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The Main Causes of Thrombotic Events in Carriage of Leiden Mutation in Women of Reproductive Age

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Abstract

The article defines the role of a combination of several acquired thrombogenic risk factors with Activated Protein C (APC) resistance which is due to carriage of FVL (1691)GA genotype to develop Venous Thromboembolic Complications (VTEC). In this regard, a prospective clinical study of 1100 women of reproductive age was conducted. The patients were divided into two groups. The main group–500 patients with FVL (1691)GA genotype, and the control group–600 women with FVL(1691)GG genotype. Based on the findings of the conducted study, it was concluded that carriage of FVL (1691)GA genotype is associated with VTEC development compared to the normozygous genotype. The highest incidence of primary thrombotic events was identified on the background of combined hormonal contraceptive administration. In the event of VTEC, carriers of Factor V Leiden (FVL (1691)GA) mutation often suffer from hypertensive disorders, varicose disease of the lower extremities, overweight and/or combinations of these pathology types. It was defined that in all cases of thrombosis episode, the value of APC resistance was ≤ 0.49 according to Normalized Ratio (NR), while episodes of VTEC were not observed in NR ≥ 0.5.

Keywords: Factor V leiden mutation; FVL (1691) GA genotype; APC resistance; Thromboembolic complications

Introduction

Venous Thrombo Embolic Complications (VTEC) which include Deep Vein Thrombosis (DVT), subcutaneous vein thrombosis and Pulmonary Embolism (PE), are interdisciplinary in nature and remain one of the important problems of clinical medicine. The value of VTEC is due to their high potential risk to health and life of patients [1,2]. It is shown that the incidence of venous thromboembolic complications is 1-1.5 cases per a thousand inhabitants annually with the frequency of PE up to 60 episodes per 100 000 population in the general population [3,4]. There is a wide range of permanent (uncontrollable) and temporary (relatively controllable) risk factors of VTEC [5]. Carriage of FV Leiden mutation [FVLG (1691)A] is traditionally described as permanent, genetically determined risk factor of thrombotic events [6,7]. However, some authors consider FVL(1691)GA genotype of Leiden mutation as low-risk thrombophilia and FVL(1691)AA genotype as high-risk thrombophilia [8-11].

In the available recommendations on the risks of Venous Thromboembolic Complications (VTEC), the meaning of this division is ambiguous due to the wide confidence intervals of thrombosis risk assessments [12-17]. Divergence of expert opinion is primarily explained by the fact that thrombosis risk in the presence of genetic thrombophilia may vary depending on the impact of additional uncontrollable and relatively controllable risk factors.

Uncontrollable risk factors include age, family and personal thrombotic history, carriage of genetic thrombophilia, not 0 blood group, systemic symptoms of angiodisplasia and many others that cannot be corrected and accompany patients for life [18,19]. Relatively controllable and temporary risk factors are more numerous and can be caused by lifestyle (bad health habits, hypodynamia, distress in mental and physical overload), individual characteristics (pregnancy), disease or pathological condition (diabetes, obesity, atherosclerosis, hypertension, heart rhythm disorders) and iatrogenesis (surgery, administration of certain medicine) [20-24]. Taking the above mentioned data into consideration, it is unclear why in clinical practice laboratory phenotype of FVL(1691)GA mutation–FVa resistance to activated protein C (APC resistance), the value of which determines the tendency to intravascular clot formation in the patients, is not taken into account when predicting the development of thrombotic events. The unclear opinion of the researchers about the importance of heterozygous carriage of FVL(1961)GA mutation, both independent and in combination with known temporary risk factors of VTEC, the lack of data on the role of laboratory phenotype in the form of APC resistance in the implementation of thrombotic events determined the purpose of this work.

Study objective

To determine the value of the combination of acquired thrombogenic risk factors with primary APC resistance which is due to heterozygous carriage of FVL(1691)GA mutation for the implementation of the tendency to develop venous thromboembolic complications.

