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## ABSTRACT

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# PREDICTORS OF ACUTE OTITIS MEDIA SEVERE COURSE IN CHILDREN AND HEAT SHOCK PROTEIN GENE HSP70-2 (RS1061581) TRANSCRIPTION ACTIVITY

**Introduction.** Acute otitis media (AOM) is one of the most common pediatric health issues worldwide. Despite extensive research on the AOM causes, there is no enough evidence of molecular-genetic and clinical progression risk-factors of AOM severe course. **Objectives**: to assess predictors of AOM severe course in children and the heat-shock protein gene (HSP70-2; rs1061581) impact, as well as its transcriptional activity.

**Materials and Methods.** A prospective cohort study involved 95 children with AOM, aged from 7 to 18 years old. Among the examined children were 34.74% (n=33) girls and 65.26% (n=62) – boys. Children were divided by age (12–18 yo/7–11 years), AOM severity (non-severe/severe), nature of the mucous membrane inflammation (purulent/catarrhal). The control group included 50 practically healthy children: 30 boys (60.0%), 20 girls (40.0%). The groups did not differ by age ($p>0.05$). HSP70-2 (rs1061581) gene genotyping was performed by qPCR method. HSP70-2 gene transcriptional activity analyzed by eQTL in the open GWAS database. Risk assessed by Risk Ratio, Odds Ratio and 95% Confidence Interval.

**Results.** The risk of a more severe clinical course of AOM elevates with eardrum perforation and otorrhea almost 15 and 10 times (OR 95%CI: 3.17–69.39 and OR 95%CI: 3.51–30.60; $p<0.001$), severe otalgia and purulent ear discharge – more than 20 and 18 times (OR 95%CI: 6.76–62.96 and OR 95%CI: 6.54–51.59; $p<0.001$), mainly with unilateral otitis localization – more than 2 times (OR 95%CI: 0.98–4.99; $p=0.055$) and otitis at pre-pubertal age (7–11 years) – more than 6 times (OR 95%CI: 1.29–29.24; $p=0.01$), respectively. The appearance of purulent discharge from the ear at the age of 7–11 years enhances the severe AOM likelihood more than 7 times (OR=7.29; OR 95%CI: 1.44–37.01; $p=0.045$). Moreover, the AOM risk increases in A-allele carriers

of the HSP70-2 gene (rs1061581) almost twice (OR=1.76; OR 95%CI: 1.02–3.05; p=0.026).

Expression quantitative trait loci of the HSP70-2 gene (rs1061581) were identified in 29 tissues and organs. The HSP70-2 gene increases the transcriptional activity of the following genes: HSP70-2 (rs11576014) ($\beta \geq 0.914$; $p=2x10^{-6}$), HSP70-1 (rs17201192/rs2763977/rs9469058) ($\beta \geq 0.029–0.67$; $p<2x10^{-10}$); SNHG32 (rs7769158/rs7765136) ($\beta \geq 0.15–0.30$; $p<9x10^{-18}$). The HSP70-2 gene causes transcriptional repression of the following genes: HSP70-1 (rs2763979/rs2763979/rs1265947) ($\beta \leq 0.09$; $p<6x10^{-6}$) and SNHG32 (rs3115674/rs35592410/rs3130476/rs34278010/rs2471980) ($\beta \leq 0.09$; $p<2x10^{-7}$).

**Conclusions.** Predictors of AOM severe course are otorrhea, purulent ear discharge, severe otalgia, perforation of the eardrum, otitis in children 7–11 years old, and the A-allele of the HSP70-2 gene (rs1061581).

**Key words:** Acute Otitis Media, HSP70-2 gene (rs1061581), predictors, children, Heat Shock Protein, risk factors, transcriptional activity, gene expression.

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## ПРЕДИКТОРИ ТЯЖКОГО ПЕРЕБІГУ ГОСТРОГО СЕРЕДНЬОГО ОТИТУ У ДІТЕЙ І ТРАНСКРИПЦІЙНА АКТИВНІСТЬ ГЕНА БІЛКА ТЕПЛОВОГО ШОКУ HSP70-2 (RS1061581)

**Актуальність.** Гострий середній отит (ГСО) є однією з найпоширеніших дитячих проблем у всьому світі. Незважаючи на широкі дослідження причин ГСО, немає достатньо доказів щодо молекулярно-генетичних і клінічних факторів ризику прогресування тяжчого перебігу ГСО. **Мета:** оцінити предиктори тяжкого перебігу ГСО у дітей та вплив гена білка теплового шоку (HSP70-2; rs1061581), а також його транскрипційну активність.

