

Agar Artsymovych¹, Olena Oshlianska¹, Olena Okhotnikova¹, Zoia Rossokha²,
Olena Popova², Nataliia Medvedeva², Victoriia Vershigora², Illya Chaikovskiy³, Olga Kryvova⁴

Received: 30.08.2021
Accepted: 07.02.2022
Published: 31.05.2022

Possibilities of using determination of allelic polymorphism of interleukin-6 *G174C* and tumour necrosis factor- α *G308A* genes for the prediction of cardiovascular disorders in children with juvenile idiopathic arthritis

Możliwości wykorzystania analizy polimorfizmu allelicznego genu interleukiny 6 *G174C* i genu czynnika martwicy nowotworów TNF α *G308A* w prognozowaniu zaburzeń układu sercowo-naczyniowego u dzieci z młodzieńczym idiopatycznym zapaleniem stawów

¹ Shpyk National University of Healthcare of Ukraine, Kyiv, Ukraine

² SI¹ Reference Centre for Molecular Diagnostics of the Ministry of Health of Ukraine, Kyiv, Ukraine

³ Glushkov Institute of Cybernetics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

⁴ International Research and Training Centre for Information Technologies and Systems of the National Academy of Sciences of Ukraine and the Ministry of Education and Science of Ukraine, Kyiv, Ukraine

Correspondence: Agar Artsymovych, Hoholivska str. 1–3, app. 20, Ukraine, Kyiv, 01054, tel.: +380663240991, e-mail: wintermdp@gmail.com

ORCID iDs

1. Agar Artsymovych <https://orcid.org/0000-0003-3891-7300>
2. Olena Oshlianska <https://orcid.org/0000-0002-9782-9709>
3. Olena Okhotnikova <https://orcid.org/0000-0003-2498-0560>
4. Zoia Rossokha <https://orcid.org/0000-0002-4767-7364>
5. Olena Popova <https://orcid.org/0000-0002-7793-021X>
6. Nataliia Medvedeva <https://orcid.org/0000-0002-8005-7220>
7. Victoriia Vershigora <https://orcid.org/0000-0003-0220-2573>
8. Illya Chaikovskiy <https://orcid.org/0000-0002-4152-0331>
9. Olga Kryvova <https://orcid.org/0000-0002-4407-5990>

Abstract

Objectives: Juvenile idiopathic arthritis is a chronic disease that affects the synovial membrane of the joints, but can also lead to secondary lesions of the cardiovascular system. The most important mechanism of myocardial damage is associated with the effect of proinflammatory cytokines. The aim of the study was to propose a method of early detection of cardiovascular system changes and lesions in patients with juvenile idiopathic arthritis based on the determination of allelic polymorphism combined with electrocardiography data. **Materials and methods:** 102 patients with juvenile idiopathic arthritis underwent a general clinical examination. The overall activity of juvenile idiopathic arthritis was assessed using the Juvenile Arthritis Disease Activity Score. In addition, the patients underwent an electrocardiographic evaluation using the software and hardware complex “Cardioplus P” which is a portable electrocardiograph providing “signal-averaged” electrocardiography performing the recognition and measurement of amplitude-time parameters, and calculation of secondary electrocardiography parameters. The genotypes of patients were additionally determined by alleles of the TNF- α (*G308A*) and IL6 (*G174C*) genes by polymerase chain reaction. **Results:** The overall number of mutations affects the course of the disease, with two or more mutations being associated with a more aggressive course of the disease, a more pronounced degree of inflammation, and a higher frequency of extra-articular lesions. The complex indicator of the functional state of the myocardium according to the electrocardiography data differed significantly ($p = 0.00001$) in clusters. **Conclusion:** Patients with juvenile idiopathic arthritis with two or more mutations in different genes of proinflammatory cytokines have a higher activity of the inflammatory process and a higher frequency of cardiovascular changes according to 4th generation electrocardiography. The determination of polymorphism may be useful in evaluating the risk of development of cardiovascular system abnormalities.

Keywords: juvenile idiopathic arthritis, allelic polymorphism, cardiovascular disorders

Streszczenie

Cele: Młodzieńcze idiopatyczne zapalenie stawów jest przewlekłą chorobą, która atakuje błonę maziową stawów, ale może także prowadzić do wtórnych uszkodzeń w obrębie układu sercowo-naczyniowego. Główny mechanizm uszkodzenia mięśnia sercowego ma związek z działaniem cytokin prozapalnych. Celem pracy jest przedstawienie proponowanej metody wczesnego wykrywania zmian i uszkodzeń w układzie sercowo-naczyniowym pacjentów z młodzieńczym idiopatycznym zapaleniem stawów na podstawie oznaczenia polimorfizmu allelicznego w połączeniu z danymi z zapisu elektrokardiograficznego.

Materiał i metody: Badaniem klinicznym objęto grupę 102 pacjentów z młodzieńczym idiopatycznym zapaleniem stawów. Aktywność choroby oceniano w skali Juvenile Arthritis Disease Activity Score (JADAS). Ponadto u pacjentów przeprowadzono ocenę elektrokardiograficzną za pomocą urządzenia Cardioplus P. Jest to przenośny elektrokardiograf, który dostarcza „uśredniony” sygnał elektrokardiograficzny, wykonując pomiar i rejestrację parametrów amplitudowo-czasowych z obliczeniami parametrów elektrokardiograficznych. Dodatkowo przeprowadzono analizę genotypów pacjentów z uwzględnieniem alleli genów TNF- α (*G308A*) i IL6 (*G174C*) metodą łańcuchowej reakcji polimerazy. **Wyniki:** Liczba mutacji wpływa na przebieg choroby, przy czym obecność dwóch lub większej liczby mutacji wiąże się z bardziej agresywnym przebiegiem choroby, bardziej nasilonym stanem zapalnym i większą częstością występowania zmian pozastawowych. Złożony wskaźnik stanu czynnościowego mięśnia sercowego według danych elektrokardiograficznych różnił się w sposób istotny statystycznie ($p = 0,00001$) w klastrach. **Wnioski:** U pacjentów z młodzieńczym idiopatycznym zapaleniem stawów, u których występują co najmniej dwie mutacje w różnych genach cytokin prozapalnych, obserwuje się wzmożoną aktywność procesu zapalnego i częstsze występowanie zmian w układzie sercowo-naczyniowym analizowanych za pomocą elektrokardiografu 4. generacji. Analiza polimorfizmów może być przydatna w ocenie ryzyka rozwoju zaburzeń układu sercowo-naczyniowego.

Słowa kluczowe: młodzieńcze idiopatyczne zapalenie stawów, polimorfizm alleliczny, zaburzenia układu sercowo-naczyniowego

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a chronic autoimmune inflammatory disease in children that primarily affects the synovial membrane of the joints, but can lead to damage to other organs and tissues⁽¹⁾, including damage to the heart muscle. To date, early diagnosis and treatment can significantly delay or prevent the disability of patients with JIA⁽²⁾, which leads to increased life expectancy and the need to prevent comorbid pathologies, primarily cardiovascular system (CVS) diseases⁽³⁾. Therefore, the question of finding an accessible and easy-to-use method for the early diagnosis of pathological processes in the CVS in patients with JIA is becoming increasingly important. Adverse changes in the CVS accompanying this disease may remain undetected for a long time⁽⁴⁾. Insufficient efficiency of standard diagnostic methods for their detection and inexpediency of routine use of such high-tech research methods as magnetic resonance imaging or coronary angiography, leave an open niche for finding a convenient and effective screening method for detecting secondary lesions in the CVS. Previous studies of the CVS status in children with rheumatic diseases have shown that the use of 4th generation electrocardiography (advanced electrocardiography, A-ECG) can detect changes in the CVS up to three times more often than a standard 12-channel ECG^(5,6), which makes it a good candidate for use as a screening method for detecting early CVS disorders in patients with JIA.