Materials and Methods

According to the target goal, a prospective clinical cohort study of 1100 women of reproductive age was conducted, the course and outcomes of 2707 pregnancies were analyzed on the basis of clinical

units of FSBEI HE ASMU (Federal State Budgetary Educational Institution of Higher Education Altai State Medical University) of Ministry of Healthcare of the Russian Federation from 2009 to 2017. Two cohorts were identified the main group-500 patients with FVL(1691)GA genotype (average age 30.2 ± 4.7 years old, the total number of completed pregnancies-1085) and the control group-600 women who were normozygous in FVL(1691)GG mutation (average age 30.3 ± 3.9 years old, the total number of completed pregnancies-1622). The groups were comparable in age ($p>0.05$) and ethnicity: the main group comprised 91.2% of Caucasian patients, the control group–89.9% ($p>0.05$).

Inclusion criteria for the main group were:

Female; carriage of FVL(1961)GA mutation; age from 18 to 45 years old.

Inclusion criteria for the control group were the same as for the main group but the patients were not carriers of FVL(1691)GA и FVL(1691)AA genes.

Exclusion criteria from the study groups were:

Autoimmune diseases including antiphospholipid syndrome; the presence of chromosomal aberrations in the patients.

The study was approved by the local Ethics Committee of FSBEI HE ASMU of Ministry of Healthcare of Russia (Protocol No. 5 of 25 June 2009). APC resistance level was investigated in all patients admitted under observation in determining FVL(1691)GA mutation. It should be noted that the laboratory analysis was carried out in the absence of heparin prophylaxis. APC resistance was determined using a set of reagents "Factor V-PC-test" (OOO Firma "Tekhnologiya-Standart", Russia) by the value of normalized ratio (NR). Statistical data processing was performed by the statistical software package MedCalc Version 17.9.7 (license BU556-P12YT-BBS55-YAH5M-UBE51). Variation series test for the normality of distribution was conducted using Shapiro-Wilk's W-test. Laboratory parameter data are presented as the median (Me), 95% confidence interval (95%CI) and interquartile range [25th and 75th percentiles]. Series comparison was performed using nonparametric methods. The absolute value and relative value were indicated in percent for indicators characterizing qualitative characteristics. Statistical hypothesis testing of the coincidence of observed and expected frequencies was carried out using the $\chi 2$ criterion and the exact Fisher criterion. Relative risk (RR) and 95% confidence interval (95% CI) were calculated for binary features. The critical level of difference significance (p) is defined as $p<0.05$. A logistic regression model with a step-by-step algorithm for predictor inclusion was used to analyze the relationship between one qualitative feature (VTEC/no VTEC), acting as a dependent, the resulting indicator, and a subset of quantitative and qualitative features. The results of the estimation of logistic regression equations are presented by a set of regression coefficients, the achieved significance levels for each coefficient, and the assessment of the concordant index of the actual patient's belonging to a particular group.

Findings

In the study of 500 patients carriers of FVL(1691)GA mutation during follow-up period, thrombotic events were reported in 70 women (14.0% from 500) *vs.* 9 (1.5% from 600) compared to normozygous FVL(1691)GG genotype that has a statistical significance [RR9.3; 95%Cl: 4.7-18.5; $p<0.0001$]. Deep vein thrombosis of the lower extremities was diagnosed in all 9 cases of thrombosis in the control group. DVT was defined in the nonpregnant state in 6 patients, it was induced by administration of combined hormonal contraceptives (CHC) in 5 cases and by conducting locked intramedullary osteosynthesis in tibial diaphyseal fracture (2nd postoperative day) in 1 case. In 3 cases, DVT was registered during pregnancy: 1 episode-in the first trimester, 2-in the postpartum period (3rd and 6th days).

1. The association of the FVL (1961) GA genotype with thromboembolic events in women of reproductive age.

In 70 patients with FVL(1961)GA mutation in different periods of their life, 98 episodes of thrombotic events were recorded: 45 (64.3% of 70) had a single episode of VTEC; 22 (31.4% of 70)-1 case of retrombosis; 3 (4.3% of 70) observations were with 2 cases of retrombosis each (Table 1).

Clinical setting	Total thrombosis, n=98			
	Primary (acute)		Rethrombosis	
	Absolute Number	Proportion in Structure	Absolute Number	Proportion in Structure
CHC administration	41	58.60%	0	0%
Pregnancy	12	17.10%	21	75.00%
Postoperative period	9	12.90%	4	14.30%
Idiopathic	7	10.00%	0	0%
ARVI in history	1	1.40%	3	10.70%
Total	70	100%	28	100%
Abbreviations: CHC-combined hormonal contraceptives, ARVI-acute respiratory viral infection				

Table 1: Venous thrombosis structure in carriage of FVL(1961)GA mutation in women of reproductive age depending on the additional risk factor influence.

