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Original Article

Tubal ectopic pregnancy occurrence is associated with high expressions of prokineticin receptors and aberrant secretion of inflammatory cytokines

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Abstract: Objective: Tubal ectopic pregnancy (TEP) remains the most common cause of maternal morbidity and mortality in the early months of pregnancy. The aim of this study is to perform the correlation between PROKRs and pro-inflammatory genes and explore the role of novel genes in pathogenesis of TEP. Methods: Here, quantitative real time PCR and immunohistochemistry were used to assess the expression of the novel genes in 120 TEP patients and 30 age-matched non-TEP patients. The correlation between PROKRs and pro-inflammatory genes were analyzed by Pearson correlation coefficient. Univariate and multivariate Cox regression analyses were used to assess the risk prediction rate of novel genes. Receiver operating characteristic was used to assess the performance of our model. Results: PROKRs (PROKR1 and PROKR2) and pro-inflammatory genes (TNF-α, IL-6, and IL-8) expression levels significantly enhanced in TEP patients, and significantly positive correlation with pro-inflammatory genes for PROKRs. A multivariate Cox regression analysis demonstrated that 2 genes (PROKR2 and IL8) had significant diagnostic value, which were associated with the occurrence and development of TEP. Conclusion: Our data further denote that dysregulation of PROKR2 and IL-8 were risk factor and played an important role in the pathogenesis of TEP.

Keywords: Tubal ectopic pregnancy, PROKRs pro-inflammatory genes, predictive and diagnostic value

Introduction

Ectopic pregnancy (EP) is a form of abnormal pregnancy and the most common implantation site is the ampullary area of the fallopian tube [1]. EP affects about 1-2% of pregnant women, and EP bleeding caused by rupture of fallopian tube is still the most common cause of maternal death in the early months of pregnancy [2, 3]. Due to improvements in early diagnosis using high-resolution transvaginal ultrasound sonography and laparoscopy, the maternal morbidity and mortality rates of EP have decreased [4]. Nevertheless, it remains the most common life-threatening early pregnancy complication. Tubal ectopic pregnancy (TEP) accounted for over 90% of EP, which may lead to tubal rupture to seriously influence the health and future fertility of pregnant woman, and increases the risk for future EP [5, 6]. Previous studies have shown that an abnormal tubal transport of the embryo, along with inflammation, activins expression, and damaged tubal environment, may cause tubal implantation [7-9]. However, the pathogenesis of TEP remains relatively unknown. A greater understanding of the pathogenesis of TEP is important for improving the preventative programs and the advancement of novel diagnosis and treatments.

The transport of the embryo is controlled by a combination of tubal epithelial, smooth muscle, and immune cells through the fallopian tube to the uterus for implantation. Prokineticin receptors (PROKR1 and PROKR2) are a regulator of smooth muscle contractility, which involved in multiple biological progression through the recruitment of different G-proteins. Current evi-

Table 1. Summary of tubal ectopic pregnancy cohort information

Characteristics	Case	Percentage/%
Age (years)		
Range	26~38	
Median	34	
Smoke		
Yes	23	19.17
No	97	80.83
History of EP		
Yes	17	14.17
No	103	85.83
History of pelvic infections		
Yes	39	32.5
No	81	67.5
History of Abortion		
Yes	25	20.83
No	95	79.17

dence suggests that cigarette smoking and *chlamydia trachomatis* infection which were the most common risk factor for TEP [10], increased PROKR1 and PROKR2 levels in the fallopian tube [11, 12]. Theses research suggested that PROKRs may play a key role in the occurrence of TEP. Previous studies have shown that EG-VEGF promoted the expressions of inflammation include cyclooxygenase (COX)-2, leukemia inhibitory factor (LIF), IL-6, and IL-11 in early human pregnancy [13]. The inflammatory environment also plays an important role in tubal implantation in women. The expression levels of IL-6 and IL-8 were up-regulated and down-regulated, respectively, in patients with TEP [14]. Leukemia inhibitory factor (LIF), a member of the interleukin-6 family of cytokines, might contribute to the development of an embryo implants at other sites [15]. Here, we speculated that PROKRs may have a correlation with pro-inflammation genes in fallopian tube from with TEP.

A previous study found that PROKRs were localized to the epithelium and smooth muscle layers of the fallopian tube, and their expression is dysregulated in fallopian tube from patients with TEP [16]. However, the correlation between PROKRs and normal secretion of pro-inflammatory genes has not been fully understood in the fallopian tube from women with TEP. In this study, we found for the first time that the significant positive correlation between PROKRs and pro-inflammation genes for women with TEP, their abnormal expression were associated with cigarette smoking habit, history of EP, history of pelvic infections, or history of abortion. A multivariate Cox regression analysis revealed that 2-genes (PROKR2 and IL-8) had significant diagnostic value, their cumulative risk score indicated that the 2 genes (PROKR2 and IL8) may play an important role in the occurrence and development of TEP.

Materials and methods

Patient recruitment

The study was approved by the Institute Research Medical Ethics Committee of the Seventh Affiliated Hospital of the Sun Yat-sen University. All participants provided informed consent before their enrollment in the study. A total of 150 patients from January 2016 to June 2018 in The Seventh Affiliated Hospital of Sun Yat-sen University were enrolled in the study, of these 120 were women with ruptured tubal ectopic pregnancy (TEP) and the remaining 30 were age-matched non-TEP, who underwent total abdominal hysterectomy for uterine fibroids or cervical cancer. TEP is diagnosed in patients with positive β-human chorionic gonadotropin (β-hCG) and the gestational sac, yolk sac, and germ located in the fallopian tube by transvaginal ultrasound or laparoscopy. The clinical history of the TEP patients are detailed in **Table 1**.

