Chapter 4

The Role of APC-Resistance for Predicting Venous Thrombosis and Pregnancy Complications in Carriers of Factor V Leiden (1691) G/A Mutation

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Abstract

This chapter presents the results of the prospective cohort study of 500 females with factor V Leiden, *FVL*, 1691 GA genotype, during 2008–2015. The association between FVL (regardless of its laboratory phenotype—factor Va resistance to activated protein C, APC resistance) and the development of VTEC (both outside of and during pregnancy) and gestational complications such as preeclampsia, fetal growth restriction, and miscarriage has been established. Additionally, the leading role of APC resistance degree in the clinical manifestation of *FVL* 1691 GA genotype as thrombotic events and pregnancy complications has been proved. Based on the data obtained, advanced approaches for the stratification of pregnant women into risk groups for the development of venous thromboembolic complications and pregnancy complications at different gestational ages adjusted for APC resistance degree are proposed. The found patterns can be useful in assessing the need for heparin prophylaxis during pregnancy from the standpoint of personalized medicine.

Keywords: APC resistance, factor V Leiden, thrombosis, preeclampsia, fetal growth restriction, miscarriage, ROC analysis

1. Introduction

In 1993, Swedish scientist Bern Dalbeck described hereditary thrombophilia that was caused by the inability of blood to react to activated protein C. A year later, in Leiden, Netherlands, Professor Roger Bertin managed to decipher the pathogenesis of this thrombophilia type.

The pathology was called "factor V Leiden" [1, 2]. Factor V Leiden is a point mutation in the proaccelerin gene, accompanied by the substitution of the guanine nucleotide with adenine at position 1691 (*FV* G1691A), which leads to the substitution of the arginine (Arg = R) with the glutamine (Gln = Q) at position 506 (FV R506Q) in the protein chain that is the product of this gene. Due to this mutation, the resistance of factor V to the activated protein C (APC resistance) is formed [3]. This genetic deficiency is one of the most common causes of hereditary thrombophilia in European countries, and it is noted in 8–15% of the white population [4, 5].

Factor V Leiden carriage (a 1691G → A substitution) is traditionally considered as a genetic, nonmodifiable risk factor for venous thromboembolic complications (VTEC) [6–8]. Moreover, the basis of risk stratification is the mutation genotype: the carriage of *FVL 1691 AA* is defined as a high risk; the carriage of *FVL* 1691 GA is defined as a moderate risk. VTEC in both carriage variants is most often associated with a precipitating factor, such as surgery, trauma, postpartum period, immobilization, hormone treatment or chemotherapy, or coexistence of other risk factors such as pregnancy, age, and comorbid conditions [7, 9–14]. The world community developed protocols and algorithms for the prevention of VTEC, depending on the degree of occurrence risk of the factor V Leiden mutation. However, the clinical manifestations of factor V Leiden are heterogeneous and can be not only in the form of thromboembolic events but also determine the risk of developing gestational complications, including the great obstetrical syndrome [15–17]. Modern ideas about the relation between *FVL* 1691 GA carriage and the risk of developing gestational complications are highly contradictory, the available studies in this direction are inadequate and ethnically heterogeneous and often contradict with each other [18–21]. According to the conclusions of world experts, it is impossible to make a final decision on the cause-effect relation between the *FVL* 1691 GA carriage and the unfavorable course of pregnancy.

Thus, it is not always possible to predict the likelihood of VTEC and obstetric complications with *FVL* 1691 GA carriage, based on the proposed and already proven risk factors. We believe that such risk depends not only and not so much on the factor V Leiden genotype but rather on its phenotype being an increase in APC resistance. However, in the existing recommendations for predicting the development of clinically significant events, the laboratory phenotype of the mutation—the APC resistance, whose magnitude actually determines the thrombosis tendency—is not taken into account.

The aim of this chapter is to outline the prospective approaches for stratifying pregnant women into risk groups for the VTEC development and for stratifying pregnancy complications based on the level of APC resistance, in order to address the issue of heparin prophylaxis during pregnancy from the standpoint of personalized medicine.

Materials and methods: Between 2008 and 2015, a prospective cohort observational study of 1100 Caucasian women was conducted, and 2707 pregnancy outcomes were analyzed in order to determine the clinical manifestation of *FVL* 1691 GA carriage in thrombotic events and gestational complications, such as preeclampsia, fetal growth restriction, and miscarriage.

The study was approved by the local research Ethics Committee of the Altai State Medical University (Protocol 5, from June 26, 2009).

Two cohorts were identified: the study group consisted of 500 patients with *FVL* 1691 GA genotype (mean age 30.2±4.7 years, the total number of completed pregnancies 1085), and the

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control group consisted of 600 women with *FVL* 1691 GG genotype (mean age 30.3±3.9 years, the total number of completed pregnancies 1622). Groups were comparable in age ($p > 0.05$).

Study group inclusion criteria:

- Female
- *FVL* 1691 GA carriage
- Age 18 to 45
- Informed consent

Control group inclusion criteria were the same as for the study group, but the patients were not carriers of *FVL* 1691 GA/AA.

Exclusion criteria:

- Age under 18 and over 45
- Autoimmune diseases, including antiphospholipid syndrome
- Chromosomal aberrations

In order to determine the relation between APC resistance with carriage of FVL and thrombotic events during pregnancy and gestational complications, within the framework of the study, APC resistance was diagnosed during pregnancy monitoring in 298 patients of the study group and 300 controls; the patients did not receive anticoagulants. To exclude the influence of confounding factors, which along with APC resistance expression can significantly affect the course and outcome of pregnancy, we defined additional inclusion/exclusion criteria for the groups.

Additional study group inclusion criteria:

- A singleton pregnancy that occurred in the natural cycle, confirmed by embryo viability at 5–6 weeks
- No abnormal development of internal genital organs
- No extragenital diseases in the stage of decompensation
- No anticoagulant therapy

Control group inclusion criteria were the same as for the study group, but the patients were not carriers of *FVL* 1691 GA/AA.

Exclusion criteria:

- Abnormal development of internal genital organs, multiple pregnancy
- Pregnancy, resulting from assisted reproductive technologies
- Extragenital diseases in the stage of decompensation

Eight points were chosen to assess APC resistance, taking into account the waves of trophoblast invasion and reflecting "critical" gestational ages: 7–8 weeks, 12–13 weeks, 18–19 weeks, 22–23 weeks, 27–28 weeks, 32–33 weeks, 36–37 weeks, and 2–3 days postpartum.

Preeclampsia was diagnosed according to the international consensus criteria: systolic blood pressure (SBP) ≥ 140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg; in women with base hypotension, an increase in the SBP by 30 mm Hg and/or DBP by 15 mm Hg compared to the base values (arterial pressure in the I trimester of pregnancy), accompanied by proteinuria: daily protein loss of 0.3 g/l or more, any proteinuria recorded in a single urine portion [22]. Fetal growth retardation was defined as a condition with the fetal body weight and/or fetal abdomen circumference being below 10% for a given gestational age and/or the morphological maturity index lag of 2 or more weeks from the true gestational age [23].

APC resistance normalized ratio (NR) value was obtained with "factor V-PC-test" detection kit ("Technology-Standard" Ltd.).

