Clinical aspects

The Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome [MIM 277000] is characterised by the congenital absence of the uterus and the upper two thirds of the vagina in 46,XX females with mostly normal ovarian function and therefore normal breast and pubic hair development. The exact description of the genital manifestation of MRKH is “uterus biphartitus solidus rudimentarius cum vagina solida” and in fact endometrium islands can be detected in a proportion of MRKH patients, leading to complications in some cases. Occasionally, the Fallopian tubes can also be affected, but the lower part of the vagina is usually unaffected. This is in good agreement with the hypothesis that the lower part of the vagina might develop from the urogenital sinus and might not be a derivative of the Müllerian ducts (MDs).

The first clinical feature is generally primary amenorrhea. Clinical examination typically reveals a normal female phenotype with breast development, axillary and pubic hair and normal external genitalia. Differential diagnosis includes isolated vaginal atresia, androgen insensitivity syndrome caused by mutations of the androgen receptor gene (AR) in XY individuals and WNT4 defects characterised by MRKH and hyperandrogenism.

Diagnostics include different methods such as transabdominal ultrasound, MRI and pelviscopy.

The incidence of MRKH is about 1:4,500 newborn girls.

The MRKH syndrome can occur as an isolated or type I MRKH or in association with extragenital malformations as type II MRKH. Upper urinary tract malformations are observed in about 40%, including unilateral renal agenesis, ectopia of one or both kidneys, renal hypoplasia, horseshoe kidneys and hydronephrosis. The most frequent skeletal anomalies include malformations of the spine in 30–40% such as Klippel–Feil anomaly or scoliosis. Müllerian hypoplasia, renal agenesis, cervicothoracic somite dysplasia (MURCS) association is the most severe form of MRKH II characterised by MD aplasia, renal dysplasia and cervical somite dysplasia. Less frequently, MRKH can be associated with hearing defects including conduction defects such as stapes fixation or sensorineural deafness. Rarely, cardiac (atrial septum defect, conotruncal defects) and digital anomalies such as syndactyly, polydactyly or ectrodactyly can occur. Occasionally, associations with situs inversus, Dandy–Walker malformation, Meckel–Gruber syndrome, Bardet–Biedl syndrome, Holt–Oram syndrome or McKusick–Kaufman syndrome have been reported, leading to the assumption that—in at least some cases—MRKH can be seen as a ciliopathy.

Embryogenetic aspects

The mammalian female and male reproductive tracts derive from the paramesonephric or MDs and mesonephric or Wolffian ducts (WDs) respectively. According to analyses of mice models, MD development includes three phases: 1) initiation, 2) cranio-caudal invagination of the coelomic epithelium into the mesonephros, and 3) elongation of the MD.

First, cells of the coelomic epithelium (Müllerian plaque) at the upper end of the urogenital ridge are specified to become MD cells. Subsequently, within the mesonephros, MD precursors invaginate into the underlying mesenchyme and migrate caudally along the length of the WDs extending posteriorly, cross the WDs until the caudal tip of the MDs reaches the urogenital sinus, which is of endodermal origin. It was suggested that the cells forming the MDs were of WD origin, but Orvis and Behringer were able to show that the elongation of the MDs is accomplished predominantly by a small group of cells proliferating at the tip of the MDs. These cells are tightly associated with the WDs and are guided by them [31]. The first phase of MD development (specification and invagination) occurs independently from the WDs, but in the next phase of elongation it is dependent on the presence of the WDs and furthermore on the expression of an elongation signal (Wnt9b, wingless-type MMTV integration site family, member 9b).

Although the MDs and WDs are of different origin, they coexist during embryogenesis in both sexes until genetic sex triggers the differentiation of the indifferent gonad into ovary or testis respectively. In females, the MDs give rise by fusion to the utero-vaginal duct, which differentiates into the uterus and the upper part of the vagina, whereas the unfused part of the MDs develops into the Fallopian tubes. In males, the testicular Sertoli cells secrete a glycoprotein, the anti-Müllerian hormone (AMH), which causes the
Table 1  Phenotypes of MRKH patients with imbalances in recurrently affected regions 1q21.1, 16q11.2, 17q12 and 22q11.21

