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Genetics of Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: advancements and implications

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Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a congenital anomaly characterized by agenesis/aplasia of the uterus and upper part of the vagina in females with normal external genitalia and a normal female karyotype (46,XX). Patients typically present during adolescence with complaints of primary amenorrhea where the diagnosis is established with significant implications including absolute infertility. Most often cases appear isolated with no family history of MRKH syndrome or related anomalies. However, cumulative reports of familial recurrence suggest genetic factors to be involved. Early candidate gene studies had limited success in their search for genetic causes of MRKH syndrome. More recently, genomic investigations using chromosomal microarray and genome-wide sequencing have been successful in detecting promising genetic variants associated with MRKH syndrome, including 17q12 (*LHX1, HNF1B*) and 16p11.2 (*TBX6*) deletions and sequence variations in *GREB1L* and *PAX8*, pointing towards a heterogeneous etiology with various genes involved. With uterus transplantation as an emerging fertility treatment in MRKH syndrome and increasing evidence for genetic etiologies, the need for genetic counseling concerning the recurrence risk in offspring will likely increase. This review presents the advancements in MRKH syndrome genetics from early familial occurrences and candidate gene searches to current genomic studies. Moreover, the review provides suggestions for future genetic investigations and discusses potential implications for clinical practice.

KEYWORDS

DNA copy number variations, genetics, genitourinary development, infertility, Mayer-Rokitansky-Küster-Hauser syndrome, MRKH syndrome, MRKHS, Müllerian aplasia

1 Introduction

In mammals, the Müllerian (paramesonephric) ducts give rise to the female reproductive tract, which consists of the Fallopian tubes (oviducts), uterus, cervix, and upper two-thirds of the vagina (1). Abnormalities in Müllerian duct (MD) development in women may result in congenital uterovaginal anomalies of various severity (2, 3).

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, also referred to as Müllerian aplasia, is a congenital disorder characterized by agenesis or aplasia of the uterus and upper part of the vagina. The patients are characterized by having a normal female karyotype (46,XX), normal external genitalia, and normal pubertal development of secondary sex characteristics (thelarche and pubarche) (4). MRKH syndrome is typically diagnosed during late adolescence when patients present with primary amenorrhea (5). The estimated birth prevalence of MRKH syndrome is 1 in 5,000 female live births (5, 6) and it is considered the second most common cause of primary amenorrhea (7). MRKH syndrome may present as an isolated anomaly (type I) or in association with extragenital malformations (type II), typically involving the kidneys, skeleton, and heart (4). Upon the diagnosis of MRKH syndrome, patients face life-impacting consequences related to their sexual identity, fear of coital difficulties, and grief of infertility (8–12), and it has been associated with a risk of depressive and anxiety symptoms (13–15). Psychological support and counseling are therefore crucial in patient care (16–18). Non-surgical and surgical treatments of vaginal hypoplasia are available to enable penetrative intercourse with non-surgical dilation considered the first-line choice (18–20). MRKH syndrome causes absolute uterine factor infertility (AUFI) but patients may achieve genetic motherhood through gestational surrogacy (21, 22). In 2014, the first baby was born following pregnancy after uterus transplantation (UTx) in Sweden, offering the first fertility treatment of AUFI achieving both gestational and genetic motherhood (23, 24).

The etiology of MRKH syndrome has long been an unanswered question in medical research, and both genetic and non-genetic factors have been considered. Despite substantial efforts to find explanations for the disorder, our current understanding of the underlying biology remains limited. However, discoveries during recent years do provide evidence for the importance of genetic factors and point towards a heterogeneous etiology with various genes involved in uterovaginal development in humans. This review presents the continuous advancements in our knowledge of genetics in MRKH syndrome, from early candidate gene studies to genome-wide gene discoveries, and provides recommendations for further progress and perspectives on potential clinical implications.

2 Embryology of the female reproductive tract

The human genitourinary system (including the kidneys, gonads, and reproductive tracts) develops from the intermediate mesoderm. At 3 weeks post gestation, the Wolffian (mesonephric) ducts, which develop into the male reproductive tracts, form and grow caudally from the primordial kidney (mesonephros) to reach the cloaca. At 5 weeks post gestation, bilateral invaginations of the coelomic epithelium of the urogenital ridges begin to form the MDs which extend caudally, guided by the Wolffian ducts, to reach the urogenital sinus in the midline (Müllerian tubercle). Here, the caudal parts of the two MDs start to fuse to form the uterus and upper vagina starting from week 8. The cranial openings of the invaginations remain and give rise to the fimbrial ends of the oviducts adjacent to the developing ovary (1, 25, 26).