Statistical data processing was carried out using MedCalc 14.8.1 statistical software package. The verification of the static series for normality was carried out using the Shapiro-Wilk's W-test. The laboratory data are presented as a median (Me), 95% confidence interval (95% CI), and interquartile range [25th and 75th percentiles]. Comparison of the series was performed using nonparametric methods (the Mann-Whitney U test). For the qualitative characteristic values, the absolute and the relative percentage values were given. The verification of statistical hypotheses on the coincidence of the observed and expected frequencies was carried out using the criterion $\chi 2$ and Fisher's exact test. For binary characteristics, relative risk (RR) and 95% confidence interval (95% CI) were calculated. Maximum p value is <0.05. To determine the predictive value of the quantitative assessment of APC resistance in the development of pregnancy complications in the given points, the ROC curve was used, followed by the determination of the area under it (AUC). According to the literature, the AUC index exceeding 0.70 is clinically/prognostically significant. Accuracy (effectiveness, significance) of the test (Ac) was calculated as the percentage of the number of true diagnostic test results to the total number of results obtained:

$$Ac = \frac{TN + TP}{FN + TN + FP + TP} \times 100 \quad (1)$$

2. Clinical manifestation of *FVL* 1691 GA carriage in thrombotic events regardless of APC resistance value

According to experts of the Royal College of Obstetricians and Gynecologists [8], factor V Leiden is considered to be a constant risk factor for thrombosis in asymptomatic women. In our study, thrombotic events were registered in 70 (14.0% out of 500) women with *FVL* 1691 GA genotype versus 9 (1.5% out of 600) with *FVL 1691 GG* genotype, which has statistical significance [RR 9.3; 95%CI, 4.7–18.5; $p < 0.0001$]. In all nine cases in the control group, deep vein thrombosis (DVT) of the lower extremities was diagnosed. In six patients DVT was diagnosed outside of pregnancy and was caused in five cases by combined oral contraceptives (COC) (calf deep vein, four cases; iliac-femoral-popliteal segment, one case) in one case by locked intramedullary flexible osteosynthesis in the setting of diaphyseal tibial fracture (the second

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day of the postoperative period). In three cases, DVT was registered during pregnancy: one episode in the first trimester and two postpartum (the third and sixth days). In 70 *FVL* 1691 GA patients, 98 thrombotic events were registered in different periods of life: in 45 women (64.3% out of 70), a single episode of VTEC; in 22 (31.4% out of 70), 1 case of rethrombosis; and in 3 (4.3% out of 70) women, 2 cases of rethrombosis. We studied primary phlebothrombosis on the background of FVL 1691 GA mutation carriage (**Figure 1**).

The analysis showed that in 71.4% (50 out of 70) of cases, the primary thrombosis was the result of iatrogenesis (41 CHC and 9 surgical intervention). As it is known, administering estrogen-containing CHC is absolutely contraindicated for *FVL* 1691 GA patients [5, 24]; however, 63 patients of the study group were offered exactly this type of elective contraception that resulted in thrombotic events in 65.1% (41 out of 63) of cases. Thrombotic patients were administered medicines containing either 30 or 20 μg of ethinylestradiol. Out of 41 episodes of CHC-induced thrombosis, in 30 cases the process developed in the area of tibial veins and in 10 cases in the iliac-popliteal-femoral segment, and in 1 case, pulmonary embolism was diagnosed. During the treatment, two patients were implanted a vena cava filter.

Taking into account that estrogen-containing medicines are widely used in gynecology (contraception, menopausal hormone therapy, ovulation stimulation cycles, etc.), we calculated the risk of developing VTEC associated with CHC in *FVL* 1691 GA patients as 9.2 [RR 9.2; 95%CI, 3.9–21.9; $p < 0.0001$].

In 13 patients, *FVL* 1691 GA manifested itself as thrombosis after surgery, with 9 patients having the first episode of thrombosis and 4 cases with rethrombosis. All women underwent "small" surgeries, and according to the combined assessment of clinical data, they had a moderate risk of VTEC in the postoperative period [25], which implies prophylactic low-molecular-weight heparin (LMWH) administration according to the dosage regimen recommended by the manufacturer for moderate-risk patients [5]. However, none of the 13 patients received heparin prophylaxis. In 7 cases (10% out of 70), the primary thrombosis cause could not be established. All seven patients with idiopathic phlebothrombosis had an episode of

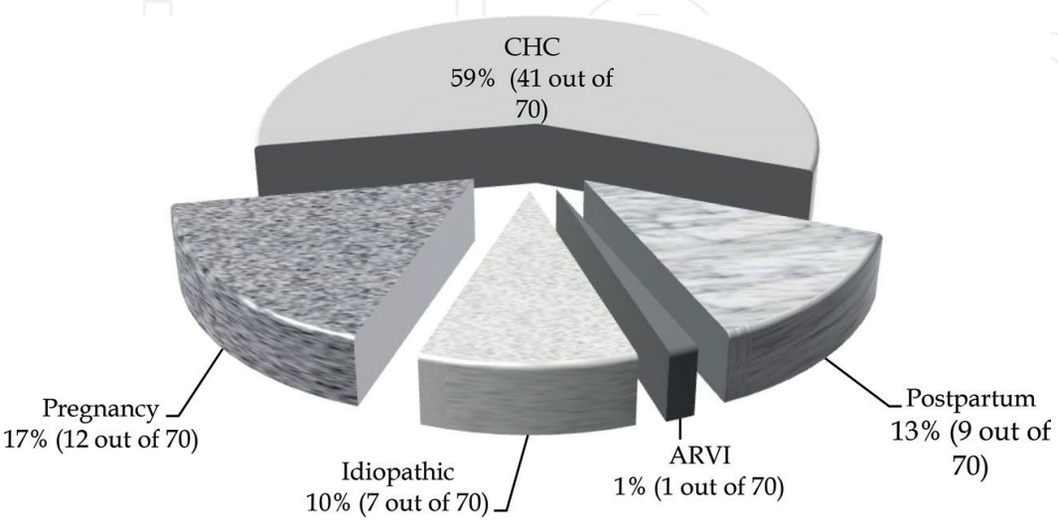

Figure 1. Primary phlebothrombosis proportion in *FVL* 1691 GA patients based on inducing factor. Abbreviations: CHC, combined hormonal contraceptives; ARVI, acute respiratory viral disease.

rethrombosis on the background of ARVI or after a surgical intervention within the first year. Viral infection, as a factor inducing primary thrombosis, was diagnosed in one case; three DVTs on the background of ARVI were recurrent.

In total, 65 thrombotic events occurred in 58 (11.6% out of 500) women outside of pregnancy; the frequency of rethrombosis was 12.1% (7 out of 65). During pregnancy, *FVL* 1691 GA was manifested in thrombotic events in 33 patients (6.6% out of 500), primary phlebothrombosis induced by pregnancy was registered in 12 patients (17.0% out of 70), and rethrombosis was registered in 21 cases.

For the convenience of perception, we identified groups of pregnant women according to the risk characteristics of VTEC development during pregnancy and postpartum given in clinical recommendations [8]:

1. Asymptomatic patients
2. A single episode of VTEC, associated with transient risk factors
3. A history with multiple episodes of VTEC

The number of pregnancies in asymptomatic *FVL* 1691 GA patients was 1027; in 58 cases thrombosis was registered before pregnancy. It should be noted that *FVL* 1691 GA genotype had not been considered as a risk factor for the development of VTEC during pregnancy and postpartum until 2015 [26]. Therefore, according to the clinical recommendations, these patients did not need antenatal VTEC prevention. In our study, the course of 12 pregnancies (1.2% of 1027) was complicated by an episode of primary VTEC, one case being vertebrobasilar basin thrombosis on the right, nine cases being tibial vein thrombosis, and two cases being iliac-femoral veins thrombosis.

According to the data obtained, we calculated the risk of *FVL* 1691 GA manifestation during pregnancy and postpartum in asymptomatic women compared to *FVL* 1691 GG patients. In our study, it is 4.7 [RR 4.7; 95%CI, 1.5–14.7; p = 0.0069].

Forty-five patients were included into the group with history of a single VTEC before pregnancy, whose thrombosis was associated with CHC or a surgical intervention. In 10 (22.2% out of 45) women, additional factors that affect the risk of developing VTEC were identified during pregnancy—a history of thrombosis in first-degree relatives up to 50 years, an implanted vena cava filter, and obesity (BMI, 25)—being the reason why they received prophylactic doses of LMWH throughout pregnancy and 6 weeks postpartum. With heparin prophylaxis, episodes of rethrombosis antenatally and/or postpartum were not registered. In 35 (77.8% out of 45) pregnant women with a history of thrombosis, the risk of VTEC was determined as intermediate, which presumed only postpartum LMWH prophylaxis for 6 weeks. In 18 (51.4% out of 35) patients who did not receive thromboprophylaxis, the course of pregnancy and postpartum were complicated by rethrombosis in the left lower extremity deep veins.

