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Влияние генетических вариантов GSTP1 и GPX4 и их межгенного взаимодействия на тяжесть течения COVID-19

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Введение. Коронавирусное заболевание 2019 (COVID-19) – высококонтагиозное заболевание, вызываемое коронавирусом SARS-CoV-2. Несколько факторов риска восприимчивости и тяжести COVID-19 связаны с окислительным стрессом. Целью настоящего исследования было изучение ассоциации однонуклеотидных полиморфизмов (SNP) генов глутатион S-трансферазы pi-1 (GSTP1) и глутатионпероксидазы 4 (GPx4) с тяжестью заболевания COVID-19.
Методы. Пациенты были разделены на две группы в зависимости от тяжести симптомов. Для генотипирования использовалась аллель-специфическая полимеразная цепная реакция в реальном времени (RT-PCR). Для исследования межгенных взаимодействий проводился MDR-анализ (multifactor dimensionality reduction).
Результаты. Показана значимая ассоциация *GPX4* rs713041 с тяжестью течения COVID-19 (p=0,035). У большинства носителей генотипа *GPX4* 718 (TT) было тяжелое течение COVID-19 (ОШ=3,50; 95% ДИ [1,18-10,35]). Трехлокусная модель взаимодействия SNP-SNP была статистически значимой (p = 0,0084, ОШ = 2,42; 95% ДИ [1,24–4,73]). Анализ неравновесия по сцеплению *GSTP1* rs1695 и rs1138272 показал высокую вероятность неравновесия по сцеплению между двумя сайтами (D' = 0,949).
Выводы. Насколько нам известно, это первое исследование ассоциаций и межгенных взаимодействий *GSTP1* rs1695, *GSTP1* rs1138272 и *GPX4* rs713041 с тяжестью симптомов COVID-19. Полученные результаты могут служить новым потенциальным фактором прогноза COVID-19.

Ключевые слова: COVID-19, окислительный стресс, однонуклеотидные полиморфизмы, *GPX4*, *GSTP1*.

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Effects of GSTP1 and GPX4 genetic variants and their gene-gene interaction on the severity of COVID-19

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Background. Coronavirus disease 2019 (COVID-19) is a highly contagious disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several risk factors of COVID-19 susceptibility and severity are associated with oxidative stress.
Aim: to investigate the association of single nucleotide polymorphisms (SNPs) of glutathione S-transferase pi-1 (*GSTP1*) and glutathione peroxidase 4 (*GPX4*) genes with the severity of COVID-19.
Methods. Study subjects were divided into two groups based on the severity of their symptoms. Allele-specific real time polymerase chain reaction (RT-PCR) was used for genotyping, and multifactor dimensionality reduction (MDR) analysis was performed to investigate the SNP-SNP interaction models.
Results. The results showed a significant association of *GPX4* rs713041 with the severity of COVID-19 (p=0.035). Most of *GPX4* 718TT carriers had a severe course of COVID-19 (OR=3.50; 95% CI [1.18-10.35]). The resulted three-locus SNP-SNP interaction model was

statistically significant (p=0.0084, OR = 2.42; 95% CI [1.24 - 4.73]). Linkage disequilibrium analysis of *GSTP1* SNPs rs1695 and rs1138272 showed a high possibility of linkage disequilibrium between the two sites (D' = 0.949).
Conclusions: To our knowledge, this is the first study to investigate the association of *GSTP1* rs1695, *GSTP1* rs1138272, and *GPX4* rs713041 and SNP-SNP interaction with the severity of COVID-19 symptoms. The obtained results may serve as a novel potential factor of COVID-19 prognosis.
Keywords: COVID-19, oxidative stress, single nucleotide polymorphisms, *GPX4*, *GSTP1*.

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1. Introduction

The novel coronavirus, named as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, 2019-nCoV), caused an atypical respiratory disease called Coronavirus disease 19 (COVID-19). It first occurred in Wuhan, China in December 2019, then spread rapidly to other areas to be later declared as a pandemic by the world health organization (WHO) [1]. As of 18 February 2024, about 774 million confirmed cases of COVID-19 globally were reported to WHO, including more than 7 million deaths.