The pathogenesis of heart disease in patients with JIA may be due to systemic inflammation, overweight, lack of physical activity, the development of atherosclerosis on the background of autoimmune inflammation, the impact of certain drugs on the myocardium, etc.^(4,7). The most important mechanism of myocardial damage is the effect of

pro-inflammatory cytokines, especially interleukin-6 (IL6). Together with tumour necrosis factor- α (TNF- α), it promotes the development of atherosclerosis, vasoconstriction, and apoptosis of endothelial cells, as well as increasing markers of oxidative stress, which leads to vascular remodelling and accelerating the catabolism of cardiomyocytes. IL6 is a marker of high risk of cardiovascular disease in various chronic inflammatory diseases in adult patients⁽⁸⁻¹⁰⁾.

IL6 and TNF- α are the most studied proinflammatory cytokines in JIA. Determination of IL6 and TNF- α serum levels is a fairly common method of assessing the activity of the inflammatory process in patients with JIA and may be helpful in the selection of treatment strategy⁽¹¹⁻¹³⁾. However, some researchers do not consider the serum level of cytokines as a reliable prognostic indicator due to the high variability and dependence on the momentary activity of systemic inflammation⁽¹⁴⁾.

Therefore, scientists are focusing their attention on the study of allelic polymorphism of genes in chronic inflammatory diseases as a potential marker of the functional activity of their encoded proteins and related features of the disease^(15,16).

Alleles are paired, functionally similar genes occupying homologous locus of chromosomes, allelic pair of genes can be homo- or heterozygous, and each gene has at least two allelic variants. The wild-type allele is the typical (“normal”) gene variant, usually the most common genotype in the natural population, occurring at a frequency of more than 95%, while the mutant allele is the result of one or more nucleotide substitutions and is usually less common⁽¹⁷⁾.

Allelic polymorphism of the TNF- α (*G308A*) and IL6 (*G174C*) genes has already been studied in diseases of the cardiovascular, digestive, and musculoskeletal systems, including JIA⁽¹⁸⁻²⁰⁾. According to previous studies, certain alleles of the *G174C* gene have been associated with the

development of a systemic variant of JIA. Other researchers have noted an increased presence of the C allele at the *G174C* locus in adult patients with rheumatoid arthritis compared with controls^(15,21). Polymorphism of the *G308A* gene has also been detected in patients with high rheumatoid arthritis activity and more severe disease course^(22,23), although in different patient populations the study data varied slightly⁽²⁴⁾. Also, according to the literature, higher frequency of detection of *G308A* polymorphism was associated with the development of aggressive forms of the seropositive polyarticular variant of JIA, while the presence of wild allele *G308A* was considered a favourable prognostic factor for response to anti-TNF- α therapy^(25,26).

However, the importance of allelic polymorphism of genes of these proinflammatory cytokines in the development of cardiovascular disorders in JIA has not been studied yet.

This led to the aim of the study, which was to investigate the interdependence of cardiovascular changes and allelic polymorphism of the genes *IL6* (*G174C*) and *TNF- α* (*G308A*) in children with JIA.

MATERIALS AND METHODS

A total of 102 patients with JIA, treated at the Department of Paediatrics No. 1 of the P.L.Shupyk National University of Health of Ukraine in the paediatric department of the State Institution "Institute of Paediatrics, Obstetrics and Gynaecology named after Academician O.M. Lukyanova NAMS of Ukraine," were examined. The diagnosis of JIA was established according to the classification criteria of the International League of Associations for Rheumatology (ILAR).

The mean age was 9.59 ± 0.47 years (2–17), the average age of onset of JIA was 6.48 ± 0.43 years (2–17 years), which corresponded to the general population of patients with JIA⁽²⁾, with 41 boys (40.2%) and 61 girls (59.8%). The oligoarticular variant of JIA (oJIA) was observed in 30 patients from the group of examined children (30%; 11 boys, 19 girls), three children were polyarticular positive for rheumatoid factor form (pJIA RF+) (2.94%; all girls), polyarticular RF-negative variant (pJIA RF-) – 23 patients (22.55%; 18 girls, five boys), enthesitis arthritis (entJIA) was present in 30 children (29.41%; 14 girls, 16 boys), psoriatic arthritis (psorJIA) was observed in four patients (3.92%; three boys, one girl), the systemic variant (sJIA) was found in eight children (7.84%; five boys, three girls), while another four children had undifferentiated JIA (nJIA) (3.92%; one boy, three girls).

All patients were examined according to the Ukrainian unified clinical protocol of medical care for children with juvenile arthritis. Patients with JIA underwent a general clinical examination. The overall JIA activity was assessed using the Juvenile Arthritis Disease Activity Score (JADAS27).

In addition, the patients underwent an ECG examination using the software and hardware complex (SHC) "Cardioplus P" which is a portable electrocardiograph providing "signal-averaged" ECG, which performs recognition

and measurement of amplitude-time parameters, and calculates secondary ECG parameters (about 180 units), thus making it possible to assess the variability of the heart rate and myocardial status⁽²⁷⁾. The information obtained during the examination using SHC "Cardio-plus P" was divided into the following parts: analysis of heart rate variability; analysis of amplitude-time indicators of ECG; analysis of heart rhythm disorders; analysis of psycho-emotional state according to the heart rate variability (HRV).

In addition to the generally accepted indicators (duration of waves, segments, intervals, complexes, amplitude), a number of additional ECG parameters were investigated including standard deviation of normal-to-normal intervals as a measure of overall heart rate variability, the level of overall adaptive potential of autonomic regulation (SDNN); heart rate fluctuation rate (a measure of sympathetic activity); periodic dispersion of RR intervals in the frequency range from 0.4 to 0.003 (3) Hz; absolute spectral power of regulation (RMSSD); triangular index (integral of the density of distribution – total number of NN – attributed to the maximum density of distribution); entropy; absolute spectral power in domain of high frequency waves, represents the measure of nuclei activity of the vagus nerve (parasympathetic activity) (HF); ratio of sympathetic and parasympathetic regulation (LF/HF); stress index (degree of centralisation in heart rate control); generalised signs of heart failure according to all sawtooths in the first lead; symmetry of the T wave. After determining the average values of the standard ECG intervals, SHC "Cardioplus P" additionally automatically calculated the integrated indicators: Macrus index, Baevsky functional state index, psycho-emotional index (ratio of the power of the spectrum of a number of R–R intervals in different ranges taking into account the coherence of the heart rhythm), and a number of other parameters. After calculating all the parameters, they automatically formed generalised indices that reflected the state of individual structures. The most relevant clinical indicators were previously identified using cluster analysis and decision-making algorithms⁽²⁸⁾.

In addition to the general clinical examination, the patients were evaluated to determine the alleles of the *TNF- α* (*G308A*) and *IL6* (*G174C*) genes by polymerase chain reaction (PCR) using specific oligonucleotide primers (Metabion, Germany), which was followed by an analysis of polymorphism of restriction fragments; the serum levels of *IL6* and *TNF- α* were determined by chemiluminescence and electrochemiluminescence.

Statistical analysis of the results was performed using the statistical software package Statistica 10.0 (StatSoft Inc., USA). Quantitative variables are presented as mean and standard deviation ($M \pm \sigma$) or median and interquartile range [Me (Q25–Q75)], while qualitative variables – in the form of detection frequencies or percentages.