As known, patients with a history of multiple VTECs belong to the group with a very high risk of recurrent VTECs. In our study, this group includes 12 women: 6 of them had rethrombosis outside of pregnancy, and 6 had thrombosis in previous pregnancies. According to the

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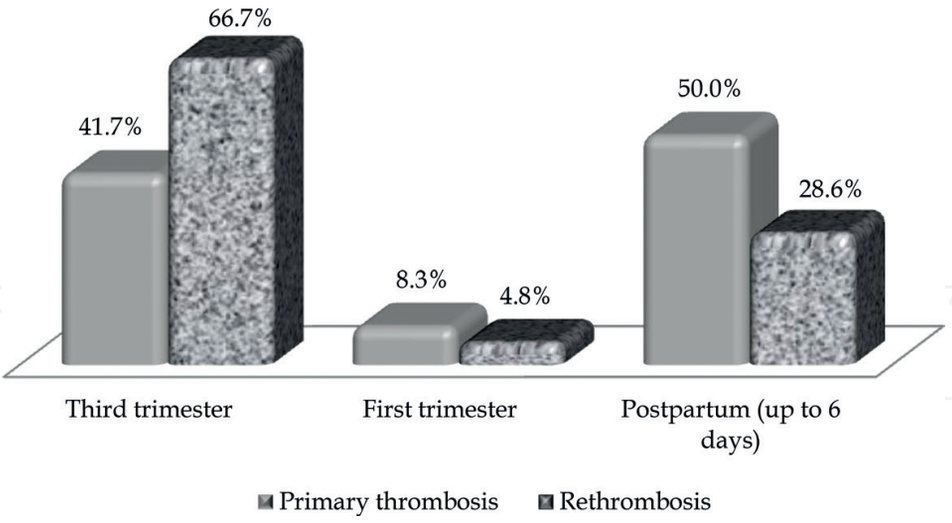

Figure 2. The periods of clinical manifestation of thrombotic events in *FVL* 1691 GA patients, depending on the personal thrombotic history.

available recommendations for VTEC prevention, patients of this group need antenatal and postpartum administration of LMWH. It should be noted that most patients did not follow the recommended continuous administration of LMWH. As a result, three (25.0% out of 12) pregnant women developed deep vein phlebothrombosis of the left lower extremity.

Interesting data were obtained during the analysis of the gestational age with a thrombotic event, depending on the personal history of thrombosis (**Figure 2**).

As can be seen, the major number of thromboses in our study was in the first trimester and postpartum. In the second trimester, thrombotic events were not registered. Perhaps this fact is due to mandatory thromboprophylaxis during the first 6 weeks after delivery in patients with a history of VTEC and without thromboprophylaxis in asymptomatic women [26].

3. Clinical manifestation of *FVL* 1691 GA in pregnancy complications regardless of changes in APC resistance

The question about the possible association between *FVL* 1691 GA and the risk of developing pregnancy complications remains controversial up to the present, despite the fact that one of the leading links in the pathogenesis of a whole range of obstetric complications is the imbalance between fibrinogenesis and fibrinolysis at the stage of trophoblast invasion, accompanied by microthrombus formation in placental vessels, by obstructive lesions of the myometrial segments of spiral arteries, and by abnormal placental perfusion.

Most of earlier studies, aimed at the chances of pregnancy complication development depending on the candidate gene and its genotype, were retrospective, ethnically heterogeneous, and sometimes not systematized. The peculiarity of our study lies in its prospective observational character and its seven-year period. Consequently, the course and outcome of 1085 pregnancies have been analyzed.

Upon completion of the study, an outcome analysis was conducted aimed at determining a possible association between *FVL* 1691 GA and its clinical manifestation in:

- Early reproductive loss (ERL)
- Preeclampsia (PE)
- Fetal growth restriction (FGR)
- Preterm birth (PB)

Early reproductive loss, as it is known, is a loss (an empty embryo sac or with an embryo) with a gestational age of up to 12 weeks [27].

The analysis of completed pregnancies showed 33.7% (366 out of 1085) of all pregnancies in *FVL* 1691 GA patients, and 10.3% (186 out of 1622) in patients with normal *FVL* 1691 GG genotype ended with early reproductive losses (ERL) [RR 3.0; 95%CI, 2.5–4.5; $p < 0.0001$].

In both groups, early reproductive losses in some patients were recurrent (**Figure 3**). The number of patients in the study group with two ERLs was 2.5 times greater than in the control group [RR 2.5; 95%CI, 1.7–3.7; $p < 0.0001$], and the number of patients with three or more ERLs, that is, those suffering from recurrent miscarriage, was 4.5 times greater in the *FVL* 1691 GA group [RR 4.5; 95%CI, 2.3–8.6; $p < 0.0001$].

We also determined the cause of reproductive losses in the groups (**Figure 4**). The proportions in spontaneous miscarriages and ectopic pregnancies were similar ($p = 0.7643$ and 0.24, respectively).

One in four pregnancies, 23.8% (258 out of 1085), with *FVL* 1691 GA ended as embryo death up to 12 weeks (70.5% of all ERL), which is nine times greater than with normal *FVL* 1691 GG genotype—2.6% (42 out of 1622) [RR 9.2; 95%CI, 6.8–12.6; $p < 0.0001$].

A more detailed analysis of non-developing pregnancies in *FVL* 1691 GA patients showed that in 65 cases (25.2% out of 258), pregnancy ceases to progress at a gestational age of 5–6 weeks, and in 193 cases (74.8% out of 258), pregnancy ceases to progress at a gestational age of 8–9 weeks, which contradicts previously obtained data stating that *FVL* 1691 GA is associated with embryo death at a gestational age of more than 14 weeks [12, 28, 29]. According to the histological investigation, anembryonic gestation was registered in 57 (22.1% out of 258) patients; in the remaining 201 (77.9% out of 258), embryo death was registered. Certainly, the knowledge of non-developing pregnancy etiology and histological evidence indicating the absence of an embryo favors a chromosomal abnormality of the embryo, but in our study, there was no karyotyping of the abortus, and therefore we cannot confirm or reject this hypothesis.

Thus, the data obtained indicate that *FVL* 1691 GA statistically significantly affects the cause and number of early reproductive losses. But, of course, this risk factor needs to be assessed in the context of additional predictors of reproductive ill-being in the individual.

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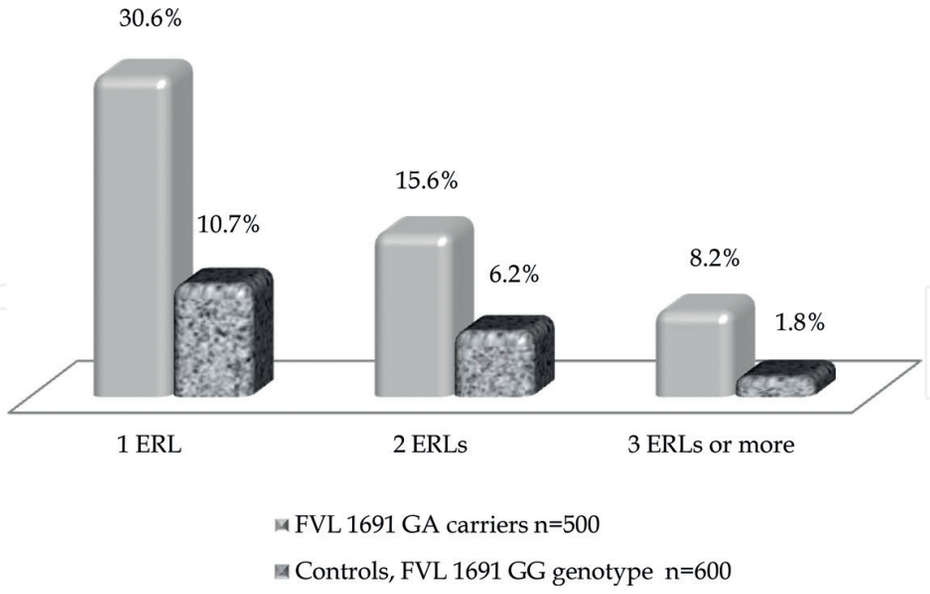

Figure 3. Proportion of women with one, two, and three or more early reproductive losses with *FVL* 1691 GA genotype and with normal *FVL* 1691 GG genotype.

