



## Original Article

# SERF2 (Hero7) Gene Polymorphism is Associated with Severe Course of COVID-19



Anna Dorofeeva, Maxim Ivenkov, Ksenia Kobzeva and Olga Bushueva\*

Research Institute for Genetic and Molecular Epidemiology, Genomic Research Laboratory, Kursk State Medical University, Kursk, Russia

Received: June 27, 2025 | Revised: August 23, 2025 | Accepted: September 15, 2025 | Published online: October 30, 2025

### Abstract

**Background and objectives:** Chaperone-like proteins are involved in the pathogenesis of coronavirus infection through regulation of the viral life cycle, immune response, and antigen presentation. A recently discovered class of chaperones, called heat-resistant obscure proteins (Hero proteins), performs functions similar to other molecular chaperones. This study aimed to investigate the association between the gene encoding the Hero protein SERF2 (Hero7) and the risk of severe COVID-19.

**Methods:** This case-control study was conducted according to the STROBE protocol. A total of 1,373 unrelated Russians (178 patients with severe COVID-19 and 1,195 controls) were recruited. Genotyping of rs4644832 in the *SERF2* gene was performed using a probe-based polymerase chain reaction approach. The effects of the single nucleotide polymorphisms (SNPs) were analyzed using bioinformatics tools, including GTExPortal, eQTLGen, HaploReg, atSNP, Gene Ontology, Lung Disease and Common Metabolic Diseases Knowledge Portals, and the STRING database.

**Results:** SNP rs4644832 in the *SERF2* gene (effect allele G) was associated with a decreased risk of severe COVID-19 in the total sample (odds ratio (OR) = 0.56, 95% confidence interval (CI) 0.39–0.81,  $P = 0.001$ ), females (OR = 0.51, 95% CI 0.31–0.87,  $P = 0.006$ ), non-smokers (OR = 0.46, 95% CI 0.29–0.74,  $P = 0.0004$ ), individuals with body mass index  $\geq 25$  (OR = 0.42, 95% CI 0.25–0.7,  $P = 0.0004$ ), individuals with low fruit and vegetable intake (OR = 0.38, 95% CI 0.22–0.67,  $P = 0.0004$ ), and individuals with low physical activity (OR = 0.41, 95% CI 0.23–0.75,  $P = 0.002$ ).

**Conclusions:** The G allele of rs4644832 in the *SERF2* gene appears to have a protective effect against severe COVID-19. Functional annotation of rs4644832 suggests that it may influence COVID-19 pathogenesis through regulation of proteostasis, ubiquitination, inflammation-induced protein aggregation, the viral life cycle, and cytoskeletal functions.

### Introduction

The coronavirus disease (COVID-19) outbreak caused by the SARS-CoV-2 virus has become one of the largest pandemics in modern history. According to the World Health Organization, as of January 2025, COVID-19 has spread to 215 countries and caused more than seven million deaths worldwide.<sup>1</sup> The spectrum of clinical forms of COVID-19 varies from asymptomatic infection to severe acute respiratory syndrome.<sup>2</sup>

It has been discovered that proteins with chaperone-like properties are actively involved in the pathogenesis of COVID-19 by regulating the immune response and viral replication,<sup>3</sup> contributing to the development of a cytokine storm,<sup>4</sup> and participating in the antigen presentation of viral proteins from infected cells.<sup>5</sup> Furthermore, SARS-CoV-2 shares immunogenic epitopes with several human chaperones, which can lead to immune hyperactivation,<sup>6,7</sup> multi-organ damage,<sup>8</sup> and post-acute sequelae of COVID-19.<sup>9</sup> In addition, as obligate intracellular parasites, viruses can seize control over the host cell metabolic machinery, including the chaperoning system, to maintain their life cycle and sustain productive infection.<sup>10</sup>

A recently discovered class of chaperones, known as heat-resistant obscure (Hero) proteins, is likely to perform functions similar to other molecular chaperones, such as maintaining proteostasis and protecting proteins from pathological aggregation.<sup>11,12</sup> Hero proteins are hydrophilic, highly charged, heat-resistant proteins with low molecular weight and disordered structures. These prop-

**Keywords:** COVID-19; Heat-resistant obscure; Hero proteins; SERF2; Hero7; SNP; Chaperones; Protein aggregation; Viral life cycle.

\***Correspondence to:** Olga Bushueva, Research Institute for Genetic and Molecular Epidemiology, Genomic Research Laboratory, Kursk State Medical University, Kursk 305041, Russia. ORCID: <https://orcid.org/0000-0003-3333-0623>. Tel: +7-4712-588147, E-mail: [olga.bushueva@inbox.ru](mailto:olga.bushueva@inbox.ru)

**How to cite this article:** Dorofeeva A, Ivenkov M, Kobzeva K, Bushueva O. *SERF2 (Hero7) Gene Polymorphism is Associated with Severe Course of COVID-19. Gene Expr* 2025;24(4):e00057. doi: 10.14218/GE.2025.00057.

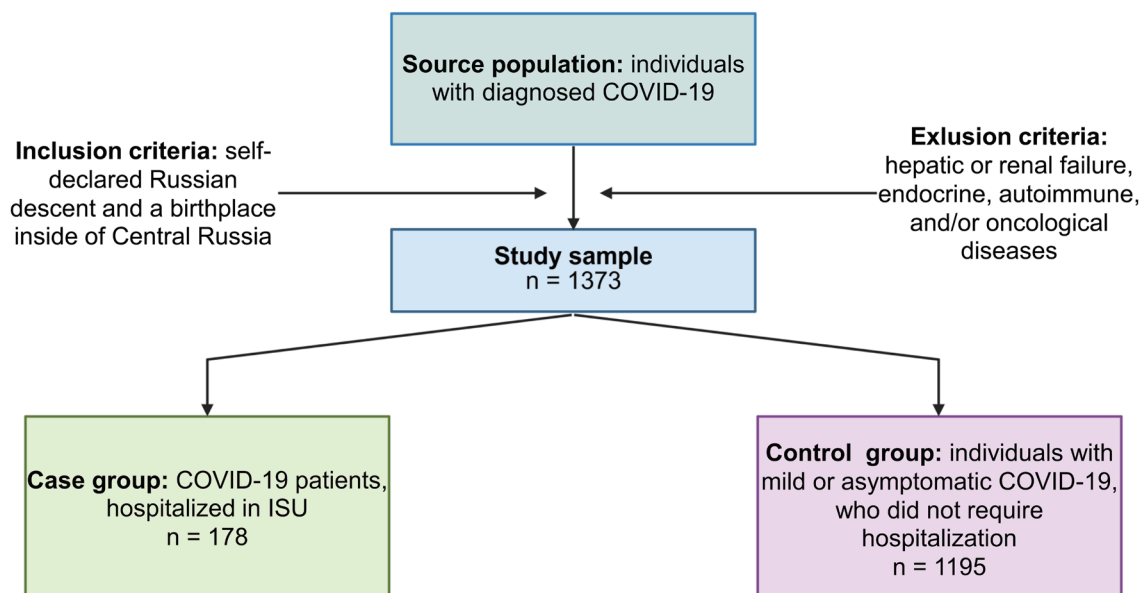


Fig. 1. Study design.

erties allow them to protect other proteins from denaturation under extreme conditions, either through simple “molecular shielding” or by promoting liquid-liquid phase separation.<sup>13</sup>

Small EDRK-rich factor 2 (*SERF2*, also known as Hero 7), a member of the Hero protein family, is known for its distinctive role in protein aggregation, which varies depending on the protein “client”.<sup>13</sup> Members of the *SERF* family perform dual functions, both promoting and preventing fibril formation of amyloidogenic proteins.<sup>14</sup> Several studies have confirmed that *SERF2* increases aggregation of huntingtin,  $\beta$ -amyloid, and  $\alpha$ -synuclein, contributing to amyloid proteotoxicity.<sup>15–17</sup> However, it also prevents aggregation of TAR DNA-binding protein 43 (TDP-43), which regulates viral RNA expression and has been actively studied in the research on COVID-19.<sup>18–20</sup>

The *SERF* protein family has an amino acid composition similar to that of DNA- and RNA-binding proteins, such as zinc-finger proteins.<sup>18,21,22</sup> Since zinc-finger proteins are heavily implicated in COVID-19 pathogenesis,<sup>23,24</sup> *SERF2* may perform similar functions, including the regulation of viral gene expression.<sup>18</sup>

In our previous studies, we identified an association between the *SERF2* SNP rs4644832 and the risk of cerebrovascular diseases.<sup>12,25</sup> Because critical illness in COVID-19 and cerebrovascular diseases share several key pathogenic mechanisms, such as increased production of proinflammatory cytokines, hypercoagulation, endothelial dysfunction, inflammation, and oxidative stress,<sup>26–34</sup> we hypothesized that SNP rs4644832 correlates with an increased risk of severe COVID-19. Therefore, we set out to investigate the association between *SERF2* SNP rs4644832 and the risk of severe COVID-19.

## Materials and methods

This study follows the criteria of a case-control study, according to the STROBE guidelines. A total of 1,373 unrelated Russians (178 patients with a severe course of COVID-19 and 1,195 controls with mild or asymptomatic COVID-19) from Central Russia were recruited for the study. The patients were enrolled during the

COVID-19 pandemic from 2020 to 2022 at the intensive care units of Kursk Regional Hospital No. 6 and Kursk Regional Tuberculosis Dispensary. The patients were recruited consecutively. Inclusion criteria for the study were self-declared Russian descent and a birthplace within Central Russia. The COVID-19 patients were enrolled under the following exclusion criteria: hepatic or renal failure, endocrine, autoimmune, and/or oncological diseases, which could alter laboratory parameters. All patients in the case group had polymerase chain reaction (PCR)-confirmed COVID-19 and required intensive care unit admission. The control group consisted of healthy volunteers who were diagnosed with COVID-19 but did not require hospitalization (Fig. 1). Baseline and clinical characteristics of the study population are listed in Table S1.