Pearson's criterion χ^2 was used to compare the frequencies of independent categorical features in conjugation tables. The significance of differences in the frequencies of the distribution of the studied traits in the groups was determined

Clinical and laboratory features of JIA		Combination of nitrogenous bases in the locus of the corresponding gene					
		<i>G174C</i> (IL6 gene)			<i>G308A</i> (TNF- α gene)		
		GG <i>n</i> = 16	GC <i>n</i> = 28	CC <i>n</i> = 14	GG <i>n</i> = 42	GA <i>n</i> = 15	AA <i>n</i> = 1
JIA markers	ANA	6 (40)	6 (25)	9 (64.29)*	17 (44.74)	3 (23.08)	0 (0)
	HLA B27	2 (13.33)	7 (29.17)	6 (42.86)*	12 (31.58)	3 (23.08)	0 (0)
	RF	0	2 (8.33)	0	0	2 (15.38)*	0 (0)
JIA subvariant	oJIA	2 (13.33)	6 (25)	5 (35.71)	11 (28.95)	2 (15.38)	0 (0)
	pJIA RF+	0	2 (8.33)	0	0	2 (15.38)	0 (0)
	pJIA RF-	5 (33.33)	4 (16.67)	2 (14.29)	9 (23.68)	2 (15.38)	0 (0)
	entJIA	3 (20)	8 (33.33)	4 (28.57)	10 (26.32)	4 (30.77)	1 (100)
	psorJIA	1 (6.67)	1 (4.17)	0	2 (5.26)	0	0 (0)
	sJIA	3 (20)*	1 (4.17)	1 (7.14)	2 (5.26)	3 (23.08)*	0 (0)
	nJIA	1 (6.67)	2 (8.33)	2 (14.29)	4 (10.53)	0	0 (0)
Clinical peculiarities of the course	Unfavourable course	5 (33.33)	17 (70.83)*	10 (71.43)*	23 (60.53)	9 (69.23)	1 (100)
	Uveitis	1 (6.67)	3 (12.5)	3 (21.43)	5 (13.16)	2 (15.38)	0 (0)
	Sacroiliitis	3 (20)	6 (25)	3 (21.43)	8 (21.05)	4 (30.77)	0 (0)
	Lesions in the hip joints	4 (26.67)	7 (29.17)	5 (35.71)	13 (34.21)	3 (23.08)	0 (0)
	Lesions in the cervical spine	2 (13.33)	3 (12.5)	2 (14.29)	5 (13.16)	2 (15.38)	0 (0)
	Hyperthermia	7 (46.67)*	4 (16.67)	5 (35.71)	12 (31.58)	4 (30.77)	0 (0)
	GI lesions	2 (13.33)	3 (12.5)	1 (7.14)	4 (10.53)	1 (7.69)	1 (100)
	Osteoporosis	4 (26.67)	9 (37.5)	4 (28.57)	12 (31.58)	5 (38.46)	0 (0)
Anaemia	4 (26.67)	4 (16.67)	3 (21.43)	7 (18.24)	2 (15.38)	1 (100)	
CVS changes according to the instrumental clinical examination	Changes in the ECG	9 (60)	15 (62.5)	8 (57.14)	24 (63.16)	9 (69.23)	0 (0)
	Changes in echo	3 (20)	5 (20.83)	6 (42.86)	10 (26.32)	4 (30.77)	0 (0)
Features of treatment	Two or more DMARDs	4 (26.67)	10 (41.67)	4 (28.57)	11 (28.95)	7 (53.85)	0 (0)
	CS per os (0.2–1 mg/kg)	8 (53.33)	12 (50)	8 (57.14)	20 (52.63)	7 (53.85)	1 (100)
	CS pulse therapy	3 (20)	3 (12.5)	2 (14.29)	6 (15.79)	2 (15.38)	0 (0)
	Intra-articular CS	7 (46.67)	9 (37.5)	8 (57.14)	20 (52.63)	3 (23.08)	0 (0)
	TNF- α blockers	5 (33.33)	6 (25)	6 (42.86)	14 (36.84)	3 (23.08)	1 (100)

G – guanine; C – cytosine; A – adenine.

*The difference is significant ($p < 0.05$) compared with other allelic groups.

Tab. 1. Allelic variants of genes of main proinflammatory cytokines in children with JIA (%)

by Pearson's criterion χ^2 with Yates correction for continuity, and using Fisher's exact t -test for tables 2×2 . Studies of the statistical relationship of quantitative traits were performed using both parametric and nonparametric criteria (correlations of Pearson, Spearman). Comparisons of two independent groups were performed by Student's t -test, or by nonparametric criteria of Wald–Wolfowitz, Mann–Whitney. When comparing several groups, the analysis of variance (ANOVA and Kruskal–Wallis ANOVA, Median Test) was used. Differences in data and correlation between data at $p < 0.05$ were considered statistically significant. Cluster analysis methods (K-means, EM with 10-fold cross-validation) were used to identify the optimal number of subgroups by traits (number of mutations, disease activity, functional state).

The study was approved by the Bioethics Committee of the Shupyk National University of Healthcare of Ukraine (protocol No. 11, dated 19 November 2018). Written informed consent was obtained from the patients and/or their parents.

RESEARCH RESULTS AND THEIR DISCUSSION

The analysis of the obtained results shows that in 34 (33.33%) examined children with JIA the onset of the disease occurred between the first and third years of life, in 25 (24.51%) children the disease began at the age of 4–7 years, in 17 (16.67%) children at the age of 8–10 years, and in 21 (20.59%) children between 11 and 16 years of life. In five patients, the exact age of onset of the disease remained unspecified.

Among the examined patients, 49 (48.04%) children had positive antinuclear antibodies (ANA), 32 patients with JIA (31.37%) were HLA B27-positive, and three (2.94%) children had a positive RF. In 29 (28.43%) patients, there was secondary systemic osteoporosis according to densitometry, in 19 (18.63%) children there was sacroiliitis, in 30 (29.41%) patients hip damage was detected, 10 (8%) developed autoimmune anterior uveitis, another 15 patients (14.71%) had a lesion of the cervical spine, 26 (25.49%) children had

hyperthermia at the onset of the disease, 11 (10.78%) children had secondary inflammatory changes of the gastrointestinal tract, while 21 (20.59%) patients had anaemia.

In 71 (69.61%) patients, ECG changes were detected, mostly in the form of diffuse metabolic changes, and changes in echo were detected in 25 (24.51%) children, presenting as minor abnormalities (mitral valve prolapse, open oval window, false chords of the left ventricle).

All treated patients received disease-modifying anti-rheumatic drugs (DMARDs) and anti-inflammatory therapy, but 35 of them (34.31%) required switching to another DMARD due to insufficient primary or secondary efficacy, 46 (45.1%) patients were prescribed corticosteroid (CS) therapy orally in doses from 0.2 mg/kg to 1 mg/kg (in cases of sJIA), while nine (8.82%) children received pulse therapy of CS due to high activity of the inflammatory process before this study. A total of 47 (46.08%) patients required intra-articular administration of CS, while 38 (37.25%) patients among the examined patients with JIA received biological therapy, one patient was switched between three biological drugs.

Overall, 62 (60.78%) children had an unfavourable course of the disease manifested as extra-articular lesions (anterior uveitis, inflammatory diseases of the gastrointestinal – GI – tract, serositis, anaemia), involvement in the pathological process of the axial skeleton and hip joints, small joints of the hands, rapid radiological progression. They noted insufficient therapeutic efficacy of one or more DMARDs, and inability to reduce the dose and withdraw the CS.

Based on the obtained results of determining the polymorphism of genes of the main proinflammatory cytokines, the patients were divided into the following groups by a combination of nitrogenous bases in the locus of the corresponding gene (Tab. 1).

The circulation of ANA and the detection of HLA B27 were most characteristic for patients with the CC (homozygous) allele of the *G174C* gene.

Echocardiography revealed the following changes: mitral valve prolapse in four patients, false chord in 10 children, open oval window in one child with IL6 174 GC gene genotype, left ventricular dilatation, tricuspid insufficiency and cardiomyopathy in one case with IL6 174 CC genotype (severe inflammatory process, extra-articular lesions), left ventricular hypertrophy – one case (174 GG).

According to the standard 12-channel ECG, the patients presented with such phenomena as sinus arrhythmia (21 children, 20.59%) and sinus bradycardia (two patients, 2%). Deviations of the electrical axis of the heart were detected in 27 (27%) children, of whom two had tachycardia (genotype 174 GC), and two more bradycardia (genotypes 174 GC and 174 CC of the IL6 gene). Two children had impaired conduction by the His bundle (one child with pJIA and one with entJIA), and six children had a potentially threatening cardiac arrhythmia in the form of a shortening of the pQ interval. In 17 (16.67%) children, there was an incomplete blockade of the right leg of the His bundle,

which occurred in almost every subvariant of JIA, but these changes could correspond to the age norm and the presence of minor abnormalities of heart development, such as mitral valve prolapse and/or abnormal left ventricular chord. An increase in bioelectric conductivity was observed in only one child with entJIA. Extrasystoles on the ECG were recorded in one child with genotype 174 CC. Signs of left ventricular hypertrophy were present in two children.

