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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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THE PREVALENCE OF C3953T IL1B GENE AND G308A TNFA GENE POLYMORPHIC VARIANTS IN THE PATIENTS WITH DIFFERENT TYPES OF ENCEPHALOPATHIES

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Abstract.

Introduction: It is essential to study disorders of the immune system in chronic encephalopathies of various genesis, considering that the mechanisms of brain damage remain unknown in their molecular basis. Among numerous inflammatory mediators, cytokines are particular in regulating immunological interactions. Many factors, including the genetic ones, determine these pro-inflammatory proteins' activity.

Aim: The aim of study was to study the prevalence of IL1β C3953T gene polymorphism and TNFα G308A gene polymorphism in patients with chronic traumatic encephalopathy (CTE), microvascular ischemic disease of the brain (or cerebral small vessel disease, (SVD)), chronic alcohol-induced encephalopathy (AIE) and postinfectious encephalopathy (PIE), and to evaluate the impact of a particular genotype presence on the occurrence and/or progression of encephalopathy.

Materials and methods: The molecular genetic study of polymorphic variants - C3953T of the IL1β gene and G308A of the TNFα gene was applied for 96 patients with encephalopathies of various genesis (CTE n=26, CAIE n=26, SVD n=18, and PIE n=26). The patients were undergoing treatment in the neurological departments of the Communal Non-commercial Enterprise "Ternopil Regional Clinical Psychoneurological Hospital" of Ternopil Regional Council (Ternopil, Ukraine) during 2021–2022. The control group consisted of 12 healthy persons, who were representative in terms of age and sex. Statistical processing of the results was carried out using the STATISTICA 10.0 software package.

Results and discussion: The frequency distribution analysis of the genotypes of the polymorphic variant C3953T of the IL1β gene and G308A of the TNFα gene in patients with CTE, SVD, CAIE, and PIE compared to individuals of the control group was performed. The statistically significant differences were found only in patients with PIE: 26.92% vs. 83.33% – carriers of the C/ C genotype, 61.54% versus 16.67% – carriers of the C/T genotype and 11.54% versus 0% – carriers of the T/T genotype and 53.85% versus 91.67% – carriers of the G/G genotype, 46 .15% versus 8.33% – carriers of the G/A genotype and 0.0% versus 0.0% – carriers of the A/A genotype, respectively. In addition, in the group of patients with PIE, the distribution of genotype frequencies of the polymorphic variant C3953T of the IL1β gene probably differed from the data of patients with CTE, SVD, and PIE ($\chi 2=28.64$; $p<0.001$), and in the group of patients with CAIE, the distribution of genotype frequencies of the polymorphic variant G308A of the TNFα gene probably differed from the data of patients with SVD and PIE ($\chi 2=24.91$; $p=0.002$). Analyzing the odds ratio and its confidence interval for the genotypes of polymorphic variants C3953T of the IL1β gene and G308A of the TNFα gene in patients with CTE, SVD, CAIE, and PIE, it was established that the presence of the C/T genotype of the IL1β gene increases the risk of encephalopathy in patients with PIE by 8.0 times, and the presence of the G/A genotype of the TNFα gene increases the risk of encephalopathy in patients with PIE by 9.4 times.

Conclusions: For the first time in the Ukrainian population, an analysis of the frequency distribution of the genotypes of the polymorphic variant C3953T of the IL1β gene and G308A of the TNFα gene in patients with chronic encephalopathies of various genesis was performed. Statistically, significant differences were found only in patients with PIE compared to healthy individuals. At the same time, the presence of the C/T genotype of the IL1β gene increases the risk of the occurrence and/or progression of PIE by 8.0 times, and the presence of the G/A genotype of the TNFα gene by 9.4 times, which indicates the feasibility of including the corresponding single-nucleotide polymorphisms in the genetic panel of the study patients with PIE.

Key words. Encephalopathy, C3953T IL1β gene, G308A TNFα gene, gene polymorphism.

Introduction.