Several risk factors for the developing and severity of COVID-19 have been identified, such as older age, male sex, ethnicity, and presence of underlying chronic diseases [2]. In addition to these factors, host genetic predisposition has been considered as a major risk factor for the severity and susceptibility of COVID-19. The association of single nucleotide polymorphisms (SNPs) of multiple candidate genes with the pathogenesis of the novel infection has gained a remarkable interest by scientists worldwide in order to better understand the possible reasons responsible for the differences in COVID-19 severity among individuals.

Oxidative stress - imbalance between the generation of reactive oxygen species (ROS) and endogenous mechanisms of detoxification, such as antioxidant enzymes [3]. Several risk factors of COVID-19 severe outcome are associated with the development of oxidative stress [4].

Glutathione (GSH) is an essential antioxidant, synthesized from cysteine, glycine, and glutamate amino acids [5]. GSH deficiency results in elevated oxidative stress due to compromised antioxidant defenses [6]. Previous studies indicate that GSH deficiency plays a crucial role in the pathogenesis of various diseases, including severe COVID-19, through oxidative stress and inflammation-related mechanisms [7]. Indeed, it has been noticed that all patients with severe COVID-19 disease and high mortality risk have low basal GSH levels [8, 9]. Glutathione peroxidases (GPx) are a family of eight different isoforms (1 through 8). All of them use GSH as a cofactor to reduce organic and inorganic peroxides to alcohols [10]. However, glutathione peroxidase 4 (GPx4) is the only member of the GPx family that protects against iron-dependent ferroptotic cell death [11]. *GPX4* gene is located on chromosome 19 (19p13.3) and expressed in most tissues. Its genetic variations could potentially alter GPX4 enzymatic function [12], therefore, could be associated with oxidative stress-related disorders. Wang and colleagues discovered that, of the numerous selenoproteins tested, SARS-CoV-2 significantly reduced the expression of *GPX4* by 69.4% [13].

Glutathione S- Transferases (GSTs) are multifunctional isoenzymes that detoxify xenobiotics and endogenous metabolites by catalyzing their conjugation with reduced glutathione (GSH) [14]. Several studies have reported their antioxidant and anti-inflammatory roles, in addition to the involvement in signaling pathways regulation [15]. Glutathione S-transferase pi-1 (GSTP1) is an important antioxidant enzyme that protects the cell by catalyzing the conjugation of GSH to reactive electrophiles produced by cytochrome P450 metabolism [16]. It is the most abundant protein subtype in GSTs [17]. The *GSTP1* gene is consisted of seven exons and located on chromosome 11 (11q13.2) [18]. Two common single nucleotide polymorphisms (SNPs) of *GSTP1* have been frequently studied. Those are rs1695 A/G and rs1138272 C/T in the fifth and sixth exons respectively [19].

The aim of the current study was to investigate the association and SNP-SNP interaction of *GPX4* rs713041 (718 C>T), *GSTP1* rs1695 (A>G) and *GSTP1* rs1138272 (C>T) with the severity of COVID-19. In addition, we

examined the linkage disequilibrium possibility, and haplotype association.

2. Methods

2.1 Patients

Enrollment of participants was after excluding all patients with comorbidities (such as diabetes and hypertension) and/or other risk factors (such as smoking) that could influence the severity of symptoms and therefore affect the validity of our results. Inclusion criteria: 1) a history of SARS-CoV-2 infection (confirmed by PCR); 2) the presence of IgG antibodies to the virus. Exclusion criteria: 1) absence of IgG antibodies to SARS-CoV-2; 2) presence of underlying diseases or risk factors that may affect the severity of COVID-19 disease. As a result, 169 COVID-19 patients were included in the study and divided according to the severity of their symptoms into 2 groups: 100 mild, and 69 severe cases. Age of participants was (18 – 70) years old, and the male/female ratio was almost the same for both groups. Blood samples were collected in "Nauka" medical center (Rostov-on-Don, Russian federation) in the period between 12.03.2021 and 08.07.2022. The study was conducted in the post-COVID period, starting from 2 months after recovery at least. The patients were classified into mild and severe groups by following the guidelines of WHO (https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2 ; 23 November 2021). The study was conducted with the approval of the Local Ethics Committee of the Academy of Biology and Biotechnology of the Southern Federal University, Rostov-on-Don, Russian Federation. All procedures performed in studies were in accordance with the ethical standards of the institutional research committee and with the Helsinki declaration (2013) and its later amendments or comparable ethical standards. Informed consents were obtained from all study participants.