TEP patients were selected according to the following criteria: age 26-38 years, tubal ampulla pregnancy following spontaneous conception, singleton pregnancy and hemodynamically stable. The exclusion criteria of TEP patients were as follows: age <18 or >40 years, multiple/heterotopic pregnancy, and symptoms and signs of hypovolemia.

Collection of fallopian tube samples

Fresh fallopian tube samples were collected from of TEP patients and non-TEP by salpingectomy or total abdominal hysterectomy. The fallopian tubes of fertilized egg implantation site (Implantation) and the adjacent fallopian tubes of the unimplanted site (Adjacent) were collected in TEP patients. The collected fallopian tube samples were divided into two parts, one of which was fixed in 4% paraformaldehyde (PFA,

Table 2. The primer sequences used in this study

Gene	Forward primer	Reverse primer
PROKR-1	GGTGTCCATCCTGATCGCCA	CAGGCCAGATCTGGCCGCAG
PROKR-2	GCTTACTTTGCAACAGAAAC	ATGAAGAGGAAGTAGGACTT
TNF-α	CAGTCAGATCATCTTCTCGA	CCGGCGGTTCAGCCACTGGA
IL-6	CCAGAGCTGTGCAGATGAGT	CTGCAGCTTCGTCAGCAGGC
IL-8	AGTGCATAAAGACATACTCC	GCTTTACAATAATTTCTGTG
GAPDH	GCTCATTTGCAGGGGGGAG	GTTGGTGGTGCAGGAGGCA

Sigma) for immunohistochemical analysis, and the other was stored in -80°C refrigerator for gene expression analysis.

Immunohistochemistry

Immunohistochemistry was performed as described previously with appropriate modifications [17]. Briefly, all fresh fallopian tubes were immediately fixed in 4% paraformaldehyde solution (PFA, Sigma) at 4°C overnight followed by dehydrated and embedded in paraffin, and then serially cut into 4-μm-thick sections. The sections were incubated with primary antibodies overnight at 4°C, including Rabbit Anti-PROKR1 antibody (1:50 in TBS containing 0.01% Triton X-100; ab140976, Abcam, USA) and Rabbit anti-PROKR2 antibody (1:50 dilution in TBS containing 0.01% Triton X-100; ab42805, Abcam, USA). After washing with TBS, the sections were incubated with horseradish peroxidase-conjugated Goat Anti-Rabbit IgG (1:500 dilution in TBS containing 0.01% Triton X-100; ab205718, Abcam, USA) for 30 min at room temperature. Then, the sections were performed with diaminaobenzide colorimetric reagent solution (Dako, Carpinteria, USA). Finally, the sections were counterstained with Mayer's hematoxylin (Sigma, St. Louis, MO, USA). Negative controls were prepared by replacing the primary antibody with TBS. Images were captured by using the Olympus light microscope (Tokyo, Japan) and assessed by using Image-Pro Plus v6.0 software (Media Cybernetics Inc., Bethesda, MD, USA).

Quantitative real-time PCR

Total RNA was extracted from the fallopian tube tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions of manufacturer. The concentration and purity of total RNA were assessed by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, San Jose, CA, USA). Then, the RNA were reverse-transcribed to cDNA using the PrimeScript RT Reagent kit with cDNA Eraser (Takara, Kyoto, Japan) according to the manufacturer's instructions. Quantitative real-time PCR were conducted using SYBR Green qPCR SuperMix (Invitrogen, Carlsbad, CA, USA) and performed on ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The results were normalized with GAPDH as an internal control. All the primers were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and showed in **Table 2**. The relative fold change expression of the genes was calculated by using the $2^{-\Delta\Delta Ct}$ method. The experiments were performed in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS version 19.0 statistical analysis package (SPSS Inc., Chicago, IL, USA). All results were expressed as the mean ± standard deviation (SD). Statistical comparisons were performed using the two-tailed Student's t-test between the two groups. Statistical comparisons among multiple groups were carried out using one-way ANOVA followed by a Tukey post-hoc test. The correlation between PROKRs (PROKR1 and PROKR2) and pro-inflammatory genes (TNF-α, IL-6, and IL-8) were analyzed by Pearson correlation coefficient. Furthermore, a standard least square regression has been carried out to calculate a regression line. Then, we detected differentially regulated PROKRs and pro-inflammatory genes with relevant clinic information content of TEP patients by carrying out two-tailed unpaired t-tests. The effects of PROKRs and pro-inflammatory genes on TEP patient were analyzed by univariate and multiple Cox regression analysis. Finally, to measure the risk prediction rate of novel genes between the two groups, the receiver operating characteristic (ROC) curves were constructed by using the "pROC" package in R software. The *P*-value less than 0.05 were considered as statistically significant.

Results

PROKR1 and PROKR2 were aberrantly upregulated in the fallopian tube tissues from women with tubal ectopic pregnancy

To investigate the potential significance of PROKR1 and PROKR2 in the progression of