Low fruit and vegetable intake was defined according to the World Health Organization guidelines as consuming less than 400 g per day.<sup>35</sup> Low physical activity was defined as engaging in less than 180 minutes per week of various physical activities.<sup>36</sup>

Genomic DNA was extracted from blood samples, and the quality of extracted DNA was assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). SNP rs4644832 in *SERF2* was genotyped using allele-specific probe-based PCR in accordance with a previously published protocol. Details of primer design, reaction solution, and PCR protocol steps have been previously published.<sup>12</sup>

STATISTICA software (version 13.3, Informer Technologies, Inc., Santa Clara, CA, USA) was used for statistical analysis. The normality of distribution for quantitative data was assessed using the Shapiro-Wilk test. Most quantitative parameters deviated from a normal distribution; thus, they were presented as the median along with the first and third quartiles [Q1 and Q3]. The Kruskal–Wallis test was applied to compare quantitative variables among three independent groups. Pairwise comparisons were then performed using the Mann-Whitney test. These tests were employed to analyze associations between rs4644832 in *SERF2* and clinical features of COVID-19 in the patient group.

To evaluate the independent contribution of the rs4644832 variant in *SERF2* to severe COVID-19 risk while adjusting for rele-

vant covariates, we performed multivariate logistic regression in R (version 4.4.1). The analysis included one SNP (rs4644832 in *SERF2*) and five covariates: age, sex, smoking status, body mass index (BMI), and vegetable intake. Unfortunately, data on physical activity levels were unavailable for the control group and could not be included as a covariate in the regression model.

The dependent variable was disease status (0 = control, 1 = severe COVID-19), and all categorical predictors (SNP genotype, sex, smoking status, vegetable intake) were encoded as factors. Genotypes were modeled under a categorical (non-additive) framework. Continuous variables (age, BMI) were used as numeric predictors. Individuals with missing values for any variable were excluded from the analysis.

The dataset was split into a training set (80%) and a testing set (20%) using the caret package to allow model evaluation. The logistic regression model was fitted using the glm function with a binomial link function. Model performance was evaluated by the likelihood ratio test against a null (intercept-only) model, Nagelkerke's pseudo- $R^2$  (calculated with the DescTools package), and classification accuracy on the training set.

To visualize significant predictors ( $P < 0.05$ ), odds ratios (OR) and 95% confidence interval (CI) were extracted using the broom package, exponentiated, and plotted on a log scale. Predictors with  $OR > 1$  were classified as "risk" factors, while those with  $OR < 1$  were classified as "protective".

Compliance of genotype distributions with the Hardy-Weinberg equilibrium was assessed using Fisher's exact test. Genotype frequencies and their correlation with disease risk were analyzed using SNPStats software (<https://www.snpsstats.net/start.htm>). A log-additive model was used for genotype association analysis. Associations within the entire group of COVID-19 patients and controls were adjusted for age and sex.

Due to the potential modifying influence of environmental risk factors on the association of genetic markers with disease, associations were analyzed based on the presence or absence of the risk factor. When information about an environmental risk factor was unavailable in the control group (for fruit/vegetable intake, physical activity levels, and BMI), the patient group was compared to the overall control group. In such cases, the Bonferroni correction was applied to account for multiple comparisons.

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

- The bioinformatic tool GTExPortal (<http://www.gtexportal.org/>) was used to analyze the expression levels of the gene in whole blood, blood vessels, and lungs, as well as to analyze the expression of quantitative trait loci (eQTLs).<sup>37</sup> The method of eQTL analysis is fully described in our previous article.<sup>38</sup>
- The eQTLGen (<https://www.eqtlgen.org/>) was applied for the examination of eQTL expression in peripheral blood.<sup>39</sup> eQTLGen incorporates 37 datasets, with a total of over 31,000 individuals.
- The HaploReg v4.2 (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) was used to assess histone modifications. Histone H3 protein lysine residues at positions 27 and 9 (H3K27ac and H3K9ac, respectively), as well as mono- and tri-methylation at position 4 (H3K4me1 and H3K4me3), were studied.<sup>40–42</sup> This resource compiles ChIP-seq data from the Roadmap Epigenomics projects.<sup>43</sup>
- The atSNP affinity test (<http://atsnp.biostat.wisc.edu/search>) was used to assess the impact of SNPs on the gene's affinity for transcription factors (TFs).<sup>44</sup> In detail, the method of TF analysis is described earlier in our study.<sup>45</sup>

- The Gene Ontology online tool (<http://geneontology.org/>) provides a systematic classification of gene functions, which we used to analyze the joint involvement of TFs linked to the reference or SNP alleles in biological processes directly related to the pathogenesis of COVID-19.<sup>46</sup>
- The Lung Disease Knowledge Portal (<https://cd.hugeamp.org/>) and Common Metabolic Diseases Knowledge Portal (<https://hugeamp.org/>) were used to analyze the correlation between SNPs and phenotypic risk factors of severe COVID-19.
- The STRING database's bioinformatic tools were used to study key functional partners of *SERF2*. Moreover, the STRING database was utilized to analyze protein-protein interactions between *SERF2* and its functional partners.<sup>47,48</sup>
- The Comparative Toxicogenomics Database (<https://ctdbase.org/>) was employed to evaluate the influence of various chemicals and hormones on *SERF2* expression.<sup>49</sup>

## Results

The distribution of genotype frequencies conformed to Hardy-Weinberg equilibrium ( $P > 0.05$ ). The log-additive model analysis was performed in the total sample. Additionally, we analyzed groups stratified by sex, BMI, smoking status, fruit and vegetable intake, and physical activity to identify associations between genetic variants and severe COVID-19 depending on the presence or absence of environmental risk factors (Table 1).

The analysis revealed significant associations between the polymorphic variant rs4644832 in *SERF2* and severe COVID-19. The G allele exhibited a protective effect in the total sample ( $OR = 0.56$ , 95% CI 0.39–0.81,  $P = 0.001$ ) (Table 1), as well as in females ( $OR = 0.51$ , 95% CI 0.31–0.87,  $P = 0.006$ ), non-smokers ( $OR = 0.46$ , 95% CI 0.29–0.74,  $P = 0.0004$ ), individuals with BMI  $\geq 25$  ( $OR = 0.42$ , 95% CI 0.25–0.70,  $P_{Bonf} = 0.0004$ ), individuals with low fruit and vegetable intake ( $OR = 0.38$ , 95% CI 0.22–0.67,  $P_{Bonf} = 0.0004$ ), and individuals with low physical activity ( $OR = 0.41$ , 95% CI 0.23–0.75,  $P_{Bonf} = 0.002$ ) (Table 1).

## Multivariable logistic regression analysis

Multivariable logistic regression adjusted for age, sex, smoking status, BMI, and vegetable intake revealed significant associations between genetic and clinical factors and severe COVID-19 (Likelihood Ratio Test  $\chi^2 = 122.5$ ,  $P < 0.001$ ) (Table S2, Fig. 2). The model explained 25% of the variance (Nagelkerke  $R^2 = 0.25$ ) and correctly classified 86% of cases.

Carriers of the SNP rs4644832 *SERF2* A/G genotype had 45% lower odds of severe COVID-19 compared to A/A homozygotes ( $OR = 0.55$ , 95% CI 0.31–0.93,  $P = 0.03$ ) (Fig. 2). The G/G genotype showed a non-significant protective trend ( $OR = 0.43$ , 95% CI 0.06–1.61,  $P = 0.3$ ). Each additional year of age increased the odds by 7% ( $OR = 1.07$ , 95% CI 1.05–1.10,  $P < 0.001$ ). Females had 60% lower odds than males ( $OR = 0.40$ , 95% CI 0.25–0.63,  $P < 0.001$ ). Regular vegetable consumption was strongly protective ( $OR = 0.38$ , 95% CI 0.25–0.58,  $P < 0.001$ ), whereas smoking showed no significant association ( $P = 0.6$ ). Each unit increase in BMI increased the odds by 7% ( $OR = 1.07$ , 95% CI 1.03–1.11,  $P = 0.001$ ) (Fig. 2).

