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Original Article

Mutation of *MDM2* gene in Chinese Han women with idiopathic premature ovarian insufficiency

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Abstract: Objective: Recent animal studies have demonstrated that the deletion of mouse double minute 2 (Mdm2) in mice leads to premature ovarian insufficiency (POI). The aim of the present study was to investigate whether mutations in the *MDM2* gene contribute to POI in Chinese Han women. Methods: The coding region of the *MDM2* gene was examined in 54 Chinese Han women with idiopathic POI and 54 Han healthy controls. Two known single nucleotide polymorphisms (SNPs), rs937283 in 5'-UTR and rs2870820 in intron 1, were compared between both POI and control groups. Results: There were no significant differences in the genotype distributions or allelic frequencies between the POI and control groups. No plausible causative mutations were identified. Conclusion: Our findings suggest that mutations in the coding region of the *MDM2* gene may not represent a risk factor in the pathogenesis of idiopathic POI among Chinese Han women. Although we fail to confirm that *MDM2* is a disease-causing gene, our study is the first to investigate the role of *MDM2* in POI patients. Further studies with larger sample size from different ethnic populations are warranted.

Keywords: *MDM2*, mutation, single nucleotide polymorphism, premature ovarian insufficiency, polymerase chain reaction

Introduction

Premature ovarian insufficiency (POI) is one of the important causes of female infertility, which is characterized by elevated gonadotrophin level (FSH>40 IU/l) and hypoestrogenism as a result of the cessation of ovarian function before the age of 40 years [1]. POI is a frequent pathologic process that affects 1-2% of women in the general population and the overall prevalence has been reported to be approximately 1-2% worldwide. POI is a heterogeneous disease caused by multiple mechanisms, such as environmental toxins, iatrogenic injuries, autoimmune diseases, infections and genetic factors [2]. Although gene screening indicates that an increasing number of genes may be causative genes, the plausible cause of POI, remains undetermined in most cases.

Murine double minute 2 (*MDM2*) is a major p53-negative regulator, which is capable of preventing the induction of apoptosis in numerous cell types [3]. *MDM2* is overexpressed in various types of carcinoma in humans [4], such as breast carcinoma [5] and soft tissue sarcomas [6]. Recently, researchers have specifically deleted *MDM2* in oocytes at different stages of folliculogenesis in mouse models. Livera *et al*. [7] have demonstrated that mice with specific *MDM2* deletion in the oocytes of growing follicles display impeded fertility and a drastic loss of secondary and more mature follicles, a strong increase in the serum FSH and LH levels, and eventually the POI phenotype. The results have been confirmed by another study [8], which further demonstrated that specific deletion of *MDM2* in the oocytes of the primordial follicles in mouse models can result in onset of infertility with a rapid loss of primordial follicles, elevated levels of serum FSH, and early termination of ovarian activity. Taken together, the specific *Mdm2* deletion at different stages of folliculogenesis in mouse models can lead to

Table 1. Primers for amplification of the coding region of *MDM2* gene

Primer ID	Sequence 5'-3'
MDM2-E1F	GTGTGGCCCTGTGTGTCGGA
MDM2-E1R	CGAAATCCCGCCCTCCTCCC
MDM2-E2F	GTAGACCTGTGGGCACGGACG
MDM2-E2R	CCACGCAGTTACGCCAGAGGTA
MDM2-E3F	GCAGGGCCTATAGTTCT
MDM2-E3R	CAATTTCTCCACATGGTCT
MDM2-E4F	TGGTTCCTGGTTGTTTACCCCTAT
MDM2-E4R	AGATGCCAGAGCTCAGGTTCTCAA
MDM2-E5F	TGGGGGTCTTCTGGTAAAGTCCA
MDM2-E5R	TCACTCCTAAACAGGAGCTTTTGAA
MDM2-E6-7F	GATAGCATATCTACTGAGT
MDM2-E6-7R	ACAGTAAACTGTGCCTGCT
MDM2-E8F	CTGCTGTAACAGTTGGACAGA
MDM2-E8R	TGGTGCAGTGGTCCACAAAT
MDM2-E9F	TCTTCTGCCCGCCGATCTCC
MDM2-E9R	AATCCTCAAGTCCACAAACCAATGT
MDM2-E10F	ATAGCTACAGGTCTCATCACAAC
MDM2-E10R	TGTTTCCCAGAAGTGACTGCC
MDM2-E11F	GCTAGCATTCCTGTGACTGAGCA
MDM2-E11R	AGGAGTTGGTGTAAAGGATGAGC

The annealing temperature of all reactions is 55°C.

phenotypes mimicking human POI due to either early ovarian reserve depletion or defects during the process of follicular growth and maturation, supporting a critical role of *MDM2* gene in ovarian function in mouse models.

