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Molecular Genetic Studies in Children With Juvenile Rheumatoid Arthritis

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Резюме: Исследования интерлейкина-17 (IL-17) выявили его ключевую роль в иммунном ответе на бактериальные и грибковые инфекции. В то же время, чрезмерная продукция IL-17 связана с аутоиммунными заболеваниями, включая ювенильный ревматоидный артрит (ЮРА). В данной работе изучается полиморфизм гена IL-17A (G-197A) у детей с ЮРА. Исследование показало, что у детей с генотипом AA наблюдается повышенный уровень IL-17A, что может указывать на предрасположенность к ЮРА. Было выявлено значительное различие в уровнях IL-17A среди генотипов G/G, G/A и A/A.

Ключевые слова: ювенильный ревматоидный артрит, IL-17A, полиморфизм, генетические маркеры, аутоиммунные заболевания.

Relevance: The interleukin-17 (IL-17) gene was first found in the cDNA of mouse T cells in 1993. IL-17 plays a key role in protecting the body from extracellular bacterial and fungal infections. However, excessive production of this protein is associated with immunoinflammatory and autoimmune diseases, such as JRA. IL-17 has become an important therapeutic target in the treatment of various human chronic inflammatory diseases [1, 5, 9].

The interleukin 17 family includes six cytokine proteins with a mass of 20–30 kDa, differing in their functional activity. The most studied members of the family are IL-17A and IL-17F, which can exist as homodimers or an IL-17A/F heterodimer. Polymorphism of the IL-17A and IL-17F genes is currently being actively studied. The relevance of such work is determined by the requirements of modern personalized medicine. A search is being conducted for mutations (genetic markers) associated with a person's predisposition to a particular disease, and possible reasons for the ineffectiveness of anti-cytokine therapy in some cases are being clarified [2, 6, 10].

Most of the substitutions are located in non-coding regions of cytokine genes. In the minor alleles rs2275913 (G/A) and rs3819024 (A/G), variations are located in the 5'-untranslated region of the IL-17A gene and may be associated with a reduced risk of developing rheumatoid arthritis [3, 7, 11]. In the rs22759133 allele, nucleotide G at position -197 is located in the promoter region of the IL-17A gene near motifs that bind to two nuclear factors of activated T cells. This region is required for the expression of the IL-17A gene; it has been shown that replacing G at this position with A leads to increased production of the cytokine. This mutation in the IL-17A gene is a reliable marker of bone tissue destruction in patients with juvenile rheumatoid arthritis [4, 8].

Purpose of the study:studying the role of IL-17A gene polymorphism in the development of juvenile rheumatoid arthritis in children.

Materials and research methods: The collection of material was carried out during 2021-2023. on the basis of the department of cardiorheumatological diseases of the clinic of the Tashkent Pediatric Medical Institute. A total of 93 patients aged from 3 to 18 years were examined. Molecular genetic research was performed on DNA isolated from peripheral blood lymphocytes after alcohol-salt treatment according to Miller. In sick and healthy children, an analysis of the polymorphism of the IL17A gene (G-197A) was carried out; the studies were carried out in the laboratory of molecular

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genetics of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan.

DNA extraction

The material for DNA extraction was venous blood from the cubital vein with a volume of 3-5 ml. (Beckton-Dickinson vacutainers with anticoagulant/preservative 15% tripotassium EDTA (Ethilen dianin-tetra acetic acid) were used for blood collection). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding +40C.

To obtain genomic DNA, a two-step method of blood cell lysis was used. By double centrifuging the entire volume of whole blood in RCLB buffer (Red cell lyses buffer) at a speed of 1500 rpm for 15-20 minutes, erythrocyte cells were lysed. The use of RCLB results in osmotic shock to the red blood cells, leading to their swelling and further destruction.

The supernatant containing destroyed red blood cells was carefully drained from the tube and the residue above the sediment was aspirated. The clot of leukocyte mixture remaining at the bottom was lysed in WCLB buffer (White cell lyses buffer, white blood cell lysis buffer) in an amount depending on the volume of the leukocyte mixture. WCLB is also a preservative even at room temperature.