## Clinical features of COVID-19 and rs4644832 *SERF2*

We analyzed associations between rs4644832 in *SERF2* and clinical features of COVID-19 among patients. In groups stratified by sex, age, smoking status, fruit and vegetable intake, and physical activity, several significant associations were observed. In females,

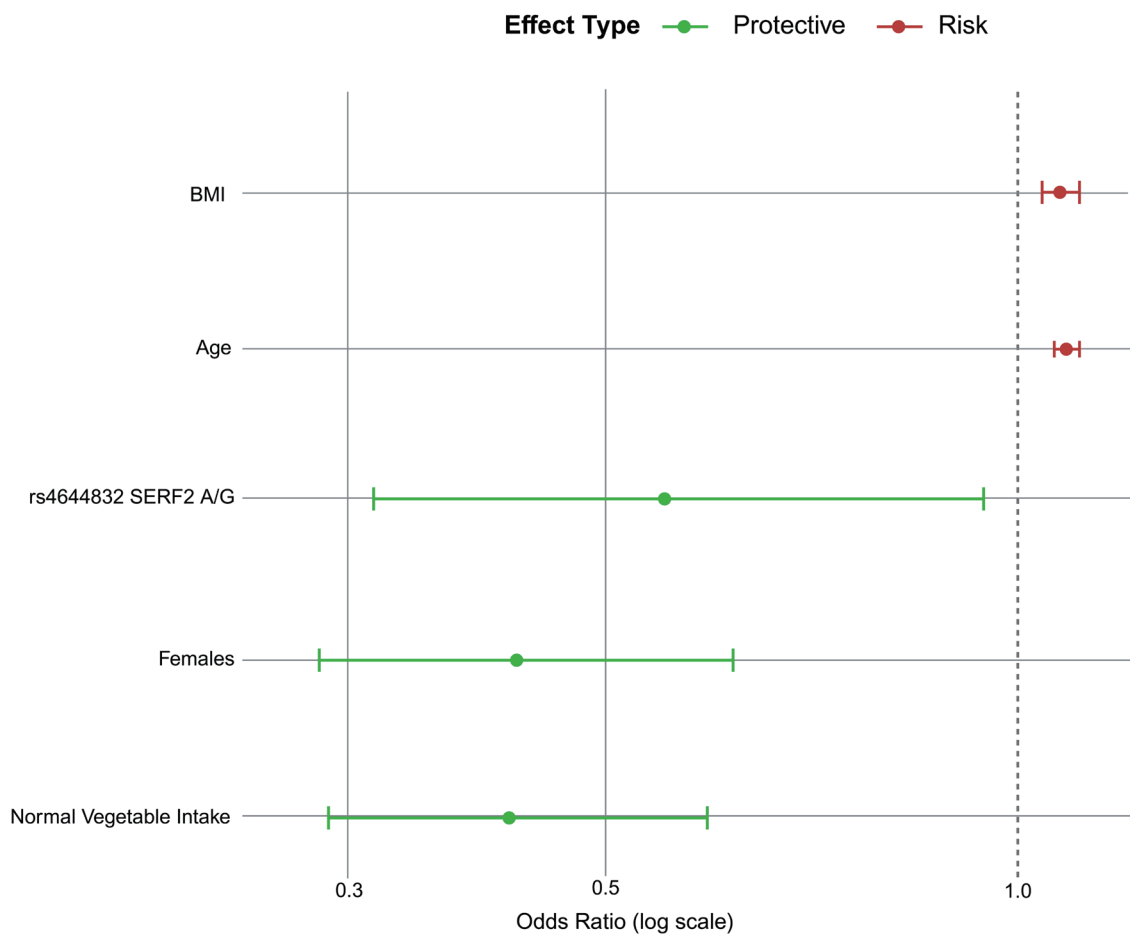
**Table 1.** Statistically significant associations of rs4644832 in *SERF2* with severe COVID-19 in subgroups stratified by sex, smoking status, fruit and vegetable intake, physical activity, and BMI

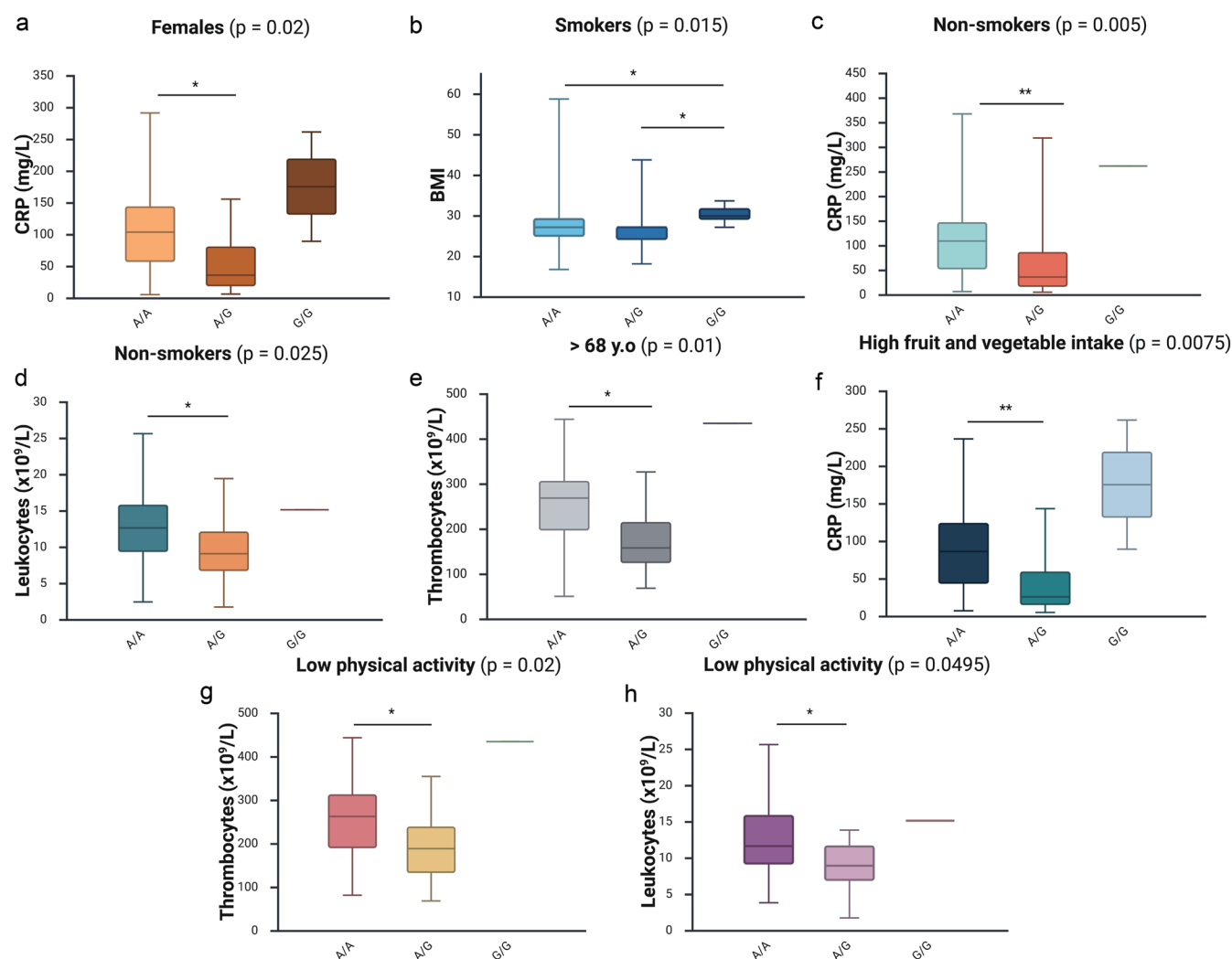
| Genetic variant                | Effect allele | Other allele | N   | OR [95%CI] <sup>1</sup> | P <sup>2</sup> (P <sub>Bonf</sub> ) | N                                 | OR [95%CI] <sup>1</sup> | P <sup>2</sup> (P <sub>Bonf</sub> ) |
|--------------------------------|---------------|--------------|-----|-------------------------|-------------------------------------|-----------------------------------|-------------------------|-------------------------------------|
| Males                          |               |              |     |                         |                                     | Females                           |                         |                                     |
| rs4644832 <i>SERF2</i>         | G             | A            | 591 | 0.62 [0.36–1.05]        | 0.059                               | 782                               | 0.51 [0.31–0.87]        | <b>0.006</b>                        |
| Smokers                        |               |              |     |                         |                                     | Non-smokers                       |                         |                                     |
| rs4644832 <i>SERF2</i>         | G             | A            | 407 | 0.83 [0.44–1.56]        | 0.55                                | 933                               | 0.46 [0.29–0.74]        | <b>0.0004</b>                       |
| Low fruit and vegetable intake |               |              |     |                         |                                     | Normal fruit and vegetable intake |                         |                                     |
| rs4644832 <i>SERF2</i>         | G             | A            | 367 | 0.38 [0.22–0.67]        | <b>0.0002 (0.0004)</b>              | 525                               | 0.87 [0.50–1.52]        | 0.63 (1.0)                          |
| Low physical activity          |               |              |     |                         |                                     | Normal physical activity          |                         |                                     |
| rs4644832 <i>SERF2</i>         | G             | A            | 443 | 0.41 [0.23–0.75]        | <b>0.001 (0.002)</b>                | 453                               | 0.71 [0.43–1.20]        | 0.19 (0.4)                          |
| BMI < 25                       |               |              |     |                         |                                     | BMI ≥ 25                          |                         |                                     |
| rs4644832 <i>SERF2</i>         | G             | A            | 201 | 0.94 [0.49–1.80]        | 0.85 (1.0)                          | 813                               | 0.42 [0.25–0.70]        | <b>0.0002 (0.0004)</b>              |

All calculations were performed relative to the minor alleles (effect allele); <sup>1</sup>odds ratio (OR) and 95% confidence interval (CI); <sup>2</sup>P-value; statistically significant differences are marked in bold. *SERF2* refers to gene encoding small EDRK-rich factor 2.

### Significant Predictors of Severe COVID-19

Odds Ratios with 95% Confidence Intervals

**Fig. 2.** Forest plot of significant predictors of severe COVID-19.



**Fig. 3.** Associations between rs4644832 in *SERF2* and C-reactive protein (CRP) levels in females (a); body mass index (BMI) in smokers (b); CRP levels (c) and leukocyte count (d) in non-smokers; thrombocyte count in the group older than 68 years (e); CRP levels in the high fruit and vegetable intake group (f); and thrombocyte (g) and leukocyte counts (h) in the low physical activity group (figure created using <https://BioRender.com>).

