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Novel Single-Nucleotide Polymorphisms of *SOHLH2* in Korean Patients with Premature Ovarian Failure

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ABSTRACT : SOHLH2 is a novel germ cell-specific transcription factor that is crucial for folliculogenesis in the ovary and spermatogenesis in the testis. *SOHLH2* represents a candidate gene for infertility with premature ovarian failure. We analyzed whether mutations in the *SOHLH2* gene in 98 Korean women with premature ovarian failure. The sequence analysis identified six novel SNPs (c.431-41G>C, c.656A>T, c.1000+27C>T, c.1000+33G>T, c1258-106G>A, and c.2094+11T>C) from Korean patients with premature ovarian failure. The c.656A>T found in exon 7 results in change of an amino acid, tyrosine to phenylalanine. Functional mutations in *SOHLH2* gene are rare in Korean women with premature ovarian failure.

Key words : Premature ovarian failure, SOHLH2, Infertility

INTRODUCTION

Infertility can be resulted from various defects including the development of reproductive system and from genetic disorders of germ-cell specific regulators. Defects in germ cells in the gonads result in premature ovarian failure (POF) in women.

Premature ovarian failure is associated with female infertility occurring in 1 to 2% of women losing follicles in the ovary before the menopause. Patients with POF show various phenotypes such as primary amenorrhea (prepubertal onset) secondary amenorrhea (postpubertal onset), hypoestrogenism, and infertility. A diagnosis of ovarian failure was made in the presence of elevated gonadotropin serum levels (FSH of >40 U/L) (Conway, 2000; Goswami & Conway, 2005). POF is caused by chromosomal, autoimmune, metabolic, infectious, and environmental causes (Goswami & Conway, 2005; Simpson & Rajkovic, 1999). However, many cases of POF remain unknown.

Recently, the genes associated with germ cell defect in the gonad have been reportedly updated in humans and mice (Matzuk & Lamb, 2008). The spermatogenesis- and oogenesis-specific basic helix-loop-helix 2 (*SOHLH2*) is located on human chromosome 13(13p13.3) and contains a basic helix-loop-helix (bHLH) domain (Ballow et al., 2006). In mice, *SOHLH2* is exclusively expressed in oogonia (Choi et al., 2008; Toyoda et al., 2009). *SOHLH2* deficiency disrupts the expression of numerous germ cell specific genes in the mouse gonads, including *SOHLH1*, *LHX8*, *NOBOX*, *FIGLA*, Zona pellucida 1 (*ZP1*), *ZP3*, *GDF9*, *KIT* and *SOX3* (Choi et al., 2008; Toyoda et al., 2009). The bHLH domain of SOHLH2 is highly conserved among species and known as a conserved DNA binding sequence, E-box (CANNTG) (Swanson & Yang, 1999). Therefore, SOHLH2 might regulate directly some of these through the E-box. Male and female mice homozygous for the null mutation of *SOHLH2* gene are sterile, due to lack of spermatogenesis and oogenesis (Choi et al., 2008; Hao et al., 2008; Toyoda et al., 2009). In addition, oocytes are rapidly lost in *SOHLH2*-

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deficient ovaries, and few are present by 14 days of postnatal life (Choi et al., 2008; Hao et al., 2008; Toyoda et al., 2009). The germ cell defect phenotype from *SOHLH2* null mice make *SOHLH2* one of the candidate genes for premature ovarian failure related to testicular- and ovarian-failure in humans.

We therefore investigated whether genetic variations in *SOHLH2* are present in Korean patients with premature ovarian failure, compared with an ethnically matched control.

MATERIALS AND METHODS

1. Patient Recruitment

Patients with either POF had been recruited at CHA Gangnam Medical Center, Fertility Center since 2004. For POF study, inclusion criteria were cessation of menstrual cycles before 40 years of age, with at least two serum FSH concentrations of >40 IU/L for POF study. 98 patients with the criteria were recruited with exclusion of abnormal chromosome structure. In addition, genetic examination included unrelated healthy women from the general population. In this study, all patient and controls were recruited with full approval from and oversight by the CHA Gangnam Medical Center at the CHA University in Seoul, the Republic of Korea. The study was approved by the Institutional Review Boards of CHA University.

