Variation analysis of *PUM1* gene in Chinese women with primary ovarian insufficiency

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Received: 17 July 2017 / Accepted: 22 December 2017 / Published online: 3 January 2018
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Abstract

Accumulating evidence has indicated that the genes involved in meiosis are highly correlated with ovarian function. Pumilio 1 (*PUM1*) is a RNA-binding protein which is involved in the meiotic process. It has been reported that the *Pum1* knockout female mice displayed subfertility due to the decrease in primordial follicle pool. The aim of our study is to investigate whether variants of the *PUM1* gene are responsible for primary ovarian insufficiency (POI) in Chinese women. We analyzed coding sequence and untranslated regions of the *PUM1* gene in 196 Han Chinese women with non-syndromic POI and 192 controls. Seven novel variants were identified, but one of them was synonymous and six were intronic. Besides, seven known single-nucleotide polymorphisms (SNPs) were found, and there were no significant differences in genotype and allele frequencies of the SNPs between patients and controls. The results suggest that the variants in *PUM1* may not contribute to POI in Han Chinese women.

Keywords Primary ovarian insufficiency · *PUM1* · Meiosis · Variant screening

Introduction

Primary ovarian insufficiency (POI) is defined as amenorrhea prior to age of 40 years with increased follicle-stimulating

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10815-017-1110-4>) contains supplementary material, which is available to authorized users.

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hormone (FSH) and decreased estrogen levels in serum [7, 13]. It affects around 1% of women by the age of 40 years [6]. Although the pathogenesis is undefined in most cases, it has been considered that the dysfunction or depletion of ovarian follicles will trigger the occurrence of POI [16]. Thus, the size of primordial follicle pool is one of the most significant elements for the female reproductive life span, which is influenced by genetic and environmental factors.

Meiosis is an essential event for establishing primordial follicles pool and must be precisely modulated by multiple genes, such as *DMC1* [2], *SGO2* [8, 9], *STAG3* [4, 8], and so on. Arresting at dictyate stage of meiosis I is one of the critical periods for maturation of oocytes [20]. Synaptonemal complex (SC), a key factor for entering into this step, is mainly composed of two lateral elements (SYCP2 and SYCP3), one central element (SYCE1, SYCE2, SYCE3, and TEX12) and linking transverse filaments (SYCP1) [1, 10, 17]. During the meiotic prophase I, the synthesis and degradation of these proteins determine the assembly and disassembly of SC, and also accompany the pairing, exchange and separation of homologous chromosomes from zygotene to diplotene stages in nearly all eukaryotes [10].

Pumilio (Pum), a posttranscriptional regulator from the PUF family [5], is well known for ovarian morphogenesis and oviposition in *Drosophila* [14]. In mammals, there are

two homologs, PUM1 and PUM2. A recent study reported a conserved role for mammalian PUM1 but not PUM2 in the establishment of the female germline. *Pum1*-deficient mice were found to have a similar phenotype to POI in humans [11]. The lack of *Pum1* in oocytes disrupted SYCP1 degradation from SC in the late meiotic prophase I and thus prevented the transition from pachytene to dictyate stage, leading to a decrease in primordial follicle pool and ultimately infertility at a younger age in mutant mice compared to wild type mice [11]. Therefore, *PUM1* may be a candidate gene for POI which is related to primordial folliculogenesis. In this study, we sequenced coding sequence and untranslated regions of *PUM1* in 196 Han Chinese women with POI to determine whether variants in this gene contribute to human POI.

Materials and methods

Patients

A cohort of 196 Han Chinese women with idiopathic POI were recruited from the Hospital for Reproductive Medicine Affiliated to Shandong University from 2013 to 2016. Inclusion criteria consisted of cessation of menstrual cycles between the age of 25 to 40, with at least two measurements of serum FSH concentration over 40 IU/L (Table 1). Patients with karyotypic abnormalities were excluded. Patients who had a history of ovarian surgery, chemotherapy or radiotherapy, immune diseases, or somatic anomalies were also excluded. A total of 192 females with normal basal serum FSH concentration and regular menstrual cycle were recruited as control group. Informed consent was obtained from each subject. The study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University on 8 May 2016 (reference number 27).

