Batumi Shota Rustaveli State University

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"Genetic Aspects of Arterial Thrombosis in the Population of Adjara "

Specialization: Genetics

For the dissertation for the degree of PhD in Biology

Annotation

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I, Sopio Garakanidze, as the author of the submitted dissertation, state that the dissertation is my original work which does not include any materials submitted for a degree, already published or not published by other authors without being referred or quoted according to proper rules.

Sopio Garakanidze

Defense of the disserration is going to be held on ----of June, 2019 at ------o'clock at Batumi Shota Rustaveli State University at the meeting of the dissertation commission formed by the Faculty of Natural Sciences and Health Care Dissertation Council.
Adress: Batumi, 35 Ninoshvili St, the 3rd floor of the university, room 328.

The dissertation can be seen in the university library and on the university website.

Approbation of the work**:** the preliminary review of the dissertation was conducted at the meeting of the Department of Biology, Batumi Shota Rustaveli State University (Protocol ----, February 22, 2019)

Introduction

Topicality of the research subject

Disease of the cardiovascular system (CVD) is one of the main causes of deaths worldwide. Atherosclerosis of blood vessels leads to myocardial infarction (MI) and ischemic stroke (IS) (S. Dimmeler, 2011). CVD accounts for 40% deaths in high-income countries and 28% in low and middle-income ones (M.S. Lee, 2012). Approximately 2200 Americans die of the diseases (on average, one death is reported every 40 seconds) and 92.1 million adolescents suffer from different forms of CVD.

CVD also causes deaths in European Union countries. It claims lives of over1.8 million people every year. 11.3 million new cases were reported in Europe in 2015.

The problem is also topical for Georgia, which is proved by "The European Heart Network" (EHN) (based in Brussels, EHN is an alliance of different countries together with non-governmental organizations and "Heart Foundations"). According to the research conducted by the alliance, in 2015, 29.007 deaths were reported in men and 33.509-in women (European Cardiovascular Disease Statistics 2017), **(** http://www.ehnheart.org/cvd-statistics.html).

Risk factors of arterial thrombosis is divided into two main classes: modifying (hyperpiesis, diabetes, heart diseases, peripheral arterial diseases) and non-modifying (age, ethnicity, sex, genetic diseases) risk factors (A. Arboix .2015).

In the 21st century, personalized medicine is of great significance. It develops a particular method of medical care according to a patient's genome. Cardiology is one of medicine areas that tries to treat and cure a patient based on the very personalized medicine (M.S. Lee, 2012). The reason why it is so important to consider a patient's genotype while treating thrombosis is that 80-90% of thromboses result from genetic disorders of homeostasis (A.Makatsaria, 2014). Thus, genetic markers are the non-modifying risk factors which need controlling regularly.

The following mutations regarded as genetic markers of arterial and vein thromboses are of great importance: a mutation in the genes for coagulation factor V known as Factor V Leiden (FVL G1691A); mutation PT (G20210A) in the genes for coagulation factor II and mutation MTHFR (C677T) and hyperhomocysteinemia caused by it.

According to World Health Organization (WHO), CVD is likely to have claimed lives of 23.6 million people by 2030 (http://www.who.int/cardiovascular_diseases/about_cvd/en/). Therefore, it is vital to study genetic aspects of arterial thromboses and take preventive measures.

The research material and methodology: The aim of our research is to study genetic aspects of arterial thrombosis in the Adjara population. Research material was collected at Batumi Referral Hospital.

There were 101 patients involved in the research. All of them were ill with arterial thrombosis. In particular, 84 patients were diagnosed with MI and 17-with IS (71.3% men, at an average age of 66.3 ± 12.1). Also, there was the control group of 113 members. An average age of the first manifestation of MI among the research group is 62.6 +/- 12.6 and 71.5+/- 11.7 in the case of IS. 12 of those patients diagnosed with myocardial infarction were affected by a recurrent episode of MI.

Patients were diagnosed with MI and IS based on the troponin test and computer tomography.

We defined how heavily a person smokes according to continuous cigarette consumption and smoking more than one per day throughout a year at least. Arterial pressure was defined with a standardized sphygmomanometer.

Polymerase Chain Reaction (PCR) was applied to identify the following genes in the blood samples of the people participating in the research: FVL G1691A, PT G20210A and MTHFR C677T .

Statistical Package (SPSS, version 21.0) (Windows - SPSS Inc, Armonk, New York) was used to process genetic and clinical data biostatistically.

Polymorphism of genes in the patients and the control group was estimated according to Hardy-Weinberg Law whose electronic version is available on the following website: www.oege.org/soft ware/hardy-weinberg.html.

