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# ASSOCIATION BETWEEN RISK OF ISCHEMIC STROKE AND THE *RS17216473* SINGLE NUCLEOTIDE POLYMORPHISM IN THE 5-LIPOXYGENASE-ACTIVATING PROTEIN GENE LOCUS

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**Ключові слова:** *однонуклеотидний поліморфізм, генетика, ішемічний інсульт, ALOX5AP*

**Abstract. Association between risk of ischemic stroke and the *rs17216473* single nucleotide polymorphism in the 5-lipoxygenase-activating protein gene locus. Pavlenko O.Yu., Strokina I.G., Drevytska T.I., Karvatsky I.M., Sokurenko L.M., Dosenko V.E.** *Multiple studies have focused on the genetic basis of stroke, in particular on single nucleotide polymorphisms. However, the contribution of single nucleotide polymorphism rs17216473 in the gene that encodes ALOX5AP to stroke has been researched too little. The purpose of the work is to study the association between the risk of ischemic stroke and the single nucleotide polymorphism rs17216473 of the gene that encodes ALOX5AP, in particular, the G/G and G/A genotypes and alleles A and G, within the Ukrainian population. DNA extracted from leukocytes of venous blood of healthy donors (control group) and patients (stroke group) was studied. The control group consisted of 110 people, including 60 men (54.5%) and 50 women (45.5%), the average age in the group was 58.8±6.2 years, mode (Mo) by group was equal to 64 years. The absence of cardiovascular pathology in the donors was confirmed by anamnesis, electrocardiography and pressure measurement. The stroke group included 109 patients, 58 men (53.2%) and 51 women (46.8%), suffering from an acute violation of cerebral blood circulation (acute ischemic stroke). The focus of brain infarction was localized in the basin of the left middle cerebral artery in 51 patients (46.8%), in the basin of the right middle cerebral artery in 39 (35.8%), in the vertebral-basilar basin in 18 (16.5%) and in the basin of the left anterior cerebral artery in 1 patient (0.9%). 21 of these patients also have a history of ischemic stroke, 15 (13.8%) of them in the same basin and 6 (5.5%) in another vascular basin. The average age of patients at the time of brain infarction was 70.3±10.0 years, mode (Mo) by group was equal to 60 years. In the context of the study of the role of single nucleotide polymorphisms in the occurrence of stroke, the contingent of patients by age and localization of the focus of brain infarction is considered as homogeneous. Healthy donors and stroke patients belonged to the older age group, they are residents of Kyiv, the percentage of men and women in the indicated groups was almost the same, therefore, according to socio-demographic indicators, the group of stroke patients and the control group can be considered homogeneous. Taking into consideration the average age and mode (Mo) in the groups (58.8±6.2 years, Mo*

*was 64 years in the control group and 70.3±10.0 years, Mo was equal to 60 years in the stroke group), the groups are statistically comparable in age. All women in both groups are postmenopausal. Real-time polymerase chain reaction and the analysis to discriminate alleles were used. The statistical analysis was performed using χ2 criteria and by χ2 criteria with Yates correction. We identified two genotypes, G/G and G/A, of the single nucleotide polymorphism rs17216473 of the gene that encodes ALOX5AP. Humans that were homozygotes for the minor allele A (carrying the A/A genotype) were not found in our study. Genotype G/G was the most prevalent in both the control and stroke groups, without significant difference between these groups. The carries of the G/A genotype were not as significantly represented as those of the G/G genotype. Simultaneously, there was no significant difference in the quantity of G/A genotype carries between the control and stroke groups. The distribution of the G allele was not significantly different between the control and stroke groups. The same trend was observed for allele A; its number in the control group was almost identical to that in the stroke group, Neither the G/G nor G/A genotypes were significantly associated with ischemic stroke risk (p>0.05). Neither allele (dominant allele G or minor allele A) of single nucleotide polymorphism rs17216473 were significantly associated with ischemic stroke risk in the Ukrainian population (p>0.05). No contribution of single nucleotide polymorphism rs17216473 (SG13S377) in the gene encoding ALOX5AP to ischemic stroke onset was observed in a Ukrainian population.*