| Locus      | Copy number/size | Causative genes(s) | Phenotype                                                                 | Reference                                      |
|------------|------------------|---------------------|---------------------------------------------------------------------------|-----------------------------------------------|
| 1q21.1     | Dup/2.7 Mb       | RBM8A               | Complete uterine + vaginal agenesis, fused external labia, ovaries undetectable; inherited by unaffected mother | Cheroki et al. (2008; [11])                   |
| 1q21.1     | Del/0.378 Mb     | RBM8A               | Müllerian aplasia type II, TAR syndrome                                   | Ledig et al. (2011; [20])                     |
| 16q11.2    | Del/0.55 Mb      | TBX6                | MURCS, hypoplasia of the wrist, disturbed psychomotor development, epilepsy, bilateral hearing loss | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.6 Mb       | TBX6                | Müllerian aplasia, short stature                                          | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.55 Mb      | TBX6                | Müllerian aplasia, short stature                                          | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.55 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.55 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 16q11.2    | Del/0.53 Mb      | TBX6                | Müllerian aplasia, long uterus horns, scoliosis, left atrophic kidney     | Nik-Zainal et al. (2011; [29])                |
| 17q12      | Del/1.2 Mb       | LHX1, HNF1B         | Müllerian aplasia, mild dysmorphic features, onychodystrophy, mental impairment, seizures | Cheroki et al. (2008; [11])                   |
| 17q12      | Del/1.5 Mb       | LHX1, HNF1B         | Müllerian aplasia, mild dysmorphic features                               | Bernardini et al. (2009; [3])                 |
| 17q12      | Del/1.5 Mb       | LHX1, HNF1B         | Müllerian malformations with right uniconuate uterus, no cavitating rudimentary left horn, right haematosalpinx and surgically corrected agenesis of the upper and middle thirds of the vagina, bilaterally multicystic kidneys | Bernardini et al. (2009; [3])                 |
| 17q12      | Del/1.8 Mb       | LHX1, HNF1B         | Müllerian aplasia                                                          | Ledig et al. (2011; [20])                     |
| 17q12      | Del/1.4 Mb       | LHX1, HNF1B         | Müllerian aplasia, unilateral kidney agenesis                             | Ledig et al. (2011; [20])                     |
| 17q12      | Del/1.4 Mb       | LHX1, HNF1B         | Müllerian aplasia                                                          | Medin et al. (2011; [29])                     |
| 17q12      | Del/1.4 Mb       | LHX1, HNF1B         | Müllerian aplasia, unilateral kidney agenesis                             | Ledig et al. (2011; [20])                     |
| 17q12      | Del/1.4 Mb       | LHX1, HNF1B         | Müllerian aplasia, unilateral kidney agenesis                             | Ledig et al. (2011; [20])                     |
| 22q11.21   | Del/2.6 Mb       | ?                   | Müllerian malformation with vaginal agenesis and rudimentary uterus, right kidney agenesis, scoliosis, mild to moderate learning disabilities, mild dysmorphic features | Cheroki et al. (2006; [10]); Cheroki et al. (2008; [11]) |
| 22q11.21   | Del/0.39 Mb      | ?                   | Müllerian aplasia                                                          | Ledig et al. (2011; [20])                     |
| 22q11.2    | Del/0.39 Mb      | ?                   | Müllerian malformation with vaginal agenesis and rudimentary uterus, right kidney agenesis, scoliosis, mild to moderate learning disabilities, mild dysmorphic features | Cheroki et al. (2006; [10]); Cheroki et al. (2008; [11]) |
| 22q11.21   | Dup/3.5 Mb       | ?                   | Müllerian malformation with vaginal agenesis and rudimentary uterus, right kidney agenesis, scoliosis, mild to moderate learning disabilities, mild dysmorphic features | Cheroki et al. (2006; [10]); Cheroki et al. (2008; [11]) |

TAR thrombocytopaenia/absent radius, MRKH Mayer–Rokitansky–Küster–Hauser syndrome, MURCS Müllerian hypoplasia, renal agenesis, cervicothoracic somite dysplasia
regression of the MD. Targeted mutagenesis in the mouse has identified several genes that are essential for proper development and differentiation of the female reproductive tract.

The mice genes required for female reproductive tract development include paired-box-gene 2 (Pax2), LIM homeobox 1 (Lhx1), wingless-type MMTV integration site family, member 4 (Wnt4), wingless-type MMTV integration site family, member 7a (Wnt7a), empty spiracles homeobox 2 (Emx2), hepatocyte nuclear factor 1-beta (Hnf1b), wingless-type MMTV integration site family, member 5a (Wnt5a), dachshund homolog 1 (Dach1), dachshund homolog 2 (Dach2), wingless-type MMTV integration site family, member 9b (Wnt9b), and genes of the abdominal B Hoxa cluster.

The homeodomain transcription factor encoding gene Pax2 is expressed in the developing kidney and in the epithelium of the MD and the WD. According to its expression mice deficient in Pax2 lack kidneys and genital ducts in both sexes [18]. MD development is initiated by the expression of Pax2 together with homeodomain coding gene Lhx1 in the coelomic epithelial cells and specifies them for a Müllerian fate. Furthermore, Pax2 is also essential for the next steps in MD development, the elongation and maintenance of the MD. Therefore, in Pax2-deficient mice the anterior portion of the MD initially forms, but then degenerates, while the urogenital sinus still gives rise to the bladder and urethra [18]. Wnt4 and Lhx1 are expressed in the embryonic mesonephric mesenchyme surrounding the newly formed MD and both are required for embryonic MD development. In Wnt4−/− female mice, the absence of MD formation and in contrast stabilization of WD suggest an essential role of Wnt4 in repressing male development in the XX gonad [45]. Furthermore, Jeays-Ward et al. showed that the masculinised phenotype of Wnt4−/− female mice originates because of the disturbance of endothelial and steroidogenic cell migration into the developing XX gonad, provoking the formation of a male-specific coelomic blood vessel and production of steroids in the female gonad [16]. Possibly, Wnt4 is acting downstream of Lhx1
and Wnt9b and initiates the MD invagination [25, 45]. Wnt4 induces expression of Wnt7a [45]. Female mice lacking Wnt7a are infertile owing to abnormal differentiation of the uterus and oviduct [33]. The LIM domain-expressing gene, Lhx1 is essential for the development of the epithelial cells of MDs and WDs. Therefore, female Lhx1 knockout mice lack the uterus and the upper part of the vagina, whereas male mice are deficient in the WD derivatives [18].

