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THE NOVEL FII C.*64_*66DEL PROTHROMBIN GENE VARIANT IN WOMEN WITH PREGNANCY LOSS

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Normal pregnancy associated with complex changes of hemostasis, leading to hypercoagulability states. The presence of acquired or genetic prothrombotic risk factors might affect the proper maternal-fetal circulation and result in pregnancy loss. Hence, the screening for the novel prothrombotic variants associated with pregnancy loss would be beneficial. Our aim was to investigate the potential association of recently reported c.*64_*66del variant in prothrombin gene with the etiology of pregnancy loss. Study included 105 women with pregnancy loss and 155 controls. Analyses in patients' plasma samples, as well as in vitro analyses on transfected Cos-7 cell line were performed in order to investigate the mechanism by which this variant could perturb the coagulation and lead to pregnancy loss. Analyses in patients' DNA and plasma samples involved: DNA sequencing and PCR-RFLP assay for detection of FII c.*64_*66del variant, routine thrombophilia screening, thrombin generation assay and Western blot analysis of prothrombin plasma level. In vitro analyses included transient transfections of Cos-7 cell line with wild-type and c.*64_*66del mutated constructs of pCIneoΔSV40 expression vector. Real-Time PCR and Western blot analysis were used to determine the effect of FII

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c.*64_*66del variant on mRNA and protein level in constructs. Three women in patients group (2.9%) were detected as heterozygous carriers of FII c.*64_*66del, while none was found among controls. The carriers routine thrombophilia parameters were in reference range and similar prothrombin plasma level in FII c.*64_*66del carriers and non-carriers were detected. The endogenous thrombin potential was slightly increased in FII c.*64_*66del carriers compared to control plasma, but this difference was not statistically significant. Results of *in vitro* analyses showed significantly decreased prothrombin mRNA and protein level for c.*64_*66del variant compared to wild-type. Results of our pilot study have shown a trend of higher prevalence of FII c.*64_*66del variant in women with pregnancy loss. However, further studies are needed to completely elucidate whether FII c.*64_*66del variant affects prothrombin expression during pregnancy and to account its potential role in etiology of pregnancy loss.

Key words: FII c.*64_*66del, pregnancy loss, 3'untranslated region, prothrombin gene, gene expression.

INTRODUCTION

Pregnancy loss is a common health problem affecting 10 to 20% of woman at their reproductive age (MICHELS *et al.*, 2007) while 1-5% of women experienced recurrent pregnancy loss (BRENNER, 2002). The most common causes of pregnancy loss include fetal chromosomal and congenital abnormalities and a wide range of maternal abnormalities including anatomical alterations of the uterus, autoimmune and endocrinological disorders, infections, thrombophilia and uncontrolled chronic illness (MICHELS *et al.*, 2007). However, the cause of pregnancy loss remains unknown in up to 50% of cases (GOLDENBERG *et al.*, 2004).

Normal pregnancy is associated with significant changes of haemostatic system toward hypercoagulable state. Most notably, the concentrations of the clotting factors are increased, while natural anticoagulants concentrations are decreased and fibrinolytic activity is reduced. Since the pregnancy is hypercoagulable state *per se*, there is a growing pool of evidence indicating that adverse pregnancy outcomes could be associated with thrombophilia (SIMCOX *et al.*, 2015). Maternal thrombophilia has been related to placental insufficiency and placental vascular dysfunction (KINZLER *et al.*, 2009; SIMCOX *et al.*, 2015). As a consequence of disturbed haemostatic balance, followed by chronic reduction in uteroplacental blood flow, placental thrombosis can result in fetal growth restriction, preeclampsia, and pregnancy loss (KINZLER *et al.*, 2009). The most common acquired thrombophilia associated with pregnancy loss is the antiphospholipid syndrome, while Factor V (FV) Leiden and prothrombin (FII) G20210A mutations account for the most frequent inherited risk factors (SIMCOX *et al.*, 2015).