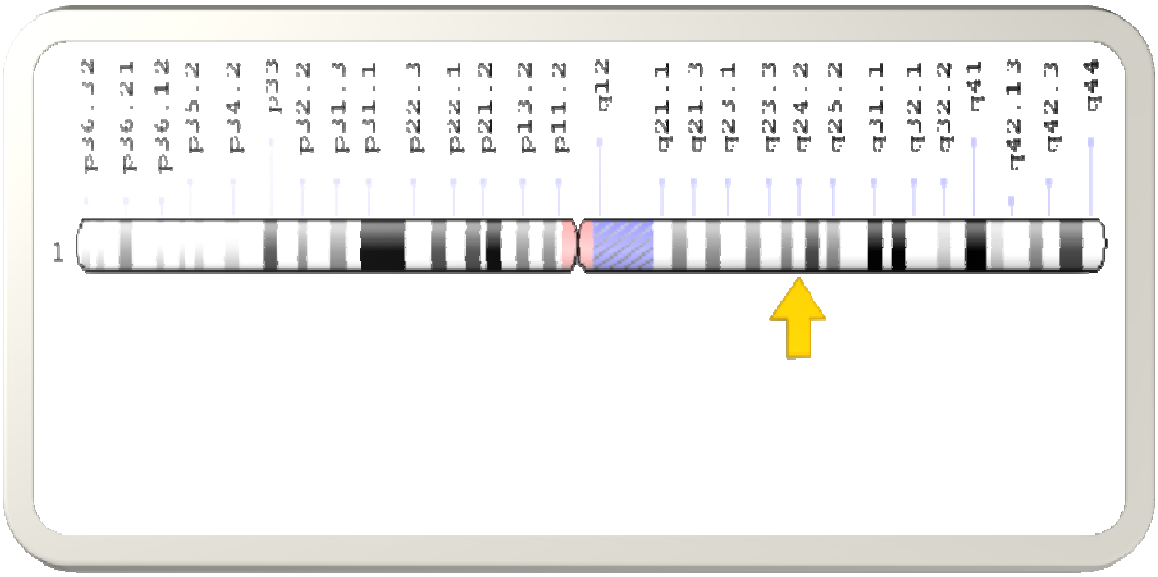
With the aim of processing clinical data, we applied Student's t and Mann-Whitney tests. Significance was accepted at $p<0.05$.

We carried out research on the above-mentioned genes using PCR method in the biochemistry laboratory of the faculty of pharmacy at University of Porto.

Literary review

Factors of blood clotting are determined genetically in a human's body and prevention of thrombosis complication depends how well genes function. Sometimes a mutation occurs in the genes which determine those factors. Because of it, the blood clotting system stops functioning normally leading to development of thrombosis.

Characterization of Factor V Leiden (G1691A): Coagulation factor V specific defect was discovered by B.Bertina while working together with co-authors (R.M. Bertina, 1994). The gene determining this factor is located in a long arm of chromosome 1 in position 24.2 (1q24.2), (Pigure 1), (https://ghr.nlm.nih.gov/gene/ F5#location),



Pigure 1

(https://ghr.nlm.nih.gov/gene/ F5#location).

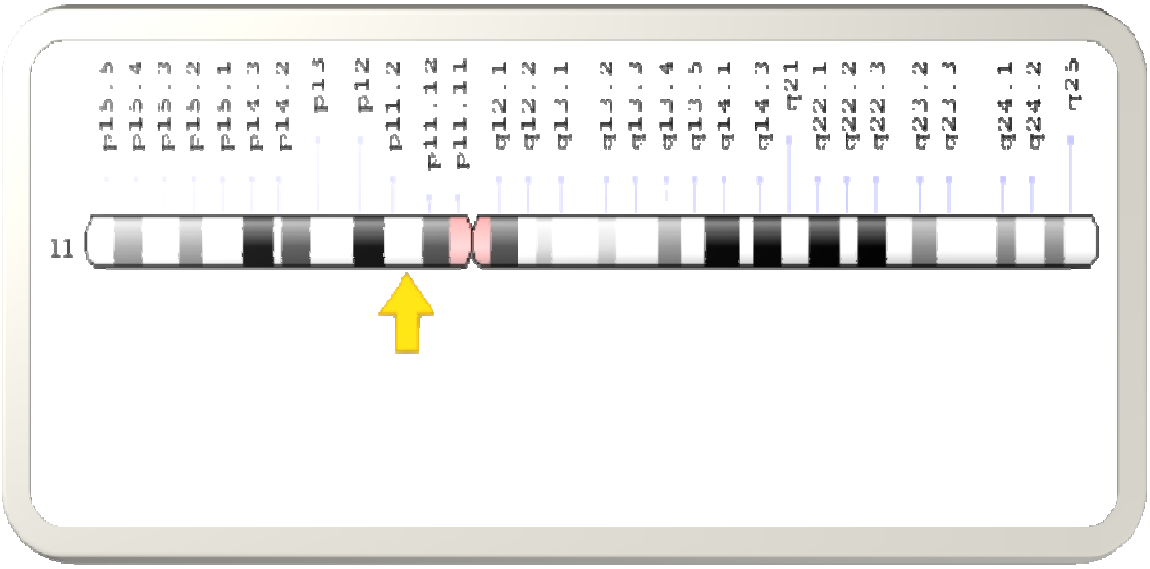
Replacing nucleotide guanine (G) with adenine (A) in position 1691 causes FVL polymorphism (SNP). Also, in factor V amino acid arginine in position 506 is replaced by glutamine because of which APC (activated protein C) cannot inactivate FV leading to hypercoagulation (M. M. Jadaon ,2011). Mutation FVL is inherited in an autosomal-dominant manner (S. Perez-Pujol, 2012).