**Реферат. Зв'язок між ризиком ішемічного інсульту та однонуклеотидним поліморфізмом у локусі *rs17216473* гена білка, що активує 5-ліпоксигеназу. Павленко О.Ю., Строкіна І.Г., Древицька Т.І., Карвацький І.М., Сокуренко Л.М., Досенко В.Є.** *Численні дослідження зосереджені на генетичній основі інсульту, зокрема на однонуклеотидних поліморфізмах. Однак внесок однонуклеотидного поліморфізму rs17216473 у гені, який кодує ALOX5AP, до інсульту досліджено занадто мало. Метою роботи було вивчити зв'язок між ризиком ішемічного інсульту та однонуклеотидним поліморфізмом rs17216473 гена, що кодує ALOX5AP, зокрема генотипів G/G та G/A та алелей A та G, в українській популяції. Досліджували ДНК, що виділяли з лейкоцитів венозної крові здорових донорів (контрольна група) та хворих (група з інсультом). Контрольна група складалася зі 110 людей, серед яких 60 чоловіків (54,5%) та 50 жінок (45,5%), середній вік у групі становив 58,8±6,2 року, мода (Мо) в групі – 64 роки. Відсутність серцево-судинної патології в донорів було підтверджено даними анамнезу, електрокардіографії та вимірювання тиску. Група з інсультом складалась зі 109 хворих, серед яких 58 чоловіків (53,2%) та 51 жінка (46,8%), що страждали на гостре порушення мозкового кровообігу (гострий ішемічний інсульт) з локалізаціями вогнища інфаркту мозку в басейні лівої середньої мозкової артерії в 51 пацієнта (46,8%); у басейні правої середньої мозкової артерії в 39 (35,8%); вертебрально-базилярному басейні у 18 (16,5%) та в басейні лівої передньої мозкової артерії в 1 пацієнта (0,9%). Серед зазначених пацієнтів 21 також мав в анамнезі ішемічний інсульт, з них 15 (13,8%) у тому ж басейні та 6 (5,5%) в іншому судинному басейні. Середній вік хворих на момент виникнення інфаркту мозку становив 70,3±10,0 років, мода (Мо) в групі – 60 років. У контексті дослідження ролі однонуклеотидидних поліморфізмів у виникненні інсульту контингент хворих за віком та локалізацією вогнища інфаркту мозку розглядається як однорідний. Здорові донори та хворі на інсульт відносилися до старшої вікової групи, були мешканцями м. Києва, відсоток чоловіків та жінок у зазначених групах був майже однаковим, тому за соціально-демографічними показниками групу хворих на інсульт та контрольну можна вважати однорідними. З урахуванням середнього віку та моди (Мо) в групах (58,8±6,2 року, Мо – 64 роки в контрольній групі та 70,3±10,0 року, Мо – 60 років у групі з інсультом), групи є статистично зіставними за віком. Усі жінки в обох групах перебували в постменопаузальному періоді. Використовували полімеразну ланцюгову реакцію в реальному часі та аналіз дискримінації алелів. Статистичний аналіз проводили за критеріями χ2 та за критеріями χ2 з поправкою Єйтса. Ми ідентифікували два генотипи, G/G і G/A, однонуклеотидного поліморфізму rs17216473 гена, який кодує ALOX5AP. Людей-гомозигот за мінорним алелем А (носіїв генотипу А/А) не було знайдено у нашому дослідженні. Генотип G/G був найбільш поширеним як у контрольній групі, так і в групі з інсультом, без істотної різниці між цими групами. Носії генотипу G/A були представлені не так значно, як носії генотипу G/G. Водночас не було суттєвої різниці у кількості носіїв генотипу G/A між групою з інсультом та контрольною. Розподіл алеля G істотно не відрізнявся між контрольною групою та групою з інсультом. Така ж тенденція спостерігалася для алеля А; його кількість у контрольній групі була майже ідентичною такій у групі з інсультом. Ані генотип G/G, ані G/A не були достовірно пов'язані з ризиком ішемічного інсульту (p>0,05). Жоден з алелів (домінантний алель G чи мінорний алель А) однонуклеотидного поліморфізму rs17216473 не був достовірно пов'язаний з ризиком ішемічного інсульту в українській популяції (p>0,05). Внеску однонуклеотидного поліморфізму rs17216473 (SG13S377) у гені, що кодує ALOX5AP, у виникнення ішемічного інсульту в українській популяції не спостерігалося.*

Stroke is one of the most prominent causes of death in different populations and is a factor that considerably decreases the quality of life. The genetic contribution to stroke onset was discussed in multiple studies [1-8], while it was suggested to separate single-gene stroke disorders from polygenic or multifactorial stroke, where single nucleotide polymorphisms (SNPs) of different genes play a significant role [6]. Numerous studies indicate that SNPs are directly associated with the development of cardiovascular diseases, particularly stroke [9-15].