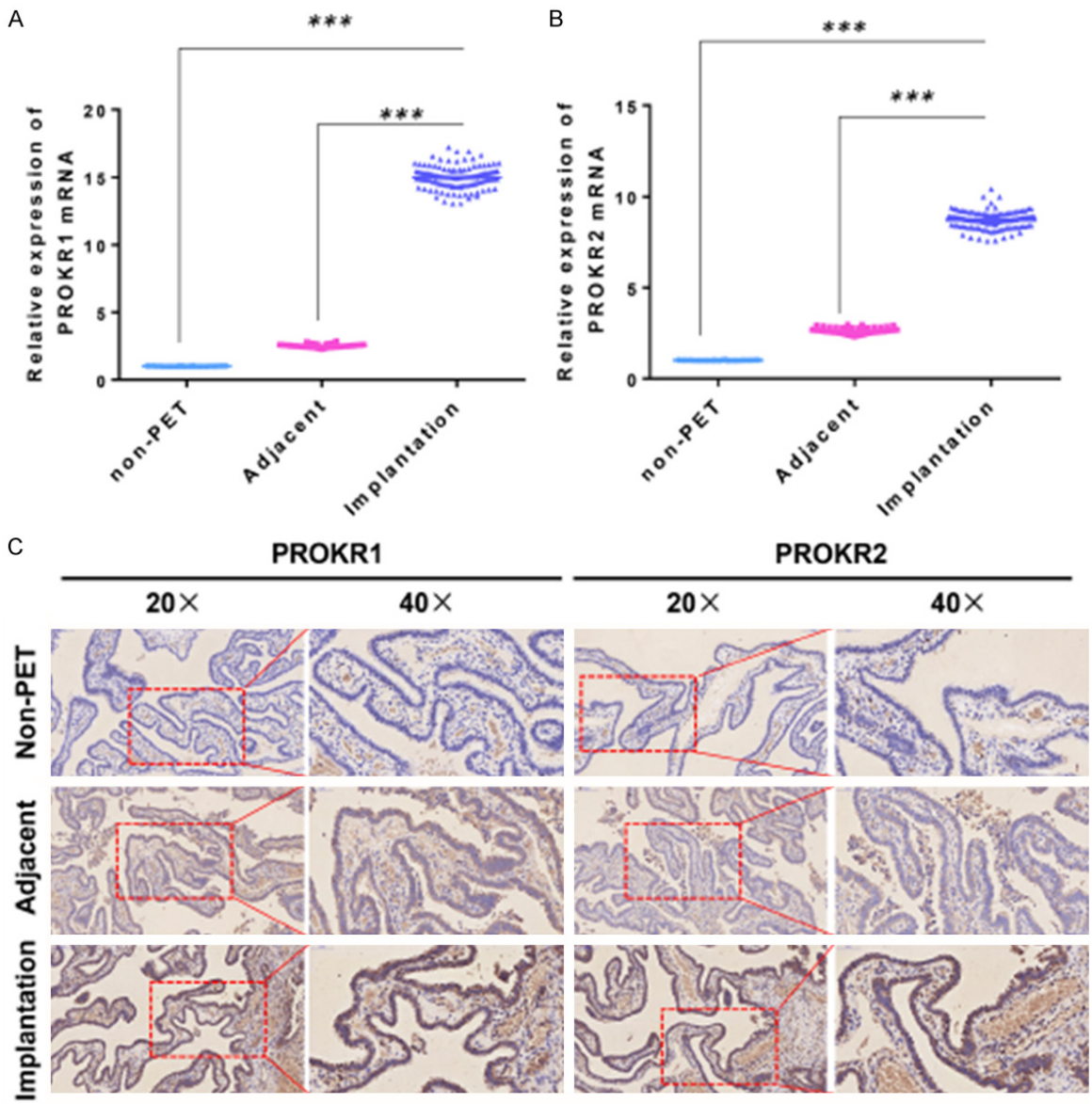

Figure 1. The mRNA expression of PROKR1 and PROKR2 were up-regulated in fallopian tube tissues from women with tubal ectopic pregnancy. A and B: The mRNA expressions of PROKR1 and PROKR2 in fallopian tube tissue were evaluated by qRT-PCR. Data are mean ± SD. One-way ANOVA followed by a Tukey post-hoc test. ***P<0.001. Non-PET, fallopian tube samples in non-PET. Adjacent, un-implantation site samples in TEP patients. Implantation, fertilized egg implantation site samples in TEP patients. C: Representative immunohistochemistry staining with PROKR1 and PROKR2 in fallopian tube tissue. Scale bar: 100 μm (20×) and 50 μm (40×). Non-PET, fallopian tube samples in non-PET. Adjacent, un-implantation site samples in TEP patients. Implantation, fertilized egg implantation site samples in TEP patients.

TEP, the expression of PROKR1 and PROKR2 were evaluated by qRT-PCR and immunohistochemistry. The mRNA expressions of PROKR1 and PROKR2 were significantly increased in fertilized egg implantation site of TEP relative to un-implantation site of TEP and non-TEP (**Figure 1A** and **1B**). Additionally, immunohistochemical analysis also showed that PROKR1 and PROKR2 were significantly higher in fertilized egg implantation site of TEP compared to un-implantation site of TEP and non-TEP (**Figure 1C**).

The expression levels of TNF-α, IL-6, and IL-8 in the fallopian tube tissues from women with TEP

To investigate the potential significance of inflammatory factors in the progression of TEP,

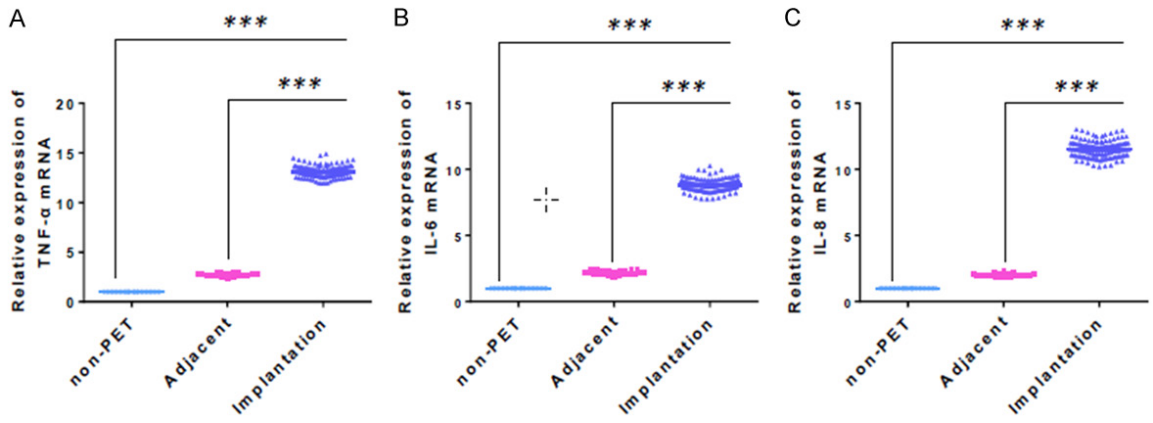

Figure 2. Pro-inflammatory genes were up-regulated in fallopian tube tissues from women with tubal ectopic pregnancy. Pro-inflammatory genes expression levels in fallopian tube tissue were measured by qRT-PCR, including TNF-α (A), IL-6 (B), and IL-8 (C). Data are mean ± SD. One-way ANOVA followed by a Tukey post-hoc test. ***P<0.001. Implantation, Non-PET, fallopian tube samples in non-PET. Adjacent, un-implantation site samples in TEP patients. Implantation, fertilized egg implantation site samples in TEP patients.