The close relationship between kidney and uterovaginal development is also reflected by the high prevalence (~30%) of kidney malformations in MRKH syndrome (5, 27). Other common extragenital anomalies include the skeleton and heart, which do also develop from the mesoderm, with the paraxial mesoderm forming the axial skeleton (28) and the lateral plate mesoderm forming the heart and appendicular skeleton (29). Therefore, genes involved in the development of mesoderm and its derived structures are relevant candidates in the etiology of MRKH syndrome.

3 Evidence for genetic etiologies in MRKH syndrome

Most cases of MRKH syndrome appear isolated with no clear indications of a familial/genetic trait (5, 30). In addition, several reports of discordant monozygotic twin pairs (5, 31–35) and patient-reported outcomes of most surrogate pregnancies also support non-Mendelian causes (36). However, it is important to consider that the disease nature of MRKH syndrome implies absolute infertility, hindering mother-to-offspring inheritance of a genetic cause, which may cause an underestimation of the genetic component of MRKH syndrome from family histories. At this point of knowledge, a single mode of inheritance to cover all cases cannot be determined and monogenic, oligogenic, polygenic, multifactorial, and environmental factors should still be considered. Hypotheses on fetal exposures disturbing uterovaginal development have included thalidomide, diethylstilbestrol, organotins, and phthalates (37–40), but there is no firm evidence for a pathological role in MRKH syndrome.

In 1973, Buchta et al. (41) reported on familial occurrences of various renal malformations following autosomal dominant inheritance and coined the term "*hereditary renal adysplasia*". One of the patients, who had eight children, was found to have left renal agenesis and a bicorn uterus during surgery for cervical carcinoma *in situ*. Interestingly, 14 years later, John M. Opitz (senior author of the first paper) reported that one daughter of this patient had been diagnosed with MRKH syndrome emphasizing the close link between uterovaginal malformations and renal malformations (42). This association was also described by Schimke & King, who instead proposed the term "*hereditary urogenital adysplasia*" (43). This pattern of both renal and MD anomalies within families, characterized by incomplete penetrance and variable sex-limited expressivity, has been reported several times in the literature (reviewed by Herlin et al. (44)). Taken together this emphasizes the importance of a detailed family history when investigating the genetics of MRKH syndrome.

Recently, the first case of mother-daughter inheritance of MRKH syndrome following gestational surrogacy was reported, when the daughter presented at 14 years old with primary amenorrhea and was diagnosed with type I MRKH syndrome as her mother. A 4 Mb deletion of 2q37.1q37.3 of unknown significance was identified in both patients, but not in the mother's parents (22). This case exemplifies the potential risk of MRKH syndrome recurrence following assisted reproduction and

with the expected increasing availability of UTx as this field moves towards clinical care (24), identifying causal variants and providing genetic counseling regarding recurrence risk and reproductive choices will become more relevant.

4 Genetic research in MRKH syndrome

One of the earliest reports describing genetic analysis in the diagnosis of MRKH syndrome is from Georges Andre Hauser in 1961. Hauser and colleagues described that sex-chromatin analysis could aid the differentiation of MRKH syndrome from Turner syndrome and defined MRKH syndrome (at the time termed 'Mayer-Rokitansky-Küster syndrome') to include normal female chromosomes (45, 46). Together with earlier anatomical descriptions of Mayer, Rokitansky, and Küster, his work led to the complete definition and its final name (4).

For over 25 years, researchers have searched for genetic alterations to cause uterovaginal agenesis in karyotypically normal women. Early studies investigated a wide range of candidate genes including *GALT* (47–49), *WT1* (50), *HNF1B* (51, 52), *AMH* (49, 53), *AMHR2* (49, 53), *CFTR* (54), *WNT7A* (55), *HOXA7-13* genes (56–58), *PBX1* (56, 59), *RARG* (60), *RXRA* (60), *CTNNB1* (61), SHOX (62–64), *PAX2* (65), *LAMC1* (66), *DLG1* (66), or *ITIH5* (67). Most of these studies had negative results and provided limited evidence for genetic factors in MRKH syndrome. This included investigations of *AMH* and *AMHR2*, encoding anti-Müllerian hormone and its receptor, respectively, involved in physiological MD regression in males (49, 53). One gene, *Wnt4*, was reported to be involved in female development in mammals with mutant female mice displaying signs of masculinization and absent Müllerian ducts, hence a relevant candidate gene in MRKH syndrome. In 2004, Biason-Lauber et al. identified a *WNT4* variant in an 18-year-old woman with Müllerian agenesis, renal agenesis, and clinical signs of hyperandrogenism. Functional analyses supported the variant pathogenicity and *WNT4* was thereby the first identified gene in uterovaginal agenesis with firm evidence for monogenic causality (discussed further below).