2.2 DNA extraction and genotyping

Venous blood samples were collected from participants and stored at -20°C. Total genomic DNA extraction was by using the AmpliSens® isolation kit RIBO-prep (AmpliSens, FBSI Central Research Institute of Epidemiology, Rospotrebnadzor, Russia). Several databases (Google Scholar, NCBI-PubMed, CyberLeninka, and eLIBRARY) were used to select candidate genes for the study. The selection was based on their potential role in redox status. This was determined using available information on the function of each gene in the Genecards human gene database (https://www.genecards.org/). Next, genetic polymorphisms of the selected genes were selected based on their functional properties and the frequency of the mutant allele. Ensembl, SNPedia, and NCBI-SNP databases were used to obtain data on mutant allele frequencies of the studied SNPs. Genes for enzymes that use glutathione for detoxification of xenobiotics (*GSTP1*) and reduction of complex hydroperoxides (*GPX4*) were selected for the study. After that, we selected polymorphic variants of these genes, characterized in detail in the literature, which cause loss-of-function effects in relation to the activity or expression of these enzymes and, as a result, may affect the processes of redox homeostasis and biotransformation. Genotyping of SNPs *GSTP1* rs1695 (313 A>G), *GSTP1* rs1138272 (341 C>T), and *GPX4* rs713041 (718 C>T) was conducted using SNP commercial qPCR kits (Syntol, Moscow, Russian Federation) and performed using the TaqMan SNP Genotyping Assay in the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The cycling conditions were: predenaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 63°C for 40 seconds. QuantStudio™ Design & Analysis Software was used to identify individual genotypes by measuring the fluorescence yield and obtaining the allelic amplification plot.

2.3 Linkage disequilibrium

Since two of the studied polymorphisms, *GSTP1* rs1695 and *GSTP1* rs1138272, are closely located on the same chromosome, linkage disequilibrium (LD) was performed using SHEsis web-based platform (online version http://analysis.bio-x.cn/myAnalysis.php, accessed 12 Nov 2023) [20]. To evaluate the possibility of recombination, Lewontin (D′) coefficient was calculated, where D'= 1 indicates complete LD, thus no evidence for recombination between the two sites, and D'= 0 indicates no LD. In this study, D' > 0.9 was considered as a strong LD.

2.4 Haplotype analysis

For the analysis of haplotype association with COVID-19 severity risk, SNPStats web tool (https://www.snpstats.net/start.htm , accessed in 12 Nov 2023) [21]. The most common haplotype was selected as reference. OR and 95% CI were calculated to estimate the degree of association between haplotypes and the risk of severe COVID-19.

2.5 SNP-SNP interaction analysis

Multifactor Dimensionality Reduction (MDR) 3.0.2 software (Computational Genetics Laboratory, Institute for Quantitative Biomedical Sciences, Dartmouth, NH,

USA) was used to study gene-gene interactions and evaluate their relation to the risk of severe COVID-19 cases. To evaluate the predictive accuracy for each model, 10-fold cross-validation was applied. In addition, the association of pairwise genotypes combinations with COVID-19 severity was also investigated.

2.6 Statistical analysis

Data were analyzed using the IBM SPSS Statistics 27.0 (IBM, Armonk, NY). Student's t-test was used to compare different variables between studied groups. Continuous variables were expressed as mean (M) ± standard deviation (SD). Chi-square ($\chi 2$) analysis was performed to assess the differences in allelic variants distribution between studied groups ($p \leqslant 0.05$ was considered statistically significant). The Bonferroni correction was applied to all experimentally determined P values to eliminate statistical errors in multiple comparisons. Odds Ratios (OR), indicated with 95% confidence interval (CI), were calculated to evaluate the risk of severe COVID-19. Hardy-Weinberg Equilibrium (HWE) was tested by Fisher's exact test χ^2 analysis and calculated using an online HWE calculator for 2 alleles (https://www.had2know.org/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html, accessed in 19 Nov 2023).