Emx2 is another homeodomain transcription factor coding gene that is expressed in the epithelial cells of the urogenital tract. Consequently, Emx2 mutant mice lack kidneys, ureters, gonads and genital tracts and die soon after birth [26]. However, in these mice, WDs initially develop, but then degenerate. The POU domain-containing Hnf1β is essential for general epithelial differentiation and is expressed in very early urogenital tract formation, continuing into adulthood [13]. Wnt5a is expressed in mesenchymal cells of the uterus, cervix and vagina and is required for the growth of the female reproductive tract [25]. Therefore, in mice deficient in Wnt5a, the cervix and the whole vagina are absent [25]. Female mice double mutant for the putative transcriptional cofactors Dach1/Dach2 show a severe disruption of MD development [14]. The adequate development, fusion and resorption of the separating wall between the MDs seem to be induced by the WDs [1]. Thus, it is known, that the WD secretes Wnt9b, which serves as a canonical Wnt signal essential for caudal MD extension [9]. In Wnt9b−deficient mice, the MDs start to invaginate, but there is no elongation caudally. Interestingly, the WDs are unaffected [9]. Different genes of the Hox family play a major role in body patterning and organogenesis and are expressed during the development of the female genital tract in different areas of the MD. The expression of the different Hox genes divides the homogeneous MD into segments along the anterior–posterior axis with each segment developing into different structures according to their 3′–5′ order in the Hox cluster: Hoxa9 is expressed in the subsequent oviduct, Hoxa10 in the developing uterus, Hoxa11 in the progenitor of the lower uterine segment and cervix and Hoxa13 in the cervix and upper vagina [47]. Mutations of either Hoxa10 or Hoxa11 result in uterine factor infertility in mice due to an implantation defect in the uterus [39].

**Genetic aspects**

Currently, the genetics of MRKH remains elusive. There is a risk of recurrence in relatives, but most cases of MRKH are sporadic. Familial cases can be explained by autosomal dominant inheritance with reduced penetrance and variable manifestation. However, oligogenic or polygenic inheritance has also been discussed [20].

There are some reports of monozygous twins discordant for MRKH, which may be explained by mosaicism or imprinting effects. Recently, insights into genetics and the pathogenesis of MRKH have come from genetic techniques such as array CGH.

Until recently, WNT4 deficiency was the only known monogenic cause for MRKH, but different groups identified by multiplex ligation-dependent probe amplification (MLPA) and genome-wide array comparative genomic hybridisation (CGH) microimbalances affecting new genes that play a role in the pathogenesis of this condition. So far, different recurrently affected chromosomal regions have been identified with the following frequencies: ~1% in 1q21.1, ~1% in 16p11.2, ~6% in 17q12 and ~4% in 22q11.21 [20, 27]. Table 1 summarises the phenotypes of MRKH patients with imbalances in the above-mentioned recurrently affected regions.

**1q21.1**

Imbalances in 1q21.1 affecting the so-called thrombocytopenia/absent radius (TAR; MIM27400) susceptibility locus have been identified in patients with or without signs of the TAR syndrome (hypomega-karyocytic thrombocytopenia, bilateral absence of the radius in the presence of both thumbs) in addition to Müllerian malformations (Table 1; [11, 20]). In an MRKH type II patient with signs of TAR syndrome a deletion affecting the TAR susceptibility locus has been identified [20]. In a second patient, a gross duplication of approximately 2.7 Mb, also overlapping the common TAR deletion interval has been described [11]. Most TAR patients carry deletions of different sizes, but always affecting a 200-kb gross common deletion interval, the TAR susceptibility locus. Rarely, malformations of the genitourinary anomalies have been observed in patients with TAR syndrome including horseshoe kidney, hypoplasia of the uterus and vagina, and renal pelvis dilatation. Furthermore, it is known from analysis of TAR patients that about 75% have inherited the deletion from an unaffected parent [17]. Therefore, the authors supposed that in addition to the rare deletion, a second frequent change, possibly a frequent variant, is needed for the phenotypic manifestation of TAR. However, mutational analysis of 10 genes, located in the minimal deletion interval, in 3 patients revealed in a first approach no second causative mutation [17]. However, recently in all patients analysed, one of two rare intronic regulatory polymorphisms in the RBM8A gene, which is located in the minimal deletion interval of the TAR susceptibility locus, have been found on the second allele [2]. Furthermore, in the case of patients with clinical signs of TAR and the causative polymorphism in the regulatory region of RBM8A, but without the deletion in 1q21.1, nonsense mutations of RBM8A in a compound heterozygous manner have been found [2]. RBM8A encodes the Y14 protein, which is one of four core components of the exon junction complex (EJC). In Drosophila melanogaster, the Y14 protein is necessary for oocyte differentiation and determination of primordial germ cells [32].