Metabolic changes in the myocardium without clinical manifestations were observed in two thirds of the children (64%), including 22 children with oJIA, 17 children with pJIA, 16 children with entJIA, six children with sJIA, three children with psorJIA and nJIA, but no changes indicated the location or nature of the lesion, or correlated with other laboratory and instrumental changes.

With the help of SHC “Cardioplus P” HRV indicators were evaluated including operative control of regulation (integral assessment of functional CVS level, deviations of NN intervals, activity levels of sympathetic and parasympathetic regulation), periodic dispersion, assessment of operative and complex myocardial condition. According to the data obtained, 86% of children had some deviation in each of the calculated parameters.

The operative control of regulation (norm 75–100%) had the lowest average value ($65 \pm 5.66\%$) in children with pJIA RF+. It was also quite reduced in patients with psoriatic arthritis and sJIA, but patients with oJIA and pJIA RF– mostly had results within the norm ($80.1 \pm 9.39\%$ and $80.5 \pm 6.61\%$). An increase in heart rate relative to the age norm was most often observed in patients with sJIA and nJIA on the background of normal body temperature. SDNN values were significantly reduced in the group of children with pJIA RF+ (30.5 ± 12.03 ms at a rate of 39 ms), and moderately reduced in the groups sJIA, entJIA and nJIA, but in children with oJIA and pJIA RF– the indicator on average was not reduced, the RMSSD (the difference between the current and previous RR intervals) again had the best results in children with oJIA and pJIA RF– (61.9 ± 33.74 and 50.5 ± 14.06 ms at a rate of 30 ms), and the lowest average was noted in the group pJIA RF+. The triangular index, on the contrary, had higher values in children with pJIA RF+ and entJIA, while in patients with oJIA and sJIA it had a lower average value (15.91 ± 14.69 vs. 9.39 ± 4.12). The activity of the vasomotor centre of regulation, which deviated from the norm by 30%, had exceeded values in children with sJIA (54.5 ± 10.61 , norm 0–37), entJIA (43.25 ± 23.68), and nJIA (58 ± 57.98). The presence of the above-mentioned norm of heart rate entropy was observed in only 21% of children, and on average, each group maintained a normal entropy.

Deviations in all the above indicators were observed in 17–34% of examined children, i.e. not more than a third of cases. The following indicators were violated in 50–86% of cases.

Thus, the stress index at normal to ≤ 120 was rejected in 69% of examined patients, in all groups without exception,

with the highest level observed in the group of nJIA (461.5 ± 125.16). The condition of regulation reserves (norm 75–100) was reduced in 87% of patients, with the lowest rate noted in the groups entJIA and pJIA RF+ (57.5 ± 4.95). Autonomic balance 1 (LF/HF) had a deviation of 78%; in one child with nJIA, this mark was abnormally high (>20 with a standard range of 1–3). Autonomic balance 2 (AIE) was changed slightly in less than of children (48%) and in children with pJIA RF+ it was below normal (50.5 ± 70 , norm 100–350), and in groups sJIA, entJIA, psorJIA and nJIA – significantly above normal (540.5 ± 51.61 in the group nJIA). The activity of the subcortical centres of regulation had a deviation of 78%, but with the exception of the group pJIA RF+ (6.5 ± 6.36 , norm 3) the deviations were not significant enough. Integral indicators of heart rate variability (norm 75–100) were reduced in more than 50% of children, significantly lower in the group nJIA (36 ± 45.45), but closest to the norm (73.75 ± 16.01) in the group entJIA. Baevsky functional state points (norm 0–2) in children significantly exceeded the interval, but it was assessed by the software as positive mark. The complex indicator of regulation (norm 75–100) was changed in 61% of children. The lowest indicators were observed in the group sJIA and nJIA (63.5 ± 6.36 and 62.5 ± 17.68).

In general, heart rate variability was more pronounced in children with sJIA, entJIA, nJIA and pJIA RF+, which corresponds to a more aggressive course in these groups, while children with oJIA and pJIA RF– usually had fewer disorders.

Regarding the indicators reflecting the state of the myocardium, only some indicators, mostly those that characterise more severe CVS disorders, in patients with JIA almost did not deviate from the norm, while the overall proportion of myocardial abnormalities was in the range of 70–95% of deviations in each group of children. Thus, the myocardial reserve was compromised in 93% of patients (62.65 ± 2.09 at a rate of 75–100), the complex indicator of functional status (CI FS, %) was reduced in 61% of children (67.71 ± 2.74), the angle of alpha QRS in the frontal plane in 80% deviated from the norm, mainly in the direction of increasing the index. The ECG phase ratio index was reduced in all patients examined with the SHC “Cardioplus P” and was 25.53 ± 5.22 at a rate of 75–100, but this may be due to the presence of a large number of patients under seven years of age who had a higher predisposition to respiratory sinus arrhythmias. One of the parameters that most often displayed deviations was the symmetry of the T-wave with the ratio of the maximum derivatives and the ratio of the planes of the triangles in most leads (usually exceeded the normative values). The integral index of the ST-T in all leads was reduced by 15–30%, however, deviations in the morphology of the T-wave were quite rare (less than 20% of cases).

Thus, with the symmetry of the T waves in different leads, a strong inverse correlation of JADAS27 parameters was found: with the symmetry of the T wave in the ratio of the

planes of the triangles in the lead I ($r = -0.89$); symmetry of the T wave in the ratio of the maximum derivatives in lead II ($r = -0.58$); symmetry of the T wave in the ratio of the maximum derivatives in lead III ($r = -0.59$); symmetry of the T wave in the ratio of the maximum derivatives in the lead augmented Vector Left (aVL) ($r = -0.59$); symmetry of the T wave in the ratio of the maximum derivatives in the lead augmented Vector Foot (aVF) ($r = -0.55$); with the morphology of the T wave ($r = -0.64$).

The examined children had lesions not only within the musculoskeletal system and CVS, but also in other organs, which affected the course of the disease and treatment. Thus, according to ultrasound evaluation, changes in parenchymal organs were detected in 42 children. Six patients had skin syndrome, mainly at the onset of the disease, while hepatosplenomegaly was observed in 16 patients. In two cases, blood was found in the stool; these children had gastrointestinal abnormalities (overall, gastrointestinal lesions including colitis and hepatitis were noted in six cases).

Other comorbid pathologies were noted in 46 cases, of which 39 were inflammatory, most often chronic tonsillitis (nine children). A relatively higher frequency of comorbidities was associated with the genotype 174 GC ($p > 0.01$). The relationship between the JIA subvariant and the genotype of IL6 and TNF genes was analysed. In the CC group of the *G174C* gene, oJIA was more often detected, followed by entJIA, with only one case of sJIA, and no cases of psorJIA and pJIA RF+ detected. However, the GG group (wild allele of the *G174C* gene) had the most cases of sJIA, as well as cases of pJIA RF–, in contrast to the cases of entJIA in this variant of the allele was the least among all subgroups. The GC group, a heterozygous state of the risk allele, was the largest of the three variants of the IL6 gene polymorphism, which coincides with previous studies^(15,18,19), and had a higher number of entJIA cases than the two parallel groups, with both cases of pJIA RF+ also belonging to this group.

The average values of JADAS27 in groups of patients with different alleles of the *G174C* gene did not differ significantly. JADAS27 was slightly higher for the GC allele of the *G174C* gene (14.39 ± 2.13) than for the other groups.

When testing the hypothesis of the independence of traits (taken as categorical traits) analysis of conjugation tables revealed a significant statistical relationship between the frequency of unfavourable course of JIA and the allelic variant of the gene IL6 (*G174C*) GC, in which the number of unfavourable cases was greater than in other variants (Pearson Chi-square = 6.65, $df = 2$, $p = 0.045$).