In total, thrombotic events occurred in 58 (11.6% of 500) patients in the nonpregnant state: a single episode of VTEC in 51 (87.9% of 58); retrombosis in 7 (12.1% of 58) women. In the carriers of FVL(1961)GA mutation, 41 episodes of primary thrombosis during CHC

administration were recorded. The process was localized in the deep veins of the lower leg in 30 cases, in 10 cases it was in the region of the iliac-femoral-popliteal segment, and 1 case was diagnosed with PE (pulmonary embolism). 2 patients were implanted with a cava filter during treatment. It should be noted that CHC were administered to the patients solely for the purpose of contraception. Rethrombosis episodes during CHC administration were not observed. Taking into account that estrogen-containing drugs are widely used in gynaecological practice (contraception, menopausal hormone therapy, cycles of ovulation induction, etc.), we calculated the risk of VTEC during CHC administration in the carriers of FVL(1961)GA mutation, which in our case amounted to 9.2 [RR9.2; 95% Cl: 3.9-21.9; p<0.0001]. Clinical implementation of the carriage of FVL(1961)GA mutation in the acute thrombosis form occurred after gynecological operations carried out by laparoscopic access (15.3% of 59 gynecological operations) in 9 women (12.9% of 70 thrombosis episodes). All 9 patients had additional risk factors: varicose disease of the lower extremities, BMI ≥ 25; episodes of reproductive losses in the history that, according to the data totality, allowed them to be included in the group of moderate risk of VTEC after surgery [25,26]. It should be noted that these patients did not get heparin prophylaxis in the postoperative period. We calculated the risk of VTEC development in patients with carriage of FVL(1961)GA mutation after gynecological operations performed by laparoscopic access, which in our case amounted to-9.6 [RR9.6; 95% Cl: 0.54-171.5; p=0.11243]-statistical significance was not determined.

The cause of primary thrombosis could not be established in 7 cases (10% of 70 episodes of acute thrombosis or 1.4% of 500 women) (determined only in the group with FVL(1961)GA mutation). All patients with idiopathic phlebothrombosis had an episode of retrombosis in the setting of ARVI (n=3) or after surgery (n=4) during the first year. Viral infection, as a factor that induces primary thrombosis, was defined in one case. Primary phlebothrombosis occurred on the background of pregnancy, was registered in 12 patients (17.1% of 70 episodes of primary thrombosis). Thrombosis of the jugular vein was diagnosed on the right in 1 case; in 9 cases thrombosis settled in the deep veins of the lower leg and 2 patients were identified with iliofemoral thrombosis. Episodes of rethrombosis during pregnancy were identified in 21 patients. In these patients their primary episode of thrombosis in personal history was realized on the background of CHC (n=10), after surgical intervention (n=1) and on the background of gestation (n=10). It should be noted that, despite the presence of an associative, statistically significant relationship between the carriage of FVL(1961)GA mutation and the risk of VTEC, it is not always possible to predict the probability degree of thrombotic event implementation under the influence of additional, temporary risk factors. We have considered the laboratory phenotype of the mutation under study-APC resistance, the value of which, as noted above, determines the tendency to intravascular clot formation, in order to determine the specific marker of possible thrombotic danger.

2. The association of the APC resistance in the carriers of FVL (1961) GA genotype with thromboembolic events in women of reproductive age.

In the framework of the present study the median of APC resistance was determined (according to NR) during thrombotic events in 27 non-pregnant patients, the carriers of the FVL(1961)GA mutation-0.44 [95%Cl: 0.41-0.49], which differed significantly (p=0.001) from the same value in the group of women without VTEC episode-0.56 [95%Cl: 0.53-0.57] and in patients with FVL(1691)GG genotype-1.00 [95%Cl: 0.85-1.4] (p<0.001) (Figure 1).

