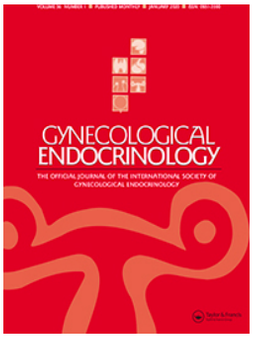

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Variation analysis of tousled like kinase 1 gene in patients with sporadic premature ovarian insufficiency

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ABSTRACT

Tousled like kinase 1 (*TLK1*), a member of DNA repair family, participates in the regulation of chromatin assembly and is associated with early menopause and premature ovarian insufficiency (POI) in European women. However, whether the sequence variant in the *TLK1* gene was causative for POI is still elusive. Here we performed direct sequencing of the *TLK1* gene in 192 patients with sporadic POI. All exons and exon–intron boundaries of *TLK1* were amplified and sequenced. Six known single-nucleotide polymorphisms were identified in POI, including rs149844334, rs11553951, rs757600673, rs2277339, rs113416007 and rs17283147. No novel variant was identified, which indicates that sequence variants in the coding region of *TLK1* might be uncommon in Chinese women with POI. The role of *TLK1* in POI pathogenesis needs to be further explored in larger cohorts from Chinese and other ethnic populations.

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KEYWORDS
Premature ovarian insufficiency; TLK1; variation analysis; polymerase chain reaction; single-nucleotide polymorphism

Introduction

Premature ovarian insufficiency (POI) is one of the commonest causes of female infertility, characterized by menstrual disturbance (amenorrhea or oligomenorrhea), high levels of gonadotropins (FSH > 25IU/L), and low estradiol in women younger than 40 years [1,2]. It affects approximately 1% of reproductive aged women [1,3]. Diverse etiologies have been responsible for POI, including chromosomal abnormality, gene mutation, autoimmune, environmental, and iatrogenic factors, however, majority of the causes are still unknown [4,5]. Over 80 genes have emerged as POI candidates, but in non-syndromic POI (manifest POI as the only phenotype, and distinct from pleiotropic Mendelian disorders may manifest POI as part of their phenotypic spectrum, e.g. Fragile X syndrome) only a minority have been proven equivocally causative by functional validation such as *NR5A1*, *BMP15*, *GDF9*, *NOBOX*, *FOXL2*, *MCM8*, *MCM9* and *CSB-PGBD3* etc. [6–14]. More genes are warranted to be explored and elucidated in patients with POI [5].

TLK1 (also known as PKU-beta KIAA0137 and PKU-BETA) is a target of the DNA damage checkpoint when DNA double-strand breaks (DSBs) generates through the ATM-Chk1-TLK pathway involved in chromatin assembly [15,16]. TLKs play important roles in processing the ends of a DSB via interaction with Rad9 [17]. TLK1 is highly conserved in both plants and animals. Knockout of Tlk1 in Drosophila and *C. elegans* resulted in an early arrest in embryonic development [18,19], while a dominant negative mutant of Tlk1 in mouse led to loss of nuclear divisions and missegregation of chromosomes [20]. HeLa cells lacking TLK1 displayed a prolonged G2/M arrest upon exposure to ionizing radiation (IR) [21]. Moreover, overexpression of TLK1 in mammalian cells protected them against IR by facilitating the repair of DSBs [22–24]. Interestingly, more and more genes involved in DNA repair were found plausible candidate genes in POI, such as MCM8, MCM9 and CSB-PGBD3 et al. [6,7,12].

A meta-analysis of 22 genome-wide association studies reported 13 novel loci susceptible for age at natural menopause. Eight candidate genes implicated in DNA repair, i.e. *EXO1*, *HELQ*, *UIMC1*, *FAM175A*, *FANCI*, *TLK1*, *POLG* and *PRIM1* [25]. Among the loci, rs10183486 in *TLK1* were identified also associated with early menopause and POI in European women [26]. Therefore, whether sequence variant in *TLK1* is responsible for patients with POI is anticipated. Here, we examined 192 patients with sporadic POI (when the index woman was the only family member affected by POI) by sequencing the coding region of *TLK1* gene to explore whether variants in this gene contribute to POI.