All of these findings suggested a strong association between RBM8A and MRKH. Therefore, by performing sequence analysis of RBM8A in a group of 116 MRKH patients, one of the two TAR-associated variants and a second undescribed intronic variant were found with higher frequencies in the patient group in contrast to the general population [43]. Interestingly, one patient carried both RBM8A
variants mentioned above, whereas another carried a gross duplication, which contains the Bardet–Biedl syndrome (BBS)-associated BBS9 gene [43]. Furthermore, in a patient with MRKH I and XX gonadal dysgenesis, a heterozygous RBM8A missense mutation was found, making RBM8A an interesting candidate for MRKH syndrome associated with ovarian dysgenesis too [43].

16p11.2

Losses in 16p11.2 have primarily been described in combination with autism spectrum disorders, but also with epilepsy, seizures, developmental delay and learning disability, dysmorphism/congenital anomalies (abnormal head size) and obesity. Furthermore, deletions in 16p11.2 were also identified in unaffected persons. However, in an array-based study of patients with isolated and syndromic Müllerian aplasia, in 4 of the 63 patients deletions of this locus were identified, suggesting a strong association of this region with MRKH syndrome (Table 1; [3, 11, 20, 29, 38]). Among the genes deleted in the common deletion interval, TBX6 seems to be a good candidate gene, as it encodes a conserved transcription factor, playing an essential role in developmental processes such as mesoderm formation and specification.

Sequence variants in TBX6 are known to cause congenital scoliosis in the Chinese Han population and spondylocostal dysostosis [15]. A mouse model, the homozygous Tbx6+/− (rib-vertebrae), show a hypomorphic phenotype, with an occasionally unilateral absence of kidneys and reduced female fertility [49]. The phenotype in this mouse model and the known association between TBX6 mutations in humans and scoliosis strongly resembles the MURCS association in humans.

Sequencing of the TBX6 gene in two studies with MRKH patients resulted in the identification of one possible pathogenic missense and one splice site mutation in a total of four patients [38, 43]. Corresponding to the phenotype seen in mice with homozygous Tbx6 mutations, two of these patients also show skeletal malformations [43]. Furthermore, two known polymorphisms could be associated with Müllerian aplasia, as they were found at a higher frequency in patients in contrast to the general population [38].

17q12

The most recurrently affected chromosomal region in MRKH is 17q12. Different array-based studies identified deletions of 1.4–1.8 Mb in size in chromosomal region 17q12 in patients with MRKH types I and II (Table 1; [3, 11, 20, 29, 38]). Associated malformations were bilaterally multicystic kidneys, mild facial dysmorphisms [3], but also severe learning disability and seizures (Table 1; [11]). Furthermore, deletions of 17q12 can also give rise to other phenotypes without any impairment of MD.

Due to expression data and mouse models, different studies favoured LHX1 and HNF1B as promising candidate genes for MRKH, but also for associated traits of the 17q12 deletion. Both genes are discussed in the following. Furthermore, the finding that the deletion size and the breakpoints observed in patients with MRKH type I are similar to those in patients with a more severe phenotype or with malformations not affecting the MD makes the involvement of other genes outside the deletion interval for this extended phenotype likely. An oligogenic mode of inheritance has also been suggested for MRKH and would explain the rare familial cases and the difficulty in identifying a single genetic cause.

LHX1

Of a total of 118 MRKH patients, we could detect in two of them heterozygosity for a frameshift mutation and a missense mutation in the LHX1 gene respectively [20, 21]. The frameshift causes a very early premature stop codon at amino acid position 33 and was detected in a type II patient with unilateral kidney agenesis [21]. The patient, who carries the LHX1 missense mutation, has a type I MRKH syndrome [20]. Furthermore, three pathogenic LHX1 mutations were found in 5 out of 112 Finnish patients with aplasia of the Müllerian ducts [38]. LHX1 on chromosome 17 belongs to the LIM homeodomain family of transcription factors, which is implicated during embryogenesis in processes such as body axis determination, in addition to tissue and regional specification. LIM homeodomain proteins contain two tandem LIM domains followed by a central homeodomain with DNA-binding activity and a C-terminal transactivation domain, which may be involved in the transcriptional regulation of target genes. The LIM domain is a cysteine-rich double zinc finger motif that binds zinc and functions as a protein adapter module that can interact with different protein domains and regulate by this the function of different components in the transcriptional complex. Female Lhx1-null mice lack a uterus and oviducts together with a complete absence of both the epithelium and the mesenchyme of the female reproductive tract, while the ovaries are unaffected [18], a phenotype strongly resembling MRKH syndrome in humans. Additionally, mice lacking Lhx1 lack kidneys and are anencephalic [40]. Most embryos deficient of Lhx1 die at embryonic day E10 because of defects in allantois differentiation, and only a few are stillborn.