Also, patients with the IL6 GC gene allele had a slightly higher activity of the disease than other *G174C* genotypes. However, in different variants of allelic polymorphism there were certain features of the joint syndrome. Thus, oJIA and pJIA RF– were found more frequently in carriers of GC (8%) and GG (10.5%) alleles of the IL6 gene.

Also, no significant difference was found between the allelic variants of the IL6 gene in terms of joint damage in disabling loci. Thus, hip lesions were found in 16 children with

JIA (32.65%), sacroiliitis in 12 patients, and cervical spine lesions in seven children, but no differences between these genotypes were observed.

On ultrasound of the joints, exudative-proliferative synovitis was relatively more often observed in patients with the GC variant of the IL6 gene (12 subjects, 25.0%).

The frequency of changes on the standard 12-channel ECG in the examined children was generally high (80.85%). However, statistically significant differences between the presence of changes in the ECG, the number of mutations, or the genotype of the IL6 gene, were not detected. Relatively higher frequency of metabolic changes recorded in the ventricular myocardium by ECG (23.81%) was characteristic of the allelic variant of the IL6 GC gene.

Polymorphism of the *G308A* gene is most often represented by the GG group (wild allele), while the AA phenotype (homozygous state of the risk allele) was detected only once. In the group of patients carrying the GA allele of the TNF gene, cases of sJIA were significantly more frequent, which corresponds to the data of other researchers^(22,23), and it included both cases of pJIA RF+.

The mean age of onset of the disease in the group of patients with JIA who were carriers of the wild GG allele of the IL6 *G174C* gene was 6.79 ± 1.07 years, and no gender-related features were noted (seven boys and eight girls). As can be seen from Tab. 1, only a third of patients in this group had an unfavourable course, which, compared with the groups of heterozygous and homozygous states of the risk allele, is more than twice less. Also, there was only one case of uveitis in this group, and lesions of the axial skeleton and hip joints were less common than in the other subgroups, while the incidence of secondary osteoporosis was lower. However, hyperthermia at the onset of the disease and anaemia of chronic inflammation occurred more often in these patients than in the other two subgroups, which may be a side clinical marker of high levels of functionally active IL6, and potentially determine the prospects of its inhibitors. They also had a slightly higher incidence of secondary lesions of the GI tract. Regarding treatment, patients in the wild allele group were less likely to require the replacement of DMARDs due to primary or secondary inefficacy of treatment, but more than 50% of them received CS orally at a therapeutic dose.

The group of patients with the heterozygous risk allele of the GC gene of the IL6 *G174C* gene did not differ significantly in terms of the age of onset (6.95 ± 0.82 years), but most of them were girls (17 girls and seven boys). Such features of the IL6 gene were found in patients with JIA most often, which coincided with the data obtained in previous studies⁽²⁹⁾. Compared with the previous group, these patients were much more likely to have an adverse course of the disease, and require replacement of DMARDs; all pJIA RF+ belonged to this group, and almost 40% had secondary systemic osteoporosis. However, the prevalence of CS use, both orally and intra-articularly or as pulse therapy, was the lowest in these children, with hyperthermia and anaemia being the least common.

The largest proportion of male patients (nine boys, four girls) was observed in the group of patients with JIA with CC homozygosity for the risk allele of the IL6 *G174C* gene. The age of onset of the disease was 6.08 ± 1.21 years; in three quarters of the patients the course of the disease was unfavourable, with a high frequency of ANA and HLA B27 detection, while in the previous two groups the frequency of their detection was much lower. It should be noted that ECG changes were observed in all three groups proportionally, but for the most part the changes were nonspecific and non-threatening in nature, as changes in echocardiography were often minor abnormalities of heart development such as additional left ventricular chord or mitral valve prolapse of the first degree. The group comprised a case of ultrasound-confirmed cardiomyopathy and dilatation of the left ventricle, possibly due to the long absence of therapy in this child with a high activity of the inflammatory process. In the study of allelic polymorphism of the *G308A* gene, the homozygous risk allele AA was detected in only one patient: a girl with the onset of the disease at the age of 13, entJIA, adverse disease course, and the development of extra-articular manifestations presenting as inflammatory changes in the GI tract.

The mean age in the *G308A* GG group (wild allele) was 8.86 ± 0.73 years, with eight boys, 20 girls, and the onset age of 6.23 ± 0.7 years. The wild allele group was three times larger than the GA group. This group was approximately twice as likely to have positive ANA, and there was no case of seropositivity for RF, but for most of the indicators evaluated in this study, both groups – GG and GA – were approximately comparable, and the difference in the frequency of complications (with some exceptions) was minor. However, significant differences were found for JADAS27 depending on the allelic polymorphism of the TNF- α gene (GA JADAS27 = 9.08 ± 2.90 but GG JADAS27 = 15.28 ± 1.18) by *t*-test ($p = 0.053$).

Given the above, it can be assumed that the use of the polymorphism of each of the two genes separately does not allow to make the previous phenotypes, despite the fact that there are small differences between the subgroups. This preceded the attempt to describe patient phenotypes based on a combination of *G308A* (TNF- α) and *G174C* (IL6) gene polymorphism variants.

Based on the obtained homo- or heterozygous variants of the alleles of both genes, the patients were divided into seven groups depending on their combinations (*G174C* GG + *G308A* GG, *G174C* GC + *G308A* GG, *G174C* CC + *G308A* GG, *G174C* GG + *G308A* GA, *G174C* GC + *G308A* GA, *G174C* CC + *G308A* GA, *G174C* GG + *G308A* AA), however, in the group of patients with JIA with the wild GG allele of the IL6 *G174C* gene and the homozygous state for the AA allele of the TNF- α *G308A* gene, there was only one child with entJIA.

Eleven children among the examined group of patients with JIA had a combination of the GG allele (wild allele) of the IL6 *G174C* gene with the wild GG allele of the TNF- α

G308A gene, which accounted for one-fifth of the total number of patients (21.15%) (50% girls), in 50% of cases there was fever at the onset of the disease, in 40% polyarticular lesions (only 20% oJIA and entJIA, 10% sJIA and psorJIA), and in 70% the course of the disease was favourable. Despite the high frequency of registration of ANA (60%) and HLA B27 (22%), they did not have extra-articular lesions such as uveitis or inflammatory bowel disease. However, 78% of children had changes on the ECG in the form of moderate metabolic changes in the myocardium. In the group with the wild GG allele of the IL6 gene *G174C* and the GA gene of the TNF- α *G308A* gene (heterozygous state of the risk allele), which included only three children (5.77% of the total number of examined patients with JIA), most of them (67%) boys and patients with sJIA (67%), the same number had secondary osteoporosis. In 100% of children, ECG changes were detected, but only in one patient they were clinically significant (shortening of the pQ interval). There were no cases of ANA+ in this group, and all patients were HLA B27-negative and negative for RF. All patients received CS therapy, including high-dose treatment in two cases of systemic JIA, and one child required anti-IL6 therapy.

The largest proportion of patients (32.69%) were 17 patients with JIA with a combination of the risk allele in the heterozygous state of the IL6 gene *G174C* (GC) and the presence of the wild allele GG *G308A*. 65% of the group consisted of girls. According to subvariants, one third of the patients had oJIA, another third – entJIA. Adverse course of the disease occurred in 76%, in 40, 63% several concomitant pathologies were registered, the activity of the disease was high (average score for JADAS27 was 17 ± 0.8 points), with a high frequency of changes on the standard 12-channel ECG (73%), including 23% potentially life-threatening (shortening the pQ interval). Secondary osteoporosis was noted in 53%. 17.6% of patients developed inflammatory changes in the gastrointestinal tract during the course of the disease. Regarding treatment, 40% of patients switched between several DMARDs due to primary or secondary ineffectiveness of therapy, four children received anti-TNF- α therapy, one child had switched between three biologicals: anti-TNF- α , Janus kinase inhibitor, anti-IL6. Nine patients (52.94%) received CS orally, three of them additionally received several courses of pulse CS therapy. Six children underwent intra-articular administration of CS.