According to the research findings, in fact, at least one thrombosis case occurs during the life of homozygotes because their genotype contains two mutated alleles. As for heterozygotes, the risk of thrombosis development is seven times higher in comparison to ones having a normal genotype (R. A. Sacher, 1999).

Mutation FVL is associated with both vein and arterial thromboses. However, the research, which was conducted on patients suffering from arterial thromboses in order to define correlation between mutation FVL and arterial thromboses, showed various results.

Characterization of prothrombin G20210A: Prothrombin plays an important role in the blood clotting system. Genetic and acquired deficiency of prothrombin complicates the clotting process, while an abundance of prothrombin leads to hypercoagulation, which is clinically revealed in the form of thromboembolism (M.M. Jadaon, 2014). When there is a rise in the concentrarion of prothrombin, more thrombin is produced that results in development of fibrin clots (S.R. Poort, 1996).

The gene determining blood coagulation factor II is located in a short arm of chromosome 11 in 1.2 (position 1), (Figure 2), (https://ghr.nlm.nih.gov/gene/F2#location),



Pigure 2

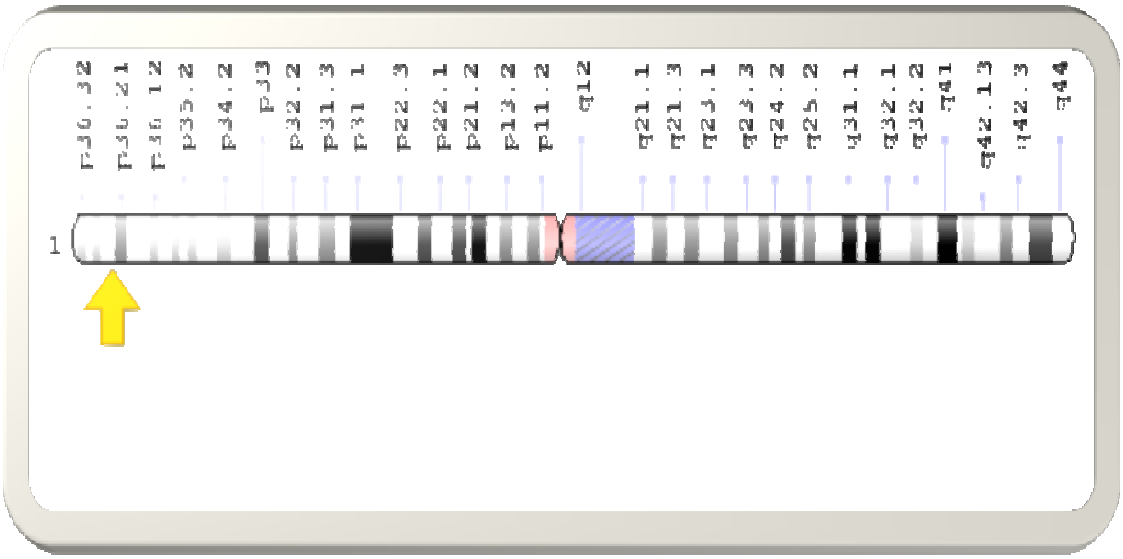
(https://ghr.nlm.nih.gov/gene/F2#location)

Mutation G20210A of prothrombin was identified by S. Poort and his co-authors using PCR-analysis. They selected 28 patients ill with vein thromboses and 5 healthy controls. 18% of the people turned out to have mutation G20210A of prothrombin in non-transmitting area 3' of a prothrombin gene (S.R. Poort, 1996).

Replacing nucleotide guanine with adenine in position 20210 causes mutation (S. SAHİN, 2012). Prothrombin gene G20210A is inherited in an autosomal-dominant manner. While mutation is in progress, the risk of thrombosis development with those having a dominant homozygous gene is higher than with heterozygotes (Jody.L.Kujovich),

(https://www.ncbi.nlm.nih.gov/books/ NBK1148/). Mutation in plasma, which results from a prothrombin rise, is regarded as the cause of deep vein thrombosis. However, mutation is asscociated with arterial thromboses as well (F.R. Rosendaal, 1998).

Characterization of gene MTHFR C667T and homocysteinemia: Gene MTHFR is located in a short arm of chromosome 1 in position 36.3 (1p36.3), (Figure 3). (https://ghr.nlm.nih.gov/gene/MTHFR#location).



Pigure 3

(**https://ghr.nlm.nih.gov/gene/MTHFR#location**).

Mutation MTHFR C677T causes nucleotide cytozine to be replaced by thymine in position 677. Also, in an enzyme, amino acid alanine is replaced by valine in position 222 (P.A. Abhinand, 2017). MTFHR C677T is inherited in an autosomal-recessive way (A. Makatsaria, 2014:440).

