ОСОБЕННОСТИ ЧАСТОТЫ ВСТРЕЧАЕМОСТИ ПОЛИМОРФИЗМА ГЕНА Т-330G *IL2* У ПАЦИЕНТОВ С COVID-19

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Резюме. Инфекция SARS-CoV-2 является этиопатогенетическим фактором новой коронавирусной инфекции. Восприимчивость к вирусу и, соответственно, заболеваемость отличается у детей и взрослых. С одной стороны, это отражает возрастные особенности иммунного ответа. С другой стороны, реализуется через выработку ряда цитокинов, в том числе IL-2, и отражает генетически-детерминированные особенности продукции цитокинов. Целью исследования был анализ частоты встречаемости полиморфных вариантов Т-330G гена *IL2* у пациентов с новой коронавирусной инфекцией. Всего было обследовано 145 пациентов, из них 31,0% детей (n = 45) и 69,0% взрослых (n = 100). Диагноз «новая коронавирусная инфекция» верифицирован методом ОТ-ПЦР подтверждающего наличие вируса SARS-CoV-2 и выявление клинических симптомов инфекции верхних дыхательных путей. Группу контроля составили 50 здоровых доноров-добровольцев. Для анализа полиморфизма Т-330G гена *IL2* использовали аллель-специфическую ПЦР с электрофоретической детекцией в 3% агарозном геле («Литех», Россия). Для сравнения частот комбинаций аллелей использовали критерий χ^2 и отношение шансов OR и (95% CI).

Доминирующим генотипом у пациентов с COVID-19 был гетерозиготный генотип GT полиморфизма Т-330G гена *IL2*. В группе детей с риском развития новой коронавирусной инфекции был ассоциирован генотип GG полиморфизма Т-330G гена *IL2* (31,1% у детей и 18,0% в группе контроля, $p < 0,05$, OR = 2,047). В то время как гомозиготный генотип TT полиморфизма Т-330G гена *IL2* являлся протективным генотипом (его частота встречаемости составила у пациентов – 26,7%, в группе контроля – 54,0%, $p < 0,05$, OR = 0,315). У взрослых с риском развития новой коронавирусной инфекции был ассоциирован гетерозиготный генотип GT полиморфизма Т-330G гена *IL2* (в группе пациентов – 44,0% против контроля – 28,0%, $p = 0,028$, OR = 2,020). Низкий риск развития заболевания был ассоциирован с гомозиготного вариантом TT полиморфизма Т-330G гена *IL2* (в группе пациентов 37,0% против контроля – 54,0%, $p = 0,024$, OR = 0,500).

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Полиморфизм Т-330G промотроной зоны гена *IL2* по-разному влияет на его продукцию. От уровня IL-2 зависит направление иммунного ответа и его эффективность. Понимание индивидуальных факторов, определяющих особенности иммунного ответа может помочь в понимании механизмов развития COVID-19-ассоциированных заболеваний и подборе подходов к персонализированным методам их лечения.

Ключевые слова: полиморфизм генов, SNP, T-330G IL2, COVID-19, дети, SARS-CoV-2

FEATURES OF THE FREQUENCY OF OCCURRENCE OF T-330G *IL2* GENE POLYMORPHISM IN PATIENTS WITH COVID-19

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Abstract. SARS-CoV-2 infection is the etiopathogenetic factor of the new coronavirus infection. Susceptibility to the virus and, accordingly, the incidence differs in children and adults. On the one hand, this reflects the age-related features of the immune response. On the other hand, it is realized through the production of a number of cytokines, including IL-2, and reflects the genetically determined features of cytokine production. The aim of the study was to analyze the frequency of occurrence of T-330G polymorphic variants of the *IL2* gene in patients with a new coronavirus infection. A total of 145 patients were examined, including 31.0% of children (n = 45) and 69.0% of adults (n = 100). The diagnosis of a new coronavirus infection was verified by RT-PCR confirming the presence of the SARS-CoV-2 virus and identifying clinical symptoms of an upper respiratory tract infection. The control group consisted of 50 healthy volunteer donors. Allele-specific PCR with electrophoretic detection in 3% agarose gel (Litech, Russia) was used to analyze the T-330G polymorphism of the *IL2* gene. To compare the frequencies of allele combinations, the χ^2 test and the odds ratio OR and (95% CI) were used.