Neurological disorders are a significant and increasing global health challenge that would significantly impair cognitive-motor function. Globally, in 2019, there were nearly 10 million deaths and 349 million disability-adjusted life years (DALYs) due to neurological disorders [1,2]. In neurological pathology, encephalopathies remain the most relevant and socially significant due to the steady increase in morbidity, the development of pronounced neuropsychological disorders, the negative impact on the quality of life, and the early disability of patients [3]. Encephalopathy (EP) is a broad term that encompasses a wide range of presentations and aetiologies. The term is often used heterogeneously, and conformity to strict definitions and confirmation of the pathophysiology can be lacking [4]. Given the fact that the pathogenetic mechanisms of brain damage, in their molecular basis, remain unknown, it is essential to study disorders of the immune system in encephalopathies of various genesis. There is data that the mechanisms of immunological response affect the course of the disease [5].

Due to the presence of the blood-brain barrier (BBB), the absence of a classic lymphatic system, and the limited penetration of peripheral immunocompetent cells into its parenchyma, the brain has traditionally been considered an immune-privileged organ. However, nonspecific, and specific immune response elements are readily organized in the central nervous system upon the action of various pathogens, autoantigens, or brain tissue damage of multiple etiologies. Astrocytes, microglia, neurons, endothelial cells of the BBB, and blood cells that

penetrate the brain parenchyma produce pro-inflammatory and anti-inflammatory molecules. Among the numerous mediators of inflammation, a unique role in regulating immunological interactions is played by cytokines, which induce or suppress their synthesis, the synthesis of other cytokines and their receptors, participating in the formation of a cytokine network [6-8].

Many factors, including genetic ones, determine the activity of the cytokine network. Cytokine genes have an extremely high degree of polymorphism, and the number of polymorphic regions in one gene can reach several dozen and be localized both in the coding regions of the gene - exons, as well as in non-coding introns and promoter regions of the gene [9].

The activity of the immune response is related to the polymorphism of genes encoding cytokines. In other words, the presence of allelic polymorphism ensures the diversity of individuals in the degree of cytokine production during the formation of cellular reactions [10-12]. An allelic pair of genes can be homozygous or heterozygous. Each gene has two or more allelic variants, with the most common allele occurring in the population at a frequency of ≤95%. Alleles can exist in two alternative states—wild and mutant. A wild-type allele is a typical ("normal") form of a gene, usually the most common phenotype in a natural population; in contrast, a mutant allele is the result of a mutation, a nucleotide substitution [13].

The aim was to study the prevalence of IL1β C3953T gene polymorphism and TNFα G308A gene polymorphism in patients with CTE, SVD, CAIE, and PIE and to assess the influence of the presence of a particular genotype of the studied genes on the occurrence and/or progression of encephalopathy.

Materials and methods.

96 patients with encephalopathies of various genesis were examined. All the patients were undergoing treatment in the neurological departments of the Communal Non-commercial Enterprise "Ternopil Regional Clinical Psychoneurological Hospital" of Ternopil Regional Council (Ternopil, Ukraine) during 2021–2022. The formation of groups of examined patients was based on the genesis of encephalopathy; in particular, the distribution by type of encephalopathies was as follows: chronic traumatic encephalopathy (CTE) – 26, chronic alcohol-induced encephalopathy (AIE) – 26, microvascular ischemic disease of the brain (or cerebral small vessel disease, (SVD)) – 18 and post-infectious encephalopathy (PIE) – 26. The control group (CG) consisted of 12 people, representative in terms of age and sex.

Considering the fact that currently there is no unified classification of encephalopathies and their stages, which would take into account the genesis and clinic of each type, the verification of various types of encephalopathies was carried out according to the criteria proposed by several authors [2-4]. Numerous factors, in particular, determine the course of each of the studied subtypes of encephalopathies, the immediate cause of encephalopathy, the influence of this cause on the development and progression of brain tissue damage and clinical manifestations, respectively, as well as the effect of concomitant diseases and the degree of their compensation. Each type of encephalopathy, depending on the severity and course of the disease, is characterized by a particular spectrum of neurological symptoms: behavior disorders, apathy, changes in memory and attention, decline in cognitive functions up to dementia, extrapyramidal disorders, pyramidal insufficiency, moderate neurological deficit.

Patient inclusion criteria were the following: age from 18 to 75 years; compliance with diagnosis criteria; availability of the patient's informed consent. Exclusion criteria: the presence of oncopathology; concomitant pathology in the stage of decompensation; use of psychoactive substances, the presence of other diseases that could be the cause of psychoneurological disorders, behavioral and mental disorders.

