BRIEF REPORT

CITED2 mutations potentially cause idiopathic premature ovarian failure

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Anomalies in gonadal development in a mouse knockout model of *Cited2* have been recently described. In *Cited2* $^{-/-}$ female gonads, an ectopic cell migration was observed and the female program of sex determination was transiently delayed. We hypothesize that, in humans, this temporary inhibition of genes should be sufficient to provoke a developmental impairment of the female gonads, conducive to premature ovarian failure (POF). To establish whether *CITED2* mutations are a common cause of the disease, we performed a mutational analysis of this gene in a panel of patients with POF and in a group of control women with normal fertility. We amplified and directly sequenced the complete open reading frame of *CITED2* in 139 patients with POF and 290 controls. This study revealed 5 synonymous and 3 nonsynonymous variants. Among these, 7 are novel. The nonsynonymous variant c.604C>A (p.Pro202Thr) was found uniquely in 1 woman from the POF group. In silico analysis of this mutation indicated a potential deleterious effect. We conclude that mutations in *CITED2* may be involved in POF pathogenesis. (Translational Research 2012;160:384–388)

Abbreviations: FSH = follicle-stimulating hormone; POF = premature ovarian failure; SGJ = serine-glycine-rich junction

Premature ovarian failure (POF) is a frequent pathology that affects approximately 1.5% of women.[1] POF is defined as at least 6 months of amenorrhea associated with elevated plasma levels of follicle-stimulating hormone (FSH) (>40 IU/mL) in women aged less than 40 years.[2] Several causative

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AT A GLANCE COMMENTARY

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Background

POF is a frequent pathology leading to infertility. The majority of cases are considered idiopathic, which suggests a genetic origin of the disease.

Translational Significance

Our results suggest a novel role of *CITED2* during human female gonadal development and POF origin.

factors have been described etiologically, such as infectious process, autoimmune conditions, iatrogeny, and anticancer treatments. Nevertheless, the majority of cases are considered idiopathic, suggesting the involvement of genetic, epigenetic, and environmental factors. Although POF molecular etiology is poorly understood, some genetic abnormalities have been identified in syndromic and nonsyndromic forms of the disease.[3,4] For example, in Turner's syndrome the haploinsufficiency of pseudoautosomal loci that escape inactivation may be the cause of the ovarian dysfunction.[5] Furthermore, sequence variants in some autosomal and X-linked genes have been associated with the etiology of nonsyndromic POF cases.[3,4]

In recent years, the study of genes identified from genetically modified mouse models displaying dysfunctional reproduction has expanded the repertoire of potential POF genes in humans.[6,7] Combes et al[8] recently studied a knockout model of *CBP/p300-interacting transactivator with ED-rich tail 2* (*Cited2*) that displays reduced levels of steroidogenic factor 1 (Sf1). During testis development, Cited2 (a transcriptional coactivator) cooperates with the Wilms tumor 1 protein (Wt1) to increase the expression of *Sf1* and *sex-determining region of the Y-chromosome* (*Sry*), 2 key genes of the male sex determination pathway.[9] In the XX gonad, the expression of specific genes such as *R-spondin* (*Rspo1*) and *wingless-type MMTV integration site family member 4* (*Wnt4*) enhance ovarian development while opposing testis development.[10] Furthermore, mesonephric cell migration (which leads to the testis cord formation) is repressed by WNT signaling in XX gonads.

Of note, in *Cited2*$^{-/-}$ female gonads, ectopic cell migration was observed and the female program of gonadal development was transiently delayed.[8] At 10.5 days post coitum, the expression levels of pro-ovarian genes, such as the *forkhead box protein L2* (*Foxl2*), *Rspo1,* and *Wnt4*, were decreased but subsequently restored at 12.5 days post coitum. In this context, we hypothesize that this temporary inhibition of ovarian–promoting genes could be sufficient to provoke a developmental impairment of the female gonads, conducive to POF.

To establish whether *CITED2* mutations are a common cause of the disease, we performed a mutational analysis of this gene on a panel of 139 patients with idiopathic POF and in a group of 290 control women with normal fertility. We identified the first mutation in *CITED2* that is potentially related to the underlying etiology. Our results argue in favor of a previously unrecognized role for *CITED2* during human female gonadal development and POF origin.

MATERIALS AND METHODS

Patients and controls. The POF patient group comprised 139 women. A total of 116 patients were of Tunisian origin and attended the Farhat Hached University Teaching Hospital (Sousse, Tunisia), and 23 patients were of Australian origin and recruited from the Royal Children's Hospital in Melbourne. All patients displayed a normal 46, XX karyotype, primary or secondary amenorrhea occurring before the age of 40 years and FSH plasma levels >40 IU/mL. Patients presenting clinical records of pelvic surgery, chemotherapy or radiotherapy treatments, or autoimmune diseases were excluded from the study. The control group included 290 women who presented menopause after the age of 50 years and lack antecedents of ovarian disease. These individuals reported regular menstrual cycles and had at least 1 child. Among these, 140 were of Tunisian origin (group A) and 150 were of Colombian origin (group B). All clinical and experimental steps of this study were approved by the institutional ethics committee of each participating institution. Patients and controls provided written informed consent.