2. Genetic Analysis

Genomic DNA was isolated from whole blood using QIAamp DNA blood kit (Applied Biosystems, Foster City,

Table 1. Oligonucleotides used for genetic screening of SOHLH2

Primer	Sequence (5'-3')	Size	EXON
Sohlh2-1	CCAGGAGCCATTAGTGCAG	265 bp	EXON1
Sohlh2-2	ACAGTTCCCCGACAATGAGA		
Sohlh2-3	TTCCTGTCCTTGAGCTGCTT	496 bp	EXON2
Sohlh2-4	GGCCCCTCAATTTGAAAAGTA		
Sohlh2-5	TTCTAAAGAAATGAAGAAAATCACTG	461 bp	EXON3,4
Sohlh2-6	TCCCTTTATTTTATTTATGCTTGTTTT		
Sohlh2-23	TGCAGCTGCTTGTTGAAGAC	655 bp	EXON 5
Sohlh2-24	CTGTTCCCCTGTGGTCAAGT		
Sohlh2-9	GCCCCTTTGTATGAAGCAAG	378 bp	EXON6
Sohlh2-10	GCTGAATGAGTGAGTGGCATC		
Sohlh2-11	TGTTAGTCCATTCTTGTGTTGCT	204 bp	EXON7
Sohlh2-12	TCTCTTGCTCCTCCTCTTGC		
Sohlh2-13	TATAGGGAGGGGATGGTTCC	619 bp	EXON8,9
Sohlh2-14	TAAGCCCCTGCACTTTCACT		
Sohlh2-15	TGGGTCATGTGTCTTTGCTC	374 bp	EXON10
Sohlh2-16	TCCTGGATTCCCTGGTTATG		
Sohlh2-17	ATAGCGGGCAGTTTATGTCG	455 bp	EXON11
Sohlh2-18	CTCTTAGGTCCCAGCCTTCC		
Sohlh2-25	GGGTGGGTTTTGTTGAATTG	500 bp	EXON12-1
Sohlh2-26	GGTCACTGAGGCCATTTGTT		
Sohlh2-27	GCAACAGTTTTGGGCGTATT	649 bp	EXON12-2
Sohlh2-28	AGCCATCGTTTCACTCAAGG		
Sohlh2-29	CCTTCTTCATTCACCATCCAA	503 bp	EXON12-3
Sohlh2-30	TGCAGATTCCATCATTTTGGT		

CA). Forty to fifty nanogram (ng) of genomic DNA was used for polymerase chain reaction (PCR). The entire coding sequence and intron-exon junctions of the *SOHLH2* gene were analyzed. The primers are designed on the basis of *SOHLH2* genome (Table 1). All of twelve exons encoding *SOHLH2* regions were amplified by PCR. PCR amplified fragments were sequenced directly on an automated sequencer, ABI Prism Sequencer 3730XL (Applied Biosystems, Foster City, CA).

RESULTS

The genetic analysis of the peripheral blood with 98 POF Korean patients revealed the presence of twelve variations including six novel single-nucleotide polymorphisms (SNP), c.431-41G>C, c.656A>T, c.1000+27C>T, c.1000+33G>T, c1258-106G>A, and c.2094+11T>C; six known SNPs, c.48+87G>A (rs3762116), c.1000+31G>C (rs1410633), c.1015G>A(rs2296968), c.1125C>T (rs2296967), c.1543A>G (rs9546567), and c.2094+14A>G (rs1328643) shown in Table 2. The five novel variations were found on various introns; intron 5 (c.431-41G>C), intron 10 (c.1000+27C>T and c.1000+33G>T), intron 11 (c1258-106G>A), and intron 12 (c.2094+11T>C). The c.656A>T variation was found in exon 7. Only c1258-106G>A variation was found in control group at frequencies that were not significantly different. However, other novel SNPs including c.431-41G>C, c.656A>T, c.1000+27C>T, c.1000+33G>T, and c.2094+11T>C are detected in heterozygotes. The SNP found on exon 7 was novel synonymous variation. The variation result in exchange from tyrosine to phenylalanine at position 218 on protein sequence. The point mutation in the *SOHLH2* gene is not found in Korean women with POF.