While functioning normally, gene MTHFR helps to produce an anzyme called methylenetetrahydrofolate reductase whose main function is to build protein molecules with amino acids. Methylenetetrahydrofolate reductase plays an important role in changing 5,10-methylenetetrahydrofolate into 5- methyltetrahydrofolate. This reaction is a multi-stage process during which amino acid homocysteine changes into amino acid methionine (https://ghr.nlm.nih.gov/gene /MTHFR).

Hyperhomocysteinemia: In 1969, Kilmer McCully made an assumption about the connection between hyperhomocysteinemia and cardiovascular pathologies (G.S. Buchanan, 2003).

Hyperhomocysteinemia is the medical condition when the level of amino acid cysteine in the blood is increased. An increase in the level of homocysteine is caused by both genetic and external factors. Hyperhomocysteinemia occurs in defect of gene encoding 5,10-methylenetetrahydrofolate reductase (E.C.1.5.1.20), (https://www. genome.jp/db get-bin/www_bget?ec:1.5.1.20), methionine synthase (E.C.1.16.1.8), (https://enzyme.Expasy.org/EC/1.16.1.8) and cystathionine β-synthase((E.C.4.2.1.22). These homozygous mutations are the main factors for severe hyperhomocysteinemia and classical homocystinuria. Hyperhomocysteinemia is caused by deficiencies of folates, vitamin B_6, vitamin B_9 (P. Ganguly, 2015). These vitamins (B_6 , B_9, B_{12} ao B_2) function as co-factors in the homocysteine metabolism (M. Hiraoka, 2017).

A lot of research has made it obvious that hyperhomocysteinemia contributes to atherosclerosis, vein and arterial thromboses (Kerkeni, 2006), peripheral arterial deseases (J.I. Spark, 2003), (S. Bhargava, 2007). It is a precedent risk factor for cardiovascular deseases (P. Ganguly, 2015).

Clinical characteristics of thromboses

Age and sex as risk factors for thrombosis: Age is one of the most relevant risk factors for vein and arterial thromboses. As a person ages, the number of thrombosis cases in both men and women increases (G.D. Lowe, 2004). Approximately 80 million Americans aged 60 or over 60(≥60) (USA population is implied) are diagnosed with at least one form of CVD which clearly indicates correlation between CVD frequency and age (A. Yazdanyar, 2009).

On a world scale, the rate of death caused by CVD varies according to sex in both men and women (https://www.paho.org/hq/ dmdocu ments/2013 /WHO -Global-Atlas-CVD-2011-eng.pdf).

Hypertension and arterial thrombosis: Epidemiological, experimental, pathophysiological and clinical studies (C.J. O'Donnell, 2002) indicate that hypertension is one of the most relevant risk factors for MI and IS. Frequency of hypertension varies by 3-70% in different populations (N.H. Zaki, 2011).

Arterial thrombosis and diabetes: Diabetes is one of the most important causes of sickness and early death (G.L. Booth, 2006) and an important risk factor for arterial thromboses. Patients

with diabetes are more at risk of endothelial dysfunction and atherosclerosis (J.D. Roberts...2009), (M.M. Al-Nozha, 2016].

Arterial thrombosis and family history: Since genetic factors together with external ones contribute to myocardial infarction and ischemic stroke, while studying a family history of thromboses, researchers have to consider the effects both factors have on our organism so that they can reach a right conclusion (R.E. Kennedy, 2012).

Arterial thrombosis, smoking and alcohol: In terms of sickness and early death, smoking is one of the most important problems in the world. There is a wide range of deseases caused by smoking, including ischemic stroke, chronical heart and peripheral vascular deseases (K, Fagerstrom , 2002).

Epidemiological research indicates that both women and men smokers are at risk of myocardial infarction and coronary arterial deseases which are likely to be fatal (J.A. Ambrose, 2004). Similarly, consuming a lot of alcohol increases the risk of CVD while an adequate intake of alcohol poses no risk under this aspect (E. Mostofsky, 2015).

The research results

Using PCR, we defined the frequency of a genotype and alleles of mutations of Factor V Leiden (G1691A), prothrombin G20210A and MTHFR genes in the samples of 101 patients who were hospitalized with a diagnosis of arterial thromboses at Batumi Referral Hospital and 113 controls.

Factor V Leiden (G1691A): Normal functioning of factor V responsible for blood clotting depends on genotype G/G, which was found with high frequency of 98.2% (n=111) in the samples of 113 controls. Correspondingly, 1.8% (n=2) of the control group had the heterozygous gene (G/A) determining Factor V Leiden, while genotype A/A (mutated) was not found in the samples of any controls (n=0) (table 1).