**Матеріали і методи.** У проспективному когортному дослідженні взяли участь 95 дітей із ГСО віком від 7 до 18 років. Серед обстежених дітей було 34,74% (n=33) дівчаток та 65,26% (n=62) хлопчиків. Дітей розподілили за віком (12–18 / 7–11 років); тяжкістю ГСО (нетяжкий / тяжкий), видом запалення слизових оболонок (гнійний / катаральний). Контрольну групу склали 50 практично здорових дітей: 30 (60,0%) хлопчиків і 20 (40,0%) дівчаток. За віком групи не відрізнялися (p>0,05). Генотипування гена HSP70-2 (rs1061581) виконали методом якісної полімеразної ланцюгової реакції. Транскрипційну активність гена HSP70-2 аналізували за локусами кількісних ознак експресії у відкритій базі GWAS. Ризик оцінювали за відношенням ризику (ВР) і шансів (ВШ) із 95% довірчим інтервалом (ДІ).

**Результати дослідження.** Ризик більш тяжкого клінічного перебігу ГСО підвищується при перфорації барабанної перетинки та отореї майже у 15 та 10 разів (ВШ 95% ДІ: 3,17–69,39 та ВШ 95% ДІ: 3,51–30,60; p<0,001), вираженій отальгії та гнійних виділеннях із вуха – у понад 20 і 18 разів (ВШ 95% ДІ: 6,76–62,96 та

ВШ 95% ДІ: 6,54–51,59; p<0,001), переважно при однобічному локалізації отиту – у понад 2 рази (ВШ 95% ДІ: 0,98–4,99; p=0,055) та у дітей передпубертатного віку (7–11 років) – більше ніж у 6 разів (ВШ 95% ДІ: 1,29–29,24; p=0,01) відповідно. Поява гнійних виділень із вуха у віці 7–11 років збільшує ймовірність тяжкого ГСО більш ніж у 7 разів (OR=7,29; OR 95%CI: 1,44–37,01; p=0,045). Крім того, ризик ГСО зростає у носіїв А-алеля гена HSP70-2 (rs1061581) майже вдвічі (OR=1,76; OR 95%CI: 1,02-3,05; p=0,026).

Локуси кількісних ознак експресії гена HSP70-2 (rs1061581) ідентифіковано в 29 тканинах і органах. Ген HSP70-2 підвищує транскрипційну активність наступних генів: HSP70-2 (rs11576014) ($\beta \geq 0,914$; $p=2x10^{-6}$), HSP70-1 (rs17201192/rs2763977/rs9469058) ($\beta \geq 0,029–0,67$; $p<2x10^{-10}$); SNHG32 (rs7769158/rs7765136) ($\beta \geq 0,15–0,30$; $p<9x10^{-18}$). Ген HSP70-2 викликає репресію транскрипції наступних генів: HSP70-1 (rs2763979/rs2763979/rs1265947) ($\beta \leq 0,09$; $p<6x10^{-6}$) і SNHG32 (rs3115674/rs35592410/rs3130476/rs34278010/rs2471980) ($\beta \leq 0,09$; $p<2x10^{-7}$).

**Висновки.** Предикторами тяжкого перебігу ГСО є оторея, гнійні виділення з вуха, виражена оталгія, перфорація барабанної перетинки, отит у дітей 7–11 років, А-алель гена HSP70-2 (rs1061581).

**Ключові слова:** гострий середній отит, ген HSP70-2 (rs1061581), предиктори, діти, білок теплового шоку, фактори ризику, транскрипційна активність, експресія генів.

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**Abbreviations:** acute otitis media (AOM), expression quantitative trait loci (eQTL), Juvenile Idiopathic Arthritis (JIA), heat shock protein (HSP), Main Histocompatibility Complex class I molecules (MHCI), confidence interval (CI), odds ratio (OR), risk ratio (RR), Rheumatoid Arthritis (RA), single nucleotide polymorphism (SNP), toll-like receptors (TLRs), transcript per million (TPM).