Thrombophilia has been associated with approximately 60% of idiopathic pregnancy losses (SARIG *et al.*, 2002). Thus, it would be beneficial to investigate whether novel prothrombotic gene variants might be involved in etiology of idiopathic pregnancy loss. Recently, the novel FII c.*64_*66del variant has been reported in the patient who suffered from idiopathic pulmonary embolism (GVOZDENOV *et al.*, 2015), but its potential association with pregnancy loss is still unknown. This variant is located within 3'untranslated region of the prothrombin gene and its mechanism has not been elucidated so far. The aim of our current study is to investigate the potential role of FII c.*64_*66del variant in the etiology of pregnancy loss.

MATERIALS AND METHODS

Patients

This study included 260 women divided into group of 105 women (34.68±5.65 years) who have suffered pregnancy loss and control group of 155 women (38.45±12.37 years) with no history of miscarriages or thrombotic events. The definition of miscarriage did not include the loss of a biochemical pregnancy (< 6 weeks). Recurrent pregnancy loss was defined in case at least two have occurred. Patients with anatomic malformations of the uterus, reproductive hormone dysfunction, diabetes mellitus, and infective disorders, as well antithrombin, protein S and protein C deficiency, lupus anticoagulant, FV Leiden and FII G20210A mutations were excluded from the study.

Peripheral blood was taken on 3.8% sodium citrate as anticoagulant. Blood samples collecting and genomic DNA purification were performed as described previously (PRUNER *et al.*, 2020). Plasma was aliquoted and frozen at - 80°C until further analysis.

DNA sequencing and PCR-RFLP

Direct DNA sequencing of the 715 bp within the 3'end of prothrombin gene included the last intron and exon, 3'utranslated region and 3'flanking region was performed as previously described (GVOZDENOV *et al.*, 2015).

The FII c.*64_*66del was detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The fragment of the prothrombin gene, where FII c.*64_*66del variant is localized, was amplified by PCR with 5'-GAAAGAATTATTTTTGTGTTTGTA-3'- forward and 5'-GAATAGCACTGGGAGCATTGA-3'- reverse primer. PCR reaction profile was: 3 cycles of 97°C/2min, 55°C/3min, followed by 38 cycles of 94°C/1 min, 60°C/1 min, 72°C/1min, and final elongation at 72°C/10 min. This was followed by digestion with Rsa I restriction enzyme (Fermentas, Waltham, Massachusetts, USA). Wild type (83 bp) and mutated (55 bp and 25 bp) alleles were distinguished by the size of the restriction fragments, using electrophoresis on 10% polyacrylamide gels and visualized by silver staining.

Thrombophilia testing

Routine thrombophilia screening included: prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin, protein C and protein S activities, lupus anticoagulant, anti-cardiolipin and anti-beta2-glycoprotein I antibodies (IgG and IgM class). Clotting activity of FII was measured in PT based assay on IL-7000 instrument (Instrumentation Laboratory, Milan, Italy) using factor II deficient plasma from the same manufacturer. FV Leiden and FII G20210A mutations are detected by PCR-RFLP analysis, as described before (DJORDJEVIC *et al.*, 2004).

Determination of plasma prothrombin level

In patients' plasma samples and standard human plasma (Siemens, Erlangen, Germany) (used for the normalization of plasma samples), as well as for prothrombin deficient plasma (HemosIL, Lexington, Massachusetts, USA) (negative control), level of prothrombin was evaluated by Western blot analysis as previously described (PRUNER *et al.*, 2020).

Thrombin generation assay

Endogenous thrombin potential (ETP) was measured with the use of commercially available assay (INNOVANCE ETP, Siemens Healthcare Diagnostics, Erlangen, Germany). The thrombin generation curve is determined by four parameters: ETP (representing a measure of the total endogenous generated thrombin (mA)); t_lag (lag phase until initiation (s)); c_max (peak thrombin generation (mA/s)); t_max (time to peak thrombin generation (s)). Commercially available Innovance ETP Standard was used for normalization. All ETP tests were performed in a series of sets where normal sample and hypercoagulable plasma sample run concurrently in every measurement (KOVAC *et al.*, 2015).

