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

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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.
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 Ab-be-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.
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
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]. Downstream of WNT/β-catenin, a network of effector factors play critical roles in urogenital system development [7].

**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 Lhx1, which is required for...
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
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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 the urogenital system.
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 Mnx1/2, 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 mosaicsisms [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 morphogenetic 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-
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 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 LHX1 is located in 17q12. During embryogenesis, it is involved in the development of several organs including the liver, the intestine, the kidney, and the reproductive tract 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.

**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
ductive tract [96]. Hhf1B θ has critical functions for kidney development regulating cell polarity and patterning of the collecting ducts [97]. HNF1B expression is also required for renal tubule regeneration in acute kidney injury repair [98]. Mutations of HNF1B 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 deciduization 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 homebox 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 Lsh1 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-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 fluorocarboxylates 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.

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