A/A homozygotes were associated with higher C-reactive protein (CRP) levels than heterozygotes ( $P = 0.02$ ).

Notably, rs4644832 influenced clinical features in both smokers and non-smokers. Among smokers, G/G homozygotes had a higher median BMI than A/A homozygotes and heterozygotes ( $P = 0.015$ ). Among non-smokers, A/A homozygotes were associated with higher CRP levels ( $P = 0.005$ ) and higher leukocyte counts ( $P = 0.025$ ) compared to heterozygotes.

In the group aged over 68 years, protective A/A homozygotes were associated with higher thrombocyte counts than heterozygotes ( $P = 0.01$ ).

In the group with high fruit and vegetable intake, A/A homozygotes had higher CRP levels than heterozygotes ( $P = 0.0075$ ). Furthermore, in the low physical activity group, A/A homozygotes showed higher thrombocyte ( $P = 0.02$ ) and leukocyte counts ( $P = 0.0495$ ) compared to heterozygotes (Fig. 3, Table S3).

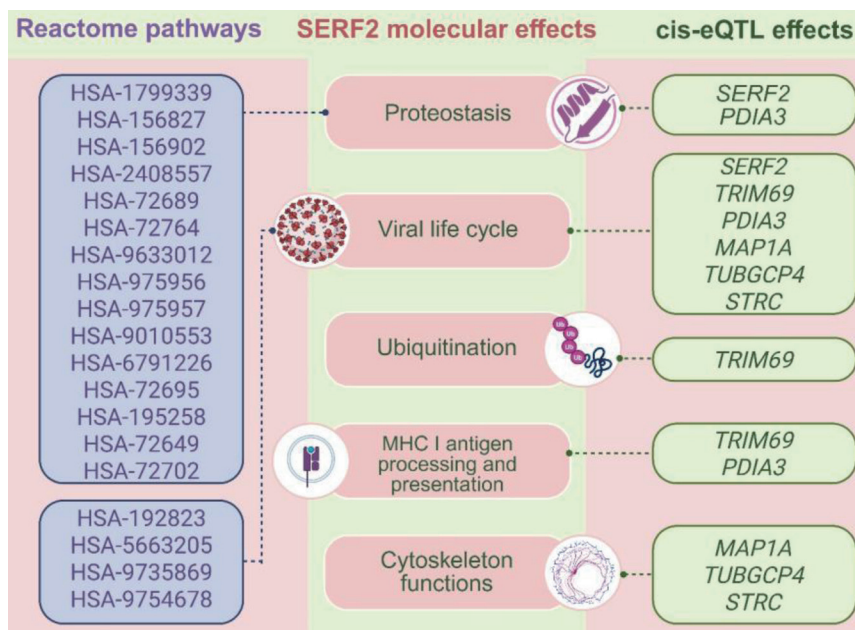
#### Molecular correlates of rs4644832 in *SERF2*

Analysis of cis-eQTL-mediated expression profiles of genes in vari-

ous tissues revealed several relevant associations. The G allele of rs4644832 in *SERF2* decreases expression of *ADAL*, *CATSPER2*, *CATSPER2P1*, *MAP1A*, *STRC*, and *STRCP1* and increases expression of *SERF2*, *ENSG00000249839*, *HYPK*, *PDIA3*, and *ZSCAN29* in lungs, blood vessels, and whole blood (Fig. 4, Table 2).

In whole blood, the G allele of rs4644832 in *SERF2* significantly decreases cis-eQTL-mediated regulation of *SERF2*, *ZSCAN29*, *TUBGCP4*, and *PDIA3*, while it increases cis-eQTL-mediated regulation of *STRCP1*, *LCMT2*, *CATSPER2*, *MAP1A*, *STRC*, *CATSPER2P1*, *ADAL*, and *TRIM69* expression (Fig. 4, Table 3).

According to HaploReg v4.2, rs4644832 in *SERF2* has a significant effect on histone modifications in COVID-19-related tissues, including blood vessels, lung, and blood cells. This variant is located within a DNA region binding to histone H3, characterized by mono-methylation at lysine 4 of histone H3 (H3K4me1), marking enhancers in blood cells, and tri-methylation at lysine 4 (H3K4me3), marking promoters in lungs, aorta, and blood cells. The effect of these histone marks is enhanced by acetylation of lysine 27 of histone H3 (H3K27ac), marking enhancers in lungs,



**Fig. 4. *SERF2* molecular effects and their interconnection with local expression quantitative trait locus (cis-eQTL) effects and Reactome pathways** (figure created using <https://BioRender.com>). Each pathway in Reactome is identified by a stable ID that starts with the specie abbreviation, HSA for homo Sapiens, and ends with a unique ID. MHC, major histocompatibility complex; *SERF2* refers to small EDRK-rich factor 2.

aorta, and blood cells, and by acetylation of lysine 9 of histone H3 (H3K9ac), marking promoters in blood cells. Furthermore, rs4644832 in *SERF2* is located within DNA regions hypersensitive to DNase-1 in blood samples (Table 4).

Bioinformatic resources including the Lung Disease Knowledge Portal (<https://lung.hugeamp.org/>) and Common Metabolic Diseases Knowledge Portal (<https://hugeamp.org/>) were used to analyze correlations between rs4644832 in *SERF2* and phenotypic risk factors for severe COVID-19. It was found that the risk allele A of SNP rs4644832 *SERF2* significantly increased the likelihood of hospitalization in COVID-19 patients. It was also associated with increased CRP levels and monocyte percentage, and decreased eosinophil count, eosinophil percentage, and insulin-like growth factor 1 levels (Table 5).

The protective G allele of rs4644832 in *SERF2* creates DNA binding sites for 46 TFs involved in the following biological processes: positive regulation of CD8-positive, alpha-beta T cell differentiation (GO:0043378; false discovery rate (FDR) = 0.00301), negative regulation of CD4-positive, alpha-beta T cell differentiation (GO:0043371; FDR = 0.0399), cellular response to glucocorticoid stimulus (GO:0071385; FDR = 0.00763), nuclear receptor-mediated steroid hormone signaling pathway (GO:0030518; FDR = 0.01), and lung development (GO:0030324; FDR = 0.0144) (Table S4).

#### Protein-protein interactions of *SERF2*

Analysis of primary functional partners of *SERF2* using the STRING database (protein-protein interactions (PPI) enrichment  $P$ -value:  $3.92 \times 10^{-5}$ ) revealed ten proteins with the most prominent interactions: actin related protein 2/3 complex subunit 2 (ARPC2), boule RNA binding protein (BOLL), huntingtin interacting protein K (HYPK), myosin light chain 6 (MYL6), ribosomal protein L23a (RPL23A), ribosomal protein lateral stalk subunit P1 (RPLP1), ribosomal protein S19 (RPS19), ribosomal protein S27 (RPS27),

signal recognition particle 14 (SRP14), and translation machinery associated 7 homolog (TMA7) (Fig. 5, Table S5).

The full list of biological processes and Reactome pathways involving *SERF2* and its PPI-partners is presented in Table S6. Key pathways include cytoplasmic translation (GO:0002181; FDR = 0.0082), ribosome assembly (GO:0042255; FDR = 0.0395), viral mRNA translation (HSA-192823; FDR = 0.00016), infectious disease (HSA-5663205; FDR = 0.0062), and SARS-CoV-2 modulation of host translation machinery (HSA-9754678; FDR = 0.0254), among others.

#### Discussion

In this study, we provide genetic evidence that rs4644832 of *SERF2* is associated with severe COVID-19. We found that the G allele of rs4644832 *SERF2* has a protective effect against severe COVID-19, and this effect is modified by sex, smoking status, level of fresh fruit and vegetable intake, physical activity, and BMI. We observed the protective effect of allele G rs4644832 *SERF2* exclusively in females and non-smokers. This can be explained by the positive regulation of *SERF2* expression by tobacco components and androgens,<sup>50–53</sup> as previously mentioned.<sup>12</sup> Moreover, several studies confirm that estrogen decreases *SERF2* expression.<sup>54,55</sup>

Additionally, the protective effect of rs4644832 *SERF2* was also observed in subgroups with low fruit and vegetable intake, low physical activity, and overweight (BMI  $\geq 25$ ). We hypothesize that the manifestation of the G allele's protective effect occurs in these subgroups with predisposing risk factors for severe COVID-19 due to the predominance of protective effects from lower BMI, normal fruit and vegetable intake, and regular physical activity.