Plasmids and cell lines

The effect of FII c.*64_*66del variant on the prothrombin protein level was assessed by *in vitro* functional analysis. The pCIneo expression vector with mutated SV40 polyadenylation signal (pCIneoΔSV40) was used for transfection (SACHCHITHANANTHAN *et al.*, 2005). We have generated two types of constructs: first, into which all fourteen exons and 3'untranslated region (3'UTR) of the human wild-type prothrombin complementary DNA (cDNA) was cloned-14eUTR construct; and the second, which contained the additional 50 bp of 3' flanking region (3'FR)- 14eUTR+FR construct. Both types of constructs were obtained in wild-type (wt) and FII c.*64_*66del mutated (mut) forms (by the PCR-mediated Site-directed Mutagenesis (PSM), QuikChange Multi Site-Directed Mutagenesis Kit, Agilent Technologies, Santa Clara, California, USA) and confirmed by DNA sequencing. The pCIneoΔSV40 vector without any insert was used as a negative control (SACHCHITHANANTHAN *et al.*, 2005). Vector pcDNA3.1(+) with human wild-type alpha-1-antitrypsin cDNA (pcDNA-AAT1-wt) (LJUJIC *et al.*, 2014) was used as a control of transfection, since alpha-1-antitrypsin is a secretory protein, as well as prothrombin.

The three independent transient cotransfections of wild-type (pCIneoΔSV40-14eUTR-wt and pCIneoΔSV40-14eUTR+FR-wt) or mutant FII c.*64_*66del prothrombin (pCIneoΔSV40-14eUTR-mut and pCIneoΔSV40-14eUTR+FR-mut) with pcDNA-AAT1-wt vector were carried out in Cos-7 cell line (ATCC® CRL-1651™). The Lipofectamine 2000 was used as DNA transfection reagent (Invitrogen, Merelbeke, Belgium) with 1.5 μg of DNA for each of the expression vectors. Cells were incubated in transfection medium for 24h, after which the medium was replaced with F12 serum free medium for additional 48h of cells incubation. Thereupon, media containing secreted proteins and cell lysates were collected.

Real-Time PCR

Total RNA was isolated from Cos-7 cells lysates (Qiagen RNeasy Plus Mini Kit protocol, Qiagen, Hilden, Germany), purified with Ambion DNA-free kit protocol (Ambion, Austin, Texas, USA) and used as a template for cDNA synthesis according to the High-Capacity cDNA Reverse Transcription Kit protocol (Applied Biosystems, Waltham, Massachusetts, USA). Taqman approach (TaqmanGene Expression Assays protocol, Applied Biosystems, Waltham, Massachusetts, USA) was applied for Real-Time PCR relative quantification of prothrombin mRNA amount on the Applied Biosystems 7500 Real-Time PCR system. As a reference gene, we used neomycin phosphotransferase I gene from the pCIneoΔSV40 expression vector

(KAPLAN *et al.*, 2005). The same cDNA template was used for prothrombin (target) and reference gene signal detection in multiplex PCR reactions. As a negative control, we used the cDNA synthesized from cells transfected with pCIneoΔSV40 without insert. The reference gene was used for the normalization of the target gene expression level. Results were analyzed by software provided with the ABI Prism 7500 (Applied Biosystems, Waltham, Massachusetts, USA).

Western blot analysis of recombinant prothrombin

Cos-7 cells media were applied to the 12% SDS-PAGE gel for the separation of prothrombin (72kDa) and alpha-1-antitrypsin (52kDa) proteins. Three independent cotransfections media were pooled and concentrated with Ambicon Ultra, Ultracel 30K Membrane (Millipore, Darmstadt, Germany). Protein separation, transfer and membrane blocking were performed as previously described (PRUNER *et al.*, 2020). Prothrombin and alpha-1-antitrypsin were detected with Thrombin K-20 (Santa Cruz Biotechnology, Dallas, Texas, USA) and alpha-1-antitrypsin (Abcam, Cambridge, UK), respectfully, both goat polyclonal primary antibody (1:10000 dilution in 2% BSA diluted in PBST buffer, incubation time 1h and 15 min). Anti-goat IgG peroxidase conjugate (Sigma, Saint Louis, Missouri, USA) was used as a secondary antibody (1:80000 dilution in 2% BSA in PBST buffer) with 1 h of incubation. Media with wild-type and mutant prothrombin were applied on SDS-PAGE in three independent experiments, each performed at least in triplicate. Protein immunoreactive bands detection and relative quantification of the amount of prothrombin and alpha-1-antitrypsin were performed as described by PRUNER *et al*. (2020).

