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Original Article

Genetics Section

Association of Interleukin-6-174G/C Gene Promoter Polymorphism with Rheumatoid Arthritis in Adolescence: A Case-control Study

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ABSTRACT

Introduction: Rheumatoid Arthritis (RA) is a chronic autoimmune disease with unknown pathogenesis. The disease is multifactorial, however, the exact causes of the occurrence, as well as the targets of the autoimmune process, are unknown. Genes candidate for a predisposition to RA are cytokine genes. Of the cytokines, IL-6 is considered a key mediator of systemic and localised inflammation in RA.

Aim: The comparative analysis of the frequency distribution of alleles and genotypes IL-6-174G/C polymorphism in patients with RA and in the control group to determine the genotype most characteristic of this disease.

Materials and Methods: This case-control study was conducted in a group of 136 children with RA aged 14 to 18 years. In the control group, there were 143 practically healthy children of similar age without RA and orthopaedic pathology. Both groups were tested for polymorphism IL-6-174G/C using real-time Polymerase Chain Reaction (real-time PCR). The results were statistically processed using the Pearson's Chi-square test.

Results: The distribution of 174G/C genotypes in the IL-6 gene was significantly different in patients with RA when compared with the control group. In patients with RA, heterozygous carriers of 174G/C (52.94%) prevailed, while in the control group the most numerous group were homozygous carriers of -174G/G (40.56%).

Conclusion: As a result of this study, it was possible to identify significant differences in distribution of genotypes 174G/C polymorphism in IL-6 gene between patients with RA and subjects of the control group. In relation to the European population of the Russian Federation, this polymorphism can serve as a diagnostic marker in the study of the pathogenesis of RA.

Keywords: Cytokines, C-Reactive protein, Diagnostic marker, Tumour necrosis factor

INTRODUCTION

Rheumatoid Arthritis (RA) is a multisystem autoimmune disease that leads to inflammatory processes in the connective tissue, mainly affecting the small joints. The incidence of this disease worldwide is approximately 1% [1]. One of the variants of the clinical course of RA is juvenile RA, which occurs in childhood and adolescence, i.e., up to 16 years of age and, unlike adult RA, does not always become chronic. Despite the fact that the aetiology of RA is multifactorial, there are genetic markers that determine the predisposition to this disease. The HLA-DR loci are the genetic factor most associated with susceptibility to RA. Recent Genome-Wide Association Studies (GWAS) have identified 101 Single Nucleotide Polymorphism (SNPs) in total, showing the highest contribution of HLA-DRB1 gene to the development of RA [2-5]. Cytokine genes are other candidate genes for susceptibility to RA. Cytokines are a broad and loose category of small proteins (~5–20 kDa) important in cell signaling. Cytokines have been shown to be involved in signaling pathways and numerous proinflammatory cytokines such as Tumour Necrosis Factor alpha (TNF-α), Interleukin-1 (IL-1) and IL-6 are mediators that are involved in the inflammatory response and play an essential role in the pathogenesis of RA.

Of all cytokines, IL-6 is considered a key mediator of systemic and localized inflammation in RA. IL-6 is a strong inducer which can cause fever, anaemia, secondary amyloidosis, elevations in acute-phase proteins {C-Reactive Protein (CRP)}. The capability of IL-6 to influence B-cell differentiation can leads to the formation of rheumatoid factor and other autoantibodies. IL-6 aids osteoclast activation in joints and induces the release of matrix metalloproteinases, thus contributing to joint damage [6-9]. Several studies have found high levels of IL-6 in the synovial fluid of inflamed joints [10,11]. Changes in IL-6 levels are regulated by several factors [12], including variants of polymorphisms in promoter regions of the IL-6 gene.