the mRNA expression of TNF-α, IL-6, and IL-8 were evaluated were evaluated by qRT-PCR. The results of qRT-PCR showed that the mRNA expression of TNF-α, IL-6, and IL-8 were significantly higher in fertilized egg implantation site of TEP relative to un-implantation site of TEP and non-TEP (**Figure 2**).

Dysregulation of PROKR1 and PROKR2 correlates with pro-inflammatory genes and clinical history in women with tubal ectopic pregnancy

To further investigate whether the key regulatory effects of PROKR1 and PROKR2 on TEP may be related to abnormal secretion of pro-inflammatory genes, we conducted correlation analysis between PROKRs and pro-inflammatory genes by Pearson correlation. The results showed significantly positive correlations with pro-inflammatory genes (TNF-α, IL-6, and IL-8) for PROKR1 (**Figure 3A-C**) and PROKR2 (**Figure 3D-F**). In addition, PROKR1, PROKR2, TNF-α, IL-6, and IL-8 levels were significantly increased in TEP patient with cigarette smoking habit, history of EP, history of pelvic infections or history of abortion compared with in TEP patient without the above clinical history, indicating that the effect of clinical history on abnormal expression of PROKRs and pro-inflammatory genes (**Table 3**).

PROKR2 and IL-8 as risk factors for tubal ectopic pregnancy

The univariate and multivariate Cox-regression models were performed to determine the risk of the PROKRs (PROKR1 and PROKR2) and pro-inflammatory genes (TNF-α, IL-6, and IL-8) in TEP. The result indicated that PROKR2 (P= 0.002) and IL-8 (P<0.001) were risk factors for TEP (**Figure 4** and **Table 4**). Further, ROC curves were used to measure the risk prediction rate of PROKR2 and IL-8 between the two groups. The AUC values of the PROKR2 and IL-8 were 0.93 and 0.98, which suggesting the highest discriminatory power as a single marker (**Figure 5A**, **5B**). Although these single PROKR2 and IL-8 may predict TEP with already good sensitivity and specificity, we evaluated whether PROKR2 and IL-8 signatures derived from supervised classification may improve test sensitivity and specificity. Therefore, we combined the information content of two selected genes (PROKR2 and IL8). The two genes signature showed extremely high diagnostic accuracy, with AUC of 1.0 (**Figure 5C**), representing a significant improvement in comparison to each single gene marker.

Discussion

Numerous studies have shown that the incidence of pathological pregnancy has been increasing worldwide in the past decades [9]. TEP may lead to tubal rupture to seriously influence the health and future fertility of pregnant woman, and increase risk for future EP [5, 6]. However, the molecular potential markers and risk factors of tubal implantation remain relatively unknown. In this study, we found that PROKRs (PROKR1 and PROKR2) and pro-in-

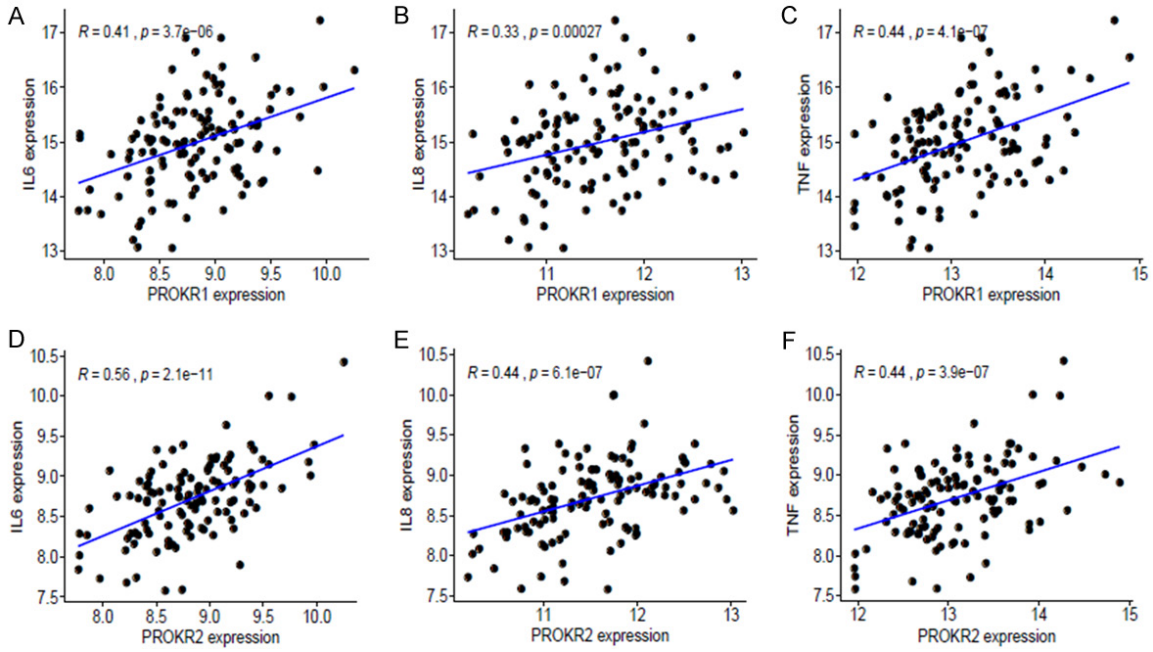

Figure 3. The PROKRs expression levels correlate with the pro-inflammatory genes expression levels. A-C: PROKR1 expression levels have significant positive correlations with pro-inflammatory genes (TNF-α, IL-6, and IL-8). D-F: PROKR2 expression levels have significant positive correlations with pro-inflammatory genes (TNF-α, IL-6, and IL-8) levels. The regression line is shown in black in the respective plots. The r is the correlation coefficient. P<0.05 was considered statistically significant difference.