Using the alcohol-salt treatment method, further purification of the leukocyte mass lysates took place (S. Milleretal, 1988) in a modernized form proposed by the Laboratory of Human Genomics of the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan (currently the Department of Genome-Cell Technologies of the Russian Research Center of Immunology of the Ministry of Health of the Republic of Uzbekistan).

After the alcohol had dried, a diluted solution of TE (Tris-EDTA) 1:3 (TE: water) pH 8.0 was added to the test tube with dried DNA.

To perform polymerase chain reaction (PCR), DNA with an approximate concentration of 0.1 µg/ml, diluted with deionized water, and the corresponding primers with a concentration of 10 pmol/µl were taken. The reaction mixture and PCR conditions are as follows: deionized water - 4.6 µl; dNTPs mixture 10x: 2 mMdATP, 2 mMdTTP, 2 mMdGTP, 2 mMdCTP - 4 µl; 10x PCR buffer (16.6 µM (NH4)2SO4, 67 mMTris-HCl (pH=8.8)) - 4 µl; MgCl2: 25 mM - 4 µl (at the required concentration of 2.5 mM) or 2.4 µl (at the required concentration of 1.5 mM); Taq polymerase - 1.33 µl; template DNA - 4 µl; primer F(+) - 2.7 µl; primer R(-) - 2.7 µl. The reaction was carried out in 35-40 µl of the reaction mixture.

Q-PCR HRM PCR technology with melting curve analysis identifies DNA fragments by detecting changes in the fluorescence level of the fragment-probe complex (labeled fluorophore oligonucleotide probe) during its denaturation and then plotting the melting curve.

To type polymorphic variants of the candidate genes under study, DNA preparations obtained from 5 ml of venous blood and paraffin blocks were used. Genotyping was carried out using the HRM-qPCR method (Stratagene M*3005P, Agilent Technologies, USA; DT-Prime, DNA-Technology, Russia). The EMBL Nucleotide Sequence Database and NCBI were used as resources for population comparisons.

Statistical processing of the results was carried out using statistical software packages Arlequin 2006 (version 3.5.2.2.), Excell 2003, SISA.

Results of the study and their discussion: We divided the children with JRA we examined into groups with increased and normal production of IL-17A.

Table 1 Average level of IL-17A in examined children with JRA

Indicators	M±SD/Me	95% CI / $Q_1 – Q_3$	n	min	max
IL-17A, Me	28.10	21.30 – 40.10	93	13.90	58.90

The table shows the results of measuring IL-17A levels in the study group of subjects. The mean (M) was 28.10 pg/mL with a standard deviation (SD) of 13.90 pg/mL. The median (Me) level of IL-17A was 21.30, and the interquartile range ($Q_1 – Q_3$) was 13.90 to 40.10. The 95% confidence interval for the mean ranges from 21.30 to 40.10. The sample size (n) was 93 people, with a minimum value of 13.90 pg/ml and a maximum value of 58.90 pg/ml (table 1).

Table 2 IL-17A production in examined children with JRA

Indicators	Categories	Abs.	%	95% CI
IL-17A level	Increased output	31	33.3	23.9 – 43.9
	Normal value	62	66.7	56.1 – 76.1

An analysis of IL-17A versus IL-17A G-197A was performed.

Increased IL-17A production was observed in 31 children (33.3%), with a 95% confidence interval of 23.9% to 43.9%. 62 children with JRA (66.7%) had normal IL-17A values, with a 95% confidence interval from 56.1% to 76.1% (table 2).

Table 3 IL-17A level depending on IL-17A polymorphism (G-197A)

Index	Categories	IL-17A			p
		M±SD	95% CI	n	
IL-17A G-197A	G/G	21.12 ± 4.09	19.90 – 22.33	46	<0.001* pG/G – G/A < 0.001 pG/G – A/A < 0.001 pG/A – A/A < 0.001
	G/A	36.52 ± 7.25	34.07 – 38.97	36	
	A/A	52.42 ± 4.95	49.09 – 55.75	eleven	

* – differences in indicators are statistically significant ($p < 0.05$)

When analyzing IL-17A depending on IL-17A G-197A, statistically significant differences were identified ($p < 0.001$) (method used: Welch's F test).