3. Results

3.1 Study subject characteristics

Patients with severe symptoms were older (53.8 ± 10.3) than patients in the mild group (43.2 ± 13.6). However, no significant difference was noticed in the gender ratio between the studied groups. Women were more than men in both mild (66%) and severe (68.1%) groups. Ceruloplasmin levels and total peroxidase activity (TPA) were also measured for all participants, and the differences between groups were statistically insignificant (p=0.0812 and 0.4136 respectively). Furthermore, lung CT scan results showed that all mild group patients had CT-1 category (Pulmonary parenchymal involvement ⩽ 25%), while severe patients had CT-3 (50-75%), and CT-4 (⩾ 75%) categories.

3.2 Association of SNPs with COVID-19 severity

GSTP1 rs1695, *GSTP1* rs1138272, and *GPX4* rs713041 genotypes were detected and their genotype distributions were all consistent with the Hardy-Weinberg equilibrium (HWE) (p>0.05) in both mild (p=0.26, 0.72, and 0.051 respectively) and severe (p=0.95, 0.95, and 0.41 respectively) groups.

Most frequent genotypes of the three polymorphisms were considered reference groups for the association studies. *GSTP1* rs1695 and rs1138272 showed no significant differences between the mild and severe groups. However, there was a significant association of *GPX4* rs713041 with the severity of COVID-19 (p=0.035). The *GPX4* 718TT genotype was more frequent in severe cases (13%) than in mild cases (6%). This means that most of TT genotype carriers had a severe course of COVID-19 (OR=3.50; 95% CI [1.18-10.35]). Genotypes and alleles frequencies of *GSTP1* rs1695, *GSTP1* rs1138272, and *GPX4* rs713041 polymorphisms in both mild and severe COVID-19 groups are presented in **Table 1**.

3.3 Gene–gene interactions

MDR algorithm was used to perform SNP-SNP interaction analysis. A three-locus model of interaction between the studied polymorphisms was established. The resulted interaction model is statistically significant (p=0.0084, OR = 2.42; 95% CI [1.24 - 4.73]), with a training balanced accuracy of 59.9%, and cross-validation consistency of 10/10 (**table 2**).

Data analysis indicated that individuals with heterozygous *GSTP1* rs1695 AG, *GSTP1* rs1138272 CT and homozygous *GPX4* rs713041 TT genotypes have a significant 4-fold higher risk of severe COVID-19 outcome. Graphical representation is shown in **fig. 1**.

According to the Fruchterman-Rheingold graph (**fig. 2**), between *GPX4* rs713041 and *GSTP1* rs1138272 a level of redundancy (-0.32%) was noticed. On the other hand, the two studied *GSTP1* SNPs showed a strong synergistic interaction (1.45%). The independent effect of *GPX4* rs713041 was significantly higher than that of *GSTP1* rs1695 and rs1138272 (2.86%, 0.26% and 0.58%, respectively).

The analysis of pairwise genotypes combinations showed that carriers of *GSTP1* (313AG)* *GPX4* (718TT) have more than 10 times higher risk of a severe COVID-19 (p=0.008). The overall results of this analysis are presented in **table 3**.

3.4 LD and Haplotypes association with COVID-19 severity

SNPs rs1695, rs1138272 are closely located within the exon 5/6 region of the *GSTP1* gene (A313G and C341T, respectively). Therefore, linkage disequilibrium analysis was performed to assess the correlation of their genetic variants. LD coefficient D' > 0.9 was considered as an evidence of high LD possibility. In our analysis, D' = 0.949, which indicated a high possibility of linkage disequilibrium between the two sites.

Haplotype association analysis was conducted for the two targeted genetic variations rs1695 and rs1138272 of

GSTP1 gene. The haplotype with the highest frequency was considered as a reference. The performed analysis showed no significant association with the severity of COVID-19 (p=0.35). Results of haplotype association analysis are presented in **table 4**.