Table 3. Effect of clinical history on PROKR1, PROKR2, TNF-α, IL-6, and IL-8 expression in women with tubal ectopic pregnancy

Clinical history	PROKR1	PROKR2	TNF-α	IL-6	IL-8
Smoke					
Yes (n=23)	15.33±0.80	9.09±0.51	14.00±0.35	9.26±0.49	11.91±0.53
No (n=97)	14.91±0.81*	8.63±0.42***	12.88±0.42***	8.72±0.42***	11.45±0.64**
Ectopic pregnancy (history)					
Yes (n=17)	16.33±0.39	9.06±0.51	13.68±0.57	9.23±0.46	11.82±0.53
No (n=103)	14.77±0.65***	8.66±0.44**	13.00±0.54***	8.76±0.45**	11.49±0.65*
Pelvic infections					
Yes (n=39)	15.35±0.85	9.21±0.32	13.37±0.62	9.11±0.48	11.82±0.49
No (n=81)	14.82±0.75**	8.48±0.33***	12.96±0.54**	8.69±0.42***	11.40±0.67***
Abortion					
Yes (n=25)	15.41±0.68	9.35±0.33	13.43±0.58	9.19±0.48	11.78±0.52
No (n=95)	14.88±0.82**	8.55±0.35***	13.01±0.57**	8.73±0.43**	11.47±0.66*

Note: Yes *vs.* No. *P<0.05, **P<0.01, and ***P<0.001.

flammatory genes (TNF-α, IL-6, and IL-8) levels were significantly enhanced in fallopian tube tissue from patients with TEP, and significant positive correlation with pro-inflammatory genes for PROKRs. Additionally, the dysregulation of these genes in TEP were promoted by cigarette smoking, history of EP, history of pelvic infections, or history of abortion. Finally, we found that PROKR2 and IL-8 were risk factors and involved in the pathogenesis of TEP.

PROKR1 (Prokineticin Receptor 1) encodes a member of the G-protein-coupled receptor family, which binds to prokineticins leading to the activation of MAPK and STAT signaling pathways [18]. PROKRs are a molecule known for its

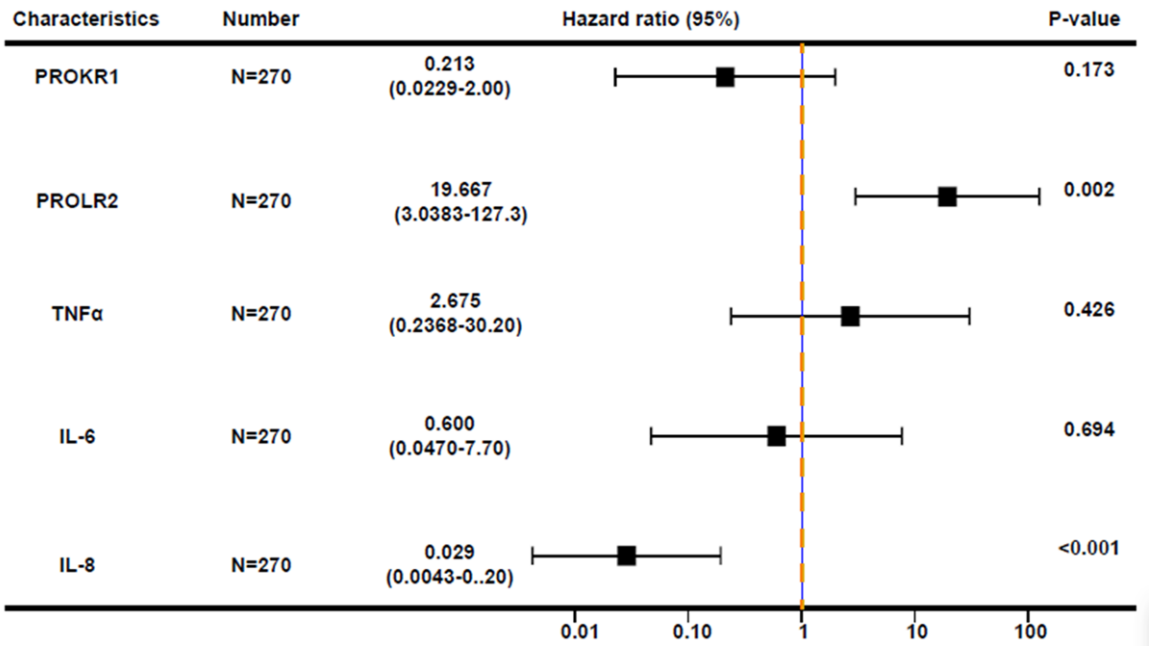

Figure 4. Multivariate analysis was performed to identify the risk factors of TEP. The P<0.05 was considered statistically significant difference. HR is expressed as Hazard ratio.

