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Article

Genes Polymorphisms and Their Association with COVID-19 Severity in Children

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Abstract: Coronavirus disease (COVID-19) and its outcomes remain one of the most challenging problems today. COVID-19 in children could be asymptomatic but can result in a fatal outcome, therefore, prediction of the disease severity is important. The goal was to investigate the human genetic factors that could be associated with COVID-19 severity in children. Single nucleotide polymorphisms of the following genes were studied: ACE2 (rs2074192), IFNAR2 (rs2236757), TYK2 (rs2304256), OAS1 (rs10774671), OAS3 (rs10735079), CD40 (rs4813003), FCGR2A (rs1801274) and CASP3 (rs113420705). In the case-control study were 30 children with mild or moderate course of the disease; 30 with severe COVID-19 symptoms and multisystem inflammatory syndrome in children (MIS -C) and 15 healthy who did not have COVID-19 infection (PCR negative, Ig G negative). The study revealed that ACE2 rs2074192 (allele T), IFNAR2 rs2236757 (allele A), OAS1 rs10774671 (allele A), CD40 rs4813003 (allele C), CASP3 rs113420705 (allele C) and male sex contribute to severe course COVID-19 infection, and MIS-C in 85.6% of cases. The World Health Organization reported new COVID-19 variants may cause previously unseen symptoms in children. Although the study has limitation due to cohort size, the findings can help provide a better understanding of SARS-CoV-2 infection and proactive pediatric patient management.

Keywords: ACE2; IFNAR2; TYK2; OAS1; OAS3; CD40; FCGR2A; CASP3, COVID-19; children

1. Introduction

Coronavirus disease (COVID-19) and its outcomes remain one of the most pressing problems today. In July 2023, the total count of confirmed COVID-19 cases reached 768 million worldwide. Notwithstanding vaccination, the mortality rate is still high. Thirteen billion persons were vaccinated against SARS-CoV-2; 66.06% of adults completed the primary series of vaccinations; 31.8% of the world population received booster or additional dosage, but during the first month of 2023, more than 220 thousand deaths related to COVID-19 were registered [1]. Despite the thought, that COVID-19 in children and adolescents has an asymptomatic course, fatal outcome is also noticed in the pediatric population. Based on UNICEF Data, in July 2023 there were 17,400 COVID-19 deaths among children and adolescents — 53% were registered in the age group 10–19 and 47% in children aged 0–9 years [2]. COVID-19 in childhood can also lead to multisystem inflammatory syndrome in children (MIS-C). Data from the USA reported an MIS-C frequency of 316 cases per million confirmed SARS-CoV-2 episodes in persons aged <21 [3]. Recent studies underline that critical illness or even

death among children with COVID-19 is most commonly seen in patients with comorbidities (respiratory, cardiovascular disorders, obesity, neurological or oncological disease) and the presence of co-infections [3–5]. In addition, age is an important risk factor for disease severity — premature babies, infants and adolescents are at greater risk for poor COVID-19 outcomes [3,5,6].

The course of any viral infection as well as COVID-19 will depend on the characteristics of the pathogen (its structure, viral load and the site of penetration into the host's cells), the type of host immune response and transmission environment with its influencing factors [6,7]. It must be mentioned that external factors (stress, sleep, nutrition and microbiota type) can undergo correction, but the host genetic factor cannot be corrected or changed and has a very significant impact on the course of the viral infection [6].

Therefore, the research goal was to investigate human genetic factors that could be associated with COVID-19 severity in the pediatric population. Single nucleotide polymorphisms (SNPs) of the next genes were studied: ACE2 (rs2074192); IFNAR2 (rs2236757); TYK2 (rs2304256); OAS1 (rs10774671); OAS3 (rs10735079); CD40 (rs4813003); FCGR2A (rs1801274) and CASP3 (rs113420705).

The angiotensin converting enzyme 2 (ACE2) gene has several single nucleotide polymorphisms that are associated with communicable and noncommunicable diseases. ACE2 alleles are the risk factors for cardiovascular diseases (hypertension, coronary artery disease, heart failure, atherosclerosis), respiratory diseases (pulmonary hypertension, chronic obstructive pulmonary disease, asthma, acute lung injury, acute respiratory distress syndrome, lung cancer, pulmonary sarcoidosis), endocrine diseases (type 2 diabetes mellitus, obesity) [8–10]. Since 2019, SARS-CoV-2 infection has become the most commonly discussed communicable disease related to ACE2 gene expression. Current studies show that intronic SNP rs2074192 (variant g42403) is related to COVID-19 severity as well as concomitant comorbidities [11].

The interferon system is extremely powerful in the context of antiviral defense. The type I interferon action is mediated by interaction with interferon alpha and beta receptor subunit 1 (IFNAR1) and interferon alpha and beta receptor subunit 2 (IFNAR2) receptors. The expression of IFNAR2 is regulated by the corresponding genes. Recent studies suggest the influence of IFNAR2 gene polymorphism on the course and severity of SARS-CoV-2 infection, especially its risk allele A [12,13].

The interferon system is closely related to tyrosine kinase 2 (TYK2). TYK2, as the part of Jak-family, is discussed as one of the key pathogenetic substances in immune-mediated inflammatory disease development [14]. Current knowledge suggests that changes in TYK2 regulation lead to deviations in interferons α and β response. Downregulation of TYK2 with other Jak-family members (Jak1, Jak2, Jak3) has a linkage with cytokines action — IL-6, IL-10, IL-11, IL-12, IL-19, IL-20, IL-22 and IL-23 [14,15]. The consequences of the TYK2 gene's (rs2304256) influence on COVID-19 are still controversial. Previous research showed discordant results. Dieter C. et al. and Benmansour R. with colleagues demonstrated a tendency in the association of the AA genotype TYK2 gene (rs2304256) with severe and lethal outcomes in adult patients with COVID-19 [16,17]. At the same time, Risi et al. reported an association of A allele carrier with mild disease course and underlined its protective properties [18]. Therefore, the influence of genotypes and alleles of the TYK2 gene (rs2304256) is still a point of concern.

Among interferon-induced enzymes, 2',5'-oligoadenylate synthetase (OAS) must be mentioned as it has a magnitude role in antiviral immunity. Continuous researches suggest that OAS1 rs10774671 with the risk allele A has a dominant role in the regulation of OAS enzymatic activity and SARS-CoV-2 elimination [19]. Single nucleotide variant rs10735079 (A > G) is responsible for gene clusters OAS1, OAS2 and OAS3 and is among COVID-19 genes [12,20]. Studies in the adult population suggest the association of rs10735079 (risk allele G) with the severity of SARS-CoV-2 infection (odds ratio (OR) = 1.3) [12,21].

MIS-C and Kawasaki disease (KD) have similarities in their pathogenesis (autoimmune pattern), clinical course (fever, rash, no purulent conjunctivitis, cardiovascular involvement) and laboratory findings (elevated pro-inflammatory markers and evidence of coagulopathy) [22]. Therefore, in differential diagnosis we assessed genome-wide significant variants associated with KD

susceptibility [22]. Current studies suggest 63 genes associated with KD [23]. Among them, four groups of genes that are related to KD susceptibility were formed: 1) genes that enhance T cell activation (ITPKC, ORAI1, STIM1); 2) genes related to dysregulation of B cell signaling (CD40, BLK, FCGR2A); 3) genes associated with decreased apoptosis (CASP3) and 4) genes related to altered transforming growth factor beta signaling (TGFB2, TGFBR2, MMP, SMAD) [23,24]. All listed genes have different risk allele frequencies in the European and Asian populations; therefore, we have focused on the genes that have a higher frequency in Europe — CD40 (CD40 molecule), Fc gamma receptor IIa (FCGR2A) and caspase 3 (CASP3) [22]. In addition, these genes are among the important regulators of antiviral immune response [25]. CD40 as the costimulatory surface receptor is expressed on B-cells, macrophages, monocytes, platelets, dendritic cells and non-hematopoietic cells — vascular endothelial cells, epithelial cells, myofibroblasts and fibroblasts [26–28]. Therefore, CD40 contributes to cellular and humoral immunity as well as to vascular remodeling [26,27,29]. FCGR2A (Fc fragment of immunoglobulin G, low-affinity IIa, receptor) is also discussed in the context of immune response and vascular remodeling [30]. Polymorphism FCGR2A rs1801274 is associated with the substitution of histidine by arginine at position 131 (H131R) [30]. As a result, binding affinity with different immunoglobulins G subclasses is changed and the autoimmune response is activated [30]. Caspase-3 is a well-known protein involved in apoptosis and it is encoded by the CASP3 gene. Activation of caspase-3 in COVID-19 patients can be caused by the enhanced production of reactive oxygen species in the presence of oxidative stress. At the same time, cytokine storm in SARS-CoV-2 infection is described as the result of apoptosis-related cellular death [31].

Therefore, CD40, FCGR2A and CASP3 genes are discussed as the genetic predispose factors of KD as well as severe COVID-19 and MIS-C in children.

Despite the number of genetic studies related to COVID-19 severity and outcome, results are controversial with different findings and associations presented. However, no previous analysis regarding the role of genetic factors in SARS-CoV-2 severity in children was reported. Therefore, it became the focus of our research.

2. Materials and Methods

A total number of 75 children were involved in the case-control study — 30 persons with mild or moderate course of the disease; 30 children with severe COVID-19 and multisystem inflammatory syndrome and 15 healthy children who did not have COVID-19 (PCR negative, Ig G negative).

Criteria of the Italian Society of Pediatric Infectious Disease [32,33] and COVID-19 Treatment Guidelines (National Institutes of Health) [34] were used to define disease severity. Mild disease severity was diagnosed in cases when upper airway symptoms without radiological/ultrasound findings were present despite the temperature level. Children with pneumonia diagnosed by imaging studies or persons with upper airway symptoms with respiratory distress were defined as patients with moderate disease severity. When patients had a fever or cough with one of the following criteria — saturation <92% in room air; severe respiratory distress or systemic symptoms (drowsiness, lethargy, seizures, dehydration) — severe disease course was diagnosed.

Diagnosis of MIS-C was done based on the World Health Organization (WHO) criteria [35]: fever duration greater than three days in children and adolescents (0–19 years) plus two of the following: 1) Rash or bilateral nonpurulent conjunctivitis or mucocutaneous inflammation signs (oral, hands or feet); 2) Hypotension or shock; 3) Features of myocardial dysfunction, pericarditis, valvulitis or coronary abnormalities (including ECHO findings or elevated Troponin/N-Terminal Pro-B-Type Natriuretic Peptide [NT-proBNP]); 4) Evidence of coagulopathy (by prothrombin time, partial thromboplastin time, elevated d-Dimers) or 5) Acute gastrointestinal problems (diarrhea, vomiting or abdominal pain). Other evaluations in MIS-C diagnosis included elevated markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein or procalcitonin, and no other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes as well as evidence of COVID-19 (real-time reverse transcription polymerase chain reaction, antigen test or serology positive) or likely contact with patients with COVID-19.