The human IL-6 gene is located on chromosome 7p21. The most frequently studied polymorphism is the SNP-174C and -174G in the promoter region, which has been associated with transcription rates of IL-6. This polymorphism is characterised by a single nucleotide substitution of guanine (G) for cytosine (C) at position -174 in the promoter region [13,14], which leads to three variants of possible genotypes- GG, GC, and CC. This polymorphism is functionally important as it affects the rate of gene transcription and the plasma concentration of the IL-6 protein.

According to number of studies, 174G/C gene polymorphism plays an important role in the pathogenesis of various diseases, such as idiopathic sick sinus syndrome, chronic hepatitis B and C, chronic HCV infection and others [15-17]. Nevertheless, each of the allelic variants of this polymorphism can play a positive, negative or neutral role in the pathogenesis of the diseases under consideration. According to studies carried out in different countries and ethnic populations, there is a wide scatter of data on the distribution of IL-6 gene genotypes both in the populations of RA patients and in controls [18-24]. Taking into account the conflicting data available in the literature, authors carried out a molecular genetic study of 174G/C polymorphism among RA patients and in control group in the Russian population (St. Petersburg) and carried out a comparative analysis of the distribution of this polymorphism among RA patients and healthy people of the same age and compared with

other populations of Northern and Southern Europe, Asia and Latin America to clarify the role of this gene in the pathogenesis of RA.

MATERIALS AND METHODS

This case-control study was conducted from September 2018 to September 2020 on patients who underwent treatment for juvenile RA at the H. Turner National Medical Research Center for Children's Orthopaedics and Trauma Surgery, St-Petersburg, Russia of the Ministry of Health of the Russian Federation. It was in accordance with the Declaration of Helsinki after being approved by the Local Ethics Committee of the Institute. Parents of the all underage patients with RA, participating in the study, gave informed consent for participation of children in the molecular genetic study of the IL-6 gene by PCR diagnostics.

Inclusion criteria for cases: A total of 136 cases of RA were included ranging in age from 14 to 18 years (50 males and 86 females), with confirmed diagnosis of juvenile RA according to the International League of Rheumatology Associations (ILAR) International Standard Definition [25], which must have occurred at least two months before enrollment at age <18 years. Arthritis of one or more joints with fever lasting for at least two weeks, swelling or limited mobility of the joint, morning stiffness and arthralgia during the day, joint pain or abnormal use of the joints were included.

Exclusion criteria for cases: Patients with active or recurrent bacterial, fungal or viral infections, including those with signs of HIV infection, hepatitis B and hepatitis C infection, patients with chronic diseases (kidney and liver disease).

Inclusion criteria for controls: The control group consisted of 143 apparently healthy males (61) and females (82) adolescents of the same age group, who were students of educational institutions of St. Petersburg, Russia without signs of RA and orthopaedic pathology according to the results of medical examinations.

Exclusion criteria for controls: It was the presence of RA and any orthopaedic diseases.

Genomic DNA Extraction and SNP Selection

2 mL Peripheral blood was taken from all patients and individuals of the control group for molecular genetic analysis and was frozen at -70°C. Genomic DNA was isolated from 100 µL blood using «ExtractDNA Blood» kit from Evrogen Company, Russia, according to the manufacturer's instructions. Analysis of the polymorphic region of the IL-6 gene was carried out using the SNP-Screen reagent kit for IL-6-174G/C polymorphism manufactured by Syntol Company, Russia, by real-time PCR on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA).

STATISTICAL ANALYSIS

Statistical data processing was carried out using online medical statistics calculators. We used the Pearson's Chi-square (χ^2) test for a comparative analysis of the distribution of allele and genotype frequencies in groups of patients with RA, as well as in the control group. Differences in indicators between the groups of RA patients and people from the control group were considered statistically significant at p<0.05. The Odds Ratio (OR) and Hardy-Weinberg Equilibrium (HWE) methods were also used.

RESULTS

Mean age of the patients was 16 years. Study results of IL-6-174G/C polymorphism in patients with RA and in the control group is presented in the [Table-Fig-1].

