MAYER ROKITANSKY SYNDROME

Mayer–Rokitansky–Kuster–Hauser syndrome: Recent clinical and genetic findings

CHARLES SULTAN¹, ANNA BIASON-LAUBER², & PASCAL PHILIBERT³

¹Unité d’Endocrinologie-Gynécologie Pédiatriques, Service de Pédiatrie I, Hôpital Arnaud de Villeneuve, CHU Montpellier, Montpellier, France, ²Universitäts-Kinderklinik, Zurich, Switzerland, and ³Service d’Hormonologie (Développement et Reproduction), Hôpital Lapeyronie, CHU Montpellier, Montpellier, France

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Abstract

Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome is characterized by Müllerian duct aplasia in an XX individual with female phenotype presenting primary amenorrhea at adolescence. Multiple abnormalities may be associated with the MRKH syndrome. Genetic investigations focused on the genes of anti-Müllerian hormone and its receptor, as well as on Wt1, Pax2, Cfr and Hox genes, have been unproductive. Only the Wnt4 gene has been clearly implicated in MRKH syndrome and found to be associated with clinical and/or biological signs of hyperandrogenism in three different works. Beside the multiple malformations that may be associated with MRKH syndrome, such as renal, skeletal, cardiac and auditory defects, MRKH and hyperandrogenism represent a new clinical and genetic disorder.

Keywords: Mayer–Rokitansky–Kuster–Hauser syndrome, adolescent amenorrhea, Wnt4 gene, hyperandrogenism

Introduction

Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome is characterized by Müllerian duct aplasia (uterus plus upper two-thirds of the vagina) in an XX individual with female phenotype (normal breast development and pubarche) presenting primary amenorrhea at adolescence. A clear delineation of the boundaries of MRKH has been difficult, however, given the multiple malformations that may be associated with it, including renal (agenesis, horseshoe kidney), skeletal (vertebral, rib, digits, palate), cardiac and auditory defects. Several classifications have thus been proposed (MRKH type I vs. type II, complete vs. incomplete, typical vs. atypical, MURCS, etc.) and other syndromes with Müllerian duct hypoplasia/aplasia as a common denominator seem to overlap with it (Vater syndrome, facioauriculovertebral syndrome, Winter syndrome, hereditary renal adysplasia).

From a molecular genetics perspective, it is nevertheless essential to work from a consensus regarding the use of a single classification system. We propose the classification suggested by Oppelt and colleagues [1]:

1. Typical MRKH = isolated uterovaginal aplasia/hypoplasia;
2. Atypical MRKH = uterovaginal aplasia/hypoplasia + renal malformation or uterovaginal aplasia/hypoplasia + ovarian dysfunction;
3. MURCS syndrome = uterovaginal aplasia/hypoplasia + renal malformation + skeletal malformation + cardiac malformation.

In a meta-analysis of 521 MRKH cases, Oppelt’s group observed that 64% showed the typical form, 24% showed an atypical form and 12% presented MURCS syndrome.

Given the heterogeneity in MRKH clinical expression, complementary explorations should be carried out in two steps:

1. Systematic, i.e. pelvic magnetic resonance imaging (MRI) + baseline hormonal work-up (plasma follicle-stimulating hormone, luteinizing hormone, testosterone and estradiol);
2. Depending on the associated symptoms, i.e. electromyogram, audiogram, skeletal X-ray or MRI, pelvic laparoscopy.
Genetic features of Mayer–Rokitansky–Kuster–Hauser syndrome

The incidence of MRKH syndrome has not been clearly established, but reports indicate a range of 1/4000 to 1/5000 live female births. Several candidate genes have been studied although no single factor has yet been identified to be responsible for MRKH.

The association of abnormalities in Müllerian duct development (uterus, vagina) with renal, skeletal, cardiac and auditory defects, among others, suggests that the major genes of fetal development and sex differentiation, such as Hox, Wnt and those encoding anti-Müllerian hormone (AMH) and its receptor, are potential candidates. We briefly describe the results of these studies and give particular attention to the one factor recognized as causing Müllerian duct abnormalities.

