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Genetic analysis of Mayer-Rokitansky-Kuster-Hauser syndrome in a large cohort of families

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Objective: To study the genetic cause of Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH). Although a few candidate genes and genomic domains for have been reported for MRKH, the genetic underpinnings remain largely unknown. Some of the top candidate genes are *WNT4*, *HNF1B*, and *LHX1*. The goals of this study were to: 1) determine the prevalence of *WNT4*, *HNF1B*, and *LHX1* point mutations, as well as new copy number variants (CNVs) in people with MRKH; and 2) identify and characterize MRKH cohorts.
Design: Laboratory- and community-based study.
Setting: Academic medical centers.
Patient(s): A total of 147 MRKH probands and available family members.
Interventions(s): DNA sequencing of *WNT4*, *HNF1B*, and *LHX1* in 100 MRKH patients, chromosomal microarray analysis in 31 North American MRKH patients, and characterization and sample collection of 147 North American and Turkish MRKH probands and their families.
Main Outcome Measure(s): DNA sequence variants and CNVs; pedigree structural analysis.
Result(s): We report finding CNVs in 6/31 people (~19%) with MRKH, but no point mutations or small indels in *WNT4*, *HNF1B*, or *LHX1* in 100 MRKH patients. Our MRKH families included 43 quads, 26 trios, and 30 duos. Of our MRKH probands, 87/147 (59%) had MRKH type 1 and 60/147 (41%) had type 2 with additional anomalies.
Conclusion(s): Although the prevalence of *WNT4*, *HNF1B*, and *LHX1* point mutations is low in people with MRKH, the prevalence of CNVs was ~19%. Further analysis of our large familial cohort of patients will facilitate gene discovery to better understand the complex etiology of MRKH. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Müllerian aplasia, MRKH, reproductive genetics, congenital absence of the uterus and vagina, gene mutation

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Mayer-Rokitanksy-Kuster-Hauser syndrome (MRKH; MIM #27700), also known as müllerian aplasia, consists of the congenital absence of the uterus and vagina. MRKH, which is the name patients prefer, accounts for 10% of the cases of primary amenorrhea (1), and it affects ~1/5,000 women (2). This anomaly constitutes the most severe malformation of the female reproductive tract, and occurs in isolation in two-thirds of patients, often referred to as type 1 (2). A subset of patients (type 2) presents with associated structural abnormalities such as unilateral renal agenesis (30%), skeletal defects (10%–15%), cardiac anomalies (2%–3%), and deafness (2%–3%). These patients have a normal 46,XX karyotype and typically exhibit normal ovarian

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function with normal development of breasts and external genitalia (2, 3).

The molecular pathways of müllerian development have been well studied in nonhuman animal models that document the importance of WNT signaling, because mice with null or mutant alleles for *Wnt9b*, *Wnt4*, *Wnt5a*, and *Wnt7a* manifest varying degrees of müllerian hypoplasia (4). In addition, other genes, such as *Pbx1*, *Pax2*, *Lhx1*, and *Emx2*, have been implicated in normal müllerian development in the mouse (5, 6). However, the genetic underpinnings of human müllerian developmental abnormalities are largely unknown. Although many cases are sporadic, some familial cases have been reported, suggesting a genetic role in the pathogenesis in some patients (7). Genetic transmission is difficult to ascertain, because affected families are often small and affected individuals are unable to have children unless they undergo surrogacy or uterine transplantation (3, 8).

A number of chromosomal regions and candidate genes have been studied in humans with MRKH, and several have been observed by different investigators, but conclusive evidence for causation is lacking (3) except for causative mutations in *WNT4* (9) and *HNF1B* (10). An *LHX1* nonsense variant has been reported, but the segregation within families and in vitro confirmation have not been documented (11). However, studies in mice suggest that mutations in *Lhx1* could lead to an MRKH-like phenotype (12–14). Chromosomal microarrays have suggested numerous copy number variants (CNVs) associated with MRKH, with 17q12 and 16p11 being two more commonly affected regions (3, 15). These CNVs contain multiple genes, and it is currently not clear if MRKH is a genomic disorder or if one or a few genes within these regions could be involved in its pathophysiology (3).