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Sciences 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). For distribution testing we used Shapiro-Wilk test. Statistical analysis was conducted using Student t-test and $\chi 2$-test. The mean values with standard deviation, OR (odds ratio) and 95%CI (confidence interval) were also calculated in SPSS. P value less than 0.05 was considered as statistically significant.

RESULTS

*Prevalence of FII c.*64_*66del variant*

The baseline characteristics of both study groups are given in Table 1. Among group of women with pregnancy loss we have detected 3 heterozygous carriers of FII c.*64_*66del variant (Figure 1), while there were no carriers observed among controls. The $\chi 2$- test showed that patient group was in Hardy-Weinberg equilibrium for FII c.*64_*66del variant.

Results shown that carriers of this variant might have an increased risk for pregnancy loss occurrence (OR=10.5, 95%CI 0.5-206.4), but the difference was not statistically significant (p=0.12) (Table 1).

For further analysis, we have collected data and plasma samples from all three detected FII c.*64_*66del carriers. In the proposita 1, the first pregnancy loss occurred at age 31 and the second a year later, both at sixth week of gestation. At the age 28, she had one successful pregnancy delivery, but the thrombus was detected on one ovary. Other chronic diseases included hypothyreosis. Proposita 2 had two pregnancy loss. The first was at age 24, during the

eight weeks of gestation due to the large hematoma. The other occurred a year later, at the twelfth week of gestation and it was caused by fetal heart arrest. After that, she had two successful pregnancy outcomes by cesarean section. Other chronic diseases were absent. Proposita 3, 40 years old, never had successful pregnancy. She got pregnant two times, but experienced pregnancy losses at seventh and eighteenth week of gestation, respectively. Both pregnancies were achieved after procedures of *in vitro* fertilization which were performed because of severe infertility in her husband. She denies any other health problem. Probands' plasma samples were used for routine thrombophilia testing, detection of prothrombin plasma level and determination of endogenous thrombin potential. Thrombophilia testing including prothrombin clotting activity assay, shown that all measured parameters were in reference range for FII c.*64_*66del variant carriers.

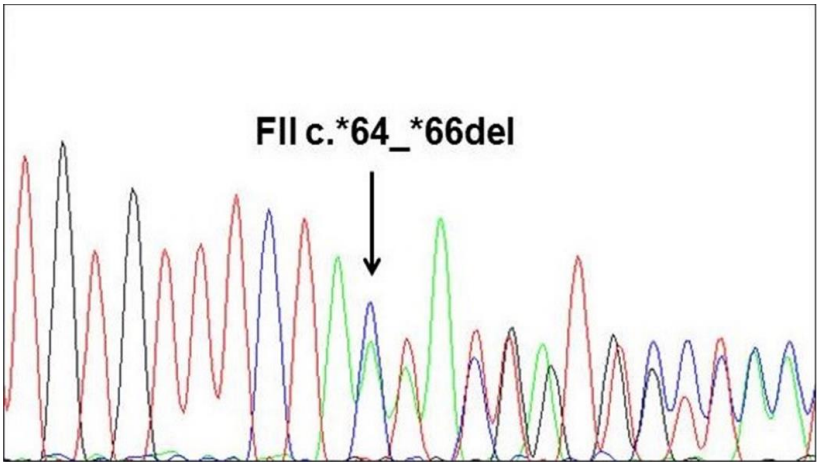

Fig. 1. Fragment of DNA sequence with FII c.*64_*66del variant

The relative quantification of Western blot analysis of prothrombin plasma level (Figure 2), after normalization with standard plasma (100%) shown that there was no significant difference in prothrombin plasma level between FII c.*64_*66del carriers (108.34±9.54) and non-carriers (111.93±8.77) (p=0.572).