The most likely candidates for this role are SNPs of 5-lipoxygenase (ALOX5) and 5-lipoxygenase activating protein (ALOX5AP) genes [9, 10, 13, 16-

21], which plays a certain role in inflammation [9, 17]. SNP of the gene encoding phosphodiesterase 4D (PDE4D) also indirectly affects the inflammatory process, [22], some SNPs of this gene are probably linked with ischemic stroke [22-24]. The involvement of SNPs in another inflammatory gene encoding C-reactive protein (CRP) is more controversial and unproven [25, 26, 27]. Different SNPs of the gene encoding methylenetetrahydrofolate reductase (MTHFR) were connected with the risk of ischemic [28, 29] but not hemorrhagic stroke [28], while plasma homocysteine levels were genotype dependent. It is suggested that SNP can influence the level of homocysteine [29].

A few studies have indicated the involvement of SNP *rs17216473* (*SG13S377*) of the ALOX5AP gene in the development of cardiovascular diseases [13, 16], however, simultaneously, certain studies have denied this claim [30].

In this study, we selected the *rs17216473* polymorphism (*SG13S377*) of the ALOX5AP gene because it is suspected to play a role in stroke. We established its contribution to myocardial infarction [16], and, on the other hand, other SNPs of ALOX5AP are associated with both stroke and heart diseases within the same population [9]. The purpose of the work was to study the association between the risk of ischemic stroke and the single nucleotide polymorphism *rs17216473* of the gene that encodes ALOX5AP, in particular, the G/G and G/A genotypes and alleles A and G, within the Ukrainian population.

**MATERIALS AND METHODS OF RESEARCH**

The study was reviewed and approved by the Biomedical Ethics Committee of O.O. Bogomolets Institute of Physiology of the National Academy of Sciences of Ukraine, excerpt from the protocol No. 3/19 of the meeting of the Biomedical Ethics Committee dated 02.04.19. Written informed consent to participate in this study was obtained from all participants. The article does not contain any information that allows to identify the research participant either from the control group or from patients. The study was carried out in the department of General and molecular pathophysiology of O.O. Bogomolets Institute of Physiology of the National Academy of Sciences of Ukraine in accordance with bioethics principals set out in "Declaration of Helsinki: Ethical principles for medical research involving human subjects", developed by World Medical Association, “Universal Declaration on Bioethics and Human Rights” (UNESCO) and European Bioethics Convention (1997).

DNA extracted from leukocytes of venous blood of healthy donors (control group) and patients (stroke group) was studied. The control group consisted of 110 people, including 60 men (54.5%) and 50 women (45.5%), the average age in the group was 58.8±6.2 years, mode (Mo) by group was equal to 64 years. The stroke group included 109 patients, 58 men (53.2%) and 51 women (46.8%), suffering from an acute disruption of cerebral blood circulation (acute ischemic stroke). The focus of brain infarction was localized in the basin of the left middle cerebral artery in 51 patients (46.8%), in the basin of the right middle cerebral artery in 39 (35.8%), in the vertebral-basilar basin in 18 (16.5%) and in the basin of the left anterior cerebral artery in 1 patient (0.9%). 21 of these patients also have a history of ischemic stroke, 15 (13.8%) of them in the same basin and 6 (5.5%) in another vascular basin. The average age of patients at the time of brain infarction was 70.3±10.0 years, mode (Mo) by group was equal to 60 years). In the context of the study of the role of single nucleotide polymorphisms in the occurrence of stroke, the contingent of patients by age and localization of the focus of brain infarction is considered as homogeneous. Healthy donors and stroke patients belonged to the older age group, they are residents of Kyiv, the percentage of men and women in the indicated groups was almost the same, therefore, according to socio-demographic indicators, the group of stroke patients and the control group can be considered homogeneous. Age in the control group ranged from 52 to 65 years, in the stroke group from 60 to 80 years, in our study the arithmetic average of the group provides objective information about age in combination with the mode (Mo). Guryanov V.G. et al. [31] note that the mode of distribution of the values of a quantitative variable is the value of the variable that occurs with the greatest frequency [31]. Taking into consideration the average age in the combination with Mo by group (58.8±6.2 years, Mo was 64 years in the control group and 70.3±10.0 years, Mo was equal to 60 years in the stroke group), the groups are statistically comparable in age. All women in both groups are postmenopausal.