***CITED2* mutational analysis.** Genomic DNA from patients and controls were extracted from whole blood samples using standard procedures. In all patients and controls, the complete open reading frame of *CITED2* was amplified by polymerase chain reaction using exon-flanking oligonucleotides. Each amplicon was sequenced with internal primers. The presence of each nonsynonymous variant was confirmed by an additional polymerase chain reaction and sequencing assay. We aligned human wild-type (GI: 270288756) and mutant sequences with those from numerous vertebrate species. The potential pathogenic effect of nonsynonymous variants was evaluated using PolyPhen2 software (v. 2.0.23). The PolyPhen2 (Harvard University, Boston, Mass) prediction values are the result of an algorithm that includes distinct features, such as

Table I. Results of the *CITED2* sequencing analysis performed in 139 patients with premature ovarian failure and 290 controls

			Allele frequency			
			Patients with POF		Controls	
NV	AA change	Allele	Tunisian	Australian	Group A	Group B
c.21C>A	p.Ala7Ala	C	198/232 (0.85)	46/46 (1)	247/280 (0.88)	251/300 (0.84)
		A	34/232 (0.15)	0/46 (0)	33/280 (0.12)	49/300 (0.16)
c.510G>C	p.Ser170Ser	G	231/232 (0.995)	46/46 (1)	279/280 (0.996)	300/300 (1)
		C	1/232 (0.005)	0/46 (0)	1/280 (0.004)	0/300 (0)
c.539G>A	p.Gly180Asp	G	232/232 (1)	46/46 (1)	280/280 (1)	299/300 (0.996)
		A	0/232 (0)	0/46 (0)	0/280 (0)	1/300 (0.004)
c.574A>G	p.Ser192Gly	A	232/232 (1)	46/46 (1)	280/280 (1)	292/300 (0.97)
		G	0/232 (0)	0/46 (0)	0/280 (0)	8/300 (0.03)
c.582C>T	p.Gly194Gly	C	231/232 (0.995)	46/46 (1)	279/280 (0.996)	300/300 (1)
		T	1/232 (0.005)	0/46 (0)	1/280 (0.004)	0/300 (0)
c.604C>A	p.Pro202Thr	C	231/232 (0.995)	46/46 (1)	280/280 (1)	300/300 (1)
		A	1/232 (0.005)	0/46 (0)	0/280 (0)	0/300 (0)
c.612C>G	p.Ser204Ser	C	232/232 (1)	46/46 (1)	280/280 (1)	299/300 (0.996)
		G	0/232 (0)	0/46 (0)	0/280 (0)	1/300 (0.004)
c.630T>A	p.Ala210Ala	T	232/232 (1)	46/46 (1)	280/280 (1)	299/300 (0.996)
		A	0/232 (0)	0/46 (0)	0/280 (0)	1/300 (0.004)

Abbreviations: AA, amino acid; *NV,* nucleotide variation; *POF,* premature ovarian failure.

comparative analysis of protein sequences from different species, physicochemical characteristics of the exchanged amino acids, and mapping of residue replacement to available 3-dimensional structures. Its positive predictive rate ranges from 73% to 92%. PolyPhen2 results are assessed as probably damaging (more confident prediction), possibly damaging (less confident prediction), or benign (nonpathogenic).[11]

RESULTS

Sequencing analysis revealed 5 synonymous and 3 nonsynonymous heterozygous variants (Table I). Among patients with POF, *CITED2* variants were found exclusively in Tunisian women. The nonsynonymous variant (c.604C>A, p.Pro202Thr) was found in 1 woman with POF and was not previously described. All these variants, except for p.Pro202Thr, are unlikely to be related to the etiology of the disease because they have been identified in control individuals of the group A. Thus, we expanded its screening to women with normal fertility from a distinct ethnical origin (control group B). Likewise, this mutation was absent in the control population of Colombian origin. Comparative protein analysis between vertebrate species demonstrated a strict conservation of the Pro residue at position 202 (Online Fig 1). Polyphen2 software classified this mutation as probably damaging. The p.Gly180Asp and Ser192Gly sequence variants present in women from the control B population were classified as possibly damaging and benign, respectively. Protein alignments showed that both glycine and serine residues are not strictly conserved among vertebrate species (Online Fig 1).

The patient carrying the heterozygous p.Pro202Thr mutation is a 34-year-old woman of Tunisian origin presenting with secondary amenorrhea. External genitalia and pubertal development were normal. Menarche appeared at 13 years of age, with 3 menstrual cycles followed by 3 years of oligomenorrhea. Amenorrhea and abnormally high levels of FSH (63 IU/mL) and luteinizing hormone (90 IU/mL) were recorded at 16 years. Pelvic ultrasonography showed a hypoplastic uterus and small ovaries lacking follicles. The 2 sisters of this patient, who did not show ovarian dysfunction, lack this p.Pro202Thr variant. Unfortunately, we could not obtain clinical data and biological samples from the mother.

