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POLYMORPHISM OF THE MTHFR GENE rs1801133: STUDY OF THE IMPORTANCE IN THE DEVELOPMENT OF VARICOSIS OF THE LEGS AND ITS ROLE IN THE OCCURRENCE OF THROMBOTIC COMPLICATIONS

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Abstract: To determine the significance of the rs1801133 polymorphism of the MTHFR gene in the development of Varicose veins of the lower extremities (VVLE) and its thrombotic complications. **Materials and methods.** The rs1801133 polymorphism of the MTHFR gene was studied in 161 patients with VVLE and its complications, including 111 with uncomplicated VVLE and 55 with venous thrombosis, compared with 155 conditionally healthy subjects by PCR. DNA isolation from peripheral blood lymphocytes was performed using the AmpliPrime RIBO-prep kit. Testing of polymorphic locus rs1801133 in the MTHFR gene was performed using a Rotor-Gene Q instrument (Qiagen, Germany), by allele-specific PCR in Real-Time format, using a kit of LLC NPF Litech (Russia). **Results**. The proportion of wild-type C/C genotype among patients with thrombotic complications was 46%, significantly lower than in the control group, whose proportion was 62.6% ($\chi2>3.84$; $p<0.05$; OR=0.5; 95%CI:0.27-0.97). The proportion of mutant homozygous genotype T/T was significantly more significant in the patients with venous thrombosis group than in the control group, accounting for 16% versus 7% of detected cases, respectively ($\chi2>3.84$; $p<0.05$; OR=3.1; 95%CI:1.11-8.49). **Conclusions**. The homozygous T/T genotype increases the risk of VBNS and its complications by venous thrombosis.

Keywords: MTHFR gene, rs1801133, Varicose veins of the lower extremities (VVLE), complications, venous thrombosis.

Varicose veins of the lower extremities belong to peripheral vascular diseases and represent a serious health problem worldwide. It is more common among the elderly and leads to vascular occlusion, especially of the lower extremities [1]. The rs1801133 (C677T (Ala 222 Val)) polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene is associated with various diseases (vascular, cancer, neurological, diabetes, psoriasis, etc.), and the epidemiology of this rs1801133 polymorphism varies depending on geography and ethnicity . In the United States, approximately 20-40% of Hispanics are heterozygous for MTHFR rs1801133 [2], this polymorphism is less common in blacks in the US (~2%), and in North America, Europe and Australia, approximately 8-20% of the population is homozygous for MTHFR rs1801133 [2]. The MTHFR gene, the enzyme methylenetetrahydrofolate reductase , plays an important role in the processing of amino acids, the building blocks of proteins. Methylenetetrahydrofolate reductase converts 5,10-

methylenetetrahydrofolate to 5-methyltetrahydrofolate. It is the main form of folic acid found in the blood and is required for the multi- step process of converting the amino acid homocysteine to another amino acid, methionine. The body uses methionine to produce proteins and other important compounds [3]. The 5,10-methylenetetrahydrofolate reductase (MTHFR) locus is mapped on chromosome 1 at the end of the short arm (1p36.6). The MTHFR gene mutation that causes the rs1801133 polymorphism is located in exon 4, resulting in the conversion of valine to alanine at codon 222, which is a common polymorphism that reduces the activity of this enzyme [4] . According to the literature data, polymorphism of the MTHFR rs1801133 locus gene is closely associated with the occurrence of a number of diseases [5]. In the studies of some authors, the MTHFR rs1801133 mutation was associated with the occurrence of deep vein thrombosis, and the T/T genotype is a genetic risk factor for deep vein thrombosis [6]. Some authors showed in their studies an association of MTHFR rs1801133 with deep vein thrombosis [7], while others reported no significant association between MTHFR rs1801133 and deep vein thrombosis [8]. Due to the lack of data on the effect of MTHFR gene polymorphism on the clinical course and the occurrence of complications in lower extremity LVLL, in our study we decided to investigate the role of the MTHFR gene in the development of LVLL and its complication with deep vein thrombosis of the lower extremities (venous thrombosis).

Target research . Determination of the significance of the rs1801133 polymorphism of the MTHFR gene in the development of varicose veins of the lower extremities (LVLV) and its thrombotic complications.