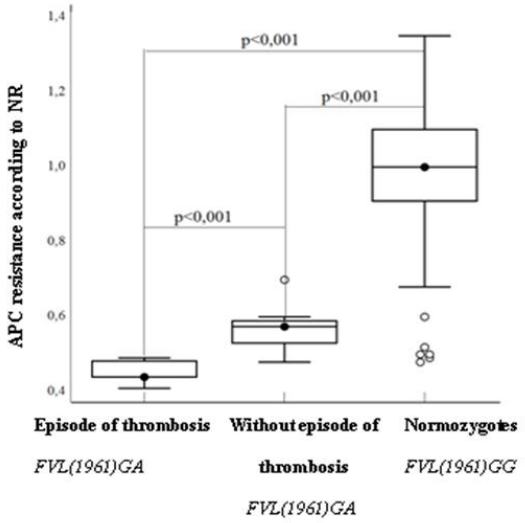

Figure 1: The value of APC resistance in acute thrombosis phase, in the absence of the carriers of FVL(1961)GA mutation and in FVL(1691)GG genotype in the nonpregnant state.

The median is a marker; the "box" is the interquartile range 25‰ to 75‰; the "whiskers" are the values corresponding to 2.5 and 97.5 percentiles; the free elements are the outlying cases. Here we also considered thrombotic history of the first-degree relatives up to 50 years old, the age of patients and somatic background, contributing to the implementation of carriage of FVL(1961)GA mutation into thrombotic events. Data analysis of the family thrombotic history demonstrated that in the presence of personal history of thrombosis, VTEC in the first-degree relatives were reported at 37.1% (26 of 70) of cases *vs.* of 26.0% (112 of 430) of cases in the group of women without burdened personal history of thrombosis in carriage of FVL(1961)GA mutation [RR1.4; 95%Cl: 1.1-2.0; p=0.0431]. The age of women older than 35 years, along with the family thrombotic history, is also defined as a statistically significant risk factor for the implementation of VTEC [RR1.8; 95%Cl: 1.2-2.6; p=0.0041] (Table 2).

Further, the relationship of comorbidity of the isolated (Table 2) states with the development of VTEC in the carriers of FVL(1961)GA mutation was studied. Somatically healthy women with episodes of VTEC in personal history in our study were not determined. Comorbid states in FVL(1961)GA mutation were identified in 95.7% (67 of 70) patients with thrombosis in personal history and in 21.4% (92 out of 430) in their absence [RR4.5; 95% Cl: 3.7-5.4; p<0.0001]. As a rule, it turned out to be a combination of hypertensive disorders and/or varicose disease of the lower extremities with overweight. A multiple logistic regression analysis was performed to rank the selected predictors in carriage of FVL(1961)GA mutation according to the connection degree with the implementation of VTEC in the nonpregnant state.

Nosological entity (code of heading according to ICD)	Personal history of thrombosis n=70		Without personal history of thrombosis n=430		Statistics		
	Absolute Number	Proportion in Structure	Absolute Number	Proportion in Structure	p	RR	95%CL
Hypertensive heart disease (I11.9)	29	41.40%	76	17.70%	<0.0001	2.3	1.6-3.3
LEVVD (I83.9)	38	54.30%	135	31.40%	0.0001	1.7	1.3-2.2
Chronic inflammatory diseases of the respiratory system (J00-J99)	38	54.30%	142	33.00%	0.0001	1.6	1.2-2.1
Obesity and other forms of nutritional redundancy (BMI ≥25) (E66)	39	55.70%	155	36.00%	0.0005	1.5	1.2-1.9
Age older than 35 years old	23	32.90%	80	18.60%	0.0041	1.8	1.2-2.6
Notes: LEVVD – Lower extremity varicose vein disease, BMI – body mass index							

Table 2: Somatic status and age of the patients with a personal history of thrombosis in carriage of FVL(1961)GA mutation.

Several models were obtained in different clinical situations. The models were formed due to step-by-step inclusion of predictor variables, which were selected as 5 risk factors, statistically significantly more often determined in the carriers of FVL(1961)GA mutation with a personal history of thrombosis: hypertensive conditions, varicose disease of the lower extremities, BMI ≥ 25, chronic inflammatory diseases of the respiratory system; family thrombotic history of the first-degree relatives up to 50 years old, and age over 35 years old. The categorical response variable is a fact of VTEC (represented as a binary value: 1-yes; 0-no). (Table 3) shows the models with concordant values of more than 80%.

