Novel variants in the *SOHLH2* gene are implicated in human premature ovarian failure

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Objective: To determine whether variants in the *SOHLH2* gene contribute to human premature ovarian failure (POF) in different ethnicities.
Design: Case-control genetic study.
Setting: University hospitals.
Patient(s): Chinese (364 cases) and Serbian (197 cases) women with nonsyndromic POF and ethnically matched controls.
Intervention(s): None.
Main Outcome Measure(s): Variation analysis of the *SOHLH2* gene.
Result(s): Eleven novel heterozygous variants were identified in cohorts of POF but were absent in matched controls. These included the nonsynonymous variants p.Glu79Lys (n = 2 cases), p.Glu105Gly, and p.Thr321Pro, which were found among four Chinese POF cases, and p.Leu120Phe (n = 3 cases) and p.Leu204Phe, which were found among four Serbian women. Protein alignments reveal that p.Glu79Lys and p.Glu105Gly involve amino acids highly conserved among mammals, both of which are predicted to be deleterious. The c.-210G>T found in the Chinese POF cohort lies in the core promoter region, which is enriched with transcription factor binding sites and CpG islands. In the Serbian cohort, the variant most likely to have a deleterious effect is c.530+6T>G, which is predicted to affect RNA splicing and result in nonsense mediated decay of transcripts. The other variants are less likely to be deleterious. Disturbing the expression, transactivation or homo-/ heterodimerization of the SOHLH2 protein could result in ovarian failure. Overall, four of the 11 novel variants seem plausible explanations for POF; the other seven variants are less likely but cannot be categorically excluded.
Conclusion(s): Our identification of novel variants in the *SOHLH2* gene, in women with POF of both Chinese and Serbian origin, strongly suggests an important role for *SOHLH2* in human POF etiology. (Fertil Steril® 2014;101:1104–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Premature ovarian failure (POF), primary ovarian insufficiency (POI), *SOHLH2*, variation, ovarian function

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Premature ovarian failure (POF), also termed primary ovarian insufficiency, is an intractable endocrine disorder of female infertility characterized by cessation of normal ovarian function before the age of 40 (1, 2). The etiology of POF is highly heterogeneous, and a genetic contribution is generally considered paramount. Well-documented chromosomal abnormalities have long been known as one explanation, accounting

for 12% of cases in a recent report (3). Increased CGG repeats in the fragile X mental retardation 1 (FMR1) gene is also considered a not uncommon explanation of POF in Caucasian populations (4). Increasing numbers of plausible causative genes involved in nonsyndromic POF have been identified; however, except for *FSHR* in Finnish women (5), none are implicated in more than 10% of POF cases in a given ethnic group. Therefore, the underlying explanation for POF remains largely to be elucidated.

Recently, several transcriptional regulators preferentially expressed in oocytes have been identified, such as *FIGLA* (6), *NOBOX* (7), *LHX8*, *SOHLH1* (8), and *SOHLH2* (9). These and other genes in specialized transcription networks regulate ovary-specific gene expression, including *BMP15*, *GDF9*, *ZP1*, *ZP2*, *ZP3*, and *POU5F1*, which are pivotal for early folliculogenesis without functional redundancy (10). *SOHLH2*, as well as *SOHLH1*, encodes a basic helix-loop-helix (bHLH) master transcription factor that is implicated in early oogenesis and spermatogenesis. SOHLH2 forms homodimers or heterodimers with SOHLH1 to coordinate transcription of key germ cell–specific genes through binding to conserved E-boxes in their promoter regions (11, 12). Its expression is uniquely confined to germ cell clusters of the embryonic ovary and oocytes of primordial to primary follicle stage when transcription of numerous oocyte-specific genes commences (9, 11). *Sohlh2*-null female mice exhibit infertility and atrophied ovaries devoid of follicles, mimicking the human POF phenotype. Oogenesis is disturbed owing to defective primordial to primary follicle transition, and thus accelerated postnatal oocyte loss occurs (11, 13). The present study was designed to determine whether *SOHLH2* variants contribute to human POF in large cohorts of women with POF of Chinese and Serbian origin.

