Mayer-Rokitansky-Küster-Hauser syndrome discordance in monozygotic twins: matrix metalloproteinase 14, low-density lipoprotein receptor–related protein 10, extracellular matrix, and neoangiogenesis genes identified as candidate genes in a tissue-specific mosaicism

Katharina Rall, M.D.,[a] Simone Eisenbeis, Ph.D.,[a] Gianmaria Barresi, M.D.,[a] Daniel Rückner, M.D.,[a] Michael Walter, Ph.D.,[b] Sven Poths,[b] Diethelm Wallwiener, M.D.,[a] Olaf Riess, M.D.,[b] Michael Bonin, Ph.D.,[b] and Sara Brucker, M.D.[a]

[a] Department of Obstetrics and Gynecology and Center for Rare Female Genital Malformations, and [b] Department of Medical Genetics, Microarray Facility, Tübingen University Hospital, Tübingen, Germany

Objective: To find a potential underlying cause for Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) discordance in monozygotic twins.
Design: Prospective comparative study.
Setting: University hospital.
Patient(s): Our study genetically analyzed 5 MRKHS-discordant monozygotic twin pairs with the unique opportunity to include saliva and rudimentary uterine tissue.
Intervention(s): Blood, saliva, or rudimentary uterine tissue from five MRKHS-discordant twins was analyzed and compared between twin pairs as well as within the same individual where applicable. We used copy number variations (CNVs) to identify differences.
Main Outcome Measure(s): CNVs in blood, rudimentary uterine tissue, and saliva, network analysis, and review of the literature.
Result(s): One duplication found in the affected twin included two genes, matrix metalloproteinase 14 (*MMP14*) and low-density lipoprotein receptor–related protein 10 (*LRP10*), which have known functions in the embryonic development of the uterus and endometrium. The duplicated region was detected in rudimentary uterine tissue from the same individual but not in saliva, making a tissue-specific mosaicism a possible explanation for twin discordance. Additional network analysis revealed important connections to differentially expressed genes from previous studies. These genes encode several molecules involved in extracellular matrix (ECM) remodeling and neoangiogenesis.

Received August 4, 2014; revised and accepted October 31, 2014; published online December 6, 2014.
K.R. was the recipient of a temporary research fellowship within the PATE, Fortune and TUFF programs (nos 1835-0-0, 2047-0-0, and 2159-0-0) of the University of Tubingen. S.E. has nothing to disclose. G.B. has nothing to disclose. D.R. has nothing to disclose. M.W. has nothing to disclose. S.P. has nothing to disclose. D.W. has nothing to disclose. O.R. has nothing to disclose. M.B. has nothing to disclose. S.B. has nothing to disclose.
M.B. and S.B. should be considered similar in author order.
Reprint requests: Katharina Rall, M.D., Department of Obstetrics and Gynecology and Center for Rare Female Genital Malformations, Tübingen University Hospital, Calwerstr. 7, 72076 Tübingen, Germany (E-mail: katharina.rall@med.uni-tuebingen.de).

Fertility and Sterility® Vol. 103, No. 2, February 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.10.053

Conclusion(s): MMP-14, LRP-10, ECM, and neoangiogenesis genes are identified as candidate genes in a tissue-specific mosaicism. The detected clusters provide evidence of deficient vascularization during uterine development and/or disturbed reorganization of ECM components, potentially during müllerian duct elongation signaled by the embryologically relevant phosphatidylinositol 3-kinase/protein kinase B pathway. Therefore, we consider these genes to be new candidates in the manifestation of MRKHS. (Fertil Steril® 2015;103:494-502. ©2015 by American Society for Reproductive Medicine.)

