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INHERITED THROMBOPHILIAS IN THROMBOSIS ADVANCEMENT IN MICROVASCULAR FLAP SURGERY

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Microvascular flap surgery is a reliable method for reconstructive surgery. To avoid and foresee free flap thrombosis advancement after microvascular flap surgery, patient assessment, flawless surgical technique, and eligible perioperative care are pivotal. In this prospective observational study, we aimed to elucidate the most common inherited single nucleotide polymorphisms (SNPs) attributable to free flap thrombosis. A total of 152 patients undergoing microvascular flap surgery during the study period of 2016–2019 were analysed for five SNPs: rs6025 in Factor V Leiden (FVL) *gene, rs1799963 in Factor II* (FII) *gene, rs2066865 in Fibrinogen Gamma Chain* gene (FGG)*, rs2227589 in* SERPINC 1 *gene and rs1801133 in Methylene Tetrahydrofolate Reductase* (MTHFR) *gene. Activated protein C resistance (aPCR), prothrombin, antithrombin (AT), fibrinogen and homocysteine plasma levels were measured to determine association with the analysed SNPs and with free flap thrombosis advancement. Our preliminary results show that carriers of* FVL *mutation were associated with aPCR, as we observed significantly lower aPCR plasma levels in carriers of genotype C/T, as compared to C/C;* p *= 0.006 (CI 95%, 0.44 to 1.19). Additionally, mean fibrinogen plasma levels were higher in carriers of* FGC *gene rs2066865 genotype A/A (5.6 ± 1.81 g/l), as compared to G/A and G/G;* p *= 0.04 (CI 95%, 0.007 to 1.09);* p *= 0.004 (CI 95%, 0.48 to 2.49), respectively. The study group included 12 patients (7.9%) with free flap thrombosis. For one patient free flap thrombosis advancement might have been related to the rs6025T –* FVL *mutation with a PCR plasma level 1.21. Lower aPCR levels was associated with carriers of* FVL *rs6025 C/T and higher fibrinogen plasma levels with carriers of* FGG *rs2066865 A/A, suggesting that these genotypes might predict higher free flap thrombosis risk, but we found no significant association between analysed SNPs and free flap thrombosis advancement.*

Key words: *polymorphisms, free flap thrombosis, Leiden factor, hyperhomocisteinemia, antithrombin deficiency, fibrinogen, prothrombin gene mutation.*

INTRODUCTION

Microvascular flap surgery is a reliable method for reconstructive surgery. Preconditions for success are thoughtful patient assessment, flawless surgical technique, and eligible perioperative care.

Post-lesion damage of endothelial cells employs its procoagulant moiety combined with other factors of the classical Virchow (1845) triad. A hypercoagulable state is an obvious predictor for thrombosis in particular groups of patients, e.g., trauma, malignancy, etc. (Biben and Atmodiwirjo, 2019; Vanags *et al.*, 2020).

Regarding inherited thrombophilia, the majority of single nucleotide polymorphisms (SNPs) are associated with defects affecting anticoagulant mechanisms (Dählback, 2008). Recent evidence suggests that five SNPs (Table 1) might influence thrombosis advancement: rs6025 in the Factor V Leiden (*FVL*) gene, rs1799963 in the Factor II (*FII*) gene, rs2066865 in the Fibrinogen Gamma Chain (*FGG*) gene,

Table 1. Relationship between analysed SNPs, coded plasma proteins and effect on coagulation

SNP	Gene	Plasma proteins	Effect on coagulation
rs6025	*FVL*	aPCR	Leads to aPCR and inactivation of factor Va by aPC
rs1799963	*FII*	Thrombin	Leads to elevated prothrombin levels and an increase of production of thrombin and thrombin activable fibrinolysis inhibitor
rs2066865	*FGG*	Fibrinogen	Leads to increase in plasma fibrinogen concentration
rs2227589	*SERPINC1*	AT	Leads to AT deficiency with increase in thrombin and factor Xa activity
rs1801133	*MTHFR*	Homocysteine	Leads to reduced activity of the MTHFR enzyme and increased plasma homocysteine level, promoting inflammation and atherosclerosis

