oviduct	-	[107,110]
EMX2	Agenesis of kidneys and FRT	p.E142X	[111,113]
TBX6	-	del 16p11.2, c.621+1G>A [splice donor]	[75]
SHOX	-	dup PAR1 region containing SHOX; dup of CNE-2 enhancer	[121]
PRKX	-	dup Xp22.33	[122]
PAX8	Dysgenesis of FRT	del 2q12.1q14.1, p.V53AfsTer24 , c.25+1G>T [splice donor], p.Y66TfsTer10, p.R108Ter, p.S181F, p.V89A, p.Ser79Cys	[75,127]
GREB1L	Agenesis of kidney and FTR	p.Q743Rfs*10], p.C646R, p.V1324Lfs*34, p.E93K, p.W235C	[7,76,128]
DACH1/2	Agenesis of FRT	-	[129]
DOCK4		p.V770M; dup 7q31.1	[73,131]

FRT: female reproductive tract

lial primordia within the mesonephric and metanephric kidneys, and the Müllerian ducts [89]. Genetic analysis in animal models has shown that *Wnt9b* is required for the caudal extension of the MDs and that *Wnt9b*-/- mice lack reproductive ducts [89]. Of note, exome sequencing analysis conducted in 442 MRKH syndrome patients and 941 controls revealed loss-of-function of *Wnt9b* in three of the cases and five of the controls [75].

A fundamental role in the canonical WNT pathway is played by the catenin beta 1 (*Ctnnb1*) gene [90]. Upon stabilization by WNT signaling, CTNNB1 accumulates in the nucleus and acts as a coactivator with TCF/LEF proteins of downstream genes [91]. In the absence of WNT, CTNNB1 undergoes ubiquitination for proteasome degradation by a multiprotein destruction complex [92]. Due to its critical role in MD development, *CTNNB1* has been suggested as a candidate gene for MRKH syndrome, but causative mutations have yet to be identified [22,93].

Although not a member of the WNT genes, *Lrp10* is an important inhibitor of the canonical WNT/β-catenin pathway, and single nucleotide and copy number variants have been found in MRKH syndrome [73,94]. However, its specific role in MD development remains unclear.

Homeobox Genes

The *LHX1* gene is located in 17q12 and encodes for a transcription factor critical for the development of the urogenital systems [95]. In the mouse, MD-specific knockout of *Lhx1* causes disruption of MD development and consequent uterine hypoplasia [95]. Deletion of 17q12 is one of the most frequent chromosomal rearrangements in MRKH syndrome and rare point mutations have also been reported [10].

HNF1β is another member of the homeodomain-containing superfamily of transcription factors and together with *LXH1* is located in 17q12. During embryogenesis, it is involved in the development of several organs including the liver, the intestine, the kidney, and the repro-

ductive tract [96]. *Hhf1β* has critical functions for kidney development regulating cell polarity and patterning of the collecting ducts [97]. *HNF1β* expression is also required for renal tubule regeneration in acute kidney injury repair [98]. Mutations of *HNF1β* gene have been reported in congenital anomalies of the kidney and the urinary tract [99].

Several Hoxa genes play fundamental roles in the development of the female reproductive tract. Located in 7q15.2, *HOXA10* regulates morphogenesis, segmentation, and differentiation processes during development [100]. In mice, *Hoxa10* loss-of-function causes anteriorly directed homeotic transformations of the uterus [101]. In addition, *Hoxa10* is expressed in the uterus during the peri-implantation period and its mutation causes a reduction in fertility [102]. A heterozygous Y57C variation was found in a genetic study of women with Müllerian anomalies [103]. HOXA11 also regulates patterning and cell positional memory along the anterior-posterior axis ensuring proper organ morphogenesis [104]. In combination with HOXD11, HOXA11 controls branching processes during kidney development, and chondrocyte differentiation during skeletogenesis [105,106]. *Hoxa11* is expressed in the MD mesenchymal cells and regulates stromal cell proliferation [107]. *Hoxa11* null mice display a partial homeotic transformation characterized by a shorter uterus lacking glands [108]. In the adult uterus, *Hoxa11* is expressed in stromal cells regulating decidualization and glandular differentiation during pregnancy [107]. In humans, a missense mutation in *HOXA11* was found to be associated with septate uterus, but it is not clear if variations of this gene play a significant role in Müllerian anomalies [109,110].

An additional member of this family, *Emx2* is expressed in the epithelial components of WD, MD, ureteric buds, and also in the gonads before sex determination [39,111,112] Ablation of *Emx2* causes the degeneration of WDs shortly after their formation resulting in failure of the ureteric bud to invade the metanephric mesenchyme. Consequently, *Emx2* null mice lack reproductive tracts and gonads, and die perinatally due to kidney agenesis [39,111,112]. It has been found that *Emx2* is regulated by PAX2, and compound heterozygous mutations of both genes cause urinary tract anomalies [111,112]. A novel mutation of *EMX2* has been found associated with uterus didelphys, suggesting a potential role of the gene in regulating Müllerian fusion during uterine development [113].

Rearrangements involving 16p11.2 are among the most frequent chromosomal aberrations found in MRKH syndrome. This region includes *TBX6*, encoding a transcription factor with critical roles in controlling cell fate determination [114]. *Tbx6* is involved in the specification of paraxial mesoderm structures [115], and in the regulation of somitogenesis by mediating *Notch* and *Mesp2* signaling [116]. In addition, TBX6 is involved in the WNT/β-catenin pathway to regulate the expression of *Dll1* during presomitic mesoderm patterning [117]. CNVs of *TBX6* have been reported in several Müllerian anomalies including MRKH syndrome [118].

