ORIGINAL ARTICLE: GENETICS

Minichromosome maintenance complex component 8 mutations cause primary ovarian insufficiency

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Objective: To investigate whether mutations in the minichromosome maintenance complex component 8 *(MCM8)* gene were present in 192 patients with sporadic primary ovarian insufficiency (POI).
Design: Retrospective case-control cohort study.
Setting: University-based reproductive medicine center.
Patient(s): A total of 192 patients with sporadic POI and 312 control women with regular menstruation (192 age-matched women and 120 women >45 years old).
Intervention(s): Sanger sequencing was performed in patients with sporadic POI, and potentially pathogenic variants were confirmed in matched controls. DNA damage was induced by mitomycinC (MMC) treatment, and DNA repair capacity was evaluated by histone H2AX phosphorylation level.
Main Outcome Measure(s): Sanger sequencing for *MCM8* was performed in 192 patients with sporadic POI, and functional experiments were performed to explore the deleterious effects of mutations identified.
Result(s): Two novel missense variants in *MCM8*, c. A950T (p. H317L), and c. A1802G (p. H601R), were identified in two patients with POI but absent in 312 controls (the upper 90% confidence limit for the proportion 2/192 is 2.24%). The HeLa cells overexpressing mutant p. H317L and p. H601R showed higher sensitivity to MMC compared with wild type. Furthermore, mutant p. H317L showed decreased repair capacity after MMC treatment with much more histone H2AX phosphorylation remaining after 2 hours of recovery.
Conclusion(s): Our result suggests novel mutations p. H317L and p. H601R in the *MCM8* gene are potentially causative for POI by dysfunctional DNA repair. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: POI, MCM8 mutation, DNA repair

Discuss: You can discuss this article with its authors and with other ASRM members at

Primary ovarian insufficiency (POI), also known as premature ovarian failure (POF[MIM 311360]), is defined as the occurrence of ovarian insufficiency before 40 years old, accompanied with elevated levels of FSH (usually >40 IU/L) and decreased levels of E_2 (1). As a heterogeneous disorder phenotypically and etiologically, the causes of POI in most patients remain unknown. The reported etiology includes chromosomal abnormalities, gene mutations, autoimmune, iatrogenic, and environmental factors (2). Evidence for genetic factors has been provided by population and candidate gene studies (1). However, at present, only a small fraction of candidate genes have been proved causative. Genes on the X chromosome can be considered to be *BMP15*, *PGRMC1*, and *FMR1* (3–5). Genes on autosomes include *FSHR*, *GDF9*, *NR5A1*, *NOBOX*, *FIGLA*, and *FOXL2* (6–11). Recently, genes related with DNA damage repair and meiosis have been identified causative in familial patients, such as *MCM8*, *MCM9*, *HFM1*, and *STAG3* (12–16).

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Xiaoyun Dou and Ting Guo should be considered similar in author order.
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TABLE 1

Clinical features of sporadic patients with POI and controls.

Characteristic	Sporadic POI	Control 1	Control 2
No. of patients	192	192	120
Age (y)	22.9 ± 4.2	22.35 ± 4.50	46.69 ± 1.67
Age at menarche (y)[a]	14.9 ± 2.3	14.8 ± 2.7	–
Age of amenorrhea (y)[a]	22.1 ± 4.4	NA	NA
FSH (IU/L)	77.8 ± 28.0	6.76 ± 1.80	8.47 ± 1.69
Patients with primary amenorrhea	33		
Patients with secondary amenorrhea	136		
Patients without amenorrhea	23		

Note: NA = not available; POI = primary ovarian insufficiency.
[a] Secondary amenorrhea.

Dou. MCM8 mutations and primary ovarian insufficiency. Fertil Steril 2016.