## INTRODUCTION

Acute otitis media (AOM) is one of the most common pediatric health issues worldwide, affecting millions of children and adolescents annually. It is a prevalent and often painful condition involving inflammation and infection of the middle ear. The condition is particularly common in children under five, with research showing that up to 80% of children experience at least one episode by the age of three [1, 2]. The World Health Organization identifies AOM as a major cause of healthcare visits and antibiotic use in young populations [3]. In the United States AOM accounts for roughly 30 million doctor visits and nearly 10 million antibiotic prescriptions each year. This widespread prevalence highlights AOM as a significant public health concern, requiring focused efforts to manage and prevent its occurrence [4-7]. Unfortunately, the mechanisms of severe AOM depending on individual immunological, anamnestic and microsocial predictors, as well as genetic predispositions, remain incompletely understood and require further research. In this context, one of the cell protection molecular systems is heat-shock proteins (HSP). The expression of these proteins and their synthesis is a response of cells to adverse factors: infections, heavy metal salts, hypoxia, oxidative stress, inflammation, radiation, etc.

HSP play a major role in the immune-inflammatory reactions of the human body, based on their chaperone function, ability to bind both whole proteins and peptides, and interaction in the active configuration formation of many immune receptors, such as toll-like receptors (TLRs) or integrins. In addition, HSPs can directly stimulate immune receptors, ensuring the participation of proteins in pro-inflammatory signaling pathways, transfer of unfolded proteins to proteasomes, presenting peptides to Main Histocompatibility Complex class I molecules (MHCI) [8-13]. HSPs are indispensable components of all antigen presentation pathways likewise. They are also

involved in a "chaperone-mediated autophagy", where they allow cytosolic proteins to enter lysosomes [14-16].

HSPs are encoded by the corresponding HSP's genes. Three genes encoding HSP70 – HSPA1A, HSPA1B, HSP-HOM are located on chromosome 6p21.3 (HLA class III subregion). The HSPA1A and HSPA1B proteins share 99% sequence identity [14, 17]. Among these, the HSPA1B gene exhibits +1267 A/G polymorphism HSP70-2 (rs1061581), which results in a silent mutation (Q351) within the coding region. This genetic variation has been linked to several diseases, including type II diabetes mellitus, autoimmune conditions like systemic lupus erythematosus [18], severity of chronic heart failure, coronary artery disease and arrhythmia [19], colienteritis, ulcerative colitis and Crohn's disease [9, 20], preeclampsia [21]. These findings highlight the potential role of HSP70 polymorphisms in influencing the pathogenesis and progression of various diseases.

Despite extensive research on the causes and clinical progression of AOM, particularly in children, there is currently no evidence of the involvement of HSP70 family genes in its pathogenesis. Genetic and molecular aspects of AOM development, especially in the context of the immune system's response, had not been explored at the start of this study. Thus, this research aimed to investigate the genetic mechanisms underlying AOM, focusing on the association between the HSP70-2 gene polymorphism (rs1061581) and other contributing risk factors.

**OBJECTIVES**. To assess predictors of AOM severe course in children and the heat-shock protein gene (HSP70-2; rs1061581) impact, as well as its transcriptional activity.

**MATERIALS AND METHODS**

The clinical data for this study was collected at the Municipal Non-Profit Enterprise "Multidisciplinary Hospital of Intensive Care" in Kitsman city during 2023-2024. A total of 100 children with AOM participated in this prospective cohort study. Screening was completed by 95 children aged from 7 to 18 years, whose parents confirmed informed consent for participation. Children underwent a comprehensive set of clinical, laboratory, and instrumental evaluations. The study adhered to ethical standards, including the principles outlined in the Council of Europe Convention on Human Rights and Biomedicine, the core tenets of Good Clinical Practice (GCP, 1996), and the World Medical Association's Declaration of Helsinki regarding ethical principles for research involving human subjects. Approval for the research was granted by the Biomedical Ethics Commission of Bukovinian State Medical University. The clinical diagnosis of AOM and the assessment of its severity were carried out in accordance with the National Unified Clinical Protocol for Primary, Secondary (Specialized), and Tertiary (Highly Specialized) Medical Care "Acute Otitis Media," as approved by the Ministry of Health of Ukraine (Order No. 688, dated April 9, 2021). Additionally, the diagnosis followed the recommendations outlined in the corresponding Clinical Guidelines "Acute Otitis Media" (2021) and international standards for AOM management [1, 2, 5, 22-24]. Supplementary imaging studies, including X-rays of the chest, paranasal sinuses, and mastoid processes were performed in two projections to support clinical evaluation, when indicated.