Variable	Coefficient(β)	Standard error	p-value	Correlated odds ratio (OR)	95% confidence interval (95% CI)
The carriers of FVL(1961)GA in the nonpregnant state					
Free term	-2.6436				
BMI≥25	1.01778	0.2861	0.0004	2.767	1.5794-4.8479
Age ≥35	0.65248	0.31067	0.0357	1.9203	1.0445-3.5304
The percentage of concordance 88.40 %					
Chi-squared -22.895; P=0.0018; AUC=0.65; 95%CI 0.61-0.70					
The carriers of FVL(1961)GA during CHC administration					
Free term	-2.8856				
CHC administration	1.34137	0.33019	<0.0001	3.8243	2.0021-7.3049
BMI≥25	1.00724	0.2931	0.0006	2.738	1.5415-4.8633
Age ≥35	0.56429	0.3217	0.0794	1.7582	0.9359-3.3030
The percentage of concordance 92.40 %					
Chi-squared - 33.057; P< 0.0001; AUC=0.71; 95%CI 0.69-0.73					

Table 3: Logit models with risk factors for the implementation of acute (primary) VTEC in carriage of FVL(1961)GA mutation in various clinical setting.

This analysis allowed to identify the most independent and permanent risk factors of the implementation of VTEC in non-pregnant carriers of FVL(1961)GA, which included age older than 35 years old and overweight. The characteristics of the model change when exposed to an additional time risk factor. For example, the inclusion of the predictor "CHC administration" in the presented model changes not only the regression coefficients for the predictors, but also the quality of the model prediction.

Discussion

The study showed that the carriage of FVL(1961)GA mutation in women of reproductive age is associated with VTE both in the

nonpregnant state and during gestation, and is implemented under the influence of additional risk factors and/or on the background of somatic pathology. In non-pregnant women, in 86.2% (50 of 58) of the cases, primary thrombosis was manifested due to iatrogenesis (CHC administration-41 and surgery-9). It is commonly known that the administration of estrogen-containing CHC is absolutely contraindicated in carriage of FVL(1961)GA [10,27]. However, 63 patients of the study group were offered this planned contraception type which was clinically realized by thrombotic events in 65.1% (41 of 63) cases, thrombosis occurred in the first 3 months of contraceptive administration. Surgical intervention initiated primary (acute) thrombosis in 9 patients with FVL(1961)GA mutation, who at the moment of examination were identified as a group of moderate risk of VTEC in the postoperative period [28], that suggests prescribing of LMWH in preventive doses and in terms recommended by the manufacturer for patients with moderate risk [10-29]. However, none of the 9 patients received heparin prophylaxis. In accordance with the data obtained, along with the main factor, that induce the implementation of thrombotic events in carriers of FVL(1961)GA, an important role belongs to background somatic pathology. At the same time, the dominant risk factors for the implementation of thrombotic events in the nonpregnant state are the age of over 35 years old and overweight. In our study, overweight as a risk factor is determined at a BMI ≥ 25, which does not contradict the data of literature sources [30,31]. The presented data on the structure of somatic pathology, in our opinion, are of practical interest from the point of view of modification possibility of these risk factors. For example, overweight is a controllable factor, weight correction can not only reducing the risk of VTEC almost 2 times, but also affect blood pressure, thereby further reducing the risk of thrombotic events.

The key points of this work include the above mentioned data on APC resistance in the carriage of FVL(1961)GA mutation in comparison with clinical manifestations, which were not previously considered by experts. It is important to note that in all cases of thrombosis episode the value of APC resistance was ≤ 0.49 [95% Cl: 0.41-0.49]. This laboratory marker in the case of carriage of FVL(1961)GA mutation can serve as an objective laboratory criterion for the state of thrombotic readiness, determining in the future, in conjunction with the clinical data, the need for heparin prophylaxis.

Conclusion

Obviously, the value of APC resistance can serve as an objective laboratory marker confirming the necessity in thromboprophylaxis along with the additional confounding risk factors of thrombosis implementation in FVL(1961)GA mutation.

We believe that within the framework of the preventive orientation of personalized medicine it is necessary to take into account that the heterozygous FVL(1961)GA carriage is:

1. A contraindication for prescribing of combined hormonal contraceptives [RR9.2; 95%Cl: 3.9-21.9; p<0.0001].

2. An indication to determine the degree of APC resistance manifestation.

3. An indication to conduct thromboprophylaxis after any surgery, including gynecological practice, when the value of APC resistance is at NR ≤ 0.49.

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