The homeobox gene *SHOX* is located in the pseudoautosomal region 1 (PAR1) of the X- (Xp22.33) and Y-chromosomes (Yp11.32) [119]. It is involved in sex and skeletal development and *SHOX* haploinsufficiency is associated with short stature in Turner syndrome [120]. *In vitro* transfection studies have suggested a potential role for SHOX, possibly following regulation by protein kinase X, a gene contained in a novel microduplication at Xp22.33 [121,122]. However, the contribution of *SHOX* variations to Müllerian anomalies is not clear and several studies have not found causative relationships [123]. Nonetheless, partial duplications of PAR1 containing *SHOX* were identified in 5 out of 30 women affected by MRKH syndrome, and a duplication of the CNE-2 enhancer was found in a patient in a cohort of 36 MRKH cases [121,124]. *PAX8* is located in 2q14.1 and is a member of the paired box (Pax) family of transcription factors. In human, PAX8 directly regulates *WT1* expression by binding to its promoter [125]. Alongside PAX2, PAX8 is involved in inducing the mesenchymal-epithelial transitions required for pronephric specification and nephric duct formation [37]. In addition, *PAX8* is expressed in normal and neoplastic Müllerian tissues, and has been proposed as an epithelial biomarker for Müllerian tumors [126]. Microdeletion of 2q12.1q14.1 involving *PAX8* has been found in two cases of MRKH syndrome associated with hypothyroidism, suggesting a possible role in MRKH syndrome especially in combination with thyroid dysfunction [127].

Additional Candidate Genes

The growth regulation by estrogen in breast cancer 1-like gene (*GREB1L*) is an androgen-regulated factor and a co-activator of the retinoic acid receptor (*RAR*). *GREB1L* has been reported as one of the most promising candidate genes of MRKH syndrome (reviewed by [7]). Due to its role in *RAR* activation, expression levels of *GREB1L* are very critical on renal system cellular differentiation, morphogenesis, and homeostasis in vertebrates [7]. Of note, variants of *GREB1L* have been reported in both sporadic and familial MRKH syndrome human patients [76] including a three-generation family of MRKH syndrome propositae [128]. In addition, variations of *GREB1L* have also been reported in isolated human cases of deafness and bilateral renal agenesis [7], which are comorbidities of MRKH type 2.

DACH2 is a transcription factor that functions redundantly with DACH1 during MD development.

Studies in the mouse suggested a critical role for MD development. Although ablation of *Dach2* alone does not cause malformations, double *Dach1/2* mutant mice show disruption in MD development [129]. This is likely due to the downregulation of key genes including *Lxh1* and *Wnt7a* [129]. The WD of these mutants form normally, suggesting a specific role in MD formation and differentiation. To date, however, no mutation of *DACH2* and/or *DACH1* has been identified in women affected by Müllerian anomalies.

Another gene that in recent years has been found associated with congenital anomalies of the female reproductive tract is *Dock4*. This membrane-associated protein participates in signal transduction by regulating small G proteins [130]. Its specific role in MD development has not been established but variations have been found in Müllerian anomalies including MRKH syndrome [73,131].

ENVIRONMENTAL ETIOLOGIES AFFECTING EMBRYONIC DEVELOPMENT

Environmental factors are believed to play a role in MRKH syndrome, likely through epigenetic modifications [132]. Normal MD development occurs in an environment free of estrogens, which are sequestered by α-fetoprotein (AFP) in rodents, and possibly by AFP peptides in humans [133]. Endocrine-disrupting chemicals (EDCs) are synthetic and naturally occurring compounds that interfere with the endocrine system signaling [133]. Hundreds of EDCs have been classified by the United States Environmental Protection Agency (EPA) as activators or blockers of estrogen and androgen receptors [134]. Due to the role of estrogens and androgens in gonadal and reproductive tract development, EDCs could have important embryological effects [133]. However, despite increasing potential concern, more data is required to understand the effect of EDCs exposure on reproductive development and function.

Diethylstilbestrol (DES)

Diethylstilbestrol (DES) is an estrogen agonist once prescribed to pregnant women to prevent miscarriage, premature labor, and other pregnancy-related complications. However, DES was later found to cause congenital anomalies in the fetus. Decades of research have shown that exposure to DES induce epigenetic modifications and result in reproductive malformations in both humans and mice [135]. The development of human fetal reproductive tracts implanted in BALB/C athymic nude mice was severely affected by administration of DES. In addition, stromal layering was inhibited in the upper tract, whereas the lower portion displayed highly glycogenated squamous epithelium [135]. In rats, fetal exposure to high doses of DES significantly reduced the uterine responsiveness to estrogen [136]. In a mouse model, *Hoxa10* was found to be repressed following administration of DES *in utero* [137]. Most importantly, a retrospective study found lower pregnancy rates, higher preterm deliveries, and higher spontaneous abortions in women exposed to DES *in utero* compared to women who were not exposed to DES [138].

Organotins

Organotins are compounds containing covalently bonded tin atoms. They are usually used in the production of pesticides and are considered to be biodegradable [139]. However, organotins have been also detected in seafood, raising concern of potential health risks [140]. In several marine species, organotins were shown to impair growth, disrupt embryonic development, and induce masculinization in females [140]. In the rat, organotins were shown to activate the retinoid X receptor (RXR), a critical factor in the RA signaling pathway regulating anteroposterior patterning and MD development. It has been suggested that these compounds may act as EDCs affecting pregnancy and uterine development [141].

Phthalate Esters

Phthalates are organic compounds mainly used as plasticizers and are among the most persistent organic pollutants in the environment [142]. In particular, both the EPA and the Chinese Environment Monitoring Centre raised health concerns over some of these compounds including the bis(2-ethylhexyl) phthalate (DEHP) [142]. In rats, fetal exposure to phthalates during the time of sex differentiation induced reproductive tract malformations similar to testicular dysgenesis in humans [143]. This phenomenon led to the term "phthalate syndrome" to describe phthalate-induced reproductive defects in rodent male offspring. In a rat model, *in utero* exposure to a mixture of phthalates containing butyl benzyl phthalate, dibutyl phthalate, diisobutyl phthalate, and DEHP has been found to induce the absence of vaginal opening and other uterine malformations similar to MRKH syndrome [144].