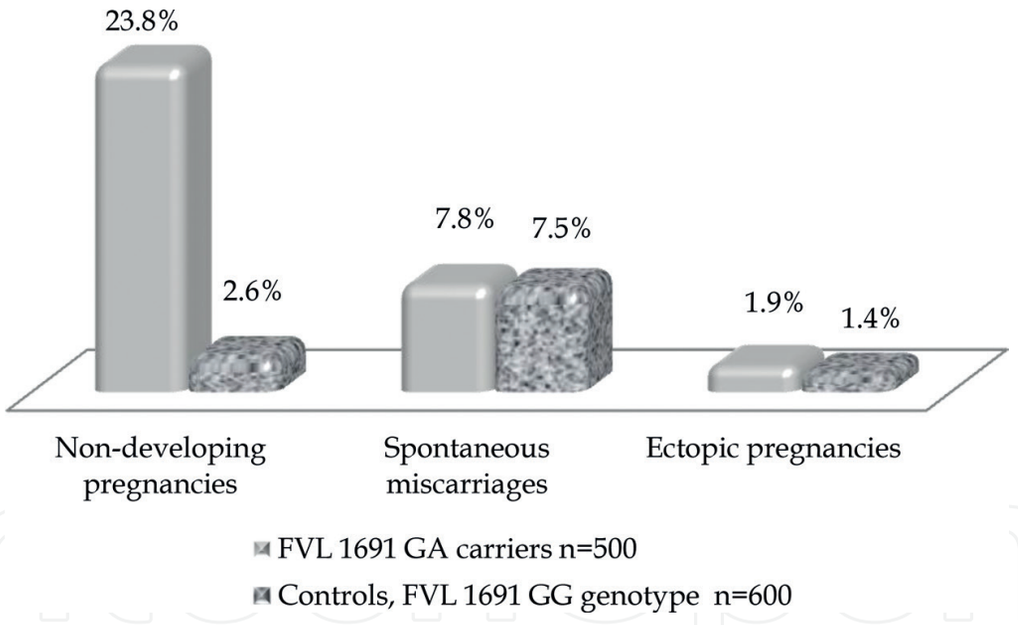

Figure 4. Proportion of ERL variants with *FVL* 1691 GA genotype and with normal *FVL* 1691 GG genotype.

Analysis of completed pregnancies in the *FVL* 1691 GA group showed that in 128 cases (11.8% out of 1085), pregnancy course was complicated by preeclampsia, which is statistically significantly greater than 52 episodes (2.9% out of 1622) with normal *FVL* 1691 GG genotype [RR 3.7; 95%CI, 2.7–5.0; $p < 0.0001$]. In this case, severe preeclampsia, as a pregnancy outcome, was registered in 28 cases (2.6% out of 1085) of the study group and in 8 cases (0.4% of 1622) of the control group [RR 5.2; 95%CI, 2.4–11.4; $p < 0.0001$].

In 130 cases (12.0% out of 1085), the course of pregnancy in the study group was complicated by FGR, which is statistically significantly greater than 64 episodes with normal *FVL* 1691 GG genotype (3.5% out of 1622) [RR 3.0; 95%CI, 2.3–4.1; $p < 0.0001$]. The weight of newborns with FGR in women of the study group was 1936.9±342.8 (95% CI 1805.9–2068.0); in the control group, it was 2124.9±274.2 (95% CI 1927.3–2321.5), thus having no statistical differences ($p = 0.1360$).

Given the pathogenesis unity of the placenta-mediated conditions, such as PE and FGR, we also analyzed the proportion of these complications in pregnancy outcomes in *FVL* 1691 GA patients.

In the study group in 33 (3.0% out of 1085) pregnancy outcomes, there was a combination of FGR and PE, versus 6 (0.3% of 1622) episodes in the control group [RR 8.2; 95%CI, 3.5–19.6; $p < 0.0001$]. In all six control group cases, the pregnancy was terminated prematurely (28–36 weeks) by an emergency abdominal birth due to life-threatening conditions of the woman and/or fetus. All six newborns were transferred to the second stage nursing; two children died during the first month of life.

With *FVL* 1691 GA genotype, the combination of FGR and PE in 7 (21.2% out of 33) resulted in antenatal fetal death (4 cases at 24–26 weeks, 3 cases at 28–30 weeks); preterm operative labor induction was performed in 27 cases (81.8% out of 33)—there were no intranatal and early perinatal losses.

Preterm birth is always considered to be an unfavorable perinatal outcome, whose degree depends not only on the gestational age but also on the causes: spontaneous or induced [30–33].

The proportion of preterm labor (PL) with *FVL* 1691 GA in our study was 7.1% (77 out of 1085), which is statistically significantly greater than 1.4% (26 of 1622) with *FVL* 1691 GG genotype [RR 4.2; 95%CI, 2.9–6.9; $p < 0.0001$]. The main PL difference is that PL before 28 weeks was only in the study group; 21 cases (27.3% out of 77) were registered. The number of PLs before 33 weeks of gestation was similar: 13 cases (16.9% out of 77) in the study group and 5 (19.2% out of 26) in the control group. There were 43 PLs (55.8% out of 77) in the study group and 21 PLs (80.8% of 26) in the control group with a gestational age of 34–36 weeks.

Structure analysis of PL is of interest. As is known, spontaneous PL is hard to manage, but it is well studied. Currently, prevention measures that have been developed and are widely used in practice are cervical incompetence correction, gestagen treatment, infection focus sanitation, and vaginal biocenosis normalization [34, 35]. PL due to medical reasons is always a catastrophe, being a condition that jeopardizes the life of a mother and/or fetus and thus ascertaining the need for early delivery regardless of the gestational age. As a rule, the main cause of indicated delivery is decompensation of placenta-dependent conditions.

In our study, the proportion of preterm labor induction with *FVL* 1691 GG genotype in our study was 26.9% (7 out of 26), which is consistent with general population data [31, 32] (**Figure 5**).

The proportion of preterm labor induction with *FVL* 1691 GA was 70.1% (54 out of 77), which is statistically significantly greater [RR 2.9; 95%CI, 1.5–5.5; $p = 0.0014$] than in the control group.

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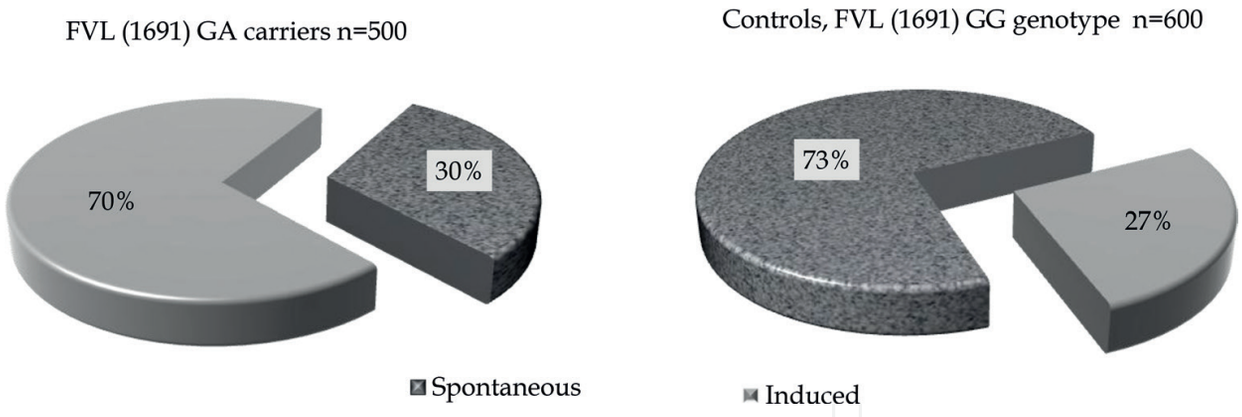

Figure 5. Preterm labor cause proportion in *FVL* 1691 GA carriers and controls.

Indications for induced PL with *FVL* 1691 GA genotype due to maternal condition were 8 cases with severe PE (10.4% out of 77) and 21 cases of placental abruption (PA) (27.3% out of 77). Due to fetal condition, there were 8 cases of progressive fetal hypoxia (10.4% out of 77) and 17 cases of antenatal fetal death (21.4% out of 77).