Table 4. Univariate Cox regression analysis of genes and clinical features

Name	HR	Z	*p*value
PROKR1	0.230	-13.506	<0.001
PROKR2	0.190	-14.340	<0.001
TNF-α	0.232	-13.989	<0.001
IL-6	0.154	-13.651	<0.001
IL-8	0.165	-12.614	<0.001
Smoke	0.988	-0.075	0.940
History of EP	0.914	-0.488	0.626
History of pelvic infections	0.895	-0.828	0.408
History of Abortion	0.908	-0.615	0.539

Note: HR, hazard ratio; Z, Z score; *p*value, *P* value.

control smooth-muscle contractility and promote inflammatory cells and immune cells migration [19], and regulation of genes important for intrauterine implantation [20]. Studies have been reported that PROKR1 expression was elevated in human decidua during early pregnancy [13]. Furthermore, PROKR1 associating with Prokineticin 1 regulated the expression of several genes with important roles in endometrial receptivity and implantation [21]. PROKR2 (Prokineticin Receptor 2) are secreted proteins that induce strong smooth muscle contraction [22]. Studies showed that PROKR2-mediated PROK2 signaling pathway has been shown to be a critical regulator of sexual maturation [23]. These findings were consistence with our results. In this study, PROKR1 and PROKR2 were identified as risk factor and played an important role in the pathogenesis of TEP. We found that PROKR1 and PROKR2 levels were increased in fallopian tube from women with TEP, but Shaw et al showed that PROKR1 and PROKR2 mRNA levels were lower in fallopian tube from women with EP compared to non-pregnant fallopian tube from the midluteal phase [16]. Our results are not consistent with the results of Shaw et al, possibly because of the different course of disease when we collected the samples. Additionally, studies have been reported that PROKR1 promoted the pro-inflammatory genes (IL-6, IL-1β, and TNF-α) expressions in murine fetal membranes, human myometrium, and cervix with leukocytes [24, 25]. However, the relationship between PROKRs and pro-inflammatory genes has not been elucidated in fallopian tube from women with TEP. In our results, pro-inflammatory genes were increased in the fallopian tube from women with TEP, suggesting these genes might play important roles in the development of TEP, including TNF-α (tumor necrosing factor alpha), IL-6 (Interleukin 6), and IL-8 (Interleukin 8). Similarly, numerous studies reported that the pro-inflammatory genes involved in the

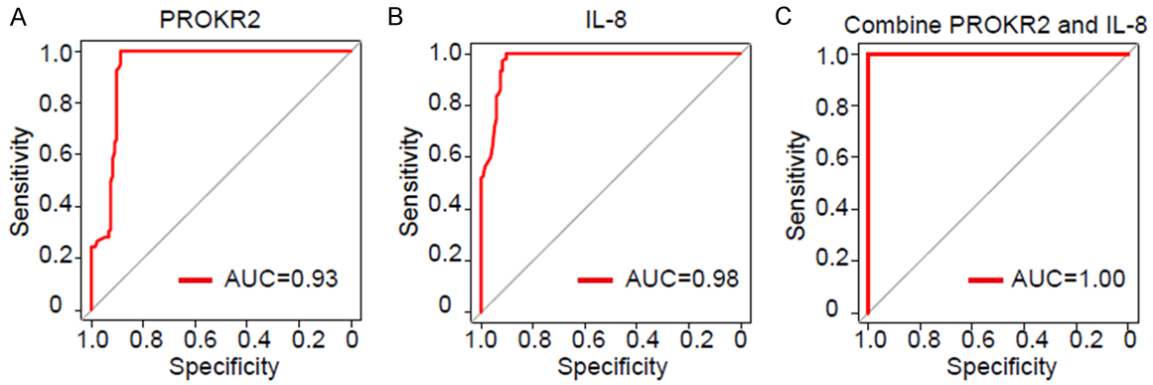

Figure 5. ROC curves demonstrated that the area under receiver operating characteristic (AUC). A: Receiver operating characteristic (ROC) curve analyses was performed for PROKR2. B: Receiver operating characteristic (ROC) curve analyses was performed for IL-8. C: ROC curves demonstrated that the area under receiver operating characteristic (AUC) of 2-genes model was 1.00, which exhibited the risk score.

pathogenesis of TEP [14, 15, 26]. For example, studies showed that TNF-α may be regarded as a prognostic inflammatory marker of infection and pregnancy [27]. In the peritoneal fluid from women with ruptured ectopic pregnancies, the TNF-α expression was elevated [28]. A polymorphism in the TNF-α gene was associated with susceptibility to recurrent pregnancy loss [29]. IL-6 encodes a cytokine that functions in inflammation [30]. Studies have been showed that IL-6 was involved in the tubal immune response against bacterial infections, as well as the pathogenesis of ectopic pregnancies [31]. IL-8 is pro-inflammatory cytokines in response to a variety of microbial and nonmicrobial agents [32]. Studies have been showed the expression levels of IL-8 of tubal epithelial and stromal cells in response to inflammatory cytokines [33]. These findings showed the roles of PRKORs and cytokines in the pathogenesis of TEP. Taken together, Our results found for the first times IL-6, IL-8, and TNF-α levels were significantly positive correlation with PROKR1 and PROKR2 levels, which suggested that PROKR1 and PROKR2 might promote the expressions of IL-6, IL-8, and TNF-α in the fallopian tube from women with TEP, similar to the effects of PROKR1 and PROKR2 on the expressions of IL-6, IL-8, and TNF-α in other physiological processes [24, 25].

Cigarette smoking habit, history of EP, history of pelvic infections, and abortion are the major risk factors of Tubal EP [34-37]. Studies have shown that smoking promoted the expression levels of PROKR1 by targeting AChRa7, and *Chlamydia trachomatis* infections increased PROKR1 levels by increasing TLR2 in the fallopian tube of patients with TEP [11, 12]. Infections could lead to increased PROKs and PROKRs levels, which would initiate inflammatory and contractile cascades and result in preterm birth [38]. In this study, we found that PROKR2 and IL-8 were major risk factors for TEP. However, cigarette smoking, history of EP, history of pelvic infections, and abortion were not risk factors, inconsistent with some studies [34-37], and may be due to the low number of samples collected. Finally, we found that these genes may be capable of discriminating TEP from control patient, and IL8 as a single marker displaying the highest discriminatory power, which provides new potential markers for clinical prevention and treatment of TEP. These results prompt that cigarette smoking habit, history of EP, history of pelvic infections, or abortion may be promote the expressions of PROKR1 and PROKR2, thereby promoting the abnormal secretion of inflammatory cytokines, and resulting in TEP, which provides a potential mechanism for the occurrence and development of TEP that were caused by cigarette smoking habit, history of EP, history of pelvic infections, or history of abortion.