Variants screening and bioinformatic analysis

Genomic DNA was extracted from peripheral blood samples of the patients using QIAamp DNA Blood Mini Kits (QIAGEN, Hilden, Germany). The entire exon sequence and intron-exon boundaries of the *PUM1* gene (Reference sequence NC_000001.11) were amplified by polymerase chain

reaction (PCR) through 23 pairs of primers (Supplementary Table 1). The PCR products were first analyzed by agarose gel electrophoresis, then purified using the method of polyethylene glycol precipitation and sequenced on ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA) using the ABI-Prism big-dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All variants were confirmed by bidirectional sequencing from at least three independent experiments.

The software NNSPLICE v.0.9 (http://www.fruitfly.org/seq_tools/splice.html) and NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>) were used to assess the possible functional impact of variant on RNA splicing. The online tool RegRNA (<http://regma.mbc.nctu.edu.tw/>) was used to analyze possible changes in the binding of microRNAs to the 3' untranslated region (UTR) of *PUM1*. The possible effect of an amino acid substitution on the function of PUM1 protein was predicted using the PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and the Sorting Tolerant From Intolerant (SIFT) algorithm (http://sift.jcvi.org/www/SIFT_enst_submit.html).

Statistics

The sequencing results were analyzed with Sequencer 4.9 software. The Fisher's exact test was used for comparison of genotype distribution and allele frequency between patients and controls. Statistical differences were considered significant when $P < 0.05$.

Results

Fourteen variants of *PUM1* identified in 196 Chinese women with POI are summarized in Table 2. Seven novel mutations were found including one synonymous mutation in exon (c.582 T > C) and six intronic variations. The novel mutation c.1792 + 7G > A did not alter RNA splicing predicted by NNSPLICE v.0.9 and NetGene2 software. The remaining seven variants were previously identified as single-nucleotide polymorphisms (SNPs) in the *PUM1* gene. Two of them were synonymous and localized in exon 15 (rs2275741) and exon 21 (rs150518018) respectively; three of them were intronic SNPs (rs2275740, rs4949187 and rs3795433); one of the seven SNPs was missense variation (rs144369611, p.Asn883Ser) which was benign predicted by Polyphen2 and SIFT software; and one SNP (rs964473189) was located in 3'UTR and did not change microRNA binding as predicted by RegRNA software. All SNPs displayed no significant differences between cases and controls in both genotype and allele frequencies.

Table 1 Clinical characteristics of 196 cases and 192 controls

Characteristic	Cases	Controls
Age (years)	32.92 ± 4.01	33.40 ± 4.91
Age at menarche (years)	14.60 ± 1.33	13.79 ± 1.52
Age at amenorrhea (years)	30.89 ± 3.93	–
Serum FSH (IU/l)	75.98 ± 30.73	5.71 ± 3.21
Estrogen (pg/ml)	15.03 ± 7.76	45.98 ± 12.23

Table 2 Variants of the *PUM1* gene identified in Chinese women with primary ovarian insufficiency

Location	dbSNP ID	Sequence variation	Amino acid variation	Allele	Allele frequency		Genotype	Genotype frequency	
					POI, %(n)	Control, %(n)		POI, %(n)	Control, %(n)
Exon 5	novel mutation	c.582 T > C	Pro194Pro	T	99.7(389)	100(384)	TT	99.5(194)	100(192)
Intron 4	novel mutation	c.492 + 238_492 + 242del	–	C	0.3(1)	0	TC	0.5(1)	0
				ATCTT	99.5(390)	100(380)	CC	0	0
				del	0.5(2)	0	ATCTT	99.0(194)	100(190)
				del	0.5(2)	0	ATCTT	1.0(2)	0
Intron 11	novel mutation	c.1507-140G > C	–	G	99.7(387)	100(384)	del del	0	0
				C	0.3(1)	0	GG	99.5(193)	100(192)
Intron 12	novel mutation	c.1792 + 7G > A	–	G	99.7(391)	100(382)	GC	0.5(1)	0
				A	0.3(1)	0	CC	0	0
Intron 16	novel mutation	c.2592-192C > T	–	G	99.7(391)	100(382)	GG	99.5(195)	100(191)
				T	0.3(1)	0	GA	0.5(1)	0
Intron 16	novel mutation	c.2592-23G > T	–	C	99.7(387)	100(384)	AA	0	0
				T	0.3(1)	0	CC	99.5(193)	100(192)
Intron 16	novel mutation	c.2592-23G > T	–	G	99.7(391)	100(384)	CT	0.5(1)	0
				T	0.3(1)	0	TT	0	0
Intron 21	novel mutation	c.3429 + 15-c.3429 + 16insGC	–	G	99.7(391)	100(384)	GG	99.5(195)	100(192)
				T	0.3(1)	0	GT	0.5(1)	0
Intron 21	novel mutation	c.3429 + 15-c.3429 + 16insGC	–	–	99.23(389)	100(384)	TT	0	0
				insGC	0.77(3)	0	–	98.5(193)	100(192)
Exon 15	rs2275741	c.2340 T > C	Asn780Asn	T	65.6(257)	67.7(260)	insGC	1.5(3)	0
				C	34.4(135)	32.3(124)	insGC	0	0
Exon 16	rs144369611	C.2648A > G	Asn883Ser	A	99.7(391)	100(384)	insGC	0	0
				G	0.3(1)	0	insGC	0.5(1)	0
Intron 17	rs2275740	c.2859 + 175G > A	–	G	49.7(195)	47.7(183)	GG	0	0
				A	50.3(197)	52.3(201)	GG	20.9(41)	20.8(40)
Intron 18	rs4949187	c.2998-70 T > A	–	T	68.4(268)	71.4(274)	GA	57.7(113)	52.8(103)
				A	31.6(124)	28.6(110)	AA	21.4(42)	26.4(49)
Intron 18	rs4949187	c.2998-70 T > A	–	T	68.4(268)	71.4(274)	TT	54.1(106)	56.3(108)
				A	31.6(124)	28.6(110)	TA	28.6(56)	30.2(58)