The performed study is a single-moment clinical study of the "case-control" type. The study protocol included screening of patients to determine compliance with inclusion and exclusion criteria, carrying out laboratory determinations, genetic research, and statistical analysis of the obtained data. All patients were informed about the purpose of the clinical study and gave written informed consent for their participation in it. Confidentiality about the patient's identity and state of health was preserved. The patient's informed consent form, examination card, and all stages of the research were approved by the bioethics commission of the Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine.

Molecular genetic study of the polymorphic variant C3953T of the IL1β gene and G308A of the TNFα gene. Its first stage was isolating DNA from whole peripheral blood on a paper blank using the commercial kit "Quick-DNA Miniprep Plus Kit" (Zymo Research, USA) according to the instructions. Molecular and genetic differentiation of the studied gene variants was carried out by the methods of allele-specific PCR or RFLP PCR (restriction fragment length polymorphism) by standard operational protocols developed in the molecular genetics laboratory of the SI "RCMD of Public Health Ministry of Ukraine"

Electrophoretic distribution was carried out in the System for horizontal electrophoresis multi–Sub Midi (Cleaver Scientific, Great Britain). The size of amplified and restriction fragments was estimated by comparison with the molecular weight marker Gene Ruler DNA Ladder (Thermo Scientific, USA) in an ethidium bromide-stained 3% agarose gel (Cleaver Scientific, UK). In the visualization process, the formed fragments for each sample were evaluated, and photofixation of the obtained images was carried out. Samples were genotyped according to facility-approved SOPs by considering the molecular weight of the restriction/amplified fragments compared to the molecular weight of the corresponding positive control samples (Table 1).

Table 1. *Molecular weight of restriction/amplified fragments.*

Gene and polymorphism, rs	**The size of the restriction/amplified fragments and the corresponding genotype**
IL-1ß C3953T, rs1143634	Genotype *CC:* 138 and 112 bp. Genotype *CT*: 250, 138 and 112 bp. Genotype *TT:* 250 bp.
TNFα G308A, rs180062	Genotype *GG:* 87 and 20 bp Genotype *GA*: 107, 87 and 20 bp Genotype *AA:* 107 bp.

Statistical analysis.

The Hardy-Weinberg law was used to assess the correspondence between the genotypes of the selected sample and the general population. Comparison of observed frequencies and expected frequencies (Pearson Chi-Square, $\chi 2$), calculated using Pearson's formula: $p2 + 2pq + q2 = 1$ (Hardy-Weinberg equilibrium), was carried out using Pearson's $\chi 2$-square. When obtaining values of the reliability coefficient $p>0.05$, we accepted the "null" hypothesis about the equality of the samples, that is, the correspondence between the selected model and the general population. Comparative analysis of frequency tables was performed using Pearson Chi-Square ($\chi 2$) and Fisher exact p, two-tailed (in those cases when the values of expected frequencies (expected frequencies) of individual indicators did not exceed 5). To assess the influence of the factor (the presence of a particular gene genotype) on the investigated feature (occurrence and progression of the disease), the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated. The influence was considered statistically probable at $p<0.05$ for the OR.

Results.

Analysis of the frequency distribution of IL1β C3953T gene genotypes according to the Hardy-Weinberg law in patients with CTE, SVD, CAIE, and PIE and assessment of compliance with population balance was performed in all observation groups and the control group. It was established that the frequency of the genotype responsible for the C/T polymorphism of the IL1β gene both in patients with various types of encephalopathies and in the control, group did not deviate significantly from the Hardy-Weinberg equilibrium ($p>0.05$) (Table 2).

Analysis of the frequency distribution of TNFα G308A gene genotypes according to the Hardy-Weinberg law in patients with the studied types of encephalopathies and assessment of compliance with population balance was carried out in all observation groups and the control group. It was established that the frequency of the genotype responsible for the G/A polymorphism of the TNFα gene both in patients with various types of encephalopathies and in the control, group did not significantly deviate from the Hardy-Weinberg equilibrium ($p>0.05$) (Table 3).