The completion of the Human Genome Project in 2003 (68, 69) together with the concomitant development of genomic technologies including chromosomal microarray (CMA) and massively parallel sequencing (also referred to as Next-Generation Sequencing [NGS]) have allowed for genome-wide ("hypothesis-free") searches of genetic variation in MRKH syndrome. CMA, including comparative genomic hybridization array and single nucleotide polymorphism (SNP) array, is used to detect copy number variations (CNVs) in the genome. These applications have identified various chromosomal imbalances (deletions/duplications) supporting the identification of candidate genes such as *HNF1B*, *LHX1*, and *TBX6* (Table 1) (30, 71, 72, 81, 82). However, recurrent chromosomal imbalances in MRKH syndrome still only apply to a minor fraction of patients (around 10%). Optical genome mapping is a newer cytogenomic method with higher resolution for the detection of both imbalanced and balanced structural variation and has recently been applied in a study by Brakta et al. (73). In more recent years, whole-exome and whole-genome sequencing analyses have been applied for genome-wide detection of genetic variation at the nucleotide level (74, 75, 96–105). Investigations have included both extended familial cases and larger patient cohorts identifying variants of interesting genes, most notably *GREB1L* (99, 101, 102) and *PAX8* (75).

The vast majority of studies to date have looked into germline genetic variation by analyzing DNA from blood samples. Due to the predominantly sporadic nature of MRKH syndrome and reports of discordant monozygotic twin pairs suggesting non-inherited genetic variation, researchers have also searched for somatic/mosaic gene variation and tissue-specific differential gene methylation/expression patterns in uterine remnants to explain the disorder (35, 98, 106–108).

5 Genetic findings in MRKH syndrome and evidence for causality

In this section, the most significant genetic findings to date and the current evidence for causality in MRKH syndrome are discussed. Table 1 summarizes the recurrent (reported in at least

TABLE 1 Recurrent[a] copy number variations associated with MRKH syndrome.

Locus	Imbalance	CNVs reported	Size range	Region of overlap[b]	Candidate genes	MRKH type	Ref.
16p11.2	Deletion	24	0.5-1.0 Mb	Chr16:29,638,676-30,188,531 (BP4-BP5) (70)	*TBX6*	Type I+II	(30, 71–79)
17q12	Deletion	21	1.4-1.9 Mb	Chr17:36,458,167-37,854,616 (80)	*HNF1B, LHX1*	Type I+II	(30, 71–73, 75, 76, 81–86)
22q11.2	Deletion	7	0.4-5.2 Mb	Various regions involved	Uncertain	Type I+II	(60, 71, 81, 85, 87–90)
	Duplication	4	0.6-3.5 Mb	Various regions involved	Uncertain	Type I	(81, 85, 91, 92)
1q21.1	Deletion	4	0.4-4.6 Mb	Chr1:145,779,056-146,019,795 (82)	*RBM8A*	Type I+II	(30, 82, 85, 93)
2q13q14.1	Deletion	2	9.8-10.8 Mb	Chr2:110,791,355-115,043,578	*PAX8*	Type II	(94, 95)

[a]Two or more reported CNVs. Non-recurrent candidate variants are listed in Supplementary Table S1.
[b]Coordinates in the GRCh38/hg38 reference human genome assembly.
BP, breakpoint; Chr, chromosome; CNV, copy number variation; Mb, megabase.

two cases) copy number variations in MRKH syndrome, whereas Table 2 lists the genes recurrently reported with sequence variants. Non-recurrent (only one case) candidate variants are listed in Supplementary Table S1.

5.1 17q12 deletions, *LHX1*, and *HNF1B*

Deletion of 17q12 was first reported in MRKH syndrome by Cheroki et al. in 2008 (81) and to this date, 21 deletions have been reported (Table 1) (30, 71–73, 75, 76, 81–86). Ledig et al. reported a 17q12 deletion in one MRKH syndrome patient (85), whose mother and sister were later reported with the same deletion and other uterovaginal anomalies (124). Deletions of 17q12 are typically 1.4 Mb in size. They are highly penetrant with variable expressivity and cause a multisystem disorder which may include kidney disease, neurocognitive impairment, endocrinological disease including *HNF1B*-related maturity-onset diabetes of the young (MODY), genital malformations, liver disease, and other manifestations (80). Two candidate genes for MRKH syndrome, *LHX1* and *HNF1B*, are located at this locus, both of them being involved in MD development.

