



Article

A Comprehensive Genetic and Bioinformatic Analysis Provides Evidence for the Engagement of COVID-19 GWAS-Significant Loci in the Molecular Mechanisms of Coronary Artery Disease and Stroke

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Abstract: Cardiovascular diseases (CVDs) significantly exacerbate the severity and mortality of COVID-19. We aimed to investigate whether GWAS-significant SNPs correlate with CVDs in severe COVID-19 patients. DNA samples from 199 patients with severe COVID-19 hospitalized in intensive care units were genotyped using probe-based PCR for 10 GWAS SNPs previously implicated in severe COVID-19 outcomes. SNPs rs17713054 *SLC6A20-LZTFL1* (risk allele A, OR = 2.14, 95% CI 1.06–4.36, $p = 0.03$), rs12610495 *DPP9* (risk allele G, OR = 1.69, 95% CI 1.02–2.81, $p = 0.04$), and rs7949972 *ELF5* (risk allele T, OR = 2.57, 95% CI 1.43–4.61, $p = 0.0009$) were associated with increased risk of coronary artery disease (CAD). SNPs rs7949972 *ELF5* (OR = 2.67, 95% CI 1.38–5.19, $p = 0.003$) and rs61882275 *ELF5* (risk allele A, OR = 1.98, 95% CI 1.14–3.45, $p = 0.01$) were linked to a higher risk of cerebral stroke (CS). No associations were observed with AH. Bioinformatics analysis revealed the involvement of GWAS-significant loci in atherosclerosis, inflammation, oxidative stress, angiogenesis, and apoptosis, which provides evidence of their role in the molecular mechanisms of CVDs. This study provides novel insights into the associations between GWAS-identified SNPs and the risk of CAD and CS.

Keywords: GWAS; COVID-19; rs17713054; rs7949972; rs61882275; rs12610495; gene–gene interactions; gene–environmental interactions; coronary artery disease; stroke



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1. Introduction

The COVID-19 pandemic has had a profound impact worldwide, claiming over 7 million lives as of May 2024 (<https://data.who.int/dashboards/covid19/deaths?n=c> accessed on 20 May 2024). As the pandemic unfolds, extensive research efforts have been directed toward unraveling the factors that contribute to the severity, outcomes, and treatment of COVID-19 infections [1–3].

Genome-wide association studies (GWAS) have identified numerous causal genes associated with severe COVID-19, including *SLC6A20*, *LZTFL1*, *IFNAR2*, *DPP9*, *CCHCR1*, and *ELF5* [4–9]. Furthermore, while the disease manifests with mild symptoms in most individuals, severe COVID-19 disproportionately affects those with comorbidities such as cardiovascular diseases (CVDs), exacerbating symptoms and elevating the risk of adverse outcomes, including mortality [10–12].

Arterial hypertension (AH) and coronary artery disease (CAD) are among the most common CVDs and are known risk factors for poor prognosis in COVID-19 patients, contributing to higher mortality rates, severe disease progression, ICU (intensive care units) admission, and overall disease progression [13]. Additionally, cerebral stroke (CS) is a

significant CVD associated with COVID-19, as the infection can trigger hypercoagulation, leading to cerebral thrombosis and/or thromboembolism [14].

Observational studies have suggested that COVID-19, AH, CAD, and CS may share common genetic variants. Several GWAS have identified specific SNPs associated with COVID-19 susceptibility, including *ACE2* and *ABO* genes, which have also been implicated in these CVDs [15–18]. Despite the significant threats posed by the intersection of COVID-19 and CVDs to patient outcomes, the shared pathology between these conditions remains largely unexplored.

This study seeks to expand on these findings by examining whether SNPs previously identified as genetic risk factors for severe COVID-19 by GWAS are also associated with CVDs, including CAD, CS, and AH in patients with severe COVID-19. By focusing on these overlapping genetic risk factors, we aim to uncover shared genetic pathways that might contribute to the comorbidities of severe COVID-19 and CVDs, therefore providing insights into their shared pathology.

2. Materials and Methods

The study involved 199 hospitalized COVID-19 patients from Central Russia. The Ethical Review Committee of Kursk State Medical University approved the study protocol (protocol No. 1 from 11 January 2022), and all participants provided written informed consent. To be included in the study, participants had to self-declare Russian ancestry and be born in Central Russia.

An outline of the study design, along with the main methods and materials used in our study, are depicted in Figure 1.

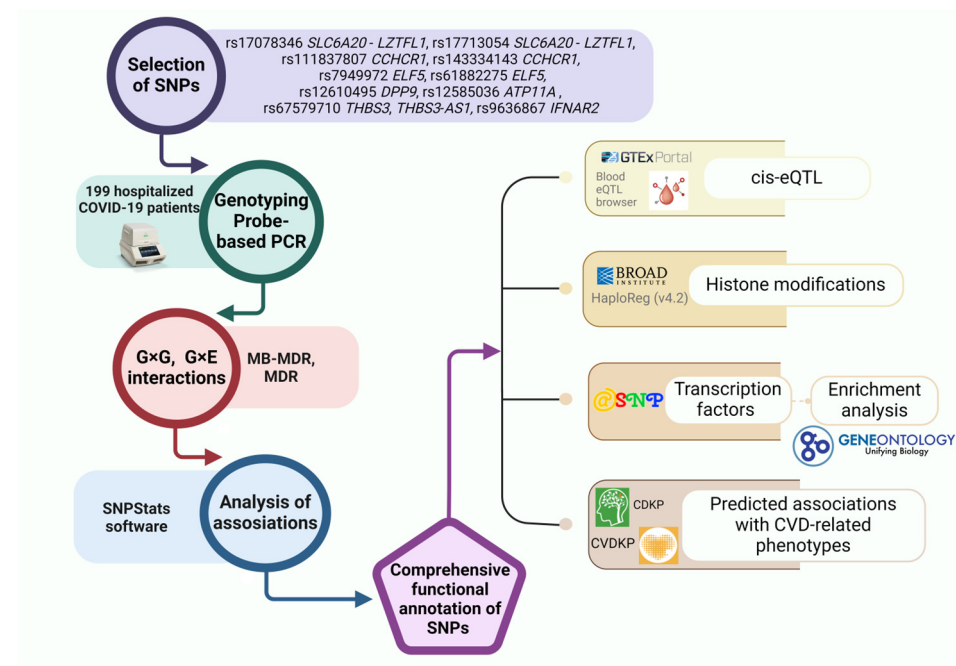


Figure 1. The outline of the study.

The patients were enrolled during the COVID-19 pandemic from 2020 to 2022 at the intensive care units (ICU) of Kursk Regional Hospital No. 6 and Kursk Regional Tuberculosis Dispensary [19]. All patients had a PCR-confirmed diagnosis of COVID-19. All patients with severe COVID-19 were divided into subgroups depending on the presence/absence of comorbid CVDs.

Table S1 presents the baseline and clinical characteristics of the studied groups, and Table 1 highlights the parameters with significant differences between studied groups.

Table 1. Baseline and clinical characteristics of the studied groups.

Baseline and Clinical Characteristics		COVID-19 Patients without CVD	COVID-19 Patients with CVD	<i>p</i> -Value
AH				
		n = 70	n = 129	
Age, Me [Q1; Q3]		56 [45; 70]	72 [65; 80]	<0.001
BMI, Me [Q1; Q3]		24.9 [22.5; 30]	31.6 [27.4; 36.5]	<0.001
Low physical activity	Yes, N (%)	23 (32.9%)	87 (67.4%)	<0.001
	No, N (%)	47 (67.1%)	42 (32.6%)	
CAD				
		n = 115	n = 84	
Age, Me [Q1; Q3]		61 [49; 71]	76.5 [67.5; 83]	<0.001
Low physical activity	Yes, N (%)	49 (42.6%)	61 (72.6%)	<0.001
	No, N (%)	66 (57.4%)	23 (27.4%)	
Cerebral stroke				
		n = 167	n = 32	
Age, Me [Q1; Q3]		67 [55; 77]	75 [66.5; 83.5]	<0.001
Low physical activity	Yes, N (%)	86 (51.5%)	24 (75%)	<0.05
	No, N (%)	81 (48.5%)	8 (25%)	

Statistically significant differences are marked in bold.

2.1. Selection of Genes and Polymorphisms

To select genes and polymorphisms for our study, we utilized data from the largest GWAS meta-analysis of severe COVID-19, published in 2023 [4]. Loci were chosen based on a significance threshold of $p \leq 1 \times 10^{-19}$, as established in this meta-analysis. We excluded SNPs with a minor allele frequency (MAF) of less than 0.05 and loci where probe development for genotyping using the TaqMan-based PCR method was not feasible due to methodological constraints (such as low CG composition, presence of GC clamps, and runs of identical nucleotides). After these exclusions, a total of 10 SNPs were included for genotyping: rs143334143 *CCHCR1*, rs111837807 *CCHCR1*, rs17078346 *SLC6A20-LZTFL1*, rs17713054 *SLC6A20-LZTFL1*, rs7949972 *ELF5*, rs61882275 *ELF5*, rs12585036 *ATP11A*, rs67579710 *THBS3*, *THBS3-AS1*, rs12610495 *DPP9*, rs9636867 *IFNAR2*.