MATERIALS AND METHODS

Patients

A total of 561 women with POF of Han Chinese (n = 364) and Serbian (n = 197) origin, were recruited from the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, People's Republic of China, and the Institute of Endocrinology, Clinical Center of Serbia, University of Belgrade, Belgrade, Serbia, respectively. All were screened for variants in the *SOHLH2* gene. Inclusion criteria consisted of secondary amenorrhea for at least 6 months before 40 years of age, with at least two serum FSH concentrations exceeding 40 IU/L. A positive family history was considered to exist if another first- or second-degree female family member had POF or early menopause (before age 45). All women with POF were karyotyped, and cases with chromosomal abnormalities were excluded. Women with a history of autoimmune disorders, such as Addison's disease, thyroid disorders, rheumatoid arthritis, psoriasis, and systemic lupus erythematosus, and those with positive results of adrenal cortex autoantibodies (Chinese POF) or the battery of autoantibodies (Serbian POF) were excluded. Women with previous chemo-/radiotherapy or ovarian surgery were excluded. Accompanying somatic anomalies, particularly any reported as associated with syndromic POF (Chinese cases: two neurosensory deafness, one blepharophimosis, and one cerebellar ataxia; Serbian cases: no anomalies), were sought and excluded. FMR1 premutation was not detected in the subjects. Ethnically matched healthy women served as controls. Chinese controls were recruited from routine health checkups, including [1] reproductive-age women with regular menses, normal estrogen and gonadotropin hormone levels, and ovarian imaging (age <40 years; 222 cases); [2] advanced-age women with regular menses and FSH <40 IU/L (age between 40 and 50 years; 178 cases). The Serbian controls were regularly menstruating women with normal FSH concentration (<10 IU/L). Ultrasound of reproductive organs was performed to verify the presence of a uterus, presence and morphologic status of ovaries, and absence of unexpected explanations for amenorrhea (e.g., neoplasia; n = 200 cases). Written informed consent was obtained from all subjects. The study was approved by the Ethics Review Board of the Center for Reproductive Medicine of Shandong University and by the Institutional Review Board of the University of Belgrade.

Variation Screening

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen; Chinese cohort) and using Oragene DNA collection kits (DNA Genotek; Serbian cohort). The entire coding sequence and intron-exon boundaries of the *SOHLH2* gene (NG_033786.1) were polymerase chain reaction (PCR) amplified. Sequencing was carried out on an ABI 3730xl DNA Analyzer (Applied Biosystems) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The novel variants identified were confirmed by bidirectional sequencing from another two independent PCR products. Descriptions of the variants identified was established according to Human Genome Variation Society (http://www.hgvs.org/mutnomen) recommendations. Primers are listed in Supplemental Table 1.

In Silico Prediction

Sequence alignments of the SOHLH2 protein among mammals were performed using ClustalW (http://www.ch.embnet.org/software/ClustalW.html). To assess the possible functional effect of amino acid variants, PolyPhen-2 v.2.2.5 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), SNPs&GO (http://snps-and-go.biocomp.unibo.it/), MutPred v.1.2 (http://mutpred.mutdb.org/), and Mutation Taster (http://www.mutationtaster.org/) were used. To assess the possible functional effects of sequence variants on RNA splicing, the following tools were used. Exonic variants were analyzed for possible disruption of splice enhancer and silencer sites with ESEfinder v.3.0 (http://rulai.cshl.edu/tools/ESE), RESCUE-ESE v.1.0 (http://genes.mit.edu/burgelab/rescue-ese), and PESX (http://cubweb.biology.columbia.edu/pesx/). Intronic variants were analyzed using Splice Site Finder (http://violin.genet.sickkids.on.ca/~ali/splicesitefinder.html), NNSPLICE v.0.9 (http://www.fruitfly.org/seq_tools/splice.html), NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/), and Human Splicing Finder v.2.4.1 (http://www.umd.be/HSF/).