The thrombin generation assay showed that all three symptomatic carriers of FII c.*64_*66del variant, in comparison to control plasma, had slightly increased ETP (398.76±27.79 mA vs. 351.54 mA, p=0.28), c_max (107.81±8.61 mA/s vs. 94.36 mA/s, p=0.31) and t_max (61.76±5.15 s vs. 53.57 s, p=0.30), while the value if t_lag did not differ among carriers and control.

Table 1. Baseline characteristics of group of women with pregnancy loss and control group

	Age mean±SD (years)	Number of FL	Family history of thrombotic events (%)	Number of heterozygous carriers of FII c.*64_*66del	p	OR (95%CI)
Women with FL (n=105)	34.68±5.65	383	38.09	3 (2.9%)	0.12	10.55 (0.54-206.43)
Control group (n=155)	38.45±12.37	None	1.29	None		

FL-pregnancy loss, n-number of participants in the group, SD-standard deviation, p-probability, OR-odds ratio, 95%CI-95% confidence interval

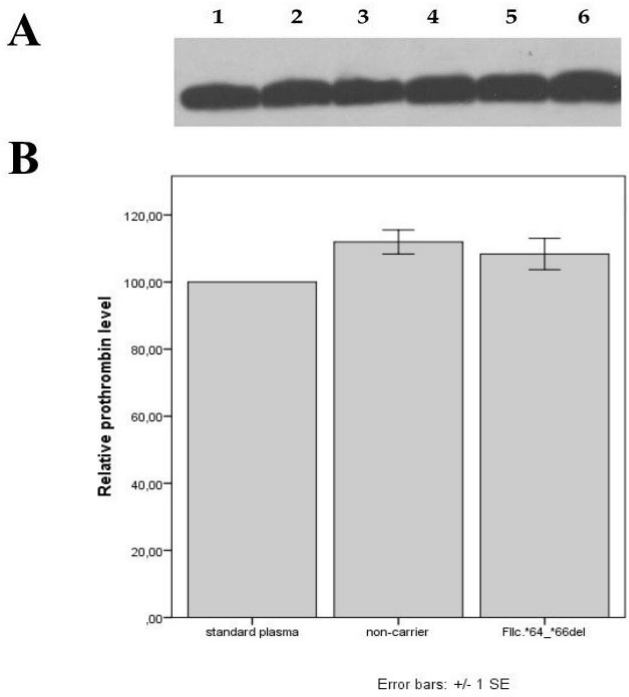

Fig. 2. Western blot analysis and relative quantification of prothrombin plasma level A) Western blot analysis: line 1, 2, 3-standard plasma; line 4, 5, 6- FII c.*64_*66del carrier; B) Relative quantification of prothrombin level in FII c.*64_*66del non-carriers and carriers compared to standard plasma. Data represent the means ± SD for 3 separate experiments.

In vitro functional analysis

The effect of FII c.*64_*66del variant on the prothrombin gene expression *in vitro* was assessed through the level of prothrombin mRNA and protein. After normalization to the level of neomycin phosphotransferase I as reference gene, relative quantification values (RQ) of prothrombin mRNA level were obtained for both types of constructs (pCIneoΔSV40-14eUTR and pCIneoΔSV40-14eUTR+FR). The results shown that the expression level of mutant FII c.*64_*66del mRNA (RQmut 1.096± 0.007) was significantly decreased (p=0.001) compared to wild-type (RQwt 1.113± 0.009) for pCIneoΔSV40-14eUTR constructs (Figure 3A), as well as for pCIneoΔSV40-14eUTR+FR constructs with additional 50 bp of 3'FR region (RQwt 1.141±0.019 vs. RQmut 1.088± 0.005, p<0.001) (Figure 3B).