Materials and methods. A total of 316 people were involved in the study, of which 161 were included in the main and 155 in the control group. The main group of patients consisted of a subgroup of patients with only LVL without thrombotic complications (n = 111), and a subgroup of patients with venous thrombosis (n = 50). To conduct a molecular genetic study, blood was taken from all subjects in vacuum tubes with EDTA (K_3 EDTA, 5 ml). Isolation of DNA from peripheral blood lymphocytes was carried out using the AmpliPrime kit. RIBO-prep ”(Interlabservice LLC, Russia).

Testing of the polymorphic locus rs1801133 in the MTHFR gene was performed on the Rotor Gene Q (Quagen, Germany), by allele-specific PCR in Real - Time format, using the kit of LLC NPF Litekh (Russia).

Statistical processing of the obtained data was carried out using the application package "OpenEpi 2009, Version 9.3".

Results of the study and their discussion. As a result of the study of the distribution frequency of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in their distribution in the main group of patients with a hereditary predisposition to VLVD and phlebothrombosis and the control sample presented in Table 1 and Figure 1, the allele C prevailed in the control group, and its frequency was 6 3.7 % versus 77.7 % (χ^2 >3.84; p <0.05; OR=0.5; 95 % CI :0.35-0.71) and the allele T prevailed in the group of patients with LVL and venous

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thrombosis, its frequency was 36.3 % versus 22.3%, respectively ($\chi 2 > 3.84$; $p < 0.05$; OR = ^{2}; 95% CI :1.40-2.83).

Thus, according to the data obtained, it can be seen that in the main group there was a predominance of the T frequency, while in the control group, C allele, the detection frequency of which was higher ($\chi^2 > 3.84$; $p < 0.05$; OR =2.0;95% CI: 1.40-2.83) (Fig. 1; Table 1).

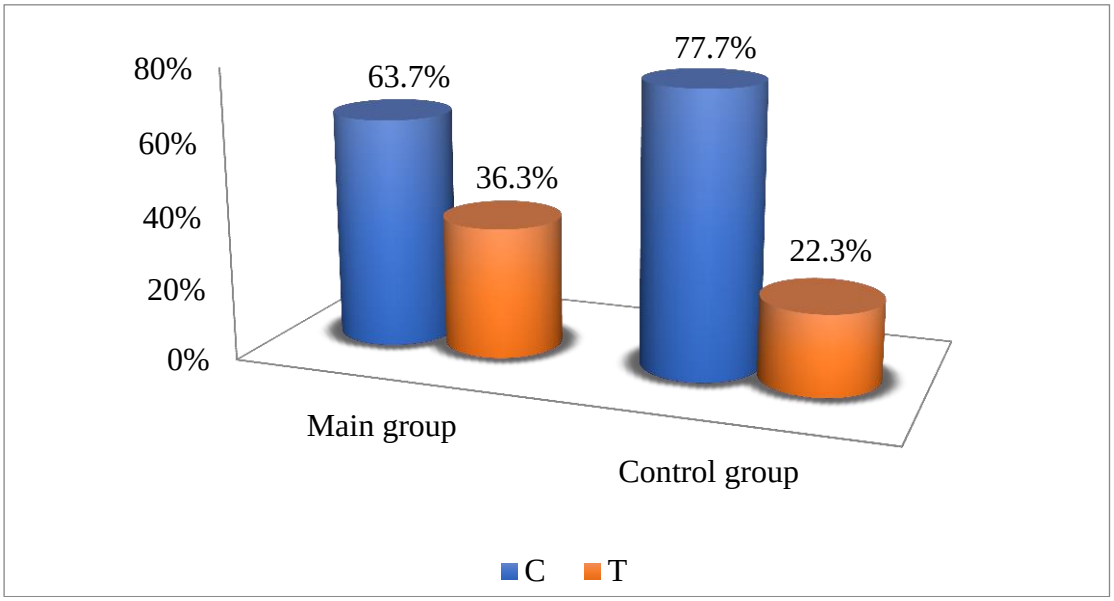

Figure 1. **The frequency of distribution of alleles of the rs1801133 polymorphism in the MTHFR gene in the main group of patients and in the control group**