The children were categorized into groups based on various factors: age: 7-11 years (81 children), 12-18 years (14 children); Severity of AOM: severe – 45.26% (43 children), Non-severe – 54.74% (52 children); Nature of mucous membrane inflammation: catarrhal – 52.63% (50 children), Purulent – 47.37% (45 children); Condition of the eardrum: pre-perforative – 81.05% (77 children), Perforative – 18.95% (18 children); Allelic status of the HSP70-2 gene (rs1061581): groups based on A1267G polymorphism were also analyzed. Among the participants, 34.74% (33 children) were girls, and 65.26% (62 children) were boys. The control group included 50 healthy children (20 girls and 30 boys) of matching age and sex distribution. Children of control group had no inflammatory diseases at the time of examination or during the prior six months. The control and study groups did not significantly differ in age ($p > 0.05$).

Genotyping of the HSP70-2 gene was conducted using qualitative Polymerase Chain Reaction (qPCR). The procedure involved separating the resulting PCR products via horizontal electrophoresis on a 3% agarose gel. The gel was stained with ethidium bromide (4 µl) to enable visualization. To analyze the fragments, a UV transluminator (Nyxtechnic, USA) was used alongside the Vitran® computed-based program for detection. A molecular mass ladder (100-1000 bp) was included to determine the size of the amplicons. The amplicons produced indicated the following genotypes based on their respective lengths: GG-genotype – fragments of 936 and 181 base pairs (bp); AA-genotype – fragment of 1117 bp; AG-genotype – fragments of 1117, 936, and 181 bp. This method allowed for precise identification of genotypic variations within the HSP70-2 gene.

Statistical analysis was conducted using Statistica 7.0 (StatSoft Inc., USA) and Excel® 2016™**.** For qualitative (categorical) data, analysis included calculating the odds ratio (OR**)** with a 95% confidence interval (CI)**.** The chi-square test ($\chi^2$) (degrees of freedom = 1) was employed, and in cases where

frequencies were less than five, Fisher's exact test was used. To further refine the analysis, a multivariate logistic regression model was applied. Results with p-values <0.05 were considered statistically significant.

This research was conducted as part of the broader project by the Family Medicine Department of Bukovinian State Medical University, titled: "Improvement of diagnosis and prediction of hypertensive-mediated certain target organ damage and symptom control in comorbid pathology considering clinical-metabolic and molecular-genetic predictors". The project is registered under State Registration Number 0124U002524 and is scheduled for implementation from 01.01.2024 to 31.12.2028.

**RESULTS**

Children with acute otitis were divided depending on the AOM severity and clinical signs (Fig. 1-2). In non-severe cases, mild or moderate otalgia with body temperature up to 39°C was recorded more often than in severe cases, by 46.16% (P<0.001), catarrhal inflammation or minor serous ear discharge – by 62.17% (P<0.001), while purulent discharge was observed more often in severe AOM cases (Fig. 1). The eardrum was perforated only in two children in mild cases, while in severe cases – eight times more often (P<0.001), perforation was not detected in the rest of the examined ones.

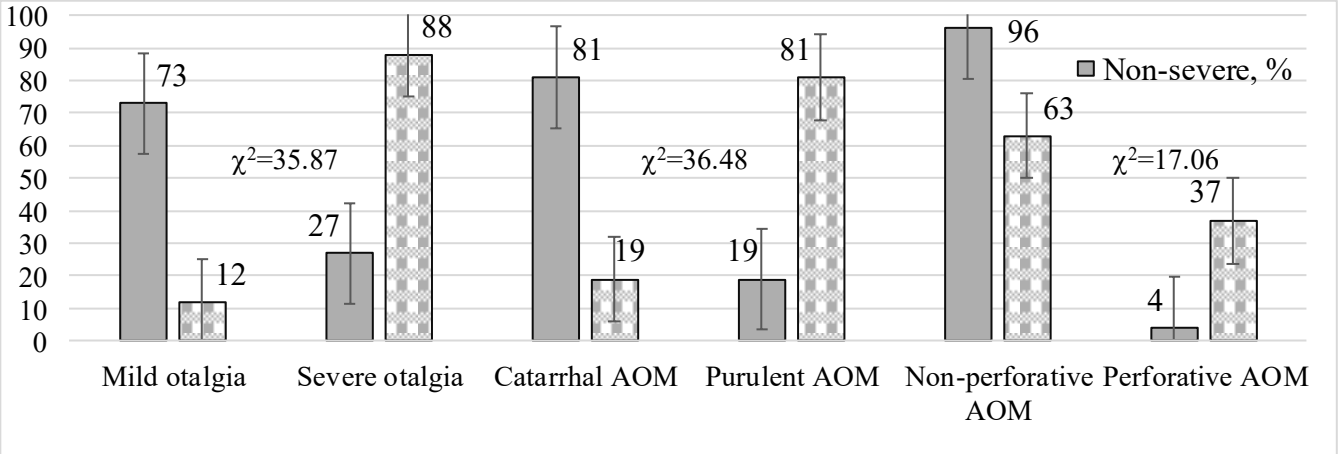


***Figure 1***. *Clinical signs of the Acute Otitis Media (AOM) in children depending on disease severity (otalgia, type of inflammatory process, eardrum pre/perforation)*