Table 1: Genotype and allelic frequency of FVL polymorphism in patients and control groups

Research contingent	N	Genotype frequency			*p* value	Allelic frequency	
		G/G n (%)	G/A n (%)	A/A n (%)		G (%)	A (%)
Controls	113	111 (98.2)	2 (1.8)	0	-	99.1	0.9

All patients	**101**	**97 (96)**	**4 (4)**	**0**	**0.424***	**98**	**2**
Myocardial infarction patients(MI)	**84**	**80 (95.2)**	**4 (4.8)**	**0**	**0.405***	**97.6**	**2.4**
Recurrent myocardial infarction patients (RMI)	**12**	**11 (91.7)**	**1 (8.3)**	**0**	**0.263***		
Ischemic stroke patients(IS)	**17**	**17**	**0**	**0**	**-**	**100**	**0**

***p value vs. controls**

The frequency of G-allele in controls is 99.1% and the frequency of A-allele –0.9%. Because of this allelic frequeny, 1.8 % of the controls were heterozygous according to FVL.

101 patients turned out to have the following frequency of FVL polymorphism genotype: G/G (a normal genotype) is found in 96% of the patients (n=97), G/A genotype was found in just 4% of the patients (n=4) and it is 2.2% more than the heterozygous controls. Genotype A/A in patients with MI and IS was not similar to one in controls.

Within the research, we expressed interest whether Factor V Leiden correlates more with MI, recurrent myocardial infarction (RMI) or IS. In order to find an answer to the question, patients diagnosed with arterial thrombosis are stratified into three groups: patients with a diagnosis of MI (n=84), patients with a diagnosis of RMI (n=12) and patients diagnosed with IS (n=17).

The above-mentioned groups have the following genotype and allelic frequency: G/G genotype frequency in MI patients equals 95.2% (n=80), while G/A heterozygous genotype was found in 4.8% of MI patients (n=4). G/G genotype in RMI patients equals 91.7% (n=11) and G/A genotype is 8.3% (n=1). As for group 3, 100% of IS patients turned out to possess normal G/G genotype, and the frequency of G and A alleles in MI patients equals 97.6% and 2.4%. Normal allelic frequency is 100% in patients with IS according to the frequency of genotype.

Based on the data we collected from the stratified groups and processed biostatistically, we conclude that all patients who turned out to have a heterozygous genotype according to Factor V

Leiden are diagnosed with MI.While comparing the results of MI and RMI patients, we also found out that RMI patients' G/A heterozygous genotype is 1.7% more than MI patients'.

We conducted a genetic analysis on FVL which showed double prevalence of mutation in patients with arterial thromboses in comparison with controls enabling us to make an assumption about the association between FVL and arterial thromboses in the population of Adjara.

Mutation of prothrombin G20210A gene: A normal genotype G/G determining prothrombin is identified in 97% of 101 patients (n=98), while the frequency of heterozygous genotype G/A equals 3% (n=3). A mutated genotype A/A of PT G20210A gene was not detected in any patients (table 2).

Table 2:-Genotype and allelic frequency of PT G20210A polymorphism in patients and control groups

Research contingent	N	Genotype frequency			*p* –value	Allelic frequency	
		G/G n (%)	G/A n (%)	A/A n (%)		G (%)	A (%)
Controls	113	108 (95.6)	5 (4.4)	0	-	97.8	2.2
All patients	101	98 (97)	3 (3)	0	0.725*	98.5	1.5
Myocardial infarction patients (MI)	84	81 (96.4)	3 (3.6)	0	0.532*	98.2	1.8
Reccurent myocardial infarction patients (RMI)	12	12 (100)	0	0	-	100	0
Ischemic stroke patients (IS)	17	17	0	0	-	100	0

***p value vs. controls**

In controls, G/G genotype equals 95.6% (n=108), heterozygous genotype G/A -4,4% (N=5) and mutated genotype A/A was not detected just like in patients. The frequency of alleles

G and A in patients is the following: 95,5% (allele G) and 1,5% (allele A), while we have a different result in controls: allele G equals 97,8% and allele A-2,2%.

96,4% of those 84 patients hospitalized with a diagnosis of MI turned out to have genotype G/G (n=81), while G/A was found in 3,6 % of the patients (n=3). Genotype AA (mutated) was not detected during the research process. 100% of the patients with a diagnosis of RMI had genotype G/G. As a result, genotype G/A was not found. Besides, we could not find mutation of prothrombin G202101 in patients diagnosed with IS. Thus, all the patients turned out to have a normal genotype G/G that determines the level of prothrombin in the blood clotting system.

The frequency of alleles in MI, RMI and IS patients is the following: in MI patients allele G equals 98,2%, and it is 100% in RMI and IS patients. Allele A is 1,8% in patients diagnosed with MI.

In sum, mutation of gene PT G20210A was found with low frequency in the patients than in controls. So, this mutation can not be considered causes of arterial thrombosis in the population of Adjara.

Mutation of gene MTHFR C677T: We conducted genetic research on 101 patients with MI and IS and on 113 controls to detect mutation of gene MTHFR C677T. A normal genotype CC of gene MTHFR is found in patients and controls with the following frequency: CC genotype in patients equals 42,6% (n=43) and in controls-61,1% (n=69). These figures indicate that there is a smaller number of a normal genotype in patients than in controls. Within the research contingent, the frequency of heterozygous genotype C/T in controls makes up 35,4% (n=40), while it is 1,4% higher in patients (48,5%) (n=49), (table 3).