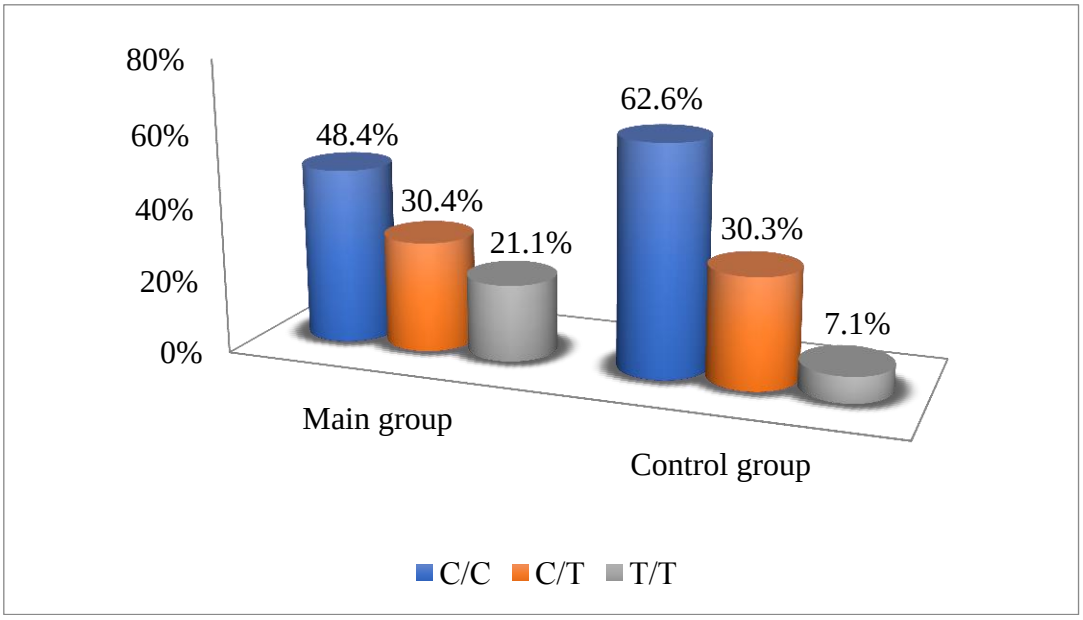

Figure 2. **Distribution frequency of rs1801133 polymorphism genotypes in the MTHFR gene in the main group of patients with VLVD and in the control group**

Table 1.

Association between the rs1801133 polymorphism in the MTHFR gene in the main group of patients with VLVL and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		odds ratio	95%CI:	χ^2	p-value
		R			
Main group (n=161)	FROM	.5	0.35 – 0.71 _	**15.1 _**	**0.0001 ***
	T	.0	1.40 – 2.83		
	S/S	.6	0.36 – 0.88 _	**6.4 _**	**0.01 ***
	S/T	.3 _	0.79 – 2.14 _	1.0	0.31 _
	T/T	.8	1.83 – 8.08	**13.7**	**0.0002 ***

Note: * - statistically significant (significant)

Frequency distribution of genotypes C/C, C/T, T/T polymorphism rs1801133 in the MTHFR gene in the main group of patients and controls were: 48.4%, 30.4% and 21.1% versus 62.6%, 30.3% and 7.1%, respectively (Fig.1.2).

wild genotype C /C was statistically significantly less frequently detected in the main group, in which its proportion was 48.4 %, against 62.6 % in the control group ($\chi^2 > 3.84$; $p < 0.05$; OR =0.6; 95% CI: 0.36-0.88).

incidence of the heterozygous C/T genotype was practically at the same level among patients of the main group and in the control group, amounting to 30.4% versus 30.3%, respectively ($\chi^2 = 1$; $p > 0.05$; OR =1.3; 95% CI: 0.79-2.14).

Proportion of unfavorable T /T genotype was significantly higher in the group of patients of the main group, compared with the control group, 21.1% vs. 7.1%, respectively ($\chi^2 > 3.84$; $p < 0.05$; OR =3.8; 95% CI: 1.83-8.08) (Table 1).

Thus, it was found that the carriage of the homozygous mutant genotype T /T increases the risk of developing LVL almost 4 times ($\chi^2 > 3.84$; $p < 0.05$; OR =3.8; 95% CI :1.83-8.08).

A study of a subgroup of patients with VLVL without thrombotic complications showed that the proportion of allele C in the control sample, which was 63.1%, was lower than among patients with VLVL, among which it was 77.7% ($\chi^2 > 3.84$; $p < 0.05$; OR =0.5; 95% CI :0.33-0.72).