Analyzing the frequency distribution of IL1β gene genotypes in patients with the studied types of encephalopathies and the control group, it was found that the C/C genotype prevailed in patients with CTE, SVD, CAIE and in the control group, while the C/T genotype prevailed in patients with PIE (Table 4). In addition, T/T genotype carriers were found only among patients with PIE. Comparing the distribution of genotypes of the IL1β gene in patients with the studied types of encephalopathies and controls, statistically significant differences were found only in patients with PIE, in whom the distribution of genotype frequencies according to the polymorphic variant of the IL1β gene was as follows: 26.92% of people were carriers of the C/C genotype, 61.54% – C/T genotype and 11.54% – T/T genotype. In addition, in the group of patients with PIE, IL1β gene genotype frequency distribution probably differed from the data of patients with CTE, SVD, and PIE ($\chi 2=28.64$; $p<0.001$).

The results of the frequency distribution of TNFα gene genotypes showed that the G/G genotype predominated in patients with CTE, SVD, CAIE, and in the control group, while in patients with PIE, the frequency distribution of G/G and G/A genotypes was even (Table 3). In patients with CTE, CAIE, PIE, and in controls, the A/A genotype of the TNFα gene was not detected. Comparing the distribution of genotypes of the TNFα gene in patients with the studied types of encephalopathies and controls, statistically significant differences were found only in patients with PIE, in whom the distribution of genotype frequencies according to the polymorphic variant of the TNFα gene was as follows: 53.85% of people were carriers of the G/G genotype, 46 .15% – G/A genotype and 0.0% – A/A genotype. At the same time, in the group of patients with CAIE, the frequency distribution of TNFα gene genotypes probably differed from the data of patients with SVD and PIE ($\chi 2=24.91$; $p=0.002$).

Analyzing the odds ratio and its confidence interval for the genotypes of the IL1β gene in patients with the studied types of encephalopathies, it was established that there is a statistically significant relationship between the carrier of the CT and CC genotypes and the risk of encephalopathy in patients with PIE (Table 5). Thus, the presence of the CT genotype increases the risk of encephalopathy in this cohort of patients by 8.0 times. In contrast, the presence of the CC genotype of the IL1β gene polymorphism has protective properties regarding the risk of encephalopathy in patients with PIE.

Analyzing the odds ratio and its confidence interval for the genotypes of the TNFα gene in patients with the studied types of encephalopathies, it was established that there is a statistically significant relationship between the carrier of the GA and GG genotypes and the risk of encephalopathy in patients with PIE (Table 6). Thus, the presence of the GA genotype increases the risk of encephalopathy in this cohort of patients by more than 9 times. In contrast, the presence of the GG genotype has protective properties regarding the risk of encephalopathy in patients with PIE. In addition, the protective properties of the GG genotype regarding the risk of encephalopathy in patients with SVD were revealed.

Discussion.

Among the numerous mediators of inflammation, cytokines play a special role in the regulation of immunological interactions, the role of which has also been proven in neurodamage. Interleukin one beta (IL1β) is the most important member of the IL-1 family. It is produced by numerous cell types, including brain parenchyma, neurons, and astrocytes after brain ischemic insult and its levels are increased after trauma. IL1β is also considered an important mediator of inflammation after cerebrovascular ischemia [14]. There is evidence that IL1β, released in the brain, contributes to the production of NO, and its levels in the brain reflect the degree of hypoxic-ischemic damage [15,16].

The gene encoding IL1β is mapped to chromosome 2 (2q14). In the region of this gene, 135 single-nucleotide substitutions were found, and the +3953C/T polymorphism is located in exon 5 of this gene, causing the substitution of cytosine (C) for thymine (T) at position +3953 of the nucleotide sequence. Analyzing the frequency distribution of the genotypes of the C3953T polymorphic variant of the IL1β gene in patients with

Table 2. *IL1β gene polymorphism according to the Hardy-Weinberg law in patients with different types of encephalopathies.*

Genotype		**CTE**		**SVD**		**CAIE**		**PIE**		**Control**	
		expected	**Available**	**expected**	**available**	**expected**	**Available**	**expected**	**Available**	**expected**	**Available**
		IL1β gene polymorphism									
Homozygotes that occur frequently	C/C	16,96	16	14,22	14	22,15	22	8,66	7	10,08	10
Heterozygotes	C/T	8,08	10	3,56	4	3,69	4	12,69	16	1,83	2
Homozygotes, which are rare	T/T	0,96	0	0,22	0	0,15	0	4,65	3	0,08	0
χ^2, p		χ^2=1,47; p>0,05		χ^2=0,28; p>0,05		χ^2=0,18; p>0,05		χ^2=1,77; p>0,05		χ^2=0,10; p>0,05	
Note. * – statistically significant result.											