Lhx1, formerly referred to as *Lim1*, encoding the transcription factor LIM homeobox 1, is involved in normal kidney and MD development (125, 126). *Lhx1*-null female mice have normal ovaries but lack their reproductive tract, which results from a disruption of MD elongation and epithelium formation (125–127). Besides deletions, six single nucleotide variants in *LHX1* (Table 2) (72, 85, 119, 120) have been reported including one missense variant with functional evidence of decreased transcriptional activity (120). Still, *LHX1* mutational analysis of larger cohorts did not report any variants, suggesting that sequence variants of *LHX1* are no major cause of MRKH syndrome (30, 128).

HNF1B has long been a candidate gene for MRKH syndrome since Lindner et al. already in 1999 reported a Norwegian family with MODY type 5 and progressive parenchymal kidney disease following autosomal dominant inheritance caused by a 75 bp in-frame deletion in exon 2 of *HNF1B*. Notably, two of four female variant carriers also had uterovaginal agenesis, supporting MRKH syndrome as part of the *HNF1B* disease spectrum (51). Subsequent studies have reported *HNF1B* variants associated with various uterovaginal malformations (52, 121, 129, 130), including a ~59 kb whole-gene deletion of *HNF1B* found by WES analysis of a 9-year-old girl presenting with precocious puberty. After the genetic result, reverse phenotyping by imaging confirmed uterus agenesis (121). Recently, Thomson et al. (83) investigated the function of *Hnf1b* following conditional ablation in mice, which resulted in a hypoplastic uterus and renal anomalies (including renal agenesis) similar to an MRKH syndrome type II phenotype. They performed single-cell RNA sequencing of the *Hnf1b*-ablated embryonic uterine tissue and found dysregulated gene expression involved in cell proliferation, patterning, and differentiation. This supports *HNF1B* as a causative gene in MRKH syndrome independent of *LHX1* involvement.

5.2 16p11.2 deletions and *TBX6*

In 2011, Nik-Zainal et al. (71) reported four cases with recurrent deletions of 16p11.2 and suggested *TBX6*, previously implicated in paraxial mesoderm development (131), as a candidate gene. To date, a large number of cases have been identified with both deletions (30, 71–79) and *TBX6* sequence variants (72, 75, 103, 105, 111, 112). The 16p11.2/*TBX6* locus is also associated with congenital scoliosis (132). The genetics hereof is complex and does not follow typical Mendelian inheritance, requiring one *TBX6*-null allele and a particular hypomorphic *trans* allele, as described in the compound inheritance gene dosage model (132, 133).

Ma et al. reported 16 rare *TBX6* variants enriched in a large MRKH syndrome patient cohort compared to controls. They performed various functional analyses of 13 missense variants and found evidence for loss-of-function in 7 variants, which together do support the role of *TBX6* variants. However, in contrast to null alleles associated with scoliosis, no second risk alleles were reported in MRKH syndrome (105). As of now, no clear biological mechanism for monoallelic *TBX6* variants causing MRKH syndrome has been established, which challenges interpretation and warrants further studies.

5.3 22q11 deletions and duplications

Both deletions (60, 71, 81, 85, 87–90) and duplications (81, 85, 91, 92) of 22q11 have been reported in MRKH syndrome patients (Table 1). The CNVs vary in size and location with no single common overlapping region, and therefore no particular candidate genes at 22q11 have been identified. Of note, 22q11 duplication has also been associated with other uterovaginal malformations (124). 22q11 deletions are often associated with DiGeorge and velocardiofacial syndromes. Features hereof are partly thought to be caused by *TBX1* haploinsufficiency, however, some of the deletions reported with MRKH syndrome do not include this particular gene. Overall, the evidence for 22q11 imbalances causing MRKH syndrome remains low.

5.4 1q21.1 and *RBM8A*

Variable-sized deletions at 1q21.1 have been associated with MRKH syndrome (30, 82, 85, 93). Duplications of 1q21.1 have also been identified in an MRKH syndrome patient (81) and one case with uterus didelphys (82). The overlapping region has been determined to be GRCh38: chr1:145,779,056-146,019,795 (82) with *RBM8A* as the proposed candidate gene in which sequence variants/polymorphisms also have been identified associated with MRKH syndrome (112).

Deletions of this region may also cause thrombocytopenia–absent radius (TAR) syndrome in compound heterozygosity with certain non-coding polymorphisms on the *trans* allele (134). Notably, TAR syndrome has previously been reported in a case with MRKH syndrome (135, 136). The possible causal role of

TABLE 2 Genes reported with recurrent[a] sequence variation associated with MRKH syndrome.