Minichromosome maintenance complex component 8 (*MCM8)*, responsible for homologous recombination and DNA double-stranded break (DSB) repairs, belongs to the evolutionarily conserved MCMs protein family. The MCM family plays a crucial role in DNA replication (17). During the S phase, six MCMs subunits, *MCM2* to *MCM7*, form a complex and function as a critical component of eukaryotic replicative helicase (18). Different from other homologues, *MCM8* is not only involved in the assembly of prereplication complex (19), but also participates in homologous recombination during meiosis and DSB repair by dimerizing with *MCM9* (20–23). Mutants of *Drosophila* Mcm8 homologue exhibit defects in meiotic crossover (24). In mice, Mcm8 knockout results in early block of follicle development and small gonads (25). Recently, a causative homozygous mutation has been found in a consanguineous family with three patients with POI (26). However, the contribution of *MCM8* in patients with sporadic POI remains elusive. Therefore, we sequenced the coding region of *MCM8* in 192 patients with sporadic POI. Two novel heterozygous mutations were found with functional impairment in DNA damage repair, indicating the potential role of *MCM8* in maintenance of ovarian function mediated by functional DNA repair.

MATERIALS AND METHODS

Subjects

In this study, 192 patients with sporadic POI and 312 control women were recruited between January 1, 2009 and September 1, 2014, in the Center for Reproductive Medicine, Shandong University. The POI inclusion criteria included primary or secondary amenorrhea, serum FSH level >40 IU/L, 46, XX karyotype, and no family history of POI. Known causes, such as autoimmune diseases, *FMR1* premutation, pelvic surgery, and chemoradiotherapy treatment were excluded. The 312 control women with regular menstruation included 192 age-matched women and 120 women >45 years, whose inclusion criteria were: [1] serum FSH level <12 IU/L and [2] three or more antral follicles remaining in bilateral ovaries. The clinical characteristics of cases and controls were shown in Table 1. Written informed consent was obtained from all participants. This study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University ([2015]IRBNo.46).

Sanger Sequencing

Polymerase chain reaction (PCR) for 19 exons of *MCM8* was performed using primers listed in Supplemental Table 1, available online. The PCR products was purified, labeled by BigDye (Terminatorv3.1 Cycle Sequencing Kits, Applied Biosystems), and sequenced by ABI 3730XL (Applied Biosystems). All of variants were confirmed by three independent PCR runs and sequenced in both forward and reverse strands.

Construction of Green Fluorescent Protein (GFP)-MCM8 Plasmid

Coding sequence of wild type was amplified by PCR (forward primer: 5′-GAAAGATCTCAATGGAAGTATAGAGGAGAG-3′; reverse primer: 5′- CGGGATCCGGTGAAGTCCTTTTACATAGTTTG-3′) with vector CH881381 as template (ViGene Biosciences). The amplicons were ligated between *Bgl*II and *Bam*HI sites in pEGFP-C3 vector (BD, Biosciences). Mutant p. H317L and p. H601R were introduced by site-directed mutagenesis using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) with primers listed in Supplemental Table 2, available online. All the plasmids were confirmed by Sanger sequencing.

MitomycinC Sensitivity Assay and Western Blot

HeLa (human cervix carcinoma cell line) were cultured in Dulbecco modified Eagle medium (DMEM) with 10% fetal bovine serum (GIBCO) at 37°C. Wild-type MCM8, mutant p. H317L and p. H601R plasmids were transfected into HeLa cells with Lipofectamine 3000 Transfection Reagent transiently (Invitrogen) and cultured for 24 hours. Then, the HeLa cells overexpressing wild-type or mutant MCM8 were exposed to 0.5 μg/mL mitomycinC (MMC; Melonepharma) for 6 hours to induce DSBs, and harvested immediately or after recovery for 2 hours in culture medium at 37°C. Then, the HeLa cells with or without MMC treatment were lysed in immunoprecipitation assay buffer with 1 mM phenylmethanesulfonyl fluoride (Beyotime). Protein lysates (50–60 μg) were diluted with 5× sodium dodecyl sulfate (SDS) loading buffer (Beyotime) and separated on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Samples were transferred to polyvinylidene fluoride membranes, blocked with 5% nonfat dry milk in Tris-buffered saline and Tween 20, and incubated with primary antibody against γH2AX, a phosphorylated histone variant H2AX at serine139 (rabbit, Cell Signaling, 1:1,000 dilution), overnight at 4°C. Membranes were washed with Tris-buffered saline and Tween 20, incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody for 2 hours at room temperature, and subjected to chemiluminescent detection with ChemiDoc MP System. The anti-β-actin antibody (rabbit, Sigma, 1:5,000 dilutions) was used as control.