The genetic component of MRKH may be complex, and complete understanding is hindered by the lack of large collections of MRKH families. Herlin et al. (16) reported one family with two MRKH probands and reviewed the literature, reporting 67 families with at least two MRKH patients or one MRKH and one with MRKH-associated anomalies. However, these families were identified from multiple publications in the literature, and there has not been any large characterization of unselected MRKH patients from a single research team. In addition, and importantly, the prevalence of gene mutations in *WNT4*, *HNF1B*, and *LHX1* has not been substantiated in a relatively large sample of MRKH women. The purpose of the present study was to: 1) collect and obtain clinical information and blood samples from a large cohort of MRKH families containing at least one MRKH patient; 2) determine the prevalence of variants in two accepted MRKH genes—*WNT4* and *HNF1B*—as well as the candidate gene *LHX1*; and 3) to determine if CNVs are present in a subset of our North American MRKH patients that are absent in their unaffected parents.

MATERIALS AND METHODS

Cohort Characterization

Patients and families were recruited to participate via ascertainment of MRKH probands through our Developmental Gene Discovery Project (DGDP). Many probands were collected by authors L.C.L. and O.M.A., but a substantial number were identified from the Beautiful You MRKH Foundation (author A.C.L.). MRKH was defined as a female with normal breast development, Tanner 5 pubic hair, and an absent vagina and uterus based on physical exam, supported by imaging (ultrasound and/or magnetic resonance imaging) and/or surgery (2, 3). All patients had a 46,XX karyotype except our one previously reported patient with a chromosomal translocation involving chromosomes 3 and 16 (17). MRKH-associated anomalies were identified by reviewing medical records and obtaining family history. Every attempt was made to collect available family members. Peripheral blood was collected for creating lymphoblastoid cell lines and extracting DNA as described previously (18). Lymphoblastoid cells were created so that a long-term supply of DNA, RNA, and protein could be available for in vitro analyses on identified genetic variants for confirmation. This study was approved by the Institutional Review Board at Augusta University, and all participating patients and available family members signed consent forms.

Sanger DNA Sequencing for *WNT4*, *LHX1*, and *HNF1B*

DNA was extracted from peripheral blood leukocytes as described previously (18, 19). Sanger sequencing was performed on a cohort of 100 MRKH patients (79 with type 1 and 21 with type 2) for the protein-coding regions and splice junctions for five exons of *WNT4* (NM_030761.4), five exons of *LHX1* (NM_005568.3), and nine exons of *HNF1B* (NM_000458.3). Each fragment was amplified by means of polymerase chain reaction (PCR) for 30 cycles consisting of a 5-minute denaturation step at 95°C followed by 30 cycles of the following: 1 minute at 95°C, 30–60 seconds at 55°C, and 30–60 seconds at 72°C—followed by a 7-minute 72°C extension step. Each fragment was resolved by agarose gel electrophoresis, and then an aliquot of the sample was subjected to dideoxy sequencing on an ABI 310 Automated DNA Sequencer as described previously (20–22). Each fragment was sequenced in the forward and reverse directions. If a variant was identified, two additional sequencing reactions were performed. The obtained sequence was blasted to the wild-type sequence.

Chromosomal Microarrays

DNA from 31 unrelated North American MRKH probands (10 with type 1 and 21 with type 2) was subjected to chromosomal microarrays. CNV analysis was performed at Harvard with the use of an Affymetrix Cytoscan HD array, which consisted of 750,000 single-nucleotide polymorphism probes and 1.9 million copy number probes to detect CNVs. The lower limit of detection for CNVs was 50 kb. One hundred nanograms of genomic DNA was labeled with the use of Cytoscan reagent kit according to the manufacturer's instructions. The array data were analyzed with Chromosome Analysis Suite software as described previously (22). Human genome hg19 assembly was used to map genomic coordinates.