Table 3: Genotype and allelic frequency of MTHFR C677T polymorphism in patients and control groups

Research contingent	N	Genotype frequncy			*p*-value	Allelic frequncy			*p*-value *
		C/C n (%)	C/T n (%)	T/T n (%)		C (%)	T (%)	Odd-ratio (95% CI)	
Controls	113	69 (61.1)	40 (35.4)	4 (3.5)	-	78.8	21.2	-	-

Patients	**101**	**43 (42.6)**	**49 (48.5)**	**9 (8.9)**	**0.016**	**66.8**	**33.2**	**1.84 (1.19-2.84)**	**0.0057**
Myocradial infarction patients (MI)	**84**	**36 (42.9)**	**42 (50.0)**	**6 (7.1)**	**0.040**	**67.9**	**32.1**	**1.76 (1.12-2.77)**	**0.0151**
Reccurent myocardial infarction patients (RMI)	**12**	**5 (41.7%)**	**5 (41.7)**	**2 (16.7)**	**0.147**	**62.5**	**37.5**	**2.23 (0.92-5.40)**	**0.0768**
Ischemic stroke patients (IS)	**17**	**7 (41.2)**	**8 (47.1)**	**2 (11.8)**	**0.210**	**64.7**	**35.3**	**2.02 (0.71-5.75)**	**0.1862**

P-value, odd ratio, CI –Confidence interval , *p value vs. Controls

An interesting and important discovery was made in patients and controls according to TT genotype frequency. The frequency of TT genotype made up 8,9% (n=9) in patients which is 2,5% higher than the one in controls whose mutated genotype frequency made up 3,5% (n=4).

The frequency of MTHFR C677T mutation varies in patients with MI, RMI and IS. According to table 3, the frequency of genotype C/C makes up 42,9% (n=36)in MI patients, while the picture is almost identical in RMI and IS patients: RMI-41,7% (n=5) and IS-41,2% (n=7).

C/T heterozygous genotype was found in the above-mentioned three groups with the following frequency: MI-50,0% (n=42), RMI-41,7% (n=5), IS-47,1% (n=8).

The highest frequency of TT mutated genotype 16,7% (n=2) was found in RMI patients, while C/T heterozygous genotypes were revealed with the highest frequency in MI patients. TT genotype frequency made up 11,8% (n=2) in IS patients and 7,1% (n=6) in MI ones.

In total, the frequency of allele T in patients makes up 33,2% and in controls-21,2%; allele C equals 66,8% in patients and in controls-78,8%.

As a result of stratifying patients into MI, RMI and IS groups, we got different results according to frequencies of alleles C and T. As we have already mentioned while characterizing frequencies of genotype, TT genotype is higher in RMI patients. Accordingly, the frequency of allele T is higher making up 37,5%. The frequency of allele C in the same group made up 62,5%.

Allele T in IS patients equals 35,3% and allele C-64,7%; In MI patients the frequencies of alleles T and C are 32,1% and 67,9%.

In conclusion, a heterozygous MTHFR gene mutation (methylenetetrahydrofolate reductase) does not affect ferment whose normal functioning depends on a normal allele existing in genotype. However, taking genetic drift into account, the probability of people being born with mutated genotype of MTHFR gene (TT) is higher when two heterozygous persons, who are either from different or the same populations, get married. As our research showed, the frequency of heterozygous genotype is high in both patients and controls along with recessive homozygous one.

The above-mentioned opinions and assumptions can be reinforced by the conclusion that A.Makatsaria made based on Spanish research results. According to the conclusion, the frequency of MTHFR recessive homozygous genotype in Spanish adolescents has become higher in recent years, which is caused by a rise in consumption of folates and decrease in spontaneous abortions (A.Makatsaria, 2014:393).

Characterizing of clinical data of patients with arterial thrombosis: We treated clinical data of 89 patients with arterial thrombosis biostatistically. Men involved in the research make up 71,3% and women - 28,7%.

An average age of the research contingent is 66, 3 +/- 12.1 years old. A diagnosis of arterial thrombosis was also made based on sex, which showed an age difference between males and females. For instance, men suffering from the desease are 60, 9+/-12.1 years old on average, and women-73.7+/-10.2 years old ($p<0,001$).

Diagnoses of MI and IS were made with a significant difference in age. For example, patients were diagnosed with MI at an average age of 62,6+/-12,9 years old, while a diagnosis of IS was made at the age of 71,5+/-11,7 ($p=0.013$).

Correlation between arterial thrombosis and sex has been proved scientifically. Within the scope of our research, we came across a sex difference among MI and IS patients. For instance, MI cases are 3.4% more in male patients than in female ones and they make up 79.2%, while the desease occurrence in women equals 20.8%. The results are different with IS patients: 58.8% of the patients diagnosed with IS are females and 41,2% -males, e.i women suffering from IS outnumber males by 1,4%. Significantly, 11 of 12 RMI patients are males against just 1 female (table 4).