The study was conducted and performed under the principles of the Declaration of Helsinki. Bioethics Committee of I. Horbachevsky Ternopil National Medical University approved this study (Protocol No 71, dated October 25, 2022). Informed consent was obtained from all children’s caregivers.

Venous blood samples for genomic study were collected in tubes with ethylenediamine tetraacetic acid. The blood sample volume for clinical care and research did not exceed the recommended WHO limit [36]. The Thermo Scientific™ GeneJET™ Whole Blood Genomic DNA Purification Mini Kit Cat. No K0781 (Thermo Fisher Scientific, USA) was used for genomic DNA extraction according to the manufacturer’s instructions. Predesigned TaqMan™ SNP Genotyping Assays, Cat. No. 4351379 (Thermo Fisher Scientific, USA) were used for next SNPs — ACE2 rs2074192, IFNAR2 rs2236757, TYK2 rs2304256, OAS1 rs10774671, OAS3 rs10735079, CD40 rs4813003, FCGR2A rs1801274 and CASP3 rs113420705. TaqMan™ Universal Master Mix II, no UNG, 1 x 5 ml, Cat. No 4440040 was used for DNA amplification using real-time polymerase chain reaction.

Statistical analysis was done with the computer software IBM SPSS Statistics 21.0. Quantitative values were presented as number (n) and frequency (%). Frequency tables 2 x 3 were compared using the chi-square test (χ^2), while for tables 2 x 2 two-tailed, Fisher exact test was used; the level of significance for each test was calculated — p-value and p_F , respectively. Correspondence for Hardy-Weinberg equilibrium was assessed for each gene. With the assumption that gene frequency corresponds to the population, one was done in a case when $p > 0.05$ in the chi-square test. For outcome prediction, OR with its 95% confidence interval (95% CI) was calculated. Logistic regression was done to determine the key predictors of COVID-19 and its severity in the pediatric population. A level of statistical significance was assumed with a p-value < 0.05 . GeneMANIA was used to assess the network cooperation between studied genes [37].

3. Results

3.1. Study Group’s Characteristic

Demographic characteristic of children involved in the study is presented in Table 1. There was no sex difference in the studied group, while age difference was revealed. Children with mild or moderate COVID-19 were significantly younger compared to the control group. Age among COVID-19 patients did not vary (Table 1).

Table 1. Demographic characteristic of children with COVID-19 (n = 60) and healthy children (n = 15).

Characteristic		Mild/Moderate COVID-19 (1)	Severe COVID-19/ MIS-C (2)	Control (3)	p
Age, years	Median (Q25; Q75)	2.5 (1.0; 7.0)	7.0 (2.0; 14.0)	8.0 (5.0; 13.0)	$p_{1-2} = 0.059$ $p_{1-3} = 0.011^*$ $p_{2-3} = 0.943$
	Min/Max	0.2/17.0	0.1/17.6	2.0/16.0	—
Sex	Boys	19 (63.33)	20 (66.67)	7 (46.67)	$\chi^2 = 1.77$; $p = 0.413$
	Girls	11 (36.67)	10 (33.33)	8 (53.33)	

Abbreviations: p_{1-2} – statistical difference between group 1 (Mild/Moderate COVID-19) and group 2 (Severe COVID-19/MIS-C); p_{1-3} – statistical difference between group 1 (Mild/Moderate COVID-19) and group 3 (control); p_{2-3} – statistical difference between group 2 (Severe COVID-19/MIS-C) and group 3 (control); χ^2 – chi-squared test, p – level of its significance. * – statistically significant result.

3.2. Correspondence to Hardy-Weinberg Equilibrium

Frequency of ACE2 rs2074192, IFNAR2 rs2236757, OAS3 rs10735079, FCGR2A rs1801274 and CASP3 rs113420705 genotypes did not follow Hardy-Weinberg equilibrium ($p < 0.05$) due to the directional selection in our study (focusing on patients with COVID-19). In COVID-19 group, TYK2 rs2304256, OAS1 rs10774671, CD40 rs4813003 and FCGR2A rs1801274 homozygotes and heterozygotes frequencies were in accordance with Hardy-Weinberg principle ($p > 0.05$). Notably,

genotype frequencies in healthy children (control group) correspond to Hardy-Weinberg proportions ($p > 0.05$) (Tables 2.1–2.3).

Table 2.1. Test for Hardy-Weinberg equilibrium for ACE2 rs2074192, IFNAR2 rs2236757, TYK2 rs2304256.

Group	Genotype	ACE2 rs2074192		IFNAR2 rs2236757		TYK2 rs2304256	
		Expected	Observed	Expected	Observed	Expected	Observed
All patients	Common						
	Homozygotes	19.3	29	19.8	25	34.7	37
	Heterozygotes	37.5	17	37.5	27	32.6	28
	Rare						
	Homozygotes	18.3	29	17.8	23	7.7	10
	χ^2 , p-value	$\chi^2 = 22.41$; $p < 0.001^*$		$\chi^2 = 5.86$; $p = 0.016^*$		$\chi^2 = 1.52$; $p = 0.218$	
COVID-19 patients	Common						
	Homozygotes	12.6	21	13.5	18	26.0	28
	Heterozygotes	29.8	13	29.9	21	27.0	23
	Rare						
	Homozygotes	17.6	26	16.5	21	7.0	9
	χ^2 , p-value	$\chi^2 = 19.06$; $p < 0.001^*$		$\chi^2 = 5.34$; $p = 0.021^*$		$\chi^2 = 1.31$; $p = 0.252$	
Control group (noninfected persons)	Common						
	Homozygotes	6.67	8	6.7	7	8.8	9
	Heterozygotes	6.67	4	6.7	6	5.4	5
	Rare						
	Homozygotes	1.67	3	1.7	2	0.8	1
	χ^2 , p-value	$\chi^2 = 2.40$; $p = 0.121$		$\chi^2 = 0.15$; $p = 0.696$		$\chi^2 = 0.07$; $p = 0.791$	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance. * – statistically significant result.

Table 2.2. Test for Hardy-Weinberg equilibrium for OAS1 rs10774671, OAS3 rs10735079.

Group	Genotype	OAS1 rs10774671)		OAS3 rs10735079	
		Expected	Observed	Expected	Observed
All patients	Common				
	Homozygotes	19.5	21	31.4	38
	Heterozygotes	38.0	33	34.3	21
	Rare				
	Homozygotes	18.5	21	9.4	16
	χ^2 , p-value	$\chi^2 = 1.31$; $p = 0.299$		$\chi^2 = 11.25$; $p < 0.001^*$	
COVID-19 patients	Common				
	Homozygotes	11.3	13	22.8	28
	Heterozygotes	29.5	26	28.4	18
	Rare				
	Homozygotes	19.3	21	8.8	14
	χ^2 , p-value	$\chi^2 = 0.83$; $p = 0.362$		$\chi^2 = 8.01$; $p = 0.005^*$	
Control group	Common	8.8	8	8.8	10

(noninfected persons)	Homozygotes				
	Heterozygotes	5.4	7	5.4	3
	Rare				
	Homozygotes	0.8	0	0.8	2
	χ^2 , p-value	$\chi^2 = 1.39$; p = 0.238		$\chi^2 = 2.92$; p = 0.088	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance. * – statistically significant result.

Table 2.3. Test for Hardy-Weinberg equilibrium for CD40 rs4813003, FCGR2A rs1801274, CASP3 rs113420705.

Group	Genotype	CD40 rs4813003		FCGR2A rs1801274		CASP3 rs113420705	
		Expected	Observed	Expected	Observed	Expected	Observed
All patients	Common						
	Homozygotes	62.6	63	25.8	30	18.8	13
	Heterozygotes	11.9	11	36.4	28	37.5	49
	Rare						
	Homozygotes	0.6	1	12.8	17	18.8	13
	χ^2 , p-value	$\chi^2 = 0.41$; p = 0.230		$\chi^2 = 3.97$; p = 0.046*		$\chi^2 = 7.05$; p = 0.008*	
COVID-19 patients	Common						
	Homozygotes	53.2	53	18.7	22	12.6	6
	Heterozygotes	6.6	7	29.6	23	29.8	43
	Rare						
	Homozygotes	0.2	0	11.7	15	17.6	11
	χ^2 , p-value	$\chi^2 = 0.63$; p = 0.230		$\chi^2 = 2.98$; p = 0.084		$\chi^2 = 11.79$; p < 0.001*	
Control group (noninfected persons)	Common						
	Homozygotes	9.6	10	7.4	8	6.7	7
	Heterozygotes	4.8	4	6.3	5	6.7	6
	Rare						
	Homozygotes	0.6	1	1.4	2	1.7	2
	χ^2 , p-value	$\chi^2 = 0.42$; p = 0.519		$\chi^2 = 0.64$; p = 0.424		$\chi^2 = 0.15$; p = 0.699	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance. * – statistically significant result.

3.3. Genotype and Allele Frequencies

3.3.1. ACE2 rs2074192

Genotype frequencies did not vary in the studied group in the codominant model (Figure 1). While analysis of dominant, recessive and overdominant models demonstrates that children with severe COVID-19 or MIS-C more often are the carriers of TT genotype ACE2 rs2074192. Genotype TT rs2074192 increases the risk of having a severe course of SARS-CoV-2 infection by 4.57 times (OR = 4.57; 95% CI 1.07–19.57; p = 0.041) (Table 3.1).