Expression of Lhx1 in the epithelium of the developing MDs in a mouse model is dynamic with onset of the expression at embryonic day 11.5 (E11.5) in the most anterior region of the urogenital ridge and caudal extension at E13.5 in both sexes [18]. The Lhx1 expression in the epithelium of the MDs becomes sexually dimorphic at E14.5, with persistent strong expression until E16.5 in females and a weaker expression in males than in females consistent with the regression of the MDs in males to this point of time. Afterwards, the Lhx1 expression also becomes downregulated in females, but persists in the differentiating oviduct. These findings correspond to an essential role of Lhx1 in the formation of the MDs in the female and suggest a further role in the development of the oviduct. Furthermore, by using a chimera assay, Kobayashi et al. showed that Lhx1 is required cell-autonomously for very early MD epithelium formation and that its expression in the Müllerian precursor cells...
is independent of Wnt7a, Pax2 and Wnt4 [18]. Lhx1 is also expressed in the WD in both sexes. In females, Lhx1 expression is lost from the anterior gonadal region around E15.25, whereas Lhx1 expression in males persists and becomes upregulated around E17.5. Interestingly, the only Lhx1-null male neonate lacks Wolffian derivatives, also suggesting that Lhx1 might play an essential role in the development of the male genital tract [18].

Furthermore, the specific knockout of Lhx1 in the WD epithelium causes the lack of the WDs, but also impairs the further development of the MDs, confirming the importance of WDs for the elongation of the MDs [19].

In addition to the malformations of the MDs, Lhx1-minus mice lack, as mentioned above, any kidneys and fail to form normal anterior head structures [18, 40]. Renal malformations such as unilateral agenesis, ectopia of kidneys or horseshoe kidneys are quite often in MRKH, but even bilateral renal agenesis has been reported (Potter sequence). Interestingly the patient who carries the mutation in HNF1B, but even bilateral renal agenesis is independent of Wnt7a, Pax2 and Wnt4 [18]. Lhx1 is also expressed in the WD in both sexes. In females, Lhx1 expression is lost from the anterior gonadal region around E15.25, whereas Lhx1 expression in males persists and becomes upregulated around E17.5. Interestingly, the only Lhx1-null male neonate lacks Wolffian derivatives, also suggesting that Lhx1 might play an essential role in the development of the male genital tract [18].

Lhx1 is expressed in the gonads and complete loss of Lhx1 has first been described to cause defective head structures and a lack of kidneys in addition to a loss of gonads in mice [40]. However, such agonadism was not found in neonate Lhx1-minus mice with mixed genetic background [18].

HNF1B Heterozygous mutations and whole gene deletions of the tissue-specific homeodomain transcription factor HNF1B gene are typically associated with renal cysts and diabetes (OMIM 137920). However, other phenotypic characteristics have also been described in association with HNF1B alterations. These are in good agreement with the tissues that express HNF1B such as kidney, pancreas, liver and the uterus. Expression of Hnf1b has been shown in MDs in the mouse embryo and in the inner epithelial layer in the adult mouse [13]. Therefore, in very few cases, HNF1B mutations have also been reported to cause, in association with renal tract malformations, abnormalities of the MDs in females [7]. However, studies analysing the HNF1B gene in patients with MRKH failed to identify mutations in HNF1B [3, 20, 21].

So far, only one case of a heterozygous HNF1B missense mutation and an isolated bicorunate uterus has been described [7]. Therefore, HNF1B mutations are associated as a cardinal feature with malformations of the renal tract and are a very rare cause of Müllerian disorders.

22q11.21

22q11.21 deletions are commonly associated with DiGeorge or velocardiofacial syndrome (DG/VCFS OMIM 188400/192430). DG/VCFS belongs to a group of related dysmorphic syndromes with highly variable clinical phenotypes encompassing congenital heart defects, hypocalcaemia, immunodeficiency, typical facial dysmorphism, learning, speech and behavioural disorders. Moreover, some publications showed an association between 22q11.21 deletions and Müllerian aplasia. Therefore, MRKH syndrome has been considered to be part of the spectrum of clinical features of the DG/VCFS. Deletions and duplications of the DiGeorge syndrome-associated region 22q11.21 have also been found in MRKH patients (Table 1; [11, 20, 29]).

Cheroki detected a gross deletion in a patient with uterus agenesis and further features present in DG/VCFS (Table 1; [10, 11]). The deletion was disrupted by a short unaffected region containing the TBX1 gene, which is responsible for some of the major clinical features of DG/VCFS. The authors suggest that the non-deletion of the TBX1 gene might be causative of the milder phenotype. A smaller deletion, also non-affecting the TBX1 gene, has been identified in a patient with type I MRKH syndrome [20]. Furthermore, an adjacent duplication of approximately 3.4 Mb has been found in another type I MRKH patient, overlapping with the distal part of the 22q11.21 microdeletion–microduplication region.

