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ARTICLE

Variation analysis of *PRIM1* gene in Chinese patients with primary ovarian insufficiency

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Abstract Insights into common genetic susceptibility between primary ovarian insufficiency (POI) and natural or early menopause have delivered an innovative way of assessing the genetic mechanisms involved in POI. *PRIM1* plays a crucial role in DNA replication by synthesizing RNA primers for Okazaki fragments. It is closely associated with age at natural menopause, early menopause and POI in European women. In this study, we aimed to investigate whether mutations in *PRIM1* contribute to POI in Chinese women. All exons and exon–intron boundaries of *PRIM1* gene were sequenced in 192 Han Chinese women with non-syndromic POI. No plausible mutations were identified. The results suggest that the perturbations in *PRIM1* gene are not a common explanation for POI in Chinese women.

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KEYWORDS: primary ovarian insufficiency, premature ovarian failure, premature menopause, early menopause, *PRIM1*, variant screening

Introduction

Primary ovarian insufficiency (POI), also known as premature ovarian failure, is established when primary or secondary amenorrhoea and hypoestrogenism with high levels of gonadotrophins occur in women before the age of 40 years. The POI prevalence in the general population was reported to be around 1% by the age of 40 years (Coulam et al., 1986). A reduction in the number of primordial follicles or acceleration of the process of follicular atresia may lead to the

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development of POI. It is a heterogeneous disorder with a multifactorial cause, including genetic, enzymatic, iatrogenic, immunological and infectious disorders (Beck-Peccoz and Persani, 2006). The cause of POI, however, remains undetermined in the most cases.

As the ovarian reserve is exhausted, menopause occurs and represents the loss of reproductive function. The overall mean age at natural menopause (AANM) was 48.8 years with considerable variation (Davis et al., 2015). Although early menopause occurs before the age of 45 years, women with POI cease menstruation before the age of 40 years (Shelling, 2010). Our previous study, undertaken to determine the correlation between POI, AANM and early menopause, revealed three significant single-nucleotide polymorphsms (SNP) in *ESR1*, *BRSK1*, and *HK3* genes, respectively, raising the attention on the common genetic susceptibility between POI and natural or early menopause (Qin et al., 2012). A meta-analysis of 22 genome-wide association studies reported 13 novel loci associated with AANM in European women (Stolk et al., 2012), many of which have been replicated in other ethnicities (Carty et al., 2013; Chen et al., 2014; Rahmani et al., 2013; Shen et al., 2013). Candidate genes located at these loci are implicated in either DNA damage repair and replication or immune function (Stolk et al., 2012). In the following study, many of these loci, including rs11668344 (*TMEM150B*), rs2517388 (*ASH2L*), rs2277339 (*PRIM1*), rs12294104, rs1046089 (*PRRC2A*), rs12461110 (*NLRP11*), rs4886238 (*TDRD3*), rs10183486 (*TLK1*), and rs2307449 (*POLG*), were also associated with early menopause and POI, further highlighting the shared cause of early menopause/POI and normal menopause (Perry et al., 2013).

According to prior genome-wide association studies, the non-synonymous SNP rs2277339 in the *PRIM1* gene is not only closely associated with AANM, but also with early menopause and POI in European women (Perry et al., 2013). During discontinuous DNA replication, PRIM1 (primase) plays a key role in the process of DNA synthesis initiation by synthesizing RNA primers for Okazaki fragments (Cloutier et al., 1997). The potential role of *PRIM1* in POI pathogenesis, however, has not been determined. Therefore, we examined 192 Han Chinese women with idiopathic POI by sequencing the coding region of *PRIM1* gene to determine whether variants in this gene contribute to human POI.

Materials and methods

Patients

Between June 2014 and September 2015, 192 Han Chinese women with secondary amenorrhoea were recruited from the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University. Inclusion criteria were well-defined as two measurements of serum FSH concentration over 40 IU/L before the age of 40 years without chromosomal abnormality. Serum FSH concentration was measured by electro-chemiluminescence immunoassay (Roche) and the normal ranges of this assay are as follows: 3.5–12.5 IU/L in follicular phase, 4.7–21.5 IU/L in ovulatory phase, 1.7–7.7 IU/L in luteal phase, and 25.8–134.8 IU/L after menopause. Patients who had undergone chemotherapy and radiotherapy, ovarian surgery, and immune diseases were excluded. Patients with other pleiotropic Mendelian disorders related to POI or somatic defects were also excluded. All the patients recruited were non-syndromic and idiopathic. The clinical characteristics of all participants are provided in **Table 1**. Informed consent for molecular studies was obtained from all participants. The genotype and allele frequencies of controls were obtained from Northern Chinese population in Ensembl database (http://asia.ensembl.org/index.html). The study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University on 3 May 2014 (reference number 39).

Table 1 Clinical characteristics of 192 patients with primary ovarian insufficiency.

Characteristic	*Mean ± SD*
Age (years)	28.2 ± 3.45
Age at menarche (years)	14.9 ± 2.22
Age at irregular menstruation (years)	20.8 ± 4.90
Age at amenorrhoea (years)	22.1 ± 4.48
Serum FSH (IU/L)	78.0 ± 28.14

Variants screening

Genomic DNA was extracted from peripheral blood samples. Thirteen pairs of primers covering the whole coding region of *PRIM1* gene were designed for sequencing (**Table 2**) according

Table 2 Primers for amplification of the coding region of the *PRIM1* gene.

Primer ID	*Sequence5'-3'*
PRIM1-E1F	CAAACTGCTGCGTCTCCC
PRIM1-E1R	TCCTCTCTCGGCCCATTTAC
PRIM1-E2F	CATGGGGAGCACAGGAAAC
PRIM1-E2R	TGGGCAACAGAGTGAGAGTCC
PRIM1-E3F	GATTTAGCAGTGTGGCAGCTCTTATA
PRIM1-E3R	GCAGGAGAATCACTTGAACCCA
PRIM1-E4F	GATTTAGCAGTGTGGCAGCTCTTATA
PRIM1-E4R	GCAGGAGAATCACTTGAACCCA
PRIM1-E5F	ACCAGGATGGGTAAAGAGATGAAG
PRIM1-E5R	GAAACTAAAGCAATGCAAAT
PRIM1-E6F	CACCTCAGCCTCCCAAAATG
PRIM1-E6R	TGAGAACATCTGTAAAGCACCT
PRIM1-E7F	TGCCAGGTTCTATGTCAGGT
PRIM1-E7R	ACTGATTTGCAACATGGCCC
PRIM1-E8F	CCACGCCCAGTCAACATTTA
PRIM1-E8R	AGGTATTGGGTGACAGAGCA
PRIM1-E9F	CCACGCCCAGTCAACATTTA
PRIM1-E9R	AGGTATTGGGTGACAGAGCA
PRIM1-E10F	AGAGTAAGGGCACAGCTAATG
PRIM1-E10R	GGGCATCGTCATCTAACCTT
PRIM1-E11F	GGCTGAGGCAGAAGAATCACT
PRIM1-E11R	TGCAGTTCCAGGACAAATGC
PRIM1-E12F	TGCATGACAGAGTGAAATCCTG
PRIM1-E12R	GTGCCTGGCCAAGTTAGTAC
PRIM1-E13F	TGTGGACTTCAGCAAATGTGT
PRIM1-E13R	CACCACACCCGACTGATTTT

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