Methoxychlor

Methoxychlor (MXC) is an organochlorine pesticide that was used as replacement for dichlorodiphenyltrichloroethane (DDT) and is one of the most studied EDCs. Although MXC is banned for use in the United States, strict regulation in other countries is lacking [145]. While MXC itself has a low binding affinity to the estrogen receptor, its secondary metabolites (HPTE [2, 2-bis-(p-hydroxyphenyl)-1, 1, 1-trichloroethane] and mono-OH MXC) have greater estrogenic, estrogen inhibitory, and androgen inhibitory effects [146]. In the rat, MXC reduc-

es estrogen receptor β (ERβ) expression in adult females by epigenetic modification of CpG islands in the promoter region [147]. In addition, exposure to MXC interfered with the estrous cycle and reduced mating rates and litter sizes [148]. Despite evidence that MXC induces epigenetic modifications and affects fertility in animal models, epidemiologic data of human exposure is lacking. In a retrospective study, Bretveld et al. (2008) reported an increased risk of spontaneous abortion and time-to-pregnancy in greenhouse female workers, selected as being likely exposed to pesticides [149]. Although the study did not investigate the type of compounds involved, these results warrant further research on the effect of pesticides including MXC.

Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

PFOA and PFOS are fluorochemicals used for coating paper products in food packaging, fabrics, upholstery, and in the carpet industry [150]. They are also used as surfactant processing aids for the production of fluoropolymers [150]. PFOA and PFOS have estimated half-lives of 3.8 and 8.7 years respectively in humans. These compounds showed developmental toxicity in rodents including pregnancy loss, delayed growth, and postnatal death [150]. In rats and mice, *in utero* exposure to PFOA caused postnatal growth retardation and compromised survival in a dose-dependent manner [146]. In a two-generation study to assess the outcome of *in utero* administration of PFOS, Deanna et al. (2005) reported no adverse effects in F0 adults and their pups for doses below 0.4 mg/kg/day. However, the study reported a decreased gestational age, reduced implantation sites, and high number of stillborn pups or post-partum mortality at doses of 3.2 mg/kg/day [151]. To our knowledge, no Müllerian ducts-related birth defects have been linked to exposure to PFOA and PFOS. However, due to their role in disrupting embryonic development and reproductive functions, specific research efforts are needed to inform exposure effects in humans.

CONCLUSION

MRKH syndrome is considered a multifactorial condition caused by both genetic and environmental factors that may interact during embryonic development resulting in a spectrum of phenotypes and severities. To date, the majority of studies have been conducted on small cohorts, often without analyzing unaffected relatives. In addition, many knockout studies in laboratory animals have not been utilized for clinical translational purposes. As a result, the etiology of MRKH syndrome remains unexplained, and the identified candidate gene variants lack proper validation to demonstrate their role in disrupting urogenital development or differentiation. Understanding the complexity of the developmental programs that are often shared among organs affected by MRKH syndrome requires a multidisciplinary approach that includes: 1) genetic testing of patients and their family members; 2) analysis of exposure history; and, most importantly, 3) functional validation using animal models. Novel approaches including whole genome/exome sequencing and genome editing will be instrumental in defining the molecular factors regulating MD development, characterizing their roles, and ultimately advancing MRKH syndrome clinical diagnosis. Creation and utilization of rare diseases registries and multicenter collaborations will enable the capacity to conduct such studies on a large scale. Acquired knowledge of genetic and environmental factors of MRKH syndrome will allow clinicians to counsel affected women who are contemplating pregnancies on the risk of transmission of the condition to their female offspring. Closing this gap between bench and bedside should be the ultimate goal of the above research.

REFERENCES

1. Herlin M, Bjørn AM, Rasmussen M, Trolle B, Petersen MB. Prevalence and patient characteristics of Mayer-Rokitansky-Küster-Hauser syndrome: a nationwide registry-based study. Hum Reprod. 2016 Oct;31(10):2384–90.
2. Committee on Adolescent Health Care. ACOG Committee Opinion No. 728: Müllerian Agenesis: Diagnosis, Management, And Treatment. Obstet Gynecol. 2018 Jan;131(1):e35–42.
3. Herlin MK, Petersen MB, Brännström M. Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: a comprehensive update. Orphanet J Rare Dis. 2020 Aug;15(1):214.
4. Mullerian aplasia | Genetic and Rare Diseases Information Center (GARD) – an NCATS Program [Internet]. [cited 2021 Sep 25]. Available from: https://rarediseases.info.nih.gov/diseases/7100/mullerian-aplasia
5. Takahashi K, Hayano T, Sugimoto R, Kashiwagi H, Shinoda M, Nishijima Y, et al. Exome and copy number variation analyses of Mayer-Rokitansky-Küster- Hauser syndrome. Hum Genome Var. 2018 Sep;5(1):27–27.
6. Strübbe EH, Willemsen WN, Lemmens JA, Thijn CJ, Rolland R. Mayer-Rokitansky-Küster-Hauser syndrome: distinction between two forms based on excretory urographic, sonographic, and laparoscopic findings. AJR Am J Roentgenol. 1993 Feb;160(2):331–4.
7. Kyei Barffour I, Kyei Baah Kwarkoh R. GREB1L as a candidate gene of Mayer-Rokitansky-Küster-Hauser Syndrome. Eur J Med Genet. 2021 Mar;64(3):104158.
8. Rall K, Eisenbeis S, Henninger V, Henes M, Wallwiener D, Bonin M, et al. Typical and atypical associated findings in a group of 346 patients with Mayer-Rokitansky-Kuester-Hauser syndrome. J Pediatr Adolesc Gynecol. 2015 Oct;28(5):362–8.
9. Carranza-Lira S, Forbin K, Martinez-Chéquer JC. Rokitansky syndrome and MURCS association—clinical features and basis for diagnosis. Int J Fertil Womens Med. 1999