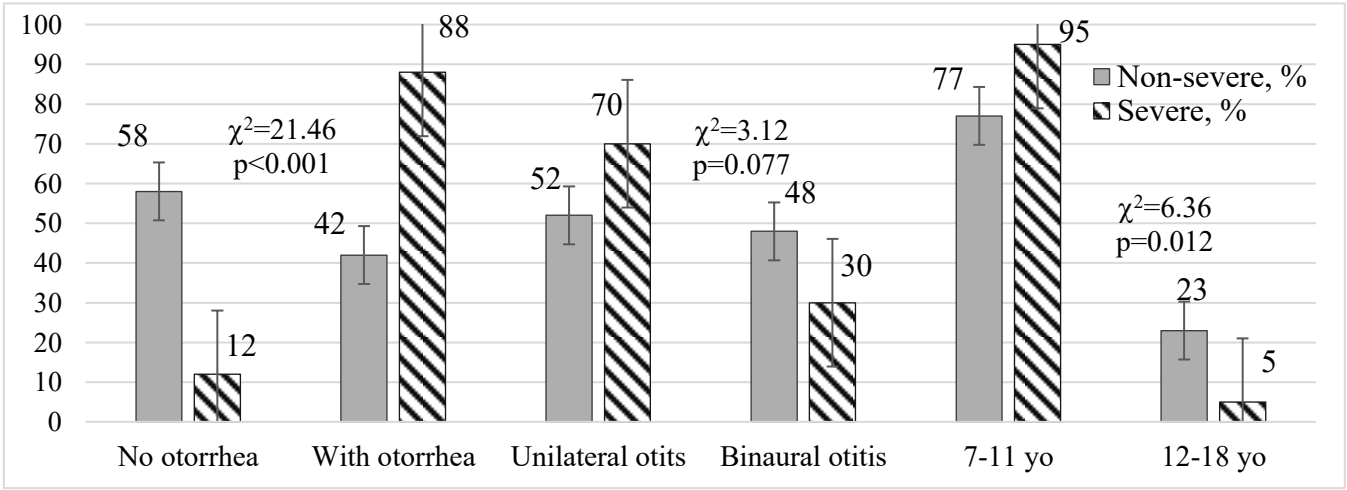


***Figure 2***. *Severity of the Acute Otitis Media (AOM) depending on clinical predictors (otitis location, presence of otorrhea, ages of children)*

Otorrhea, as excessive secretory activity of the mucous membranes of the middle ear in mild cases was encountered 15.37% less often than its absence, while in severe cases of AOM, on the contrary, otorrhea prevailed – by 76.74% (p<0.001) (Fig. 2). The mild course was accompanied by almost parity localization of the process (p>0.05), while in severe AOM, unilateral otitis dominated by 39.54% (p<0.001) over binaural otitis. In general, among the patients, children of pre-adolescent age prevailed over adolescents by 70.92% (p<0.001): 85.26% (n=81) versus 14.34% (n=14), which, however, had a significant significance in severe cases of AOM (p=0.012).

Epidemiological analysis showed an increased risk of a more severe AOM clinical course (Table 1) in presence of severe otalgia and purulent ear discharge more than 20 and 18 times (OR=20.63; OR 95%CI: 6.76-62.96 and OR=18.37; OR 95%CI: 6.54-51.59; p<0.001), perforation of the eardrum and otorrhea – almost 15 and 10 times (OR=14.81; OR 95%CI: 3.17-69.39 and OR=10.36; OR 95%CI: 3.51-30.60; p<0.001), mainly with unilateral localization – more than 2 times (OR=2.14; OR 95%CI: 0.98-4.99; p=0.055), and in prepubertal age (7-11 years) – more than 6 times (OR=6.15; OR 95%CI: 1.29-29.24; p=0.01), respectively.