During the statistical processing of the results of the distribution of genotypes between RA patients and the control group, the following indicators were obtained: the value of the χ^2=6,488 with the number of degrees of freedom equal to 2. Significance level p=0.04, that

IL-6-174G/C genotypes, alleles	RA patients (n=136)	%	Control group (n=143)	%	OR (95% CI)
G/G	42	30.88	58	40.56	0.655 (0.400-1.073)
G/C	72	52.94	54	37.76	1.854 (1.151-2.988)
C/C	22	16.18	31	21.68	0.697 (0.381-1.277)
G	156	57.35	170	59.44	χ2=0.250 p=0.6
C	116	42.65	116	40.56	

[Table/Fig-1]: Distribution of genotypes and alleles in study groups.
p-value for genotypes=0.04

is, the relationship between the factorial and effective traits was statistically significant with an acceptable value of p<0.05. As can be seen from the table, as a result of PCR genotyping, in patients with RA (n=136), heterozygous carriers of 174G/C (52.94%) prevailed, while in the control group (n=143) the group of homozygous carriers of -174 G/G (40.56%) was the most numerous.

At the same time, no significant difference was observed in the ratio of the number of G and C alleles between RA patients and the control group. Here, the value of the χ^2=0.250 and the significance level was p=0.617, which is much higher than p<0.05.

When calculating the HWE index, it can be seen that in patients and in controls there were significant deviations from the expected equilibrium in opposite directions. Thus, in the case of heterozygous genotype 174 (G/C), the deviation from the expected value of HWE in RA patients occurred by 4.04% upwards, and in the control group by 10.44% downwards [Table/Fig-2].

IL-6-174G/C genotypes	RA patients, % (n=136)	HWE	Control group, % (n=143)	HWE
G/G	30.88	32.9	40.56	35.3
G/C	52.94	48.9	37.76	48.2
C/C	16.18	18.2	21.68	16.5

[Table/Fig-2]: Hardy-Weinberg test (HWE) for the RA group and control group.

DISCUSSION

Our study of the IL-6-174G/C polymorphism in patients with juvenile RA showed the following regularities: in the group of RA patients, heterozygous (G/C) carriage of this polymorphism predominates (52.94%). At the same time, in the control group of healthy volunteers, the major genotype G/G was most represented (40.56%), as in many other populations.

This distribution of genotypes in patients with RA in North-West Russia is similar in nature to patients in the North European populations [21,23]. The most numerous group was the group of heterozygous GC carriers [Table-Fig-3].

IL-6-174G/C genotypes	RA, % Great Britain [21] n=383	Control group, % Great Britain [21] n=422	RA, % Poland [23] n=98	Control group, % Poland [23] n=105	RA, % Russia, present study	Control group, % Russia, present study
G/G	35.2	35.1	26.5	23.8	30.88	40.56
G/C	46.0	50	54.1	55.2	52.94	37.76
C/C	18.8	14.9	19.4	21.0	16.18	21.68
	p=0.29		p=0.9		p=0.04	

[Table/Fig-3]: Distribution of genotypes and alleles in the group of patients with RA and among residents of northern Europe.
p<0.05 is considered to be significant

In the countries of southern Europe, the proportion of heterozygous GC carriers decreases and the GG genotype becomes dominant [Table-Fig-4] [20,22].

In Latin American and Asian populations, the GG genotype is even more prevalent, and the C allele has an extremely low representation [Table-Fig-5] [19,24].