4. Discussion

Genome-wide association studies (GWAS) and comparative genome sequencing analyses have been carried out globally to uncover genetic variants linked to COVID-19 severity [22]. Oxidative stress has been suggested as a key player in the SARS-CoV-2 pathogenesis, by mediating various processes, including binding, viral replication, cytokine production, and inflammation [23]. Therefore, genetic variations of redox-related enzymes have been considered as potential study targets, to assess their association with the severity of COVID-19 infection. Based on the above mentioned importance of oxidative stress, we started investigating the role of antioxidant enzymes' genetic variants with

Таблица 1. Частоты аллелей и генотипов *GSTP1* rs1695, *GSTP1* rs1138272 и *GPX4* rs713041 в группах пациентов с легкой и тяжелой формами COVID-19.

Table 1. *GSTP1* rs1695, *GSTP1* rs1138272, and *GPX4* rs713041 in mild and severe COVID-19 groups

Genotype/Allele	mild (n)	severe (n)	P value	OR (95% CI)
GSTP1 rs1695 (313 A>G)				
AA	50 (50%)	34 (49.3%)		Reference
AG	38 (38%)	29 (42%)	0.74	1.12 (0.59-2.15)
GG	12 (12%)	6 (8.7%)		0.74 (0.25-2.15)
AG+GG	50 (50%)	35 (50.7%)	0.93	1.03 (0.56-1.90)
A	138 (69%)	97 (70.3%)	0.89	1.06 (0.66-1.70)
G	62 (31%)	41 (29.7%)		
GSTP1 rs1138272 (341 C>T)				
CC	84(84%)	53 (76.8%)		Reference
CT	15 (15%)	15 (21.7%)	0.51	1.58 (0.72-3.51)
TT	1 (1%)	1 (1.5%)		1.58 (0.10-25.88)
CT+TT	16 (16%)	16 (23.2%)	0.24	1.58 (0.73-3.44)
C	183 (91.5%)	121 (87.7%)	0.25	0.66 (0.32-1.34)
T	17 (8.5%)	17 (12.3%)		
GPX4 rs713041 (718 C>T)				
CC	42 (42%)	26 (37.7%)		Reference
CT	52 (52%)	30 (43.5%)	**0.035***	0.93 (0.48-1.81)
TT	6 (6%)	13 (18.8%)		**3.50 (1.18-10.35)**
CT+TT	58 (58%)	43 (62.3%)	0.57	1.20 (0.64-2.24)
C	136 (68%)	82 (59.4%)	0.10	1.45 (0.92-2.27)
T	64 (32%)	56 (40.6%)		

OR= Odds Ratio, CI= Confidence Interval, *p<0.05

Таблица 2. MDR-анализ межгенных взаимодействий

Table 2. MDR analysis of gene-gene interactions

Model	CV-Consistency	Accuracy	Sensitivity	Specificity	OR (95% CI)	P value
GSTP1 rs1695, rs1138272, and *GPX4* rs713041	10/10	59.9%	42%	77%	2.42 (1.24 - 4.73)	**0.0084***