Gene	Chromosomal location	Zygosity	Variants reported	Variant type	MRKH type	Other phenotypes/entities associated with gene	Ref.
GREB1L	18q11.1-q11.2	Monoallelic	30	Missense (19) Frameshift (5) Splice-site (3) Stop-gain (2) Deletion (1)	Type II (22) Type 1 (8)	CAKUT (renal agenesis/renal hypodysplasia [OMIM #617805]), hearing loss (OMIM #619274), heart malformation, other UVMs	(98, 99, 101, 102, 109, 110)
TBX6	16p11.2	Monoallelic/ biallelic[b] (111)	21	Missense (16)[c] Splice-site (4) Stop-gain (1)	Type II (13) Type I (12)	Scoliosis, spondylocostal dysostosis (OMIM #122600), CAKUT	(72, 75, 103, 105, 111, 112)
PAX8	2q14.1	Monoallelic	11	Missense (6) Frameshift (2) Stop-gain (2) Splice-site (1)	Type I (11)	Thyroid hypoplasia/dysgenesis (OMIM #218700)	(75, 98)
SHOX	Xp22.33	Monoallelic	10	Duplication (8) Missense (2)	Type I (6) Type II (4)	Leri-Weill dyschondrosteosis (OMIM #127300)	(62, 64, 103)
WNT9B	17q21.32	Monoallelic/ biallelic[b] (113)	9	Missense (7) Stop-gain (1) Regulatory (1)	Type I (8)	CAKUT, cleft lip/palate, other UVMs	(98, 103, 113, 114)
WNT4	1p36.12	Monoallelic	7	Missense (7)	Type I (6) Type II (1)	Müllerian aplasia and hyperandrogenism (OMIM #158330), other UVMs	(103, 115–118)
LHX1	17q12	Monoallelic	6	Missense (5) Frameshift (1)	Type I (2) Type II (1) NS (3)	–	(72, 85, 119, 120)
LRP10	14q11.2	Monoallelic/ biallelic[b]	5	Missense (5)	Type I (3) Type II (1)	–	(74, 103)
HNF1B	17q12	Monoallelic	4	In-frame deletion (2) Frameshift (1) Deletion (1)	Type II	Renal cysts and diabetes syndrome/ MODY5 (OMIM #137920)	(51, 52, 121)
LAMC1	1q25.3	Monoallelic	4	Missense (4)	Type I (3) Type II (1)	–	(103)
BMP4	14q22.2	Monoallelic	3	Stop-gain (2) Splice-site (1)	Type I (2) Type II (1)	Microphthalmia (OMIM #607932, cleft lift/palate (OMIM #600625)	(75)
CTNNA3	10q21.3	Monoallelic	3	Deletion (3)	Type II (2) Type I (1)	Arrhythmogenic right ventricular dysplasia (OMIM #615616)	(73)
ESR1	6q25.1-q25.2	Monoallelic	3	Missense (2) Regulatory (1)	Type I	Estrogen resistance (OMIM #615363), breast cancer	(122)
MMP14	14q11.2	Monoallelic	3	Missense (2) Duplication (1)	Type I (2) Type II (1)	–	(35, 103)
RARA	17q21.2	Monoallelic/ biallelic[b]	3	Missense (3)	Type I	Acute promyelocytic leukemia	(103)
BMP7	20q13.31	Monoallelic	2	Frameshift (1) Splice-site (1)	Type I	–	(75)
DLG5	10q22.3	Monoallelic	2	Missense (1) Stop-gain (1)	Type I+II	CAKUT	(104, 123)
HOXA10	7p15.2	Monoallelic	2	Missense (1) Frameshift (1)	Type I+II	Other UVM	(75, 103)
KMT2D	12q13.12	Monoallelic	2	Missense (2)	Type I+II	Kabuki syndrome (OMIM# 147920)	(104)
MKKS	20p12.2	Monoallelic	2	Missense (2)	Type 2	Bardet-Biedl syndrome (OMIM #605231), McKusick-Kaufman syndrome (OMIM #236700)	(74)

(Continued)

TABLE 2 Continued

Gene	Chromosomal location	Zygosity	Variants reported	Variant type	MRKH type	Other phenotypes/entities associated with gene	Ref.
MYCBP2	13q22.3	Monoallelic	2	Missense (2)	Type I	–	(97)
PKD1	16p13.3	Monoallelic	2	Missense (1) Stop-gain (1)	Type I+II	Polycystic kidney disease (OMIM #173900)	(123)
SPECC1L	22q11.23	Monoallelic	2	Missense (2)	Type 2	Teebi hypertelorism syndrome (OMIM #145420)	(74)
TBC1D1	4p14	Monoallelic	2	Missense (1) Frameshift (1)	Type I+II	CAKUT	(104)

[a]Two or more reported sequence variations in the gene. Non-recurrent candidate variants are listed in Supplementary Table S1.
[b]Two variants reported in one case. Phasing was not done to determine *trans* or *cis* configuration.
[c]Including two polymorphisms (rs56098093 and rs201231713).
CAKUT, congenital anomalies of the kidneys and urinary tracts; MODY5, maturity-onset diabetes of the young type 5; NS, not stated; OMIM, Online Mendelian Inheritance in Man; UVM, uterovaginal malformations.