Summarizing the data presented above, we can note the following patterns:

1. The relative risk of developing VTEC outside of pregnancy in *FVL* 1691 GA patients is 9.3 [RR 9.3; 95%CI, 4.7–18.5; $p < 0.0001$]; when administering CHC it is 9.2 [RR 9.2; 95%CI, 3.9–21.9; $p < 0.0001$].
2. The risk of developing VTEC during pregnancy and postpartum in asymptomatic *FVL* 1691 GA patients compared to *FVL* 1691 GG patients is 4.7 [RR 4.7; 95%CI, 1.5–14.7; $p = 0.0069$].
3. *FVL* 1691 GA carriage is statistically significantly associated with early reproductive losses, increasing their number threefold [RR 3.0; 95%CI, 2.5–3.5; $p < 0.0001$], compared to normal *FVL* 1691 GG genotype.
4. With *FVL* 1691 GA, 70.5% of ERLs are non-developing pregnancies when embryo dies at 8–9 weeks, whose number is statistically significantly greater than with *FVL* 1691 GG genotype [RR 9.2; 95%CI, 6.8–12.6; $p < 0.0001$].
5. *FVL* 1691 GA is associated with the development of placenta-mediated conditions, increasing the risk of preeclampsia [RR 3.7; 95%CI, 2.7–4.0; $p < 0.0001$] and FGR [RR3.0; 95%CI, 2.3–4.1; $p < 0.0001$].
6. *FVL* 1691 GA is associated with a higher frequency of preterm birth, increasing their number 4.2-fold compared to normal *FVL* 1691 GG genotype [RR 4.2; 95%CI, 2.9–6.9; $p < 0.0001$].
7. With *FVL* 1691 GA, 70.1% of PLs are induced deliveries, which are statistically significantly greater than in patients with normal genotype [RR 2.8; 95%CI, 1.5–5.5; $p = 0.0014$].

A summary report on the clinical manifestation of *FVL* 1691 GA as thrombotic events and pregnancy complications is presented in **Table 1**.

Variable	Relative risk (RR)	95% confidence interval (95% CI)	P-value
VTEC outside of pregnancy in asymptomatic women	9.3	4.7–18.5	<0.0001
With CHC	9.2	3.9–21.9	<0.0001
VTEC during pregnancy in asymptomatic women	4.7	1.5–14.7	0.0069
Fetal growth restriction	3.0	2.3–41	<0.0001
Preeclampsia	3.7	2.7–4.0	<0.0001
Severe preeclampsia	5.2	2.4–11.4	<0.0001
Early reproductive losses	3.0	2.5–3.5	<0.0001
Non-developing pregnancy	9.2	6.8–12.6	<0.0001
Preterm labor	4.2	2.9–6.9	<0.0001
Induced labor	2.8	1.5–5.5	0.0014

A tabular report on the association of *FVL* 1691 GA with the risk of VTEC development and gestational complications.

Table 1. The relative risk of VTEC and pregnancy complications with ***FVL*** 1691 GA genotype.

The obtained data are consistent with the results of previously published meta-analyses and clinical recommendations [17–19, 21]. Nevertheless, despite an associative, statistically significant relation between *FVL* 1691 GA and the risk of VTEC development and pregnancy complications, up to now there are no international recommendations for the prevention of pregnancy complications in *FVL* 1691 GA patients. In order to determine a universal laboratory marker for possible ill-being, both thrombotic and gestational, we have attempted to examine the laboratory phenotype of the studied mutation as APC resistance, whose magnitude actually determines the tendency to thrombosis.

4. Relation between APC resistance in *FVL* 1691 AG patients and VTEC and pregnancy complications

The analysis of the NR, characterizing the degree of APC resistance, based on the genotype (*FVL* 1691 GA or *FVL* 1691 GG), showed that the APC resistance NR value median with normal genotype fluctuated at the study time points from 1.0 to 0.86 [95% CI 1.2–0.8]. At the same time, in pregnant women with *FVL* 1691 GA genotype, regardless of the pregnancy course, this value was significantly lower ($p < 0.0001$)—from 0.53 to 0.48 [95% CI 0.55–0.43] (**Table 2**).

According to the received data, the APC resistance NR value tends to decrease throughout pregnancy in both groups analyzed, regardless of the presence of a pathological allele. The nadir is at 28 weeks of gestation, when there is decrease of interstitial cytotrophoblast-invasive potencies, and gestational changes in the myometrial and endometrial segment of radial arteries of the uteroplacental area are completed. Further growth of placenta and fetus directly depends on the adequate remodeling of radial arteries and uteroplacental-fetal blood flow formation [10, 36].

Group	Statistics	Control endpoints of the study							
		7–8 weeks	**12–13 weeks**	**18–19 weeks**	**22–23 weeks**	**27–28 weeks**	**32–33 weeks**	**36–37 weeks**	**Postpartum**
FVL 1691 GG	Me (95% CI)	1	0.95	0.9	0.88	0.86	0.86	0.88	0.9
		0.9–1.2	0.9–1.0	0.85–0.95	0.8–0.9	0.85–0.90	0.85–0.88	0.85–0.90	0.89–0.91
	25th–75th	0.9–1.05	0.9–1.0	0.85–1.0	0.8–0.95	0.83–0.90	0.8–0.90	0.8–0.95	0.8–0.91
FVL 1691 AG	Me (95% CI)	0.53	0.51	0.51	0.51	0.48	0.49	0.51	0.5
		0.52–0.55	0.50–0.53	0.50–0.53	0.44–0.51	0.43–0.51	0.48–0.50	0.5–0.52	0.48–0.51
	25th–75th	0.47–0.56	0.47–0.54	0.45–0.54	0.44–0.54	0.44–0.52	0.45–0.52	0.5–0.52	0.46–0.53
Mann-Whitney U		0	0	0	0	0	3	26	36
Test statistic Z		17.138	15.133	14.899	15.596	14.807	15.623	14.189	11.377
Two-tailed probability (p)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. APC resistance (NR value) depending on the presence of a pathological allele (***FVL*** 1691 AG or GG) in the groups at different gestational ages and 2–3 days postpartum.

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With *FVL* 1691 GG genotype, the decrease in APC resistance NR value by 28 weeks in this study was 14.0% ($p < 0.0001$); with the heterozygous variant (*FVL* 1691 AG), it is 9.4% ($p < 0.0001$). Closer to the due date, there was an insignificant increase in the NR value: by 2.3% in the control group ($p = 0.1767$) and by 4.1% in *FVL* 1691 AG carriers ($p = 0.1265$) (**Figure 6**).

Here and below, the median is a marker; values corresponding to 95% confidence interval are the lower and upper vertical bars.

We conducted a study of APC resistance in 17 patients, whose pregnancy was complicated by vein thrombosis of the lower extremities. The NR value median showing the degree of APC resistance and preceding the episode of phlebothrombosis in the first trimester (7–8 weeks) was 0.49 [95%CI, 0.43–0.49]; in the third trimester (32 weeks), it was 0.48 [95%CI, 0.46–0.49]; 2–3 days postpartum, it was 0.44 [95%CI, 0.43–0.48], thus being statistically significantly lower than in the group with a normal pregnancy course (**Figure 7**).

Here and below, the median is a marker; values corresponding to 95% CI are the lower and upper vertical bars inside the "box"; the "box" is interquartile range between 25th and 75th percentiles; mustache is values corresponding to 2.5th and 97.5th percentiles; free elements are outliers.

At the next step, the APC resistance was analyzed depending on the course of pregnancy. In most cases in *FVL* 1691 GA patients with APC resistance between 0.58 and 0.5, pregnancy proceeded normally and ended with due date delivery. Moderate and severe PE were characterized by APC resistance between 0.48 and 0.43; with FGR the values were between 0.49 and 0.45. It should be noted that the increase in APC resistance in these outcomes was registered in 7–8 weeks of gestation already, when chorionic blood exchange develops and the first portions of uteroplacental artery blood enter the intervillous space [37, 38] (**Table 3**).