SNP, single nucleotide polymorphism; FVL, Factor V Leiden; FII, Factor II; FGG, Fibrinogen Gamma; MTHFR, methylene tetrahydrofolate reductase; AT, antithrombin; aPCR, activated protein C resistance; aPC, activated protein C

rs2227589 in the *SERPINC1* gene and rs1801133 in the Methylene Tetrahydrofolate Reductase (*MTHFR*) gene (Rees *et al*., 1995; El-Galaly *et al.*, 2013; Pathare *et al*., 2004). However, there is not much literature demonstrating associations of mentioned SNPs and occurrence of free flap thrombosis in microvascular flap surgery patients.

Therefore, the aim of our study was to analyse the most common inherited SNPs that might have an effect on free flap thrombosis advancement after microvascular flap surgery.

Factor V Leiden gene, a mutation of *FVL*, is the most widespread autosomal dominant genetic mutation (Rees *et al*., 1995; Rosendaal *et al*., 1995; Ridker *et al*., 1997). *FVL* mutation is a missense mutation in the FV gene (NM_000130.4(F5):c.1601G>A (p.Arg534Gln), rs6025 (c.1691C>T) where in position R506 arginine is replaced by glutamine (Zoller and Dahlbäck, 1994; Friedman *et al*., 2010). It is known as activated protein C resistance (aPCR), which is attributable to the *FVL* mutation in up to 95% of cases (Segers, 2007).

Factor II gene. *FII* or the prothrombin mutation (NM_000506.4(F2):c.97 G>A, rs1799963 G/A), was found to occur in 3–17 % of patients with VTE and about 1–8 % of healthy controls (Rosendaal *et al*., 1998). Carriers of the rs1799963A allele have a higher plasma prot hrombin level and 2.8-fold higher risk of VTE (Poort *et al*., 1996; Meltzer *et al*., 2010).

Fibrinogen gamma chain gene. Fibrinogen is a plasma glycoprotein synthesised by hepatocytes. Fibrinogen molecules are comprised of two sets of three polypeptide chains — A alpha; B beta; and gamma (Henschen *et al.*, 1983). The three chains are encoded by three separate genes, fibrinogen alpha (FG>A), fibrinogen beta (*FGB*), and fibrinogen gamma (*FGG*). The rare allele T of rs20066865 polymorphism (NM_000509.5(FGG):c.216 GA, rs2066865G/A) in the *FGG* gene is described as a reason for alterations in the coagulation system (Drizlionoka *et al*., 2019).

A polymorphism in *SERPINC1* gene (NM_000488.3(*SERPINC1*):c.41+141C>T, rs2227589 C/T) is recognised as causing antithrombin (AT) deficiency and is associated with the highest risk of VTE of all known inherited thrombophilias. Inherited AT deficiency is an uncommon autosomal dominant disorder. Prevalence in general population is 1 : 2000 to 1 : 5000 and is associated with a 10-fold to 20-fold increased risk of VTE (Patnaik and Moll, 2008). Most of the VTE episodes are unprovoked for those with AT deficiency (Patnaik and Moll, 2008).

Methylene Tetrahydrofolate Reductase gene, a polymorphism in *MTHFR* gene (NM_005957.4(*MTHFR*):c.665 G>A (p.Ala222Val), results in reduced activity of this enzyme (Rosenberg *et al.*, 2002). An increased level of homocysteine has been reported as a risk factor for coronary artery disease (Rozen, 1997; Stanger *et al*., 2004), especially in young/middle aged Caucasians with an increased risk for myocardial infarction (Xuan *et al*., 2011), cerebral and peripheral artery disease (Welsch *et al*., 1997).

MATERIALS AND METHODS

Subject and perioperative management. In an observational prospective case series study, we enrolled 152 adult consecutive patients who had microvascular flap surgery in the Centre of Plastic and Reconstructive Microsurgery of Latvia during 2016–2019.

All patients undergoing microvascular flap surgery were enrolled during the study period after exclusion criteria were applied. The informed consent form including the request to donate genetic material and the protocol was approved by the Latvian Central Ethics Committee (No. 1/28-11-16). All patients provided written, informed consent.