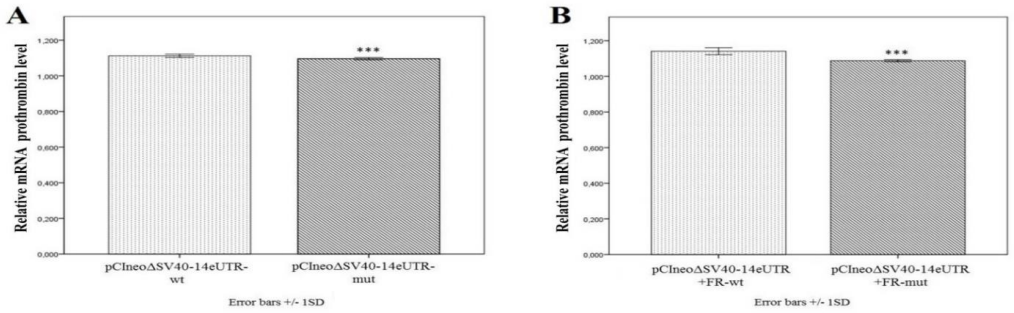

Fig. 3. Relative quantification of mRNA prothrombin level from Cos-7 transfected cells by Real-time PCR. Data represent the means ± SD for 3 separate experiments A) Relative mRNA level for wild-type and FII c.*64_*66del mutant pCIneoΔSV40-14eUTR pair of constructs; B) Relative mRNA level for wild-type and FII c.*64_*66del mutant pCIneoΔSV40-14eUTR+FR pair of constructs

Western blot analysis of collected cell media, which contained prothrombin secreted from Cos-7 transfected cells, was shown on Figure 4A and 4B. Data obtained for prothrombin were normalized to the level of alpha-1-antitrypsin. The observed FII c.*64_*66del mutant prothrombin level was decreased for pCIneoΔSV40-14eUTR (0.89±0.14, p=0.028) (Figure 4C), as well as for pCIneoΔSV40-14eUTR+FR (0.73±0.17, p=0.595) (Figure 4D) compared to wild-type (1.02±0.13; 0.81±0.28, respectfully), but this decrease was significant only for pCIneoΔSV40-14eUTR pair of constructs.

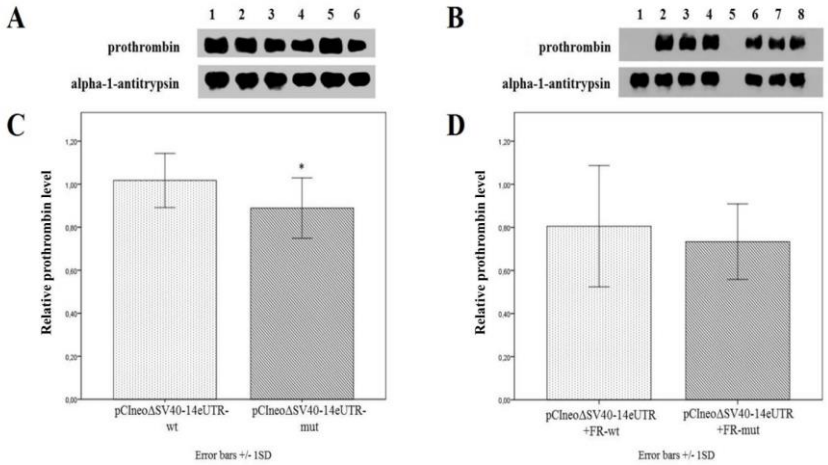

Figure 4. Western blot analysis and relative quantification of wild-type and FII c.*64_*66del mutant prothrombin level. Data represent the means ± SD for 3 separate experiments A) Western blot analysis for pCIneoΔSV40-14eUTR pair of constructs: line 1, 2, 3-pCIneoΔSV40-14eUTR-wt prothrombin; line 4, 5, 6-pCIneoΔSV40-14eUTR-mut prothrombin; B) Western blot analysis for pCIneoΔSV40-14eUTR+FR pair of constructs: line 1-pCIneoΔSV40 vector with no insert; 2, 3, 4-pCIneoΔSV40-14eUTR+FR-wt prothrombin; line 5-Cos-7 untransfected cells; line 6-pCIneoΔSV40-14eUTR+FR-mut prothrombin; C) Relative quantification of wild-type wt and FII c.*64_*66del mutant prothrombin level in pCIneoΔSV40-14eUTR pair of constructs; D) Relative quantification of wild-type wt and FII c.*64_*66del mutant prothrombin level in pCIneoΔSV40-14eUTR+FR pair of constructs

DISCUSSION

The 3'utranslated region (3'UTR) is an important regulatory element involved in post-transcriptional regulation of gene expression by determining the stability, localization, translation and degradation of mRNA. Therefore, any mutation or perturbation of the regulatory elements architecture within this region may strongly affect mRNA stability, the RNA folding, mRNA localization and translation of protein, causing aberrant gene expression and leading to different pathologies (HAMEED *et al.*, 2014; LAKKA *et al.*, 2000; MATOULKOVA *et al.*, 2012; REAMON-BUETTNER *et al.*, 2007).