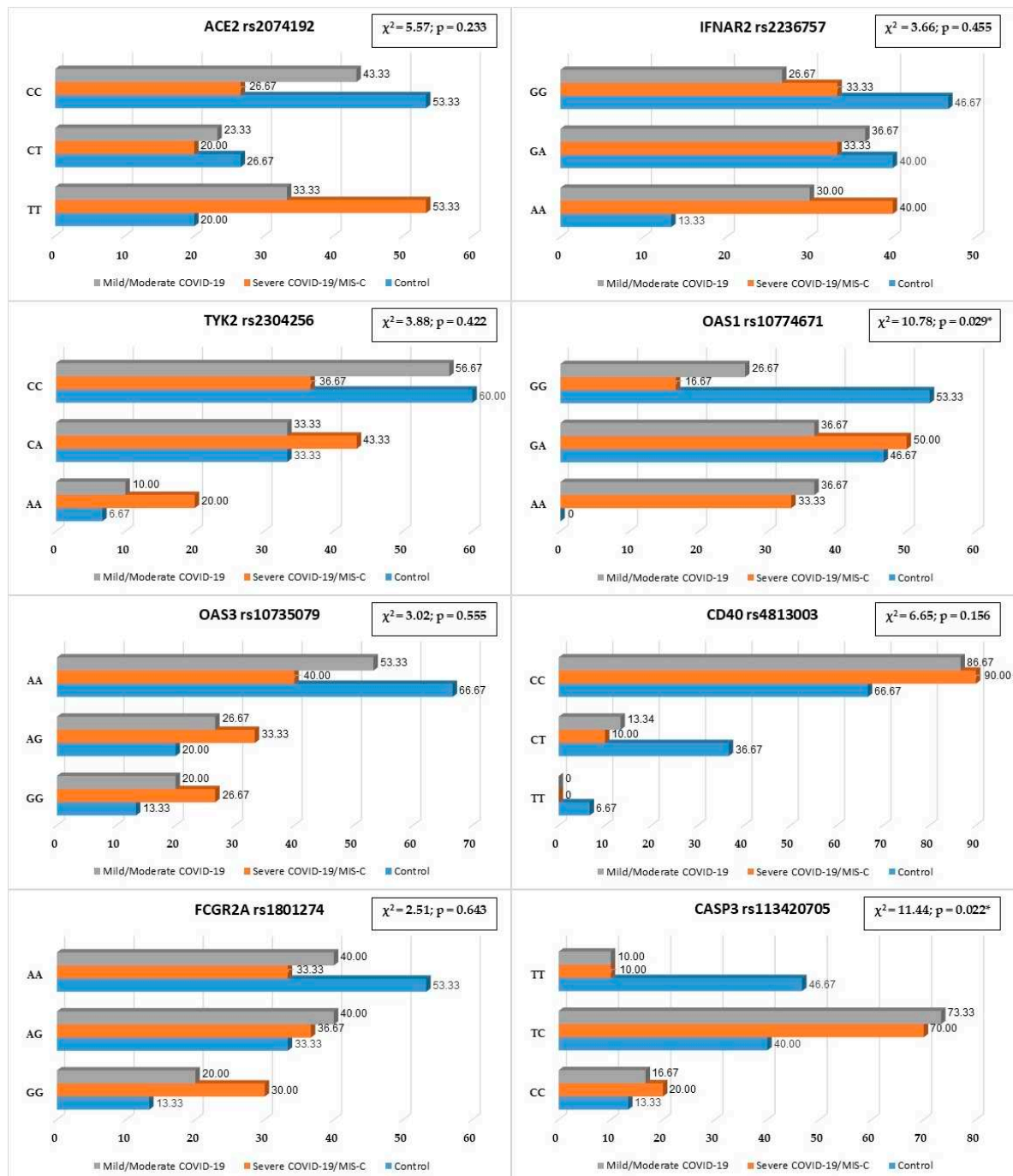


Figure 1. Genotype frequencies in the codominant model for genes ACE2 rs2074192, IFNAR2 rs2236757, TYK2 rs2304256, OAS1 rs10774671, OAS3 rs10735079, CD40 rs4813003, FCGR2A rs1801274 and CASP3 rs113420705 in children with COVID-19 and noninfected children (ACE 2 – angiotensin converting enzyme 2; IFNAR2 – interferon alpha and beta receptor subunit 2; TYK2 – tyrosine kinase 2; OAS1 – 2'-5'-oligoadenylate synthetase 1; OAS3 – 2'-5'-oligoadenylate synthetase 3; CD40 – CD40 molecule; FCGR2A – Fc gamma receptor IIa; CASP3 – caspase 3).

Children with severe COVID-19 course and MIS-C significantly often are the allele T carriers ACE2 rs2074192 compared to healthy persons ($p = 0.017$). Risk allele T increases of severe disease course by 3.45 times ($p = 0.009$); while allele C has protective properties ($OR = 0.29$; $0.12-0.73$) (Table 4).

Comparison of our results with data from the project “ALFA: Allele Frequency Aggregator” [38] showed that the frequency of alleles C and T in healthy children corresponds to the European

population frequency ($p = 0.061$), while patients with COVID-19 often were the carriers of risk allele T compared to the general population (0.54 versus 0.45; $p = 0.048$) (Table 5).

Notably, the genotype frequency varied significantly between girls and boys in the COVID-19 group — boys have genotype TT more often compared to girls. In healthy children, such sex difference was not revealed (Table 5). At the same time, a comparison of healthy boys and boys with COVID-19 showed a significant difference in ACE2 rs2074192 genotype frequencies ($\chi^2 = 6.94$; $p = 0.031$); healthy and infected by SARS-CoV-2 girls did not differ by genotype frequencies ($\chi^2 = 0.68$; $p = 0.711$) (Table 6). Allele T frequency varied between groups — in 60.26% of infected boys compared to 14.29% of noninfected boys ($p = 0.002$) (Table 5).

Table 3.1. Dominant, recessive and overdominant models for ACE2 rs2074192.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	CC	13 (43.33)	8 (26.67)	8 (53.33)	$\chi^2 =$ 3.46	0.32 (0.09–1.16)	0.084
	CT-TT	17 (56.67)	22 (73.33)	7 (46.67)	$p =$ 0.177	3.14 (0.86–11.50)	
Recessive	CC-CT	21 (70.00)	14 (46.67)	12 (80.00)	$\chi^2 =$ 5.28	0.22 (0.05–0.93)	0.041*
	TT	10 (33.33)	16 (53.33)	3 (20.00)	$p =$ 0.071	4.57 (1.07–19.57)	
Overdominant	CC-TT	23 (76.67)	24 (80.00)	11 (73.33)	$\chi^2 =$ 0.27	1.45 (0.34–6.22)	0.613
	CT	7 (23.33)	6 (20.00)	4 (24.67)	$p =$ 0.875	0.69 (0.16–2.94)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation). * – statistically significant result.

3.3.2. IFNAR2 rs2236757

Rare homozygous AA was seen in 40% of patients with severe COVID-19 and MIS-C, while in the control group it was observed in 13.33% of cases. Despite the higher frequency, this difference did not reach the level of statistical significance ($p = 0.071$) (Figure 1). In the recessive inheritance model, genotype AA IFNAR2 rs2236757 showed a tendency to increase disease severity more than four times (OR = 4.33; 95% CI 0.83–22.75; $p = 0.083$) (Table 3.2).

IFNAR2 rs2236757 risk A allele was revealed significantly more often among patients with severe COVID-19 and MIS-C compared to the control group — 56.67% vs. 33.33% ($p = 0.046$). Carriers of risk allele A are more prone to suffer from severe COVID-19 or MIS-C (OR = 2.62; $p = 0.039$) (Table 4).

It is important to emphasize that allele A is registered significantly more often in children with COVID-19 compared to data in the general European population ($p < 0.001$), while alleles G and A frequencies in healthy children correspond to it ($p = 0.497$) (Table 5).

There were no sex differences in genotype or allele frequencies in the COVID-19 group and control group ($p > 0.05$). But comparison via the same sex demonstrates a higher frequency of risk allele A in boys with SARS-CoV-2 compared to healthy boys — 50% and 21.43% ($p = 0.049$); in girls such difference was not revealed ($p = 0.365$) (Table 6).

Table 3.2. Dominant, recessive and overdominant models for IFNAR2 rs2236757.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	GG	10 (33.33)	8 (26.67)	7 (46.67)	$\chi^2 =$ 1.80	0.42 (0.11–1.52)	0.185
	GA-AA	20 (66.67)	22 (73.33)	8 (53.33)	p = 0.406	2.41 (0.66–8.81)	
Recessive	GG-GA	21 (70.00)	18 (60.00)	13 (86.67)	$\chi^2 =$ 3.54	0.23 (0.04–1.21)	0.083
	AA	9 (30.00)	12 (40.00)	2 (13.33)	p = 0.187	4.33 (0.83–22.75)	
Overdominant	GG-AA	19 (63.33)	20 (66.67)	9 (60.00)	$\chi^2 =$ 0.20	1.33 (0.37–4.80)	0.660
	GA	11 (36.67)	10 (33.33)	6 (40.00)	p = 0.904	0.75 (0.21–2.70)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation)).

3.3.3. TYK2 rs2304256

There were no differences in TYK2 rs2304256 genotype frequencies via codominant, dominant, recessive and overdominant models ($p > 0.05$) (Figure 1, Table 3.3).

Alleles C and A frequencies also did not vary between groups of different COVID-19 severity and healthy children (Table 4). Risk allele A was seen in 23.33% of healthy persons vs. 41.67% in patients with severe COVID-19/MIS-C and 26.67% in the mild/moderate group ($p > 0.05$). Allele frequencies in the COVID-19 group were similar to population frequency (Table 5).

TYK2 rs2304256 genotype and allele frequencies were similar between boys and girls as well as between infected and noninfected children of the same sex ($p > 0.05$) (Table 6).

Table 3.3. Dominant, recessive and overdominant models for TYK2 rs2304256.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	CC	17 (56.67)	11 (36.67)	9 (60.00)	$\chi^2 =$ 3.26	0.39 (0.11–1.38)	0.143
	CA-AA	13 (43.33)	19 (63.33)	6 (40.00)	p = 0.197	2.59 (0.73–9.25)	
Recessive	CC-CA	27 (90.00)	24 (80.00)	14 (93.33)	$\chi^2 =$ 2.02	0.29 (0.03–2.62)	0.268
	AA	3 (10.00)	6 (20.00)	1 (6.67)	p = 0.364	3.50 (0.38–32.14)	
Overdominant	CC-AA	20 (66.67)	17 (56.67)	10 (66.67)	$\chi^2 =$ 0.77	0.65 (0.18–2.38)	0.520
	CA	10 (33.33)	13 (43.33)	5 (33.33)	p = 0.681	1.53 (0.42–5.58)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation)).

3.3.4. OAS1 rs10774671

The codominant model's study revealed differences in genotype frequencies for gene OAS1 rs10774671 ($p < 0.05$) (Figure 1). Genotype AA OAS1 rs10774671 was registered in 35% of patients infected by SARS-CoV-2, while in healthy children it was not noticed ($p = 0.022$). Study results demonstrate that homozygous GG is more than five times less likely to have a severe COVID-19 course (OR = 0.18) compared to carriers of allele A (heterozygous GA and homozygous AA) — OR = 5.71 ($p < 0.05$) (Table 3.4).