Sep-Oct;44(5):250–5.
10. Ledig S, Wieacker P. Clinical and genetic aspects of Mayer-Rokitansky-Küster-Hauser syndrome. Med Genetik. 2018;30(1):3–11.
11. Hentrich T, Koch A, Weber N, Kilzheimer A, Maia A, Burkhardt S, et al. The endometrial transcription landscape of MRKH syndrome. Front Cell Dev Biol. 2020 Sep;8:572281.
12. Duru UA, Laufer MR. Discordance in Mayer-von Rokitansky-Küster-Hauser Syndrome noted in monozygotic twins. J Pediatr Adolesc Gynecol. 2009 Aug;22(4):e73–5.
13. Panici PB, Ruscito I, Gasparri ML, Maffucci D, Marchese C, Bellati F. Vaginal reconstruction with the Abbè-McIndoe technique: from dermal grafts to autologous in vitro cultured vaginal tissue transplant. Semin Reprod Med. 2011 Jan;29(1):45–54.
14. Nodale C, Vescarelli E, D'Amici S, Maffucci D, Ceccarelli S, Monti M, et al. Characterization of human vaginal mucosa cells for autologous in vitro cultured vaginal tissue transplantation in patients with MRKH syndrome. BioMed Res Int. 2014;2014:201518.
15. Borruto F, Chasen ST, Chervenak FA, Fedele L. The Vecchietti procedure for surgical treatment of vaginal agenesis: comparison of laparoscopy and laparotomy. Int J Gynaecol Obstet. 1999 Feb;64(2):153–8.
16. Friedler S, Grin L, Liberti G, Saar-Ryss B, Rabinson Y, Meltzer S. The reproductive potential of patients with Mayer-Rokitansky-Küster-Hauser syndrome using gestational surrogacy: a systematic review. Reprod Biomed Online. 2016 Jan;32(1):54–61.
17. Brännström M, Diaz-Garcia C, Johannesson L, Dahm-Kähler P, Bokström H. Livebirth after uterus transplantation. Lancet. 2015;385:2352–3. https://doi.org/10.1016/S0140-6736(14)61728-1.
18. Johannesson L, Testa G, Putman JM, McKenna GJ, Koon EC, York JR, et al. Twelve live births after uterus transplantation in the Dallas UtErus Transplant Study. Obstet Gynecol. 2021 Feb;137(2):241–9.
19. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017 Sep;108(3):393–406.
20. Fesahat F, Montazeri F, Hoseini SM. Preimplantation genetic testing in assisted reproduction technology. J Gynecol Obstet Hum Reprod. 2020 May;49(5):101723.
21. Petrozza JC, Gray MR, Davis AJ, Reindollar RH. Congenital absence of the uterus and vagina is not commonly transmitted as a dominant genetic trait: outcomes of surrogate pregnancies. Fertil Steril. 1997 Feb;67(2):387–9.
22. Drummond JB, Rezende CF, Peixoto FC, Carvalho JS, Reis FM, De Marco L. Molecular analysis of the β-catenin gene in patients with the Mayer-Rokitansky-Küster-Hauser syndrome. J Assist Reprod Genet. 2008 Nov-Dec;25(11-12):511–4.
23. Visser JA. AMH signaling: from receptor to target gene. Mol Cell Endocrinol. 2003 Dec;211(1-2):65–73.
24. Colvin JS, Green RP, Schmahl J, Capel B, Ornitz DM. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. Cell. 2001 Mar;104(6):875–89.
25. Liu F, van den Broek O, Destrée O, Hoppler S. Distinct roles for Xenopus Tcf/Lef genes in mediating specific responses to Wnt/β-catenin signalling in mesoderm development. Development. 2005 Dec;132(24):5375–85.
26. Prummel KD, Nieuwenhuize S, Mosimann C. The lateral plate mesoderm. Development. 2020 Jun 19;147(12):dev175059. doi: 10.1242/dev.175059.
27. James RG, Schultheiss TM. Bmp signaling promotes intermediate mesoderm gene expression in a dose-dependent, cell-autonomous and translation-dependent manner. Dev Biol. 2005 Dec;288(1):113–25.
28. Wilm B, James RG, Schultheiss TM, Hogan BL. The forkhead genes, Foxc1 and Foxc2, regulate paraxial versus intermediate mesoderm cell fate. Dev Biol. 2004 Jul;271(1):176–89.
29. Wahl MB, Deng C, Lewandoski M, Pourquié O. FGF signaling acts upstream of the NOTCH and WNT signaling pathways to control segmentation clock oscillations in mouse somitogenesis. Development. 2007 Nov;134(22):4033–41.
30. Dubrulle J, McGrew MJ, Pourquié O. FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. Cell. 2001 Jul;106(2):219–32.
31. Moreno TA, Kintner C. Regulation of segmental patterning by retinoic acid signaling during Xenopus somitogenesis. Dev Cell. 2004 Feb;6(2):205–18.
32. Marose TD, Merkel CE, McMahon AP, Carroll TJ. β-catenin is necessary to keep cells of ureteric bud/Wolffian duct epithelium in a precursor state. Dev Biol. 2008 Feb;314(1):112–26.
33. Krause M, Rak-Raszewska A, Pietilä I, Quaggin SE, Vainio S. Signaling during kidney development. Cells. 2015 Apr;4(2):112–32.
34. Haegel H, Larue L, Ohsugi M, Fedorov L, Herrenknecht K, Kemler R. Lack of beta-catenin affects mouse development at gastrulation. Development. 1995 Nov;121(11):3529–37.
35. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature. 1994 Dec;372(6507):679–83.
36. Marose TD, Merkel CE, McMahon AP, Carroll TJ. Beta-catenin is necessary to keep cells of ureteric bud/Wolffian duct epithelium in a precursor state. Dev Biol. 2008 Feb 1;314(1):112–26.
37. Bouchard M, Souabni A, Mandler M, Neubüser A, Busslinger M. Nephric lineage specification by Pax2 and Pax8. Genes Dev. 2002 Nov;16(22):2958–70.
38. Kobayashi A, Shawlot W, Kania A, Behringer RR. Requirement of Lim1 for female reproductive tract development. Development. 2004 Feb;131(3):539–49.
39. Boualia SK, Gaitan Y, Murawski I, Nadon R, Gupta IR, Bouchard M. Vesicoureteral reflux and other urinary tract malformations in mice compound heterozygous for Pax2 and Emx2. PLoS One. 2011;6(6):e21529.
40. Donovan MJ, Natoli TA, Sainio K, Amstutz A, Jaenisch R, Sariola H, et al. Initial differentiation of the metanephric mesenchyme is independent of WT1 and the ureteric bud. Dev Genet. 1999;24(3-4):252–62.
41. Grote D, Boualia SK, Souabni A, Merkel C, Chi X, Costantini F, et al. Gata3 acts downstream of β-catenin