In summary, the present study suggested that dysregulation of PROKR2 and IL8 were risk factors for TEP, which were associated with cigarette smoking habit, history of EP, history of pelvic infections, or history of abortion, and 2-gene signature may be independent diagnostic signatures and play an important role in

the occurrence and development of TEP. In addition, this present study has several limitations. Such as whether cigarette smoking habit, history of EP, history of pelvic infections, or history of abortion leads to the development of TEP through PROKR2 and IL8 remain unclear. Therefore, we will further investigate the potential mechanisms by which PROKRs promote TEP by regulating the secretion of inflammatory factors.

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Disclosure of conflict of interest

None.

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References

[1] Shaw JL, Diamandis EP, Horne AW, Barnhart K, Bourne T and Messinis IE. Ectopic pregnancy. Clin Chem 2012; 58: 1278-1285.

[2] Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med 2009; 361: 379-387.

[3] Khan KS, Wojdyla D, Say L, Gulmezoglu AM and Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet 2006; 367: 1066-1074.

[4] Richardson A, Gallos I, Dobson S, Campbell BK, Coomarasamy A and Raine-Fenning N. Accuracy of first-trimester ultrasound in diagnosis of tubal ectopic pregnancy in the absence of an obvious extrauterine embryo: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2016; 47: 28-37.

[5] Fernandez H, Capmas P, Lucot JP, Resch B, Panel P and Bouyer J. Fertility after ectopic pregnancy: the DEMETER randomized trial. Hum Reprod 2013; 28: 1247-1253.

[6] de Bennetot M, Rabischong B, Aublet-Cuvelier B, Belard F, Fernandez H, Bouyer J, Canis M and Pouly JL. Fertility after tubal ectopic pregnancy: results of a population-based study. Fertil Steril 2012; 98: 1271-1276, e1-3.

[7] Shao R, Nutu M, Karlsson-Lindahl L, Benrick A, Weijdegård B, Lager S, Egecioglu E, Fernandez-Rodriguez J, Gemzell-Danielsson K, Ohlsson C, Jansson JO and Billig H. Downregulation of cilia-localized Il-6R alpha by 17beta-estradiol in mouse and human fallopian tubes. Am J Physiol Cell Physiol 2009; 297: C140-151.

[8] Gebeh AK, Willets JM, Marczylo EL, Taylor AH and Konje JC. Ectopic pregnancy is associated with high anandamide levels and aberrant expression of FAAH and CB1 in fallopian tubes. J Clin Endocrinol Metab 2012; 97: 2827-2835.

[9] Patel MA. Scar ectopic pregnancy. J Obstet Gynaecol India 2015; 65: 372-375.

[10] Horne AW, Brown JK, Nio-Kobayashi J, Abidin HB, Adin ZE, Boswell L, Burgess S, Lee KF and Duncan WC. The association between smoking and ectopic pregnancy: why nicotine is BAD for your fallopian tube. PLoS One 2014; 9: e89400.

[11] Shaw JL, Oliver E, Lee KF, Entrican G, Jabbour HN, Critchley HO and Horne AW. Cotinine exposure increases Fallopian tube PROKR1 expression via nicotinic AChRalpha-7: a potential mechanism explaining the link between smoking and tubal ectopic pregnancy. Am J Pathol 2010; 177: 2509-2515.

[12] Shaw JL, Wills GS, Lee KF, Horner PJ, McClure MO, Abrahams VM, Wheelhouse N, Jabbour HN, Critchley HO, Entrican G and Horne AW. Chlamydia trachomatis infection increases fallopian tube PROKR2 via TLR2 and NFkappaB activation resulting in a microenvironment predisposed to ectopic pregnancy. Am J Pathol 2011; 178: 253-260.

[13] Evans J, Catalano RD, Morgan K, Critchley HO, Millar RP and Jabbour HN. Prokineticin 1 signaling and gene regulation in early human pregnancy. Endocrinology 2008; 149: 2877-2887.

[14] Rajendiran S, Senthil Kumar GP, Nimesh A, Dhiman P, Shivaraman K and Soundararaghavan S. Diagnostic significance of IL-6 and IL-8 in tubal ectopic pregnancy. J Obstet Gynaecol 2016; 36: 909-911.

[15] Krishnan T, Winship A, Sonderegger S, Menkhorst E, Horne AW, Brown J, Zhang JG, Nicola NA, Tong S and Dimitriadis E. The role of leukemia inhibitory factor in tubal ectopic pregnancy. Placenta 2013; 34: 1014-1019.

[16] Shaw JL, Denison FC, Evans J, Durno K, Williams AR, Entrican G, Critchley HO, Jabbour HN and Horne AW. Evidence of prokineticin dysregulation in fallopian tube from women with ectopic pregnancy. Fertil Steril 2010; 94: 1601-1608, e1.

[17] Han C, Gao L, Zhao L, Sheng Q, Zhang C, An Z, Xia T, Ding Y, Wang J, Bai H and Dou X. Immu-

nohistochemistry detects increased expression of Aldo-Keto reductase family 1 member B10 (AKR1B10) in early-stage hepatocellular carcinoma. Med Sci Monit 2018; 24: 7414-7423.