Leukocytes of whole venous blood from healthy individuals (control group) and individuals with acute ischemic stroke (stroke group) were collected by medical staff at medical institutions, Kyiv City Blood Center and Kyiv City Clinical Hospital No. 4, I and II Neurological Departments under the supervision of employees of P.L. Shupyk National Medical Academy of Postgraduate Education with the written informed consent of all participants. The absence of cardiovascular pathology, including congenital, in the donors was almost completely confirmed by anamnesis, external examination, auscultation, electrocardiography and pressure measurement.

The reagents and genotyping methods used in this study were identical to those in a previous study [16]. The following reagents were used: the DIAtom DNA Prep kit (Isogene Lab LTD), containing lysing reagent (guanidine isothiocyanate), sorbent (*NucleoS*), saline solution to wash off DNA, ExtraGene to elute DNA from the sorbent, and a mixture of probes for TaqMan® PCR. Deionized water was used to prepare the water solutions [16]. DNA was extracted from blood samples using the DIAtom DNA Prep kit (Isogene Lab LTD). The kit contains the lysing reagent guanidine isothiocyanate, sorbent *NucleoS*, saline solution, and ExtraGene to elute DNA from the sorbent. This method is based on the use of guanidine isothiocyanate as a lysing reagent for cell lysis, solubilization of cell debris, and denaturation of cell nucleases. DNA is actively being absorbed on *NucleoS*™ under the presence of lysing reagent and then easily washed off from proteins with saline solution and ethanol. Subsequently, the DNA was extracted from the sorbent and transferred to sterile DNA-free and RNA-free microtest tubules (Eppendorf, Humburg, Germany). The obtained DNA was used for real-time PCR.

Allele polymorphisms of the ALOX5AP gene (G→A) were determined using TaqMan® SNP Assay C_11599359_10. Amplification was performed with a 7500 Fast Real-time PCR System (Applied Biosystems, Forster City, CA, USA).

To determine which of the nucleotides, A or G, was present at the *rs17216473* locus of ALOX5AP, we obtained the genotypes in the study groups (the stroke and control groups) by allele discrimination analysis using a 7500 Fast Real-time PCR System Software (Applied Biosystems, Foster City, CA, USA), and the number of alleles and the number of individuals having the corresponding genotype (A/A; A/G; G/G) within the control group (healthy) and patients with stroke were counted. The contributions of the two genotypes and the A and G alleles were analyzed in the current study [16].

The data obtained in the study were processed using the Microsoft Excel for Microsoft 365 MSO license program, license ID: CWW_8345bc9a-868e-426b-9026-8222e41cea63_8345bc9a-868e-426b-9026-8222e41cea63_d3a12f339eddfc0edd, Microsoft Excel for Mac version 16.86 (24060916) License ID: CWW_8345bc9a-868e-426b-9026-8222e41cea63_ 8345bc9a-868e-426b-9026-8222e41cea63_ 74bc5ead496badbe37.

Analyzed data were: absolute number (in individuals) and relative (in percentage) carriers of genotypes A/A, A/G and G/G at the polymorphic locus *rs17216473* of the ALOX5AP gene in the group of stroke patients and in the control group, respectively, and the absolute number of alleles A and G and relative (in percentages) in the indicated groups.

We verified the distribution of the genotypes in stroke patients and in the control group for consistency with Hardy-Weinberg law [32, 33]. Then the results was analyzed using the $\chi 2$ criteria [32]. To evaluate the association between the G/G and A/G genotypes and the alleles A and G in the *rs17216473* polymorphic locus of the ALOX5AP gene and the risk of stroke, we compared the control group with the stroke group using $\chi^2$ and $\chi^2$ with Yates correction criteria [31, 32]. No A/A genotype carriers were identified in our study. The statistical significance was estimated using $\chi^2$ criteria (Pearson criteria). The association was considered statistically significant at $p<0.05$ [16].