The dominant genotype in patients with COVID-19 was the heterozygous GT genotype of the T-330G polymorphism of the *IL2* gene. In the group of children at risk of developing a new coronavirus infection, the GG genotype of the T-330G polymorphism of the *IL2* gene was associated (31.1% in children and 18.0% in the control group, $p < 0.05$, OR = 2.047). While the homozygous TT genotype of the T-330G polymorphism of the *IL2* gene was a protective genotype (its occurrence rate was 26.7% in patients, 54.0% in the control group, $p < 0.05$, OR = 0.315). In adults, the heterozygous GT genotype of the T-330G polymorphism of the *IL2* gene was associated with the risk of developing a new coronavirus infection (in the group of patients – 44.0% *versus* control – 28.0%, $p = 0.028$, OR = 2.020). A low risk of developing the disease was associated with the homozygous TT variant of the T-330G polymorphism of the *IL2* gene (in the group of patients 37.0% *versus* control – 54.0%, $p = 0.024$, OR = 0.500).

The T-330G polymorphism of the promoter zone of the *IL2* gene differently affects its production. The direction of the immune response and its effectiveness depend on the level of IL-2. Understanding the individual factors that determine the features of the immune response can help in understanding the mechanisms of development of COVID-19-associated diseases and the selection of approaches to personalized methods of their treatment.

Keywords: polymorphism of gene, SNP, T-330G IL2, COVID-19, children, SARS-CoV-2

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Introduction

Infants and children under 5 years of age have a unique immunophenotype and respond differently to SARS-CoV-2 infection compared to adults who are more often to develop a cytokine storm [4, 9]. For children SARS-CoV-2 infection develops mild (27%) or asymptomatic disease (66%) [2, 5, 7, 8]. Moderate and severe severity is extremely rare in children (5 and 2%, respectively) [6].

Comparison of adult patients and children also demonstrates differences in both the level of

production and the functional characteristics of the immune response. Increasing evidence has demonstrated that interleukins played an important role in the progression of COVID-19. Compared to mild COVID19 cases, serum interleukins levels increased greatly in severe and critical patients [3]. Pro-inflammatory cytokines induce active production of cytokines and the immune response. Interleukin 2 (IL-2) is a monomeric glycoprotein with a molecular weight of approximately 15 kDa, produced by Th-1 cells. IL-2 plays a critical role in the differentiation and survival of regulatory T cells, thus ensuring their importance in the control of the immune response. One of the best achievements in this direction was to highlight the important role of single nucleotide polymorphisms (SNPs) of those genes involved in the immune regulatory mechanism. **The aim of the study** was to analyze the frequency of occurrence of T-330G *IL2* polymorphic genes in patients with COVID-19.

Materials and methods

In total, 145 patients with a new coronavirus infection were examined, represented by a group of "children" and a group of "adults". The group of "children" (n = 45) – patients admitted to the department of the Republican Children's Infectious Diseases Clinical Hospital with a new coronavirus infection of moderate severity at the age of 0 to 14 years (n = 45). Distribution of children depending on the age of the group 0-4 years old – 40.0% (n = 18), 5-9 years old – 22.2% (n = 10) and 10-14 years old – 37.8% (n = 17).

The group of "adults" (n = 100) – patients admitted to the pulmonology department of the Institution of Healthcare of the Republic of Crimea "Academic Research Institute of Physical Methods of Treatment, Medical Climatology and Rehabilitation named after I.M. Sechenov" for the purpose of sanatorium treatment after a new coronavirus infection. Women made up 74.07% (n = 80) of the study population, 25.93% (n = 28) were men.

Inclusion criteria for the study were: previous novel coronavirus infection. Signed informed consent for inclusion in the study. The exclusion criteria were: the presence of complicated forms of viral pneumonia in the presence of severe functional pulmonary and extrapulmonary disorders, age over 75 years.