Candidate-gene studies

Genetic investigations first focused on the genes of AMH and its receptor, given the role of this hormone in the fetal regression of the Müllerian ducts. No abnormal expression of Amh was found, nor any activating mutation of its receptor [2].

The genes implicated in early embryonic development, such as Wt1 (Wilms tumor 1) [3] and Pax2 (paired box gene 2) [4], were also studied, yet none was identified as leading to the abnormalities of MRKH syndrome.

The association of MRKH with galactosemia prompted the search for an abnormality in Galt, the gene for galactose-1-phosphate uridyl transferase, without result [5]. Similarly, the association of MRKH with cystic fibrosis led to an analysis of Cftr, the gene coding for the chloride channel cystic fibrosis transmembrane conductance regulator. This study was negative as well [6].

The candidate-gene approach has been unproductive in that none of the studies clearly pointed to the implication of any of these genes in MRKH syndrome.

The homeobox genes

The homeobox genes comprise a large family of 39 genes categorized into four classes: Hoxa, Hoxb, Hoxc and Hoxd. In mouse, Müllerian duct development implicates only two groups, Hoxa and Hoxd, and more particularly the genes Hoxa7, Hoxa9 to -13 and Hoxd9 to -13, expressed in the Müllerian ducts (Figure 1 [7]). Abnormalities in the development of Müllerian structures are nevertheless observed only in mice deficient in Hoxa10, Hoxa11 and Hoxa13 (homozygous invalidation). These genes are also expressed in the kidney and are required for adequate bone development. It should be recalled that renal and skeletal malformations can be associated with MRKH syndrome [8]. Despite these elements, however, no abnormality in the Hox genes has been identified in women with MRKH syndrome [9].

It thus remains quite difficult to determine the genetic cause or causes of MRKH syndrome. Although Lindner and associates [10] found Müllerian aplasia, expressed as vaginal aplasia and rudimentary uterus, in two females in a family with a novel syndrome characterized by maturity-onset diabetes of the young renal dysfunction and genital malformation due to partial deletion of the pseudo-POU domain of Tcf2 (the gene encoding hepatocyte nuclear factor-1β), the finding remained isolated.
Only the \textit{Wnt4} gene has been clearly implicated in atypical MRKH (i.e. associated with hyperandrogenism).

\textbf{Personal experience: atypical Mayer–Rokitansky–Kuster–Hauser syndrome and Wnt4 gene mutation}

Some of the genes belonging to the family of secreted WNT proteins are also implicated in Müllerian duct development. \textit{Wnt4}, \textit{Wnt5a} and \textit{Wnt7a} are strongly expressed during the development of the internal genital structures of female mice. Moreover, homozygous invalidation of these genes leads to severe abnormalities in the formation of the fallopian tubes, uterus and vagina.

To date, only the \textit{Wnt4} gene has been implicated in women with abnormalities of the Müllerian ducts, with two such cases reported [11,12]. The adolescent girls presented with primary amenorrhea and abnormalities in uterine and vaginal development associated with signs of hyperandrogenism.

In a collaborative French study, we were able to study 28 patients with MRKH syndrome. The purpose of the study was to look for a mutation in the \textit{Wnt4} gene [13]. Of the 28 patients, we identified a new \textit{Wnt4} gene mutation in a patient presenting uterine hypoplasia associated with clinical (acne) and biological signs of hyperandrogenism. This mutation causes a substitution of a leucine in position 12 by a proline and is localized at the level of a potential secretory signal (Figure 2).

In order to confirm the implication of this mutation in the observed phenotype, we performed an \textit{in vitro} functional study. As can be seen in Figure 3, the mutation removes the inhibition of the enzymes implicated in steroidogenesis (3\textbeta-hydroxysteroid dehydrogenase and 17\textalpha-hydroxylase), leading to exaggerated androgen production. Moreover, the \textit{Wnt4} L12P mutation reduces the intranuclear level of \textit{\beta}-catenine, a key transcription factor in Müllerian duct development.