The exclusion criteria were: pregnancy, peripartum period; transfusion of allogeneic blood components, and/or coagulation factors within 72 h perioperatively, proven left ventricular failure, allogenic bone marrow transplantation, liver failure, liver transplantation, and end-stage kidney disease.

General anaesthesia (GA) was provided for all patients according to the guidelines for free flap transfer microsurgery. Peripheral nerve blocks under ultrasound guidance were performed for analgesia requirement when applicable. For those without a peripheral nerve block, continuous intravenous infusion of fentanyl 0.5–1 mcg/kg/h was administered. Fluid management, oxygen supply and transfusion manage-

ment were provided in the post-anaesthesia care unit with tight haemodynamic monitoring and followed local guidelines for microvascular flap surgery post-operative care.

During the study, a total of 152 microvascular flap surgeries were performed by highly trained specialists. The venue of free flap thrombosis (i.e., arterial, venous, or both) was assured by direct visualisation during the revision of anastomosis.

For patients on direct oral anticoagulants, medication was stopped 72 h prior to sample collection to avoid any inaccuracy in testing. For patients taking a vitamin K antagonist, medication was stopped according to the patient's international normalised ratio (INR) level, 4 to 5 d prior to sample collection. Patients with a high thrombosis risk were switched to low-molecular-weight heparin (LMWH) and use was discontinued 12 h prior to the surgery.

Each patient was genotyped to determined SNPs: rs6025 in the *FVL* gene, rs1799963 in the *FII* gene, rs2066865 in the *FGG* gene, rs2227589 in the *SERPINC1* gene and rs1801133 in the *MTHFR* gene. Plasma concentrations of aPCR, prothrombin, fibrinogen, AT and serum homocysteine were measured one time pre-operatively.

Laboratory workup and genotyping. Blood samples were drawn on the day of microsurgery prior to the induction of GA and any crystalloid infusion. All tests were processed within an hour.

To determine potential alteration in plasma protein levels associated with the analysed SNPs, the following laboratory measurements were performed: aPCR was measured using the clotting method with a Sysmex CS2100i, UK LTD (reference range ≥ 1.8). Prothrombin was measured using the clotting time method (for prothrombin added tissue thromboplastin ISI < 1.5) with a Sysmex system, CS-2100i, UK LTD (reference range 70–130%). Plasma AT was measured using a chromogenic method with a Sysmex CS-2100i, UK LTD (reference range 75–125%). Total plasma fibrinogen concentration was measured by Clauss method in citrated plasma using a STA-R COMPACT Diagnostika Stago, Asnières-sur-Seine, France (reference range 2–4 g/l). Homocysteine was measured using the immunochemical luminescence method on a ADVIA, Centaur, Siemens Healthcare GmbH, Germany (reference range 5.00–12.00 mkmol/l).

Genomic DNA was extracted using the standard phenol-chloroform extraction protocol. Extracted DNA was dissolved in water. Genotyping was performed by Taqman Pre-Designed SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA) on a Viia7 Real-Time polymerase chain reaction (PCR) system (Applied Biosystems) (Livak, 1999) according to the supplier's recommendations.

In addition to genetic and coagulation laboratory workout, patients were interviewed to register co-morbidities, particularly focusing on previous thrombotic events. We also registered demographic data, localisation of soft tissue defect, type of free flap transfer and the duration of surgery.

Statistical analysis. We compared variables with independent-sample (unpaired) t-tests using SPSS 23 Statistics software (IBM Korea, Seoul, Korea). The Kolmogorov–Smirnov test was used to check whether the variables followed a normal distribution. Normally distributed, continuous variables were presented as means ± standard deviation (M ± SD) and categorical variables as percentages (%). The frequency of alleles was tested against Hardy–Weinberg equilibrium. Odds ratios and 95% confidence intervals were calculated to evaluate factor impacts between groups. Comparisons between genotype groups were performed with Kruskal–Wallis H tests for nonparametric variables and with ANOVA for parametric variables. Pearson's and two-tailed Fisher exact tests were used for data comparison, depending on the number of cases. The Spearman's rank correlation coefficient was used where applicable. Statistical significance was assumed as two-tailed with $p < 0.05$.