Table 3. *Polymorphism of the TNFα gene according to the Hardy-Weinberg law in patients with various types of encephalopathies.*

Genotype		**CTE**		**SVD**		**CAIE**		**PIE**		**Control**	
		expected	**available**	**expected**	**available**	**expected**	**available**	**expected**	**available**	**expected**	**available**
		***TNFα* gene polymorphism**									
Homozygotes that occur frequently	G/G	18,62	18	8	9	22,15	22	15,38	14	11,02	11
Heterozygotes	G/A	6,77	8	8	6	3,69	4	9,23	12	0,96	1
Homozygotes, which are rare	A/A	0,62	0	2	3	0,15	0	1,38	0	0,02	0
χ^2, p		χ^2=0,86; p>0,05		χ^2=1,13; p>0,05		χ^2=0,18; p>0,05		χ^2=2,34; p>0,05		χ^2=0,02; p>0,05	
Note. * – statistically significant result.											

Table 4. *IL1β and TNFα gene polymorphisms in patients with different types of encephalopathies.*

	CTE		**SVD**		**CAIE**		**PIE**		**Control**	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
IL1β gene polymorphism										
C/C	16	61,54	14	77,78	22	84,62	7	26,92	10	83,33
C/T	10	38,46	4	22,22	4	15,38	16	61,54	2	16,67
T/T	0	0	0	0	0	0	3	11,54	0	0
χ^2 (EP/CG), p	χ^2=1,80; p=0,179		χ^2=0,14; p=0,709		χ^2=0,01; p=0,920		χ^2=10,71; p=0,005*		–	
χ^2, p	χ^2=28,64; p<0,001*; $p_{1-4,\ 2-4',\ 3-4}$<0,05*									
TNFα gene polymorphism										
G/G	18	69,23	9	50,00	22	84,62	14	53,85	11	91,67
G/A	8	30,77	6	33,33	4	15,38	12	46,15	1	8,33
A/A	0	0	3	16,67	0	0	0	0	0	0
χ^2 (EP/CG), p	χ^2=2,29; p=0,131		χ^2=5,80; p=0,055		χ^2=0,36; p=0,550		χ^2=5,22; p=0,022*		–	
χ^2, p	χ^2=24,91; p=0,002*; $p_{2-3',\ 3-4}$<0,05*									
Note. * – statistically significant result.										

Table 5. *Odds ratios for IL1β genotypes in patients with different types of encephalopathies.*

Type of encephalopathy	***IL1β* gene polymorphism**					
	C/C		**C/T**		**T/T**	
	OR	**95 % CI**	**OR**	**95 % CI**	**OR**	**95 % CI**
CTE	0,32	0,06−1,77	3,13	0,56−17,30	0,47	0,01−25,18
SVD	0,70	0,11−4,59	1,43	0,22−9,38	0,68	0,01−36.35
CAIE	1,10	0,17−7,03	0,91	0,14−5,81	0,47	0,01−25,18
PIE	0,07*	0,01−0,42	8,00*	1,44−44,30	3,72	0,18−77,97
Note. * – statistically significant result.						

Table 6. *Odds ratios for TNFα genotypes in patients with different types of encephalopathies.*

Type of encephalopathy	***TNFα* gene polymorphism**					
	GG		**GA**		**AA**	
	OR	**95 % CI**	**OR**	**95 % CI**	**OR**	**95 % CI**
CTE	0,20	0,02−1,86	4,89	0,54−44,57	0,47	0,01−25,18
SVD	0,09*	0,01−0,86	5,50	0,57−53,22	5,65	0,27−119,85
CAIE	0,50	0,05−5,03	2,00	0,20−20,10	0,47	0,01−25,18
PIE	0,11*	0,01−0,95	9,43*	1,06−84,04	0,47	0,01−25,18
Note. * – statistically significant result.						