2.2. Genetic Analysis

The Laboratory of Genomic Research at the Research Institute for Genetic and Molecular Epidemiology of Kursk State Medical University (Kursk, Russia) performed genotyping. Up to 5 mL of venous blood from each participant was collected from a cubital vein, put into EDTA-coated tubes, and kept at -20°C until it was processed. Defrosted blood samples were used to extract genomic DNA using the standard methods of phenol/chloroform extraction and ethanol precipitation. The purity, quality, and concentration of the extracted DNA samples were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Genotyping of the SNPs was performed using allele-specific probe-based polymerase chain reaction (PCR) according to the protocols designed in the Laboratory of Genomic Research at the Research Institute for Genetic and Molecular Epidemiology of Kursk State Medical University. The Primer3 software was used for primer design. A real-time PCR procedure was performed in a 25 μL reaction solution containing 1.5 units of Hot Start Taq DNA polymerase (Biolabmix, Novosibirsk, Russia), approximately 10 ng of DNA, and following concentrations of reagents: 0.25 μM of each primer; 0.1 μM of each probe; 250 μM of each dNTP; 3 mM MgCl_2 for rs7949972, 3.5 mM MgCl_2 for rs61882275, 2 mM MgCl_2 for

rs12610495 and 2.5 mM MgCl₂ for the remaining SNPs; 1xPCR buffer (67 mM Tris-HCl, pH 8.8, 16.6 mM (NH₄)₂SO₄, 0.01% Tween-20). The PCR procedure comprised an initial denaturation for 10 min at 95 °C, followed by 39 cycles of 92 °C for 30 s and 57 °C, 59 °C, 60 °C, 61 °C, 62 °C, 63 °C, 65 °C, 66 °C for 1 min (for rs12610495, rs17078346, rs17713054, rs111837807, rs9636867, rs143334143 and rs7949972, rs12585036 and rs61882275, rs67579710, respectively). A total of 10% of the DNA samples were genotyped twice, blinded to the case-control status, to assure quality control. Over 99% of the data were concordant.

2.3. Statistical and Bioinformatic Analysis

The statistical analysis was performed using STATISTICA software (v13.3, USA). The normality of quantitative data distribution was assessed with the Shapiro–Wilk test. As most quantitative parameters deviated from a normal distribution, they were presented as medians (Me) with first and third quartiles [Q1, Q3]. For comparisons between two independent groups, the Mann–Whitney test was also used. Categorical variables were analyzed for statistical significance using Pearson’s chi-squared test with Yates’s correction for continuity.

Genotype distributions were checked for Hardy-Weinberg equilibrium using Fisher’s exact test. Genotype frequencies and their associations with disease risk were analyzed using SNPStats software (<https://www.snptest.net/start.htm>; accessed on 31 May 2024). The log-additive model was considered in the genotype association analysis. Associations in the entire group of COVID-19 patients and controls were adjusted for covariates, which included variables showing differences in the general biological characteristics of the study groups.

Considering the significant role of epistatic interactions, as well as the joint influence of genetic and environmental factors on the risk of developing multifactorial diseases [20–23], we used the MB-MDR analysis for testing two-, three-, and four-level genotype combinations ($G \times G$) and genotype-environment combinations ($G \times E$). The empirical p -value (p_{perm}) for each model was estimated using a permutation test, with models considered statistically significant if $p_{\text{perm}} < 0.05$. All calculations were adjusted for covariates, and the most robust models (averaging 3–4 models per level) based on Wald statistics and significance levels were included in the study. Additionally, the MB-MDR method identified individual genotype combinations associated with the studied phenotypes ($p < 0.05$). Calculations were performed using the MB-MDR program for the R software environment (version 3.6.3).

The most significant $G \times G$ and $G \times E$ models were further analyzed using the MDR method, implemented in the MDR program (v3.0.2) (<http://sourceforge.net/projects/mdr>; accessed on 10 June 2024). The MDR method assessed interaction mechanisms (synergy, antagonism, additive interactions) and interaction strength (contribution of individual genes/environmental factors to trait entropy and interaction contributions). Results from the MDR analysis were visualized as graphs to illustrate these interactions.

The following bioinformatics resources were used to analyze the functional effects of GWAS SNPs:

- GTEx Portal (<http://www.gtexportal.org/>; accessed on 4 June 2024): this tool was used to analyze the expression levels of the studied genes in whole blood, blood vessels, brain tissue, and heart. It also helped in analyzing the expression of quantitative trait loci (eQTLs) associated with these genes [24].
- eQTLGen (<https://www.eqtlgen.org/>; accessed on 4 June 2024): This resource was used to examine the binding of GWAS SNPs to eQTLs in peripheral blood [25].
- HaploReg (v4.2), a bioinformatics tool available at <https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>; accessed on 4 June 2024). This tool assessed the associations between GWAS SNPs and specific histone modifications that indicate promoters and enhancers. These modifications included acetylation of lysine residues at positions 27 and 9 of the histone H3 protein, as well as monomethylation at position 4 (H3K4me1) and tri-methylation at position 4 (H3K4me3) of the histone H3

protein. Additionally, HaploReg was used to investigate SNP positioning in DNase hypersensitive regions, regulatory motif sites, and binding locations for regulatory proteins [26].

- atSNP Function Prediction (atSNP Search human version 1.0.0) (<http://atsnp.biostat.wisc.edu/search>; accessed on 3 June 2024): This online tool was utilized to evaluate the impact of GWAS SNPs on gene affinity to transcription factors (TFs) depending on the presence of reference or alternative alleles. TFs were included based on the degree of influence SNPs have on their interaction with DNA, calculated using a positional weight matrix [27].
- Gene Ontology (<http://geneontology.org/>; accessed on 3 June 2024): This tool enabled the analysis of the joint involvement of TFs linked to the reference or SNP alleles in overrepresented biological processes directly related to the pathogenesis of cardiovascular diseases (CVDs) [28]. Biological functions controlled by transcription factors associated with GWAS SNPs were used as functional groups.
- Cerebrovascular Disease Knowledge Portal (CDKP) (<https://cd.hugeamp.org/>; accessed on 4 June 2024) and Cardiovascular Disease Knowledge Portal (<https://cvd.hugeamp.org/>; accessed on 4 June 2024): These online tools, which combine and analyze results from genetic associations by the largest consortia studying cardio- and cerebrovascular diseases, were used for bioinformatic analyses of the associations of GWAS SNPs with atherosclerosis-associated diseases, intermediate phenotypes, and CVD risk factors such as total cholesterol, LDL, and BMI [29].

3. Results

3.1. Assosiations of GWAS-Significant SNPs with the Risk of CVDs in Severe COVID-19 Patients

Upon analyzing the associations between GWAS SNPs and CVD risk, we observed no correlation with AH but identified associations with CAD and CS (Table S2). In the overall analysis, SNP rs17713054 *SLC6A20-LZTFL1* (risk allele A, OR = 2.14, 95% CI 1.06–4.36, $p = 0.03$) and rs12610495 *DPP9* (risk allele G, OR = 1.69, 95% CI 1.02–2.81, $p = 0.04$) were found to increase the risk of CAD (Table 2). Additionally, rs61882275 *ELF5* was associated with an increased risk of CS (risk allele A, OR = 1.98, 95% CI 1.14–3.45, $p = 0.01$). Notably, allele T rs7949972 *ELF5* was associated with an elevated risk of both CAD (OR = 2.57, 95% CI 1.43–4.61, $p = 0.0009$) and CS (OR = 2.67, 95% CI 1.38–5.19, $p = 0.003$) (Table 2).

Table 2. The significant associations between GWAS SNPs and CVDs in the entire group.

Genetic Variant	Effect Allele	Other Allele	N	OR [95% CI] ¹	<i>p</i> ²	OR [95% CI] ¹	<i>p</i> ²	OR [95% CI] ¹	<i>p</i> ²
			AH		CAD		CS		
rs12610495 <i>DPP9</i>	G	A	183	1.84 [0.28–11.91]	0.52	1.69 [1.02–2.81]	0.04	1.41 [0.80–2.48]	0.24
rs61882275 <i>ELF5</i>	A	G	185	2.64 [0.59–11.92]	0.18	1.51 [0.96–2.39]	0.073	1.98 [1.14–3.45]	0.01
rs7949972 <i>ELF5</i>	T	C	185	4.25 [0.39–46.54]	0.2	2.57 [1.43–4.61]	0.0009	2.67 [1.38–5.19]	0.003

All calculations were performed relative to the minor alleles (Effect allele) with adjustment for age and physical activity; ¹—odds ratio and 95% confidence interval; ²— p -value; statistically significant differences are marked in bold.