signaling to prevent ectopic metanephric kidney induction. PLoS Genet. 2008/12/30. 2008;4(12):e1000316.
42. Mullen RD, Behringer RR. Molecular genetics of Müllerian duct formation, regression and differentiation. Sex Dev. 2014;8(5):281–96.
43. Roly ZY, Backhouse B, Cutting A, Tan TY, Sinclair AH, Ayers KL, et al. The cell biology and molecular genetics of Müllerian duct development. Wiley Interdiscip Rev Dev Biol. 2018 May;7(3):e310.
44. Atsuta Y, Takahashi Y. Early formation of the Müllerian duct is regulated by sequential actions of BMP/Pax2 and FGF/Lim1 signaling. Development. 2016 Oct;143(19):3549–59.
45. Orvis GD, Behringer RR. Cellular mechanisms of Müllerian duct formation in the mouse. Dev Biol. 2007 Jun;306(2):493–504.
46. Kobayashi A, Behringer RR. Developmental genetics of the female reproductive tract in mammals. Nat Rev Genet. 2003 Dec;4(12):969–80.
47. Gruenwald P. The relation of the growing Müllerian duct to the Wolffian duct and its importance for the genesis of malformations. Anat Rec. 1941;81(1):1–19.
48. Dohr G, Tarmann T, Schiechl H. Different antigen expression on Wolffian and Müllerian cells in rat embryos as detected by monoclonal antibodies. Anat Embryol (Berl). 1987;176(2):239–42.
49. Kashimada K, Koopman P. Sry: the master switch in mammalian sex determination. Development. 2010 Dec;137(23):3921–30.
50. Welsh M, Sharpe RM, Walker M, Smith LB, Saunders PT. New insights into the role of androgens in wolffian duct stabilization in male and female rodents. Endocrinology. 2009 May;150(5):2472–80.
51. Ottolenghi C, Pelosi E, Tran J, Colombino M, Douglass E, Nedorezov T, et al. Loss of Wnt4 and Foxl2 leads to female-to-male sex reversal extending to germ cells. Hum Mol Genet. 2007 Dec;16(23):2795–804.
52. Tanwar PS, Kaneko-Tarui T, Zhang L, Rani P, Taketo MM, Teixeira J. Constitutive WNT/beta-catenin signaling in murine Sertoli cells disrupts their differentiation and ability to support spermatogenesis. Biol Reprod. 2010 Feb;82(2):422–32.
53. Deutscher E, Hung-Chang Yao H. Essential roles of mesenchyme-derived beta-catenin in mouse Müllerian duct morphogenesis. Dev Biol. 2007 Jul;307(2):227–36.
54. Vainio S, Heikkilä M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. Nature. 1999 Feb;397(6718):405–9.
55. Miller C, Sassoon DA. Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. Development. 1998 Aug;125(16):3201–11.
56. Mericskay M, Kitajewski J, Sassoon D. Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. Development. 2004 May;131(9):2061–72.
57. Taylor HS, Vanden Heuvel GB, Igarashi P. A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. Biol Reprod. 1997 Dec;57(6):1338–45.
58. Warot X, Fromental-Ramain C, Fraulob V, Chambon P, Dollé P. Gene dosage-dependent effects of the Hoxa-13 and Hoxd-13 mutations on morphogenesis of the terminal parts of the digestive and urogenital tracts. Development. 1997 Dec;124(23):4781–91.
59. Pourquié O. The segmentation clock: converting embryonic time into spatial pattern. Science. 2003 Jul 18;301(5631):328–30.
60. Gomez C, Özbudak EM, Wunderlich J, Baumann D, Lewis J, Pourquié O. Control of segment number in vertebrate embryos. Nature. 2008 Jul;454(7202):335–9.
61. Gilbert SF. Developmental biology. Sinauer Associates, Incorporated; 1997.
62. Fomenou MD, Scaal M, Stockdale FE, Christ B, Huang R. Cells of all somitic compartments are determined with respect to segmental identity. Dev Dyn. 2005 Aug;233(4):1386–93.
63. Miller S, Runner M. Tissue specificity for incorporation of thymidine by the 10- to 12-somite mouse embryo: alteration by acute exposure to hydroxyurea. Embryol exp Morph. 1978;44:181–9.
64. Tam PP, Trainor PA. Specification and segmentation of the paraxial mesoderm. Anat Embryol (Berl). 1994 Apr;189(4):275–305.
65. Peters H, Wilm B, Sakai N, Imai K, Maas R, Balling R. Pax1 and Pax9 synergistically regulate vertebral column development. Development. 1999 Dec;126(23):5399–408.
66. Herbrand H, Pabst O, Hill R, Arnold HH. Transcription factors Nkx3.1 and Nkx3.2 (Bapx1) play an overlapping role in sclerotomal development of the mouse. Mech Dev. 2002 Sep;117(1-2):217–24.
67. Marongiu M, Marcia L, Pelosi E, Lovicu M, Deiana M, Zhang Y, et al. FOXL2 modulates cartilage, skeletal development and IGF1-dependent growth in mice. BMC Dev Biol. 2015 Jul;15(1):27.
68. Akiyama H. Control of chondrogenesis by the transcription factor Sox9. Mod Rheumatol. 2008;18(3):213–9.
69. Sauka-Spengler T, Meulemans D, Jones M, Bronner-Fraser M. Ancient evolutionary origin of the neural crest gene regulatory network. Dev Cell. 2007 Sep;13(3):405–20.
70. Herlin M, Højland AT, Petersen MB. Familial occurrence of Mayer-Rokitansky-Küster-Hauser syndrome: a case report and review of the literature. Am J Med Genet A. 2014 Sep;164A(9):2276–86.
71. Rall K, Eisenbeis S, Barresi G, Rückner D, Walter M, Poths S, et al. Mayer-Rokitansky-Küster-Hauser syndrome discordance in monozygotic twins: matrix metalloproteinase 14, low-density lipoprotein receptor–related protein 10, extracellular matrix, and neoangiogenesis genes identified as candidate genes in a tissue-specific. Fertil Steril. 2014/12/11. 2015;103(2):494-502. e3.
72. Guerrier D, Mouchel T, Pasquier L, Pellerin I. The Mayer-Rokitansky-Küster-Hauser syndrome (congenital absence of uterus and vagina)—phenotypic manifestations and genetic approaches. J Negat Results Biomed. 2006 Jan;5(1):1–8.
73. Backhouse B, Hanna C, Robevska G, van den Bergen J, Pelosi E, Simons C, et al. Identification of candidate genes for Mayer-Rokitansky-Küster-Hauser syndrome using genomic approaches. Sex Dev. 2019;13(1):26–34.