The role of *SERF2* in human pathology remains to be fully elucidated. Our research group recently discovered a link between rs4644832 *SERF2* and ischemic stroke.<sup>12</sup> In our previous study, we suggested that *SERF2* influences ischemic stroke development

**Table 2.** Effect of the A allele of rs4644832 in *SERF2* on gene expression (cis-eQTL) in various tissues (<https://gtexportal.org>)

| SNP                     | Effect allele | Gene expressed         | P-value                | Effect (NES) | Tissue            |
|-------------------------|---------------|------------------------|------------------------|--------------|-------------------|
| rs4644832, <i>SERF2</i> | A             | <i>ADAL</i>            | 0.00002                | ↓ (-0.29)    | Artery - Aorta    |
|                         |               |                        | $2.00 \times 10^{-7}$  | ↓ (-0.26)    | Artery - Tibial   |
|                         |               | <i>CATSPER2</i>        | $5.30 \times 10^{-7}$  | ↓ (-0.2)     | Artery - Tibial   |
|                         |               |                        | $3.20 \times 10^{-7}$  | ↓ (-0.27)    | Lung              |
|                         |               |                        | 0.00052                | ↓ (-0.12)    | Whole Blood       |
|                         |               | <i>CATSPER2P1</i>      | 0.0001                 | ↓ (-0.22)    | Artery - Tibial   |
|                         |               | <i>ENSG00000249839</i> | $1.00 \times 10^{-16}$ | ↑ (0.68)     | Artery - Aorta    |
|                         |               |                        | $1.90 \times 10^{-8}$  | ↑ (0.6)      | Artery - Coronary |
|                         |               |                        | $2.80 \times 10^{-10}$ | ↑ (0.41)     | Artery - Tibial   |
|                         |               |                        | $3.40 \times 10^{-8}$  | ↑ (0.4)      | Lung              |
|                         |               | <i>HYPK</i>            | $8.1 \times 10^{-6}$   | ↑ (0.1)      | Lung              |
|                         |               | <i>MAP1A</i>           | $7.8 \times 10^{-6}$   | ↓ (-0.17)    | Artery - Aorta    |
|                         |               | <i>PDIA3</i>           | $2.70 \times 10^{-7}$  | ↑ (0.1)      | Whole Blood       |
|                         |               | <i>SERF2</i>           | $1.1 \times 10^{-6}$   | ↑ (0.14)     | Artery - Aorta    |
|                         |               |                        | 0.00015                | ↑ (0.16)     | Artery - Coronary |
|                         |               |                        | $8.60 \times 10^{-7}$  | ↑ (0.088)    | Artery - Tibial   |
|                         |               |                        | $6.40 \times 10^{-7}$  | ↑ (0.11)     | Lung              |
|                         |               |                        | $1.60 \times 10^{-10}$ | ↑ (0.1)      | Whole Blood       |
|                         |               | <i>STRC</i>            | $1.30 \times 10^{-8}$  | ↓ (-0.32)    | Artery - Aorta    |
|                         |               |                        | $3.90 \times 10^{-7}$  | ↓ (-0.27)    | Artery - Tibial   |
|                         |               |                        | $2.30 \times 10^{-11}$ | ↓ (-0.37)    | Lung              |
|                         |               | <i>STRCP1</i>          | $2.50 \times 10^{-7}$  | ↓ (-0.31)    | Artery - Aorta    |
|                         |               |                        | $5.00 \times 10^{-8}$  | ↓ (-0.28)    | Artery - Tibial   |
|                         |               |                        | 0.00002                | ↓ (-0.24)    | Lung              |
|                         |               | <i>ZSCAN29</i>         | 0.00014                | ↑ (0.13)     | Lung              |
|                         |               |                        | 0.00004                | ↑ (0.1)      | Whole Blood       |

NES, normalized effect size; *SERF2*, gene encoding small EDRK-rich factor 2; SNP, single nucleotide polymorphism; ↑, increase the n effect; ↓, decrease in the effect.

**Table 3.** Effect of the G allele of rs4644832 in *SERF2* on gene expression (cis-eQTL) in whole blood (<https://www.eqtigen.org/cis-eqtls.html>)

| SNP                     | Allele | Gene expressed    | Z-score      | P-value                 | FDR   |
|-------------------------|--------|-------------------|--------------|-------------------------|-------|
| rs4644832, <i>SERF2</i> | G      | <i>ZSCAN29</i>    | ↓ (-46.5249) | $3.27 \times 10^{-310}$ | <0.05 |
|                         |        | <i>SERF2</i>      | ↓ (-26.1112) | $2.72 \times 10^{-150}$ | <0.05 |
|                         |        | <i>TUBGCP4</i>    | ↓ (-23.7631) | $8.03 \times 10^{-125}$ | <0.05 |
|                         |        | <i>STRCP1</i>     | ↑ (20.6369)  | $1.28 \times 10^{-94}$  | <0.05 |
|                         |        | <i>LCMT2</i>      | ↑ (18.2518)  | $2.01 \times 10^{-74}$  | <0.05 |
|                         |        | <i>CATSPER2</i>   | ↑ (11.8697)  | $1.70 \times 10^{-32}$  | <0.05 |
|                         |        | <i>PDIA3</i>      | ↓ (-10.7944) | $3.65 \times 10^{-27}$  | <0.05 |
|                         |        | <i>MAP1A</i>      | ↑ (9.1256)   | $7.13 \times 10^{-20}$  | <0.05 |
|                         |        | <i>STRC</i>       | ↑ (9.0382)   | $1.59 \times 10^{-19}$  | <0.05 |
|                         |        | <i>CATSPER2P1</i> | ↑ (8.2227)   | $1.99 \times 10^{-16}$  | <0.05 |
|                         |        | <i>ADAL</i>       | ↑ (6.7174)   | $1.85 \times 10^{-11}$  | <0.05 |
|                         |        | <i>TRIM69</i>     | ↑ (6.2919)   | $3.14 \times 10^{-10}$  | <0.05 |

eQTL, expression of quantitative trait locus; FDR, false discovery rate; SNP, single nucleotide polymorphism; ↑, increase in Z-score; ↓, decrease in Z-score.

**Table 4.** Tissue-specific effects of rs4644832 in *SERF2* on histone modifications (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>)

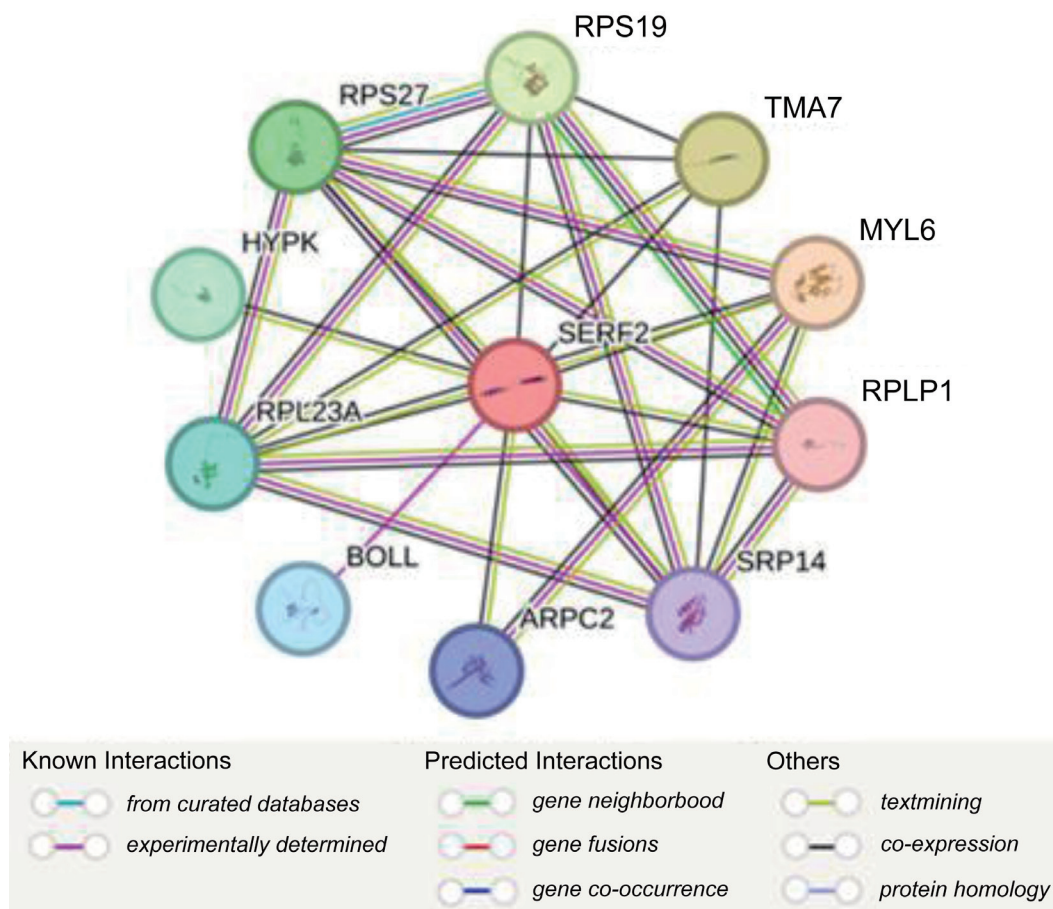
| Tissue          | H3K4me1 | H3K4me3 | H3K27ac | H3K9ac | DNase |
|-----------------|---------|---------|---------|--------|-------|
| Lung            | –       | Pro     | Enh     | –      | –     |
| Vessels – aorta | –       | Pro     | Enh     | –      | –     |
| Blood           | Enh     | Pro     | Enh     | Pro    | DNase |

Enh, enhancer; Pro, promoter.