1q21.1 deletions/*RBM8A* gene variants in MRKH syndrome is, however, still unclear warranting further studies to establish causality.

5.5 2q13q14.1 deletions and *PAX8*

Pathogenic variants in *PAX8* are an established monogenic cause of congenital hypothyroidism due to thyroid dysgenesis (CH, OMIM #218700), first described in 1998 by Macchia et al. (137). In an experimental study, *Pax8*-deficient female mice were reported with infertility independently of thyroid replacement therapy, and a possible role in MD development was suggested (138).

Years later, Ma et al. reported on the investigation of a 12-year-old girl with global developmental delay, MD agenesis, and hypothyroidism, found to carry a large 10.79 Mb deletion of 2q13q14.2 (94). A partially overlapping 2q12.1q14.1 deletion was reported in another case with CH, atrial septal defect, intellectual disability, and MD agenesis with anterior displacement of the anus (95). The two deletions share an overlapping region of 4.3 Mb spanning *PAX8* as the proposed candidate gene for MRKH syndrome.

Most recently, Chen et al. (75) reported on a mutational burden analysis of 19 candidate genes based on WES data from 442 cases and 941 controls. Among cases, they found enrichment for predicted loss-of-function variants in *PAX*8. When including results from a replication cohort (n=150), a CH cohort (n=5), and missense variants, a total of 11 *PAX8* variants were found associated with MRKH syndrome. In three cases with available parental DNA, paternal inheritance was confirmed, showing a sex-limited expressivity of infertility. Functional analysis of the five missense variants by luciferase reporter assay found evidence for loss-of-function in two variants. Finally, reverse-phenotyping of five female CH cases, revealed uterovaginal aplasia in one individual, emphasizing the pleiotropy of *PAX8*. This confirms MRKH syndrome as a part of the *PAX8* disease spectrum in females, which the authors refer to as CH-MRKHS (75).

5.6 *GREB1L*

In 2017, three unrelated studies identified *GREB1L* variants as a new autosomal dominant cause of congenital anomalies of the kidney and urinary tract (CAKUT, OMIM #617805) (109, 110, 139). Some of the female cases, mainly fetuses affected by bilateral renal agenesis, were also described with uterovaginal malformations supporting a possible link to MD development (109, 110).

In 2019, Herlin et al. (101) reported on the investigation of a three-generational family with four cases of renal agenesis, including two adult female cousins with MRKH syndrome type II with unilateral renal agenesis. Whole-exome sequencing analysis in this family identified a segregating missense variant in *GREB1L*, supporting *GREB1L* variants as a novel monogenic cause of MRKH syndrome associated with incomplete penetrance and sex-limited expressivity and a phenotype mirroring the early descriptions of *hereditary renal/urogenital adysplasia* (41–43, 140, 141). Jacquinet et al. (102) identified *GREB1L* variants in 5 of 63 (7.9%) sporadic MRKH syndrome patients and segregating variants in four multiplex families presenting renal and uterovaginal malformations. Buchert et al. reported a stop-gain variant in a monozygotic twin-pair discordant for MRKH syndrome with the other twin having unilateral renal agenesis (98). Most recently, Jolly et al. performed an unbiased rare variant enrichment analysis based on WES data from a large American-European cohort (n=148), identifying *GREB1L* as the only gene approaching exome-wide significance based on seven detected variants. From a replication cohort of 442 Han Chinese cases, additional variants were found, including in six cases with type I MRKH syndrome. Of note, besides kidney and uterovaginal malformations, *GREB1L* variants have also been associated with inner ear malformations and deafness as well as complex congenital heart disease (142, 143).

GREB1L is considered to be involved in retinoic acid signaling, although its protein remains poorly characterized (109). *Greb1l*-knockdown in zebrafish causes abnormal pronephros morphogenesis (110, 139), and injection of wild-type human mRNA has been shown to rescue the phenotype (110). Knock-in mutagenesis of one missense variant in mice has also been shown to cause renal agenesis (139). Homozygous knock-out of *Greb1l* in mice has been shown to cause absence of the kidneys, Wolffian ducts, and Müllerian ducts (109). However, *GREB1L* variants reported in humans are monoallelic and predominantly missense variants (Table 2), and the pathogenic mechanism of how these missense variants cause MRKH syndrome is still unknown requiring further functional analysis.