The group with the combination of GC (heterozygous risk allele of the IL6 *G174C* gene) and GA (heterozygous risk allele of the TNF- α *G308A* gene) included only seven children (13.46% of all patients with JIA), most of whom (86%) were girls. In this group, there was a relatively older age of onset of the disease (7.7 ± 3.04 years). This group included all cases of pJIA RF+. However, no cases of inflammatory lesions of the gastrointestinal tract (hepatitis, colitis) and uveitis were observed in this group. Changes in the ECG were detected slightly less frequently than in the previous groups, and were not threatening. 57% of these

patients had switched between DMARDs, and the same number received biologic therapy.

In contrast, in the JIA group that had a homozygous state of the CC risk allele of the IL6 *G174C* gene in combination with the wild GG allele of the TNF- α *G308A* gene, the majority of the group were male patients (70%), who also had an adverse course in 70% as uveitis, hip damage associated with ANA positivity (86%) and HLA B27 positivity (56%), and required anti-TNF- α therapy in 55% of cases. Changes in ECG and ultrasound of the heart were noted with a high frequency in this group of children.

In a small group of three patients (including two boys) with JIA (5.77% of all JIA) who were carriers of the homozygous allele of CC risk of the IL6 *G174C* gene at the same time as heterozygosity for the GA allele of the TNF- α *G308A* gene, 100% had an unfavourable course, all had changes on the standard 12-channel ECG in the form of moderate metabolic changes in the myocardium, and all required the introduction of biological therapy.

According to the laboratory parameters in the examined children with JIA, among these indicators, only the activity of ALT was higher in cases with the genotype 174 GG compared with 174 CC (36.41 ± 3.9 U/L vs. 17.44 ± 1.6 U/L).

The content of the main proinflammatory cytokines in patients with JIA did not differ significantly, depending on the allelic variant of the *G174C* gene, because it reflected the overall disease activity and corresponded to certain features of cytokine imbalance in different subvariants of JIA. However, cases with extremely high levels of TNF- α in the serum of patients with JIA (1,040–2,115 pg/mL) were observed only in the GA phenotype of the TNF- α *G308A* gene, which could be a manifestation of overdominance – a phenomenon in which the dominant allele in the heterozygote is more pronounced than in the homozygote⁽¹⁷⁾. Interestingly, in the presence of a complete mutation of *G174C* in this group, the lowest level of IL6 in serum was observed (5.63 ± 4.04 pg/mL), which does not contradict the data reported by previous researchers on the highest level of IL6 in carriers of the wild allele *G174C*⁽²⁹⁾.

The content of IL6 in the serum according to nonparametric tests was the highest in cases with the GC genotype of the IL6 gene: median 13.60 for 174 GC compared to 8.89 for CC, and 6.98 for GG.

According to the median test, some differences in IgM content in patients with JIA depending on the allelic polymorphism of the IL6 gene were identified, so, most of its content depended on the level of IL6 in patients with JIA with the CC allele of the IL6 gene [CC ($n = 5$) 61.02 ± 123.91 (1.50–282.60); GC ($n = 15$) 16.44 ± 20.43 (1.50–81.20); GG ($n = 8$) 31.08 ± 147.71 (1.50–135.40)].

A significant correlation was found between the content of IL6 and TNF- α in the serum of patients with JIA – Spearman correlation coefficient $R = 0.57$, $p = 0.03$ ($r = -0.065$), which indicated the feasibility of taking into account their ratio as a significant indicator in the processes of immune regulation.

Variable	Aggregate results of descriptive statistics								
	Cluster	Valid n	Mean	Minimum	Maximum	SD	Median	Lower quartile	Upper quartile
JADAS27, score	1,000	10	9.1	0	23	6.7	9.5	5.00	12.00
n of mutations	1,000	10	1.5	0	5	2.0	0	0	3
CI FS, %	1,000	10	77.7	76	79	0.94	78.0	77.00	78.00
JADAS27, score	2,000	15	12.9	0	27	6.9	14.0	10.00	16.00
n of mutations	2,000	15	2.53	0	6	2.19	3	0	4
CI FS, %	2,000	15	61.9	47	74	9.1	62.0	56.00	71.00

Tab. 2. Distribution of patients with JIA in cluster groups by the number of mutations in the genes of proinflammatory cytokines IL6 G174C and TNF-α G308A

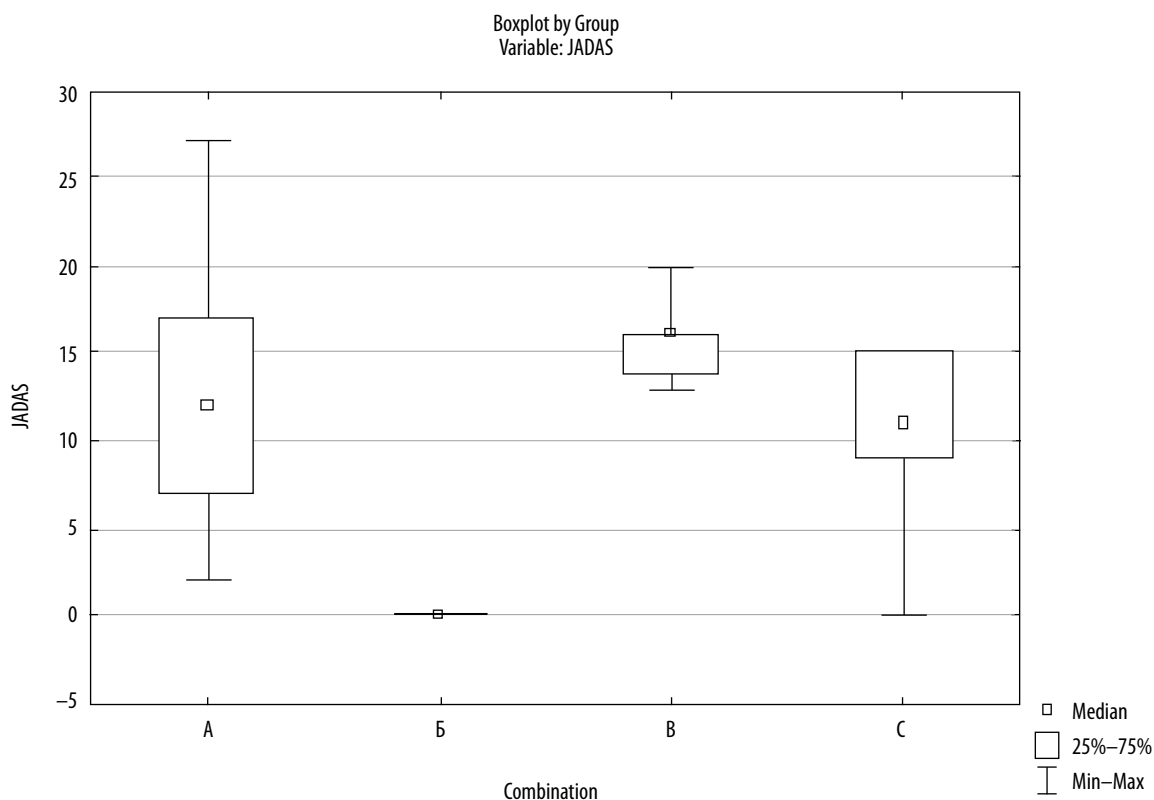
However, the variability in the serum levels of interleukins in patients with JIA in each group was very high, and significantly elevated levels of TNF-α were observed in patients in drug remission, so it can be assumed that determining only the content of cytokines in peripheral blood is not informative enough for predicting further course of JIA and the development of certain complications.

To further assess the informativeness of the prediction of cardiovascular lesions in patients with JIA, an in-depth cluster analysis of A-ECG data was performed with other data, including allelic polymorphism of these genes of the main proinflammatory cytokines (IL6, TNF-α).

According to the indicators of disease activity, the number of mutations and the complex indicator of the functional state of the myocardium, the optimal number

of cluster groups was determined, namely, two clusters (Tab. 2). K-means and EM algorithms were used. The number of children who finally entered this stage of the analysis was 25. The number of mutations that were identified as a quantitative variable was marked as “0” where there was no data.