3.2. Gene–Gene and Gene–Environmental Interactions

3.2.1. Gene–Gene Interactions Associated with Coronary Artery Disease Risk in Patients with Severe COVID-19 (MB-MDR, MDR Modeling)

Using the MB-MDR approach, the nine most significant gene–gene interaction patterns associated with CAD in patients with severe COVID-19 were identified: 3 two-locus, 3 three-locus, and 3 four-locus ($p_{\text{perm}} \leq 0.05$) (Table 3). In total, the best models of $G \times G$ interactions included seven polymorphic loci: rs7949972 *ELF5*, rs17078346 *SLC6A20-LZTFL1*,

rs12610495 *DPP9*, rs9636867 *IFNAR2*, rs61882275 *ELF5*, rs12585036 *ATP11A*, rs17713054 *SLC6A20-LZTFL1*; all were involved in 2 or more of the best models of gene–gene interactions. In the next step, we analyzed the interactions between these genetic variants and smoking using the multifactor dimensionality reduction (MDR) method (Figure 2).

Table 3. Gene–gene interactions associated with coronary artery disease in patients with severe COVID-19 (MB-MDR modeling).

Gene–Gene Interaction Models	NH	beta H	WH	NL	beta L	WL	Wmax	p_{perm}
The best two-locus models of gene–gene interactions (for $G \times G$ models with $p_{\text{min.}} < 0.001$, 1000 permutations)								
rs7949972 <i>ELF5</i> × rs17078346 <i>SLC6A20-LZTFL1</i>	2	0.2778	13.34	1	−0.1453	3.980	13.34	0.003
rs9636867 <i>IFNAR2</i> × rs12610495 <i>DPP9</i>	3	0.2362	13.13	1	−0.2002	7.132	13.13	0.006
rs7949972 <i>ELF5</i> × rs12610495 <i>DPP9</i>	2	0.2397	11.71	2	−0.2051	10.747	11.71	0.017
The best three-locus models of gene–gene interactions (for $G \times G$ models with $p_{\text{min.}} < 2 \times 10^{-4}$, 1000 permutations)								
rs7949972 <i>ELF5</i> × rs61882275 <i>ELF5</i> × rs12610495 <i>DPP9</i>	2	0.2807	14.63	2	−0.2132	9.971	14.63	0.005
rs9636867 <i>IFNAR2</i> × rs61882275 <i>ELF5</i> × rs17078346 <i>SLC6A20-LZTFL1</i>	4	0.4438	16.20	2	−0.2596	7.125	16.20	0.019
rs9636867 <i>IFNAR2</i> × rs12610495 <i>DPP9</i> × rs12585036 <i>ATP11A</i>	2	0.4049	15.09	2	−0.2640	7.089	15.09	0.028
The best four-locus models of gene–gene interactions (for $G \times G$ models with $p_{\text{min.}} < 5 \times 10^{-6}$, 1000 permutations)								
rs7949972 <i>ELF5</i> × rs9636867 <i>IFNAR2</i> × rs61882275 <i>ELF5</i> × rs17078346 <i>SLC6A20-LZTFL1</i>	6	0.4660	25.32	2	−0.2633	7.358	25.32	< 0.001
rs9636867 <i>IFNAR2</i> × rs12610495 <i>DPP9</i> × rs12585036 <i>ATP11A</i> × rs17713054 <i>SLC6A20-LZTFL1</i>	4	0.4245	23.36	2	−0.2907	6.638	23.36	0.006
rs7949972 <i>ELF5</i> × rs9636867 <i>IFNAR2</i> × rs12610495 <i>DPP9</i> × rs17713054 <i>SLC6A20-LZTFL1</i>	6	0.4511	22.59	3	−0.3020	8.573	22.59	0.017

Note: NH is the number of interacting high-risk genotypes, beta H—regression coefficient for high-risk interactions identified at the 2nd stage of analysis, WH—Wald statistics for high-risk interactions, NL—number of interacting low-risk genotypes, beta L—regression coefficient for low-risk interactions identified at the 2nd stage of analysis, WL—Wald statistics for low-risk interactions, Wmax—maximum values of the Wald statistics, p_{perm} —permutational significance levels for models (all models are adjusted for age and physical activity); Loci included in 2 or more best $G \times G$ models are indicated in bold.

The MDR method showed that, first, the genetic variants included in the best $G \times G$ models are characterized predominantly by antagonism and independent (additive) effects, with the exception of interactions between SNPs rs17078346 *SLC6A20-LZTFL1* and rs9636867 *IFNAR2*, rs7949972 *ELF5* and rs12585036 *ATP11A*, which exhibit additive (independent) effects in interaction with each other). Second, rs12610495 *DPP9* is characterized by a maximum mono-effect (6.22% contribution to the entropy of CAD), exceeding the mono-effects of other SNPs (maximum indicators—2.33%), and exceeding the effects of intergenic interactions (maximum contribution of intergenic interactions to the entropy of the trait—2.59%). Thirdly, the mono-effects of all other (except for rs12610495) SNPs (contribution to entropy 0.05–2.33%) are comparable to the effects of intergenic interactions (0.03–2.59% contribution to entropy). Fourthly, the following combinations of genotypes of polymorphic gene variants have the strongest correlations with coronary artery disease in patients with severe COVID-19: rs7949972 *ELF5* T/C × rs17078346 *SLC6A20-LZTFL1* C/A (Beta = 0.24961; $p = 0.00722$); rs9636867 *IFNAR2* A/G × rs12610495

DPP9 A/A (Beta = −0.2002; p = 0.008286); rs7949972 *ELF5* T/C × rs12610495 *DPP9* G/A (Beta = 0.18543; p = 0.01752); rs7949972 *ELF5* T/C × rs618822758 *ELF5* A/G × rs12610495 *DPP9* G/A (Beta = 0.23456; p = 0.005411); rs9636867 *IFNAR2* G/G × rs618822758 *ELF5* A/G × rs17078346 *SLC6A20-LZTFL1* C/A (Beta = 0.342103; p = 0.02595); rs9636867 *IFNAR2* A/G × rs12610495 *DPP9* G/A × rs12585036 *ATP11A* C/C (Beta = 0.39442; p = 0.00112); rs7949972 *ELF5* T/C × rs9636867 *IFNAR2* G/G × rs618822758 *ELF5* A/G × rs17078346 *SLC6A20-LZTFL1* C/A (Beta = 0.338995; p = 0.02709); rs9636867 *IFNAR2* A/G × rs12610495 *DPP9* G/A × rs12585036 *ATP11A* C/C × rs17713054 *SLC6A20-LZTFL1* G/G (Beta = 0.3989; p = 0.005714); rs7949972 *ELF5* C/C × rs9636867 *IFNAR2* A/G × rs12610495 *DPP9* G/G × rs17713054 *SLC6A20-LZTFL1* G/G (Beta = −0.66382; p = 0.02712) (Table S3).

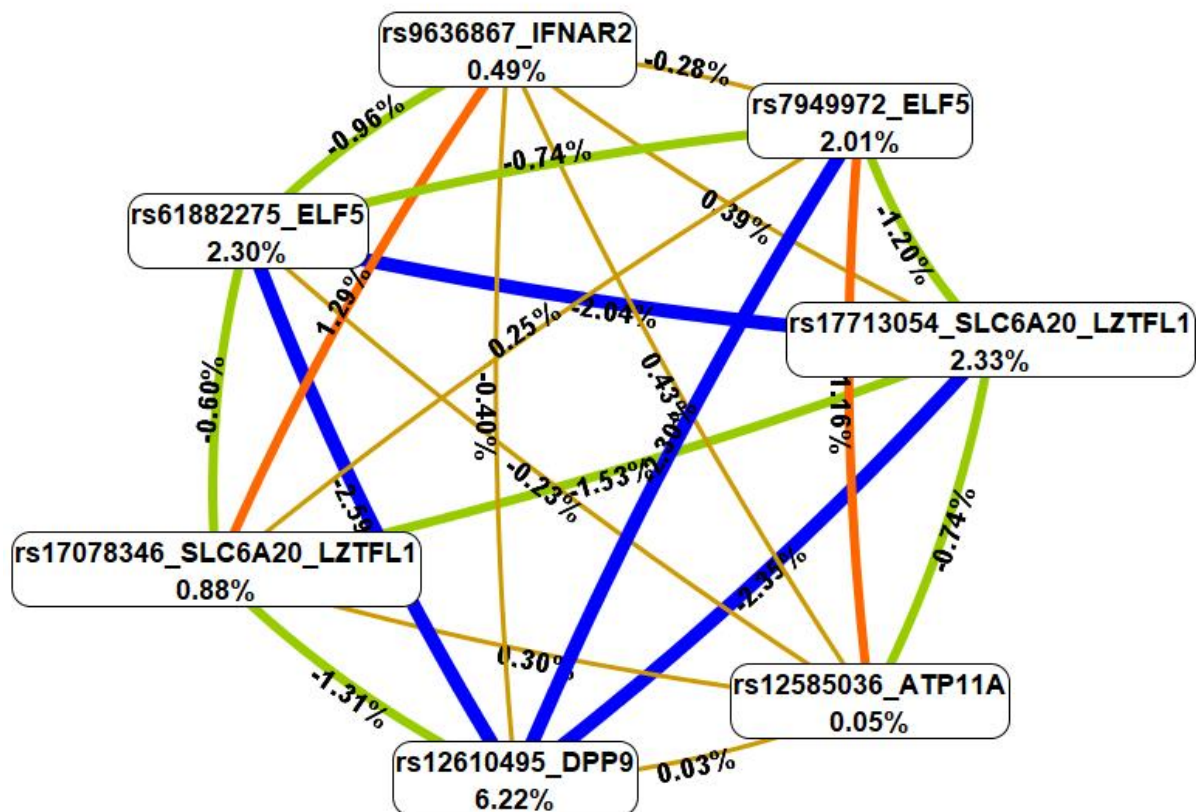


Figure 2. Graph reflecting the structure and power of the most significant $G \times G$ interactions of GWAS loci associated with CAD in severe COVID-19 patients. Notes: the color of the lines reflects the nature of the interaction: orange lines mean moderate synergism, brown means additive (independent) effects; blue and green represent strong and moderate antagonism, respectively; % reflects the strength and direction of the phenotypic effect of gene–gene interaction (% of entropy).