RESULTS

We analysed 152 patients scheduled for microvascular flap surgery (35 female and 117 males), who met the inclusion criteria. Table 2 displays demographic and clinical variables of the studied group. History of personal or family thrombosis was observed in 5.2% and 13.8% of female and male cases, respectively. Most patients had surgery after more than 30 days post-traumatic injury and defect localisation was mostly on a lower extremity. Correlation was not observed between SNPs and metabolic disturbances and smoking.

Relationships between SNPs and coded protein plasma levels. To analyse each SNP, patients were classified by their *FVL* gene C/T, *FII* gene G/A, *FGG* gene G/A, *SERPINCI1* gene C/T and *MTHFR* gene G/A characteristics and subdivided into three groups, according to the genotype of each gene polymorphism. The genotype results of analysed SNPs were all in Hardy-Weinberg equilibrium. Table 3 shows protein plasma levels in relation to the analysed SNPs.

rs6025 *FVL* gene. 96% of the studied population had the C/C genotype of the *FVL* gene rs6025 polymorphism. We observed significantly a lower aPCR plasma level in carriers of genotype C/T, as compared to C/C; $p = 0.006$ (CI 95%, 0.44 to 1.19). None of the analysed patients had genotype T/T.

rs2066865 *FGG* gene. Analysis of the association between *FGG* gene rs2066865 polymorphism and fibrinogen plasma levels showed that mean fibrinogen plasma levels were higher in carriers of the genotype A/A, compared to G/A and G/G; $p = 0.04$ (CI 95%, 0.007 to 1.09); $p = 0.004$ (CI 95%, 0.48 to 2.49), respectively. The largest number of patients were carriers of G/G and A/G genotypes (in 60% and 34% of cases) and only 6% of the studied patients had the

Table 2. Characteristics of the studied group

n = 152	n (%)
Age, years (mean ± SD)	45.1 (14.9)
Sex, female	35 (23)
History of thrombosis	10 (5.2)
Family history of thrombosis	21(13.8)
Smoking	64 (42.1)
Metabolic disturbances[?]	15 (9.9)
Defect aetiology:	
Trauma	70 (46.1)
Recent trauma, (< 30 days)	25 (16.4)
Polytrauma	12 (7.9)
Chronic inflammation	34 (22.4)
Malignancy	30 (19.7)
Combustion	6 (3.9)
Defect localisation:	
Lower extremity	82 (53.9)
Upper extremity	36 (23.6)
Trunk	3 (2.00)
Head/orofacial	29 (19.07)
Abdomen	2 (1.3)

Data are presented as mean ± SD or number (n) and percentage (%).

*Diabetes mellitus; adiposities (BMI > 25)

A/A genotype, which was associated with a significantly higher mean fibrinogen plasma level (5.6 ± 1.81 g/l).

rs2227589 *SERPINC1* gene. Although carriers with the C/C genotype (81%) of the *SERPINC1* gene rs2227589 polymorphism had a tendency to have a lower AT plasma level, we did not observe a significant difference of AT plasma level between homo- and heterozygous allele carriers; $p = 0.09$ (CI 95%, 1.37 to 18.02).

rs1799963A in *FII* gene. As for the above gene, mean prothrombin plasma level displayed no significant difference between *FII* gene rs1799963A polymorphism genotype groups; $p = 0.8$ (CI 95%, 20.31 to 26.23 between G/G and G/A genotype carriers).

rs1801133 *MTHFR* gene. Carriers of the *MTHFR* gene rs1801133 genotype G/A and A/A presented with slightly higher plasma levels of homocysteine, as compared with carriers of genotype G/G, but this difference did not reach significance; $p = 0.07$ (CI 95%, 0.26 to 5.6) between G/G and G/A; $p = 0.26$ (CI 95%, 2.39 to 8.26) between G/G and A/A.