As can be seen from Tab. 2, in cluster 1 (ten children, group 1) the higher level of a complex indicator of a functional condition of a myocardium and smaller degree of disease activity is noted, while in cluster 2 (15 children, group 2) high general activity of the disease is observed together with a decrease in the complex indicator of the functional state (CI FS, %) of the myocardium. It is notable that in the second group the onset of the disease occurred relatively earlier, but the difference is insignificant.



66 Fig. 1. JIA activity according to JADAS27 in two comparison clusters (differences between groups according to Wald-Wolfowitz test, $p = 0.033$; according to non-parametric Mann-Whitney U test $U = 43.0$, $p = 0.08$)

Variable	Mean			Normal range
	Cluster 1	Cluster 2	<i>p</i>	
Heart rate per min	76.9000	90.0000	0.10	60–100
Fractal index	0.80	0.68	0.07	≥ 0.75
Baevsky index of functional state, points	3.60	4.73	0.08	0–2
Immediate control of the condition of myocardium	62.30	46.13	0.01	75–100
Integral indicator of ST-T form (lead I)	77.40	53.20	0.02	75–100
Dislocation of ST segment (lead I), mV	0.021	–0.002	0.496	–0.7–0.7
T wave (high) (lead I)	0.55	1.11	0.31	0.143–0.333
T wave symmetry based on derivatives ratio (lead I)	0.59	1.16	0.28	0.45–0.7
Integral indicator of ST-T form (lead II)	64.70	52.87	0.076	75–100
Dislocation of ST segment (lead II), mV	0.09	0.01	0.10	0.7–0.7
T wave (high) (lead II)	0.38	0.36		0.125–0.25

Tab. 3. Main A-ECG parameters registered by SHC “Cardioplus P” in cluster groups (by the number of mutations in the genes of the main proinflammatory cytokines) in patients with JIA

These cluster groups of patients with JIA showed a number of significant differences in general clinical laboratory parameters: the activity of AST in the blood of patients with JIA was 29 ± 3.1 IU/L in the first group against 24 ± 2.2 IU/L in the second group ($p = 0.07$ Wald–Wolfowitz), serum creatinine was higher in the first group 68.8 ± 11.0 $\mu\text{mol/L}$ (against 57.0 ± 9.3 $\mu\text{mol/L}$ in the second group; $t = -2.24$, $p = 0.044$ significantly), also in the second group according to the general analysis of blood there was leukocytosis ($11.6 \pm 4.1 \times 10^9$ vs. $6.69 \pm 0.8 \times 10^9$, $p = 0.075$ Wald–Wolfowitz) (Fig. 1). According to the data obtained from cluster analysis, the total number of mutations in the genes IL6 and TNF- α

determined the characteristics of the course of JIA. In cases with a larger number of mutations, a more aggressive course of the disease, a more pronounced degree of inflammation, and a higher frequency of extra-articular lesions, including changes in the CVS, were noted. The data of the leading A-ECG indicators in the selected cluster groups of patients with JIA were analysed (Tab. 3).

The generalised A-ECG indicators (operative state of the myocardium, the integrated indicator of the form of ST-T in the first lead, etc.) differed significantly in clusters, and patients with JIA who had a higher total number of mutations in genes encoding proinflammatory cytokines also

Variable	The indicators in cluster groups and Student's t-test values				
	Mean 1,000	Mean 2,000	t value	df	<i>p</i>
Condition of myocardium reserves	63.10	61.13	0.51	23	0.615980
Amplitude-areas index (lead I)	54.70	50.67	0.71	23	0.487060
P amplitude (lead I), μV	42.90	37.87	0.33	23	0.740707
Q amplitude (lead I), μV	–98.70	–219.93	1.54	23	0.138180
R amplitude (lead I), μV	263.80	244.93	0.16	23	0.878188
T amplitude (lead I), μV	90.80	–18.45	1.61	23	0.120690
Area P, $\mu\text{V}^*\text{s}$	0.002	0.001	1.24	23	0.228547
Area QRS, $\mu\text{V}^*\text{s}$	0.012	0.013	–0.43	23	0.670298
Area T, $\mu\text{V}^*\text{s}$	4.67	2.25	1.5	23	0.147685
J point dislocation, μV	23.40	13.50	0.33	22	0.744656
Amplitude-areas index (lead II)	64.60	49.33	1.67	23	0.108170
T amplitude (lead II), μV	211.40	50.57	1.58	22	0.127668
Index of ECG intervals duration, ms	81.90	87.67	–1.55	23	0.135191
Myocardial index of stationarity	93.50	56.57	1.93	11	0.079206
Advanced ECG analysis (12 leads)	70.60	56.60	2.47	23	0.021131
3D QRS-T angle, $^\circ$	45.80	91.36	–2.44	22	0.023289
Complex indicator of the condition of myocardium (12 leads)	62.90	53.27	2.25	23	0.034127
Psycho-emotional index	72.80	72.20	0.39	23	0.698974
CI FS, %	77.70	61.93	5.40	23	0.000017

Tab. 4. Differences in main A-ECG parameters in cluster groups (depending on the number of mutations in the genes of the main proinflammatory cytokines) in patients with JIA according to Student's t-test

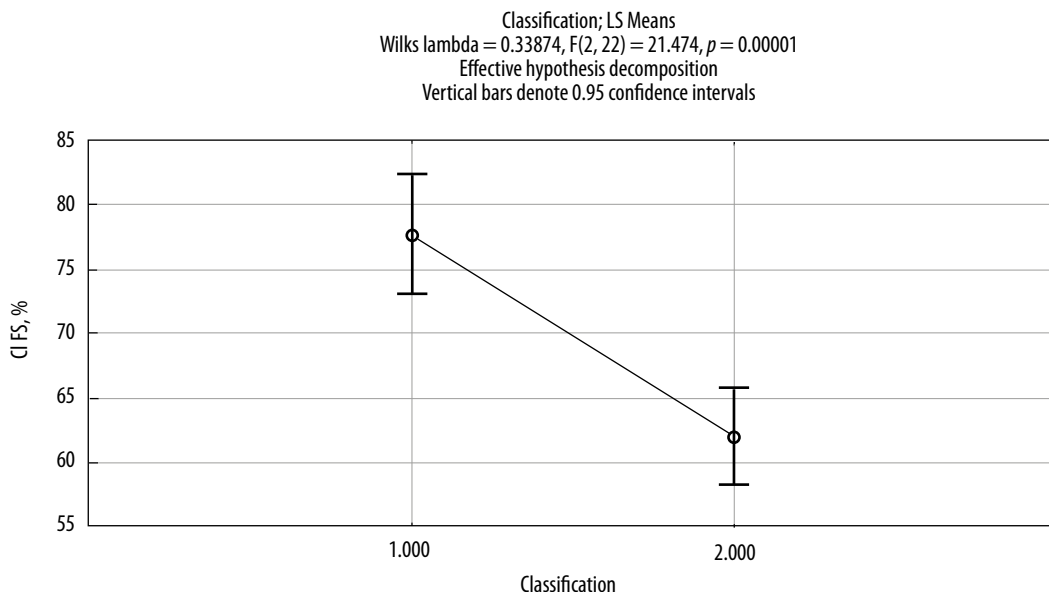


Fig. 2. RMS values of the complex indicator of the functional state of the myocardium in cluster groups (depending on the number of mutations in the genes of the main proinflammatory cytokines) in patients with JIA

had more pronounced deviations from the norm of several indicators of the state of the myocardium. To a lesser extent, the Baevsky functional state and fractal index differed in these comparison groups as well.

Comparison of the A-ECG parameters in patients with JIA from different cluster groups (selected by the number of mutations in the genes of the main proinflammatory cytokines IL6 and TNF- α) using the Student's *t*-test is given in Tab. 4.

