ISSN 0233–7657. Biopolymers and Cell. 2013. Vol. 29. N 5. P. 402–405 doi: 10.7124/bc.000830

BIOMEDICINE

UDC 575.11+577.21

The role of *IL6* and *ESR1* gene polymorphisms as immunological factors of pregnancy maintenance

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Aim. *The study is aimed at the evaluation of the association of IL6 gene -174G/C polymorphism and ESR1 gene -397C/T polymorphism with recurrent pregnancy loss (RPL) pathogenesis and at the investigation of the ESR1 gene -397C/T variant regulatory significance for the IL6 gene function.* ***Methods***. *A case group of 75 women with RPL history and a control group of 106 unrelated healthy women, who have given birth to at least one child conceived in natural way, were genotyped by a PCR based restriction fragment length polymorphism assay.* ***Results***. *There was no significant difference in IL6 -174G/C or ESR1 -397C/T genotype and allele frequencies between the case and control groups. Combined genotype distribution analysis showed significantly ($p < 0.05$) lower frequency of individuals homozygous for both IL6 -174G and ESR1 -397C alleles in case group (0.026) comparing to control (0.094).* ***Conclusions***. *Genotype comprising IL6 -174G and ESR1 -397C alleles in homozygous state may be considered as a genetic marker of successful pregnancy maintenance during gestation early stages.*

Keywords: recurrent pregnancy loss, interleukin, polymorphism.

Introduction. Recurrent pregnancy loss (RPL) is the occurrence of three or more consecutive miscarriages in the first trimester of gestation. RPL affects 2–5 % of women in reproductive age [1]. The most common types of etiology of pregnancy loss are endocrine, immunological, anatomical and idiopathic [2]. RPL is a distressing condition for affected couples and deciphering the molecular effects of the various types of etiology associated with recurrent miscarriage is of great importance.

Immunological factors are estimated to be the leading cause of miscarriage in up to 40 % cases [2]. The state of maternal immune system is crucial for pregnancy establishment and maintenance. During gestation pro- and anti-inflammatory environment prevalence periods emerge in precise sequence critical for pregnancy tolerance [3]. This regularity is stipulated by a vast amount of cytokines present in gestational tissues [2]. Interleukin 6 (IL6) is a pleiotropic cytokine and a key immune response regulator with both pro- and anti-inflammatory properties. It plays a role in acute and chronic inflammatory processes as well as in autoimmunity [4]. Highly regulated fluctuations of IL6 level in both blood and gestational tissues are crucial for pregnancy maintenance, though the specific essential role of this interleukin has not been clearly identified [3].

IL6 is encoded by *IL6* gene which is located in 7p21 locus and consists of five exons and four introns [5]. The regulation of IL6 level is mainly provided by regulatory elements in the promoter region of *IL6* gene [6]. A common G/C polymorphism of the *IL6* promoter on -174 position has been shown to influence the *in vivo* protein expression [7].

It has been investigated in a wide variety of pathological conditions, including coronary heart disease

[8], bone mineral density [9] and spontaneous abortion [10].

However, the results of association studies are contradictory. The experiments *in vitro* showed that some of the *IL6* promoter regulatory elements including -174 G/C region are sensitive to the estrogen-estrogen receptor complex regulation [11]. Consequently, the functional state of this complex is crucial for the IL6 expression. An estrogen receptor α is a common part of the estrogen-estrogen receptor complex in gestational tissues [12]. This protein is encoded by *ESR1* gene located in 6q25.1 region and consisting of 8 exons [13]. In a lot of studies more than 4 thousand SNPs in *ESR1*gene have been identified [14] including 1 intron -397C/T variant which is proven to affect the protein expression [15]. This polymorphic variant has shown the association with a number of reproductive pathologies, including endometriosis [16], and impaired ovarian reserve [17].

Taking into account a functional significance of mentioned polymorphic variants, which change the respective genes expression, we focused our study on the evaluation of association of the latter with RPL pathogenesis. We also intended to investigate probable regulatory significance of *ESR1* gene -397 C/T polymorphism for the *IL6* gene function.

Materials and methods. A case group comprised 75 unrelated women with history of RPL (at least two cases of miscarriage in the first trimester) undergoing observation in the SI «Institute of Pediatrics, Obstetrics and Gynecology of NAMS of Ukraine». A control group consisted of 106 unrelated healthy women who have given birth to at least one child conceived in natural way. All participants have given their informed consent prior to clinical examination and genotyping. The study has been approved by The Bioethical Committee of Institute of Molecular Biology and Genetics of NAS of Ukraine.

The material of the study was the genomic DNA extracted from peripheral blood samples of patients and control group individuals using standard phenol–chloroform technique. Genotyping for *IL6* gene -174 G/C and *ESR1* gene -397 C/T polymorphic variants was performed by the PCR based restriction fragment length polymorphism assay as described previously [7, 18]. A statistical analysis has been performed using GenePop and OpenEpi statistical packages [19, 20]. Fisher's exact test (Mid-P method) was used to estimate the difference in genotype and allelic distribution. In order to assess the association of certain genotype with RPL, OR index was calculated. P-value less than 0.05 was regarded as significant.

Results and discussion. The results of genotyping for both studied polymorphic variants are presented in Table.

Both *IL6* gene -174 G/C and *ESR1* gene -397 C/T genotype frequencies showed no significant deviation from those expected according to Hardy-Weinberg equilibrium. There was no significant difference in *IL6* -174 G/C genotype and allele frequencies between the case and control groups. The same was observed when comparing the *ESR1* -397 C/T genotype and allele frequencies in both studied groups.