Case series. In Table 4, we demonstrate data for 12 patients (7.89%) of 152 who presented with free flap thrombosis. Only one of the twelve patients had all wild type alleles of analysed SNPs. The other eleven had one or a combination of two of the analysed SNPs. Polymorphism rs1801133 in the *MTHFR* gene G/A genotype was most often observed in combination or alone (Table 4). For all of those patients, the homocysteine plasma level was higher than normal, with a mean value 13.3 ± 0.74 mKmol/l. Two patients had solely a in *MTHFR* gene. One presented with free flap thrombosis in

Table 3. Determined SNPs and coded protein plasma levels

Type of SNP	Coded protein plasma levels, normal range (min.-max.)	*p*
rs6025 *FVL* (C > T)	aPCR (>1.8)	
C/C (n = 146)	2.02 ± 0.32*	*0.006
C/T (n = 6)	1.19 ± 0.17*	
T/T (n = 0)	–	
rs1799963 *FII* (G > A)	Prothrombin (70–130%)	
G/G (n = 148)	96.38 ± 20.23	NS
G/A (n = 3)	99.33 ± 15.94	
A/A (n = 1)	106.00	
rs2066865 *FGG* (G > A)	Fibrinogen (2–4 g/l)	
G/G (n = 91)	4.1 ± 1.3 #	* 0.04
G/A (n = 52)	4.6 ± 1.7 *	# 0.004
A/A (n = 9)	5.6 ± 1.8 *#	
rs2227589 *SERPINC1* (C > T)	AT (75–125%)	
C/C (n = 123)	89.56 ± 14.14	NS
C/T (n = 28)	97.89 ± 14.51	
T/T (n = 1)	92.10	
rs1801133 *MTHFR* (G > A)	Homocysteine (5.00–12.00 mkmol/lL)	
G/G (n = 81)	10.56 ± 4.05	NS
G/A (n = 61)	13.23 ± 5.55	
A/A (n = 10)	13.50 ± 5.81	

Values are presented as mean, ± SD; sig. 2- tailed $p < 0.05$. SNP, single nucleotide polymorphism; FVL, Factor V Leiden; FII, Factor II; FGG, Fibrinogen Gamma; MTHFR, methylene tetrahydrofolate reductase; AT, antithrombin; aPCR, activated protein C resistance; NS, nonsignificant; n, number; G, guanine; A , adenine; T, thymine; C, cytosine

arterial and the other in venous flow. The rs2066865 polymorphism in the *FGG* gene G/A genotype was most often detected, and associated with a higher mean fibrinogen plasma level (4.7 ± 0.35 g/l). Only one patient had a homozygote form of A/A genotype, with a fibrinogen plasma level of 4.7 g/l. None, except one, had perioperative thromboprophylaxis. Most of the patients (10 of 12, 83%) with free flap thrombosis had a defect with localisation on a lower extremity.

The first patient in Table 4 was a carrier of the *FVL* gene rs6025 polymorphism C/T genotype who presented with a markedly lower aPCR plasma level (1.21) and underwent microvascular flap surgery twice in two years. Both of these transfers were complicated by free flap thrombosis. Free flap thrombosis might have been related to the rs6025T *FVL* mutation in this case.

When comparing patients with (n = 12) and without (n = 140) free flap thrombosis, we were not able to find any specific factor or their combinations that independently affected advancement of free flap thrombosis.

DISCUSSION

In this observational case series study we evaluated the association of hereditary thrombophilia with free flap thrombosis advancement in microvascular flap surgery. We fo-