74. Morcel K, Guerrier D, Watrin T, Pellerin I, Levêque J. [The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: clinical description and genetics]. J Gynecol Obstet Biol Reprod (Paris). 2008 Oct;37(6):539–46.
75. Chen N, Zhao S, Jolly A, et al. Perturbations of genes essential for Müllerian duct and Wölffian duct development in Mayer-Rokitansky-Küster-Hauser syndrome. Am J Hum Genet. 2021;108(2):337–345. doi:10.1016/j.ajhg.2020.12.014.
76. Jacquinet A, Boujemla B, Fasquelle C, Thiry J, Josse C, Lumaka A, et al. GREB1L variants in familial and sporadic hereditary urogenital adysplasia and Mayer-Rokitansky-Kuster-Hauser syndrome. Clin Genet. 2020 Aug;98(2):126–37.
77. Hugo Gene Nomenclature committee. Symbol report for Wnt4 [Internet]. 2020. Available from: https://www.genenames.org/data/gene-symbol-report/#!/hgnc_id/12783
78. Naylor RW, Jones EA. Notch activates Wnt-4 signalling to control medio-lateral patterning of the pronephros. Development. 2009 Nov;136(21):3585–95.
79. Mandel H, Shemer R, Borochowitz ZU, Okopnik M, Knopf C, Indelman M, et al. SERKAL syndrome: an autosomal-recessive disorder caused by a loss-of-function mutation in WNT4. Am J Hum Genet. 2008 Jan;82(1):39–47.
80. Philibert P, Biason-Lauber A, Rouzier R, Pienkowski C, Paris F, Konrad D, et al. Identification and functional analysis of a new WNT4 gene mutation among 28 adolescent girls with primary amenorrhea and müllerian duct abnormalities: a French collaborative study. J Clin Endocrinol Metab. 2008/01/10. 2008;93(3):895–900.
81. van Amerongen R, Fuerer C, Mizutani M, Nusse R. Wnt5a can both activate and repress Wnt/β-catenin signaling during mouse embryonic development. Dev Biol. 2012 Sep;369(1):101–14.
82. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development. 1999 Mar;126(6):1211–23.
83. Wu K, Chang X, Wei D, Xu C, Qin Y, Chen ZJ. Lack of association of WNT5A mutations with Müllerian duct abnormalities. Reprod Biomed Online. 2013 Feb;26(2):164–7.
84. St-Jean G, Boyer A, Zamberlam G, Godin P, Paquet M, Boerboom D. Targeted ablation of Wnt4 and Wnt5a in Müllerian duct mesenchyme impedes endometrial gland development and causes partial Müllerian agenesis. Biol Reprod. 2019 Jan;100(1):49–60.
85. Carmon KS, Loose DS. Secreted frizzled-related protein 4 regulates two Wnt7a signaling pathways and inhibits proliferation in endometrial cancer cells. Mol Cancer Res. 2008 Jun;6(6):1017–28.
86. Parr BA, McMahon AP. Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. Nature. 1998 Oct;395(6703):707–10.
87. Vandenberg AL, Sassoon DA. Non-canonical Wnt signaling regulates cell polarity in female reproductive tract development via van gogh-like 2. Development. 2009 May;136(9):1559–70.
88. Ravel C, Lorenço D, Dessolle L, Mandelbaum J, McElreavey K, Darai E, et al. Mutational analysis of the WNT gene family in women with Mayer-Rokitansky-Kuster-Hauser syndrome. Fertil Steril. 2009 Apr;91(4 Suppl):1604–7.
89. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell. 2005 Aug;9(2):283–92.
90. Preda V, Larkin SJ, Karavitaki N, Ansorge O, Grossman AB. The Wnt signalling cascade and the adherens junction complex in craniopharyngioma tumorigenesis. Endocr Pathol. 2015 Mar;26(1):1–8.
91. Ikeda T, Yoshinaga K, Semba S, Kondo E, Ohmori H, Horii A. Mutational analysis of the CTNNB1 (beta-catenin) gene in human endometrial cancer: frequent mutations at codon 34 that cause nuclear accumulation. Oncol Rep. 2000 Mar-Apr;7(2):323–6.
92. Herington JL, Bi J, Martin JD, Bany BM. β-catenin (CTNNB1) in the mouse uterus during decidualization and the potential role of two pathways in regulating its degradation. J Histochem Cytochem. 2007 Sep;55(9):963–74.
93. Deutscher E, Hung-Chang Yao H. Essential roles of mesenchyme-derived beta-catenin in mouse Müllerian duct morphogenesis. Dev Biol. 2007 Jul;307(2):227–36.
94. Rall K, Eisenbeis S, Barresi G, Rückner D, Walter M, Poths S, et al. Mayer-Rokitansky-Küster-Hauser syndrome discordance in monozygotic twins: matrix metalloproteinase 14, low-density lipoprotein receptor-related protein 10, extracellular matrix, and neoangiogenesis genes identified as candidate genes in a tissue-specific mosaicism. Fertil Steril. 2015 Feb;103(2):494–502.e3.
95. Huang CC, Orvis GD, Kwan KM, Behringer RR. Lhx1 is required in Müllerian duct epithelium for uterine development. Dev Biol. 2014 May;389(2):124–36.
96. Igarashi P, Shao X, McNally BT, Hiesberger T. Roles of HNF-1β in kidney development and congenital cystic diseases. Kidney Int. 2005 Nov;68(5):1944–7.
97. Desgrange A, Heliot C, Skovorodkin I, Akram SU, Heikkilä J, Ronkainen VP, et al. HNF1B controls epithelial organization and cell polarity during ureteric bud branching and collecting duct morphogenesis. Development. 2017 Dec;144(24):4704–19.
98. Ogata K, Shimamura Y, Hamada K, Hisa M, Bun M, Okada N, et al. Upregulation of HNF-1β during experimental acute kidney injury plays a crucial role in renal tubule regeneration. Am J Physiol Renal Physiol. 2012 Sep;303(5):F689–99.
99. Bascur PM, Ceballos OM, Farfán UM, Gajardo HI, López CJ. [Detection of mutations of the HNF1B gene in children with congenital anomalies of the kidney and urinary tract]. Rev Chil Pediatr. 2018;89(6):741–6.
100. Zanatta A, Rocha AM, Carvalho FM, Pereira RM, Taylor HS, Motta EL, et al. The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: a review. J Assist Reprod Genet. 2010 Dec;27(12):701–10.
101. Lalwani S, Wu HH, Reindollar RH, Gray MR. HOXA10 mutations in congenital absence of uterus and vagina. Fertil Steril. 2008 Feb;89(2):325–30.
102. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects

implantation. Gene Ther. 2000 Aug;7(16):1378–84.
103. Cheng Z, Zhu Y, Su D, Wang J, Cheng L, Chen B, et al. A novel mutation of HOXA10 in a Chinese woman with a Mullerian duct anomaly. Hum Reprod. 2011 Nov;26(11):3197–201.
104. Blanco MJ, Misof BY, Wagner GP, Blanco MJ, Misof BY, Wagner GP. Heterochronic differences of Hoxa-11 expression in Xenopus fore- and hind limb development: evidence for lower limb identity of the anuran ankle bones. Dev Genes Evol. 1998 Jun;208(4):175–87.
105. Patterson LT, Pembaur M, Potter SS. Hoxa11 and Hoxd11 regulate branching morphogenesis of the ureteric bud in the developing kidney. Development. 2001 Jun;128(11):2153–61.
106. Gross S, Krause Y, Wuelling M, Vortkamp A. Hoxa11 and Hoxd11 regulate chondrocyte differentiation upstream of Runx2 and Shox2 in mice. PLoS One. 2012;7(8):e43553.
107. Wong KH, Wintch HD, Capecchi MR. Hoxa11 regulates stromal cell death and proliferation during neonatal uterine development. Mol Endocrinol. 2004 Jan;18(1):184–93.
108. Wong KH, Wintch HD, Capecchi MR. Hoxa11 regulates stromal cell death and proliferation during neonatal uterine development. Mol Endocrinol. 2004 Jan;18(1):184–93.
109. Cheng Z, Zhu Y, Su D, Wang J, Cheng L, Chen B, et al. A novel mutation of HOXA10 in a Chinese woman with a Mullerian duct anomaly. Hum Reprod. 2011 Nov;26(11):3197–201.
110. Chen X, Li G, Qin Y, Cui Y, You L, Chen ZJ. Mutations in HOXA11 are not responsible for Müllerian duct anomalies in Chinese patients. Reprod Biomed Online. 2014 Jun;28(6):739–42.
111. Pellegrini M, Pantano S. Lucchini, Fumi M, Forabosco A. Emx2 developmental expression in the primordia of the reproductive and excretory systems. Reprod Biomed Online. 2014;196(6):739–42.
112. Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S. Defects of urogenital development in mice lacking Emx2. Development. 1997 May;124(9):1653–64.
113. Liu S, Gao X, Qin Y, Liu W, Huang T, Ma J, et al. Nonsense mutation of EMX2 is potential causative for uterus didelphysis: first molecular explanation for isolated incomplete müllerian fusion. Fertil Steril. 2015;103(3):769-774. e2. https://doi.org/10.1016/j.fertnstert.2014.11.030.
114. White PH, Farkas DR, McFadden EE, Chapman DL. Defective somite patterning in mouse embryos with reduced levels of Tbx6. Development. 2003 Apr;130(8):1681–90.
115. Pruitt KD, Tatusova T, Klimke W, Maglott DR. NCBI Reference Sequences: current status, policy and new initiatives. Nucleic Acids Res. 10/16. 2009;37(Database issue):D32–6. https://doi.org/10.1093/nar/gkn721.
116. Yasuhiko Y, Haraguchi S, Kitajima S, Takahashi Y, Kanno J, Saga Y. Tbx6-mediated Notch signaling controls somite-specific Mesp2 expression. Proc Natl Acad Sci USA. 2006 Mar;103(10):3651–6.
117. Hofmann M, Schuster-Gossler K, Watabe-Rudolph M, Aulehla A, Herrmann BG, Gossler A. WNT signaling, in synergy with T/TBX6, controls Notch signaling by regulating Dll1 expression in the presomitic mesoderm of mouse embryos. Genes Dev. 2004 Nov;18(22):2712–7.
118. Milsom SR, Ogilvie CM, Jefferies C, Cree L. Discordant Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome in identical twins - a case report and implications for reproduction in MRKH women. Gynecol Endocrinol. 2015;31(9):684–7.
119. Ogata T. SHOX: pseudoautosomal homeobox containing gene for short stature and dyschondrosteosis. Growth Horm IGF Res. 1999 Jun;9 Suppl B:53–7.
120. Clement-Jones M, Schiller S, Rao E, Blaschke RJ, Zuniga A, Zeller R, et al. The short stature homeobox gene SHOX is involved in skeletal abnormalities in Turner syndrome. Hum Mol Genet. 2000 Mar;9(5):695–702.
121. Pontecorvi P, Bernardini L, Capalbo A, Ceccarelli S, Megiorni F, Vescarelli E, et al. Protein-protein interaction network analysis applied to DNA copy number profiling suggests new perspectives on the aetiology of Mayer-Rokitansky-Küster-Hauser syndrome. Sci Rep. 2021 Jan;11(1):448.
122. Pontecorvi P, Megiorni F, Camero S, Ceccarelli S, Bernardini L, Capalbo A, et al. Altered Expression of Candidate Genes in Mayer-Rokitansky-Küster-Hauser Syndrome May Influence Vaginal Keratinocytes Biology: A Focus on Protein Kinase X. Biology (Basel). 2021 May;10(6):450.
123. Sandbacka M, Painter J, Puhakka M, Halttunen M, Laivuori H, Aittomäki K. Does the Y chromosome have a role in Müllerian aplasia? Fertil Steril. 2010 Jun;94(1):120–5.
124. Morcel K, Dallapiccola B, Pasquier L, Watrin T, Bernardini L, Guerrier D. Clinical utility gene card for: Mayer-Rokitansky-Küster-Hauser syndrome. Eur J Hum Genet. 2012 Feb;20(2):246.
125. Fraizer GC, Shimamura R, Zhang X, Saunders GF. PAX 8 regulates human WT1 transcription through a novel DNA binding site. J Biol Chem. 1997 Dec;272(49):30678–87.
126. Ozcan A, Liles N, Coffey D, Shen SS, Truong LD. PAX2 and PAX8 expression in primary and metastatic müllerian epithelial tumors: a comprehensive comparison. Am J Surg Pathol. 2011 Dec;35(12):1837–47.
127. Smol T, Ribero-Karrouz W, Edery P, Gorduza DB, Catteau-Jonard S, Manouvrier-Hanu S, et al. Mayer-Rokitansky-Künster-Hauser syndrome due to 2q12.1q14.1 deletion: PAX8 the causing gene? Eur J Med Genet. 2020 Apr;63(4):103812.
128. Herlin MK, Le VQ, Højland AT, Ernst A, Okkels H, Petersen AC, et al. Whole-exome sequencing identifies a GREB1L variant in a three-generation family with Müllerian and renal agenesis: a novel candidate gene in Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. A case report. Hum Reprod. 2019 Sep;34(9):1838–46.
129. Davis RJ, Harding M, Moayedi Y, Mardon G. Mouse Dach1 and Dach2 are redundantly required for Müllerian duct development. Genesis. 2008 Apr;46(4):205–13.
130. Yajnik V, Paulding C, Sordella R, McClatchey AI, Saito M, Wahrer DC, et al. DOCK4, a GTPase activator, is disrupted during tumorigenesis. Cell. 2003 Mar;112(5):673–84.
131. Ledig S, Tewes AC, Hucke J, Römer T, Kapczuk K, Schippert C, et al. Array-comparative genomic hybridization analysis in patients with Müllerian fusion anomalies. Clin Genet. 2018 Mar;93(3):640–6.
132. Steinkampf MP, Dharia SP, Dickerson RD. Monozygotic