**RESULTS AND DISCUSSION**

We first verified the distribution of the genotypes in stroke group and in the control group for consistency with Hardy-Weinberg law. The results are fully included in the divisions of the Hardy-Weinberg law (Table 1), according to it, $\chi 2$ max is more than $\chi 2$ for both stroke group and control group respectively. Therefore, the results can be analyzed using the $\chi 2$ criteria.

*Table 1*

**Distribution of genotypes in stroke group and in the control group according to the Hardy-Weinberg law. The number of genotype carries in absolute units (n)**

| Genotype | Control group n=110 | Stroke group n=109 |
|---|---|---|
| G/G the number of genotype carries in the group, individuals (n) | 89 | 83 |
| G/A the number of genotype carries in the group, individuals (n) | 21 | 26 |
| A/A the number of genotype carries in the group, individuals (n) | 0 | 0 |
| $\chi 2$ | 1.2 | 2.0 |
| $\chi 2$ max | 3.8 | 3.8 |

**Notes:** $\chi 2$ max is more than $\chi 2$ for both stroke group and control group respectively. Therefore, the results can be analyzed using the $\chi 2$ criteria.

Different statistical methods have been developed to estimate SNPs heritability, which measures the proportion of phenotypic variance explained by all measured SNPs in the data [34, 35]. We focus on the accordance between phenotypic manifestation of stroke and SNP *rs17216473*. There are two variants of nucleotides in the SNP, G or A nucleotides, which can give G/G, G/A or A/A genotypes in corresponding locus. G allele is dominant one; A allele is minor allele and it was assumed to play a role in the stroke onset. In other words, the carries of A/A genotype should have had phenotypic manifestations of stroke with a high probability, the carries of G/A genotype should have had phenotypic manifestations of stroke with a moderate probability, the carries of G/G genotype should have had it with low probability or absence of stroke.

We found two genotypes in the polymorphic locus of *rs17216473* in the ALOX5AP gene: G/G and G/A. We did not identify carriers of the A/A genotype in the stroke and control group, in contrast to the study of peripheral artery disease, where all genotypes A/A, G/G and G/A in the locus of *rs17216473* were present [11]. The percentage distribution of G/G and G/A genotype carriers in the control group corresponded to 80.9% and 19.1%, respectively, in the stroke group percentages of G/G and G/A genotype carriers were 76.1% and 23.9%, respectively (Table 2). Genotype G/G was the most prevalent in both the control and stroke groups. The G/G genotype distribution in the stroke group was almost identical to that in the control group, whereas that in the myocardium infarction group was significantly lower than that in the control group [16]. That is, an essential difference between the G/G genotype distribution of the specified SNP in patients with stroke and myocardial infarction is assumed [16]. In our previous study, we proposed the use of the G/G genotype as a good prognostic indicator to avoid myocardial infarction [16]. However, we cannot say the same for stroke, as the G/G genotype does not contribute to stroke prevention.

*Table 2*

**Distribution of genotypes G/G, G/A, and A/A in the control and stroke group. The number of genotype carries is in absolute units (n), frequency of genotypes obtained from the sample (percentage of genotypes carriers in the group, %±m%)**

| Genotypes | Control group n=110 | Stroke group n=109 | Total number of control and stroke groups, n=219 |
|---|---|---|---|
| Number of G/G genotype carriers in the group, individuals (n) | 89 | 83 | 172 |
| Percentage of G/G genotype carriers in the group, (%±m%) | 80.9±3.8 | 76.1±4.1 | 78.5±2.8 |
| Number of G/A genotype carriers in the group, individuals (n) | 21 | 26 | 47 |
| Percentage of G/A genotype carriers in the group, (%±m%) | 19.1±3.8 | 23.9±4.1 | 21.5±2.8 |
| Number of A/A genotype carriers in the group, individuals (n) | 0 | 0 | 0 |
| Percentage of A/A genotype carriers in the group, (%±m%) | 0 | 0 | 0 |

**Notes:** n – the number of genotype carries in the group; % – the frequency of genotype obtained from the sample (percentage of genotypes carriers in the group); m – the standard error of the frequency of the qualitative variable.