Patients with COVID-19 often were the carrier of allele A (56.67%) compared to healthy children (23.33%) ($p = 0.001$) (Table 4). Notably, allele A OAS1 is associated with severe COVID-19 course (OR = 4.60; 95% CI 1.71–12.37; $p = 0.003$). It should be noted that patients in the control group did not correspond to the European population according to OAS1 rs10774671 allele G and A frequencies (Table 5).

Genes located on the autosome OAS1 rs10774671 genotype and allele frequencies were similar for both sexes (Table 6).

Table 3.4. Dominant, recessive and overdominant models for OAS1 (rs10774671).

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	GG	8 (26.67)	5 (16.67)	8 (53.33)	$\chi^2 =$ 6.71	0.18 (0.04–0.71)	0.015*
	GA–AA	22 (73.33)	25 (83.33)	7 (46.67)	$p =$ 0.035*	5.71 (1.41–23.10)	
Recessive	GG–GA	19 (63.33)	20 (66.67)	15 (100.00)	$\chi^2 =$ 7.37	0.06 (0.01–1.16)	0.063
	AA	11 (36.67)	10 (33.33)	0	$p =$ 0.025*	15.88 (0.86–292.28)	
Overdominant	GG–AA	19 (63.33)	15 (50.00)	8 (53.33)	$\chi^2 =$ 1.14	0.88 (0.25–3.03)	0.833
	GA	11 (36.67)	15 (50.00)	7 (46.67)	$p =$ 0.567	1.14 (0.33–3.96)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation). * – statistically significant result.

3.3.5. OAS3 rs10735079

There were no statistical differences between OAS3 rs10735079 genotypes and allele frequencies between children with different COVID-19 severity and healthy kids (Figure 1, Table 3.5). Nevertheless, it should be noticed that frequencies of allele G and genotypes with allele G (GG + GA) are two times higher in persons with severe COVID-19 course compared to the control group — 43.33% vs. 23.33% for alleles ($p = 0.065$) and 60% vs. 33.33% for genotypes ($p = 0.095$).

OAS3 rs10735079 allele frequencies in noninfected children differ significantly from data in the European population ($p = 0.024$) (Table 5). Sex differences were not typical for rs10735079 (Table 6).

Table 3.5. Dominant, recessive and overdominant models for OAS3 rs10735079.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
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Dominant	AA	16 (53.33)	12 (40.00)	10 (66.67)	$\chi^2 =$ 2.99	0.33 (0.09–1.22)	0.098
	AG–GG	14 (46.67)	18 (60.00)	5 (33.33)	$p =$ 0.225	3.00 (0.82–10.99)	
Recessive	AA–AG	24 (80.00)	22 (73.33)	13 (86.67)	$\chi^2 =$ 1.11	0.42 (0.08–2.30)	0.320
	GG	6 (20.00)	8 (26.67)	2 (13.33)	$p =$ 0.573	2.36 (0.43–12.87)	
Overdominant	AA–GG	22 (73.33)	20 (66.67)	12 (80.00)	$\chi^2 =$ 0.93	0.50 (0.11–2.19)	0.357
	AG	8 (26.67)	10 (33.33)	3 (20.00)	$p =$ 0.629	2.00 (0.46–8.75)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation).

3.3.6. CD40 rs4813003

The research did not reveal significant differences in codominant, dominant, recessive and overdominant models between study groups for gene CD40 rs4813003 (Figure 1, Table 3.6). Importantly, genotype CC was typical for 90% of children with severe COVID-19 and MIS-C, while in a healthy group it was registered in 66.67% of cases ($p = 0.056$). Correspondingly, allele C significantly dominates in the COVID-19 group compared to noninfected children – 94.17% vs. 80% ($p = 0.024$). Based on our study results, allele C increases the risk of severe COVID-19 course or MIS-C in 4.75 times ($p = 0.037$) (Table 4).

It is important to note that allele C and T frequencies were significantly different compared not only to the control but also to the general European population ($p = 0.011$). In noninfected children, both allele C and T frequencies correspond to the general population frequency ($p > 0.05$) (Table 5).

Comparison between boys and girls did not show any difference in genotype and allele frequency ($p > 0.05$) (Table 6).

Table 3.6. Dominant, recessive and overdominant models for CD40 rs4813003.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	CC	26 (86.67)	27 (90.00)	10 (66.67)	$\chi^2 =$ 4.32	4.50 (0.90–22.40)	0.066
	CT–TT	4 (13.33)	3 (10.00)	5 (33.33)	$p =$ 0.116	0.22 (0.04–1.11)	
Recessive	CC–CT	30 (100.00)	30 (100.00)	14 (93.33)	$\chi^2 =$ 4.05	6.31 (0.24–164.57)	0.268
	TT	0	0	1 (6.67)	$p =$ 0.132	0.16 (0.01–4.13)	
Overdominant	CC–TT	26 (86.67)	27 (90.00)	11 (73.33)	$\chi^2 =$ 2.29	3.27 (0.63–17.09)	0.160
	CT	4 (13.33)	3 (10.00)	4 (26.67)	$p =$ 0.318	0.31 (0.06–1.60)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation).

3.3.7. FCGR2A rs1801274

Analysis of all genetic models of inheritance did not reveal significant changes in FCGR2A rs1801274 genotype frequencies between those infected by the SARS-CoV-2 virus and noninfected persons (Figure 1, Table 3.7).

Allele A and G frequencies in patients with COVID-19 did not differ significantly from the control group as well as from the general population ($p > 0.05$) (Tables 4 and 5). At the same time, our study results demonstrated a deviation in allele frequencies between healthy children and the European population ($p < 0.05$) (Table 5)

Genotype and allele frequencies in the male group correspond to the same parameters in the female group in COVID-19 patients and healthy children ($p > 0.05$) (Table 6).

Table 3.7. Dominant, recessive and overdominant models for FCGR2A rs1801274.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	AA	12 (40.00)	10 (33.33)	8 (53.33)	$\chi^2 =$ 1.67	0.44 (0.12–1.55)	0.201
	AG+GG	18 (60.00)	20 (66.67)	7 (46.67)	p = 0.435	2.29 (0.64–8.11)	
Recessive	AA+AG	24 (80.00)	21 (70.00)	13 (86.67)	$\chi^2 =$ 1.79	0.36 (0.07–1.93)	0.232
	GG	6 (20.00)	9 (30.00)	2 (13.33)	p = 0.409	2.79 (0.52–14.96)	
Overdominant	AA+GG	18 (60.00)	19 (63.33)	10 (66.67)	$\chi^2 =$ 0.20	0.86 (0.23–3.19)	0.826
	AG	12 (40.00)	11 (36.67)	5 (33.33)	p = 0.905	1.16 (0.31–4.27)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation).

3.3.8. CASP3 rs113420705

CASP3 rs113420705 genotype TT was registered significantly more often among healthy children (46.67%) compared to patients with COVID-19 of different severity (10%) ($p < 0.05$) (Figure 1). Notably, TT genotype rs113420705 is protective against severe COVID-19 or MIS-C in the pediatric population (OR = 0.13; $p = 0.010$) (Table 3.8). Carriers of allele C in both heterozygous TC and rare homozygous CC have an increasing risk of severe SARS-CoV-2 infection (OR = 7.88) (Table 3.8). Important to note is that despite the absence of a statistically significant difference between COVID-19 groups and control, the study noted a significantly higher prevalence of allele C in COVID-19 pediatric patients and European alleles' frequencies (Table 4 and Table 5).

Sex differences were not typical for CASP3 rs113420705 genotypes and allele frequencies (Table 6).

Table 3.8. Dominant, recessive and overdominant models for CASP3 rs113420705.

Model	Genotype	Mild/ Moderate COVID- 19	Severe COVID- 19/ MIS- C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
Dominant	TT	3 (10.00)	3 (10.00)	7 (46.67)	$\chi^2 =$ 11.26	0.13 (0.03–0.61)	0.010*

	TC+CC	27 (90.00)	27 (90.00)	8 (53.33)	p = 0.004*	7.88 (1.65–37.69)	
Recessive	TT+TC	25 (83.33)	24 (80.00)	13 (86.67)	$\chi^2 = 0.33$	0.62 (0.11–3.50)	0.584
	CC	5 (16.67)	6 (20.00)	2 (13.33)	p = 0.850	1.63 (0.29–9.23)	
Overdominant	TT+CC	8 (26.67)	9 (30.00)	9 (30.00)	$\chi^2 = 5.39$	0.29 (0.08–1.04)	0.058
	TC	22 (73.33)	21 (70.00)	6 (40.00)	p = 0.068	3.50 (0.96–12.78)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation). * – statistically significant result.

Table 4. Allele frequencies for genes ACE2 rs2074192, IFNAR2 rs2236757, TYK2 rs2304256, OAS1 rs10774671, OAS3 rs10735079, CD40 rs4813003, FCGR2A rs1801274, CASP3 rs113420705 in children with different COVID-19 severity and noninfected children.

Gene	Allele	Mild/ Moderate COVID-19	Severe COVID-19/ MIS-C	Control	χ^2 , p- value	OR (95% CI)	p- value for OR
ACE2 rs2074192	C	33 (55.00)	22 (36.67)	20 (66.67)	$\chi^2 = 8.20$; p = 0.017*	0.29 (0.12–0.73)	0.009*
	T	27 (45.00)	38 (63.33)	10 (33.33)		3.45 (1.37–8.69)	
IFNAR2 rs2236757	G	31 (51.67)	26 (43.33)	20 (66.67)	$\chi^2 = 4.36$; p = 0.113	0.38 (0.15–0.95)	0.039*
	A	29 (48.33)	34 (56.67)	10 (33.33)		2.62 (1.05–6.53)	
TYK2 rs2304256	C	44 (73.33)	35 (58.33)	23 (76.67)	$\chi^2 = 4.40$; p = 0.111	0.43 (0.16–1.15)	0.091
	A	16 (26.67)	25 (41.67)	7 (23.33)		2.35 (0.87–6.31)	
OAS1 rs10774671	G	27 (45.00)	25 (41.67)	23 (76.67)	$\chi^2 = 10.80$; p = 0.005*	0.22 (0.08–0.58)	0.003*
	A	33 (55.00)	35 (58.33)	7 (23.33)		4.60 (1.71–12.37)	
OAS3 rs10735079	A	40 (66.67)	34 (56.67)	23 (76.67)	$\chi^2 = 3.67$; p = 0.159	0.40 (0.15–1.07)	0.068
	G	20 (33.33)	26 (43.33)	7 (23.33)		2.51 (0.94–6.75)	
CD40 rs4813003	C	56 (93.33)	57 (95.00)	24 (80.00)	$\chi^2 = 6.19$; p = 0.045*	4.75 (1.10–20.57)	0.037*
	T	4 (6.67)	3 (5.00)	6 (20.00)		0.21 (0.05–0.91)	
FCGR2A rs1801274	A	36 (60.00)	31 (51.67)	21 (70.00)	$\chi^2 = 2.85$; p = 0.241	0.46 (0.19–1.16)	0.100
	G	24 (40.00)	29 (48.33)	9 (30.00)		2.18 (0.86–5.54)	
CASP3 rs113420705	T	28 (46.67)	27 (45.00)	20 (66.67)	$\chi^2 = 4.20$; p = 0.122	0.41 (0.165–1.02)	0.056
	C	32 (53.33)	33 (55.00)	10 (33.33)		2.44 (0.98–6.10)	

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance; OR – odds ratio (calculated for the outcome “Severe COVID-19/MIS-C”; 95% CI – 95% confidence interval (severe COVID-19/MIS-C – outcome in OR calculation). * – statistically significant result.