Table 4. Case series of patients with free flap thrombosis

Free flap transfer	Cause	Defect localisation	Thrombosis venue	SNP and genotype	Laboratory parameters
Scapular/ Parascapular	osteomyelitis	lower extremity	venous *	rs6025 *FVL* C/T rs2066865 in *FGG* G/A	aPCR 1.21 Fibrinogen 4.24 g/l
Medial plantar artery	osteomyelitis	lower extremity	arterial	rs2227589 in *SERPINC1* C/T rs1801133 in *MTHFR* G/A	AT 76% Homocysteine 13.3 mkmol/l
Lateral arm	trauma	lower extremity	venous	rs2066865 in *FGG* G/A	Fibrinogen 4.4 g/l
Scapular/ Parascapular	osteomyelitis	lower extremity	venous	rs1801133 in *MTHFR* G/A	Homocysteine 12.8 mkmol/l
Scapular/ Parascapular	polytrauma	lower extremity	venous	rs2066865 in *FGG* G/A	Fibrinogen 4.7 g/l
Serratus anterior muscle	trauma	lower extremity	arterial and venous	rs2066865 in *FGG* G/A rs2227589 in *SERPINC1* C/T rs1801133 in *MTHFR* G/A	Fibrinogen 5.1 g/l
Osteocutaneus	trauma	lower extremity	arterial and venous	rs2227589 in *SERPINC1* C/T rs1801133 in *MTHFR* G/A	AT 81% Homocysteine 14.1 mkmol/l
Osteocutaneus	osteomyelitis	lower extremity	arterial and venous	rs2066865 in *FGG* G/A rs1801133 in *MTHFR* G/A	Fibrinogen 5.1 g/l Homocysteine 13.2 mkmol/l
Serratus anterior muscle	osteomyelitis	lower extremity	arterial and venous	rs2227589 in *SERPINC1* C/T	AT 111.8%
Radial forearm	malignancy	orofacial	arterial	rs1801133 in *MTHFR* G/A	Homocysteine 12.2 mkmol/l
Lateral arm	trauma	upper extremity	venous	rs2066865 in *FGG* A/A rs1801133 in *MTHFR* G/A	Fibrinogen 4.5 g/l Homocysteine 14.4 mkmol/l
Scapular/ Parascapular	trauma	lower extremity	venous	-	no alteration in laboratory data

SNP, single nucleotide polymorphism; FVL, Factor V Leiden; FII, Factor II; *FGG*, Fibrinogen Gamma; MTHFR, methylene tetrahydrofolate reductase; AT, antithrombin; aPCR, activated protein C resistance; NS, nonsignificant; n, number; G, guanine; A, adenine; T, thymine; C, cytosine; * patient with noticed association between SNP rs6025 in *FVL* and aPCR and free flap thrombosis

cused on five SNPs, which were mentioned in literature most frequently in relation with their coded plasma protein levels and free flap thrombosis advancement of diverse localisation, either venous or arterial origin, in 152 microvascular flap surgery patients. Our study group was relatively young (mean age 45.1 years), and only 5.2 per cent had a history of previous thrombotic events and 13.8 per cent had a positive family history of thrombosis. This study revealed that a significantly lower aPCR plasma level occurred in carriers of *FVL* gene genotype C/T in the analysed polymorphism. We also noticed a highest fibrinogen plasma level in carriers of *FGG* gene genotype A/A in the analysed polymorphism. Correspondingly, for one patient, advancement of free flap thrombosis was most likely associated with one of our analysed SNP where aPCR was detected.

In microvascular flap surgery both arterial and venous circulation systems are involved. Therefore, there is still lack of a haemostatic tool that would allow evaluating who would benefit from perioperative thromboprophylaxis without increasing risk of haemorrhage. Although has not yet been evaluated for microvascular flap surgery, the 2005 Caprini Risk Assessment Model could be applicable for those patients (Pannuci *et al*., 2015).

It has been mentioned that approximately 5 per cent of aPCR could occur by different mechanisms (Segers, 2007), particulary in FVL gene rs6025T C/T genotype carriers. We recognised only one patient with aPCR who had a C/T genotype combined with a markedly lower aPCR plasma level and who underwent microvascular flap surgery twice in two years' time period, both with eventual free flap thrombosis. aPCR can be detected in 20% of patients wih VTE and in more than a half of selected families with thrombophilia (Rees *et al*.,1995; Rosendaal *et al*., 1995). The prevalence of the homozygous T/T genotype form is 1.5 per cent of the general population (Rees *et al*., 1995). Thus, we can explain why none of the patients in our study group had this genotype of the FVL gene for the analysed SNP.