The 3'end of prothrombin gene has a specific non-canonical organization, which is characterized by an efficient upstream sequence element (USE) and low efficiency cleavage site and downstream sequence element (DSE). All the aforesaid makes 3'end of prothrombin gene susceptible to generation of gain-of-function variants, which could thus affect the prothrombin gene expression and contribute to thrombophilia (DANCKWARDT *et al.*, 2004; DANCKWARDT *et*

al., 2008; GEHRING *et al.*, 2001). The FII c.*64_*66del variant has been previously associated with thrombophilia (GVOZDENOV *et al.*, 2015), but its potential role in the etiology of pregnancy loss is still unknown. Also, localization of FII c.*64_*66del variant within the 3'UTR and scarce data on the role of this region in the regulation of prothrombin gene expression, results in still not completely understood mechanism of this variant.

In order to investigate the potential association of this variant with pregnancy loss, we have carried out a study which included group of 105 women with idiopathic pregnancy loss and 155 controls, among which we have detected 3 patients who were heterozygous carries of FII c.*64_*66del variant and none among controls. After detailed medical data of these three carriers have been collected, it was noticed that they had recurrent pregnancy losses. Our results shown that FII c.*64_*66del variant was more frequent in patients group (OR 10.5, 95%CI 0.5-206.4), but the difference was not statistically significant compared to control group (p=0.12). The limitation of our association study was a small sample size, so the larger studies should be conducted to confirm our results and to further asses the association of this variant with the etiology of pregnancy loss.

Since the mechanism of this variant is still completely unknown, our aim was to perform functional studies in order to give the first insights into it. Results of our study showed that the presence of FII c.*64_*66del variant does not affect the level of prothrombin (carriers (108.34±9.54) (111.93±8.77) as well as that prothrombin clotting activity values were in reference range. The thrombin generation assay shown slightly increased thrombogenic potential of FII c.*64_*66del variant. Since these assays were performed on small number of samples, larger study group is needed to evaluate whether the FII c.*64_*66del has a significant thrombogenic potential.

The functional analysis on Cos-7 cells was performed in order to assess the effect of FII c.*64_*66del variant on mRNA by Real-Time PCR and protein level by Western blot analysis. Two types of constructs were generated in wild-type and FII c.*64_*66del mutant form in order to investigate whether the FII c.*64_*66del expression profile depends on the presence or absence of additional 50 bp of 3'FR. Danckwardt et al. have previously shown that the presence of prothrombin 3'FR sequence caused 3.9 times lower expression of β-globin gene-prothrombin 3'UTR-prothrombin 3'FR construct compared to β-globin gene-prothrombin 3'UTR- β-globin 3'FR construct (DANCKWARDT *et al.*, 2004). This is in accordance with the non-canonical model of 3'end of prothrombin gene which is caracterized by weak activity of DSE element due to the its decreased ability to bind CstF complex (DANCKWARDT *et al.*, 2007). However, Sachchithananthan et al. shown that construct which contained only 3'UTR had slighlty lower expression (1.5) compared to constrict which contained both, 3'UTR and DSE (1.7) (SACHCHITHANANTHAN *et al.*, 2005). In our study, relative quantification of Real-Time PCR shown that prothrombin mRNA level was significantly decreased in the presence of FII c.*64_*66del variant compared to wild-type for both types of constructs. In the assessment of FII c.*64_*66del effect on protein level, it has been shown that the amount of mutant FII c.*64_*66del prothrombin level was significantly decreased compared to wild-type for pCIneoΔSV40-14eUTR (0.89±0.14 mut vs. 1.02±0.13 wt, p=0.028), while for pCIneoΔSV40-14eUTR+FR constructs this decrease was not statistically significant (0.73±0.17 mut vs. 0.81±0.28 wt, p=0.595). The decreased level of c.*64_*66del mutant recombinant prothrombin,

while similar plasma level in carriers and non-carriers, might be explained by the homozygosity of recombinant compared to heterozygous FII c.*64_*66del prothrombin observed in patients.