**Table 5.** Summary of associations between rs4644832 in *SERF2* and phenotypes linked to a severe course of COVID-19

| No | SNP                           | Phenotype                                              | P-value                | Beta (OR)                         | Sample Size |
|----|-------------------------------|--------------------------------------------------------|------------------------|-----------------------------------|-------------|
| 1  | rs4644832, <i>SERF2</i> (A/G) | Hospitalized vs non-hospitalized COVID-19 <sup>1</sup> | 0.008                  | OR $\blacktriangle$ 1.0641        | 52 246      |
| 2  |                               | Eosinophil percentage <sup>2</sup>                     | $2.27 \times 10^{-16}$ | Beta $\blacktriangledown$ -0.0033 | 1 334 180   |
| 3  |                               | Eosinophil count <sup>2</sup>                          | $2.00 \times 10^{-12}$ | Beta $\blacktriangledown$ -0.0027 | 1 794 490   |
| 5  |                               | Insulin-like growth factor (IGF-1) <sup>2</sup>        | 0.00001                | Beta $\blacktriangledown$ -0.0045 | 445 573     |
| 7  |                               | Plasma C-reactive protein <sup>2</sup>                 | 0.0002                 | Beta $\blacktriangle$ 0.0052      | 1 215 140   |
| 9  |                               | Monocyte percentage <sup>2</sup>                       | 0.03                   | Beta $\blacktriangle$ 0.0053      | 853 181     |

The effect allele is marked in bold. <sup>1</sup>Data from Lung Disease Knowledge Portal; <sup>2</sup>Data from Common Metabolic Diseases Knowledge Portal. OR, odds ratio; *SERF2*, gene encoding small EDRK-rich factor 2; SNP, single nucleotide polymorphism.



**Fig. 5.** Predicted functional partners of *SERF2* according to the STRING database (PPI enrichment  $P$ -value:  $3.92 \times 10^{-5}$ ). ARPC2, actin related protein 2/3 complex subunit 2; BOLL, boule RNA binding protein; HYPK, huntingtin interacting protein K; MYL6, myosin light chain 6; RPL23A, ribosomal protein L23a; PPI, protein-protein interactions; RPLP1, ribosomal protein lateral stalk subunit P1; RPS19, ribosomal protein S19; RPS27, ribosomal protein S27; SRP14, signal recognition particle 14; TMA7, translation machinery associated 7 homolog.

through its ability to maintain proteome quality,<sup>12</sup> which is also essential in the context of COVID-19.<sup>56</sup>

*SERF2* plays a dual role in proteostasis. On one hand, it accelerates amyloid formation.<sup>15–17</sup> Persistent fibrin amyloid microclots obstruct small vessels and inhibit tissue oxygenation, potentially leading to thrombotic complications and post-acute sequelae of COVID-19.<sup>57</sup> On the other hand, *SERF2* prevents TDP-43 aggregation.<sup>13</sup> TDP-43 acts as a regulator of viral RNA expression due to its ability to interact with ribonucleoprotein complexes.<sup>19,58,59</sup> Viral infections trigger TDP-43 aggregation; for instance, the SARS-CoV-2 main protease induces neurotoxic TDP-43 aggregation,<sup>20</sup> and its spike S1 protein receptor-binding domain (SARS-CoV-2 S1 RBD) binds to TDP-43,<sup>60</sup> suggesting TDP-43 involvement in the neurological symptoms of COVID-19.<sup>19</sup>

In cis-eQTL analysis, we have found that the risk allele A of rs4644832 upregulates *SERF2* expression in blood vessels, lung, and whole blood. Therefore, it may be associated with increased proteotoxicity and amyloid clot formation. Furthermore, increased TDP-43 stability caused by high *SERF2* expression can promote the viral life cycle.<sup>59</sup>

Some genes influenced by rs4644832 *SERF2* cis-eQTL effects are linked to ubiquitination, which is essential for protein synthesis, signaling, and innate and adaptive immune responses.<sup>61</sup> Efficient ubiquitination followed by degradation of SARS-CoV-2 spike protein ensures an antiviral response.<sup>62</sup> Recent studies have shown that high ubiquitination levels are associated with a favorable COVID-19 prognosis due to proper immune regulation and prevention of immune damage.<sup>63</sup> The G allele of rs4644832 *SERF2* upregulates *TRIM69*, which encodes a human E3 ubiquitin-protein ligase critical for antiviral immunity, mediating major histocompatibility complex class I (MHC I) antigen processing and presentation.<sup>64–67</sup> It is worth mentioning that *SERF2* itself, conversely, prevents degradation of ubiquitinated proteins.<sup>68</sup>

According to GTExPortal data, the risk allele A of rs4644832 *SERF2* increases *PDIA3* expression via cis-eQTL effects. *PDIA3* encodes a molecular chaperone that is a core component of major histocompatibility complex class I and is heavily implicated in antigen presentation to cytotoxic T lymphocytes.<sup>69–72</sup> Overexpression of *PDIA3* enhances Wnt/ $\beta$ -catenin signaling,<sup>73,74</sup> which is involved in triggering the cytokine storm.<sup>75</sup> Furthermore, recent studies confirm that *PDIA3* disulfide isomerase activity participates in the viral life cycle<sup>76</sup>; its deletion significantly decreases viral load and inflammatory cytokine levels.<sup>77,78</sup> Considering that the G allele of rs4644832 *SERF2* reduces *PDIA3* levels, this may explain the SNP's protective effect against a severe course of COVID-19.

Moreover, in cis-eQTL analysis, we discovered that rs4644832 of *SERF2* modulates the expression of several genes involved in maintaining cytoskeleton function, including *MAP1A*,<sup>79</sup> *TUBGCP4*,<sup>80</sup> and *STRC*.<sup>81</sup> The cytoskeleton ensures the structural integrity of the endothelial barrier; thus, endothelial dysfunction, one of the major pathological features of severe COVID-19,<sup>27,28</sup> is believed to be associated with cytoskeleton disorganization.<sup>82</sup> Viruses frequently depend on microtubules at multiple stages of their life cycle. SARS-CoV-2 employs the host cytoskeleton for virion transport, cell-to-cell spread, and disruption of the immune response.<sup>83</sup>

Notably, *SERF2* and its functional partners in the PPI network are associated with viral mRNA translation and modulation of the host translation machinery by SARS-CoV-2. This discovery provides evidence of *SERF2*'s pivotal role in COVID-19 pathogenesis.

We accessed data from the Lung Disease Knowledge Portal and the Common Metabolic Diseases Knowledge Portal to iden-

tify associations between rs4644832 *SERF2* and phenotypical risk factors for severe COVID-19. It was found that the risk A allele is associated with an increased likelihood of hospitalization in COVID-19 patients. Moreover, the A allele of rs4644832 *SERF2* increases certain laboratory traits typical of severe COVID-19, such as monocyte percentage and CRP levels.

Elevation in monocyte proportion can be linked to the COVID-19-associated cytokine storm. The cytokine storm in COVID-19 differs considerably from the canonical cytokine storm seen in macrophage activation syndrome in other infectious diseases.<sup>84</sup> In COVID-19, the atypical cytokine storm is orchestrated mainly by monocytes, whereas in macrophage activation syndrome, macrophages play the predominant role.<sup>85</sup>

Our analysis of COVID-19 clinical features corresponds with data from the Common Metabolic Diseases Knowledge Portal. It shows a correlation between the protective G allele of rs4644832 *SERF2* and decreased CRP levels in females and non-smokers. CRP is one of the most accurate and sensitive markers of inflammation.<sup>86,87</sup> It is synthesized in the liver in response to pro-inflammatory cytokines, such as interleukin-6, interleukin-1 $\beta$ , and tumor necrosis factor- $\alpha$ .<sup>88</sup> In COVID-19, CRP levels have an indisputable prognostic and diagnostic value.<sup>89</sup>

To further explore the molecular mechanisms by which rs4644832 *SERF2* influences COVID-19 pathogenesis, we analyzed TFs that bind to the DNA region containing the studied SNP and their associated biological processes. These TFs are involved in positive regulation of CD8-positive, alpha-beta T-cell differentiation (GO:0043378), negative regulation of CD4-positive, alpha-beta T-cell differentiation (GO:0043371), cellular response to glucocorticoid stimulus (GO:0071385), nuclear receptor-mediated steroid hormone signaling pathway (GO:0030518), and lung development (GO:003032).

CD8+ T-cells, or cytotoxic T-cells, are involved in eliminating cells infected by intracellular pathogens.<sup>90</sup> CD4+ T-cells, or T-helper cells, play an important role in the adaptive immune response against intracellular pathogens.<sup>91</sup>

The efficacy of glucocorticoids in managing hyperimmune responses in COVID-19 patients is well established. They are effective immunosuppressors and are widely used to treat hypoxic respiratory failure and ARDS.<sup>92</sup> Glucocorticoids reduce inflammation by regulating cytokine gene transcription and proinflammatory pathways.<sup>93</sup> Activation of the cellular response to glucocorticoid stimulus and the nuclear receptor-mediated steroid hormone signaling pathway, promoted by the protective G allele of rs4644832 *SERF2*, may consequently enhance the efficacy of glucocorticoid treatment in severe COVID-19 cases.

Our study has several limitations. Firstly, since a COVID-19 diagnosis can be based on clinical evidence, the control group includes both PCR-confirmed and clinically confirmed cases, which may lead to misclassification. Secondly, our study is limited to the Russian population; therefore, confirming our results requires studies in diverse populations. Furthermore, we did not measure gene expression levels in the blood of *SERF2* genotype carriers. Instead, we used bioinformatic tools to assess cis-eQTL effects of SNPs on gene expression. Similarly, histone modifications and TFs binding were analyzed exclusively using bioinformatic resources.

## Conclusions

The present study reveals the protective effect of the G allele of rs4644832 *SERF2* against severe COVID-19. These results provide novel insights into the involvement of Hero proteins in the

pathogenesis of viral infections. Further studies may help uncover the detailed role of SERF2 in COVID-19 pathogenesis.

### Acknowledgments

None.

### Funding

No funding was received to assist with the preparation of this manuscript.

### Conflict of interest

All authors declare no conflicts of interest.