DISCUSSION

From a genetic point of view, it has been demonstrated that the *SRY* gene located on the Y chromosome is responsible for activating the molecular pathway leading to the formation of the male gonads.[12] In addition, some specific pro-ovarian molecules have been identified in recent years.[13] Those genes that play a critical role in early ovary development have been shown to have mutations that can cause POF.[3,4] The recent findings of Combes and coworkers[8] revealed a temporary delay in the female pathway caused by the ablation of the *Cited2* gene in mouse. This strongly suggests that *CITED2* may have a potential involvement in human POF pathogenesis.

In the present study, sequence analysis of the *CITED2* coding region in a panel of women with POF and control individuals revealed 8 sequence variants (Table I). Most of these sequence variants are unlikely to be related to the etiology of the disease because they have been identified in individuals of control group A or they are silent substitutions. However, the c.604C>A (p.Pro202Thr) variant was observed in 1 patient with POF and was absent in control individuals of 2 distinct ethnical origins (groups A and B). The 2 sisters of this patient, who did not show ovarian dysfunction, lack this p.Pro202Thr mutation. Although we could not definitely establish the parental origin of the variant, these features suggest its potential role during POF pathogenesis. Furthermore, in silico analysis of the mutant protein argues in favor of a pathogenic effect because the proline residue at position 202 is strictly conserved among vertebrate species, suggesting its crucial functional role (Online Fig 1). The presence of a Pro instead a Thr is predicted to alter both structure and hydrophobicity of the encoded protein. Indeed, proline is a nonpolar hydrophobic residue, and threonine is a polar amino acid possessing hydrophilic properties. These differences might alter the protein folding and its function. Accordingly, the PolyPhen2 bioinformatic tool displayed a score compatible with a potential pathogenic effect.

The *CITED2* p.Pro202Thr mutation found in our case is located in the vicinity of the serine-glycine-rich junction (SGJ) of the protein (residues 162–199), which has been described as a mutational hotspot. Indeed, 3 heterozygous mutations in this region (p.Ser170_Gly178del, p.Gly178_Ser179ins9, and p.Ser198_Gly199del) have been functionally characterized.[14] In normal conditions, hypoxia-inducible factor 1, alpha subunit and transcription factor activation protein-2 gamma coactivation is modulated by Cited2.[15,16] In the study by Sperling and coworkers,[14] all 3 mutant proteins displayed a reduction in hypoxia-inducible factor 1, alpha subunit transcriptional repressive capacity of CITED2. Moreover, the p.Ser170_Gly178del mutation was associated with diminished levels of transcription factor activation protein-2 gamma coactivation. These results led to the proposal that mutations of the SGJ could alter the capacity of the CITED2-EP300 binding domain to recruit or interact with essential cofactors. Likewise, the mutation found in our case might interfere with the normal folding of the SGJ or the protein conformation itself, modifying its transrepression/transactivation properties when coupled with transcription factors. Of note, 2 controls of Colombian origin presented nonsynonymous mutations (p.Gly180Asp and p.Ser192Gly) that were not identified in patients with POF. Because Polyphen2 and comparative protein analysis did not display results related with deleterious effects, we estimate that they lack functional relevance and may constitute common specific SNPs present in the Colombian population.

To date, mutations located in the SJS region have been uniquely associated with cardiac abnormalities.[14] Therefore, it is possible that the p.Pro202Thr, as well as other CITED2 mutations located on the SGJ region, possesses differential pathogenic effects during cardiac or ovarian development that are due to a distinct proteomic environment between these tissues. Thus, it would be interesting to know whether the patients described by Sperling and coworkers[14] display reproductive dysfunctions.

In the ovarian context, the phenotype observed in our case argues in favor of a drastic functional effect of the p.Pro202Thr mutation because the patient presented with secondary amenorrhea at the age of 16 years. Thus, we hypothesize that the haploinsufficiency of *CITED2* might affect the normal expression of further essential factors that participate in the female gonadal differentiation program, such as SF1, FOXL2, RSPO1, and WNT4. This assumption is plausible because it has been demonstrated that mammalian gonadal development (and the Cited2-Wt1-Sf1 regulatory cascade) is extremely sensitive to gene dosage.[9,13] Furthermore, mutations in *SF1* and *FOXL2* have been related with nonsyndromic and idiopathic POF cases.[17,18]

CONCLUSIONS

Taken together, our results suggest that mutations in *CITED2* may be involved in POF pathogenesis. Although it has been established that in *Cited2*$^{-/-}$ animals, ovaries show cellular and molecular defects at fetal stages,[8] the consequences of *Cited2* mutation for the long-term function or pathogenesis of the ovary in adult mice are currently unknown. *Cited2*$^{-/-}$ females are fertile, indicating that a certain level of ovarian function is retained. Further studies would be required to determine how long fertility is maintained compared with wild-type female mice, or whether the adult females become susceptible to any reproductive disorder(s), such as POF. The present results may provide the impetus for exploring this issue further.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.trsl.2012.05.006.

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