Taken together, the current evidence with 30 reported variants (Table 2) including disease-segregating variants in extended pedigrees, epidemiological evidence of rare variant enrichment in larger cohorts, and functional evidence from knock-out mice, suggest *GREB1L* as a major causative gene in MRKH syndrome.

5.7 *SHOX*

Gervasini et al. (62) first reported on the association of partial *SHOX* duplications in MRKH syndrome in 2010. They investigated 30 cases and 53 controls and identified 5 cases with *SHOX* duplications including a sib-pair with MRKH syndrome. No duplications were identified among controls. Guerrier and Morcel (64) reported similar findings with three *SHOX* duplications and one duplication downstream of the gene. In contrast, Sandbacka et al. found no duplications among 101 Finnish cases questioning the role of *SHOX* in MD development (63). More recently, Mikhael et al. reported two missense variants from an investigation of 72 candidate genes in 111 cases (103). The functional role of these duplications (including their insertion sites) and missense variants is unknown and causality has not been established.

5.8 *WNT9B*

Wnt9b encodes a protein involved in MD development and *Wnt9b* knock-down in mice causes uterovaginal and renal agenesis. *Wnt9b* is expressed in the Wolffian duct epithelium providing signals guiding MD elongation (144). *WNT9B* has therefore been considered a candidate gene in MRKH syndrome and has been investigated in several studies identifying a total of 9 sequence variants in MRKH syndrome type I, of these 7 missense variants (Table 2) (98, 103, 113, 114). One patient has been reported with two variants (114). Other studies found no variants in MRKH syndrome patients (145, 146). *WNT9B* variants have also been reported in other uterovaginal malformations (114, 146). The functional role of these variants remains to be ascertained.

5.9 *WNT4*

As previously described, *WNT4* was the first gene identified as a monogenic cause of MD agenesis in females (OMIM #158330) (115). Since its discovery, a total of seven missense variants have been reported (103, 115–118). A missense variant of *WNT4* has also been reported with another uterovaginal anomaly with renal agenesis (Herlyn-Werner-Wunderlich syndrome) (147).

Importantly, MD agenesis caused by *WNT4* variants is associated with clinical and biochemical hyperandrogenism, representing a phenotype distinct from MRKH syndrome in general as described by Biason-Lauber et al. (116), and is often considered a separate entity. This is also supported by several investigations reporting no variants in larger MRKH syndrome and MD anomaly cohorts (30, 148, 149).

5.10 *LRP10*

Lrp10 encodes low-density lipoprotein receptor-related protein 10, which has been proposed as a negative regulator of Wnt/β-catenin signaling (150), a pathway involved in MD development (151). In 2015, Rall et al. reported on a SNP-array analysis of CNVs in discordant twin pairs. In one affected twin, a 585 kb duplication at 14q11.2 spanning *LPR10* was identified, not present in the other twin, and this gene was suggested as a candidate gene for MRKH syndrome. Two subsequent studies have reported a total of five missense variants in *LRP10*, hereof two variants in the same patient (74, 103). Functional evidence for these variants is lacking and causality has not been established.

6 Discussion

As described, knowledge of genetic variation in MRKH syndrome has increased considerably during recent years enabled by WES/WGS analysis. In the following, thoughts on how to continue the search for genetic causes are presented and potential implications for future clinical care are discussed.

6.1 Family history is key

The development of the field shows the importance of detailed family history in the genetic assessment, as it may include important information to suggest an underlying genetic trait or perhaps provide information on the genetic nature of the family's trait. The early descriptions of hereditary urogenital adysplasia families, reported long before molecular genetic testing became available, is a good example hereof suggesting an autosomal dominant trait with sex-limited expressivity (41–43, 140, 141). In recent years, *GREB1L* variants have been identified as a major cause of MRKH syndrome, particularly in families with a hereditary urogenital adysplasia-like presentations (101, 102).

Family histories should not only include incidents of MRKH syndrome but also associated extragenital malformations and other uterovaginal malformations in family members. The subtle nature of many renal and uterovaginal malformations is, however, important to consider, as these may not yet have been revealed in asymptomatic relatives, requiring radiological imaging. The relevance of other uterovaginal malformations in the genetic assessment of MRKH syndrome patients is highlighted by the many genes reported in both phenotypes, including 17q12 deletion (85, 124), *EMX2* (75, 152), *GREB1L* (102, 109, 110), *HNF1B* (52, 129, 130), *HOXA10* (58, 75, 153), *SPECC1L* (74, 154, 155), *WNT4* (115, 147), and *WNT9B* (114). This could suggest a spectrum of uterovaginal anomalies with shared causes.