Based on the findings obtained from the rodent models, we speculated that *MDM2* was a candidate gene for human POI. Therefore, the objective of the present study was to investigate for the first time that whether *MDM2* mutation contributes to the incidence of human POI by screening the coding region of *MDM2* in Chinese Han women with idiopathic POI.

Materials and methods

Patients

A total of 54 Chinese Han women with idiopathic POI and 54 healthy Chinese Han female controls admitted to Reproductive Medicine Department of Dezhou People's Hospital and Weihai Second Municipal Hospital of Qingdao University between 2016 and 2018 were recruited in this study. The inclusion criteria for POI were amenorrhea for at least 6 months occurring before age of 40 and at least two serum FSH concentrations exceeding 40 IU/l with 4-6 weeks interval. Patients with known pelvic surgery, autoimmune diseases, chromosomal abnormality, or anticancer treatment were excluded. The control group was composed of 54 healthy Chinese Han women under 40 years old with regular menstrual cycles, normal ovary morphology by pelvic ultrasound and proven fertility. The study procedures were approved by the Ethics Committee of the Reproductive Medicine Department of Dezhou People's Hospital and Weihai Second Municipal Hospital of Qingdao University. Written informed consents were obtained from all participants prior to the study.

Mutational analysis

Genomic DNA was extracted from the peripheral leukocytes using EZ-10 Spin Column Blood Genomic DNA Miniprep Kit (Bio Basic, Canada) strictly according to the manufacturer's instructions. The whole coding region and exon-intron boundaries of MDM2 were amplified by polymerase chain reaction (PCR) with ten pairs of primers (**Table 1**). PCR assay was carried out in a 25 μl reaction mixture containing 20 ng of DNA, 10 pmol/μl of each primer, 2.5 mmol/l $MgCl_2$, 10 mmol/l dNTP, and 2.5 U Taq DNA polymerase (Thermo Scientific, China). The thermal cycler parameters were as follows: initial denaturation at 95°C for 5 min, 35 amplification cycles of 95°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for 40 s (elongation) and a final elongation step at 72°C for 7 min. After confirmation by agarose gel electrophoresis, the PCR products were sequenced directly on an automated sequencer (3730XL; Applied Biosystems). All of the sequence variants were confirmed by three independent PCR runs, followed by sequencing of both the forward and reverse strands.

Statistical analysis

The Sequencer 4.9 software was utilized for the analysis of sequencing results. Statistical Package for Social Sciences (SPSS) version 16.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The chi-square test or Fisher's exact test (two tailed) were employed where appropriate. A *P* value of less than 0.05 was considered significant.

Table 2. Known single nucleotide polymorphisms in *MDM2* gene identified between the POI and control groups

Location	Sequence Variation	Genotype	Genotype frequency in POF, n (%)	Genotype Frequency in Control, n (%)	*P*-value	Allele	Allele Frequency in POF, n (%)	Allele Frequency in Control, n (%)	*P*-value
5'-UTR	rs937283 c.-707A>G	AA	28	25	NS	A	79	76	NS
		AG	23	26					
		GG	3	3		G	29	32	
Intron 1	rs2870820 c.-545C>T	CC	28	25	NS	C	79	76	NS
		CT	23	26					
		TT	3	3		T	29	32	

NS = not statistically significant.

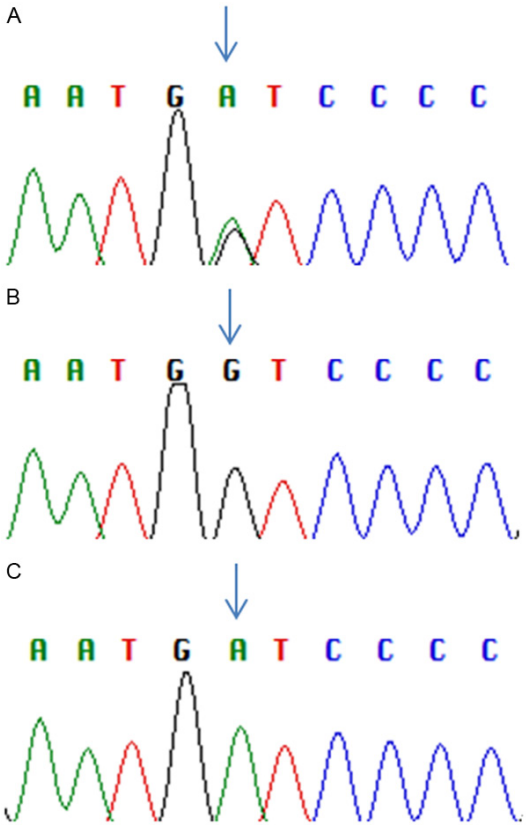

Figure 1. Sequencing map of rs937283 in MDM2. A. AG; B. GG; C. AA.