A more detailed analysis showed that the peak of APC resistance in the setting of FGR was at 18–19 weeks of gestation 6.2% ($p = 0.0239$); in the setting of PE, it was at 22–23 weeks 8.5% ($p < 0.0001$). In normal pregnancy course, the peak of APC resistance was at 28–29 weeks of gestation—8.9% ($p < 0.0001$) (**Figure 8**).

In order to predict placenta-mediated conditions with the APC resistance, we used the ROC analysis, which determined the threshold value of the predictor and the gestational age when the APC resistance has maximum chances to predict PE and FGR development. In this study, the following criteria for selecting the cutoff value have been defined: method sensitivity is ≥80%, the maximum total sensitivity and specificity of the diagnostic value. We also calculated the accuracy of the method (test effectiveness), which shows how many results were predicted correctly using this research method.

Table 4 summarizes the results of NR value ROC analysis assessing APC resistance at different time points as a predictor for the development of PE and FGR.

In accordance with the obtained results, we have determined the optimal cutoff for the APC resistance NR value in *FVL* 1691 AG patients for predicting PE and FGR, which was ≤0.49 for all studied gestational ages. The area under the ROC curve (AUC) at 8, 12, and 18 weeks of gestation proved high prognostic strength and clinical significance of this laboratory marker

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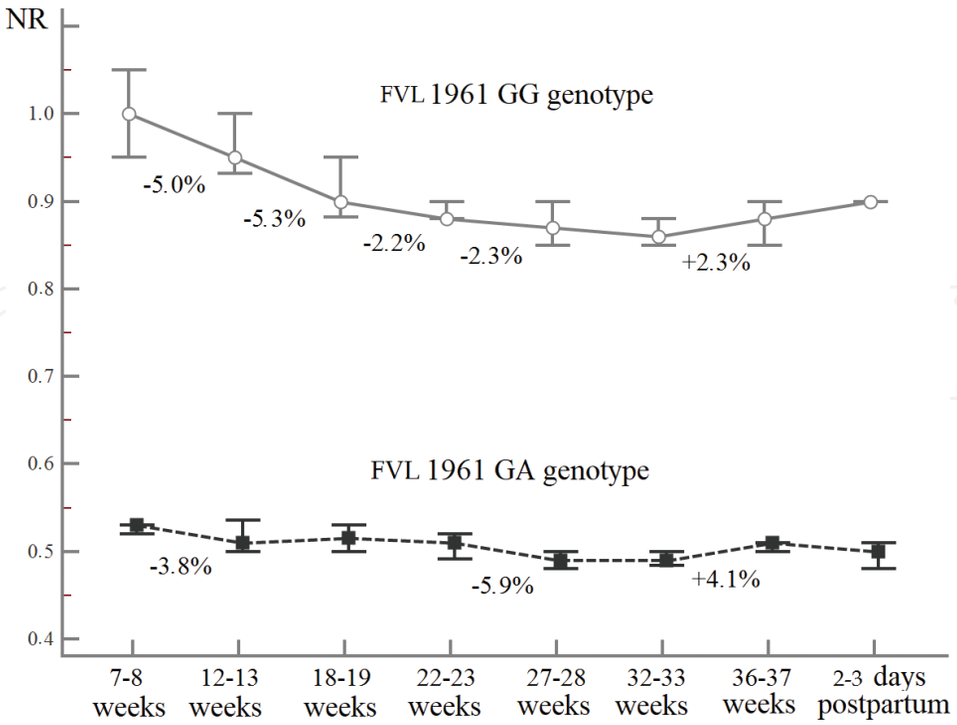

Figure 6. APC resistance depending on the presence of a pathological allele (*FVL* 1691 AG or GG) in the groups at different gestational ages and 2–3 days postpartum.

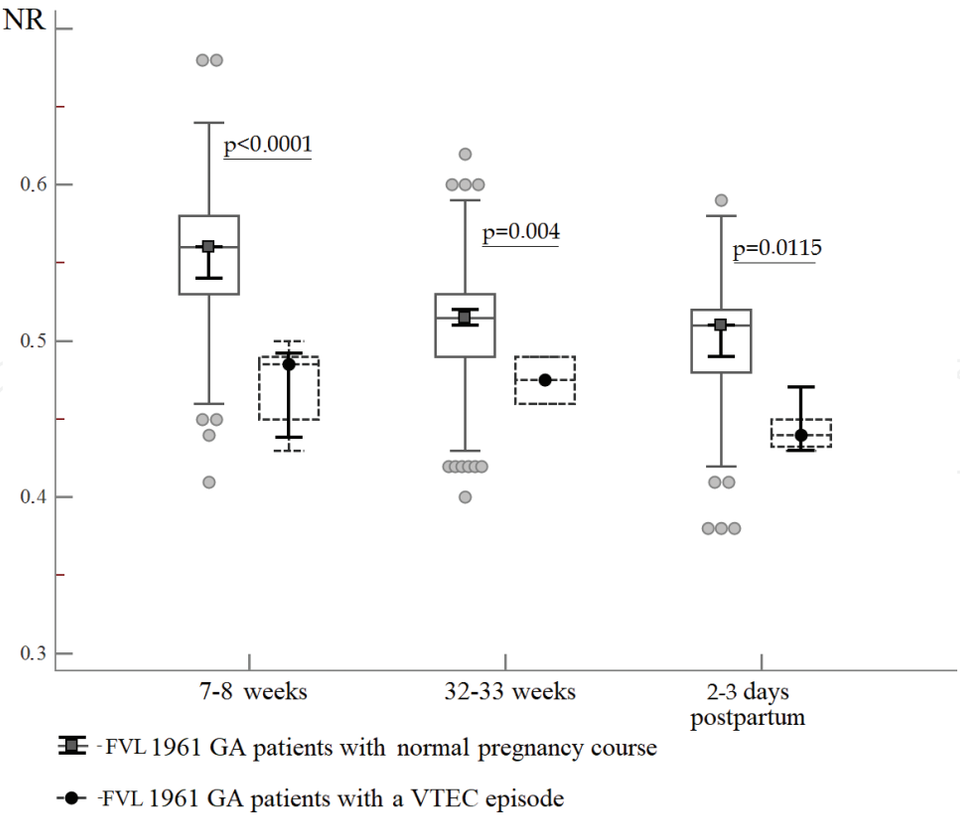

Figure 7. Values of APC resistance at the time points preceding thrombotic events in *FVL* 1691 GA patients.

Group	Statistics	Study points							
		7–8 weeks	12–13 weeks	18–19 weeks	22–23 weeks	27–28 weeks	32–33 weeks	36–37 weeks	Postpartum
NC n = 185 (1)	Me (95% CI)	0.56	0.54	0.54	0.53	0.51	0.51	0.51	0.51
		0.54–0.56	0.53–0.55	0.53–0.55	0.52–0.53	0.51–0.53	0.51–0.52	0.51–0.52	0.49–0.51
	25–75‰	0.53–0.58	0.51–0.58	0.52–0.55	0.51–0.54	0.50–0.53	0.50–0.53	0.50–0.52	0.48–0.52
PE n = 45 (2)	Me (95% CI)	0.47	0.45	0.45	0.43	0.43	0.44	0.49	0.51
		0.44–0.47	0.44–0.48	0.43–0.47	0.41–0.45	0.42–0.44	0.42–0.45	0.48–0.50	0.49–0.52
	25–75‰	0.44–0.48	0.43–0.48	0.43–0.48	0.41–0.45	0.41–0.45	0.41–0.46	0.47–0.50	0.48–0.52
FGR n = 58 (3)	Me (95% CI)	0.48	0.47	0.45	0.45	0.45	0.46	0.5	0.5
		0.47–0.48	0.46–0.48	0.43–0.46	0.44–0.45	0.42–0.46	0.45–0.47	0.48–0.50	0.49–0.51
	25–75‰	0.45–0.49	0.44–0.48	0.42–0.47	0.43–0.46	0.41–0.48	0.44–0.48	0.48–0.51	0.48–0.51
Mann-Whitney U (1/2)		925	254.5	328.5	267.5	95	436.5	748	473
Test statistic Z (1/2)		8.098	7.597	7.83	6.795	8.361	7.948	5.971	0.66
Two-tailed probability (p) (1/2)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0031	0.5096
Mann-Whitney U (1/3)		1267	493.5	506	556	248	957.5	1220	909.5
Test statistic Z (1/3)		8.778	8.552	8.492	8.057	8.677	7.052	5.267	0.545
Two-tailed probability (p) (1/3)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0611	0.5857

Abbreviations: NC, normal course; PE, preeclampsia; FGR, fetal growth restriction.