***Table 1*** *– Predictors of more severe acute otitis media in the examined children*

| Potential predictors | OR | OR 95% CI | RR | RR 95% CI | p |
|---|---|---|---|---|---|
| Severe otalgia | 20.63 | 6.76-62.96 | 6.28 | 2.71-14.56 | <0.001 |
| Non-severe otalgia | 0.05 | 0.02-0.15 | 0.16 | 0.07-0.37 | |
| Purulent AOM | 18.37 | 6.54-51.59 | 4.86 | 2.53-9.35 | <0.001 |
| Catarrhal AOM | 0.05 | 0.02-0.15 | 0.21 | 0.11-0.40 | |
| Perforative AOM | 14.81 | 3.17-69.39 | 2.54 | 1.79-3.58 | <0.001 |
| Non-perforative AOM | 0.07 | 0.01-0.32 | 0.39 | 0.28-0.56 | |
| AOM with otorrhea | 10.36 | 3.51-30.60 | 4.43 | 1.93-10.21 | <0.001 |
| AOM without otorrhea | 0.10 | 0.03-0.28 | 0.23 | 0.10-0.52 | |
| Binaural AOM | 0.47 | 0.20-1.09 | 0.65 | 0.39-1.08 | 0.055 |
| Unilateral AOM | 2.14 | 0.98-4.99 | 1.54 | 0.93-2.55 | |
| Age 12-18 years old | 0.16 | 0.03-0.77 | 0.28 | 0.08-1.04 | 0.01 |
| Age 7-11 years old | 6.15 | 1.29-29.24 | 3.54 | 0.96-13.01 | |

*Note. RR – risk ratio; OR – odds ratio; CI – Confidence Interval; P - significance of differences*

The Heat Shock Protein gene HSP70-2 (rs1061581) A1267G polymorphism genotypes distribution in the examined cohort was as follows: in patients AA-, AG-, GG-genotypes - 8 (8.42%), 52 (54.74%) and 35 (36.84%), *G*-allele – 122 (64.21%), *A*-allele – 68 (35.79%) respectively; in control group – one (2.0%), 22 (44.0%), 27 (54.0%) ($\chi^2$=3.94; p=0.047), *G*-allele – 76 (76.0%), *A*-allele – 24 (24.0%) ($\chi^2$=4.20; p=0.04), respectively. In A-allele carriers significantly increases the risk of AOM developing, with odds nearly doubling (OR = 1.76; 95% CI: 1.02–3.05; p = 0.026). Conversely, individuals carrying the G-allele, particularly in a homozygous state (GG-genotype), show a reduced risk of AOM development by approximately half (OR = 0.50; 95% CI: 0.25–0.99; p = 0.035).

Some Expression quantitative trait loci (eQTL) for SNP rs1061581 using the open GWAS database (GWAS Catalog) are shown in Table 2. The HSP70-2 gene increases the transcriptional activity of the following genes: HSP70-2 (rs11576014) interacting through the A-allele (β≥0.914; p=2x10$^{-6}$) in the peroxidation reaction; HSP70-1 (rs17201192 – T-allele; rs2763977 – A-allele; rs9469058 – A-allele) in lymphocytes, in the effect on adjusted body mass index (BMI) through waist-to-hip ratio (WHR), neonatal blood flow, complement C4b level (β≥0.029-0.67; p<2x10$^{-10}$); SNHG32 gene (rs7769158 – T-allele and rs7765136 – A-allele) in the influence on the sulfatase-modifying factor 2 activity, complement C4 content and neonatal blood circulation (β≥0.15-0.30; p<9x10$^{-18}$).

The HSP70-2 gene (rs1061581) causes transcriptional repression of the following genes: HSP70-1 (rs2763979 – T-allele, rs2763979 – A-allele, rs1265947 – C-allele) in prostate cancer, systemic lupus erythematosus (SLE), inguinal hernia (β ≤ 0.09; p < 6x10$^{-6}$); the SNHG32 gene (rs3115674-T-allele,

rs35592410-T, rs3130476-C, rs34278010-G-allele, rs2471980-C-allele) – in SLE, inguinal hernia, prostate cancer and at the level of lymphocytes ($\beta \leq 0.09$; $p < 2x10\text{-}7$) (Table 2).

To analyze the transcriptional activity of the HSP70-2 gene, we evaluated eQTL in the open GWAS catalog database. The expression of the HSP70-2 gene in organs and tissues, as well as genes close in terms of transcriptome activity, is shown in Figure 3. The highest transcriptional activity of the HSP70-2 gene was found in the vagina (5383 Transcript per million (TPM)), breast (1457 TPM), mitral and tricuspid valves of the heart (1042 and 1170 TPM), medulla oblongata (576 TPM), substantia nigra of the midbrain – extrapyramidal system (572 TPM), dorsal thalamus (434 TPM).