The description of our case is similar to that of the two previously reported cases (Table I) and thus confirms the implication of \textit{Wnt4} in atypical MRKH syndrome, i.e. associated with hyperandrogenism. Moreover, we observed a diminution in the number of follicles in the left ovary in our patient, which agrees with the observation of no follicles in

\begin{table}[h]
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\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Clinical features} & \textbf{Case 1 (Biason-Lauber et al. [11] as reported in [13])} & \textbf{Case 2 (Biason-Lauber et al. [12] as reported in [13])} & \textbf{Case 3 (present report)} \\
\hline
\textbf{Age} & 18 years 7 months & 19 years 6 months & 16 years 8 months \\
\textbf{Weight (kg)} & 47.0 & 69.2 & 62.0 \\
\textbf{Height (cm)} & 148.0 & 148.6 & 165.0 \\
\textbf{BMI (kg/m$^2$)} & 18.8 & 31.6 & 22.8 \\
\textbf{Acne (treatment)} & yes (antiandrogen) & yes (n/a) & yes, severe (OC) \\
\textbf{Hirsutism} & n/a & mild facial & no \\
\textbf{Clitoris size} & normal (0.5 cm) & normal (0.7 cm) & normal \\
\textbf{Breasts and pubic hair} & Tanner 5 & Tanner 5 & Tanner 4 \\
\textbf{Age at adenarche (years)} & 12 & 10 & 11 \\
\textbf{Karyotype} & 46,XX (20 cells) & 46,XX (20 cells) & 46,XX (20 cells) \\
\hline
\textbf{Hormonal data} & & & \\
\textbf{T (nmol/l)} & 4.6 (0.3–3.4) & 6.86 (0.3–3.4) & 1.8 (0.3–2.0) \\
\textbf{Free T (pmol/l)} & 19.9 (2.4–12.4) & n/a & 6.4 (1.4–8.7) \\
\textbf{A (nmol/l)} & 25.4 (2.8–8.0) & 8.53 (1.4–8.9) & 7.15 (1.7–6.9) \\
\textbf{DHEA (pmol/l)} & 11.8 (2.2–9.2) & 5.0 (2.2–9.0) & 4.0 (1.3–5.4) \\
\textbf{P (nmol/l)} & 16.2 & 1.9 (1–64) & n/a \\
\textbf{17OHP (nmol/l)} & 5.4 & 7.2 (0.5–9.5) & 3.3 (0.3–5.0) \\
\textbf{E$_2$ (pmol/l)} & 179 & 216 (76–1285) & 367 (73–550) \\
\textbf{LH (IU/l)} & 10.0 & 5.3 (4.5–25.0) & 5.5 (1.5–6.0) \\
\textbf{FSH (IU/l)} & 6.4 & 4.5 (4.0–20.0) & 3.1 (3.0–8.0) \\
\textbf{LHRR test} & n/a & n/a & ↑ response of LH (45 U/l) \\
\hline
\textbf{Pelvic ultrasonography} & & & \\
\textbf{Ovary size} & normal/ecotopic & normal/ecotopic & right subnormal/left rare follicles \\
\textbf{Uterus} & absent & agenesis & hypoplastic \\
\textbf{Fallopian tubes} & normal & normal & normal \\
\textbf{Kidneys} & right aplastic & normal & normal \\
\textbf{Mutation} & E226G & R83C & L12P \\
\hline
\end{tabular}
\caption{Clinical and biological description of three cases of atypical Mayer–Rokitansky–Kuster–Hauser syndrome resulting from a \textit{Wnt4} mutation (adapted from [13]).}
\end{table}

BMI, body mass index; T, testosterone; A, androstenedione; DHEA, dehydroepiandrosterone; P, progesterone; 17OHP, 17-hydroxyprogesterone; E$_2$, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; LHRH, luteinizing hormone-releasing hormone; n/a, not available.
the Wnt4 ε/ε mouse. It thus seems likely that Wnt4 is also implicated in ovarian function.

**Conclusion**

MRKH syndrome does not present with clearly identified genetic causes, with the exception of an atypical MRKH associated with signs of hyperandrogenism. In this specific case, it is legitimate and necessary to look for a modification in the Wnt4 gene, the only gene identified to date as causing abnormalities in the Müllerian ducts.

Beside the multiple malformations that may be associated with MRKH syndrome, such as renal, skeletal, cardiac and auditory defects, MRKH and hyperandrogenism represent a new clinical and genetic disorder.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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