3.2.2. Gene–Environmental Interactions Associated with Coronary Artery Disease Risk in Severe COVID-19 Patients (MB-MDR, MDR Modeling)

Using the MB-MDR approach, the seven most significant gene–environment interaction patterns associated with severe COVID-19 were identified: 1 two-level, 3 three-level, and 3 four-level (Table 4). In total, the best models of $G \times E$ interactions included smoking in interaction with 6 SNPs, four of which—rs17078346 *SLC6A20-LZTFL1*, rs17713054 *SLC6A20-LZTFL1*, rs61882275 *ELF5*, rs9636867 *IFNAR2*—were involved in 2 or more of the most significant models of $G \times E$ interactions. It is noteworthy that all the best models of gene–environment interactions involved a single environmental risk factor: smoking. In the next step, we analyzed the interactions between these genetic variants and smoking using the multifactor dimensionality reduction (MDR) method (Figure 3).

Table 4. Gene–environmental interactions associated with coronary artery disease in patients with severe COVID-19 (MB-MDR modeling).

Gene–Gene Interaction Models	NH	beta H	WH	NL	beta L	WL	Wmax	<i>p</i> _{perm}
The best two-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 0.001, 1000 permutations)								
rs12610495 <i>DPP9</i> × SMOKE	1	0.2256	6.458	1	−0.2236	12.038	12.04	0.003
rs17078346 <i>SLC6A20-LZTFL1</i> × SMOKE	1	0.1599	4.122	1	−0.2143	12.085	12.09	0.004
rs17713054 <i>SLC6A20-LZTFL1</i> × SMOKE	1	0.1641	4.642	1	−0.2132	11.988	11.99	0.005
The best three-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 0.0003, 1000 permutations)								
rs61882275 <i>ELF5</i> × rs17078346 <i>SLC6A20-LZTFL1</i> × SMOKE	2	0.3420	14.902	1	−0.1674	4.220	14.90	0.017
rs7949972 <i>ELF5</i> × rs17713054 <i>SLC6A20-LZTFL1</i> × SMOKE	2	0.2891	8.943	2	−0.2364	14.471	14.47	0.011
The best four-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 8 × 10 ^{−6} , 1000 permutations)								
rs9636867 <i>IFNAR2</i> × rs61882275 <i>ELF5</i> × rs17078346 <i>SLC6A20-LZTFL1</i> × SMOKE	6	0.5166	25.77	2	−0.2424	7.451	25.77	0.004
rs9636867 <i>IFNAR2</i> × rs61882275 <i>ELF5</i> × rs17713054 <i>SLC6A20-LZTFL1</i> × SMOKE	5	0.5910	22.24	2	−0.2400	7.539	22.24	0.013

Note: NH is the number of high-risk interactions, beta H—regression coefficient for high-risk interactions identified at the 2nd stage of analysis, WH—Wald statistics for high-risk interactions, NL—number of interacting low-risk interactions, beta L—regression coefficient for low-risk interactions identified at the 2nd stage of analysis, WL—Wald statistics for low-risk interactions, Wmax—maximum values of the Wald statistics, *p*_{perm}—permutational significance levels for models (all models are adjusted for age); Loci included in 2 or more best G × E models are indicated in bold.

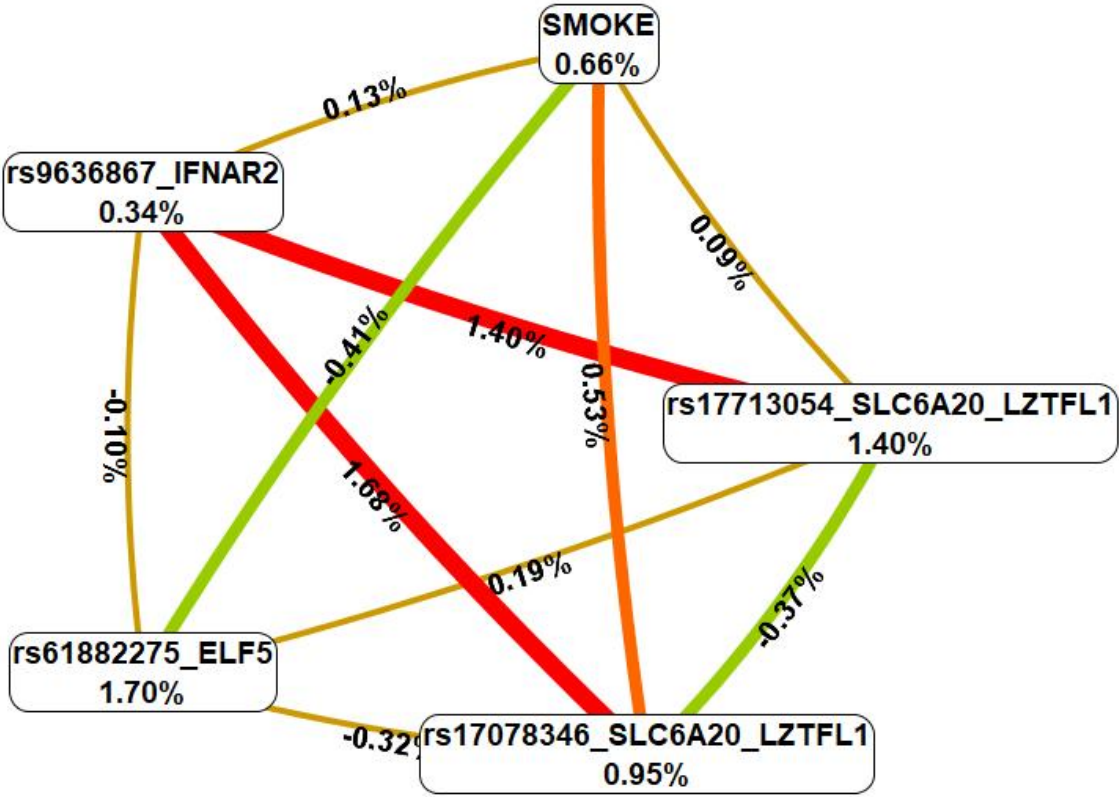


Figure 3. Graph reflecting the structure and power of the most significant G × E interactions of GWAS loci associated with CAD in severe COVID-19 patients. Notes: the color of the lines reflects the nature of the interaction: orange and red mean moderate and strong synergism, green —moderate antagonism; brown means additive (independent) effects; % reflects the strength and direction of the phenotypic effect of gene–environmental interaction (% of entropy).

First, MDR revealed that the mono-effect of smoking (0.66% contribution to entropy) is comparable to the mono-effects of SNPs included in the best gene-environment interaction models (0.34–1.70% contribution to entropy). Second, the effects of gene-environment interactions (0.09–0.53% contribution to entropy) are comparable to the mono-effect of smoking (0.66% entropy). Thirdly, smoking is characterized by multidirectional effects in interaction with genetic variants in the most significant $G \times E$ models: additive (independent effects in interaction with rs9636867 *IFNAR2* and rs17713054 *SLC6A20-LZTFL1*); moderate synergism in interaction with rs17078346 *SLC6A20-LZTFL1* and moderate antagonism in interaction with rs61882275 *ELF5*.

Fifth, the strongest associations with CAD in severe COVID-19 patients have the following gene-environmental interactions: rs12610495 *DPP9* A/A $\times$ 0 (Beta = -0.22356 ; $p = 0.000656$); rs17078346 *SLC6A20-LZTFL1* A/A $\times$ 0 (Beta = -0.21431 ; $p = 0.000638$); rs17713054 *SLC6A20-LZTFL1* G/G $\times$ 0 (Beta = -0.21315 ; $p = 0.000671$); rs61882275 *ELF5* A/G $\times$ rs17078346 *SLC6A20-LZTFL1* C/A $\times$ 0 (Beta = 0.401734 ; $p = 0.002264$); rs7949972 *ELF5* C/C $\times$ rs17713054 *SLC6A20-LZTFL1* G/G $\times$ 0 (Beta = -0.18079 ; $p = 0.02385$); rs9636867 *IFNAR2* A/G $\times$ rs61882275 *ELF5* A/G $\times$ rs17078346 *SLC6A20-LZTFL1* A/A $\times$ 1 (Beta = 0.58464 ; $p = 0.01801$); rs9636867 *IFNAR2* A/G $\times$ rs61882275 *ELF5* A/G $\times$ rs17713054 *SLC6A20-LZTFL1* G/G $\times$ 1 (Beta = 0.584637 ; $p = 0.01801$) (Table S4).