However, the analysis of combined *IL6* -174 G/C and *ESR1* -397 C/T genotype frequencies revealed a significantly ($p < 0.05$) lower frequency of individuals homozygous[for both *IL6* -174 G and *ESR1* -397 C alleles in [the] case group (0.026) comparing to control group (0.094). As *IL6* -174 G allele is associated with normal and high levels of IL6, the results observed in our study are consistent with the experimental data which show that elevated levels of IL6 during early pregnancy are necessary for its maintenance. The greatest challenge to the maternal immune response occurs at the outset of pregnancy, when the embryo implants into the maternal endometrium and placenta development commences.

Although the precise role of IL6 signaling during implantation process is yet to be determined, the *in vitro*, experiments with mouse embryos suggest that *IL6* can promote the pre-implantation embryo development and increase blastocyst cell number [21]. A vast amount of studies corroborate the role of IL6 in trophoblast proliferation and placental morphogenesis [22]. The studies on the fetal-placental tissue of abortion-prone mice lines showed that it secrets a decreased level of IL6 comparing to the tissues from normal pregnant mice [23]. A reduced IL6 production by endometrial and placental tissue has also been observed in women prone to recurrent spontaneous abortion [24].

The result observed in our study is consistent with the experimental data demonstrating that elevated levels of

Genotype and allelic distribution of IL6 -174 G/C and ESR1 -397 C/T gene polymorphic variants in studied groups

Locus	RPL group, *n*	RPL group, %	Control group, *n*	Control group, %
IL6 -174 G/C				
Genotype				
GG	25	33.33	38	35.85
GC	34	45.33	42	39.62
CC	16	21.33	26	24.53
Allele				
G	84	56.00	118	55.70
C	66	44.00	94	44.30
ESR1 -397C/T				
Genotype				
CC	18	24.00	21	19.81
CT	37	49.33	53	50.00
TT	20	26.67	32	30.19
Allele				
C	73	48.70	95	44.80
T	77	51.30	117	55.20

IL6 during early pregnancy are necessary for its maintenance. Normally, the estrogen-estrogen receptor complex is known to inhibit the IL6 production in gestational tissues, but the study on the genetic constructs containing different alleles of *IL6* -174G/C polymorphism has shown otherwise. The presence of estrogen-estrogen receptor complex makes the *IL6* promoters with -174G allele sensitive to other expression stimulating factors thus resulting in the elevated IL6 level [11].

Thus, bearing in mind that *ESR1* -397 C results in normal ERα production, the combination of these alleles in homozygous state in one genotype may be suggested to lead to well-regulated relatively high level of IL6 in gestational tissues providing conditions for successful pregnancy.

Conclusions. On the basis of established statistically significant difference in combined genotype distribution, the genotype comprising *IL6* -174G and *ESR1* -397C alleles in homozygous state might be considered as a genetic marker of successful pregnancy maintenance during early stages of gestation.

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Поліморфізми генів *IL6* і *ESR1* як імунологічні чинники підтримання вагітності

Резюме

Мета. *Встановити асоціацію поліморфних варіантів -174 G/C гена IL6 і -397 C/T гена ESR1 з патогенезом звичного невиношування вагітності (ЗНВ) та дослідити ймовірний регуляторний вплив поліморфізму -397 C/T гена ESR1 на функціонування гена IL6.* ***Методи***. *Досліджувану (75 жінок з історією ЗНВ) та контрольну (106 неспоріднених здорових жінок, які народили хоча б одну дитину, зачату природним шляхом) групи прогенотиповано методом ПЛР з наступним аналізом поліморфізму довжини рестрикційних фрагментів.* ***Результати***. *Достовірної різниці частот генотипів та алелів за поліморфізмами IL6 -174 G/C і ESR1 -397 C/T не встановлено. Аналіз комбінованих генотипів виявив статистично достовірно ($p < 0,05$) нижчу частоту осіб, гомозиготних за алелями IL6 -174G та ESR1 -397C у досліджуваній групі (0,026) порівняно з контрольною (0,094).* ***Висновки***. *Генотип, до складу якого входять алелі IL6 -174G та ESR1 -397C у гомозиготному стані, можна розглядати як генетичний маркер успішного підтримання вагітності на ранніх строках гестації.*

Ключові слова: звичне невиношування вагітності, інтерлейкін, поліморфізм.

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Полиморфизмы генов *IL6* и *ESR1* как иммунологические факторы поддержания беременности

Резюме

Цель. *Установить ассоциацию полиморфных вариантов -174 G/C гена IL6 и -397 C/T гена ESR1 с патогенезом привычного невынашивания беременности (ПНБ), а также исследовать вероятный регуляторный эффект полиморфизма -397 C/T гена ESR1 на функционирование гена IL6.* ***Методы***. *Исследуемая (75 женщин с историей ПНБ) и контрольная (106 неродственных женщин, родивших хотя бы одного ребенка, зачатого естественным путем) группы прогенотипированы методом ПЦР с последующим анализом полиморфизма длины рестрикционных фрагментов.* ***Результаты***. *Достоверной разницы в частоте генотипов и аллелей по полиморфизмам IL6 -174 G/C и ESR1 -397 C/T не установлено. Анализ комбинированных генотипов выявил статистически достоверно ($p < 0,05$) меньшую частоту индивидов, гомозиготных по аллелям IL6 -174 G и ESR1 -397 C в исследуемой группе (0,026) по сравнению с контрольной (0,094).* ***Выводы***. *Генотип, в состав которого входят аллели IL6 -174G и ESR1 -397C в гомозиготном состоянии, можно рассматривать в качестве генетического маркера успешного поддержания беременности на ранних сроках гестации.*

Ключевые слова: привычное невынашивание беременности, интерлейкин, полиморфизм.

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Received 02.04.13