Table 4. ***Distribution of myocardial infarction and ischemic stroke patientsby gender***

	Male n (%)	Female n (%)	Total	p-value
Myocardial infarction (MI)	**57 (79.2)**	**15 (20.8)**	**72**	**0.005**
Ischemic stroke (IS)	**7 (41.2)**	**10 (58.8)**	**17**	
Total	**64**	**25**	**89**	

With the aim of defining association between arterial thrombosis and diabetes, we processed data of MI and IS patients statistically. 23,6% of MI patients are diabetics and 76,4%- are not diabetic; 17,6% of IS patients have diabetes and 82,4% of them are not diabetic. The total number of diabetics is 22,5%. In terms of diabetes, we found no significant difference between MI and IS groups (table 5).

Table 5. Distribution of myocardial infarction and ischemic stroke patients based on diabetic status

	Diabetes n (%)	No diabetes n (%)	Total	p-value
Myocardial infarction (MI)	**17(23.6)**	**55 (76.4)**	**72**	**0.433**
Ischemic stroke (IS)	**3 (17.6)**	**14 (82.4)**	**17**	
Total	**20**	**69**	**89**	

In order to define correlation between arterial thrombosis and hypertension, we studied data collected from 89 MI and IS patients. The research contingent was stratified into two groups: patients with and without hypertension. 75% of MI patients suffer from high arterial pressure. Hypertension was derected in 64,7% of IS patients. 25% of MI and 35,3% of IS patients do not have hypertension.

In total 73% (n=65) of patients have hypertension. In terms of hypertension, we found no significant difference between MI and IS patients (table 6).

Table 6. Distribution of myocardial infarction and ischemic stroke patients with hypertension

	Hypertension		total	p-value
	Yes n (%)	No n (%)		
Myocardial infarction (MI)	**54 (75.0)**	**18 (25.0)**	**72**	**0.283**
Ischemic stroke (IS)	**11 (64.7)**	**6 (35.3)**	**17**	
Total	**65 (73.0)**	**24 (27.0)**	**89**	

While studying clinical data, we paid particular attention to the patients having a family history of thromboses. In total there were 69 patients (n=69).

According to patient's medical records, 49.3% of them seem to have a family history full of thrombosis cases. In particular, 50.8% of MI patients have a family history of thromboses; IS patients with heritable thrombosis make up 33.3%. Considering the results, there is a slight difference between the two groups (MI and IS). In total 50. 7% of MI and IS patients have a family history of arterial thrombosis (table 7).

Table 7. Family history of thrombosis in myocardial infarction and ischemic stroke patients

	Family history of thrombosis		Total	p-value
	Yes n (%)	No n (%)		
Myocardial infarction (MI)	**32 (50.8)**	**31 (49.2)**	**63**	**0.673**
Ischemic stroke (IS)	**2 (33.3)**	**4 (66.7)**	**6**	
Total	**34 (49.3)**	**35 (50.7)**	**69**	

With the aim of defining association between arterial thrombosis and smoking in MI and IS patients, we counted smokers and non-smokers within the research contingent.

With regard to smoking, we statistically processed data collected from 71 patients. 60,7% of MI patients smoke, while just 39,3% -do not. As for IS patients, 80% of them are smokers against 20% of non-smokers. The research revealed a slight difference between MI and IS patients who smoke (table 8).

Table 8. Distribution myocardial infarction and ischemic stroke patients who consume alcohol

	Alcohol consummation		Total	p-value
	Yes n (%)	No n (%)		
Myocardial infarction (MI)	**21 (37.5)**	**35 (62.5)**	**56**	**0.474**
Ischemic stroke(IS)	**2 (20.0)**	**8 (80.0)**	**10**	
Total	**23 (34.8)**	**43 (65.2)**	**66**	

Within the scope of the research, we studied the frequency of drinking alcohol in 66 patients diagnosed with arterial thrombosis. It is worth mentioning that all of the patients are males and they make up 34,5%. 37,5% of MI patients drink alcohol and 65,6 of them-do not; 20% of IS patients consume alcohol and 80% of them do not drink any alcoholic drinks. Thus, we found no significant difference between MI and IS patients in terms of drinking alcohol (table 9).

Table 9. Distribution myocardial infarction and ischemic stroke patients who consume alcohol

	Alcohol consumption		**Total**	**p-value**
	Yes n (%)	**No n (%)**		
Myocardial infarction (MI)	**21 (37.5)**	**35 (62.5)**	**56**	**0.474**
Ischemic stroke (IS)	**2 (20.0)**	**8 (80.0)**	**10**	
Total	**23 (34.8)**	**43 (65.2)**	**66**	

Based on the statistical analysis of clinical data collected from patients with arterial thrombosis in the population of Adjara, we have made the following conclusions: men involved in the research are 2,5% more than women. Besides, an average age of MI patients is 62 years old, and patients over 71 years old were diagnosed with IS (p=0.013). Also, while making a diagnosis, there is an age difference according to sex. For instance, on average, men are over 60 and women-over 73 years old (p<0.001). MI male patients outnumber female ones by 3,4%, whereas women with ishemic stroke are 1,4% more than males (p=0.005). Considering the statistical analysis of data on consuming alcohol, smoking, a family history of diabetes, hypertension and thrombosis, we do believe that it is important to collect data from a lot more patients so that we can get much more authentic results.