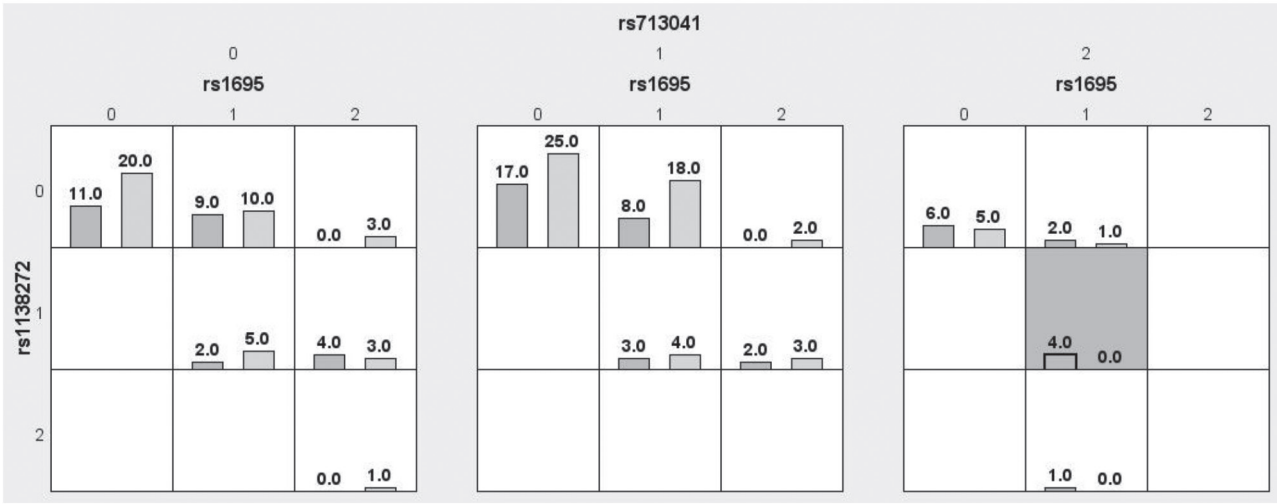

Рис. 1. MDR-анализ. Трехлокусная модель (*GSTP1* rs1695, rs1138272 и *GPX4* rs713041).

Примечание. Темным и светлым фоном обозначены комбинации высокого и низкого риска соответственно. Цифры обозначают генотипы: 0 – гомозиготный по аллелю дикого типа, 1 – гетерозиготный и 2 – гомозиготный по мутантному аллелю. В правых столбцах показаны значения в группе с легким течением COVID-19, в левых столбцах – в группе с тяжелым течением.

Fig. 1. Multifactor dimensionality reduction (MDR) analysis. The summary of the three-factor model (*GSTP1* rs1695, rs1138272 and *GPX4* rs713041).

Note. Dark and light backgrounds represent high-risk and low-risk combinations respectively. Numbers represent genotypes: 0 – homozygous for wild-type allele, 1- heterozygous, and 2- homozygous for mutant allele. Right columns are for mild COVID-19 cases, whereas left columns are for severe cases.

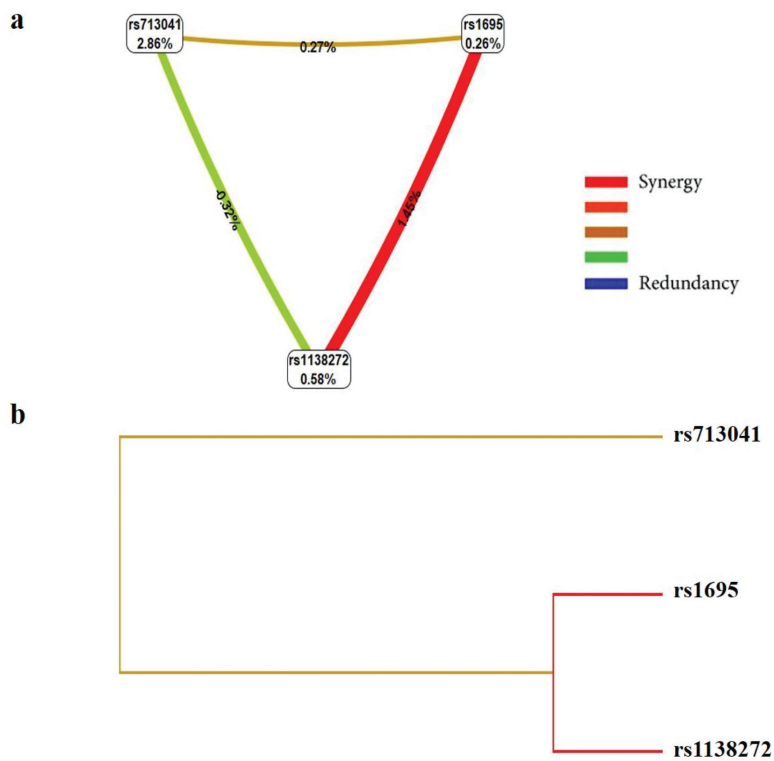

Рис. 2. MDR-анализ для модели взаимодействия SNP *GSTP1-GPX4*.