Verification of the diagnosis of novel coronavirus infection was based on confirmation of the SARS-CoV-2 virus by RT-PCR and the presence of clinically identifiable symptoms or signs of an upper respiratory tract infection, namely throat congestion, sore throat and fever, and on x-ray. All patients signed an informed consent for the study.

The control group consisted of 50 relatively healthy respondents, 32 women (64.0%) and 18 men (36.0%), whose average age was 44.3±5.23 years.

The study was conducted in accordance with the rules of the Helsinki Declaration of 1975, revised in 2013 and approved by the Ethics Committee of V.I. Vernadsky.

Methods

To analyze the T-330G polymorphism of the *IL2* gene, an allele-specific polymerase chain reaction with electrophoretic detection was used. DNA was isolated from the whole blood of patients with a new coronavirus infection and healthy volunteers using the DNA-express blood kit according to the manufacturer's instructions. Allele-specific PCR was performed using "T-330G *IL2*" kits (Liteh, Russia) according to the manufacturer's instructions. Detection of amplification products was carried out by horizontal electrophoresis in 3% agarose gel.

The study was conducted at the Collective Center for the use of scientific equipment "Molecular Biology" CFU named after V.I. Vernadsky.

The data obtained were analyzed using the Statistica 8.0 software package. The expected allele frequency was calculated based on the Hardy–Weinberg law. To compare the frequencies of allele combinations, the χ^2 test was used with the Yates correction for continuity. The association of polymorphisms with novel coronavirus infection was analyzed by determining the odds ratio (OR) test and 95% confidence interval (95% CI), $p < 0.05$.

Results and discussion

We found all the studied IL-2 mutations in accordance with the Hardy–Weinberg law ($p > 0.05$). As a result of the study, it was shown that the distribution of polymorphic variants of IL-2 genotypes differed in all the research groups. The dominant genotype in patients with COVID-19 was the heterozygous GT genotype of the T-330G *IL2* gene polymorphism (Table 1). Its frequency was statistically significantly higher in patients than in the control group ($p < 0.05$). In the group of healthy donors, the most common genotype was homozygous TT of the T-330G *IL2* gene polymorphism. Its frequency of occurrence was statistically significantly lower in children and adults (Figure 1, Table 1, $p < 0.05$). The frequency of occurrence of GG homozygous of the T-330G *IL2* gene polymorphism in the adult group was comparable to that in the control group, while in children it was statistically significantly lower than in adults ($p < 0.05$).

The analysis showed that in children with the risk of a new development of coronavirus infection, the GT genotype of the T-330G *IL2* gene polymorphism is associated. While the TT polymorphism of the *IL2* T-330G gene is a protective genotype (Table 1). In adults, the GT genotype of the polymorphism of the T-330G *IL2* gene is associated with the risk of developing a new coronavirus infection. While the

TABLE 1. MULTIPLICATIVE INHERITANCE MODEL (χ^2 TEST, df = 1)

Genotypes	Case n (%)	Control n (%)	χ^2	p	OR	
					values	95% CI
Children						
TT	12 (26.7)	27 (54.0)	14.0	0.001	0.315	0.1740-0.5690
GT	19 (42.2)	14 (28.0)	3.714	0.054	–	–
GG	14 (31.1)	9 (18.0)	3.892	0.049	2.047	1.054-3.973
Adult						
TT	37 (37.0)	27 (54.0)	5.162	0.024	0.500	0.284-0.880
GT	44 (44.0)	14 (28.0)	4.883	0.028	2.020	1.122-3.640
GG	19 (19.0)	9 (18.0)	0	1.0	–	–

TABLE 2. COMPARISON OF THE FREQUENCY OF ALLELES AND GENOTYPES OF T-330G *IL2* POLYMORPHIC VARIANTS (rs2069762) COMPARED WITH THE FREQUENCY OF OCCURRENCE IN POPULATIONS

Population	Sample size	Reference allele	Alternative allele
Study groups			
Total	145	0.55	44.4
Children	45	0.478	0.522
Adult	100	0.556	0.444
Control	50	0.680	0.320
According to the website rs2069762 RefSNP Report – dbSNP – NCBI (nih.gov)			
African	4876	0.9219	0.0781
African American	4730	0.9207	0.0793
Asian	216	0.671	0.329
European	53642	0.69843	0.30157
Latin American 2	4672	0.6803	0.3197