[18] Li QF, Zhu HY, Yang YF, Liu J, Xiao FJ, Zhang QW, Wu CT, Wang H and Wang LS. Prokineticin-1/endocrine gland-derived vascular endothelial growth factor is a survival factor for human multiple myeloma cells. Leuk Lymphoma 2010; 51: 1902-1912.

[19] Sasaki S, Baba T, Muranaka H, Tanabe Y, Takahashi C, Matsugo S and Mukaida N. Involvement of Prokineticin 2-expressing Neutrophil Infiltration in 5-Fluorouracil-induced aggravation of breast cancer metastasis to lung. Mol Cancer Ther 2018; 17: 1515-1525.

[20] Brouillet S, Murthi P, Hoffmann P, Salomon A, Sergent F, De Mazancourt P, Dakouane-Giudicelli M, Dieudonne MN, Rozenberg P, Vaiman D, Barbaux S, Benharouga M, Feige JJ and Alfaidy N. EG-VEGF controls placental growth and survival in normal and pathological pregnancies: case of fetal growth restriction (FGR). Cell Mol Life Sci 2013; 70: 511-525.

[21] Macdonald LJ, Sales KJ, Grant V, Brown P, Jabbour HN and Catalano RD. Prokineticin 1 induces Dickkopf 1 expression and regulates cell proliferation and decidualization in the human endometrium. Mol Hum Reprod 2011; 17: 626-636.

[22] Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M and Moerman A. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2006; 2: e175.

[23] Traboulsi W, Brouillet S, Sergent F, Boufettal H, Samouh N, Aboussaouira T, Hoffmann P, Feige JJ, Benharouga M and Alfaidy N. Prokineticins in central and peripheral control of human reproduction. Horm Mol Biol Clin Investig 2015; 24: 73-81.

[24] Lannagan TR, Wilson MR, Denison F, Norman JE, Catalano RD and Jabbour HN. Prokineticin 1 induces a pro-inflammatory response in murine fetal membranes but does not induce preterm delivery. Reproduction 2013; 146: 581-591.

[25] Gorowiec MR, Catalano RD, Norman JE, Denison FC and Jabbour HN. Prokineticin 1 induces inflammatory response in human myometrium: a potential role in initiating term and preterm parturition. Am J Pathol 2011; 179: 2709-2719.

[26] Hoenderboom BM, van Benthem BHB, van Bergen JEAM, Dukers-Muijrers NHTM, Götz HM, Hoebe CJPA, Hogewoning AA, Land JA, van der Sande MAB, Morré SA and van den Broek IVF. Relation between Chlamydia trachomatis infection and pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility in a Dutch cohort of women previously tested for chlamydia in a chlamydia screening trial. Sex Transm Infect 2019; 95: 300-306.

[27] Singh KP, Shakeel S, Naskar N, Bharti A, Kaul A, Anwar S, Kumari S, Kumar A, Singh JK and Kumari N. Role of IL-1beta, IL-6 and TNF-alpha cytokines and TNF-alpha promoter variability in Plasmodium vivax infection during pregnancy in endemic population of Jharkhand, India. Mol Immunol 2018; 97: 82-93.

[28] Sadovsky Y, Pineda J and Collins JL. Tumor necrosis factor alpha is elevated in the peritoneal fluid from women with ruptured ectopic pregnancies. Gynecol Obstet Invest 1991; 32: 157-159.

[29] Li HH, Xu XH, Tong J, Zhang KY, Zhang C and Chen ZJ. Association of TNF-alpha genetic polymorphisms with recurrent pregnancy loss risk: a systematic review and meta-analysis. Reprod Biol Endocrinol 2016; 14: 6.

[30] Hunter CA and Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015; 16: 448-457.

[31] Refaat B, Ashshi AM, Batwa SA, Ahmad J, Idris S, Kutbi SY, Malibary FA and Kamfar FF. The prevalence of Chlamydia trachomatis and Mycoplasma genitalium tubal infections and their effects on the expression of IL-6 and leukaemia inhibitory factor in Fallopian tubes with and without an ectopic pregnancy. Innate Immun 2016; 22: 534-545.

[32] Swami SK, Vijay A, Nagarajan G, Kaur R and Srivastava M. Molecular characterization of pro-inflammatory cytokines interleukin-1beta and interleukin-8 in asian elephant (Elephas maximus). Anim Biotechnol 2016; 27: 66-76.

[33] Mulayim N, Palter SF, Selam B and Arici A. Expression and regulation of interleukin-8 in human fallopian tubal cells. Am J Obstet Gynecol 2003; 188: 651-656.

[34] Hyland A, Piazza KM, Hovey KM, Ockene JK, Andrews CA, Rivard C and Wactawski-Wende J. Associations of lifetime active and passive smoking with spontaneous abortion, stillbirth and tubal ectopic pregnancy: a cross-sectional analysis of historical data from the Women's Health Initiative. Tob Control 2015; 24: 328-335.

[35] Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L and Job-Spira N. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol 2003; 157: 185-194.

[36] Goller JL, De Livera AM, Guy RJ, Low N, Donovan B, Law M, Kaldor JM, Fairley CK and Hock-

ing JS. Rates of pelvic inflammatory disease and ectopic pregnancy in Australia, 2009-2014: ecological analysis of hospital data. Sex Transm Infect 2018; 94: 534-541.

[37] NeamȚu SD, DiȚescu D, ForȚofoiu M, Stanca L, Tigae C, Niculescu M, NeamȚu CO, Manolea MM, Siminel MA, Şurtea LE, NeamȚu AV, Novac MB, Vasile L and Gluhovschi A. Correlation of clinical and biological evidence - a dominant therapeutic element of succeeding in ectopic pregnancy. Rom J Morphol Embryol 2017; 58: 167-174.

[38] Catalano RD, Lannagan TR, Gorowiec M, Denison FC, Norman JE and Jabbour HN. Prokineticins: novel mediators of inflammatory and contractile pathways at parturition? Mol Hum Reprod 2010; 16: 311-319.