3.2.3. Gene-Gene Interactions Associated with Cerebral Stroke Risk in Patients with Severe COVID-19 (MB-MDR, MDR Modeling)

Using the MB-MDR approach, nine of the most significant patterns of gene-gene interactions associated with cerebral stroke in patients with severe COVID-19 were identified: 2 two-locus, 1 three-locus, and 2 four-locus ($p_{\text{perm}} \leq 0.05$) (Table 5). In total, the best models of $G \times G$ interactions included seven polymorphic loci, three of which—rs12610495 *DPP9*, rs7949972 *ELF5*, rs9636867 *IFNAR2*—were involved in 2 or more best models of intergenic interactions. It should be noted that the combination rs7949972 *ELF5* $\times$ rs9636867 *IFNAR2* $\times$ rs12610495 *DPP9* was the basis for the formation of all the most significant models of “high-order” intergenic interactions (three-locus and four-locus). In the next step, we analyzed the interactions between these genetic variants and smoking using the multifactor dimensionality reduction (MDR) method (Figure 4).

Table 5. Gene-gene interactions associated with cerebral stroke in patients with severe COVID-19 (MB-MDR modeling).

Gene-Gene Interaction Models	NH	beta H	WH	NL	beta L	WL	Wmax	p_{perm}
The best two-locus models of gene-gene interactions (for $G \times G$ models with $p_{\text{min.}} < 0.001$, 1000 permutations)								
rs17078346 <i>SLC6A20-LZTFL1</i> $\times$ rs12610495 <i>DPP9</i>	1	0.2915	14.82	1	-0.1521	4.812	14.82	0.014
rs12610495 <i>DPP9</i> $\times$ rs17713054 <i>SLC6A20-LZTFL1</i>	1	0.2638	12.59	1	-0.1576	5.300	12.59	0.046
The best three-locus models of gene-gene interactions (for $G \times G$ models with $p_{\text{min.}} < 1 \times 10^{-8}$, 1000 permutations)								
rs7949972 <i>ELF5</i> $\times$ rs9636867 <i>IFNAR2</i> $\times$ rs12610495 <i>DPP9</i>	5	0.4991	38.48	0	NA	NA	38.48	0.004
The best four-locus models of gene-gene interactions (for $G \times G$ models with $p_{\text{min.}} < 1 \times 10^{-11}$, 1000 permutations)								
rs7949972 <i>ELF5</i> $\times$ rs9636867 <i>IFNAR2</i> $\times$ rs12610495 <i>DPP9</i> $\times$ rs143334143 <i>CCHCR1</i>	6	0.6534	60.64	0	NA	NA	60.64	0.005
rs7949972 <i>ELF5</i> $\times$ rs9636867 <i>IFNAR2</i> $\times$ rs12610495 <i>DPP9</i> $\times$ rs11183780 <i>CCHCR1</i>	5	0.6379	53.99	0	NA	NA	53.99	0.012

Note: NH is the number of interacting high-risk genotypes, beta H—regression coefficient for high-risk interactions identified at the 2nd stage of analysis, WH—Wald statistics for high-risk interactions, NL—number of interacting low-risk genotypes, beta L—regression coefficient for low-risk interactions identified at the 2nd stage of analysis, WL—Wald statistics for low-risk interactions, Wmax—maximum values of the Wald statistics, p_{perm} —permutational significance levels for models (all models are adjusted for age and physical activity); Loci included in 2 or more best $G \times G$ models are indicated in bold.

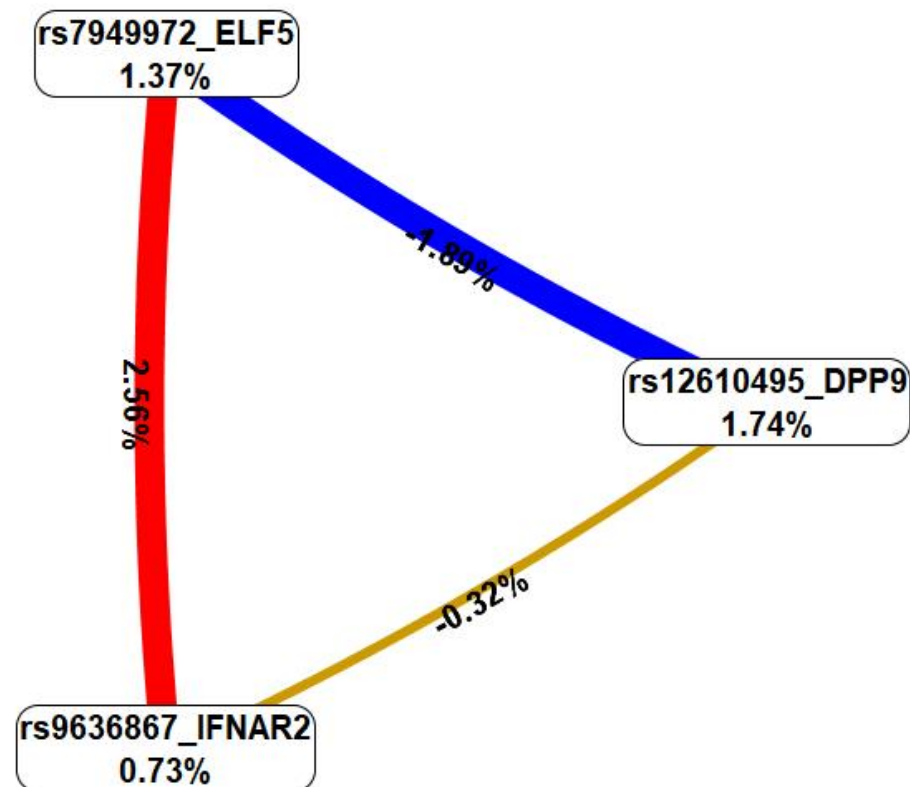


Figure 4. Graph reflecting the structure and power of the most significant $G \times G$ interactions of GWAS loci associated with CS in severe COVID-19 patients. Notes: the color of the lines reflects the nature of the interaction: red mean strong synergism, blue—pronounced antagonism; brown means additive (independent) effects; % reflects the strength and direction of the phenotypic effect of gene–gene interaction (% of entropy).

First, MDR showed that the genetic variants included in the best $G \times G$ models are characterized by multidirectional effects: synergism, antagonism, and independent (additive) effects. Second, the effects of intergenic interactions (contribution to the entropy of the trait—0.32–2.56%) are comparable to the mono-effects of SNPs (0.73–1.74% of the entropy of cerebral stroke). Thirdly, the following combinations of genotypes of polymorphic gene variants have the strongest associations with cerebral stroke in patients with severe COVID-19: rs17078346 *SLC6A20-LZTFL1* A/A $\times$ rs12610495 *DPP9* G/A (Beta = 0.29; $p = 0.0002$); rs12610495 *DPP9* G/A $\times$ rs17713054 *SLC6A20-LZTFL1* G/G (Beta = 0.26; $p = 0.0005$); rs7949972 *ELF5* T/C $\times$ rs9636867 *IFNAR2* A/G $\times$ rs12610495 *DPP9* G/A (Beta = 0.4091; $p = 0.0008$); rs7949972 *ELF5* T/T $\times$ rs9636867 *IFNAR2* A/G $\times$ rs12610495 *DPP9* G/A $\times$ rs143334143 *CCHCR1* A/G (Beta = 1.8; $p = 3.45 \times 10^{-5}$); rs7949972 *ELF5* T/T $\times$ rs9636867 *IFNAR2* A/G $\times$ rs12610495 *DPP9* G/A $\times$ rs11183780 *CCHCR1* C/T (Beta = 1.8; $p = 3.62 \times 10^{-5}$) (Table S5).

3.2.4. Gene–Environmental Interactions Associated with Cerebral Stroke Risk in Severe COVID-19 Patients (MB-MDR, MDR Modeling)

Using the MB-MDR approach, the four most significant models of gene–environment interactions associated with cerebral stroke in severe COVID-19 patients were identified: 1 two-level, 1 three-level, and 2 four-level (Table 6). In total, the best models of $G \times E$ interactions included smoking and low consumption of fresh vegetables and fruit in interaction with 5 SNPs, four of which—rs12610495 *DPP9*, rs61882275 *ELF5*, rs12585036 *ATP11A*, rs11183780 *CCHCR1*, along with smoking and level of consumption of fresh vegetables and fruit—were involved in 2 or more of the most significant $G \times E$ interaction patterns. In the next step, we analyzed the interactions between these genetic variants

and environmental factors using the multifactor dimensionality reduction (MDR) method (Figure 5).

Table 6. Gene–environmental interactions associated with cerebral stroke in patients with severe COVID-19 (MB-MDR modeling).