***Table 2*** *– Quantitative signs of process-associated transcriptional activity (expression, repression) of the HSP70-2 gene (rs1061581)*

| Gene interaction | Rs variant - allele interaction | The studied process, pathology | β | OR [95% CI] | P |
|---|---|---|---|---|---|
| HSP70-2 (HSPA1B) | rs11576014 - A | Peroxidase activity | ≥0.914 | [0.53-1.30] | $2 \times 10^{-6}$ |
| HSP70-1 (HSPA1A) | rs2763979 - A | Systemic lupus erythematosus | - | 1.30 | $6 \times 10^{-6}$ |
| | rs2763979 - T | Prostate cancer | ≤0.09 | [0.05-0.12] | $2 \times 10^{-14}$ |
| | rs9469058 - A | Neonatal circulation, C4 complement | ≥0.67 | [0.63-0.71] | $5 \times 10^{-183}$ |
| | rs17201192 - T | Lymphocyte level | ≥0.058 | [0.04-0.07] | $2 \times 10^{-14}$ |
| | rs2763977 - A | C4b complement | ≤0.346 | [0.26-0.43] | $3 \times 10^{-16}$ |
| | | Adjusted BMI (WHR) | ≥0.029 | [0.02-0.04] | $4 \times 10^{-10}$ |
| | rs1265947 - C | Inguinal hernia | - | 1.11 | $2 \times 10^{-9}$ |

*Note. BMI - body mass index; WHR – waist-to-hip ratio; β - Strength of effect*

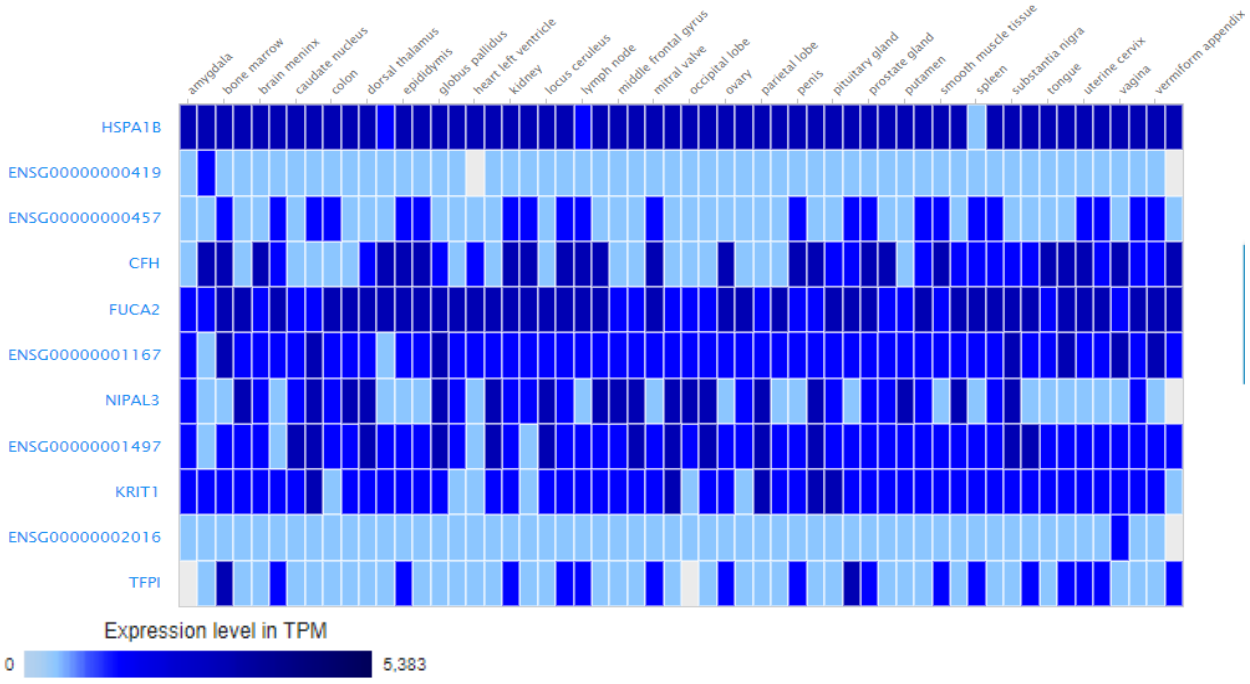


***Figure 3.*** *Transcriptional activity of the HSP70-2 gene (rs1061581) in organs and tissues*

*Note. TPM (Transcript per million) – level of relative normalized transcriptome gene expression in transcripts per million*