None of our patients with a mutation in the *FII* gene had free flap thrombosis. All patients had prothrombin plasma levels within the normal range. It has been observed that when the prothrombin cut off value reached 95.19%, risk of thrombosis increased by 2.34 times (Drizlionoka *et al*., 2019). This is consistent with observations made by previous investigators. Patients with a prothrombin plasma level higher than 1.15 U/ml had a 2.1-fold higher risk of VTE than those in the reference category < 0.95 U/ml (Poort *et al*., 1996).

We believe that increased risk of free flap thrombosis might be associated with increased plasma levels of fibrinogen. Carriers of the FGG gene rs2066865 genotype A/A had sig-

nificantly higher levels of fibrinogen, compared with carriers of genotype G/G. We also noticed that plasma levels of fibrinogen are higher for FGG gene heterozygotes A/G. The latter genotype was found in 5 of 12 patients with free flap thrombosis, as shown in Table 4. Undas and Casini (2019) previously reported that patients with hereditary dysfibrinogenemia are highly heterogeneous in clinical manifestation. Correlations between genotype and phenotype may sometimes not be established, mainly due to involvement and overlapping of a variety of mechanisms like changes in fibrin network stability, strength, architecture and impairment in fibrinogen binding sites, resulting in qualitative and quantitative alteration. Therefore, we speculate that A/G genotype carriers with consequently higher plasma fibrinogen levels might have an association with an increased free flap thrombosis incidence and could be considered as a genetic marker associated with free flap thrombosis development (Drizlionoka *et al*., 2019).

Interestingly, lower AT concentration does not inevitably ensure thrombosis. In a study of a family with inherited AT deficiency, there were few cases of pedigrees with low AT plasma levels and with no convincing history of thrombosis (van der Meer *et al.*, 1973). We did not recognise a difference in plasma AT level either in patients carrying a heterozygous or homozygous form of SNP rs2227589 in the *SERPINC1* gene with or without free flap thrombosis. Both patients with the heterozygous form of SNP and free flap thrombosis had age under 50 years, which agrees with data showing that the first thrombotic event usually occurs by the age of 30 years and by the age of 50 years, and that 50% of patients having AT deficiency will have had thrombosis episode, generally DVT.

Seven out of the 12 analysed patients in the free flap thrombosis case series were carriers of the *MTHFR* gene rs1801133 G/A genotype and had a higher serum homocysteine plasma level (> 12 mkmol/l). Two patients had solely a mutation in the *MTHFR* gene. One eventually had free flap thrombosis in arterial and the other in venous flow. There have been controversies about whether an increase in serum homocysteine levels is only associated with arterial thrombosis, or also has an effect on VTE (Falcon *et al*., 1994; Ray *et al*., 2002; Pathare *et al*., 2004). Thus, although no general agreement has been reached, we support the idea that a higher homocysteine plasma levels might be associated with higher risk of free flap thrombosis, taking into account that both arterial and venous blood flow are involved.

There were few limitations in the study. The main limitation was the rather small sample size; therefore, we were not able to obtain strong findings by means of genotype intergroup analysis. In the Centre of Plastic and Reconstructive Microsurgery of Latvia about forty surgeries are performed per year, limiting the sample size. Moreover, the analysed clinical outcome in our study was free flap thrombosis. The average incidence of free flap thrombosis varies between 8 and 10% (Vanags *et al*., 2020). In a recent study (Vanags *et al*., 2020), free flap thrombosis developed in 15.5% of microvascular flap surgery patients. It explains the rather small sample size of patients with free flap thrombosis in our study. Therefore, we were not able to find statistical significant differences in coded plasma proteins and SNPs between those with and without free flap thrombosis, most likely due to incomparable unequal sample size groups and unequal distribution of genotype groups for each analysed polymorphism.

Additionally, we were not able to assess the function of coded plasma proteins related with analysed SNPs. Most importantly for AT and fibrinogen, it would be advisable to distinguish between qualitative or quantitative inherited thrombophilia disorders. AT function in order to understand whether patients are carrying SNP rather than I type AT deficiency would be essential.