However, the unfavorable allele T prevailed in the subgroup of patients, relative to the control group, where its frequency was 36.9 % versus 22.3%, respectively ($\chi^2 > 3.84$; $p < 0.05$; OR =2; 95% CI: 1.40-3.00) (Fig. 2; table .2).

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Genotypes C/C, C/T, T/T polymorphism rs1801133 of the MTHFR gene among patients with VLVL and in the control group were distributed as follows: 49.5%, 27.0% and 23.4% versus 62%, 30% and 7%, respectively.

Proportion of wild genotype C/C was significantly lower among patients with uncomplicated VLLE, accounting for 49.5%, relative to apparently healthy individuals in the control group, where its proportion was 62.6% (χ^2 >3.84; p <0.05; OR =0.6; 95% CI: 0.36 -0.96).

In the study of the distribution of the C/T genotype, no statistically significant differences were found - there was an insignificant excess in the frequency of detection of this genotype among conditionally healthy patients, compared with the group of patients with VLVL, where they were detected in 27.0% and 30.3%, respectively (χ^2 = 0.2; p >0.05; OR =1.1; 95% CI: 0.64-1.98).

The proportion of the mutant T/T genotype was statistically significantly higher among LVL patients without venous thrombosis, amounting to 23.4% versus 7.1 % in the control sample (χ^2 >3.84; p <0.05; OR =4.2; 95% CI: 1.91-9.08). (Fig. 3.4; Table 2).

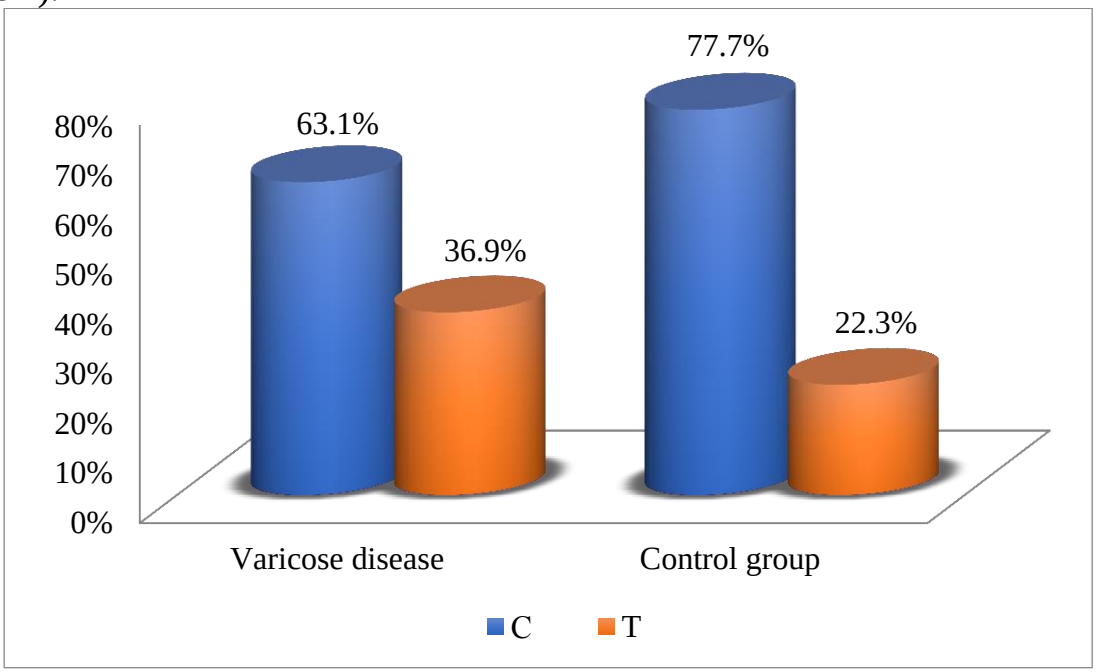

Figure 3 . **The frequency of distribution of alleles of the rs1801133 polymorphism in the MTHFR gene in the group of patients with VLVL and in the control group**