Table 5. Comparison of allele frequencies in children infected by SARS-CoV-2 compared to the general European population.

Gene	Allele	Allele Frequency			$p_{\text{COVID-19-EUR}}$	$p_{\text{Control-EUR}}$
		Children with COVID-19	Healthy children	European population		
ACE2	C	0.46	0.67	0.55	0.048*	0.061
rs2074192	T	0.54	0.33	0.45		
IFNAR2	G	0.48	0.67	0.71	<0.001*	0.497
rs2236757	A	0.52	0.33	0.29		
TYK2	C	0.66	0.77	0.72	0.144	0.390
rs2304256	A	0.34	0.23	0.28		
OAS1	G	0.43	0.77	0.36	0.110	<0.001*
rs10774671	A	0.57	0.23	0.64		
OAS3	A	0.62	0.77	0.63	0.818	0.024*
rs10735079	G	0.38	0.23	0.37		
CD40	C	0.94	0.80	0.86	0.011*	0.342
rs4813003	T	0.06	0.20	0.14		
FCGR2A	A	0.56	0.70	0.51	0.271	0.039*
rs1801274	G	0.44	0.30	0.49		
CASP3	T	0.46	0.67	0.72	<0.001*	0.542
rs113420705	C	0.54	0.33	0.28		

Abbreviations: $p_{\text{COVID-19-EUR}}$ – level of statistical significance for comparison of COVID-19 group with general European population; $p_{\text{Control-EUR}}$ – level of statistical significance for the comparison control group (healthy children) with general European population. * – statistically significant result.

Table 6. Sex differences in genotype frequencies among those infected with SARS-CoV-2 and noninfected children.

Gene	Genotype/ Allele	COVID-19 (n = 60)		χ^2 , p/ p_F	Control (n = 15)		χ^2 , p/ p_F
		Boys	Girls		Boys	Girls	
ACE2 rs2074192	CC	13 (39.13)	8 (38.10)	$\chi^2 = 6.95$; p = 0.031* $p_F = 0.085$	5 (71.43)	3 (37.50)	$\chi^2 = 3.45$; p = 0.178 $p_F = 0.058$
	CT	5 (12.82)	8 (38.10)		2 (28.57)	2 (25.00)	
	TT	21 (53.85)	5 (23.81)		0	3 (37.50)	
	C	31 (39.74)	24 (57.14)		12 (85.71)	8 (50.00)	
	T	47 (60.26)#	18 (42.86)		2 (14.29)	8 (50.00)	
IFNAR2 rs2236757	GG	12 (30.77)	6 (28.57)	$\chi^2 = 0.97$; p = 0.615 $p_F = 0.566$	4 (57.14)	3 (37.50)	$\chi^2 = 2.09$; p = 0.352 $p_F = 0.260$
	GA	15 (38.46)	6 (28.57)		3 (42.86)	3 (37.50)	
	AA	12 (30.77)	9 (42.86)		0	2 (25.00)	
	G	39 (50.00)	18 (42.86)		11 (78.57)	9 (56.25)	
	A	39 (50.00)	24 (57.14)		3 (21.43)	7 (43.75)	
TYK2 rs2304256	CC	20 (51.28)	8 (38.10)	$\chi^2 = 1.25$; p = 0.536 $p_F = 0.548$	5 (71.43)	4 (50.00)	$\chi^2 = 1.25$; p = 0.535 $p_F = 0.399$
	CA	13 (33.33)	10 (47.62)		2 (28.57)	3 (37.50)	
	AA	6 (15.38)	3 (14.29)		0	1 (12.50)	
	C	53 (67.95)	26 (61.90)		12 (85.71)	11 (68.75)	
	A	25 (32.05)	16 (38.10)		2 (14.29)	5 (31.25)	
	GG	8 (20.51)	5 (23.81)	$\chi^2 = 0.10$;	4 (57.14)	4 (50.00)	

OAS1 rs10774671	GA	17 (43.59)	9 (42.86)	$p = 0.953$	3 (42.86)	4 (50.00)	$p_F =$
	AA	14 (35.90)	7 (33.33)		0	0	1.000
	G	33 (42.31)	19 (45.24)	$p_F = 0.847$	11 (78.57)	12 (75.00)	$p_F =$
	A	45 (57.69)	23 (54.76)		3 (21.43)	4 (25.00)	1.000
OAS3 rs10735079	AA	19 (48.72)	9 (42.86)	$\chi^2 = 1.06;$	4 (57.14)	6 (75.00)	$\chi^2 = 0.67;$
	AG	10 (25.64)	8 (38.10)	$p = 0.588$	2 (28.57)	1 (12.50)	$p = 0.715$
	GG	10 (25.64)	4 (19.05)		1 (14.29)	1 (12.50)	
	A	48 (61.54)	26 (61.90)	$p_F = 1.000$	10 (71.43)	13 (81.25)	$p_F =$
	G	30 (38.46)	16 (38.10)		4 (28.57)	3 (18.75)	0.675
CD40 rs4813003	CC	34 (87.18)	19 (90.48)	$p_F = 1.000$	5 (71.43)	5 (62.50)	$\chi^2 = 1.94;$
	CT	5 (12.82)	2 (9.52)		1 (14.29)	3 (37.50)	$p = 0.379$
	TT	0	0		1 (14.29)	0	
	C	73 (93.59)	40 (95.24)	$p_F = 1.000$	11 (78.57)	13 (81.25)	$p_F =$
	T	5 (6.41)	2 (4.76)		3 (21.43)	3 (18.75)	1.000
FCGR2A rs1801274	AA	12 (30.77)	10 (47.62)	$\chi^2 = 3.00;$	5 (71.43)	3 (37.50)	$\chi^2 = 2.65;$
	AG	18 (46.15)	5 (23.81)	$p = 0.223$	2 (28.57)	3 (37.50)	$p = 0.266$
	GG	9 (23.08)	6 (28.57)		0	2 (25.00)	
	A	42 (53.85)	25 (59.52)	$p_F = 0.570$	12 (85.71)	9 (56.25)	$p_F =$
	G	36 (46.15)	17 (40.48)		2 (14.29)	7 (43.75)	0.118
CASP3 rs113420705	TT	3 (7.69)	3 (14.29)	$\chi^2 = 2.05;$	4 (57.14)	3 (37.50)	$\chi^2 = 0.75;$
	TC	27 (69.23)	16 (76.19)	$p = 0.358$	2 (28.57)	4 (50.00)	$p = 0.687$
	CC	9 (23.08)	2 (9.52)		1 (14.29)	1 (12.50)	
	T	33 (42.31)	22 (52.38)	$p_F = 0.339$	10 (71.43)	10 (62.50)	$p_F =$
	C	45 (57.69)	20 (47.62)		4 (28.57)	6 (37.50)	0.709

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance. * – statistically significant result.

3.3.9. Alleles Associations and Interrelationships in Children with COVID-19

Our research revealed that children noninfected by SARS-CoV-2 often are the carriers of two or three risk alleles ($p < 0.001$). However, 30% of COVID-19 patients are carriers of 7–8 risk alleles, which was not typical for healthy children ($p = 0.034$) (Table 7).

Table 7. Association of risk alleles in children with COVID-19 and healthy control.

Combination of risk alleles	COVID-19	Control	p	χ^2 , p
1 risk alleles	0	0	p < 0.001*	χ^2 = 24.40; p < 0.001*
2 risk alleles	0	2 (13.33)		
3 risk alleles	1 (1.67)	4 (26.67)		
4 risk alleles	10 (16.67)	3 (20.00)	p = 0.750	
5 risk alleles	14 (23.33)	2 (13.33)		
6 risk alleles	17 (28.33)	4 (26.67)		
7 risk alleles	14 (23.33)	0	p = 0.034*	
8 risk alleles	4 (6.67)	0		

Abbreviations: χ^2 – chi-squared test, p-value – level of its significance. * – statistically significant result.

Logistic regression model for COVID-19 prediction based on the child's sex and alleles was statistically significant ($\chi^2 = 45.96$; $p < 0.001^*$) (Table 8). The proposed model explained 41.7% (Nagelkerker R Square) of the variation of COVID-19 susceptibility and correctly predicted 85.3% of cases. Therefore, based on the model the key component of COVID-19 susceptibility in childhood are alleles of genes IFNAR2 rs2236757, OAS1 rs10774671, OAS3 rs10735079, CD40 rs4813003 and CASP3 rs113420705.

Table 8. Logistic regression analysis for COVID-19 susceptibility prediction in the pediatric population.

Variable	B	S.E.	Wald	df	p	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Constant	4.33	1.28	11.44	1	0.001*	75.77		
Sex Male/Female	1.01	0.53	3.63	1	0.057	2.73	0.97	7.70
ACE2 rs2074192 C/T	-1.05	0.54	3.73	1	0.053	0.35	0.12	1.02
IFNAR2 rs2236757 G/A	-1.19	0.58	4.15	1	0.042*	0.31	0.10	0.96
TYK2 rs2304256 C/A	-0.32	0.63	0.26	1	0.611	0.73	0.21	2.51
OAS1 rs10774671 G/A	-2.14	0.62	12.02	1	0.001*	0.12	0.04	0.40
OAS3 rs10735079 A/G	-1.26	0.63	4.00	1	0.046*	0.28	0.08	0.98
CD40 rs4813003 C/T	1.99	0.82	5.93	1	0.015*	7.30	1.47	36.14
FCGR2A rs1801274 A/G	-0.40	0.55	0.51	1	0.475	0.67	0.23	1.99
CASP3 rs113420705 T/C	-2.33	0.64	13.11	1	<0.001*	0.10	0.03	0.34

Abbreviations: B – predictor's logistic coefficient; S.E. – standard error for B; Wald – Wald chi-square value; df – degree of freedom; p – level of statistical significance; Exp(B) – odds ratio for indicators – sex "Male" and dominant gene's allele; 95% CI for Exp(B) – 95% confidence interval for Exp(B). * – statistically significant result.