Methods

Patients

Total of 192 patients with sporadic POI were recruited from the Center for Reproductive Medicine, Shandong University. POI was defined as sporadic when the index woman was the only family member affected by POI. Recruitment criteria comprised cessation of menstrual cycles or oligomenorrhea before 40 years of age and at least two serum follicle stimulating hormone (FSH) concentrations exceeding 25 IU/L. Women with chromosomal abnormalities, family history, pelvic surgery, and chemoradiotherapy treatment were excluded. The clinical characteristics of all participants were shown in Table 1.

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Supplemental data for this article can be accessed here.

DNA extraction and sequencing

Peripheral blood was obtained and genomic DNA was isolated from whole blood samples using DNeasy Blood & Tissue Kit (QIAGEN Inc, Mississauga, Ontario, Canada). Polymerase chain reaction (PCR) for the coding exons and exon/intron boundaries of *TLK1* was performed using primers listed in Supplementary Table 1. PCR conditions were as follows: pre-denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and elongation at 72 °C for

Table 1. Clinical features of patients with sporadic POI.

	Sporadic POI		
Characteristic	Primary amenorrhea	Secondary amenorrhea	Without amenorrhea
No. of patients	29	133	30
Age (y)	27.65 ± 4.46	29.00 ± 4.70	28.73 ± 4.15
Age at menarche (y)	NA	14.41 ± 1.69	14.17 ± 1.77
Age of amenorrhea (y)	NA	23.87 ± 4.79	NA
FSH (IU/L)	73.72 ± 24.75	76.46 ± 25.83	63.06 ± 16.45
Family history	No	No	No
Autoimmune diseases history	No	No	No
Pelvic surgery history	No	No	No
Chemo-/radiotherapy treatment	No	No	No
Somatic anomalies	No	No	No

The sporadic POI that we recruited were characterized by primary amenorrhea, secondary amenorrhea or without amenorrhea, younger than forty years old, FSH concentrations exceeding 25 IU/L and without chromosomal abnormalities, family history, pelvic surgery, and chemoradiotherapy treatment.
NA: not available; POI: primary ovarian insufficiency; FSH: follicle stimulating hormone.

Table 2. Genotype frequency of Variants identified in *TLK1* gene in patients with sporadic POI.

Location	dbSNP ID	POI case	Variation	Genotype	(*n*, %)	(*n*, %)[a]	*p* value
Exon 8	rs149844334	192	Synonymous	TT	(191,99.5)	(101,99.1)	.28
	C.666T>C		variant	TC	(1,0.5)	(2,1.9)	
				CC	(0,0)	(0,0)	
Exon 8	rs11553951	192	Synonymous	CC	(168,87.5)	(87,84.5)	.47
	C. 690C>T		variant	CT	(24,12.5)	(16,15.5)	
				TT	(0,0)	(0,0)	
Exon 8	rs771244101	192	Synonymous	AA	(169,88.0)	(87,84.5)	.39
	C. 681A>G		variant	GA	(23,12.0)	(16,15.5)	
				GG	(0,0)	(0,0)	
Exon21	rs3731993	192	Synonymous	TT	(87,45.3)	(44,42.7)	.48
	C.2262T>G		variant	TG	(92,47.9)	(55,53.4)	
				GG	(13,6.8)	(4,3.9)	
Intron1	rs113416007	192	Intron variant	GG	(155,80.7)	(88,85.4)	**.01**
	c.139 + 44G>A			AG	(21,10.9)	(15,14.6)	
				AA	(16,8.4)	(0,0)	
Intron6	rs17283147	192	Intron variant	AA	(166,84.9)	(87,84.5)	.65
	c.954 + 70A>T			AT	(25,13.0)	(16,15.5)	
				TT	(1,0.5)	(0,0)	

Six known SNPs, rs149844334, rs11553951, rs757600673 in exon8, rs2277339 in exon 21, rs113416007 in intron 1, and rs17283147 intron 6 were identified in 192 patients. There were no differences in genotype frequencies except for rs113416007, located in intron 1 between patients and controls.
TLK1: Tousled like kinase 1; POI: Premature ovarian insufficiency; SNP: single-nucleotide polymorphism.
[a]International HapMap-CHB project database.
$P < 0.05$ was considered statistically significant.