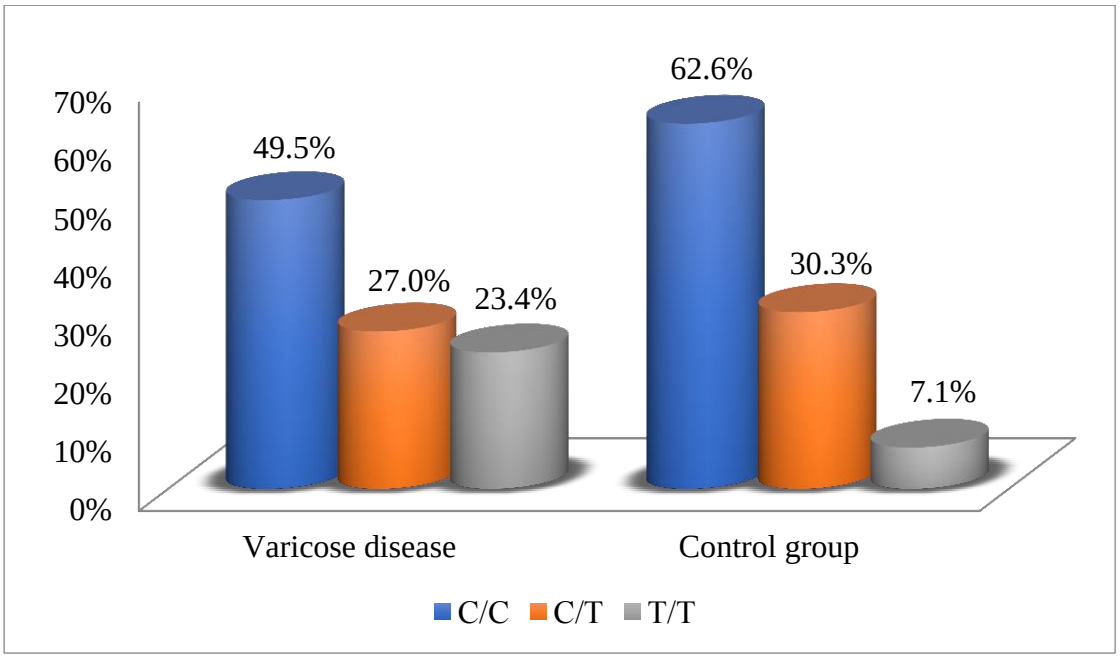

Figure 4 **Distribution frequency of rs1801133 polymorphism genotypes in the MTHFR gene in the group of patients with VLVL and in the control group**

Table 2.

Association between the rs1801133 polymorphism in the MTHFR gene in groups of patients with VLVL and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		odds ratio		χ^2	p-value
		OR	95%CI:		
Varicose disease (n=111)	FROM	0.5 _	0.33 - 0.72	**13.7 ***	**0.0002 ***
	T	2.0	1.40– 3.00		
	S/S	0.6	0 . 36-0 . _ _ 96	**4.5 ***	**0.03 ***
	S/T	1.1	0.64– 1.98 _	**0.2 ***	0.68 _
	T/T	4.2 _	1.91– 9.08	**14.1 ***	**0.0002 ***

Note: the same as in Table. one.

Thus, in the course of the study of the frequency of distribution of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in the group of patients with uncomplicated venous thrombosis of the form of VLVL and the control sample, it was found that the homozygous genotype T /T can more than quadruple the risk of developing structural changes in the wall of the veins and the development of VLEV ($\chi 2 > 3.84$; $p < 0.05$; OR =4.2; 95% CI: 1.91 - 9.08).

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In the study of the distribution frequency of alleles and genotypes of the rs1801133 polymorphism in the MTHFR gene for differences in the studied groups, it was found that the C allele was less frequently detected among patients with venous thrombosis, compared with apparently healthy individuals in the control sample, where they were detected in 65 .0 % and 77.7 % cases, respectively (χ^2 =6.5; p >0.01; RR =0.6; 95% CI: 0.33-0.87; OR =0.5), which indicates the protective nature of this allele.

At the same time, carriers of the allele T were more common among patients with venous thrombosis, in contrast to conditionally healthy subjects - 35.0 % versus 22.3 %, respectively (χ^2 >3.84; p <0.05; OR =1.9; 95% CI:1.15-3.07).