When analyzing the G/G distribution in comparison with G/A, it was seen that G/G was also prevalent in both the control and stroke groups, totaling 76.1% against 23.9% for G/A in the stroke group and 80.9% against 19.1% for G/A in the control group (Table 2). In the study of peripheral artery disease in the locus of *rs17216473* the G/G genotype is also the most common, the second is the G/A genotype, and the least common is the A/A genotype. However, the frequency of G/G is less than that in our study, and G/A, on the contrary, is greater [11].

Simultaneously, in our study, there was no significant difference in G/A genotype between the control and stroke groups (21 individuals (19.1%) and 26 individuals (23.9%), respectively) (Table 2). The A/A genotype was absent in both groups (Table 2). This was the next significant difference between stroke and myocardial infarction in which we found homozygotic individuals with myocardial infarction [16]. At the same time, Jin S. et al. [11] found A/A genotype in the same SNP ALOXAP.

The distribution of the G allele was not significantly different between the control and stroke groups. The same trend was observed for allele A; its number in the control group was almost identical to that in the stroke group (Table 3). The difference between alleles G and A was essential, which was expected, considering the absence of carriers with the A/A genotype in the study groups and the fact that the number of G/G carriers was larger than that of G/A carriers. Jin S. et al. [11] detected significantly more percentage of A allele in the same ALOXAP SNP that we did, which can be explained with the presence of A/A genotype in their study.

*Table 3*

**Distribution of G and A alleles in the control and stroke groups. The number of allele is in absolute units (n), frequency of alleles obtained from the sample (percentage of allele in the group, %±m%)**

| Alleles | Total number of G and A alleles | |
|---|---|---|
| | control group n=220 | stroke group n=218 |
| Number of G allele in the group, (n) | 199 | 192 |
| Percentage of G allele in the group, (%±m%) | 90.5±2.0 | 88.1±2.2 |
| Number of A allele in the group, (n) | 21 | 26 |
| Percentage of A allele in the group, (%±m%) | 9.5±2.0 | 11.9±2.2 |

**Notes:** n – the number of alleles in the group; % – the frequency of the allele obtained in the sample (percentage of the allele in the group); m – the standard error of the frequency of the qualitative variable.

We then tried to understand the contribution of the nucleotide G substitution for A in the current SNP to stroke, by counting the links between G/G and G/A genotypes and stroke using Pearson criteria ($\chi^2$). Subsequently, the same procedure was performed for determining the association between G and A alleles and stroke.

Statistical analysis of the links between the G/G and G/A genotypes and stroke showed no statistically significant connections between these genotypes and stroke ($p>0.05$); specifically, the G/G and G/A genotypes were not associated with stroke (Table 4).

*Table 4*

**Association between G/G and G/A genotypes and stroke using Pearson criteria ($\chi^2$). The number of genotype carries is in absolute units (n)**

| Genotype | With stroke (number of genotype carries, individuals (n) | Without stroke (number of genotype carries, individuals (n) | $\chi^2$ (Value of criteria/ level of significance) | $\chi^2$ with Yates correction (Value of criteria/ level of significance) |
|---|---|---|---|---|
| G/G | 83 | 89 | 0.737**/ p=0.391*** | 0.481**/ p=0.488*** |
| G/A | 26 | 21 | | |

**Notes:** association between G/G and G/A genotypes and stroke was estimated by Pearson criteria ($\chi^2$), it is statistically significant when $p<0.05$; ** – the values of $\chi^2$ criteria and $\chi^2$ with Yates correction; *** – the level of significance p.

We also found no statistically significant connection between the A and G alleles and stroke ($p>0.05$) (Table 5). Opposite results were obtained by Shah S.H. et al. [13]. SNP *rs17216473* of the ALOX5AP gene was associated with in-stent restenosis after percutaneous coronary intervention. The authors note that atherosclerosis risk factors coincide with the risk of in-stent restenosis [13]. At the same time, the A allele contributed to restenosis, while the G allele, on the contrary, played a protective role [13], just as the G allele of this polymorphism reduced the

risk of myocardial infarction, and the A allele increased it in our previous study [16].