The complex indicator of the functional state of the myocardium according to the A-ECG evaluation, which is its key indicator, in the comparison clusters differed significantly (with a high level of significance, $p = 0.00001$) (Fig. 2). In addition, a number of other parameters were identified, the difference between which in the clusters was highly reliable, including in-depth ECG analysis ($p = 0.02$), 3D QRS angle and a complex indicator of myocardial status. When calculating the significance of the difference between the A-ECG parameters previously assessed as the most significant⁽²⁸⁾, in all cases, the indicators in patients with JIA who carried more mutant genes of proinflammatory cytokines (IL6 and TNF- α) were notably worse (T amplitude in lead I, the area T in lead I and the amplitude-areas index in lead II). Summarising the above, it should be noted that allelic gene polymorphism certainly affects the structure and functional properties of proinflammatory cytokines in patients with JIA. According to this study, the number of mutations in the IL6 and TNF- α genes is essential for predicting further course of the disease, as the combination of homozygous or heterozygous mutations of both *G174C* and *G308A* genes potentiates the development of greater inflammatory activity, adverse heart disease, and negative changes in cardiomyocytes. This can lead to different responses to the routinely

prescribed protocol-based treatment of children. Therefore, we believe that attempts to predict further course of the disease taking into account certain genetic features of TNF- α *G308A* and IL6 *G174C* genes by using A-ECG are promising and useful for the selection of treatment strategy in patients with JIA. This is necessary to prevent adverse events later in life, especially from the cardiovascular system.

CONCLUSIONS

1. The presence of mutant alleles of the IL-6 (*G174C*) GC gene in patients with JIA promotes the release of the largest amount of interleukin 6 and immunoglobulin M in the serum and causes a more severe course of the disease.
2. The combination of the GC allele of the IL6 *G174C* gene and the GA allele of the TNF- α *G308A* gene is the most common, occurs in all JIA subvariants, and is unfavourable for the development of CVS lesions.
3. Patients with JIA who carry two or more mutations in different genes of proinflammatory cytokines have a higher activity of the inflammatory process and a higher frequency of cardiovascular changes according to A-ECG evaluation.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations which might negatively affect the content of this publication and/or claim authorship rights to this publication.

References

1. Ravelli A, Martini A: Juvenile idiopathic arthritis. *Lancet* 2007; 369: 767–778.
2. Sherry DD: Juvenile idiopathic arthritis. *Medscape* 2021. Available from: <https://www.medscape.com/answers/1007276-43052/how-does-the-prevalence-of-juvenile-idiopathic-arthritis-jia-vary-by-age>.
3. Peters MJL, Symmons DPM, McCarey D et al.: EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis* 2010; 69: 325–331.
4. Ahmad HS, Othman G, Farrag SE et al.: Subclinical heart failure in juvenile idiopathic arthritis: a consequence of chronic inflammation and subclinical atherosclerosis. *Egypt Rheumatol Rehabil* 2016; 43: 78–83.
5. Oshlianska OA, Chaikovskiy IA, Artsymovych AG et al.: Assessment of the cardiovascular system in patients with rheumatic diseases of children using the software and hardware complex Cardio-Plus P. *Modern Pediatrics* 2017; 8: 59–67.
6. Signal-averaged electrocardiography. *J Am Coll Cardiol* 1996; 27: 238–249.
7. Amaya-Amaya J, Montoya-Sánchez L, Rojas-Villarraga A: Cardiovascular involvement in autoimmune diseases. *Biomed Res Int* 2014; 2014: 367359.
8. Engin AB, Engin A: *Endothelium: Molecular Aspects of Metabolic Disorders*. CRC Press, Boca Raton, FL 2013.
9. Smart NA, Larsen AI, Le Maitre JP et al.: Effect of exercise training on interleukin-6, tumour necrosis factor alpha and functional capacity in heart failure. *Cardiol Res Pract* 2011; 2011: 532620.
10. Panichi V, Maggiore U, Taccola D et al.: Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients. *Nephrol Dial Transplant* 2004; 19: 1154–1160.
11. Kishimoto T, Akira S, Taniuchi T: Interleukin-6 and its receptor: a paradigm for cytokines. *Science* 1992; 258: 593–597.
12. van den Ham HJ, de Jager W, Bijlsma JWJ et al.: Differential cytokine profiles in juvenile idiopathic arthritis subtypes revealed by cluster analysis. *Rheumatology (Oxford)* 2009; 48: 899–905.
13. Kaminiarczyk-Pyzalka D, Adamczak K, Mikos H et al.: Proinflammatory cytokines in monitoring the course of disease and effectiveness of treatment with etanercept (ETN) of children with oligo- and polyarticular juvenile idiopathic arthritis (JIA). *Clin Lab* 2014; 60: 1481–1490.
14. Woo P: Cytokines and juvenile idiopathic arthritis. *Curr Rheumatol Rep* 2002; 4: 452–457.
15. Amr K, El-Awady R, Raslan H: Assessment of the $-174G/C$ (rs1800795) and $-572G/C$ (rs1800796) *interleukin 6* gene polymorphisms in Egyptian patients with rheumatoid arthritis. *Open Access Maced J Med Sci* 2016; 4: 574–577.
16. Ulhaq ZS, Garcia CP: Inflammation-related gene polymorphisms associated with Parkinson's disease: an updated meta-analysis. *Egypt J Med Hum Genet* 2020; 21: 14.
17. Singh RS, Kulathinal RJ: in Brenner's *Encyclopedia of Genetics*. 2nd ed., Academic Press, 2013: 4367.
18. Pignatti P, Vivarelli M, Meazza C et al.: Abnormal regulation of interleukin 6 in systemic juvenile idiopathic arthritis. *J Rheumatol* 2001; 28: 1670–1676.
19. Ogilvie EM, Fife MS, Thompson SD et al.: The $-174G$ allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. *Arthritis Rheum* 2003; 48: 3202–3206. doi:10.1002/art.11300
20. Zhang YJ, Li MH, Yang SH et al.: Correlation of TNF- α G308A polymorphism and rheumatic heart disease: a meta-analysis. *Chinese Journal of Evidence-Based Medicine* 2012; 12: 1209–1212.
21. Siniauskaya E, Kuzhir T, Yagur V et al.: IL6 $-174G/C$ (rs1800795) polymorphism rather than IL6R (rs2228145 and rs4845618) polymorphisms is associated with susceptibility to rheumatoid arthritis in the Belarusian population. *J Genet Genomic Sci* 2020; 5: 015.
22. Mourão AF, Caetano-Lopes J, Costa P et al.: Tumour necrosis factor- α -308 genotypes influence inflammatory activity and TNF- α serum concentrations in children with juvenile idiopathic arthritis. *J Rheumatol* 2009; 36: 837–842.
23. Modesto C, Patiño-García A, Sotillo-Piñeiro E et al.: TNF- α promoter gene polymorphisms in Spanish children with persistent oligoarticular and systemic-onset juvenile idiopathic arthritis. *Scand J Rheumatol* 2005; 34: 451–454.
24. Ozen S, Alikasifoglu M, Bakkaloglu A et al.: Tumour necrosis factor α G \rightarrow A -238 and G \rightarrow A -308 polymorphisms in juvenile idiopathic arthritis. *Rheumatology (Oxford)* 2002; 41: 223–227.
25. Scardapane A, Breda L, Lucantoni M et al.: TNF- α polymorphisms in juvenile idiopathic arthritis: which potential clinical implications? *Int J Rheumatol* 2012; 2012: 756291.
26. Petty RE: Prognosis in children with rheumatic diseases: justification for consideration of new therapies. *Rheumatology (Oxford)* 1999; 38: 739–742.
27. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; 93: 1043–1065.
28. Chaikovskiy I, Oshlianska O, Artsymovych A et al.: Using of Data Mining methods to evaluate the myocardial damage in children with juvenile idiopathic arthritis. *IEEE 40th International Conference on Electronics and Nanotechnology (ELNANO)*, 2020: 391–395. DOI: 10.1109/ELNANO50318.2020.9088843.
29. Li B, Xiao Y, Xing D et al.: Circulating interleukin-6 and rheumatoid arthritis: a Mendelian randomization meta-analysis. *Medicine (Baltimore)* 2016; 95: e3855.