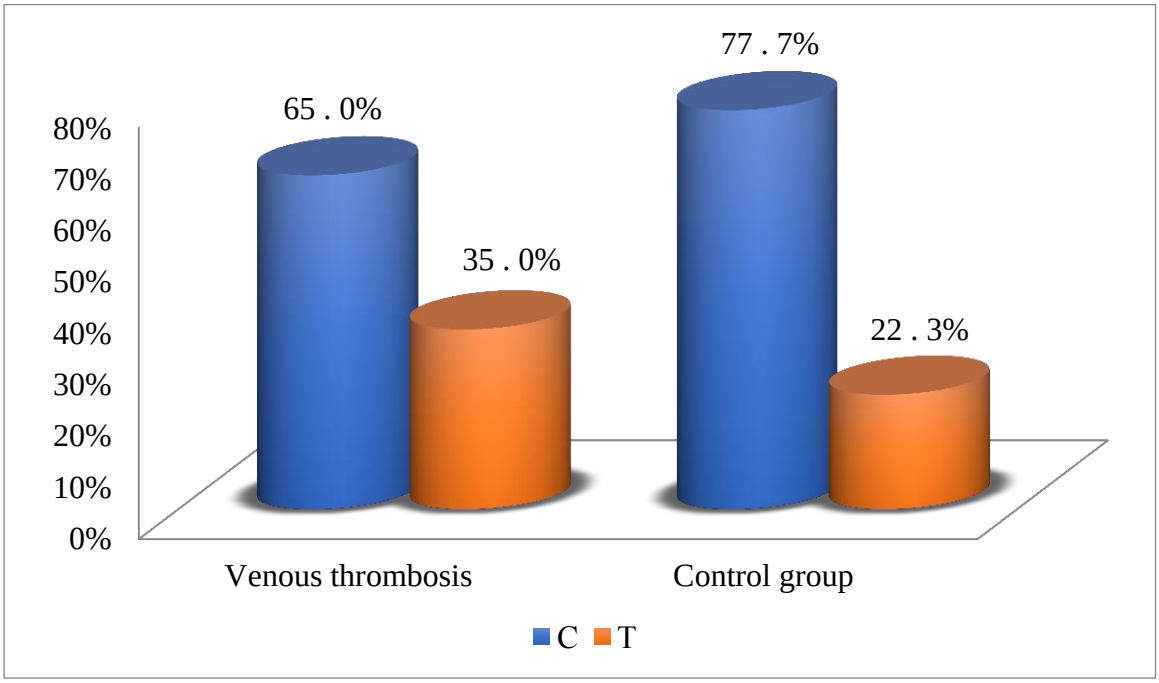

Figure 5. **The frequency of distribution of alleles of the rs1801133 polymorphism in the MTHFR gene in the group of patients with venous thrombosis and in the control group**

The frequencies of C/C, C/T, T/T genotypes of the rs1801133 polymorphism in the MTHFR gene in the group of patients with venous thrombosis and in the control group were: 46%, 38% and 16%, versus 63%, 30% and 7%, respectively (Figure 5, 6, Table 3).

Proportion of wild genotype C /C among patients with thrombotic complications was 46.0%, which was significantly lower than in the control group, where its proportion was 62.6% (χ^2 >3.84; p <0.05; OR =0.5; 95% CI: 0.27 -0.97).

heterozygous C/T genotype in patients with venous thrombosis was found statistically insignificantly more often than in the control group, where they were detected in 38.0% and 30.3% of cases, respectively (χ^2 =2.2; p >0.01; OR =1.7; 95% CI: 0.85-3.43).