Currently, there are isolated data in public databases on the contribution of *rs17216473* of the ALOX5AP gene to the development of stroke. Zheng J.H. et al. [30] reported about the absence of association between haplotype B containing SNP *rs17216473* together with other SNPs and ischemic stroke risk, which correlates with our results. On the contrary, the data of Shah S.H. et al. [13] can be considered in favor of the role of this polymorphism in the stroke development, because atherosclerosis is a significant factor in the ischemic stroke onset [10, 13].

*Table 5*

**Association between A and G alleles and stroke using Pearson criteria ($\chi^2$). The number of A and G alleles is in absolute units (n)**

| Allele | With stroke (number of alleles (n) | Without stroke (number of alleles (n) | $\chi^2$ (value of criteria/ level of significance) | $\chi^2$ with Yates correction (value of criteria/ level of significance) |
|---|---|---|---|---|
| A | 26 | 21 | 0.648**/ p=0.421*** | 0.423**/ p=0.516*** |
| G | 192 | 199 | | |

**Notes:** association between G/G and G/A genotypes and stroke was estimated by Pearson criteria ($\chi^2$), it is statistically significant when $p<0.05$; ** – the values of $\chi^2$ criteria and $\chi^2$ with Yates correction; *** – the level of significance p.

The role of other ALOX5AP polymorphisms in the stroke onset is studied much more. Thus, it is noted that there is a connection between stroke and other SNPs ALOX5AP in an Icelandic population [9] and a group of Scottish people [10], in both studies, an association with stroke was established for ALOX5AP SNPs grouped in haplotype A, while for ALOX5AP SNPs belonging to haplotype B, no reliable link with stroke was found [9, 10]. Bie X. et al. [17] showed that 5 haplotypes of the promoter region of the ALOX5AP gene locus *rs4073259* were statistically more frequent in ischemic stroke patients compared to controls. Shah S.H. et al. [13] also reported about the connection of SNPs ALOX5AP *rs17222814* allele G, *rs10507391* allele A and *rs17222814* allele A with ischemic stroke, with *rs17222814* allele A being protective, in contrast to the other two SNPs [13]. In favor of the participation of SNPs ALOX5AP in the stroke development, the increased level of ALOX5AP gene expression in patients with ischemic stroke in the study of the T-allele of SNP SG13S114 is also evidenced. It has been shown that this allele is a risk factor for ischemic stroke [18]. There are also data on an increased level of ALOX5AP gene expression in the *rs4073259* locus and mRNA expression in patients with ischemic stroke [17]. A significant relationship between SNPs SG13S114 and SG13S100 of the ALOX5AP gene and stroke [19], a connection between SNP *rs4073259* of the A allele of the ALOX5AP gene and the risk of cerebral infarction [20], and an association between subclinical manifestations of atherosclerosis and SNPs of ALOX5AP in patients with diabetes mellitus [21].

At the same time, there are studies disproving the role of ALOX5AP gene SNPs in the occurrence of stroke. Thus, according to Lemaitre R.N. et al. [36], no correlation was established between SNPs of the ALOX5AP gene and stroke. Zheng J.H. et al. [30] reported about the absence of association between ALOX5AP gene SNPs *rs10507391, rs4769874, rs9551963, rs17222814, rs17222919, rs4073259*, and also Haplotype A and B and ischemic stroke risk.

Jin S. et al. [11] showed that SNPs of ALOX5AP (except A/A in *rs17216473*, A/A in *rs10507391*, G/G in *rs4769874*, and A/A in *rs9551963*) were associated with the prevalence of peripheral arterial disease and also some genotype combinations of ALOX5AP could be a risk factor for peripheral arterial disease. At the same time, the prevalence of peripheral arterial disease increases with fewer genotypes A/A in *rs17216473*, A/A in *rs10507391*, G/G in *rs4769874*, and A/A in *rs9551963* [11].