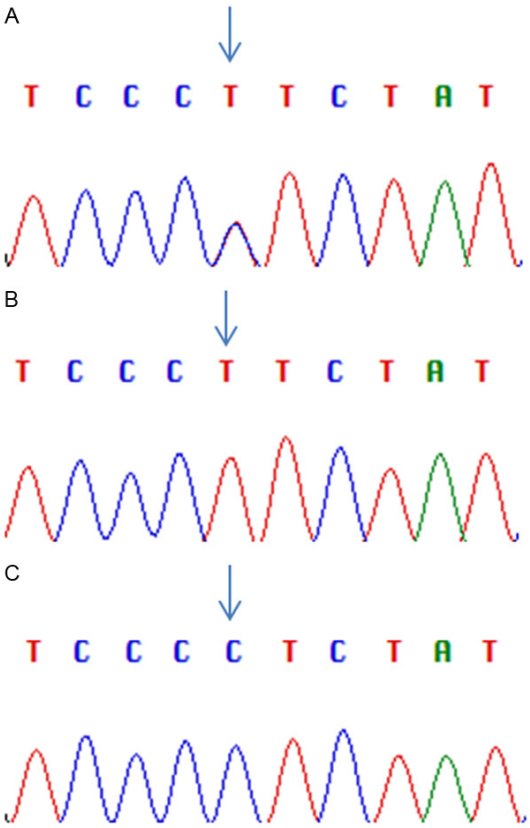

Figure 2. Sequencing map of rs2870820 in MDM2. A. CT; B. TT; C. CC.

Results

The coding region of the *MDM2* gene was examined in 54 Chinese Han women with idiopathic POI and 54 Han healthy female controls. Two known single nucleotide polymorphisms (SNP) including *rs937283* in the 5'-UTR and *rs2870820* in the intron 1, were identified in both the POI and control groups.

Statistical comparison of the genotype and allelic frequencies between POI cases and healthy controls showed no significant differences in either of the two SNPs (*rs937283* in the 5'-UTR and *rs2870820* in the intron 1), as illustrated in **Table 2**. Moreover, no additional polymorphisms or mutations were subsequently identified, as illustrated in **Figures 1**, **2**.

Discussion

POI is a heterogenetic disorder with an acknowledged genetic component. However, the pathogenesis of POI remains unexplained in a major-

ity of cases in clinical practice. The fact that variants were identified by other than genome-wide association study (GWAS) on ovarian aging indicates an inherent limitation of GWAS to identify all the genetic variants to POI [9]. Consequently, screening of the candidate variants should help to explore the underlying genetic pathogenesis of POI. Desai *et al*. have explored the frequency of variants in *MCM8* and *MCM9* in 155 participants with primary POI under the age of 40 and identified a large quantity of novel variants in *MCM8* and *MCM9* genes among the enrolled POI women, and explored a multi-allelic association with variants in the *MCM8* and *MCM9* interactome genes [9-11].

Previous studies have confirmed that increased levels of the MDM family members MDM2 and MDM4 are correlated with the occurrence of breast cancer. In particular, MDM2 is a pivotal regulator of tumor suppressor P53 activity in the breast. The disruption of the function of MDM2 probably results in the failure of effectively repairing or eliminating the damaged DNA, eventually leading to the onset of breast cancer [5, 12].

MDM2, the main negative regulatory component of *p53*, is considered to play an indispensable role in maintaining the ovarian function in the knock-out mouse models. However, the exact role of *MDM2* in human reproduction has not been studied. In the present study, we evaluated the *MDM2* gene mutation in Chinese women with idiopathic POI. No plausible causal mutation was identified except for two known SNPs (*rs937283* in the 5'-UTR and *rs2870820* in the intron 1). In addition, there were no statistical differences in the genotypes and allelic frequencies between the POI group and the control group. To the best of our knowledge, this is the first study to investigate the association between *MDM2* gene and the risk of POI. Although the sample size in the current study was not large and the presence of mutations in other regions, such as the promoter, introns, or other regulatory regions cannot be excluded, our results can also indicate that the mutations occurring in the coding region of *MDM2* gene may not have a direct association with the pathogenesis of POI in Chinese Han women diagnosed with POI.

In conclusion, our findings suggest that the mutations in the coding region of the *MDM2* gene may not represent a risk factor in the pathogenesis of idiopathic POI among Chinese Han women. Although we failed to obtain any evidence that *MDM2* is a disease-causing gene, our study is the first to investigate the role of *MDM2* gene in POI patients. Further studies with larger sample sizes from different ethnic populations are warranted to validate the preliminary findings.

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Disclosure of conflict of interest

None.

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