Примечание. (A) Круги взаимодействия с парными связями между SNP. Значение энтропии (%) в каждом поле указывает на независимый эффект изучаемого полиморфизма, а значения и цвета между узлами относятся к эффектам взаимодействия. Цвет линии указывает на тип взаимодействия. (B) Дендрограмма уровня взаимодействия между тремя оцененными SNP.

Fig. 2. Multifactor dimensionality reduction (MDR) for *GSTP1-GPX4* SNPs interactions model.

Note. (A) Interaction circle graph with pairwise connections among SNPs. Entropy value (%) in each box indicates the independent effect of studied polymorphism, while values and colors between nodes refer to the interaction effects. A synergistic effect is represented by positive values and redundancy by negative values. The color of the line indicates the type of interaction. (B) The dendrogram graph to show the level of interaction between the three evaluated SNPs.

the severity of symptoms in Rostov region population. Our previous study showed a significant association of paraoxonase 1 *PON1* rs662 (A575G) and nitric oxide synthase 3 *NOS3* rs2070744 (T786C) with COVID-19 severity [24].

Two regularly occurring polymorphisms within the *GSTP1* gene's exon 5/6 region, rs1695 (A313G, IIe105Val) and rs1138272 (C341T, Ala114Val), have been linked to the emergence and progression of certain disorders [25]. These polymorphic variants are associated with alterations in the catalytic and regulatory roles of GSTP1, and therefore may have clinical significance regarding disease susceptibility and response to oxidative stress [26]. For example, *GSTP1* rs1695 polymorphism is associated with the risk of Alzheimer's disease [27], esophageal cancer [28], malignant melanoma [29] and pregnancy loss [30]. The *GSTP1* rs1138272 polymorphism has been also linked to the risk of various cancers, such as non-small cell lung cancer [31] and prostate cancer [26].

The *GPX4* rs713041 polymorphism is located at position 718 (C718T) in the 3'UTR region, which is a regulatory region needed for incorporation of selenium into selenoproteins [32]. This genetic variation has been associated with several cancer types, including colorectal cancer [33], breast cancer [34], lung and laryngeal cancer [35]. More-

Таблица 3. Ассоциация парных комбинаций генотипов с тяжестью заболевания COVID-19

Table 3. The association of pairwise genotypes combinations with COVID-19 severity

Genotypes combinations	Mild (n=100) (%)	Severe (n=69) (%)	P value	OR (95% CI)
GSTP1(313AA)**GSTP1*(341CC)	50 (50)	34 (49.28)	0.99	0.97 (0.53-1.75)
GSTP1(313AG)**GSTP1*(341CC)	29 (29)	19 (27.50)	0.86	0.93 (0.46-1.78)
GSTP1(313AG)**GSTP1*(341CT)	9 (9)	9 (13.4)	0.45	1.51 (0.60-3.79)
GSTP1(313GG)**GSTP1*(341CT)	6 (6)	6 (8.70)	0.55	1.49 (0.44-5.03)
GSTP1(313AA)**GPX4*(718CC)	20 (20)	11 (15.94)	0.54	0.75 (0.35-1.70)
GSTP1(313AA)**GPX4*(718CT)	25 (25)	17 (24.64)	0.99	0.98 (0.49-1.99)
GSTP1(313AA)**GPX4*(718TT)	5 (5)	6 (8.70)	0.35	1.81 (0.50-5.48)
GSTP1(313AG)**GPX4*(718CC)	15 (15)	11 (15.94)	0.99	1.07 (0.48-2.46)
GSTP1(313AG)**GPX4*(718CT)	22 (22)	11 (15.94)	0.43	0.67 (0.31-1.47)
***GSTP1*(313AG)**GPX4*(718TT)**	**1 (1)**	**7 (10.14)**	**0.008***	**11.18 (1.86-127.0)**
GSTP1(313GG)**GPX4*(718CC)	7 (7)	4 (5.80)	0.99	0.81 (0.25-2.72)
GSTP1(313GG)**GPX4*(718CT)	5 (5)	2 (2.90)	0.70	0.56 (0.11-2.77)
GSTP1(341CC)**GPX4*(718CC)	33 (33)	20 (28.99)	0.61	0.82 (0.42-1.63)
GSTP1(341CC)**GPX4*(718CT)	45 (45)	25 (36.23)	0.27	0.69 (0.36-1.27)
GSTP1(341CC)**GPX4*(718TT)	6 (6)	8 (11.59)	0.25	2.05 (0.65-6.33)
GSTP1(341CT)**GPX4*(718CC)	8 (8)	6 (8.70)	0.99	1.09 (0.35-3.44)
GSTP1(341CT)**GPX4*(718CT)	7 (7)	5 (7.25)	0.99	1.03 (0.35-3.51)