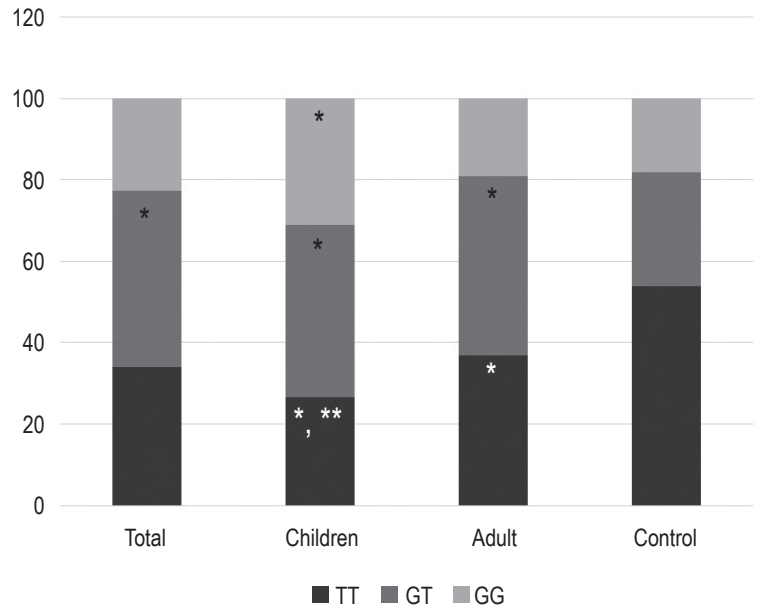

Figure 1. Genotype frequency of T-330G polymorphism of the *IL2* gene in the studied group

Note. *, significant differences from the groups of patients with control; **, significant differences from the group of children with adult.

TT polymorphism of the *IL2* T-330G gene is also protective.

When detecting the frequency of occurrence of alleles with a number of populations, a definite was revealed. The frequency of T alleles of the T-330G *IL2* polymorphism both in the control group and in patients with a new coronavirus infection was considered similar to that in Asians, Caucasians and Latin American, but was lower than in Africans and African Americans (Table 2). Allele C of the T-330G *IL2* polymorphism was marked by an increased frequency of occurrence in Asians, Caucasians and Latin American, and was higher than in Africans and African Americans.

Since the beginning of the COVID-19 outbreak, an increasing number of cases of COVID-19 have been confirmed. Various aspects of COVID-19 disease are described, taking into account risk factors such as age, gender, and pre-existing metabolic conditions such as diabetes, obesity, and hypertension. However, the genetic background has often been overlooked to end up being watched as a major player by COVID-19.

The cytokine storm arose with COVID-19, and interleukins and IFNγ were involved in the process of hyperinflammation. Immune mediators, including interleukins, have been shown to play an important role in the development of COVID-19 [3]. It has been noted that genotypes correlate with susceptibility to COVID-19, mortality, and immune response activity. When comparing our data on the frequency of distribution of genotypes, that in adult patients of the Caucasian race and lived in the Trans-Baikal Territory the chance of developing SARS-COV-2 increased in carriers of the allele T and the TT genotype of *IL2* gene [1].

Conclusion

The dominant genotype in children and adults with COVID-19 was the TT polymorphism T-330G *IL2* gene. The GG genotype of the T-330G *IL2* gene polymorphism in children is statistically significantly lower than in adults, whose level was comparable to the control group. In children, GC genotype of the T-330G *IL2* gene polymorphism is associated with the risk of developing a SARS-COV-2 infection. In adults at risk SARS-COV-2, GT genotype of the T-330G *IL2* gene polymorphism is associated. While TT of the T-330G *IL2* gene polymorphism in both children and adults is protective. T-330G *IL2* SNPs may be associated with a higher risk of COVID-19 infection. Understanding individual-specific polymorphisms may help better explain COVID-19 outcomes in genetic profiling to create personalized COVID-19 therapies.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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