Table 2 (continued)

Location	dbSNP ID	Sequence variation	Amino acid variation	Allele	Allele frequency		Genotype	Genotype frequency	
					POI, %(n)	Control, %(n)		POI, %(n)	Control, %(n)
Intron 20	rs3795433	c.3245 + 26A > G	–	A	68.9(270)	72.8(282)	AA	17.3(34)	13.5(26)
				G	31.1(122)	27.2(102)	AG	49.0(96)	57.3(110)
							GG	39.8(78)	32.3(62)
Exon 21	rs150518018	c.3285G > A	Thr1095Thr	G	99.7(391)	100(384)	GG	11.2(22)	10.4(20)
				A	0.3(1)	0	GA	99.5(195)	100(192)
							AA	0.5(1)	0
3'UTR	rs964473189	c.3561 + 807del	–	A	99.7(367)	100(370)	AA	0	0
				del	0.3(1)	0	Adel	99.5(183)	100(185)
							del del	0.5(1)	0
								0	0

Each SNP was tested separately and no statistically significant differences were found
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Discussion

Numerous studies have shown that genetic factors are highly linked with gonadal function and POI [3, 12, 18]. With the development of molecular genetic approaches, the candidate genes of POI have been discovered gradually [8].

Pumilio is a RNA-binding protein which binds to the 3' untranslated region (3'UTR) of its target mRNA to mediate translational repression and/or mRNA decay [19, 21]. While Pum1 can suppress multiple activators of p53 to safeguard spermatogenesis in male mice [5], the subfertility of knockout female mice was attributed to a decrease in ovarian reserve because the significant proportion of oocytes were arrested at pachytene stage without the disassembly of SYCP1 protein in meiosis I [11]. Therefore, PUM1 is involved in primordial folliculogenesis and, more specifically, in controlling the transition of oocyte from pachytene to dictyate stage via facilitating homologous chromosome desynapsis by downregulating SYCP1 protein during the late meiotic prophase I [11]. Our study was performed to investigate whether mutations in *PUM1* could lead to human POI. We sequenced 196 Chinese women with POI and found no causative variants. Up to now, the prevalence of single gene mutations being causative in POI was very low and varied with ethnicity [15].

The information for the *PUM1* gene in ExAC database (<http://exac.broadinstitute.org/gene/ENSG00000134644>) indicates that the number of observed protein affecting variants is less than the expected for this gene and loss of function variants are not tolerated in human. These imply that *PUM1* gene is essential for cell function and their loss of function variant can be lethal or affect early embryo development. The discrepancy in phenotype between mouse and human may exist. Although the results may be limited by the sample size, we conclude that the variants in *PUM1* may not contribute to POI in Han Chinese women. Future studies in larger cohorts and other ethnic populations may determine the exact role of *PUM1* in human POI.

Funding information This research was supported by grants from the National Key Research and Developmental Program of China (2017YFC1001100), National Natural Science Foundation of China (81522018, 81471509, 81571505 and 81571406), Young Scholars Program of Shandong University (2016WLJH26), and the Fundamental Research Funds of Shandong University.

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