Gene–Gene Interaction Models	NH	beta H	WH	NL	beta L	WL	Wmax	<i>p</i> _{perm}
The best two-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 0.01, 1000 permutations)								
rs12610495 <i>DPP9</i> × SMOKE	1	0.2671	8.424	1	−0.1162	2.849	8.424	0.047
The best three-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 1 × 10 ^{−4} , 1000 permutations)								
rs61882275 <i>ELF5</i> × rs12585036 <i>ATP11A</i> × SMOKE	1	0.7640	22.49	0	NA	NA	22.49	0.016
The best four-order models of gene- interactions (for G × E models with <i>p</i> _{min.} < 3 × 10 ^{−8} , 1000 permutations)								
rs61882275 <i>ELF5</i> × rs12610495 <i>DPP9</i> × rs11183780 <i>CCHCR1</i> × VEGET	2	1.7321	36.28	0	NA	NA	36.28	0.002
rs9636867 <i>IFNAR2</i> × rs12585036 <i>ATP11A</i> × rs11183780 <i>CCHCR1</i> × VEGET	2	1.3997	35.31	0	NA	NA	35.31	0.002

Note: NH is the number of high-risk interactions, beta H—regression coefficient for high-risk interactions identified at the 2nd stage of analysis, WH—Wald statistics for high-risk interactions, NL—number of interacting low-risk interactions, beta L—regression coefficient for low-risk interactions identified at the 2nd stage of analysis, WL—Wald statistics for low-risk interactions, Wmax—maximum values of the Wald statistics, *p*_{perm}—permutational significance levels for models (all models are adjusted for age); Loci included in 2 or more best G × E models are indicated in bold.

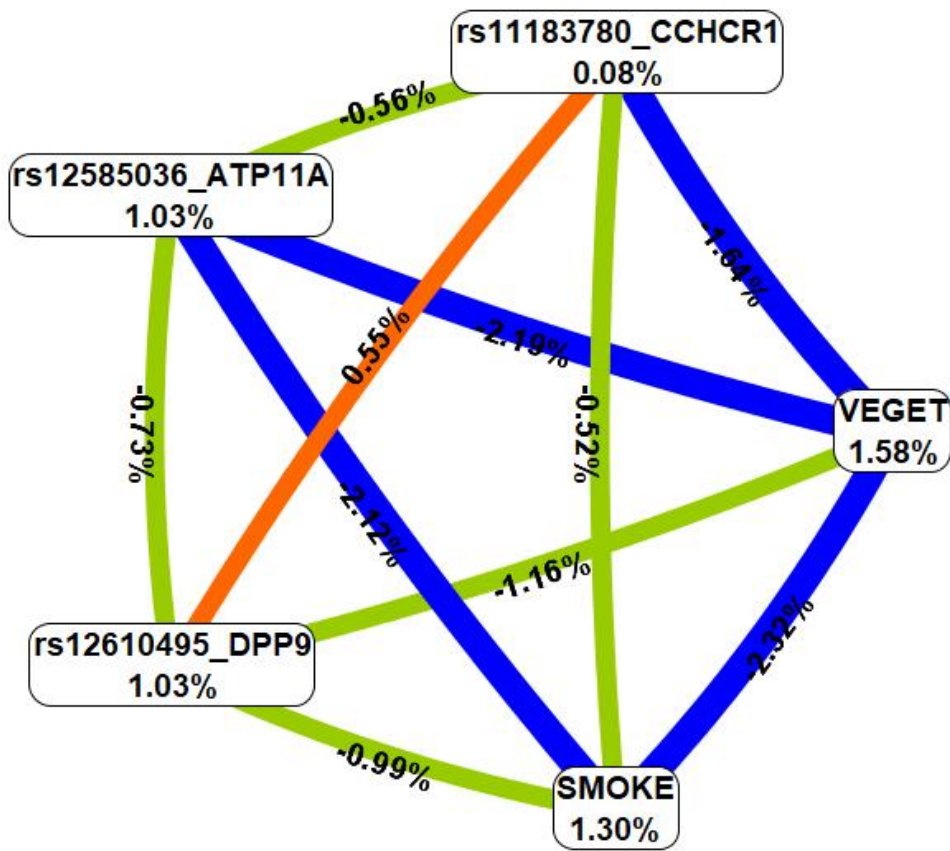


Figure 5. Graph reflecting the structure and power of the most significant G×E interactions of GWAS loci associated with CS in severe COVID-19 patients. Notes: the color of the lines reflects the nature of the interaction: orange mean moderate synergism, green and blue—moderate and pronounced antagonism; % reflects the strength and direction of the phenotypic effect of gene–gene interaction (% of entropy).

First, MDR revealed that environmental risk factors such as smoking and the level of consumption of fresh vegetables and fruit are characterized by moderate and pronounced antagonism in interaction with all SNPs included in the 2 or more most significant gene-environment interactions. Second, smoking and the level of consumption of fresh vegetables and fruit interact antagonistically. Thirdly, all SNPs characterizing the best $G \times E$ models are characterized by antagonism in their interaction with each other, except for rs12610495 *DPP9* and rs11183780 *CCHCR1*, which are characterized by moderate synergism in their interaction with each other. Fourth, the mono-effects of smoking and the level of consumption of fresh vegetables and fruit (1.30–1.58% contribution to entropy) are comparable to the mono-effects of SNPs (1.03%), except for rs11183780 *CCHCR1*, which is characterized by the least significant mono-effect (0.08% contribution to entropy). Fifth, the effects of gene-environment interactions (0.52–2.19%) exceed/are comparable to the mono-effects of SNPs in the best $G \times E$ models (0.08–1.03%). Sixth, the strongest associations with CS in severe COVID-19 patients have the following gene-environmental interactions: rs12610495 *DPP9* $G/A \times 1$ (Beta = 0.27; $p = 0.004$); rs618822758 *ELF5* $A/A \times rs12585036$ *ATP11A* $C/C \times 1$ (Beta = 0.8; $p = 4.36 \times 10^{-6}$); rs618822758 *ELF5* $A/A \times rs12610495$ *DPP9* $G/A \times rs11183780$ *CCHCR1* $C/T \times 1$ (Beta = 1.75; $p = 6.01 \times 10^{-5}$); rs9636867 *IFNAR2* $A/G \times rs12585036$ *ATP11A* $C/C \times rs11183780$ *CCHCR1* $C/T \times 1$ (Beta = 1.24; $p = 6.29 \times 10^{-5}$) (Table S6).

3.3. Functional Annotation of CVDs-Associated SNPs

3.3.1. Subsubsection

The SNP rs17713054 *SLC6A20-LZTFL1* was found to reduce the expression levels of *FLT1P1*, *CCR3*, *CCR1*, *SACM1L*, *CCR5*, *CCR2*, *RP11-24F11.2*, and *CXCR6* in blood, as well as *CXCR6* in tibial tissue, while it was associated with an increase in the expression of *CCR9* in whole blood (Table S7). SNP rs7949972 *ELF5* was identified as decreasing the expression levels of *CAT* in whole blood, the heart (left ventricle, atrial appendage), and the tibial artery, with *ABTB2* expression reduced in whole blood due to the influence of this SNP (Table S7). Additionally, rs61882275 *ELF5* was linked to reduced expression of *CAT* in blood and the tibial artery, as well as *ABTB2* in blood (Table S7). Regarding SNP rs12610495 *DPP9*, it was observed to elevate the expression levels of *TNFAIP8L1* in whole blood while reducing the expression of *DPP9* in the aorta, tibial artery, brain (cerebellum), and whole blood (Table S7).

3.3.2. Histone Modifications

Our study investigated histone modifications associated with CVD-related SNPs (Table 7).

Table 7. The impact of GWAS SNPs on histone tags in various tissues.

SNP (Ref/Alt Allele)	Tissues Marks	Vessels—Aorta	Heart	Blood
rs17713054 (G/A) <i>SLC6A20-LZTFL1</i>	H3K4me1	Enh	Enh	-
	H3K27ac	Enh	Enh	-
rs12610495 (A/G) <i>DPP9</i>	H3K4me1	-	Enh	Enh
	H3K4me3	-	-	-
	H3K27ac	-	Enh	-
	H3K9ac	-	-	-

H3K4me1—monomethylation at the 4th lysine residue of the histone H3 protein; H3K4me3—tri-methylation at the 4th lysine residue of the histone H3 protein; H3K9ac—the acetylation at the 9th lysine residues of the histone H3 protein; H3K27ac—acetylation of the lysine residues at N-terminal position 27 of the histone H3 protein; effect alleles are marked in bold. Enh—histone modification in the enhancer region.

SNP rs17713054 *SLC6A20-LZTFL1* is within a DNA-binding region associated with histone H3 monomethylation at the 4th lysine residue (H3K4me1) in the aorta and heart.

The effect of these histone tags is increased by the H3K27ac, marking enhancers in the aorta and heart.

SNP rs12610495 *DPP9* is in a DNA-binding region associated with H3K4me1 in the heart and blood. Additionally, the impact of these histone modifications is further enhanced by the presence of H3K27ac in the heart.

3.3.3. Transcription Factors

The risk allele A of rs17713054 *SLC6A20-LZTFL1* creates DNA-binding sites for 48 transcription factors (TFs) (Table S8). These TFs are implicated in transforming the growth factor beta receptor signaling pathway (GO:0007179; FDR = 4.94×10^{-2}). On the other hand, the protective allele G of rs17713054 creates binding sites for 24 TFs, jointly involved in response to hypoxia (GO:0001666; FDR = 2.8×10^{-2}), myoblast differentiation (GO:0045445; FDR = 2.56×10^{-3}), and response to growth factor (GO:0070848; FDR = 1.79×10^{-3}).