In study of population diversity using known SNPs, we identified that several SNPs, c.48+87G>A (rs3762116), c.1015G>A(rs2296968), and c.1125C>T (rs2296967) are high frequency in Asian population compared to European (Table 3). However, other two SNPs, c.1543A>G (rs9546567), and c.2094+14A>G (rs1328643) show similar frequency among population.

DISCUSSION

In the present study we report the first screening of *SOHLH2* gene in 98 unrelated Korean women with POF.

Table 2. SOHLH2 sequencing results in 98 Korean women with POF

Sequence variation	Location	Amino acid variation	dbSNP ID	Allele frequency (x/98)					
				Patients with POF			Control		
				Wild type	Hetrozygote	Homozygote	Wild type	Hetrozygote	Homozygote
c.48+87G>A	Intron 2	Synonymous	rs3762116	34/98(34.7)	44/98(44.9)	20/98(20.4)	*28/88(31.8)	*54/88(61.4)	*6/88(6.8)
c.431-41G>C	Intron 5	Synonymous	Novel	96/98(98)	2/98(2)	0	98/98(100)	0	0
c.656A>T	EXON 7	Tyr218Phe	Novel	97/98(99)	1/98(1)	0	101/101(100)	0	0
c.1000+27C>T	Intron 10	Synonymous	Novel	97/98(99)	1/98(1)	0	107/107(100)	0	0
c.1000+31G>C	Intron 10	Synonymous	rs1410633	98/98(100)	0	0		N/A	
c.1000+33G>T	Intron 10	Synonymous	Novel	97/98(99)	1/98(1)	0	107/107(100)	0	0
c.1015G>A	EXON 10	p.Thr339Ala	rs2296968	29/98(29.6)	51/98(52)	18/98(18.4)	*20/90(22.2)	*50/90(55.6)	*20/90(22.2)
c.1125C>T	EXON 10	Synonymous	rs2296967	87/98(88.8)	11/98(11.2)	0	*70/90(77.8)	*20/90(22.2)	*0
c.1258-106G>A	Intron 11	Synonymous	Novel	86/98(87.8)	12/98(12.2)	0	74/84(88.1)	10/84(11.9)	
c.1543A>G	EXON 11	Synonymous	rs9546567	95/98(96.9)	3/98(3.1)	0	*88/90(97.8)	*2/90(2.2)	*0
c.2094+11T>C	Intron 12	Synonymous	Novel	97/98(99)	1/98(1)	0	105/105(100)	0	0
c.2094+14A>G	Intron 12	Synonymous	rs1328643	90/98(91.8)	8/98(8.2)	0	*66/90(73.3)	*24/90(26.7)	*0

* This data derived from independent sample in the Chinese HapMap Project.
N/A: not available.

Genetic analysis identified six novel SNPs (c.431-41G>C, c.656A>T, c.1000+27C>T, c.1000+33G>T, c1258-106G>A, and c.2094+11T>C) from Korean patients with POF. In POF patient, twelve SNPs were identified on *SOHLH2* gene (Table 2). Among those, twelve SNPs were found on various locations. The c.431-41G>C was found on intron2. The c.656A>T was found on intron 5. The intron 10 contains three SNPs, c.1000+27C>T, c.1000+31G>C and c.1000+33G>T. The c1258-106G>A was located on intron 11. c.2094+11T>C and c.2094+14A>G (rs1328643) were found in intron 12. Exon contains several variations. The c.656A>T was found on exon 7. The variation leads to exchange of one of amino acids, tyrosine to phenylalanine. The exon 10 contains two SNPs, c.1015G>A (rs2296968) and c.1125C>T (rs2296967). The c.1015G>A(rs2296968) resulted in exchange from threonine to alanine at position 339 of protein sequence of SOHLH2. The c.1125C>T (rs2296967) was synonymous. The c.1543A>G (rs9546567)