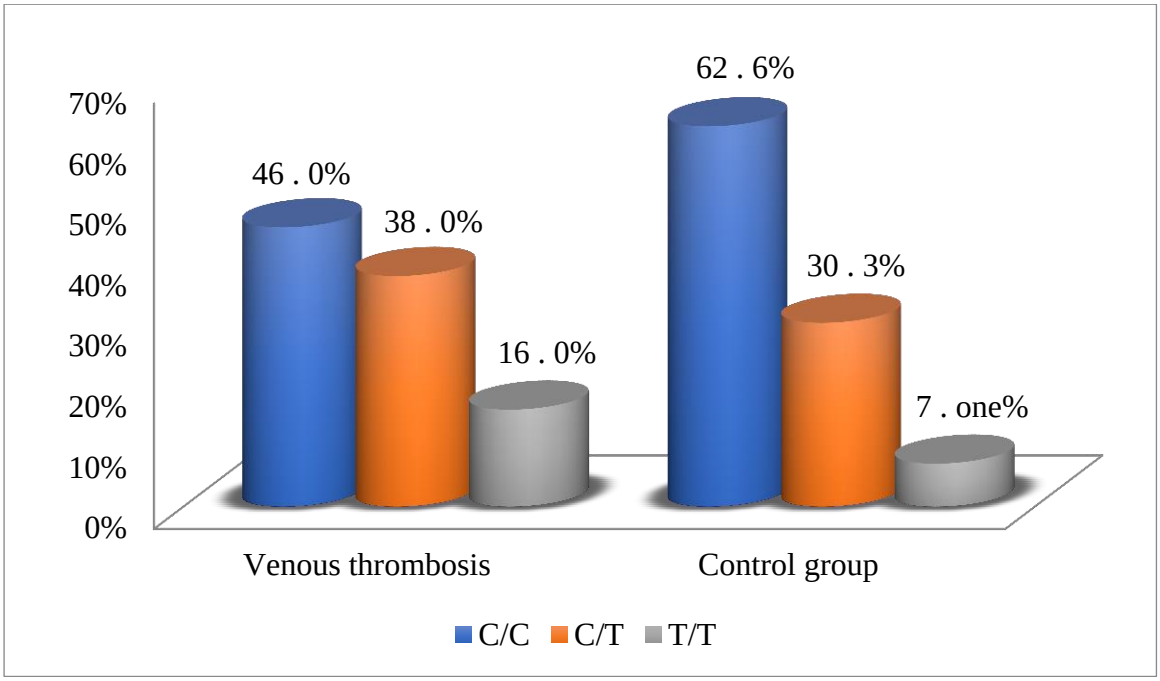

Figure 6. **Distribution frequency of rs1801133 polymorphism genotypes in the MTHFR gene in the group of patients with venous thrombosis and in the control group**

Table 3

Association between the rs1801133 polymorphism in the MTHFR gene in groups of patients with venous thrombosis and in the control group

Study Groups	Alleles and genotypes	Statistical difference			
		odds ratio		χ^2	p-value
		R	95%CI:		
VBNC with venous thrombosis (n=50)	FROM	.5	0.3 3–0.8 7 _	**.5 ***	**0.01 ***
	T	.9 _	1.1 5–3.0 7		
	S/S	.5 _	0 .2 7–0.9 7	**.3 ***	**0.04 ***
	S/T	.7	0.8 5–3.4 3	.2	0.1
	T/T	.1 _	1.1 1–8.4 9	**.0***	**0.02 ***

Note: the same as in Table. one.

The proportion of the mutant homozygous genotype T / T significantly prevailed in the group of patients with venous thrombosis, amounting to 16.0% versus 7.1% of detected cases in the control group (χ^2 >3.84; p <0.05; OR =3.1; 95% CI: 1.11-8.49) (Figure 5, 6, Table 3).

Discussion. In our work, we studied the molecular genetic aspects of the rs1801133 polymorphism in the MTHFR gene among patients with VLVL and venous thrombosis in order to determine the mutation frequency of this gene in Uzbekistan, since according to some data [9], the prevalence of this gene depends on the geographical distribution and ethnicity in all over the world. The results of our study showed that the frequency rs1801133 polymorphism in the MTHFR gene among patients with VLVL and venous thrombosis is quite high. Some studies have confirmed a weak association between an increased risk of venous thromboembolism and polymorphism rs1801133 of the MTHFR gene, however, this relationship was more often population-based and was not found in North America, which may be associated with high dietary intake of riboflavin and folic acid [10]. In Turkish and Iranian populations, the prevalence of this gene was 49.6% and 67.0%, respectively [11. 12]. Based on the results of Li A. Et all. (2020) the rs1801133 polymorphism of the MTHFR gene was expressed in patients with carotid atherosclerosis [13]. According to the authors, genetic polymorphisms rs1801133 and A1298C gene MTHFR were associated with cerebral venous sinus thrombosis [14]. In addition, these loci have been associated with increased susceptibility to the development of venous thromboembolic disease, which includes deep vein thrombosis and pulmonary embolism [15].

Conclusion. Thus, in the course of the study frequencies of distribution of alleles and genotypes of the rs1801133 polymorphism of the MTHFR gene for differences in the group of patients with VLVL and in the control sample , it was found that the homozygous genotype T /T increases the risk of developing both LVL and its complication by venous thrombosis.

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