These findings suggest that genes other than the TBX1 gene in the 22q11.21 deletion syndrome might play a role in uterine malformations. Interestingly, analysis of MRKH patients with a commercially available DGS and DGS-like MLPA kit revealed imbalances in 22q11.2 and in the DGS-like phenotype associated regions 4q34–qter, 8p23.1 and 10p14 [28].

In addition to recurrent aberrations, array CGH-based studies also identified various interesting private losses and gains such as 2p24.1–24.3, 7p14.3 and Xq21.31 [11, 20, 29].
Members of the WNT family

**WNT4**

WNT4, which maps to human chromosome 1, is a member of the WNT family of structurally related and highly conserved genes that encode secreted signalling factors that regulate a broad range of developmental processes, but has also been implicated in carcinogenesis. WNT4 is known to be essential for the development of the female reproductive tract, whereas it has been shown to play in the development of the female gonad a double role, on the one hand by controlling the female development and on the other hand by preventing testes formation.

Heterozygous mutations in the WNT4 gene have been associated with MRKH in humans [4, 5, 35, 36]. The 4 patients described so far displayed an agenesis or hypoplasia of the Müllerian derivatives, but also clinical or biochemical signs of hyperandrogenism (hirsutism, acne, elevated plasma testosterone levels). These findings are in good agreement with the phenotype found in Wnt4-deficient mice, which fail to develop MD and are masculinised. However, unilateral renal agenesis has been identified in females with heterozygous WNT4 mutations. Functional analysis of the WNT4 mutations revealed failure of post-transcriptional lipid modification, misfolding and formation of intractable aggregates, defects in receptor-binding and partial deregulation of enzymes involved in ovarian androgen biosynthesis [6, 36].

Studies involving classical MRKH patients failed to identify WNT4 mutations [10, 12]. Therefore, it has been suggested that MRKH syndrome with signs of androgenisation due to heterozygous WNT4 mutations is a distinct clinical entity that can be delineated from typical or classic MRKH. Moreover, because of its role in gonadal development, folliculogenesis can also be disturbed in affected women [35].

**WNT9B**

However, alterations in other members of the complex WNT signalling pathway have been suggested as being causative, but no mutation in WNT5A, WNT7A and WNT9B could be detected in 11 MRKH patients [37].

Despite these findings, WNT9B seemed to be a good candidate, as Wnt9b−/− female mice have no uterus and upper part of the vagina, but have normal ovaries, which is comparable to the MRKH phenotype in women [9]. Furthermore, Carroll et al. showed in the same work that Wnt9b acts upstream of Wnt4 in the development of the urogenital tract and is essential for the development of mesonephric and metanephric tubules and caudal extension of the Müllerian ducts in mice.

A first association between WNT9B and MRKH was found in a Chinese study with 42 patients, in which two possible pathogenic WNT9B mutations were detected in one patient [46]. Although it was unknown if these two mutations had been in cis or trans, the authors suggested a synergistic effect. In contrast, another Chinese study found no association between anomalies of the Müllerian ducts and mutations in WNT9B [42].

However, by analysing WNT9B in a group consisting of 59 MRKH and 50 MRKH II patients, Waschk et al. identified in five of the MRKH I patients potential pathogenic mutations (one nonsense and four missense mutations) [48], but no WNT9B mutation was detected in MRKH II patients. Interestingly, previous studies showed that two of the patients with a WNT9B mutation carried either an additional deletion of LHX1 or a missense mutation in TBX6 [20, 43], suggesting digenic inheritance in MRKH. Interestingly, it was shown that the expression of Wnt9b in Lhx1−/− deficient mice is markedly altered [34]. All of these findings suggest a common pathway in MRKH syndrome with WNT9B acting upstream of WNT4 and LHX1.

Furthermore, in the past, the possible involvement of other genes in the pathogenesis of MRKH has been tested. Mutations affecting AMH, which initiates regression of MDs during male embryonic development, anti-Müllerian hormone receptor (AMHR) and various homeobox (HOX) genes, have been excluded as causative factors for MRKH syndrome [30]. Furthermore, mutational analysis of HOXA10 and HOXA11 in a small group of patients with malformations of the female genital tract revealed only one missense variant of unknown pathogenicity, which was also present in the patients’ unaffected mother [23].

Finally, it should be considered that exogenous factors such as diethylstilbestrol (DES), which functions as a strong oestrogen, may be involved in the pathogenesis of MRKH syndrome.

**Conclusion**

Mayer–Rokitansky–Küster–Hauser syndrome is a phenotypically and genetically very heterogeneous disorder and has an incidence of 1:4,500 newborn females. Most of the cases are sporadic, but analyses of the few reported familial cases suggest an autosomal-dominant inheritance with reduced penetrance. As array CGH analyses in MRKH patients identified recurrent aberrations in chromosomal regions 1q21.1, 16p11.2, 17q12 and 22q11.21 respectively, array CGH analyses should be performed in women with the suspected diagnosis of MRKH syndrome. These recurrent aberrations are associated with highly variable clinical phenotypes and can also cause further disorders, e.g. in the case of 22q11.21 deletion heart defects or 17q12 deletion maturity-onset diabetes of the young (MODY) due to deletions of HNF1B.