Table 3. APC resistance (NR value), depending on the course of pregnancy at different gestational ages and 2–3 days postpartum in ***FVL*** 1691 AG patients.

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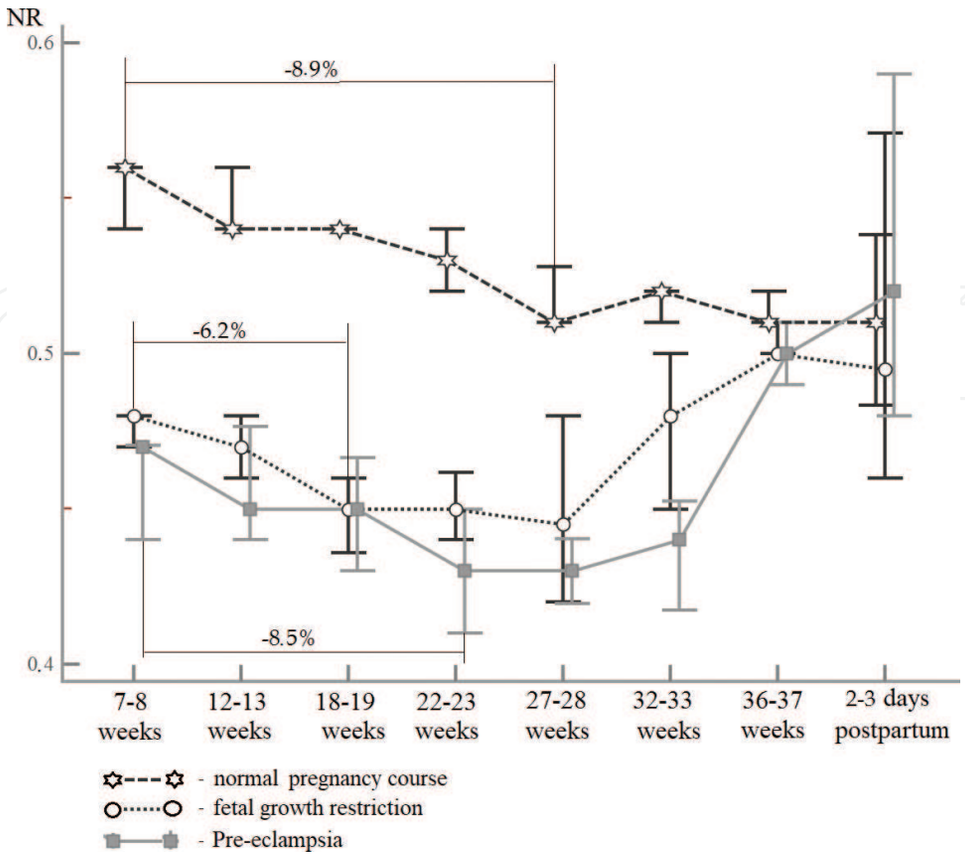

Figure 8. APC resistance in FVL (1691) GA patients with or without pregnancy complications.

for PE and FGR. The best AUC values (0.839 (95% CI [0.776–0.896], $p < 0.0001$) and 0.867 (95% CI [0.806–0.928], $p < 0.0001$) for PE and FGR, respectively) and the accuracy of the method (PE 86.2% and FGR 85.4%) were at 7–8 weeks (**Table 4**). ROC curves with maximum test effectiveness are shown in **Figure 9**.

In 25 *FVL* 1691 GA patients with embryo death before 12 weeks of gestation, APC resistance was also assessed. In all cases, the embryo death was registered at 8–9 weeks of gestation. NR median of APC resistance at 7–8 weeks of gestation in this group was 0.48 (95% CI, 0.41–0.49) and was statistically significantly lower than with further prolongation of pregnancy with a normal outcome—Me 0.56 (95% CI, 0.54–0.56, $p < 0.0001$) (**Figure 10**).

A critical disorder of uteroplacental area perfusion leads to decompensation of the placenta and, as a rule, is a reason for labor induction. We analyzed APC resistance in 29 women, whose pregnancy ended with indicated preterm birth. In 6 (20.7% out of 29) cases, preterm birth was due to decompensation of the intrauterine fetal condition, in 7 cases (24.1% out of 29), it was due to severe preeclampsia, and 16 (55.2% out of 29) cases were premature detachment of normally situated placenta.

Moreover, APC resistance at the time points before preterm birth was assessed, median NR of which had the lowest values (22–23 weeks 0.41 [95%CI, 0.40–0.43], 27–28 weeks, Me 0.42 [95%CI, 0.41–0.43]) in comparison with the same values at the studied time points with the development of PE and FGR (**Figure 11**).

Preeclampsia							
Statistical values	**8 weeks n = 45**	**12 weeks n = 45**	**18 weeks n = 45**	**22 weeks n = 45**	**28 weeks n = 43**	**32 weeks n = 40**	**37 weeks n = 38**
APC resistance NR value cutoff	≤0.49	≤0.48	≤0.49	≤0.48	≤0.48	≤0.49	≤0.49
Sensitivity	95.45	86.96	95.65	95.45	95.45	87.50	60.00
Specificity	66.38	69.64	66.02	68.93	66.31	64.91	66.67
The area under the ROC curve (AUC)	0.839	0.802	0.836	0.799	0.795	0.755	0.666
95% CI for AUC	0.767–0.896	0.716–0.900	0.764–0.907	0.749–0.830	0.738–0.831	0.679–0.815	0.530–0.802
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0170
Accuracy of the test %	86.2	78.3	84.4	76.7	76.1	70.1	68.7
Fetal growth restriction							
Statistical values	8 weeks n = 58	12 weeks n = 58	18 weeks n = 58	22 weeks n = 57	28 weeks n = 56	32 weeks n = 52	37 weeks n = 49
APC resistance NR value cutoff	≤0.49	≤0.49	≤0.49	≤0.49	≤0.49	≤0.49	≤0.49
Sensitivity	96.77	97.06	96.67	85.29	90.00	93.33	61.06
Specificity	78.95	63.37	71.30	73.53	53.85	40.74	64.57
The area under the ROC curve (AUC)	0.867	0.815	0.805	0.766	0.769	0.679	0.651
95% CI for AUC	0.806–0.928	0.746–0.884	0.723–0.887	0.678–0.854	0.679–0.859	0.580–0.779	0.515–0.762
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0180
Accuracy of the test %	85.4	78.6	82.7	79.2	72.3	64.2	63.1

Table 4. Results of ROC analysis in predicting PE and FGR at different gestational ages, using the APC resistance NR value in ***FVL*** 1691 AG patients as a marker.