The protective allele A of rs12610495 *DPP9* generates DNA-binding sites for 39 TFs (Table S9). These TFs participate in positive regulation of endothelial cell migration (GO:0010595; FDR = 0.04), positive regulation of angiogenesis (GO:0045766; FDR = 0.01), cardiac muscle tissue regeneration (GO:0061026; FDR = 0.002), cardiac right ventricle morphogenesis (GO:0003215; FDR = 0.03), and response to xenobiotic stimulus (GO:0009410; FDR = 0.03).

Regarding the risk allele C of rs7949972 *ELF5*, it generates DNA-binding regions for 31 TFs (Table S10), which are involved in two overrepresented biological processes: positive regulation of CD8-positive, alpha-beta T-cell differentiation (GO:0043378; FDR = 0.002) and negative regulation of CD4-positive, alpha-beta T-cell differentiation (GO:0043371; FDR = 0.03). The protective allele T rs7949972 *ELF5* creates DNA-binding sites for 32 TFs, implicated in glial cell fate commitment (GO:0021781; FDR = 0.02) and regulation of neuron apoptotic process (GO:0043523; FDR = 0.04).

Lastly, the protective allele G rs61882275 *ELF5* creates DNA-binding sites for 32 TFs, implicated in the regulation of neurogenesis (GO:0050767; FDR = 0.003) (Table S11).

3.3.4. Bioinformatic Analysis of the Relationship between GWAS SNPs and CVD-Related Phenotypes

According to the bioinformatic resources Cerebrovascular Disease Knowledge Portal (CDKP) and Cardiovascular Disease Knowledge Portal (CVDKP), which combines and analyzes the results of genetic associations of the largest consortia for the study of cardio- and cerebrovascular diseases, the GWAS SNPs associated with CAD and CS, are associated with several CVD-related intermediate phenotypes and (Table 8).

Table 8. Results of aggregated bioinformatic analyses of associations between SNPs, cardio- and cerebrovascular diseases, and their intermediate phenotypes.

No.	SNP	Phenotype	p-Value	Beta (OR)	Sample Size
1	rs17713054 <i>SLC6A20-LZTFL1</i> (G/A)	¹ Long QT syndrome	0.007	OR▼0.786	5673
2		¹ Atrial fibrillation or flutter	0.012	OR▼0.9531	130,776
3		¹ LDL cholesterol	0.018	Beta▼−0.0048	2,783,500
4		¹ Non-HDL cholesterol	0.039	Beta▼−0.006	1,087,880
5		¹ Triglycerides	0.04	Beta▼−0.0042	2,412,380
6	rs7949972 <i>ELF5</i> (C/T)	² Coronary artery disease (CAD)	0.012	OR▲1.0111	1,250,150
7		² TOAST other undetermined	0.012	OR▲1.07	39,776
8		² Pulse pressure	0.029	Beta▼−0.0031	910,329
9		¹ Total cholesterol	0.0004	Beta▲0.0019	2,664,070
10		¹ LDL cholesterol	0.0007	Beta▲0.0028	3,137,790

Table 8. Cont.

No.	SNP	Phenotype	p-Value	Beta (OR)	Sample Size
11		¹ Serum ApoB	0.001	Beta▲0.0059	436,068
12		¹ Non-HDL cholesterol	0.0105	Beta▲0.0039	1,085,390
13		¹ Long QT syndrome	0.02	OR▼0.9116	5673
14	rs61882275 ELF5 (G/A)	² TOAST other undetermined	0.035	OR▲1.0649	39,604
15	rs12610495 DPP9 (A/G)	¹ Total cholesterol	6.97×10^{-9}	Beta▼−0.0066	2,655,840
16		¹ LDL cholesterol	2.47×10^{-7}	Beta▼−0.0060	3,106,200
17		¹ Non-HDL cholesterol	2.53×10^{-6}	Beta▼−0.0082	1,026,040
18		¹ Serum ApoB	0.005	Beta▼−0.006	436,068
19		¹ Hypertrophic cardiomyopathy	0.0086	OR▼0.9178	11,942
20					
21		¹ Left ventricular stroke volume	0.014	Beta▲0.0154	77,177
22		¹ Long QT syndrome	0.016	OR▼0.895	5673

¹—data obtained using the bioinformatic resource Cardiovascular Disease Knowledge Portal (<https://cvd.hugeamp.org/>; accessed on 4 June 2024); ²—data obtained using the bioinformatic resource Cerebrovascular Disease Knowledge Portal (<https://cd.hugeamp.org/>; accessed on 4 June 2024); Effect alleles are marked in bold.

4. Discussion

Cardiovascular diseases are well-known major risk factors for severe COVID-19, contributing to worsened symptoms, higher risk of adverse outcomes, and elevated mortality rates [30,31]. Our findings corroborate this, demonstrating that the presence of underlying coronary artery disease (CAD) or cerebral stroke (CS) significantly increases the risk of mortality from COVID-19 (Table 1). Moreover, in the context of multisystem inflammatory syndrome (MIS-C), SARS-CoV-2 leads to multiorgan damage, including significant effects on the heart. The inflammatory changes observed in the heart during MIS-C differ from those caused by typical cardiotropic viral infections [32]. These differences are likely due to the virus binding to ACE2 receptors, excessive cytokine release, T-cell dysregulation, microvascular damage, and endothelial dysfunction [33]. Crucially, MIS-C almost always results in thromboembolic events, including potential coronary thrombosis and embolism in the CNS [34]. This has important implications for treatment and drug selection, emphasizing that myocarditis, while serious, may be less critical than other symptoms that require more urgent attention.

Here, we report that severe COVID-19-related GWAS SNPs—rs17713054 *SLC6A20-LZTFL1*, rs12610495 *DPP9*, and rs7949972 *ELF5*—are linked to the risk of CAD. Additionally, we found that rs61882275 *ELF5* and rs7949972 *ELF5* are associated with cerebral stroke risk. Notably, our study did not find an association between these GWAS loci and arterial hypertension, suggesting that these risk SNPs do not influence vascular tone but rather modify the risk of stroke and CAD, predominantly through pro-atherosclerosis mechanisms. Our bioinformatic analysis supports this hypothesis (Figure 6).

First, our analysis of transcription factors (TFs) indicates molecular mechanisms by which GWAS SNP rs17713054 *SLC6A20-LZTFL1* potentially contributes to the development of CAD. The protective allele G of rs17713054 *SLC6A20-LZTFL1* is involved in response to hypoxia and myoblast differentiation (Table S5). Conversely, the risk allele A rs17713054 is implicated in the transforming growth factor beta (TGFβ) receptor signaling pathway. The TGFβ family is crucial in regulating both normal and abnormal vascular functions, including angiogenesis (Table S5). Dysregulation of the TGFβ pathway can lead to vascular dysfunction and pathologies, including atherosclerosis and vascular calcification [35].

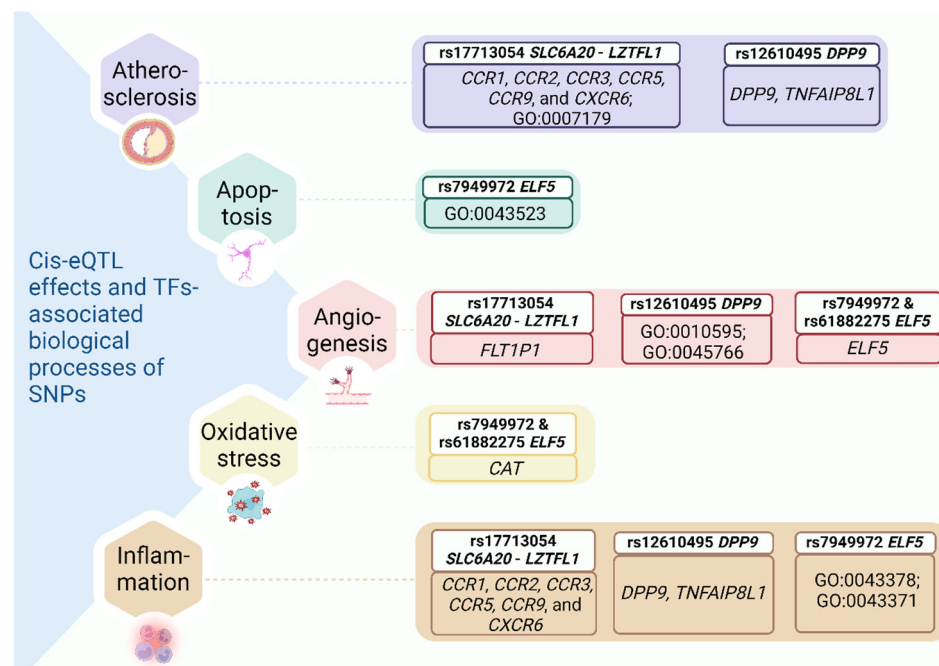


Figure 6. cis-eQTL effects and TFs-associated biological processes of GWAS SNPs linked to CVDs.