In children with the G/G genotype (46 people), the average level of IL-17A was 21.12 ± 4.09 pg/ml (95% CI: 19.90 – 22.33), in children with the G/A genotype (36 people) this figure was higher and amounted to 36.52 ± 7.25 pg/ml (95% CI: 34.07 – 38.97), and in children with the A/A genotype (11 people) the level of IL-17A was even higher higher and amounted to 52.42 ± 4.95 pg/ml (95% CI: 49.09 – 55.75). Differences in IL-17A levels between groups were statistically significant ($p < 0.001$) (Table 3).

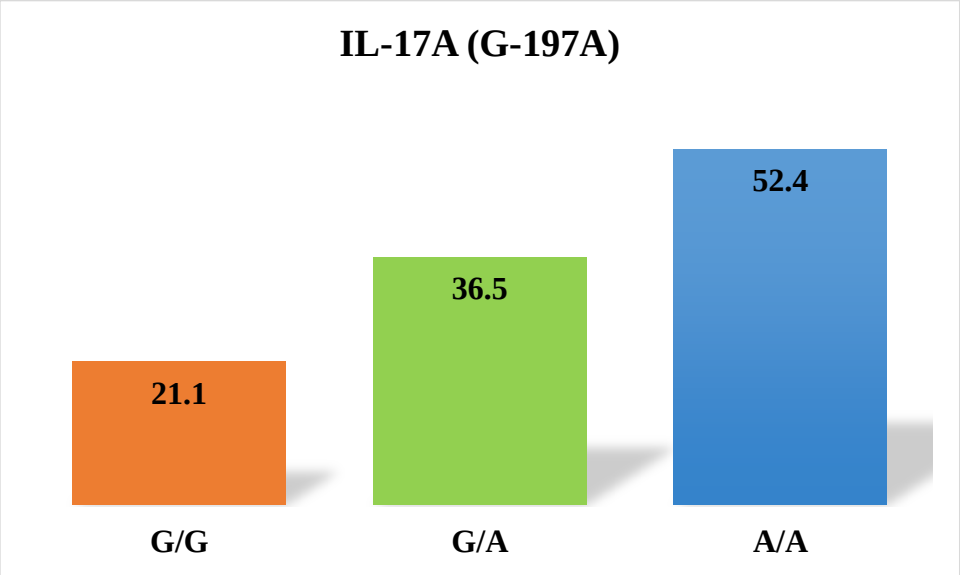

Fig. 1 – Analysis of IL-17A depending on IL-17A (G-197A)

As can be seen in Figure 1, in the group of children with the AA genotype, the highest concentrations of IL-17A levels are observed, which is significantly higher than in the group of children with the GG genotype by 2.48 times.

Table 4 IL-17A level depending on IL-17A polymorphism (G-197A)

Index	Categories	IL-17A G-197A			p
		G/G	G/A	A/A	
Level Up IL-17A	Increased output	0 (0.0)	20 (55.6)	11 (100.0)	<0.001* pG/G – G/A < 0.001 pG/G – A/A < 0.001 pG/A – A/A = 0.006
	Normal values	46 (100.0)	16 (44.4)	0 (0.0)	

** – differences in indicators are statistically significant (p < 0.05)*

According to the presented table, when comparing the level of increase depending on IL-17A G-197A, statistically significant differences were identified (p < 0.001) (method used: Pearson Chi-square).

In this table, we present data on the level of IL-17A in children with juvenile rheumatoid arthritis (JRA) depending on the G-197A genotype and its relationship with the level of increase. In the group of children with the G/G genotype (n=46), an increased level of IL-17A was not detected, while in the groups with the G/A (n=36) and A/A (n=11) genotypes an increased level was detected level in 20 (55.6%) and 11 (100.0%) children, respectively. Analysis of relationships between genotypes showed that comparisons between groups G/G and G/A, G/G and A/A, as well as G/A and A/A showed statistically significant differences (p G/G – G/A < 0.001 , p G/G – A/A < 0.001, p G/A – A/A = 0.006) (table 4).