IL-6-174G/C genotypes	RA, % North Macedonia, Trajkov D et al., 2009 [20] n=85	Control group, % North Macedonia, Trajkov D et al, 2009 [20] n=301	RA, % Spain, Pascual M et al., 2000 [22] n=163	Control group, % Spain, Pascual M et al., 2000 [22] n=157	RA, % Russia, present study	Control group, % Russia, present study
G/G	45.2	47.8	46.0	46.5	30.88	40.56
G/C	35.7	43.9	44.2	42.0	52.94	37.76
C/C	19.1	8.3	9.8	11.5	16.18	21.68
	p=0.068		p=0.864		p=0.04	

[Table/Fig-4]: Distribution of genotypes and alleles in the group of patients with RA and among residents of southern Europe.
p<0.05 is considered to be significant

IL-6-174G/C genotypes	RA, % Mexico, Zavaleta-Muñiz SA et al., 2013 [24] n=137	Control group, % Mexico, Zavaleta-Muñiz SA et al., 2013 [24] n=102	RA, %, China, Li F et al., 2014 [19] n=256	Control group, % China, Li F et al., 2014 [19] n=331	RA, % Russia, present study	Control group, % Russia, present study
G/G	77.4	78.4	96.5	99.4	30.88	40.56
G/C	21.9	19.6	2.7	0.3	52.94	37.76
C/C	0.7	2	0.8	0.3	16.18	21.68
	p=0,651		p=0.049		p=0.04	

[Table/Fig-5]: Distribution of genotypes and alleles in the group of patients with RA and among residents of Asia and Latin America.
p<0.05 is considered to be significant.

At the same time, it should be emphasised that in the Russian Federation, due to the fact that it is a multinational country, there are significant differences in the distribution of genotypes and alleles in regions where the majority of the population are either Caucasian (Slavic) or Mongoloid (Asian) population. Nevertheless, a more important indicator, in our opinion, is the assessment of the significance of differences in the distribution of genotypes between patients and the control group. However, there is also mixed evidence in various studies. So, in RA groups in Egypt, China, Macedonia [18-20], these differences were significant, and in RA groups in UK, Spain, Poland [21-23] there were no significant differences.

There can be several explanations for these facts. First, it is logical to assume that with such a wide scatter of data, the 174G/C polymorphism does not play a significant role in the aetiology and pathogenesis of the RA, but there are other, more significant factors, possibly epigenetic methylation factors [26]. Second, where the difference between RA patients and the control group was significant, the situation changes, and obviously, this factor takes a more important place, while the importance of other genetic and epigenetic determinants decreases. Some of the increased risk is due to environmental factors such as acute viral or bacterial infections, prolonged hypothermia and other stochastic influences. Smoking is the best defined risk and interacts with HLA-DR to amplify the risk of developing RA. Although the search for genetic variance continues, we have likely reached the point of diminishing returns. For example, the disease concordance rate between identical twins is surprisingly low. If one twin develops RA the chance that the second twin will develop the disease is only 12% to 15% [27]. In this regard, in order to understand the determining genetic and epigenetic factors of the aetiology and pathogenesis of RA, it is necessary to search for factors that would have an equally high penetrance for worldwide populations. In this regard, we continue to look for such factors, expanding our research and investigating genes for other proinflammatory cytokines, as well as genetic and epigenetic mechanisms leading to increased expression of HLA-antigens.

Limitation(s)

Due to limited possibilities, the authors did not conduct a study of the IL-6 level in the blood and tissues, which could supplement the information on the ratio of IL-6 genotypes with the concentration distribution of IL-6 in tissues.

CONCLUSION(S)

This study revealed significant differences in the distribution of genotypes 174G/C of the IL-6 gene between patients with RA and a control group in the Russian population (St. Petersburg). This indicates that this polymorphism can serve as a diagnostic marker when studying the pathogenesis of RA in the European population of the Russian Federation. The authors are currently continuing the research on other proinflammatory cytokines along with investigating other possible genetic, pharmacogenetic and epigenetic markers of the aetiopathogenesis of RA. In particular, genes associated with apoptosis and genes of the folate cycle are being investigated.

The main recommendations for future studies of RA lie in the plane of epigenetic modifications in inflammatory processes that leads to the triggering of autoimmune switching in a predisposed person. The presented work makes a certain contribution to the accumulation of population information on the role of proinflammatory cytokines in the pathogenesis of various diseases.

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