## DISCUSSION

HSPs modulate the inflammatory process by producing pro-inflammatory cytokines. HSP-mediated expression of IL-10 contributes to the anti-inflammatory role through TLR2 and TLR4-dependent mechanisms. Necroptosis, a caspase-independent programmed apoptosis, plays an important role in the progression of many inflammatory processes, and its main components are HSP-dependent [25]. Moreover, extracellular HSP70, a so-called "chaperokine", stimulates the expression of pro-inflammatory genes in monocytes, macrophages, and neutrophils and increases the activity of natural killer cells and the phagocytic activity of macrophages. Importantly, increased HSP70 levels enhance stress tolerance and may exert paracrine and autocrine activities. It is thought that low levels of HSP70 may contribute to the suppression of the inflammatory response, whereas higher levels may potentiate it [25, 26].

The activity and synthesis of HSP family proteins are encoded by the corresponding HSP genes. A number of genetic studies have shown that the single nucleotide polymorphism Ps+I of the HSP70-2 gene is associated with a severe clinical course of Crohn's disease [27]. At the same time, the A-allele was detected more often in patients than in controls (61% vs. 52%, P=0.047, OR=1.423, 95% CI: 1.004-2.015). Other studies have shown that there was no significant difference in the distribution of HSP70-2 gene genotypes (A1267G) between groups of healthy individuals and patients with ulcerative colitis [20, 28]. In our study GG genotype and G-allele of HSP70-2 gene (rs1061581) were more frequently observed in the control group, than in children with AOM by 17.16% ($\chi^2$=3.94; p=0.047) and 11.79% ($\chi^2$=4.20; p=0.04), respectively. On the other hand, the mutational A-allele occurred more often in children with AOM, than in the healthy subjects by 11.79% (p<0.05).

HSPs are immunodominant proteins and immunomodulatory agents which closely linked in modulating of inflammatory and autoimmune responses. Their expression is significantly elevated in various inflammatory conditions, including arthritis, colitis, and transplant rejection models: Juvenile Idiopathic Arthritis (JIA) – enhanced Hsp60 expression has been observed in synovial and mononuclear cells of JIA patients [29, 30]; Rheumatoid Arthritis (RA) – elevated levels of inducible HSP70 and Heat Shock Factor 1 (HSF1), as well as proinflammatory cytokines are present in inflamed joints of RA patients [31-33]. Moreover, BiP (an endoplasmic reticulum-specific HSP70 family protein) and constitutive HSPc70 are also overexpressed in the synovium of RA patients, suggesting their potential involvement in disease pathogenesis [34, 35].

These findings highlight the role of Hsp70 proteins in the inflammatory process, likely through their chaperone activity, immune modulation, and possible cytokine-like effects in regulating immune responses. The specific mechanisms underlying these effects are still being explored, offering a promising avenue for understanding inflammation and developing targeted therapies.

## CONCLUSIONS

Severe clinical course of acute otitis media occurs more often in children aged 7-11 years – by 18.43% (p=0.012), A-allele carriers of the HSP70-2 gene (A1267G) – by 21.90% (p=0.039), with purulent discharge from the ears and more pronounced otalgia with a fever ≥39°– by 62.17% (p<0.001) and 61.46% (P<0.001). In severe AOM course unilateral otitis media dominates over binaural – by 39.54% (p<0.001), eardrum perforation and otorrhea occurs 8 times more often (p<0.001) and by 76.74% (p<0.001) respectively. Predictors of AOM severe course are otorrhea (OR=10.36; p<0.001), purulent ear discharge (OR=18.37; p<0.001), severe otalgia (OR=20.63; p<0.001), perforation of the eardrum (OR=14.81; p<0.001), otitis in children 7-11 years old (OR=6.15; p=0.01), and the A-allele of the HSP70-2 gene (rs1061581) (OR=1.76; p=0.026). Expression quantitative trait loci of the HSP70-2 gene (rs1061581) were identified in 29 tissues and organs.

## PROSPECTS FOR FUTURE RESEARCH

Prospects for further research include studying anamnestic and microsocial concomitant risk factors for acute otitis media and the nonspecific immune defence state in children.

## AUTHOR CONTRIBUTIONS

*Sydorchuk LP* – contributed to the study conception and design, data extraction and analysis, the drafting of the manuscript, and final critical revision and approval.

*Sakovets OP* – data extraction contributed to the interpretation and analysis of the descriptive data, critical revision, and final approval.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors declare that they did not use artificial intelligence to prepare the manuscript for publication.

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