Key Words: Uterovaginal aplasia, MRKH syndrome, CNV, monozygotic discordant twins, mosaicism

The Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS; OMIM 277000), also referred to as müllerian aplasia or vaginal agenesis, affects at least 1 in 4,500 females. MRKHS is characterized by congenital absence of the uterus and the upper two-thirds of the vagina in otherwise phenotypically normal females with normal secondary sexual characteristics and a 46,XX karyotype (1, 2). During mammalian fetal development, the female and male reproductive tracts develop from the müllerian ducts (MD) and the wolffian ducts, respectively. In the mouse, genes required for initial MD formation have been identified by targeted mutagenesis. The association of abnormalities in MD development with other defects suggests that crucial genes of fetal development and sex differentiation, such as *HOX* and *WNT*, are potential candidates (2–4). However, no structural abnormalities or variations in *HOX* genes or in hormones regulating *HOX* expression have been identified in women with MRKHS (5–7).

Heterozygous mutations of *WNT4* have been detected in a distinct clinical entity, müllerian aplasia and hyperandrogenism (8–16). This is in good agreement with findings in Wnt4-null mutant mice, which lack MD formation but exhibit wolffian duct formation (8, 12–16).

In a previous study, we used, for the first time, a combined whole-genome expression and methylation approach to investigate the etiology of the MRKHS. The findings suggested that the abnormal development of the female reproductive tract might be due to either deficient estrogen receptors or the ectopic expression of certain *HOXA* genes (2).

In addition to genetic factors, chemicals with estrogen-like effects have been discussed as candidate causes of MRKHS. There are a number of examples of such "endocrine disruptors" that have a negative effect on uterine development by adversely affecting MD development. Diethylstilbestrol (DES), for example, can alter cell proliferation in the developing reproductive tract of the female rat by disrupting normal expression of epidermal growth factor and estrogen receptors 1 and 2 (17). On exposure of fetal mice to DES, MD cell proliferation in the proximal epithelium and mesenchyme is increased but in the caudal epithelium is decreased (18).

MD formation occurs in three phases: initiation, invagination, and elongation (19). The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway plays a central role in MD development. Signaling or interaction between the MD epithelium and the surrounding extracellular matrix (ECM) may be the key to proper development (20). The PI3K/AKT pathway in the MD is also required for the activation of matrix metalloproteinases (MMPs) (20–22). MMPs not only degrade structural components of the ECM, thereby facilitating cell migration, but also affect cellular signaling and functions (23). The inter-alpha-trypsin inhibitor heavy chain 5 gene (*ITIH5*) belongs to a family of genes that encode proteins involved in the dynamics of the ECM. In 2012, Morcel et al. reported the first case of a 10p14 deletion associated with uterovaginal aplasia, a finding that supports *ITIH5* as a putative candidate gene for MRKHS (24).

Reported cases of MRKHS-discordant monozygotic (MZ) twins have challenged a purely genetic basis for this syndrome (25–29). To date, attempts to identify a genetic locus with the use of standard genetic linkage analysis have been mainly unsuccessful.

Recently, several recurrent copy number variants (CNVs) have been reported in patients with isolated and syndromic müllerian aplasia and other disorders of sexual development (30–36). With the use of array comparative genomic hybridization (array CGH), different chromosomal regions have been found to be associated with MRKHS. However, none of the CNVs occurred consistently in a larger group of patients.

CNVs account for much of the genome and are strongly polymorphic and relatively unstable (37, 38). De novo CNVs and CNV mosaicism may be partly responsible for phenotypic discordance in MZ twins (37, 39–42). Bruder et al. showed that CNVs exist in pairs of MZ twins with concordant and discordant phenotype, suggesting that CNV analysis in phenotypically discordant monozygotic twins may be valuable in identifying disease-predisposing loci (41).

The advantage of analyzing a number of monozygotic discordant twins is that even a single pair of twins could be sufficient to reveal the genetic cause. Additionally, we had the unique opportunity to include saliva and rudimentary uterine tissue and not only blood, a fact that increases the chance of finding tissue-specific mosaicisms. We here report on the first study conducted to identify candidate genes for MRKHS in five pairs of discordant MZ twins, a considerable number in view of the infrequency of the syndrome.