twins discordant for vaginal agenesis and bilateral tibial longitudinal deficiency. Fertil Steril. 2003 Sep;80(3):643–5.
133. Hong H, Branham WS, Dial SL, Moland CL, Fang H, Shen J, et al. Rat α-Fetoprotein binding affinities of a large set of structurally diverse chemicals elucidated the relationships between structures and binding affinities. Chem Res Toxicol. 2012 Nov;25(11):2553–66.
134. Zota AR, Shamasunder B. The environmental injustice of beauty: framing chemical exposures from beauty products as a health disparities concern. Am J Obstet Gynecol. 2017 Oct;217(4):418.e1–6.
135. Robboy SJ, Taguchi O, Cunha GR. Normal development of the human female reproductive tract and alterations resulting from experimental exposure to diethylstilbestrol. Hum Pathol. 1982 Mar;13(3):190–8.
136. Alworth LC, Howdeshell KL, Ruhlen RL, Day JK, Lubahn DB, Huang TH, et al. Uterine responsiveness to estradiol and DNA methylation are altered by fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: effects of low versus high doses. Toxicol Appl Pharmacol. 2002 Aug;183(1):10–22.
137. Bromer JG, Wu J, Zhou Y, Taylor HS. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. Endocrinology. 2009 Jul;150(7):3376–82.
138. Kaufman RH, Adam E, Hatch EE, Noller K, Herbst AL, Palmer JR, et al. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. Obstet Gynecol. 2000 Oct;96(4):483–9.
139. Wang F, Ma J, Xie F, Li L, Chen J, Fan J, et al. Organic Cation-Dependent Degradation Mechanism of Organotin Halide Perovskites. Adv Funct Mater. 2016 May;26(20):3417–23.
140. Antizar-Ladislao B. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. a review. b_antizar@hotmail.com. Environ Int. 2008 Feb;34(2):292–308.
141. Ema M, Miyawaki E, Kawashima K. Suppression of uterine decidualization as a cause of implantation failure induced by triphenyltin chloride in rats. Arch Toxicol. 1999 Apr-May;73(3):175–9.
142. Gao DW, Wen ZD. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci Total Environ. 2016 Jan;541:986–1001.
143. Fisher J. Macpherson, Marchetti N, Sharpe R. Human "testicular dysgenesis syndrome": a possible model using in-utero exposure of the rat to dibutyl phthalate. Hum Reprod. 2003;18(17):7.
144. Hannas BR, Howdeshell KL, Furr J, Gray LE Jr. In utero phthalate effects in the female rat: a model for MRKH syndrome. Toxicol Lett. 2013 Dec;223(3):315–21.
145. Das Neves J, Barnhoorn IE, Wagenaar GM. The effects of environmentally relevant concentrations of aldrin and methoxychlor on the testes and sperm of male Clarias gariepinus (Burchell, 1822) after short-term exposure. Fish Physiol Biochem. 2018 Dec;44(6):1421–34.
146. Gaido KW, Leonard LS, Maness SC, Hall JM, McDonnell DP, Saville B, et al. Differential interaction of the methoxychlor metabolite 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane with estrogen receptors α and β. Endocrinology. 1999 Dec;140(12):5746–53.
147. Zama AM, Uzumcu M. Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. Endocrinology. 2009 Oct;150(10):4681–91.
148. Zama AM, Uzumcu M. Epigenetic effects of endocrine-disrupting chemicals on female reproduction: an ovarian perspective. Front Neuroendocrinol. 2010 Oct;31(4):420–39.
149. Bretveld RW, Hooiveld M, Zielhuis GA, Pellegrino A, van Rooij IA, Roeleveld N. Reproductive disorders among male and female greenhouse workers. Reprod Toxicol. 2008 Jan;25(1):107–14.
150. Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, et al. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006 Apr;90(2):510–8.
151. Luebker DJ, Case MT, York RG, Moore JA, Hansen KJ, Butenhoff JL. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology. 2005 Nov;215(1-2):126–48.