Such indicators as male sex, ACE2 rs2074192 allele T, IFNAR2 rs2236757 allele A, OAS1 rs10774671 allele A, CD40 rs4813003 allele C and CASP3 rs113420705 allele C can predict severe COVID-19 course and MIS-C in the pediatric population in 85.6% of cases (Table 9). The proposed model is significant ($\chi^2 = 56.85$; $p < 0.001$) and demonstrates strong goodness of fit – Nagelkerke R square 0.65.

Table 9. Logistic regression analysis for severe COVID-19 course and MIS-C prediction in the pediatric population.

Variable	B	S.E.	Wald	df	p	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Constant	2.54	1.61	2.51	1	0.113	12.71		
Sex Male/Female	2.68	1.05	6.48	1	0.011*	14.59	1.85	114.80
ACE2 rs2074192 C/T	-2.08	0.86	5.82	1	0.016*	0.13	0.02	0.68
IFNAR2 rs2236757 G/A	-2.98	1.06	7.86	1	0.005*	0.05	0.01	0.41
TYK2 rs2304256 C/A	-0.65	0.93	0.49	1	0.486	0.52	0.08	3.24
OAS1 rs10774671 G/A	-2.47	0.98	6.33	1	0.012*	0.09	0.01	0.58
OAS3 rs10735079 A/G	-1.20	0.82	2.15	1	0.143	0.30	0.06	1.50
CD40 rs4813003	5.58	1.93	8.36	1	0.004*	264.57	6.03	11601.05

C/T								
FCGR2A rs1801274	-1.19	0.89	1.78	1	0.182	0.31	0.05	1.74
A/G								
CASP3 rs113420705	-3.72	1.11	11.31	1	0.001*	0.02	0.01	0.21
T/C								

Abbreviations: B – predictor's logistic coefficient; S.E. – standard error for B; Wald – Wald chi-square value; df – degree of freedom; p – level of statistical significance; Exp(B) – odds ratio for indicators – sex "Male" and dominant gene's allele; 95% CI for Exp(B) – 95% confidence interval for Exp(B). * – statistically significant result.

4. Discussion

4.1. Genes Interactions

Conducted research suggests the importance of host genetic factors in the antiviral immunity against SARS-CoV-2 in children. For sure, genetic patterns are stable during whole life; despite this, influence of SNPs on COVID-19 severity was studied first. We focused on the key genes, which play the most significant role in the immune response in COVID-19 and we studied genes related to Kawasaki disease. Network data from GeneMANIA [37] confirmed the interrelationship between studied genes (Figure 2). It should be emphasized that in immune protection many genes are involved. With the exception of eight studied genes (ACE2, IFNAR2, TYK2, OAS1, OAS3, CD40, CASP3, FCGR2A), we also must be focused on the next genes: angiotensin I converting enzyme (ACE), angiotensinogen (AGT), collectrin, amino acid transport regulator (CLTRN), ghrelin and obestatin prepropeptide (GHRL), 2'-5'-oligoadenylate synthetase 2 (OAS2), 2'-5'-oligoadenylate synthetase like (OASL), poly(A) polymerase beta (PAPOLB), poly(A) polymerase gamma (PAPOLG), terminal nucleotidyltransferase 4B (TENT4B), TNF receptor associated factor 2 (TRAF2), TNF receptor associated factor 3 (TRAF3), CASP8 and FADD like apoptosis regulator (CFLAR), thioredoxin (TXN), neogenin 1 (NEO1), glycoprotein VI platelet (GP6), NADH: ubiquinone oxidoreductase core subunit S1 (NDUFS1), Janus kinase 1 (JAK1), Janus kinase and microtubule interacting protein 1 (JAKM1P1), receptor for activated C kinase 1 (RACK1), interleukin 12 receptor subunit beta 1 (IL12RB1) (Figure 2).

Physical interactions between genes in the proposed network model were registered in 35.92% of cases, co-expression in 30.46%, shared protein domains in 17.70%, co-localization in 4.55% and predicted functional relationships between genes in 11.37% of cases (Table 10).

Table 10. Gene network interactions.

Co-expression	Physical interactions	Shared protein domains	Co-localization	Predicted functional relationships
ACE2- ACE	ACE2 – ACE	ACE2 – ACE	ACE – CFLAR	ACE2 – AGT
ACE2 – CLTRN	ACE2 – AGT	ACE2 – CLTRN	IFNAR2 – IL12RB1	ACE2 – GHRL
IFNAR2 – TRAF3	AGT – ACE	IFNAR2 – IL12RB1	IFNAR2 – RACK1	IFNAR2 – JAK1
OAS1 – OAS3	IFNAR2 – TYK2	TYK2 – JAK1	IFNAR2 – TRAF2	IFNAR2 – TYK2
OAS1 – OAS2	IFNAR2 – JAK1	OAS1 – OAS3	OAS1 – OAS3	TYK2 – JAK1
OAS1 – OASL	IFNAR2 – RACK1	OAS1 – OAS2	OAS1 – OAS2	IFNAR2 – RACK1
OAS1 – FCGR2A	TYK2 – JAK1	OAS1 – OASL	OAS3 – OAS2	OAS1 – OASL
OAS2 – OAS3	TYK2 – RACK1	OAS2 – OASL	JAK1 – CFLAR	OAS3 – OAS2

OAS2 – OASL	TYK2 – IL12RB1	OAS3 – OASL	OAS2 – OASL
OAS2 – IFNAR2	TYK2 – JAKMIP1	OAS3 – OAS2	FCGR2A – GP6
OAS2 – NEO1	JAKMIP1 – JAK1	OAS1 – TENT4B	CD40 – TRAF2
OAS2 – FCGR2A	RACK1 – JAK1	OAS2 – TENT4B	CD40 – TXN
FCGR2A – CFLAR	CD40 – TRAF2	OAS3 – TENT4B	CASP3 – TRAF3
FCGR2A – CD40	CD40 – TRAF3	OAS1 – PAPOLG	TRAF3 – CFLAR
CD40 – JAK1	CASP3 – NDUFS1	OAS2 – PAPOLG	
CD40 – TXN	CASP3 – NEO1	OAS3 – PAPOLG	
CD40 – OAS1	CASP3 – TXN	OAS1 – PAPOLB	
CD40 – OAS3	CASP3 – CFLAR	OAS2 – PAPOLB	
CD40 – CFLAR	CFLAR – TRAF2	OAS3 – PAPOLB	
CFLAR – JAK1		PAPOLG – TENT4B	
CFLAR – TXN		PAPOLB – TENT4B	
CFLAR – TRAF2		PAPOLB – PAPOLG	
		FCGR2A – GP6	
		CASP3 – CFLAR	
		TRAF2 – TRAF3	

Our study results confirmed genes' interrelationship in order to predict course of SARS-CoV-2 infection. Regression analysis showed that COVID-19 susceptibility in children increases in cases of interactions of IFNAR2 rs2236757, OAS1 rs10774671, OAS3 rs10735079, CD40 rs4813003 and CASP3 rs113420705. While crucial patterns for development of severe COVID-19 or MIS-C are sex (male gender), ACE2 rs2074192, IFNAR2 rs2236757, OAS1 rs10774671, CD40 rs4813003 and CASP3 rs113420705.

More often pediatric patients with COVID-19 were the carriers of a combination of seven or eight risk alleles. Noninfected children were more likely to be the carriers of three or less risk alleles compared to patients with COVID-19 of different severity.

Importantly, our study has demonstrated the significant prevalence of risk alleles among pediatric COVID-19 group compared to relevant data in European population. These frequency differences were revealed for four studied genes — ACE2 rs2074192 allele T (54% vs. 45%), IFNAR2 rs2236757 allele A (52% vs. 29%), CD40 rs4813003 allele C (94% vs. 86%) and CASP3 rs113420705 allele C (54% vs. 28%). This finding is of great importance for confirmation of genetic susceptibility to SARS-CoV-2 infection.