Taking into account that mutations within 3'UTR might alter the binding of miRNAs to their targets, as well as that some estrogen responsive miRNAs regulate coagulation during pregnancy (TAY *et al.*, 2015), we hypothesize that the mechanism of FII c.*64_*66del variant might be estrogen-dependent. The increase in estrogen level during pregnancy might affect the expression of miRNAs' network which is involved in regulation of haemostatic factors and lead to haemostatic disorders (TAY *et al.*, 2015). Miura et al showed that among 82 miRNAs, which were predominantly expressed in placenta, the concentrations of 24 of them in maternal plasma significantly decreased after delivery (MIURA *et al.*, 2010). These miRNAs are determined as pregnancy-associated miRNAs and considered as potential new biomarkers for monitoring pregnancy-associated diseases (MIURA *et al.*, 2010). It was also described that decreased levels of miR-21 and miR-126 might increase the risk of recurrent pregnancy loss (EL-SHORAFA *et al.*, 2013), while placental miR-17 and miR-19a are implicated in early pregnancy loss (TSOCHANDARIDIS *et al.*, 2015). However, it is still unknown whether pregnancy-associated miRNAs might affect the expression of prothrombin gene and consequently lead to pregnancy loss.

In conclusion, this is the first study in which FII c.*64_*66del variant has been detected among women who experienced pregnancy loss. However, the mechanism of this variant and its association with the etiology of pregnancy loss is not fully elucidated yet. Further studies should be conducted in order to investigate the potential role of miRNAs in the regulation of prothrombin expression and their association with the mechanism of FII c.*64_*66del variant.

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NOVA FII C.*64_*66DEL VARIJANTA PROTROMBINSKOG GENA KOD ŽENA SA GUBITAKOM TRUDNOĆE

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Izvod

Trudnoća je sama po sebi prirodno hiperkoagulaciono stanje, tako da prisustvo dodatnih stečenih ili genetskih protrombotičkih faktora rizika može značajno uticati na pravilnu cirkulaciju između majke i fetusa i time dovesti do spontanog pobačaja. Ispitivanje potencijalne povezanosti nedavno prijavljene varijante c.*64_*66del u genu za protrombin sa etiologijom spontanog pobačaja. Studija je obuhvatila 105 ispitanica koje su imale spontani pobačaj i 155 kontrola. Urađene su analize u uzorcima plazme ispitanica kao i in vitro analize u ćelijskoj kulturi kako bi se ispitalo da li varijanta FII c.*64_*66del može dovesti do poremećaja koagulacije i spontanog pobačaja. Tri ispitanice u grupi bolesnica (2,9%) bile su heterozigotni nosioci FII c.*64_*66del, dok među kontrolama ova varijanta nije detektovana. Kod svih nosilaca ove varijante parametri rutinskih testova za trombofiliju bili su u referentnom opsegu i u uzorcima plazme su detektovani su slični nivoi protrombina u odnosu na ne-nosioce. Endogeni potencijal trombina je bio blago povišen kod nosilaca FII c.*64_*66del varijante u poređenju sa kontrolnom plazmom, ali ova razlika nije bila statistički značajna. Ekspresija gena za protrombin, na nivou iRNK i proteina, bila je statistički značajno smanjena kada je varijanta c.*64_*66del bila prisutna u odnosu na vrednosti detektovane za neizmenjenu sekvencu. Rezultati studije pokazali su trend veće učestalosti FII c.*64_*66del varijante kod ispitanica sa spontanim pobačajima. Međutim, potrebne su dodatne studije kako bi se u potpunosti razjasnilo da li varijanta FII c.*64_*66del utiče na ekspresiju protrombina tokom trudnoće i koji je njen značaj u etiologiji spontanih pobačaja.

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