Thus, it was revealed that in children with JRA genotype AA of the IL-17A gene (G-197A) there is an increased production of the pro-inflammatory cytokine IL-17A, which indicates a predisposition.

Many studies have shown a positive relationship between the degree of activity of clinical processes in the joints and the content of pro-inflammatory and anti-inflammatory cytokines in the patient's blood serum [6, 11].

Next, we conducted molecular genetic studies of IL-17A gene polymorphism (G-197A) in a group of seropositive and seronegative forms of JRA.

Table 5. IL-17A gene polymorphism (G-197A) in the group seropositive form of JRA

YURA	n =38	%	Counter.	n=62	%	OR	χ2	p
G	48	66.7	G	107	86.3	0.422	4,964	00.3
A	24	33.3	A	17	13.7	2,368	4,964	0.005
GG	19	52.8	GG	46	74.19	0.384	5,056	0.001
GA	10	27.8	GA	15	24.2	2,591	4,505	0.05
A.A.	7	19.4	A.A.	1	1.61	5,071	3,363	0.05

* – differences in indicators are statistically significant (p < 0.05)

When analyzing the results obtained in the group with the seropositive form of JRA, it was revealed that in this sample of children with JRA, allele A was a significantly significant marker of predisposition with a high significance index (OR = 2.368, χ2 =4,964, Wald 95% CI: 1.456 > 4.149 > 9.332). In turn, marker G was found less frequently in the group of patients compared to practically healthy individuals (OR=0.422, χ2 =4,964, Wald 95% CI: 0.198 > 0.342 > 0.7). The GG genotype was more common in the healthy group compared to the group of children with JRA (OR=0.384, χ2 =5,056Wald 95% CI: 0.106 >0.308> 0.612), while the GA genotype was more common in the group of patients (OR=2,591, χ2 =4,505, Wald 95% CI: 0.32 >2.98 > 7.981). The AA genotype has the highest relative risk indicators, this indicator is significant (OR = 5.071, χ2 =3,363, Wald 95% CI: 0.411 >5.521 > 9.471) (table 5).

Table 6. IL-17A gene polymorphism (G-197A) in the group seronegative form of JRA

YURA	n =57	%	Counter.	n = 62	%	OR	χ2	p
G	89	78.0	G	107	86.3	0.241	9,478	0.002
A	25	22.0	A	17	13.7	4.149	9,478	0.002
GG	35	61.4	GG	46	74.19	0.216	9.124	0.002
GA	19	33.3	GA	15	24.2	3,980	6,584	0.01
A.A.	3	5.60	A.A.	1	1.61	5,071	2,066	0.15

* – differences in indicators are statistically significant (p<0.05)

A comparative analysis of data on the frequency of occurrence of the IL-17A gene polymorphism (G197A) in the group with a seronegative form of JRA and the control group revealed that allele A was also a significantly significant marker of susceptibility with a high OR=4.149, χ2 =9,478, Wald 95% CI: 1.117>2.452>5.474. Marker G was found less frequently in the group of patients compared with practically healthy individuals (OR=0.241, χ2 =9,478, Wald 95% CI: 0.153>0.312>0.873). The GG genotype was more common in the patient group compared to the healthy group (OR=0.216, χ2 =9.124Wald 95% CI: 0.157 >0.415>0.928). The GA genotype had the highest relative risk in the group of patients, which indicates its predisposing significance (OR=3,980, χ2 =6,584Wald 95% CI: 1.147>3.284>7.429). The AA genotype was more common in the patient group compared to the control group (OR=5,071, χ2 =2,066, Wald 95% CI: 0.104 >2.897>8.21), but did not reach true significance (table 6.)

Thus, in the group with the seropositive form of JRA, allele A, heterozygous genotype GA and homozygous genotype AA G197A of the IL-17A gene are a risk prognostic genotype for JRA. In the seronegative form, the risk prognostic genotype is the A allele and the heterozygous GA genotype.

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