Reproductive BioMedicine Online (2011) 23, 697–703

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Identification of novel missense mutations of the *TGFBR3* gene in Chinese women with premature ovarian failure

Chun-rong Qin [a,b], Shi-ling Chen [a,*], Ji-long Yao [b], Wei-qing Wu [c], Jian-sheng Xie [c]

[a] *Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, 1838 North Guangzhou Avenue, Guangzhou 510515, PR China;* [b] *Department of Obstetrics and Gynecology, The Affiliated Shenzhen City Maternity and Child Healthcare Hospital of Southern Medical University, Shenzhen, Guangdong Province, PR China;* [c] *Department of Central Laboratory, The Affiliated Shenzhen City Maternity and Child Healthcare Hospital of Southern Medical University, Shenzhen, Guangdong Province, PR China*
[*] Corresponding author. *E-mail address:* chensl_92@163.com (S-l Chen).

Professor Shi-ling Chen obtained her MD degree in 1989. From 2000 to 2003, she was a visiting scholar in University of Medicine and Dentistry of New Jersey. Professor Shi-ling Chen is a well-known infertility specialist in China and she is an American Medical Association member. Her research interest include the relationship of epigenetics (imprinted gene) and assisted reproductive techniques, optimized ovarian stimulation protocols, prevention of IVF complications, diminished ovarian reserve and premature ovarian failure.

Abstract The aim of this study was to assess the association between human transforming growth factor β receptor, type III (*TGFBR3*) and idiopathic premature ovarian failure (POF) in a Chinese population. A total of 112 Chinese women with idiopathic POF and 110 normal controls were examined. DNA samples prepared from blood leukocytes were used as templates for polymerase-chain reaction amplification of DNA fragments from *TGFBR3*. The gene fragments were sequenced. Web-based programs, including PolyPhen, Sorting Intolerant from Tolerant (SIFT), Prediction of Pathological Mutations (PMUT), ScanProsite and ClustalW2, were used to predict the potential functional and structural impacts of the missense variants of *TGFBR3*. A total of 11 novel variants were identified. Among them, six were found only in the POF patients. Two missense variants, p.E459G and p.P825L, which are conserved in primates, were predicted to have functional and structural impacts on the *TGFBR3* protein. The other four variants (c.381+12A>C, c.2431−7A>G, p.S172S and p.C220C) were considered benign. However, further functional studies are necessary to confirm these findings.

KEYWORDS: infertility, mutation, premature ovarian failure, *TGFBR3*

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2011.07.021

Introduction

Premature ovarian failure (POF) is defined as the cessation of ovarian function with elevated gonadotrophins and low oestrogen concentration before the age of 40. This condition affects approximately 1% of reproductive-age women (Panay and Kalu, 2009). Besides the health risks associated with reduced oestrogen concentrations over many years, such as osteoporosis, the major concern for affected women is typically infertility. POF is a heterogeneous disorder caused by a variety of mechanisms and although the cause remains unexplained in the majority of cases, data indicate that POF has a strong genetic component (van Kasteren et al., 1999; Vegetti et al., 1998). Few gene targets, however, have been positively correlated with POF, which necessitates further candidate screening.

Taking into account that transforming growth factor (TGF) β receptor, type III (*TGFBR3*) is an essential component in mediating the specific antagonist action of inhibins toward activins and bone morphogenetic proteins (BMP) (Lewis et al., 2000; Wiater and Vale, 2003), which is crucial for normal ovarian function, genetic variants in *TGFBR3* could affect the risk of POF.

TGFBR3 are transmembrane proteins that modulate TGF-β-mediated cellular responses. They have large extracellular domains and serine/threonine-rich cytoplasmic regions. Lacking a signalling component, *TGFBR3* consists of two ligand-binding regions, the endoglin-related (Lopez-Casillas et al., 1993) and uromodulin-related domains (Esparza-Lopez et al., 2001). Although TGF-β interacts with both domains, inhibin binding is restricted to the uromodulin-related domain, where it binds with high affinity.