Furthermore, the clinical overlap of MRKH syndrome with different ciliopathies suggests that MRKH syndrome might also be a ciliopathy.

By analysing candidate genes, being located in these aberrations, mutations in genes such as LHX1, RBM8A and TBX6 have been identified as being causative of MRKH syndrome. However, in WNT4, which is associated with a distinct clinical entity of MRKH syndrome and signs of hyperandrogenism, and in its family member WNT9B, causative mutations have also been detected. Furthermore, in some patients with fusion anomalies of the MDs such as uterus didelphis, the same causes as for MRKH syndrome, e.g. deletions in 17q12, duplications of chromosomal region 22q11.21, and vari-
In fusion anomalies of the uterus, the increasing number of genes responsible for MRKH syndrome might have a partially common aetiology [22, 43, 48].

New insights can be expected from studies on large cohorts of well-characterised patients in combination with technologies such as next-generation sequencing.

Aspects of genetic counselling

- MRKH syndrome is a rare, heterogeneous disease characterised by the absence of a uterus and the upper two thirds of the vagina in 46,XX females.
- Most cases are sporadic, but familial occurrence is well documented and indicates autosomal-dominant inheritance with variable manifestation.
- MRKH syndrome is frequently associated with malformations, especially of the kidneys (unilateral renal agenesis 30%), skeleton (10–15%), cardiac anomalies (2–3%) and deafness (2–3%).
- Siblings of MRKH patients can also show, for example, malformations of the MDs or associated anomalies.
- In about 10%, causative microdeletions and microduplications can be detected by array CGH.
- The detection of a microdeletion in chromosomal region 16p11.2, 17q12 or 22q11.2 in a MRKH patient can also have implications for other family members. Notably, those imbalances can be inherited by unaffected parents and can be associated with autism (16p11.2), MODY (17q12) or cardiac malformations (22q11.21), which can also occur in male family members.
- An increasing number of genes responsible for MRKH syndrome such as LHX1, TBX6, WNT9B, and WNT4 have been identified.
- In fusion anomalies of the uterus, the same causes of MRKH syndrome can be identified. Both MRKH syndrome and fusion anomalies of the uterus can be observed in the same family.
- Genetic diagnosis includes array CGH and next-generation sequencing.
- Various surgical procedures are available for building a neovagina. In a few cases, uterus transplantation has been performed to enable pregnancy.

Compliance with ethical guidelines

Conflict of interest.

S. Ledig and P. Wlecker declare that they have no competing interests.

This article does not contain any studies with human participants or animals performed by any of the authors.

Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Ácién P, Sánchez del Campo F, Mayol MJ, Ácién M (2011) The female genitubucalum: role in the embryology and development of the genital tract and in the possible genesis of malformations. Eur J Obstet Gynecol Reprod Biol 159:426–432
2. Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smithhurst PA, Jolley JD, Cvejic A, Kostadima M, Bertone P, Breuning M, Debili N, Deloukas P, Favier R, Fiedler J, Hobbs CM, Huang N, Hurles ME, Kiddie G, Kramps I, Norden P, Ruivenkamp CAL, Sambrook JGSK, Stemple DL, Strauss G, Thys C, van Geet C, van Geet C, van Geet C, Newbury-Ecob R, Ouwehand WH, Ghevaert C (2012) Compound inheritance of low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RRM8B causes TAR syndrome. Nat Genet 44:435–441
3. Bernardini L, Gimelli S, Gervassi C, Carella M, Baban A, Frontino G, Barbano G, Divizia MT, Fedele L, Novelli A, Bena F, Lalatta F, Miozzo M, Dallapiccola B (2009) Recurrent microdeletion at 17q12 as a cause of Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome: two case reports. Orphanet J Rare Dis 4:25
4. Biason-Lauber A, Konrad D, Nanavati F, Schoenle EJ (2004) WNT4 mutation associated with Mullerian duct regression and virilization in a 46,XX woman. N Engl J Med 351:792–798
5. Biason-Lauber A, De Filippo G, Konrad D, Scarno G, Zazzaro A, Schoenle EJ (2007) WNT4 deficiency—a clinical phenotype distinct from the classic Mayer-Rokitansky-Kuster-Hauser syndrome: a case report. Hum Reprod 22:224–229
6. Biason-Lauber A, Konrad D (2008) WNT4 and sex development. Sex Dev 2:210–218
7. Bingham C, Ellard S, Cole TR, Jones KE, Allen LI, Goodship JA, Goodship TH, Bakalnina-Pugh D, Russell GI, Woolf AS, Nicholls AJ, Hattersley AT (2002) Solitary functioning kidney and diverse genital tract malformations associated with hepatocyte nuclear factor-1b mutations. Kidney Int 61:1243–1251
8. Bousifha N, Errahay S, Saadi H, Ouldik D, Bouchikhi C, Banani A (2010) Gonadal dysgenesis 46,XX associated with Mayer-Rokitansky-Kuster-Hauser syndrome: one case report. Obstet Gynecol Int. https://doi.org/10.1155/2010/847370
9. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP (2005) Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell 9:283–292
10. Cheroki C, Krepschi-Santos AC, Rosenberg C, Jehee FS, Mongroni-Netto RC, Pavanello Filho I, Zanforlin Filho S, Kim CA, Bagnoli VR, Mendonca BB, Suzhai K, Otto PA (2006) Report of a 22q11T1 in a patient with Mayer-Rokitansky-Kuster-Hauser syndrome: one case report. J Med Genet A 140:1339–1342
11. Cheroki C, Krepschi-Santos AC, Suzhai K, Bremner Y, Kim CA, Otto PA, Rosenberg C (2008) Genomic imbalances associated with mullerian aplasia. J Med Genet 45:228–232
12. Clement-Ziza M, Khen N, Gonzales J, Cretolle-Vastel C, Picard JY, Tullio-Pelet A, Besmond C, Munchik A, Lyonnet S, Nhoul-Fekeete C (2005) Exclusion of WNT4 as a major gene in Rokitansky-Kuster-Hauser anomaly. Am J Med Genet A 137:98–99
13. Coffnier C, Barra J, Babinet C, Yaniv M (1999) Expression of the vHNF1/HNF1beta homeoprotein during mouse organogenesis. Mech Dev 89:211–213
14. Davis RJ, Harding M, Moayed Y, Mardon G (2008) Mouse Dach1 and Dach 2 are redundantly required for Mullerian duct development. Genesis 46:205–213
15. Fei Q, Wu Z, Wang H, Zhou X, Wang N, Ding Y, Wang Y, Ou G (2010) The association analysis of TBX6 polymorphism with susceptibility to congenital scoliosis in a Chinese Han population. Spine 35:983–988
16. Jeays-Ward K, Hoyle C, Brennan J, Dandonneau M, Allus G, Capel B, Swain A (2003) Endothelial and steroidogenic cell migration are regulated by WNT4 in the developing mullerian gonad. Development 130:3663–3670
17. Kloppock E, Schulze H, Strauss G, Ott CE, Hall J, Trotter F, Fleischhauer S et al (2007) Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thorombycopenia-absent radius syndrome. Am J Hum Genet 80:232–240
18. Kobayashi A, Shawlot W, Kania A, Behringer RR (2004) Requirement of LIM1 for female reproductive tract development. Development 131:539–549
19. Kobayashi A, Kwan KM, Carroll TJ, McMahon AP, Mendelsohn CL, Behringer RR (2005) Distinct and sequential tissue-specific activities of the LIM-class homeobox-gene lim1 for tubular morphogenesis during kidney development. Development 132:2809–2823
Von Gen zur Therapie
Antisense-Technik bremst selte ne neurologische Erkrankungen

„Der Schlüssel zur Behandlung Seltener Erkrankungen liegt oft in ihren genetischen Ursachen“, sagt Professor Christine Klein, Neurogenetikerin aus Lübeck und Stellvertretende Präsidentin der Deutschen Gesellschaft für Neurologie (DGN).

Mit der Einführung des „next generation sequencing“ konnten die Aufklärungsrate bei Patienten mit seltenen erbliechen Erkrankungen in den letzten Jahren von 5 auf circa 40% erhöht werden.

Antisense-Therapie
Kürzlich erhielt das Medikament Nusinersen zur Behandlung der Spinalen Muskellatrophi e (SMA) die europäische Zulassung – ein Durchbruch für die neurologische Forschung, denn gegen die seltene schwere Erkrankung gab es zuvor keine Therapieoption. Das Medikament verlangsamt das Fortschreiten der Erkrankung. Die neuromuskuläre Erkrankung betrifft vor allem Kinder. Aufgrund eines Gendefekts gehen Nervenzellen zugrunde, die Bewegungen kontrollieren, weshalb die Muskulatur, einschließlich der Schluck- und Atemmuskulatur, degeneriert.

Die Antisense-Technik ist auch für andere seltenere Erkrankungen eine vielversprechende Option. Derzeit laufen Therapiestudien bei der Huntington-Krankheit. Für die Muskeldystrophie Typ Duchenne ist eine Therapie bereits auf Basis von Antisense-Nukleotiden zugelassen, woraus sich erstmals eine kausale Therapieoption ergibt.

Literatur:
Klein CJ, Foroud TM. Neurology Individualized Medicine: When to Use Next-Generation Sequencing Panels. Mayo Clin Proc. 2017 92(2):292–305. 10.1016/j.mayocp.2016.09.008 Maeder ML, Gersbach CA. Genome- editing Technologies for Gene and Cell Therapy. Mol Ther. 2016 24(3):430–46. 10.1038/mt.2016.10 Prof. Dr. med. Hans-Christoph Diener, Pressesprecher der DGN