*Pairwise genotype combination significantly associated with COVID-19 severity is shown in **bold**.

Таблица 4. Анализ ассоциации гаплотипов SNP *GSTP1* rs1695 (313 A>G) и rs1138272 (341 C>T)

Table 4. Haplotype association analysis of *GSTP1* SNPs rs1695 (313 A>G) and rs1138272 (341 C>T)

Haplotype (Alleles)	Frequencies			P value	OR (95%CI)
	Total	Mild	Severe		
GSTP1 313A * *GSTP1* 341C	0.69	0.69	0.69	-	1
GSTP1 313G * *GSTP1* 341C	0.21	0.23	0.18	0.36	0.77 (0.43 – 1.36)
GSTP1 313G * *GSTP1* 341T	0.10	0.08	0.13	0.47	1.32 (0.62-2.80)

over, it has been linked to other diseases, such as autoimmune thyroid diseases [36], multiple sclerosis [37], obesity [38] and depression [39].

In this study, the associations of *GSTP1* rs1695 (A>G), rs1138272 (C>T) and *GPX4* rs713041 (718 C>T) SNPs with the severity of COVID-19 were investigated. There was a significant association of *GPX4* rs713041 with COVID-19 severity (p=0.035). Carriers of *GPX4* rs713041 TT genotype were shown to have a significantly higher risk of severe COVID-19 course. This result can be supported by the findings of previous studies regarding the effect of the targeted SNP on the regulation of GPx4 synthesis and function. For example, C allele of *GPX4* rs713041 was shown to have a protective role against oxidative DNA damage in *in vitro* studies [40]. Furthermore, *Méplan et al.* showed that when selenium intake falls, GPx4 concentrations in lymphocytes are better maintained in carriers of CC genotype than in carriers of TT genotype, suggesting that *GPX4* SNP rs713041 affects the concentration of lymphocyte GPx4 and other selenoproteins *in vivo*, thus may influence susceptibility to diseases [41].

The MDR analysis showed that the carriers of *GSTP1* rs1695 AG * *GSTP1* rs1138272 CT * *GPX4* rs713041 TT had a significantly higher risk of severe COVID-19 outcome. *GPX4* rs713041 had the highest independent effect, therefore the most significant association with the risk of COVID-19 severity, confirming the results of genotype association analysis presented in Table 1. These findings were further confirmed by the results of haplotype association analysis, which showed that the presence of mutant alleles of the targeted polymorphisms increases the risk of a severe COVID-19.

LD analysis showed an evidence of high linkage disequilibrium possibility between the two sites of *GSTP1* SNPs rs1695 (A313G) and rs1138272 (C341T). Although the *GSTP1* genetic variations in our study showed no significant association with COVID-19 severity, their suggested co-inheritance can be helpful for the further studies of their association with other diseases.

Limitations of the current study must be mentioned, such as small sample size, and the need for other subsequent analyses to support the findings. Therefore, further research, with a larger sample size and biochemical analyses of the studied enzymes, is required.

5. Conclusion

To our knowledge, this is the first study to investigate the effect of *GSTP1* rs1695, rs1138272, *GPX4* rs713041 and their SNP-SNP interaction with the severity of COVID-19 symptoms. The obtained results may serve as a novel potential factor of COVID-19 prognosis.

Data availability statement

All datasets generated for this study are included in the article.

Ethics Approval and informed consent:

The study was conducted with the approval of the Local Ethics Committee of the Academy of Biology and Biotechnology of the Southern Federal University, Rostov-on-Don, Russian Federation. Informed consents were obtained from all study participants.

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