### Author contributions

Study design (OB), performance of experiments, analysis and interpretation of data, statistical analysis (AD, MI, KK), manuscript writing (AD), critical revision, administrative, technical, and material support, and study supervision (OB). All authors have made significant contributions to this study and have approved the final manuscript.

### Ethical statement

The study was conducted according to the guidelines of the Declaration of Helsinki (as revised in 2024) and was approved by the Ethical Review Committee of Kursk State Medical University (protocol №1 from January 11, 2022). Informed consent was obtained from all individual participants included in the study.

### Data sharing statement

No additional data are available.

### References

- [1] World Health Organization. COVID-19 deaths | WHO COVID-19 dashboard. COVID-19 Cases, World. Available from: <https://data.who.int/dashboards/covid19/cases>. Accessed January 12, 2025.
- [2] Montazersaheb S, Hosseiniyan Khatibi SM, Hejazi MS, Tarhriz V, Farjani A, Ghasemian Sorbeni F, *et al*. COVID-19 infection: an overview on cytokine storm and related interventions. *Viral J* 2022;19(1):92. doi:10.1186/s12985-022-01814-1, PMID:35619180.
- [3] Bolhassani A, Agi E. Heat shock proteins in infection. *Clin Chim Acta* 2019;498:90–100. doi:10.1016/j.cca.2019.08.015, PMID:31437446.
- [4] Danladi J, Sabir H. Innate immunity, inflammation activation and heat-shock protein in COVID-19 pathogenesis. *J Neuroimmunol* 2021;358:577632. doi:10.1016/j.jneuroim.2021.577632, PMID:34186336.
- [5] Binder RJ. Functions of heat shock proteins in pathways of the innate and adaptive immune system. *J Immunol* 2014;193(12):5765–5771. doi:10.4049/jimmunol.1401417, PMID:25480955.
- [6] Cappello F, Gammazza AM, Dieli F, Conway de Macario AJ. Does SARS-CoV-2 Trigger Stress-Induced Autoimmunity by Molecular Mimicry? A Hypothesis. *J Clin Med* 2020;9(7):2038. doi:10.3390/jcm9072038, PMID:32610587.
- [7] Lucchese G, Flöel A. SARS-CoV-2 and Guillain-Barré syndrome: molecular mimicry with human heat shock proteins as potential pathogenic mechanism. *Cell Stress Chaperones* 2020;25(5):731–735. doi:10.1007/s12192-020-01145-6, PMID:32729001.
- [8] Angileri F, Legare S, Marino Gammazza A, Conway de Macario E, JL Macario A, Cappello F. Molecular mimicry may explain multi-organ damage in COVID-19. *Autoimmun Rev* 2020;19(8):102591. doi:10.1016/j.autrev.2020.102591, PMID:32535095.
- [9] Marino Gammazza A, Légaré S, Lo Bosco G, Fucarino A, Angileri F, Oliveri M, *et al*. Molecular mimicry in the post-COVID-19 signs and symptoms of neurovegetative disorders? *Lancet Microbe* 2021;2(3):e94. doi:10.1016/S2666-5247(21)00033-1, PMID:35544159.
- [10] Paladino L, Vitale AM, Caruso Bavisotto C, Conway de Macario E, Cappello F, Macario AJL, *et al*. The Role of Molecular Chaperones in Virus Infection and Implications for Understanding and Treating COVID-19. *J Clin Med* 2020;9(11):3518. doi:10.3390/jcm9113518, PMID:33143379.
- [11] Morimoto E, Tsuboyama K, Tomari Y. Fusion with heat-resistant obscure (Hero) proteins have the potential to improve the molecular property of recombinant proteins. *PLoS One* 2022;17(6):e0270097. doi:10.1371/journal.pone.0270097, PMID:35714106.
- [12] Belykh AE, Soldatov VO, Stetskaya TA, Kobzeva KA, Soldatova MO, Polonikov AV, *et al*. Polymorphism of SERF2, the gene encoding a heat-resistant obscure (Hero) protein with chaperone activity, is a novel link in ischemic stroke. *IBRO Neurosci Rep* 2023;14:453–461. doi:10.1016/j.ibneur.2023.05.004, PMID:37252629.
- [13] Tsuboyama K, Osaki T, Matsuura-Suzuki E, Kozuka-Hata H, Okada Y, Oyama M, *et al*. A widespread family of heat-resistant obscure (Hero) proteins protect against protein instability and aggregation. *PLoS Biol* 2020;18(3):e3000632. doi:10.1371/journal.pbio.3000632, PMID:32163402.
- [14] Qi S, Peng Y, Wang G, Zhang X, Liu M, He L. A tale of dual functions of SERF family proteins in regulating amyloid formation. *ChemBiochem* 2024;25(5):e202300727. doi:10.1002/cbic.202300727, PMID:38100267.
- [15] Cleverley K, Lee WC, Mumford P, Collins T, Rickman M, Cunningham TJ, *et al*. A novel knockout mouse for the small EDRK-rich factor 2 (Serf2) showing developmental and other deficits. *Mamm Genome* 2021;32(2):94–103. doi:10.1007/s00335-021-09864-6, PMID:33713180.
- [16] van Ham TJ, Holmberg MA, van der Goot AT, Teuling E, Garcia-Arencibia M, Kim HE, *et al*. Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. *Cell* 2010;142(4):601–612. doi:10.1016/j.cell.2010.07.020, PMID:20723760.
- [17] Falsone SF, Meyer NH, Schrank E, Leitinger G, Pham CL, Fodero-Tavolletti MT, *et al*. SERF protein is a direct modifier of amyloid fiber assembly. *Cell Rep* 2012;2(2):358–371. doi:10.1016/j.celrep.2012.06.012, PMID:22854022.
- [18] Sahoo BR, Bardwell JCA. SERF, a family of tiny highly conserved, highly charged proteins with enigmatic functions. *FEBS J* 2023;290(17):4150–4162. doi:10.1111/febs.16555, PMID:35694898.
- [19] Rahic Z, Buratti E, Cappelli S. Reviewing the Potential Links between Viral Infections and TDP-43 Proteinopathies. *Int J Mol Sci* 2023;24(2):1581. doi:10.3390/ijms24021581, PMID:36675095.
- [20] Yang J, Li Y, Wang S, Li H, Zhang L, Zhang H, *et al*. The SARS-CoV-2 main protease induces neurotoxic TDP-43 cleavage and aggregates. *Signal Transduct Target Ther* 2023;8(1):109. doi:10.1038/s41392-023-01386-8, PMID:36894543.
- [21] Bartas M, Červeň J, Guziurová S, Slychko K, Pečinka P. Amino Acid Composition in Various Types of Nucleic Acid-Binding Proteins. *Int J Mol Sci* 2021;22(2):922. doi:10.3390/ijms22020922, PMID:33477647.
- [22] Sahoo BR, Kocman V, Clark N, Myers N, Deng X, Wong EL, *et al*. Protein G-quadruplex interactions and their effects on phase transitions and protein aggregation. *Nucleic Acids Res* 2024;52(8):4702–4722. doi:10.1093/nar/gkae229, PMID:38572746.
- [23] Nchioua R, Kmiec D, Müller JA, Conzelmann C, Groß R, Swanson CM, *et al*. SARS-CoV-2 Is Restricted by Zinc Finger Antiviral Protein despite Preadaptation to the Low-CpG Environment in Humans. *mBio* 2020;11(5):e01930–20. doi:10.1128/mBio.01930-20, PMID:33067384.
- [24] Esposito S, D'Ambrosia G, Antolak A, Pedone PV, Isernia C, Malgieri G. Host and Viral Zinc-Finger Proteins in COVID-19. *Int J Mol Sci* 2022;23(7):3711. doi:10.3390/ijms23073711, PMID:35409070.
- [25] Shilenok VV, Shilenok IV, Soldatov VO, Orlov YL, Kobzeva KA, Deykin