The data we obtained, which indicate the relation between the APC resistance degree and the severity of the placenta-mediated conditions, bring us back to the second wave of cytotrophoblast invasion (18–28 weeks), whose quality depends on the completeness of myometrial radial arteries remodeling and intervillous space increase. Evidently, maternal coagulation disorder, which is also due to increased APC resistance, can lead to blood stasis, thrombosis on the

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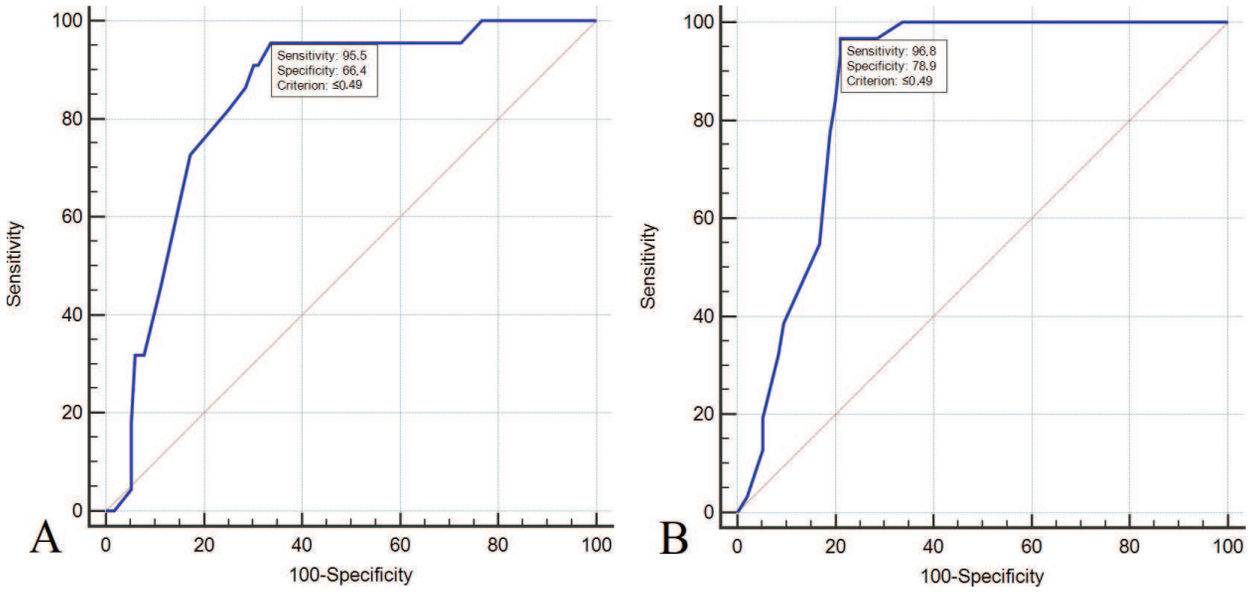

Figure 9. ROC curve for predicting the development of the placenta-mediated pregnancy complications based on the level of APC resistance in *FVL* 1691 GA patients. (A) The relation between APC resistance at 7–8 weeks of gestation and the development of PE. (B) The relation between APC resistance NR value at 7–8 weeks of gestation and the development of FGR.

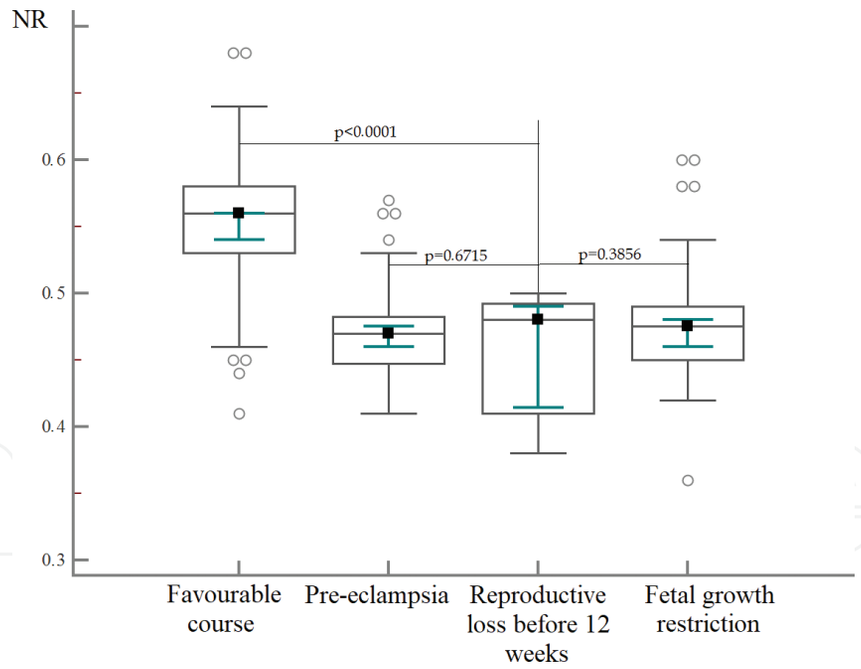

Figure 10. Median NR of APC resistance at 7–8 weeks of gestation in *FVL* 1691 GA patients depending on pregnancy outcomes.

surface of the syncytiotrophoblast microvilli, ischemia, damage, and invasive ability disorder. Clinically, this process can manifest itself either in ischemic placental disease [39] with FGR and PE or with the induction of microthrombosis from the area of destroyed microvilli and thrombosis increase in the intervillous space, followed by hematoma and placental abruption.

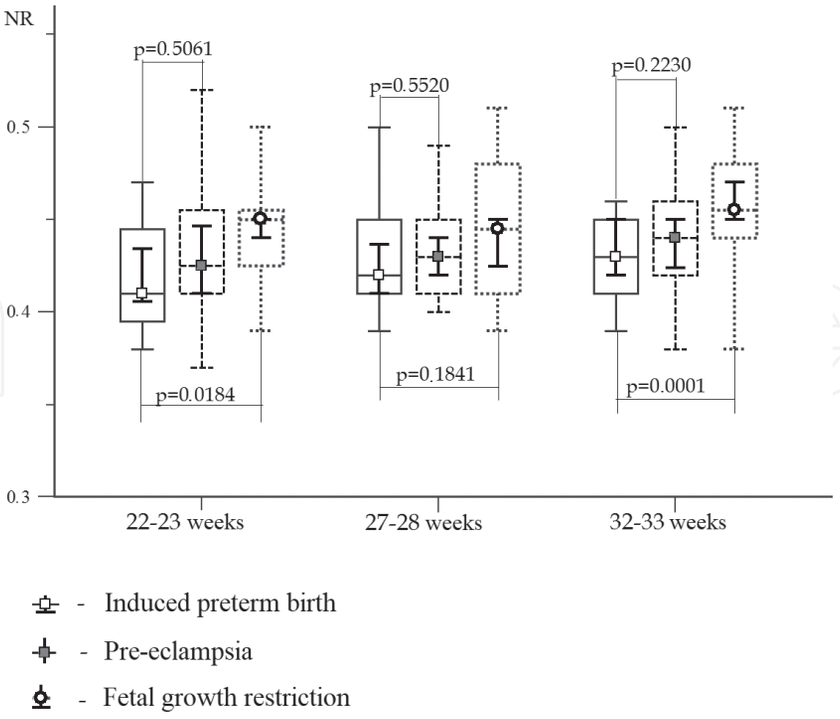

Figure 11. Values of APC resistance at gestational ages before the induced preterm birth in *FVL* 1691 GA patients.

Of course, APC resistance value cannot be considered the only and main factor of pathological processes. But its role in the development of placenta-mediated pregnancy complications is undeniable, which is confirmed by our study.

5. Conclusion

Heterogeneous carriage of FVL 1691 GA genotype, according to numerous studies, is associated with both thrombosis and pregnancy complications [17–19, 21, 40, 41]. In the study, *FVL* 1691 GA genotype clinical manifestation was close to that presented in the literature. In particular, outside of pregnancy the risk of developing VTEC increased 9.3-fold and 9.2-fold with CHC administration. At the same time, the risk of early reproductive losses increased 3-fold, 70.5% of which were missed abortions with embryo death at 8–9 weeks; the risk of developing preeclampsia increased 3.7-fold. There has been a 3-fold increase in the risk of fetal growth restriction, a 4.2-fold increase in preterm birth risk, and a 2.8-fold increase in indicated preterm delivery risk.

Despite the proven association of thrombosis and/or obstetric complications with FVL, the need for heparin prophylaxis remains questionable for such patients, as this mutation is not always clinically apparent. In our opinion, when making a decision on the use of anticoagulants during pregnancy, an additional criterion, APC resistance, can be considered, whose presence and degree, as are known, determine the tendency to thrombosis. We came to this conclusion after analyzing the frequency of clinically significant complications in pregnant women, with the normalized ratio values of APC resistance within the ranges of ≤0.49 to ≥0.50.

Thus, the presented data give grounds for considering the level of APC resistance as an objective laboratory criterion that allows not only stratifying patients into risk groups for thrombotic and pregnancy complications but also recommending antenatal heparin prophylaxis for FVL patients from the standpoint of personalized medicine.

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