As reported previously (Vanags *et al*., 2020), although all patients in our study received standardised treatment, and received operations by experienced plastic surgeons who routinely perform microvascular flap surgery, we consider that technical surgical factors are one of most important limitations. We were also not able to avoid the multifactorial nature of a thrombotic event and the human factor in surgery and post-operative care. Moreover, for patients that had more than one SNPs, it was almost impossible to evaluate the impact on one particular SNP in such a small study population. Inherited thrombogenic factors and acquired thrombogenic factors overlap, making it infeasible to distinguish the role of the SNP *per se*. Population bearing more than one risk factor, either genetic or acquired, are at higher risk and VTE is considered as a multigenetic/multifactorial disease (Dahlbäck, 2005).

Finally, low incidence of rare allele SNPs and free flap thrombosis rate revealed a rather wide confidence interval.

CONCLUSIONS

Our preliminary results showed that a lower aPCR level was associated with *FVL* rs6025 C/T and higher fibrinogen plasma levels with *FGG* rs2066865 A/A, suggesting that these genotypes might predict higher free flap thrombosis risk. However, we found no significant association between the analysed SNPs and free flap thrombosis advancement.

We further aim to increase the study group to explore the associations between the determined SNPs and free flap thrombosis rate.

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IEDZIMTO TROMBOFĪLIJU LOMA TROMBOZES ATTĪSTĪBĀ MIKROVASKULĀRĀ LĒVERU ĶIRURĢIJĀ

Mikrovaskulārajā ķirurģijā defektu slēgšanai no muskuļaudiem, kaulaudiem un/vai saistaudiem tiek veidots brīvais lēveris. Neraugoties uz uzkrātām zināšanām, prasmēm un labu tehnisko nodrošinājumu, brīvā lēvera trombozēšanās joprojām ir aktuāla problēma. Lēveru trombozēšanos, iespējams, būtiski varētu ietekmēt iedzimtās trombofīlijas, kuru loma ir aprakstīta citu trombožu — kā venozo, tā arteriālo — attīstībā. Šajā prospektīvajā pētījumā mēs 152 pacientiem, kuriem veica mikrovaskulāro lēveru ķirurģiju, noteicām piecus biežākos literatūrā aprakstītos polimorfismus: rs6025 FV Leidena (*FVL*) gēnā, rs1799963 FII gēnā, rs2066865 Fibrinogēna Gamma (*FGG*) gēnā, rs2227589 *SERPINC1* gēnā un rs1801133 *MTHFR* gēnā, kā arī novērtējām šo polimorfismu ietekmi uz to regulēto proteīnu izmaiņām plazmā. Dati tika vākti laika posmā no 2016. līdz 2019. gadam Latvijas Mikroķirurģijas centrā Rīgā. Pirmie rezultāti uzrādīja, ka pacientiem ar C/T genotipu rs6025 *FVL* gēnā ir ievērojami zemāks rezistentā aktivētā proteīna C (aPCR) līmenis plazmā, nekā tiem ar C/C genotipu; $p = 0.006$ (CI 95%, 0.44–1.1). Papildus novērojām, ka pacientiem ar A/A genotipu rs2066865 *FGG* gēnā ir salīdzinoši augstāks fibrinogēna līmenis plazmā (1.81 ± 5.6 g/l), nekā tiem ar G/A un G/G genotipiem; $p = 0.04$ (CI 95%, 0.007–1.09); $p = 0.004$ (CI 95%, 0.48–2.49). Brīvā lēvera trombozi konstatēja 12 pacientiem (7.89 %). Tikai vienam no tiem varēja atrast sakarību starp brīvā lēvera trombozēšanos un zemu aPCR līmeni plazmā, pieņemot, ka lēvera trombozēšanos noteica mutācija rs6025 *FVL* gēnā. Secinājums: kaut gan zemāks aPCR līmenis plazmā korelēja ar C/T genotipu rs6025 *FVL* gēnā un augstāks fibrinogēna līmenis — ar A/A genotipu rs2066865 *FGG* gēnā, kas varētu liecināt par augstāku trombozēšanās risku, mēs neatradām ticamu saistību starp noteikto gēnu polimorfismiem un brīvā lēvera trombozēšanos.