According to the TOAST classification (Trial of Org 10172 in Acute Stroke Treatment), based on the etiology of stroke, cerebral ischemic stroke has 5 main pathogenetic subtypes: 1) large-artery atherosclerosis (LAA), 2) cardioembolism (CE), 3) small-vessel occlusion (SVO), 4) stroke of other determined etiology (ODE), 5) stroke of undetermined etiology (UDE) [37]. It can be assumed that in the pathophysiological changes inherent in different types of acute cerebral stroke, SNPs of different genes should play a certain role, since ischemic stroke is a poly-

genic disease [6, 10] and even one subtype is most likely caused by SNPs of different genes. Thus, the LAA subtype was observed in the study of SNPs of the gene ALOX5AP [10, 19], SNPs of the gene encoding PDE4D [22, 24], SNPs of the gene encoding CRP [26], SNPs of the gene encoding MTHFR [29]; CE subtype was registered in the study of SNPs of the ALOX5AP gene [10, 19] and SNPs of the gene encoding PDE4D [6, 22, 24]; SVO in the study of SNPs of the gene encoding CRP [27] and the ALOX5AP gene [10, 19]. Two other subtypes of ischemic stroke, ODE and UDE, were observed in in the study of ALOX5AP SNPs [10, 19]. All of the above SNPs were not only observed but also correlated with ischemic stroke. SNPs of numerous genes play a role in the occurrence of various subtypes of ischemic stroke [4], however, SNPs of ALOX5AP, SNPs of genes encoding PDE4D, CRP, and MTHFR are closely associated with inflammation and atherosclerosis. Atherosclerosis, in turn, is a chronic vascular inflammatory disease [38]. The role of "pro-inflammatory" genes in subjects with ischemic stroke in immunoinflammatory activation of the acute phase of stroke is noted [7].

ALOX5AP SNPs increase leukotriene B4 production in stimulated neutrophils, thereby contributing to vascular inflammation in myocardial infarction and stroke [9]. Possible consequences of inflammation are atrial fibrillation [22] and the development of atherosclerosis [22, 39]. Atrial fibrillation is one of the causes of CE subtype [40] and was observed in LAA, CVO and CE subtypes [41]. Stroke most often occurs on the basis of atherosclerosis [10, 40], especially the LAA subtype. One of the causes of atherosclerosis is an increased level of homocysteine [6, 42], patients with ischemic stroke have a significantly higher level of homocysteine compared to controls [43]. In turn, hyperhomocysteinemia can be caused by a genetic defect of the MTHFR enzyme [42]. Jin M. et al. [29] noted that one of the SNPs in the gene encoding MTHFR could affect homocysteine levels and, either alone or in combination with other factors, increases the risk of LAA [29].

SNPs of the gene encoding phosphodiesterase 4D (PDE4D) were significantly associated with CE and LAA subtypes [24]. PDE4D specifically degrades cyclic adenosine monophosphate (cAMP) and is one of the main enzymes of the cAMP signaling pathway in inflammatory cells. A decrease in cAMP leads to inflammation [22]. SNPs of the gene encoding CRP can theoretically also be involved in the development of atherosclerosis. C-reactive protein is an indicator of inflammation [39, 44], its plasma concentration increases in response to injury, infection, and inflammation [45]. SNPs of the gene encoding CRP can be associated with LAA [26] and SVO [27] subtypes, but its association with ischemic stroke is more controversial and unproven [25, 26, 27, 46].

As can be seen, the data are contradictory and insufficient, which requires further study of the role of the ALOX5AP SNP in stroke onset. Our study is one of the attempts to detect genetic predisposition to ischemic stroke disease or risk factors.

**CONCLUSIONS**

1. No statistically significant association was observed between stroke and G/G and G/A genotypes or G and A alleles of rs17216473 (SG13S377) of the gene encoding ALOX5AP in a Ukrainian population.
2. No contribution of single nucleotide polymorphism rs17216473 (SG13S377) in the gene encoding ALOX5AP to ischemic stroke onset was observed in a Ukrainian population.

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**Contributions**:

Pavlenko O.Yu. – formal analysis, investigation, writing – original draft, writing – review and editing the manuscript;

Strokina I.G. – formal analysis, writing – original draft, manuscript translation into English, writing – review and editing the manuscript;

Drevytska T.I. – investigation, writing – original draft, writing – review and editing the manuscript;

Karvatsky I.M. – computing resources, formal analysis;

Sokurenko L.M. – writing – original draft, writing – review and editing the manuscript;

Dosenko V.E. – conceptualization, data curation, methodology, formal analysis, investigation, writing – original draft, writing – review and editing the manuscript, supervision, project administration.

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