Table 3. Population diversity in genotype frequency of known SNPs

Sequence variation	dbSNP ID	Population	Individual group	Genotype		
				G/G	G/A	A/A
c.48+87G>A	rs3762116	HapMap-CEU	European	19.00%	55.20%	25.90%
		HapMap-HCB	Asian	31.80%	6.14%	6.80%
		HapMap-JPT	Asian	36.40%	54.50%	9.10%
		HapMap-YRI	Sub-Saharan African	65%	31.70%	3.30%
				C/C	C/G	G/G
c.1000+31G>C	rs1410633	ENSEMBL_Watson		100%	0%	0%
				A/A	A/G	G/G
c.1015G>A	rs2296968	HapMap-CEU	European	5%	43.30%	51.70%
		HapMap-HCB	Asian	22.20%	55.60%	22.20%
		HapMap-JPT	Asian	22.20%	48.90%	28.90%
		HapMap-YRI	Sub-Saharan African	3.30%	21.70%	75%
				C/C	C/T	T/T
c.1125C>T	rs2296967	HapMap-CEU	European	76.70%	21.70%	1.70%
		HapMap-HCB	Asian	77.80%	22.20%	0%
		HapMap-JPT	Asian	88.90%	11.10%	0%
		HapMap-YRI	Sub-Saharan African	100%	0%	0%
				A/A	A/G	G/G
c.1543A>G	rs9546567	HapMap-CEU	European	50.80%	40.70%	8.50%
		HapMap-HCB	Asian	97.80%	2.20%	0%
		HapMap-JPT	Asian	100%	0%	0%
		HapMap-YRI	Sub-Saharan African	70%	30%	0%
				A/A	A/G	G/G
c.2094+14A>G	rs1328643	HapMap-CEU	European	81.70%	18.30%	0%
		HapMap-HCB	Asian	73.30%	26.70%	0%
		HapMap-JPT	Asian	77.30%	22.70%	0%
		HapMap-YRI	Sub-Saharan African	89.80%	10.20%	0%

was found in exon 11. The mutation in c.1015G>A (rs2296968) was found in the control. However one of novel variation found in exon 7, c.656A>T was found in POF patient. The exon7 of *SOHLH2* gene contains DNA binding domain. Therefore, the exchange from tyrosine to phenylalanine might lead to change of SOHLH2 protein structure resulting in affecting its transcriptional activity, even if phenylalanine is similar to tyrosine. It needs further study to examine the mutated protein on the target promoter in the future. Endogenous targets might be from the list of microarray analysis using *SOHLH2* deficient mice. It might include numerous target gene such as *SOHLH1*, *LHX8*, *NOBOX*, *FIGLA*, Zona pellucida 1 (*ZP1*), *ZP3*, *GDF9*, *KIT* and *SOX3* (Choi et al., 2008; Toyoda et al., 2009). These contains specific DNA binding element(s), E-Box which is known as basic helix-loop-helix containing transcription factor (Data not shown here).

In addition, we identified that three SNPs, c.48+87G>A (rs3762116), c.1015G>A(rs2296968), and c.1125C>T (rs2296967) are high frequency in Asian population compared to European (Table 3). It suggests that Korean and other Asian might have closer than European.

In conclusion, our study shows first the relationship between *SOHLH2* gene and Korean patient with either POF. These SNPs data could give us a better understanding on finding causes of POF. Relationship between these SNPs and infertility with POF need further study in the future.

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