CTE, SVD, CAIE and PIE compared to individuals of the control group, statistically significant differences were found only in patients with PIE, among whom the most carriers of the C/T genotype and the least of the T/T genotype were found. According to the data obtained by Aguet F. and coauthors (2020), the expression of IL-1β is highest in TT genotype and lowest in CC [17]. Licastro F. et al. investigated whether IL-1β polymorphisms affected neuro-pathological features and clinical status of Alzheimer's disease (AD) patients with autopsy confirmed diagnosis. AD patients (n=133) were genotyped for the polymorphic regions in the apolipoprotein E ε (APOE ε) and interleukin-1β (IL-1β) genes. The IL-1β +3953 polymorphism influenced survival in AD patients and those with the TT genotype and without the APOE ε4 allele showed the shortest cumulative survival [18].

TNF-α is a pro-inflammatory cytokine with a wide range of biological functions, in particular, it induces the synthesis of macrophages and dendritic cells, pro-inflammatory cytokines IL-1β, IL-6, IL8, and TNFα activate cells of innate immunity. In addition, TNFα increases the cytotoxic properties of NK cells and the production of IFNγ, which activates various cells of innate immunity and induces the differentiation of T cells according to the Th1 pathway. The human TNF-α gene is located on chromosome 6p21.1–21.3 within the highly polymorphic region of the major histocompatibility complex [19]. The TNFα G308A polymorphism – a single nucleotide polymorphism involving guanine to adenine substitution at position 308 in the promoter region of the TNFα gene. This polymorphism is functional, that is, it causes changes in the level of TNF-α production [20].

Analyzing the frequency distribution of the genotypes of the polymorphic variant G308A of the TNFα gene showed that in patients with CTE, SVD, CAIE, PIE compared to the control group, statistically significant differences were found only in patients with PIE, among whom no carriers of the A/A genotype were found, and the number of genotype carriers G/G and G/A was parity. Wang T. conducted a meta-analysis that showed that TNF-alpha G308A polymorphism may be associated with the increased risk of AD in Chinese and decreased risk of AD in northern European populations [21].

Our results regarding statistically significant differences in the frequency distribution of genotypes of polymorphic variants of the studied proinflammatory cytokine genes only in patients with PIE can be related to neuroinflammation, which is a common feature of encephalopathies associated with infectious diseases. In the brain, cytokines are able to activate glial cells, modulate the metabolism of neurotransmitters and lead to neurotoxic cascades [22]. After exposure to proinflammatory stimuli, microglia undergo morphological and functional changes and organize an immune response in the CNS. The proinflammatory environment also leads to several pathological changes in astroglia. This reactive astrogliosis is characterized by hypertrophy, a modified secretome, and increased expression of intermediate filament proteins, especially glial fibrillary acidic protein, and vimentin [23].

Conclusion.

1. Analyzing the frequency distribution of genotypes of the C3953T polymorphic variant of the IL1β gene in patients with CTE, SVD, CAIE, PIE relative to healthy individuals, statistically significant differences were found only in patients with PIE (26.92% vs. 83.33% - carriers of the C/C genotype, 61, 54% versus 16.67% – carriers of the C/T genotype and 11.54% versus 0% – carriers of the T/T genotype). In addition, in the group of patients with PIE, the distribution of genotype frequencies probably differs from the data of patients with CTE, SVD, and PIE ($\chi2=28.64$; $p<0.001$).

2. Analyzing the frequency distribution of genotypes of the polymorphic variant G308A of the TNFα gene showed that in patients with CTE, SVD, CAIE, PIE compared to healthy individuals, statistically significant differences were found only in patients with PIE (53.85% vs. 91.67% - carriers of the G/G genotype, 46.15% versus 8.33% – carriers of the G/A genotype and 0.0% versus 0.0% – carriers of the A/A genotype). At the same time, in the group of patients with CAIE, the distribution of genotype frequencies probably differed from the data of patients with SVD and PIE ($\chi2=24.91$; $p=0.002$).

3. Analyzing the odds ratio and its confidence interval for the genotypes of polymorphic variants C3953T of the IL1β gene and G308A of the TNFα gene in patients with CTE, SVD, CAIE, and PIE, it was established that the presence of the C/T genotype of the IL1β gene increases the risk of occurrence and/or progression of encephalopathy in patients with PIE by 8.0 times, and the presence of the G/A genotype of the TNFα gene increases the risk of occurrence and/or progression of encephalopathy in patients with PIE by 9.4 times, which indicates the feasibility of including the corresponding single nucleotide polymorphisms of the IL1β and TNFα genes in the genetic panel of patients with PIE to prescribe adequate therapy to prevent disease progression.

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