Conclusions

1. It was the first time they had studied MTHFR C677T, FVL G1691A and PTG20210A genes polymorphism in the Adjarian population;
2. We detected mutation of MTHFR C677T (methylenetetrahydrofolate reductase) gene encoding the ferment which regulates homocysteine metabolism;
3. One of the causes of arterial thrombosis in people with MTHFR C677T mutation can be a disorder of homocysteine metabolism;
4. We found heterozygous and homozygous polymorphism of MTHFR gene;
5. According to the research results, there was double prevalence of Factor V Leiden in patients in comparison to controls;
6. No connection was found between PT G202010A mutation and arterial thrombosis in the research contingent;
7. We found a significant difference between an average age of developing arterial thrombosis and sex (men-60,9+/-12,1 years old, women-73,7+/-10,2 years old).
8. There was a significant difference between developing myocardial infarction and ischemic stroke and age (MI-62,6+/-12,9 and IS - 71.5 +/- 11.7 years old);
9. We found a significant difference between the number of patients with myocardial infarction and ischemic stroke and sex (MI males-79,2%, females-20,8%);
10. The study showed that the stratified groups (myocardial infarction and ischemic stroke patients) experience the following characteristics: diabetes, hypertension, a family history of thrombosis, alcohol consumption and smoking with almost the same frequency.

Articles

1. Garakanidze Sopio , Elisio Costa , Elsa Bronze-Rocha , Alice Santos-Silva , Nikolaishvili Giorgi , Glonti Salome , Kakauridze Nona and Koridze Marina. FACTOR V LEIDEN G1691A AND PROTHROMBIN G20210A POLYMORPHISMS IN GEORGIAN ARTERIAL THROMBOSIS PATIENTS. DOI:10.21474/IJAR01/4825

2. Garakanidze S[1], Costa E[2], Bronze-Rocha E[2], Santos-Silva A[2], Nikolaishvili G[3], Nakashidze I[1], Kakauridze N[4], Glonti S[3], Khukhunaishvili R[1], Koridze M[1], AhmadS[5]. Methylenetetrahydrofolate Reductase Gene Polymorphism (C677T) as a Risk Factor for Arterial Thrombosis in Georgian Patients. Clin Appl Thromb Hemost. 2018 Oct; 24(7):1061-1066. Doi: 10.1177/1076029618757345. Epub 2018 Feb 13.

3. Sopio Garakanidze[1], Elísio Costa[2], Elsa Bronze-Rocha[2], Alice Santos-Silva[2], Giorgi Nikolaishvili[3], Irina Nakashidze[1,3], Nona Kakauridze[5], Salome Glonti[4], Rusudan Khukhunaishvili[1], Marina Koridze[1*], Sarfraz Ahmad[6*]. Characterization of a Cohort of Patients with Arterial Thrombosis from the Georgian Adjarian Population.

Abstracts

1. S. Garakanidze, M.Koridze, E. Manuel Sousa Costa, N. Kakauridze, G. Nikolaishvili, Marina Koridze. Batumi 6th International School-Seminar „Actual Issues of Biomedicine ‘‘„FACTOR V LEIDEN G1691A AND PROTHROMBIN G20210A POLYMORPHISMS IN GEORGIAN ARTERIAL THROMBOSIS PATIENTS‘‘.

2. Sopio Garakanidze, Elisio Costa, Nona Kakauridze, Giorgi Nikolaishvili, Marina Koridze. International Scientific Conference Future Technology and Quality of l Life. „The association between MTHFR C677T Polymorphism and Arterial Thrombosis‘‘.

3. Sopio Garakanidze[1], Elisio Costa[2], Nona Kakauridze[3], Rusudan Khukhunaishvili[1], Marina Koridze[1].The First International Conference in Georgia-Human Genome and Health. „ The Association Between Genetic Markers and Arterial Thrombosis‘‘.

4. Marina Koridze[1], Sopio Garakanidze[1], Elísio Costa[2], Elsa Bronze Rocha[2], Alice Santos Silva[2], Irina Nakashidze[1], Giorgi Nikolaishvili[1], Rusudan Khukhunaishvili[1], Aleko Kalandia[1], Salome Glonti[1], Nona Kakauridze[3], Sarfraz Ahmad4. European biotechnology Congress 2019. FVL G1691A and PT G20210A genes mutation and family history of thrombosis in Georgian myocardial infarction and ischemic stroke patients.

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- https://ghr.nlm.nih.gov/gene /MTHFR
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