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LHX8 Mutation Analysis in Premature Ovarian Failure

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Abstract

LHX8 (LIM homeobox 8) gene encodes a LIM homeodomain transcriptional regulator preferentially expressed in germ cells and critical for mammalian oogenesis. We investigated whether nucleotide changes were present in the *LHX8* gene of Caucasian women with premature ovarian failure (POF) as compared to control women. We sequenced 95 Caucasian women with POF, and discovered two novel single nucleotide polymorphisms (SNPs) in intron 3 (c.769+10G>T) and 3′ untranslated region (c.1787A>G) of the *LHX8* gene. These polymorphisms were also found in controls (N=94) with frequencies that were not statistically different from POF women. Mutations in the *LHX8* exons are uncommon in Caucasian women with POF.

Keywords

Premature Ovarian Failure; LHX8; homeobox; LIM domain; oogenesis

Premature ovarian failure (POF) is defined as ovarian failure before the age of 40 years. It is characterized by secondary amenorrhea, infertility, hypoestrogenism and elevated gonadotropin serum levels (FSH>40 U/L) (1). Mechanisms long invoked in pathogenesis of POF include chromosomal abnormalities of the X or autosomes, autoimmune or infectious aberration, and environmental causes (2,3). POF is heritable in up to 30% of women with POF (4), and predicted to be genetically heterogeneous (5). For selective cases a genetic basis has been shown, involving *FMR1* (6), *FSHR* (7), *POF1B* (8), *FOXL2* (9) or *BMP15* (10); functional data support these mutations being causative. Heterozygous missense mutations and polymorphisms have also been described for *GDF9* (11), *FOXO3A* (12), *FOXO1A* (12), and *INHA* (13); however, functional data for these mutations is lacking. It is likely that mutations in other genes preferentially expressed in the ovaries account for a subset of women with POF.

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Capsule and Narrative Abstract

Two novel single SNPs in the *LHX8* gene were found among women with premature ovarian failure and controls population. Mutations in *LHX8* are uncommon in Caucasian women with POF.

*LHX*8 is a member of the LIM homeobox gene family, a gene family that through transcriptional regulation, plays critical roles in the control of pattern formation and specification during embryonic development (14). *LHX8* in particular has been proposed as a candidate gene for POF based on studies in the murine orthologue *Lhx8* (15). Murine *Lhx8* transcripts localize to oocytes from germ cell cysts through antral follicles, and adult *Lhx8* null ovaries lack germ cells (15). Like other members of the LIM homeobox gene family, *LHX8* contains two tandemly repeated LIM domains, which are characterized by cysteine-rich, double-zinc finger motifs and a distinct homeodomain. Homeobox genes encode proteins that bind DNA and function as transcription factors to control development and differentiation of tissues (16). Murine *Lhx8* protein shares 93% identity with its human orthologue. Encoded by ten exons, human *LHX8* resides on chromosome 1p31.1.

In the present study we investigated for the presence of *LHX8* mutations in 95 American Caucasian women with POF, whose DNA had been collected at Baylor College of Medicine since 2001. Inclusion criteria were cessation of menstrual cycles before 40 years of age, with at least two serum follicle stimulating hormone (FSH) concentrations exceeding 40 IU/L. Women with known chromosomal abnormalities were excluded. Ninety-four (94) women with no evidence of POF served as the control group. Informed consent for molecular studies was obtained from all subjects. The study was approved by the Institutional Review Board of Baylor College of Medicine.

LHX8-specific primers for coding regions including LIM and homeodomain were designed from the human sequence NC_000001 (National Center for Biotechnology Information). All *LHX8* primers are numbered from three to ten according to the exons predicted in NC_000001. The two LIM and one homeodomain regions are ecoded by exons 4-5, 6 and 7-9 respectively. All of the eight exons, encoding *LHX8* conserved LIM and homeodomain regions, were amplified using polymerase chain reaction (PCR). All *LHX8* primers and detailed information of the PCR conditions are available upon request. PCR products were sequenced directly on an automated sequencer, ABI Prism Sequencer 3730XL (Applied Biosystems, Foster City, CA).

Genomic DNA was obtained from peripheral blood. In the POF subjects two novel variants were found in intron 3 and 3′ UTR region respectively. Neither affected the amino acid sequence. The two novel SNPs were also found in controls at frequencies that did not differ significantly ($p>0.05$). We also found a known synonymous variation, rs941032 in exon9, in both POF and control subjects (Table 1).

Our study is the first to investigate the role of *LHX8* gene in the etiology of POF. Although we found two novel SNPs among 95 POF subjects, these SNPs were also present in control women and therefore unlikely to be causative of POF. We conclude that mutations in the coding region of *LHX8* are not common in Caucasian women with POF.

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Table 1

Genotype frequencies of SNPs found in the 95 POF and the 94 controls

Location	Variation	POF (n=95)	Control (n=94)	dbSNP ID
Intron3	c.769+10G>T*			Novel
	GG	47/95 (49.5%)	50/94 (53.2%)	
	GT	37/95 (38.9%)	35/94 (37.2%)	
	TT	11/95 (11.6%)	9/94 (9.6%)	
Exon9	c.1513C>T			rs941032 (synonymous)
	CC	33/95 (34.7%)	35.6%[a]	
	CT	46/95 (48.4%)	50.8%[a]	
	TT	16/95 (16.9%)	13.6%[a]	
3′ UTR	c.1787A>G**			Novel
	AA	91/95 (95.8%)	93/94 (98.9%)	
	AG	4/95 (4.2%)	1/94 (1.1%)	

[a] International HapMap Project Database (CSHL- HAPMAP-CEU)

* Allelic frequencies between the general population and POF cases show no significant differences (Chi square, $p > 0.05$; Fisher's Exact test, $p > 0.05$)