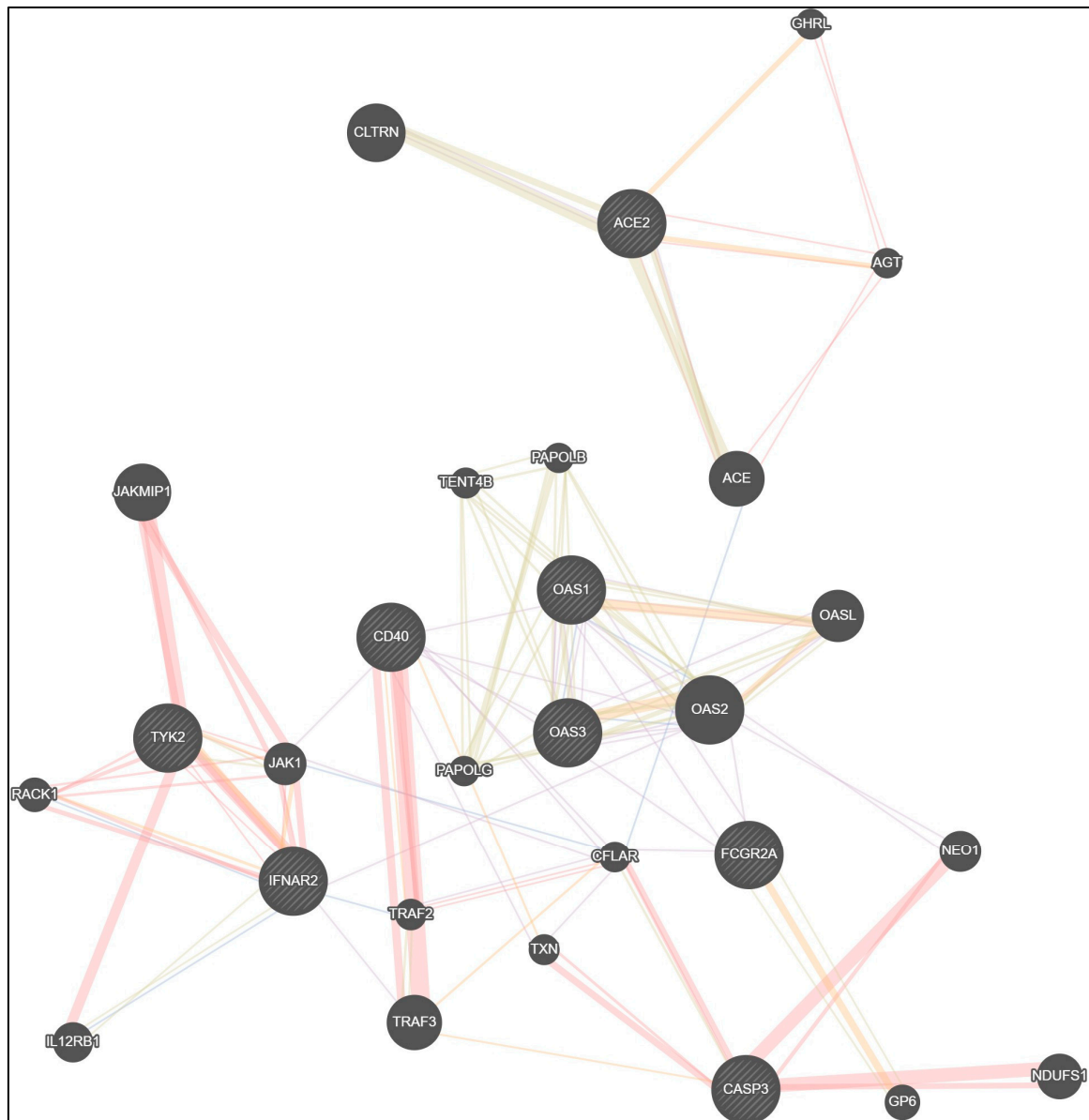


Figure 2. Network data for gene interactions between studied genes ACE2, IFNAR2, TYK2, OAS1, OAS3, CD40, CASP3, FCGR2A and other genes generated by GeneMANIA.

4.2. ACE2 rs2074192

Gene ACE2 encodes cognominal type 1 membrane protein ACE2 [8]. ACE2 gene is located on the X chromosome (Xp22.2), locus NG_012575 [39,40]. Numerous studies suggest that single-nucleotide polymorphisms (SNPs) in the gene ACE2 are related to the expression of ACE2 and the following binding affinity of SARS-CoV-2 [41,42]. Virus binding to the transmembrane ACE2 protein leads to the interruption of the whole ACE2/Ang1-7/MasR axis and impairs ACE2 enzyme activity. In such conditions, upregulation of ACE/AngII/AT1R is noticed, which leads to the vasoconstriction and activation of pro-inflammatory state [9].

Our research revealed sex differences in risk allele T frequency — boys are more often the carriers of it; and therefore, are more susceptible to SARS-CoV-2 infection. Such sex differences can be explained by the location of ACE2 rs2074192 on the X chromosome. The study suggests that such Xp22 gene location encompasses an area, which is not under the physiological X-inactivation [8]. X-inactivation normally happens in females to ensure population basis gene distribution. Escaping from X-inactivation leads to different phenotypic patterns and sex tissue-specific differences [8].

Allele T of ACE2 rs2074192 shows higher frequency in symptomatic COVID-19 patients compared with the asymptomatic group and it is more often associated with severe outcomes [43]. Previous meta-analyses done by K. Gupta et al. demonstrated genotype contrast between TT vs. CT genotype ACE2 rs2074192 in severe COVID-19 prediction in adult population, but no allele differences [44]. Our research received similar results — genotype differences between studied groups with TT genotype prevalence in children with severe COVID-19/MIS-C. However, our study also demonstrated allele differences and risk allele T influence on the SARS-CoV-2 infection course in children. While in L. E. Martinez-Gomez et al. study codominant, dominant and recessive model study did not reveal significant differences between severe and critical COVID-19 patients [45]. Research in Spain demonstrates protective effect of ACE2 rs2074192 regarding female hospitalization during COVID-19 [40].

Therefore, data regarding ACE2 rs2074192 impact on SARS-CoV-2 are still controversial and future studies must be carried out.

4.3. IFNAR2 rs2236757

SARS-CoV-2 virus has a direct stimulatory effect on interferon stimulated genes (ISG), and also causes the activation of immune cells. ISGs cause an increase in the expression of pro-inflammatory genes. Type I interferon response is among key pathogenetic basis for antiviral host defense. The course of COVID-19 is characterized by reduced production of IFN-I in the early stages of the disease, which in turn causes excessive viremia, which leads to an increase in the virulence of the pathogen. Increased viral replication is a trigger factor for excessive production of pro-inflammatory cytokines. At the same time, studies emphasized the role of the interferon system in inducing a “cytokine storm” by activating the synthesis of TNF/IL-1 β [46]. Hadjadj et al. demonstrated that patients with severe COVID-19 have downregulation of IFN-stimulated genes (MX1, IFITM1 and IFIT2) compared to patients with mild and moderate disease severity [47–49]. Research suggested that low type I IFN response manifests with worsening of clinical condition up to critical state [17,47]. At the same time, endogenous or exogenous steroids can suppress IFN signaling that manifest in impaired antiviral immune response and increasing disease severity. It is crucial for understanding SARS-CoV-2 course, because glucocorticoids are used in the medical management of MIS-C, acute respiratory distress syndrome and sepsis [50]. Therefore, INF pathway and its genetic control should be assessed in pediatric population.

Intron variant IFNAR2 rs2236757 is located on chromosome 21q22.1 (chr21:34,624,917) [12]. Fricke-Galindo et al. showed association of IFNAR2 rs2236757 with disease severity and mortality risk in adults with COVID-19 [13]. Study of genetic mechanisms of critical COVID-19 also underlined that low expression of IFNAR2 is associated with life-threatening disease, while its high expression has reduced odds of severe COVID-19 [12]. Association of AA genotype rs2236757 with intensive care admission was shown in adult patients infected by SARS-CoV-2 [16,17].

Despite the fact that mortality rate and intensive care unit admission were not in the focus of our research, our findings in pediatric population were in line with public data regarding disease severity — allele A increases the risk of severe COVID-19 and MIS-C in single-gene study and in cases of multigene interaction.

4.4. TYK2 rs2304256

Tyrosine kinase 2 (TYK2) is a protein functioning as Janus kinase/signal transducer and activator of transcription (Jak-STAT) pathways [14]. The Jak-STAT pathway has an important role in the intracellular cell conduction of signals as well as in cytokine release and response [14]. The role of TYK2 in the immune response regulation is confirmed by the experiments, which prove that TYK2 deficiency is associated with an imbalance in the IL-12 and IFN- α/β system [15]. TYK2 activation is carried out by IFN- α , which leads to the phosphorylation of STAT1 and STAT2 and the subsequent dimerization of activated STATs. Subsequent translocation of dimerized STATs in the cell nucleus leads to induction of interferon-stimulated gene expression and activation of antiviral defense [15,51]. TYK2 also stimulates nontraditional pathways of antiviral protection, such as NF- κ B signaling and

the mitogen-activated protein kinase pathway [51]. The role of TYK2 in macrophage activation is also important. At the same time, TYK2 regulates the activity and function of T-helpers of type 1 and T-helpers of type 17 through a functional connection with IL-12 and IL-23 [15,52]. The differentiation of CD4⁺ T cells, activated by the interaction of IL-23 with TYK2/Jak2, occurs with the participation of IL-6, IL-1 β and TGF- β , which is extremely important in the context of the cytokine storm of COVID-19 [52]. Research showed that the pathogenetic connection of IL-23 with TYK2 also supports the subsequent release of pro-inflammatory cytokines as IL-17 and IL-22. In turn, IL-17 stimulates neutrophils and keratinocytes with the subsequent activation of dendritic cells and release of IL-23, which forms a “closed circle” in the inflammatory process that has arisen [52]. Based on current studies, TYK2 is of great importance in the context of antiviral protection and cytokine releasing.

TYK2 (rs2304256) is a gene that encodes a non-receptor tyrosine-protein kinase; it is a nonsynonymous variant that is located on chromosome 19 in exon 8 (chr19:10,350,533-10,380,608) [53,54]. It is suggested that TYK2 gene is associated with susceptibility to inflammatory and autoimmune disorders [53]. In European population the protective role of minor allele A was demonstrated in systemic lupus erythematosus, type 1 diabetes, psoriasis and idiopathic inflammatory myopathies [53]. Association with autoimmune diseases can be explained by rs2304256-related modification of expression of less common disease variants rs34536443 (P1104A) and rs12720356 (I684S) [53].

Our research did not find any differences in genotype or allele frequencies in TYK2 rs2304256 depending on COVID-19 severity. It can be explained by previously reported absence of altered TYK2 function after acid substitution caused by rs2304256 [53]. Therefore, based on our study TYK2 did not impact COVID-19 outcome or the autoimmune regulatory mechanism that can be defined in MIS-C, but studies are ongoing and other regulatory pathways could be found.

4.5. *OAS1 rs10774671 and OAS3 rs10735079*

OAS gene family are closely related with interferon stimulated genes (ISG) [46]. OAS genes are located on chromosome 12 (12q24.13 region) and encode the synthesis of oligoadenylate OAS1, OAS2 and OAS3 [19].

Activated by viral RNA (mainly double-stranded) oligoadenylate catalyzes ATP polymerization and activation of ribonuclease latent (RNase L) [55]. Direct RNase L action leads to viral RNA destruction [19,56]. OAS–RNase L cleaves viral messenger-RNA and as the result, next viral replication is impossible [55]. RNase L activation leads to the ribosomal and mitochondrial RNA degradation and next apoptosis [57]. It should be emphasized that expression of oligoadenylate synthetases (OAS) is induced by IFN [57]. In turn, OAS–RNase L leads to the amplification of IFN signaling, which again confirms OAS–IFN interaction [57].

Last studies suggest that single-gene inborn errors of the OAS–RNase L leads to the uncontrolled production of pro-inflammatory cytokines by mononuclear phagocytes, which can predispose MIS-C development [58]. Lee et al. revealed that approximately 1% of patient with MIS-C had autosomal recessive deficiencies of OAS1, OAS2 or RNase L [58].

Results of our study demonstrated protective effect of GG genotype OAS1 (rs10774671) in dominant inheritance model regarding severe SARS-CoV-2 (OR = 0.18; $p < 0.05$), while carriers of allele A have higher risk for worse COVID-19 outcome (OR = 5.71; $p < 0.05$). Influence of allele A OAS1 rs10774671 in COVID-19 susceptibility and its severe course were confirmed by two logistic models. Our data corresponds to the previously presented data in adult population where involvement of rs10774671 in SARS-CoV-2 pathogenesis was demonstrated [55]. Presence of allele A is associated with two mRNA variants — p48 and p52 with low OAS activity. While allele G leads to the production of p46 form with high OAS activity [55].