Inhibins are the most important factors directly involved in the down-regulation of FSH via a negative feedback mechanism. Failure to modulate FSH concentrations results in a diminished ovarian reserve and is clinically characterized as hypergonadotrophic, hypogonadism or POF (Welt et al., 2005; Zintzaras, 2009). The regulation of FSH concentrations is maintained by opposing actions of both inhibins and activins. Lewis et al. (2000) reported *TGFBR3* as an inhibin-specific receptor that satisfies all the receptor criteria. The activins bind their type-II receptors (ActRIIA and ActRIIB), which are located on the gonadotroph cell surface. The activin/ActRII complex further recruits type-I receptor ALK4. This activin/ActRII/ALK4 complex in turn activates Smad2/3 signalling and results in elevated FSH production. Conversely, inhibins bind *TGFBR3*, located on the gonadotrophs. *TGFBR3* also enhances the inhibin-binding affinity to activin and BMP type-II receptors (ActRII and BMPRII), thus preventing ligand binding and recruitment of type-I receptor, therefore competing with activin-mediated signalling. This inhibin-mediated phenomenon is incompetent to initiate Smad signalling, resulting in the down-regulation of FSH production (Lewis et al., 2000). Because inhibins bind with low affinity to type-II receptors in the absence of *TGFBR3*, it has an essential, direct role in potentiating inhibin antagonism of activin and BMP signalling.

Two studies have reported an association between POF and the gene *TGFBR3*. First, an Indian study (Dixit et al., 2006) reported genotypic distribution of three variants (c.382−81C>T, c.382−77T>C and c.1200G>A) that was significantly different between the patients and the controls. The second, a New Zealand study (Chand et al., 2007), targeted mutational screening of *TGFBR3* (exons 11 and 12) and demonstrated an increased occurrence of the exon 12 194969T>C variant in POF patients, compared with controls, indicating that *TGFBR3* might be a new candidate gene involved in POF. The study aimed to investigate a possible association between *TGFBR3* polymorphisms and POF in the Chinese Han population.

Materials and methods

Patient and control recruitment

One hundred and twelve patients with idiopathic POF were recruited between January 2009 and July 2010 at the Affiliated Shenzhen City Maternity and Child Healthcare Hospital of Southern Medical University, Shenzhen, PR China. The study was approved by the University's Institutional Ethics Committee and informed consent was obtained from all participants. The diagnostic criteria for POF was as follows: at least 6 months of amenorrhoea before the age of 40, with at least two serum FSH concentrations of >40 IU/l. Controls (n = 110) were individuals under 40 with proven fertility, normal menstrual cycles, normal FSH concentrations and ovary morphology, with no history of subfertility treatment. Each patient and control were assessed clinically, with a complete medical and gynaecological history, including the history of menses, age at menopause, LH and FSH concentrations (twice at 1-month intervals) and pelvic ultrasound. Patients with associated endocrinopathies, autoimmune disorders, iatrogenic agents, such as pelvic surgery, chemotherapy and radiotherapy, and infections, were excluded. Karyotyping with high-resolution GTG banding to check for chromosomal anomalies was performed in all patients and controls. Those with abnormalities were excluded from the study.

DNA extraction and karyotyping

A 5-ml aliquot of peripheral blood was collected in EDTA vacutainers for genomic DNA isolation, and another 5 ml of peripheral blood was collected in heparin vacutainers for chromosomal analysis. Genomic DNA was extracted from lymphocytes using standard proteinase K/chloroform extraction methods (Shelling et al., 2000). Chromosomal analysis was performed on phytohaemagglutinin-stimulated peripheral lymphocyte cultures using standard conventional cytogenetic methods.

PCR protocol

TGFBR3 is comprised of 16 exons. Primers for all the exons were designed using Genefisher software (http://bibiserv.techfak.uni-bielefeld.de/genefisher). All PCR conditions included initial denaturation at 98°C for 3 min, cyclic denaturation at 94°C for 45 s and extension at 72°C for 45 s with a total of 35 cycles. The primer sequences, with their corresponding annealing temperatures, are summarized in **Table 1**. Annealing time was 45 s. The presence of all sequence variants was confirmed by performing three independent PCRs and subsequent DNA sequencing.