Additionally, allele A rs17713054 influences the expression of other genes through cis-eQTL effects, such as reducing the expression of *FLT1P1* in blood, potentially disrupting vascular endothelial growth factor receptor 1 (*VEGFR1*) expression [36]. *VEGFR1* is vital for angiogenesis and vascular permeability, acting as a decoy to sequester VEGF and prevent intracellular signaling initiation (Figure 6). *VEGFR1* knockout mice exhibit higher mortality due to heart failure, cardiac hypertrophy, and cardiac dysfunction [37,38].

The SNP rs17713054 also affects the expression levels of several chemokine receptors (*CCR1*, *CCR2*, *CCR3*, *CCR5*, *CCR9*, and *CXCR6*), with dysregulated expression of these receptors linked to atherosclerosis, a major risk factor for CAD (Figure 6) [39–41]. Moreover, the rs17713054 downregulates *SACM1L* expression. Deficiency in *SACM1L* has been shown to block the autophagosome fusion with vacuoles/lysosomes, a critical step in autophagy [42]. Autophagy is essential for preventing atherosclerosis [43,44] and maintaining cardiac cellular homeostasis [45,46].

It is noteworthy that rs17713054 characterized the best $G \times G$ models of intergenic interactions, predominantly interacting antagonistically with other loci of GWAS-significant genes for severe COVID-19. It also determined the formation of the most significant models of gene-smoking interactions. SNP rs17713054 is in the *SLC6A20-LZTFL1* intergenic region. Interestingly, smoking affects the expression of both of them: there is evidence in the literature that tobacco smoke pollution results in decreased expression of *LZTFL1* mRNA and affects the expression of *SLC6A20* mRNA [47]. This provides further support for the significant role of interactions between rs17713054 *SLC6A20-LZTFL1* and smoking.

Second, SNP rs12610495 *DPP9* was associated with an increased risk of CAD. Interestingly, when analyzing intergenic interactions, this genetic variant has the most pronounced mono-effect (6.22% contribution to CAD entropy), exceeding the mono-effects of other SNPs and the effects of intergenic interactions. SNP rs12610495 influences several key processes through its interaction with TFs. The protective allele A of rs12610495 *DPP9* is involved in the positive regulation of endothelial cell migration, angiogenesis, cardiac muscle tissue regeneration, cardiac right ventricle morphogenesis, and response to xenobiotic stimuli (Figure 6, Table S6). These processes are crucial for heart health, as they are regenerative and restorative [48,49].

DPP9, a member of the dipeptidyl peptidase family, is known for its role in regulating inflammasomes and pyroptosis, implicating it in autoinflammatory diseases [50,51],

including atherosclerosis [52,53]. SNP rs12610495 downregulates *DPP9* expression in blood and arteries through cis-eQTL effects while upregulating *TNFAIP8L1* (TNF-alpha-induced protein 8-like 1) in blood. The tumor necrosis factor (TNF)-alpha-induced protein 8 (TNFAIP8 or TIPE) family is important for maintaining immune homeostasis [54,55]. Notably, *Tnfaip8l2* expression is significantly induced in mouse models of atherosclerosis, severe hypercholesterolemia, and obesity (Figure 6) [56]. These findings underscore the multifaceted role of rs12610495 *DDP9* in CAD risk, highlighting its impact on endothelial function, inflammation, and immune regulation.

Thirdly, SNPs rs61882275 and rs7949972 in the *ELF5* gene were associated with an increased risk of cerebral stroke, with rs7949972 also linked to a higher risk of CAD. It is important to note that polymorphic *ELF5* loci were also involved in the most significant gene–gene interactions associated with CAD and CS (Tables 3 and 5). A previous study found that rs7949972 *ELF5* was associated with the comorbid conditions of bronchial asthma, arterial hypertension, and CAD [57]. Moreover, data from CDKP and CVDKP underscore the significance of rs7949972 *ELF5* SNP in increasing the risk of CAD and stroke, as well as contributing to a worsened lipid profile by elevating levels of total cholesterol, LDL cholesterol, and non-HDL cholesterol. Additionally, rs61882275 *ELF5* is associated with an increased risk of stroke, particularly TOAST, and otherwise undetermined.

ELF5, an epithelial-specific member of the Ets transcription factor family, regulates various cellular processes, including keratinocyte terminal differentiation, trophoblast differentiation, and epithelial–mesenchymal transformation in tumor cells [58]. Recent research has shown that increased *ELF5* expression promotes angiogenesis following ischemia-induced cardiac injury [59].

Our bioinformatic analysis of *ELF5* SNPs reveals key molecular mechanisms that may contribute to the development of CAD or CS. Transcription factors binding to the risk allele C of rs7949972 *ELF5* are involved in the positive regulation of CD8-positive alpha-beta T-cell differentiation and the negative regulation of CD4-positive alpha-beta T-cell differentiation, indicating a role in immune regulation and inflammation processes. Additionally, *ELF5* SNPs influence neuronal cell regulation: the protective allele T of rs7949972 *ELF5* regulates glial cell fate commitment and the neuronal apoptotic process, while the protective allele G of rs61882275 *ELF5* regulates neurogenesis (Figure 6, Tables S7 and S8).

Importantly, *ELF5* SNPs downregulate the expression levels of catalase in blood, arteries, and heart via cis-eQTL effects. Oxidative stress can arise from the overproduction of reactive oxygen species (ROS) or inadequate antioxidant defenses [60,61]. Catalase (CAT), a mitochondrial enzyme, plays a crucial role in ROS metabolism and cellular defense against oxidative stress (Figure 6) [62]. Given that lipid peroxidation is a major predictor of CVD [63,64], the role of antioxidant enzymes like catalase in CVD pathogenesis is well-documented [65–67]. A large body of evidence shows that oxidative stress directly induces inflammatory cascades and accelerates the oxidation of LDL-C [68,69], leading to atherosclerotic plaque instability and the worsening of CAD [70–72].

Additionally, rs61882275 *ELF5*, when interacting with smoking, was implicated in the most significant gene–environment interactions associated with CAD. Previous studies have shown that tobacco smoke pollution decreases the expression of *ELF5* mRNA [47]. This additional evidence of *ELF5* downregulation due to smoking suggests a potential worsening of oxidative stress in patients who smoke and carry risk *ELF5* alleles.

Thus, we further underscore the significant impact of *ELF5* SNPs on cardiovascular health through their influence on oxidative stress and immune responses.

5. Conclusions

We are the first in the world to investigate the associations between GWAS-significant loci and the risk of CVD, including AH, CAD, and CS, in patients with severe COVID-19. Our study identified the most significant intergenic and gene–environment interactions and performed a comprehensive functional annotation of SNPs linked to CVD to analyze their involvement in the molecular mechanisms of these diseases. Our findings suggest

that several GWAS loci, including rs17713054, rs7949972, and rs12610495, which were previously linked to severe COVID-19, are associated with an increased risk of CAD in hospitalized patients with severe COVID-19. Additionally, intergenic interactions involving rs9636867, rs7949972, rs17713054, rs12585036, rs12610495, rs17078346, and rs61882275, as well as gene-environment interactions of rs17713054, rs17078346, rs61882275, and rs9636867 with smoking, are also associated with this increased risk of CAD. Similarly, the GWAS loci rs7949972 and rs61882275, intergenic interactions of rs7949972, rs12610495, and rs9636867, and gene-environment interactions of rs61882275, rs12610495, rs12585036, and rs11183780 with smoking and low fresh fruit and vegetable intake, are linked to a higher risk of CS in hospitalized patients with severe COVID-19. Using bioinformatics tools, we established the high regulatory potential of the studied SNPs and identified mechanisms through which they can influence the risk of CAD and CS, primarily through their binding to transcription factors and expression of quantitative trait loci.

6. Study Limitations

First, the study faced limitations due to the small number of cases, as obtaining informed consent from severely ill hospitalized patients was challenging. This constraint may have reduced the statistical power, possibly leading to missed associations with the risk of CVD. Second, our research was confined to 10 GWAS-significant SNPs without exploring other genes implicated in the progression of severe COVID-19. Thirdly, we lacked data on crucial biochemical parameters for CVD, such as total cholesterol, triglyceride levels, lipoproteins, and history of CVDs, which prevented us from assessing the correlations between these parameters and the GWAS SNPs.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jmp5030026/s1>, Table S1: The most significant gene–gene combinations associated with coronary heart disease in patients with severe COVID-19. Table S2: The most significant gene–environmental combinations associated with coronary heart disease in patients with severe COVID-19. Table S3: The most significant gene–gene combinations associated with cerebral stroke in patients with severe COVID-19. Table S4: The most significant gene–environmental combinations associated with cerebral stroke in patients with severe COVID-19. Table S5: Analysis of the effect of rs17713054 *SLC6A20-LZTFL1* on the binding of DNA to transcription factors. Table S6: Analysis of the effect of rs12610495 *DPP9* on the binding of DNA to transcription factors. Table S7: Analysis of the effect of rs7949972 *ELF5* on the binding of DNA to transcription factors. Table S8: Analysis of the effect of rs61882275 *ELF5* on the binding of DNA to transcription factors.

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