Table 3. Allele frequency of variants identified in *TLK1* gene in patients with sporadic POI.

Location	dbSNP ID	POI case	Variation	Allele	(*n*, %)	(*n*, %)[a]	*p* value
Exon 8	rs149844334	192	Synonymous	T	(383,99.7)	(204,99.0)	.28
	c.666T>C		variant	C	(1,1.3)	(2,1.0)	
Exon 8	rs11553951	192	Synonymous	C	(360,93.8)	(190,92.2)	.49
	c.690C>T		variant	T	(24,6.2)	(16,7.8)	
Exon 8	rs771244101	192	Synonymous	A	(361,94.0)	(121387,99.98)	.41
	c.681A>G		variant	G	(23,6.0)	(21.0.02)	
Exon21	rs3731993	192	Synonymous	T	(266,69.3)	(143,69.4)	.97
	c.2262T>G		variant	G	(118,30.7)	(63,30.6)	
Intron1	rs113416007	192	Intron	G	(331,86.2)	(191,92.7)	**.02**
	c.139 + 44G>A		variant	A	(53,13.8)	(15,7.3)	
Intron6	rs17283147	192	Intron	A	(357,93.0)	(190,92.2)	.06
	c.954 + 70A>T		variant	T	(53,7.0)	(16,7.8)	

Six known SNPs, rs149844334, rs11553951, rs757600673 in exon8, rs2277339 in exon 21, rs113416007 in intron 1, and rs17283147 intron 6 were identified in 192 patients. There were no differences in allele frequencies except for rs113416007, located in intron 1 between patients and controls.
TLK1: Tousled like kinase 1; POI: Premature ovarian insufficiency; SNP: single-nucleotide polymorphism
[a]International HapMap-CHB project database.
$P < 0.05$ was considered statistically significant.

1 min. PCR products were purified, labeled by BigDye (Terminatorv3.1 Cycle Sequencing Kits, Applied Biosystems), and sequenced directly on an automated sequencer, ABI Prism Sequencer 3730XL (Applied Biosystems). All of variants were confirmed by three independent PCR runs and sequenced in both forward and reverse strands.

Statistics

The sequencing results were analyzed with Sequencer 4.9 software. The continuous data were checked for normality using the Kolmogorov–Smirnov test and described as mean ± standard deviation (SD). Categorical data were tested by Pearson's x^2 test or Fisher's exact test. A two sided $P < 0.05$ was considered statistically significant. Statistical analyses were performed with Statistical Package for Social Sciences version 18.0(SPSS 18.0; SPSS, Chicago, IL, USA).

Results

No novel variants were found in 192 patients. As shown in Tables 2 and 3, six known SNPs, rs149844334, rs11553951, rs757600673 in exon8, rs2277339 in exon 21, rs113416007 in intron 1, and rs17283147 intron 6 were identified. Comparisons of genotype and allele frequencies showed significant difference in rs113416007 between cases and general population.

Discussion

To our knowledge, this is the first study about sequencing *TLK1* in Chinese patients with sporadic POI. Six known SNPs, rs149844334, rs11553951, rs757600673, rs2277339 and rs17283147, were identified with no difference in genotype and allele frequencies except for rs113416007, located in intron 1 region, with significant difference between patients and controls. Most studies so far largely focus on coding variants. However, only 1.5% of the genome is protein-coding. Non-coding variants must be more robustly interrogated. So rs113416007 may be associated with POI. Rs10183486, was proved related with early menopause and POI in Europe women, but was not identified in the 192 patients with sporadic POI that we recruited from Chinese population. The inconsistence may be explained by ethnic difference or limited sample size in this study and its role in the etiology of POI needs more confirmation.

Conclusion

In conclusion, sequence variants in the coding region of *TLK1* might be uncommon in Chinese women with POI. The role of *TLK1*, especially rare SNP rs113416007 in POI pathogenesis needs to be further explored in larger cohorts from Chinese and other ethnic populations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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