6.2 Future genetic studies

Exome and genome sequencing have proven useful in the discovery of new genes during recent years, including *GREB1L* (99, 101, 102) and *PAX8* (75). With the continuously developing field of genomic sequencing technologies and multi-omics analyses, there is reason to believe that additional genetic causes remain to be identified. Recently, the first complete telomere-to-telomere genome reference was published, which will support even better detection of genetic variation in future genetic studies (156). Also, our understanding of the role of non-coding parts in the genome such as topologically associated domains and long non-coding RNAs is increasing (157, 158). Genome sequencing today is typically based on short-read sequencing due to its cost-effectiveness and accuracy. Long-read sequencing has increased in use and provides some advantages in terms of *de novo* assembly, read-mapping, detection of structural variants, phasing of variants, and transcript isoform identification (159). Another technology for improved analysis of structural variation is optical genome mapping, which has been applied by Brakta et al. (73). All along the technical developments, it will still be relevant to revisit sequencing data of unsolved cases, as new knowledge emerges supporting variant interpretation. Sequenced MRKH syndrome cohorts will increase in size, as will available genomic data resources, which will improve rare variant discovery from association analyses.

It is also relevant to look for somatic variation or differential gene expression in affected tissues as previously performed on surgically-removed uterine remnants (35, 98, 106–108). This may also be helpful in functional analyses of candidate variants of unknown significance to provide evidence for pathogenicity. However, it should be considered that organogenesis in the embryo is an accurately orchestrated process with precise spatiotemporal control of gene expression (160). Therefore, gene expression in adult uterine remnants may not be representative of the gene expression occurring during embryonic development, which in that case would require mouse modeling. As the causal effect of many gene variants remains unknown, functional analyses will be important for further progress.

Finally, it will be important to investigate candidate variants in extended pedigrees to understand the mode of inheritance and disease segregation, also considering more complex mechanisms in developmental genomics as proposed in the compound inheritance gene dosage model (161).

6.3 Importance of a genetic diagnosis – possible implications for clinical practice

Identifying genetic causes of MRKH syndrome is of immense importance including both academic pursuits towards improving our understanding of genetic factors underlying human uterovaginal development as well as establishing the necessary evidence to provide informed care and counseling for the individual patient and their families in clinical settings. Rare disease patients often undergo lengthy diagnostic odysseys from the first suspicion until the time of (genetic) diagnosis. Although the diagnostics in MRKH syndrome primarily involve finding the gynecological cause for primary amenorrhea, questions of "*why did this happen*?" remain and feelings of guilt and responsibility are prevalent among patients and their parents (11, 12). Identifying the underlying cause of malformation syndromes may reduce the burden for patients and families, even if it does not lead to changes in treatment (162).

Genetic counseling and management following the identification of a gene variant depends on the 'clinical actionability' of the variant, which requires both a valid gene-condition association and sufficient evidence for pathogenicity (163). As evidence for genetic causes in MRKH syndrome continues to increase, it may contribute to better disease classification in the future based on molecular causes instead of the current clinical distinction of type I and II. It can also be expected to guide future patient management such as examinations for associated diseases and anomalies specific to the particular gene. Clinically actionable variants may lead to genetic testing of family members at risk, and variant carriers may then be referred to relevant specialists for further examination. Finally, genetic counseling may address the recurrence risk if patients/couples with an identified genetic cause wish to pursue genetic parenthood either through gestational surrogacy or UTx. The possibility of recurrence in offspring was demonstrated in a recent report, describing the first case of mother-to-daughter inheritance following surrogacy (22). As UTx approaches clinical implementation (24) and the evidence for genetic causes increases, questions about offspring recurrence risk and reproductive choices will likely become more prevalent and so will requests for preimplantation genetic testing as part of the *in vitro* fertilization preceding the UTx procedure.

However, in the current state of knowledge, many reported variants associated with MRKH syndrome are still to be considered as variants of uncertain significance, which warrant cautious interpretations and counseling in clinical care.

7 Conclusion

In conclusion, recent advancements provide evidence for genetic causes of MRKH syndrome. The combined evidence points towards a heterogeneous etiology with various genes implicated. With the identification of monogenic causes in MRKH syndrome and increasing fertility options allowing couples to pursue genetic parenthood, the need for genetic counseling will likely increase.

Author contributions

MKH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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