Studies show that the presence of the risk allele G rs10735079 gene leads to decrease in the OAS1 level, which proves the existence of a negative inverse relationship between the severity of the course of COVID-19 and the level of OAS1 [21]. The level of OAS1 is directly related to the hospitalization frequency in cases of diagnosed pneumonia [5,21]. However, the role of oligoadenylate synthetases in the elimination of single-stranded viral RNAs remains debatable and requires further study. Still

now, current results regarding OAS3 rs10735079 are controversial. Horowitz et al. have performed comparisons between COVID-19 positive patients vs. COVID-19 negative or unknown and reported that allele G was the effecting allele in COVID-19 pathogenesis [59]. At the same time, Pairo-Castineira et al. as well as Pietro et al. presented the allele A rs10735079 influence on COVID-19 manifestations in adults and in children [5,12].

Our study did not reveal any differences in the genetic models between OAS3 rs10735079 studied groups. However, in case of gene interaction, significant impact of OAS3 rs10735079 was shown on COVID-19 susceptibility, but it was not involved in the prediction of MIS-C or severe COVID-19 development. Importantly, that minor allele G frequency in our research was significantly lower in healthy children compared to the European population. This diversity can be explained by small group sample in control group ($n = 15$). Therefore, next comparisons between allele's frequencies in infected and noninfected groups were difficult.

4.6. CD40 rs4813003

CD40 is a 48-kDa type I transmembrane protein and belongs to the tumor necrosis factor (TNF) receptor (TNFR) family [26]. It was revealed that cytokine production is stimulated by the CD40 engagement on the surface of dendritic cells [26]. Cell immunity is also mediated through CD40 signaling and as a result, T-cell activation and differentiation are achieved [26]. It is reported that CD40-CD40L interaction leads to the development of CD8+ cytotoxic T lymphocytes, which is crucial in antiviral and anticancer immunity [60]. CD40L is a natural ligand for CD40 also known as CD154 and it is a type II, 39-kDa transmembrane glycoprotein [60,61]. At the same time, CD40 is involved in the regulation of the humoral immune response. B-cell activation by CD40 stimulates immunoglobulin (Ig) isotype switching and Ig somatic hypermutation [26]. As a result, the affinity of Ig to antigen is increased. CD40-CD40L pathway is known as the regulator of the production of IL-10 and IL-12 by monocytes and macrophages [61]. Notwithstanding this, CD40-CD40L signaling is related to the formation of memory B cells and long-lived plasma cells and their survival [26,60]. Also, CD40 has an important role in maintaining the life cycle of B cells, dendritic cells and endothelial cells [26]. Therefore, dysregulation of CD40 is associated with impaired immune response and autoimmune processes. Based on the current knowledge, autoimmunity can be caused by impaired CD40 expression or its abnormal function and as a result the expansion of autoreactive B cells instead of their elimination [61]. It is also shown that activation of the CD40-CD40L pathway is related to sepsis, cardiovascular disease, kidney graft rejection, inflammageing, cancer and cognitive decline [61].

CD40 molecule is encoded by intergenic variant rs4813003 and is located on chromosome 20 (20:46134645; cytogenetic region 20q13.12) [62]. Current research suggest rs4813003 TT genotype reduces the risk to have KD (OR = 0.64), while genotypes CC and CT increase it [28]. Studies confirmed the association of CD40 with KD is mainly related to the East Asian population where risk allele C increased the risk of KD by 1.41 times [23,27,63]. In our research, CD40 rs4813003 genotype frequencies did not differ between study groups and control, while allele C was seen significantly more often among patients with COVID-19 compared with control ($p < 0.05$). Our study results also demonstrate significant impact of allele C on severe COVID-19 course or MIS-C in both single allele analysis (OR = 4.75; $p = 0.037$) and in case of allele interactions (OR = 264.57; $p = 0.004$).

Risk allele C increases not only CD40 function but also increases expression of its ligand CD40L [23,26]. Soluble CD40L is associated with vasculitis and vascular remodeling [64]. Most of the soluble sCD40L is of platelet origin, which indicates the activation of platelets in the lungs' microcirculation during SARS-CoV-2 infection. sCD40L is able to locally activate endothelial cells, pericytes and smooth muscle cells, stimulate the expression of FGF-2 and, as a result the vascular wall remodeling happened [64]. In this regard, the study of the CD40 rs4813003 polymorphism is important in COVID-19 and MIS-C because cardiovascular system involvement is typical for both of them, especially MIS-C.

4.7. FCGR2A rs1801274

Missense variant rs1801274 encodes transmembrane glycoprotein Fc gamma (γ) receptor IIa and is located on chromosome 1 (1:161505430-161524013, cytogenetic region 1q23.3) in the EC2 domain [62,65,66].

Fc γ receptor (Fc γ R) presents on the immune cells and can bind with specific antibodies. Complex Fc γ R and Ig G stimulate the release of cytokines, the production of reactive oxygen species as well as antibody-dependent cellular cytotoxicity and phagocytosis [65,66]. Based on the affinity to different Ig G subtypes, Fc γ R is divided into three types: FCGR1, FCGR2 and FCGR3. FCGR2 has the lowest affinity to Ig G. Three different subtypes of FCGR2 were revealed — A, B and C. FCGR2 is presented on a variety of immune competent cells, such as natural killers, macrophages and neutrophils [65]. Variable response to infection depends on the Ig F binding affinity. Polymorphism in the rs1801274 variant leads to the formation of two different isoforms of FCGR2 — Fc γ RIIA-Arg and Fc γ RIIA- His [66]. These two forms can be made via the substitution of histidine for arginine at position 131 of the FCGR2A protein [67]. Fc γ RIIA- His have a higher affinity to Ig G1 and Ig G2 compared to Fc γ RIIA-Arg, while the binding capacity of Ig G3 and Ig G4 is almost the same in both isoforms [66]. Therefore, any deviations in FCGR2A expression can lead to the implicated Ig G binding affinity and, consequently, to immune dysregulation. It is suggested that FCGR2A is responsible for the modulation of severity of infection and autoimmune response, it is not only the marker of KD [66]. It was also proposed to use genetic studies of FCGR in disease management, especially in the case of the prescription of therapeutic antibodies [66].

Study results showed that risk allele G of gene FCGR2A rs1801274 increase the risk of KD either under the homozygous (AA) or heterozygous (AG) model in Asian and Caucasian populations, while allele A has a protective effect [65,68]. At the same time, controversial results were demonstrated by Zhang et al. that the estimation of the association between the FCGR2A rs1801274 polymorphism in Asian and Caucasian populations revealed that risk allele A is related to KD (OR = 1.41; $p < 0.001$) [69]. Research of adults of European ancestry demonstrated that allele G carriers (Fc γ RIIA-Arg) are more prone to have lethal outcome from COVID-19 compared to homozygous AA (OR = 2.2; $p = 0.01$) [66]. Greek colleagues (Chatzikiyriakidou et al.) did not reveal any genotype or allele differences between groups that suffered from KD and healthy control [30]. Cardiovascular events were also not related to rs1801274 polymorphism [30]. Analysis of the previous thirteen case-control studies did not reveal a significant association between FCGR2A rs1801274 and overall pneumonia risk [67].

Our results correspond to European studies. The absence of difference between the control group and the COVID-19 group, as well as the absence of difference between COVID-19 patients and European FCGR2A allele frequencies, demonstrate that this gene is not related to disease severity.

4.8. CASP3 rs113420705

Caspase 3 (CASP3) is a protein in the cysteine-aspartic acid protease (Caspase) family [66]. CASP3 with caspase-2, -6, -7, -8, -9 belongs to the apoptotic caspases [70]. Apoptosis is a well-known mechanism of programmed cell death [70]. Apoptosis is crucial in the viral life cycle; both RNA and DNA viruses cause apoptosis in infected cells to maintain their replication [70]. Pathogen-induced apoptosis may increase apoptosis and viral pathogenicity [71]. Simultaneously, host organisms through IFN-stimulated genes expression try to overcome viral-induced apoptosis as well as maintain antiviral immune defense [70]. An increase in CASP3 level is associated with increased apoptosis [70]. CASP3 could restrain IFN production. CASP3 deficiency is related to enhanced type I IFN secretion and stimulation of innate immune response and as a result, makes cells virally resistant [70]. CASP3 is also responsible for cell growth and differentiation as well as for cytokine expression [68,72].

The CASP3 is the gene located on chromosome 4 (4:184627696-184650062; cytogenetic region 4q35.1) that encodes protein caspase 3 [62]. Alteration in CASP3 expression leads to increased susceptibility to KD in European American and Asian populations [68]. A case-control study in North India demonstrates that persons with CT genotype rs113420705 of CASP3 are more prone to have KD and carriers of minor allele C most often have coronary artery aneurysm compared with T allele

carriers [73]. Our study showed significantly higher frequency of allele C rs113420705 in children with COVID-19 compared to European ancestry data.

CASP3 gene is also important for injury prevention caused by viral infection (SARS-CoV-2, hepatitis C virus, Enterovirus 71 infection, H-1 parvovirus) because CASP3 maintains cellular homeostasis and viability [72]. Therefore, it is suggested that caspase's level and its gene expression could predict viral disease severity. In addition, hematological and immunological parameters in COVID-19 can be associated with dysregulated caspase activation [69]. In COVID-19 patients, CASP3 activity is upregulated in red blood cells and platelets cell death can also be related to CASP3 [71].

Results of our study showed significant impact of CASP3 rs113420705 on the COVID-19 susceptibility and a worse outcome development in children.

5. Conclusions

SARS-CoV-2 infection in children has different severity and outcomes. The clinical course varies from asymptomatic, mild forms to severe COVID-19 symptoms, even MIS-C. Among the influencing factors, genetics is one of the most important. The allele T ACE2 rs2074192 (OR = 3.45; $p = 0.009$), allele A IFNAR2 rs2236757 (OR = 2.62; $p = 0.039$), allele A OAS1 rs10774671 (OR = 4.60; $p = 0.003$), allele C CD40 rs4813003 (OR = 4.75; $p = 0.037$) are associated with severe illness of COVID-19 and could advance to MIS-C. In the multifactorial model, it was shown that males in addition to ACE2, IFNAR2, OAS1, CD40 genes, as well as CASP3 rs113420705 gene (allele C) make the clinical outcome of COVID-19 worse. The carriers of pathogenic risk alleles are more prone to suffer from severe illness of COVID-19. These findings can help in the management of SARS-CoV-2 infections in children and in COVID-19 prevention.

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