- AV, *et al.* Bioinformatic analysis of the regulatory potential of tagging SNPs provides evidence of the involvement of genes encoding the heat-resistant obscure (Hero) proteins in the pathogenesis of cardiovascular diseases. *J Integr Bioinform* 2025;22(1):20240043. doi:10.1515/jib-2024-0043, PMID:40459864.
- [26] Chernyak BV, Popova EN, Prikhodko AS, Grebenchikov OA, Zinovkina LA, Zinovkin RA. COVID-19 and Oxidative Stress. *Biochemistry (Mosc)* 2020;85(12):1543–1553. doi:10.1134/S0006297920120068, PMID:33705292.
- [27] Fodor A, Tiperciuc B, Login C, Orasan OH, Lazar AL, Buchman C, *et al.* Endothelial Dysfunction, Inflammation, and Oxidative Stress in COVID-19-Mechanisms and Therapeutic Targets. *Oxid Med Cell Longev* 2021;2021:8671713. doi:10.1155/2021/8671713, PMID:34457119.
- [28] McAlpine LS, Zubair AS, Maran I, Chojecka P, Lleva P, Jasne AS, *et al.* Ischemic Stroke, Inflammation, and Endotheliopathy in COVID-19 Patients. *Stroke* 2021;52(6):e233–e238. doi:10.1161/STROKEAHA.120.031971, PMID:33966492.
- [29] Valencia I, Lumpuy-Castillo J, Magalhaes G, Sánchez-Ferrer CF, Lorenzo Ó, Peiró C. Mechanisms of endothelial activation, hypercoagulation and thrombosis in COVID-19: a link with diabetes mellitus. *Cardiovasc Diabetol* 2024;23(1):75. doi:10.1186/s12933-023-02097-8, PMID:38378550.
- [30] Soldatov V, Venediktov A, Belykh A, Piavchenko G, Naimzada MD, Ogneva N, *et al.* Chaperones vs. oxidative stress in the pathobiology of ischemic stroke. *Front Mol Neurosci* 2024;17:1513084. doi:10.3389/fnmol.2024.1513084, PMID:39723236.
- [31] Loktionov AV, Kobzeva KA, Karpenko AR, Sergeeva VA, Orlov YL, Bushueva OY. GWAS-significant loci and severe COVID-19: analysis of associations, link with thromboinflammation syndrome, gene-gene, and gene-environmental interactions. *Front Genet* 2024;15:1434681. doi:10.3389/fgene.2024.1434681, PMID:39175753.
- [32] Kobzeva KA, Gurtovoy DE, Polonikov AV, Pokrovsky VM, Patrakhanov EA, Bushueva OY. Polymorphism in Genes Encoding HSP40 Family Proteins is Associated with Ischemic Stroke Risk and Brain Infarct Size: A Pilot Study. *J Integr Neurosci* 2024;23(12):211. doi:10.31083/j.jin2312211, PMID:39735968.
- [33] Shilenok I, Kobzeva K, Stetskaya T, Freidin M, Soldatova M, Deykin A, *et al.* SERPINE1 mRNA Binding Protein 1 Is Associated with Ischemic Stroke Risk: A Comprehensive Molecular-Genetic and Bioinformatics Analysis of SERBP1 SNPs. *Int J Mol Sci* 2023;24(10):8716. doi:10.3390/ijms24108716, PMID:37240062.
- [34] Loktionov A, Kobzeva K, Dorofeeva A, Sergeeva V, Bushueva O. GWAS-Identified Loci are Associated with Obesity and Type 2 Diabetes Mellitus in Patients with Severe COVID-19. *Front Biosci (Schol Ed)* 2024;16(3):14. doi:10.31083/j.fbs1603014, PMID:39344392.
- [35] World Health Organization. Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* 2003;916:i-viii, 1-149, backcover. PMID:12768890.
- [36] World Health Organization. Physical activity. Available from: <https://www.who.int/europe/news-room/fact-sheets/item/physical-activity>. Accessed February 11, 2025.
- [37] GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* 2020;369(6509):1318–1330. doi:10.1126/science.aaz1776, PMID:32913098.
- [38] Shilenok I, Kobzeva K, Soldatov V, Deykin A, Bushueva O. C11orf58 (Hero20) Gene Polymorphism: Contribution to Ischemic Stroke Risk and Interactions with Other Heat-Resistant Obscure Chaperones. *Biomedicines* 2024;12(11):2603. doi:10.3390/biomedicines12112603, PMID:39595169.
- [39] Vösa U, Claringbould A, Westra HJ, Bonder MJ, Deelen P, Zeng B, *et al.* Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet* 2021;53(9):1300–1310. doi:10.1038/s41588-021-00913-z, PMID:34475573.
- [40] Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40(Database issue):D930–D934. doi:10.1093/nar/gkr917, PMID:22064851.
- [41] Kobzeva KA, Soldatova MO, Stetskaya TA, Soldatov VO, Deykin AV, Freidin MB, *et al.* Association between HSPA8 Gene Variants and Ischemic Stroke: A Pilot Study Providing Additional Evidence for the Role of Heat Shock Proteins in Disease Pathogenesis. *Genes (Basel)* 2023;14(6):1171. doi:10.3390/genes14061171, PMID:37372351.
- [42] Kobzeva KA, Shilenok IV, Belykh AE, Gurtovoy DE, Bobyleva, Kraviva AB, *et al.* C9orf16 (BBLN) gene, encoding a member of Hero proteins, is a novel marker in ischemic stroke risk. *Research Results in Biomedicine* 2022;8(3):278–292. doi:10.18413/2658-6533-2022-8-3-0-2.
- [43] Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* 2015;518(7539):317–330. doi:10.1038/nature14248, PMID:25693563.
- [44] Shin S, Hudson R, Harrison C, Craven M, Keleş S. atSNP Search: a web resource for statistically evaluating influence of human genetic variation on transcription factor binding. *Bioinformatics* 2019;35(15):2657–2659. doi:10.1093/bioinformatics/bty1010, PMID:30534948.
- [45] Shilenok I, Kobzeva K, Deykin A, Pokrovsky V, Patrakhanov E, Bushueva O. Obesity and Environmental Risk Factors Significantly Modify the Association between Ischemic Stroke and the Hero Chaperone C19orf53. *Life (Basel)* 2024;14(9):1158. doi:10.3390/life14091158, PMID:39337941.
- [46] The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. *Nucleic Acids Res* 2019;47(D1):D330–D338. doi:10.1093/nar/gky1055, PMID:30395331.
- [47] Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, *et al.* The STRING database in 2025: protein networks with directionality of regulation. *Nucleic Acids Res* 2025;53(D1):D730–D737. doi:10.1093/nar/gkae1113, PMID:39558183.
- [48] Stetskaya TA, Kobzeva KA, Zaytsev SM, Shilenok IV, Komkova GV, Goryainova NV, *et al.* HSPD1 gene polymorphism is associated with an increased risk of ischemic stroke in smokers. *Research Results in Biomedicine* 2024;10:175–186. doi:10.18413/2658-6533-2024-10-2-0-1.
- [49] Davis AP, Wiegiers TC, Johnson RJ, Sciaky D, Wiegiers J, Mattingly CJ. Comparative Toxicogenomics Database (CTD): update 2023. *Nucleic Acids Res* 2023;51(D1):D1257–D1262. doi:10.1093/nar/gkac833, PMID:36169237.
- [50] Takami T, Kaposi-Novak P, Uchida K, Gomez-Quiroz LE, Conner EA, Factor VM, *et al.* Loss of hepatocyte growth factor/c-Met signaling pathway accelerates early stages of N-nitrosodiethylamine induced hepatocarcinogenesis. *Cancer Res* 2007;67(20):9844–9851. doi:10.1158/0008-5472.CAN-07-1905, PMID:17942915.
- [51] Jiang CL, He SW, Zhang YD, Duan HX, Huang T, Huang YC, *et al.* Air pollution and DNA methylation alterations in lung cancer: A systematic and comparative study. *Oncotarget* 2017;8(1):1369–1391. doi:10.18632/oncotarget.13622, PMID:27901495.
- [52] Gonzalez-Rivera JC, Baldrige KC, Wang DS, Patel K, Chuvalo-Abraham JCL, Hildebrandt Ruiz L, *et al.* Post-transcriptional air pollution oxidation to the cholesterol biosynthesis pathway promotes pulmonary stress phenotypes. *Commun Biol* 2020;3(1):392. doi:10.1038/s42003-020-01118-6, PMID:32699268.
- [53] Bereketoglu C, Modig C, Pradhan A, Andersson PL, Stasinopoulou S, Mitsiou DJ, *et al.* The brominated flame retardants TBEC and DPTE alter prostate growth, histology and gene expression patterns in the mouse. *Reprod Toxicol* 2021;102:43–55. doi:10.1016/j.reprotox.2021.04.002, PMID:33848595.
- [54] Gertz J, Reddy TE, Varley KE, Garabedian MJ, Myers RM. Genistein and bisphenol A exposure cause estrogen receptor 1 to bind thousands of sites in a cell type-specific manner. *Genome Res* 2012;22(11):2153–2162. doi:10.1101/gr.135681.111, PMID:23019147.
- [55] Wang Q, Gao S, Chen B, Zhao J, Li W, Wu L. Evaluating the Effects of Perinatal Exposures to BPSIP on Hepatic Cholesterol Metabolism in Female and Male Offspring ICR Mice. *Environ Health Perspect* 2024;132(9):97011. doi:10.1289/EHP14643, PMID:39298647.
- [56] Khomari F, Nabi-Afjadi M, Yarahmadi S, Eskandari H, Bahreini E. Effects of Cell Proteostasis Network on the Survival of SARS-CoV-2. *Biol Proced Online* 2021;23(1):8. doi:10.1186/s12575-021-00145-9, PMID:33618659.
- [57] Pretorius E, Venter C, Laubscher GJ, Kotze MJ, Oladejo SO, Watson LR, *et al.* Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with Long COVID/Post-Acute Sequelae of COVID-19 (PASC). *Cardiovasc Diabetol* 2022;21(1):148. doi:10.1186/s12933-022-01579-5, PMID:35933347.

- [58] Fiesel FC, Weber SS, Supper J, Zell A, Kahle PJ. TDP-43 regulates global translational yield by splicing of exon junction complex component SKAR. *Nucleic Acids Res* 2012;40(6):2668–2682. doi:10.1093/nar/ gkr1082, PMID:22121224.
- [59] Avendaño-Vázquez SE, Dhir A, Bembich S, Buratti E, Proudfoot N, Baralle FE. Autoregulation of TDP-43 mRNA levels involves interplay between transcription, splicing, and alternative polyA site selection. *Genes Dev* 2012;26(15):1679–1684. doi:10.1101/gad.194829.112, PMID:22855830.
- [60] Idrees D, Kumar V. SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration. *Biochem Biophys Res Commun* 2021;554:94–98. doi:10.1016/j. bbr.2021.03.100, PMID:33789211.
- [61] Oh E, Akopian D, Rape M. Principles of Ubiquitin-Dependent Signaling. *Annu Rev Cell Dev Biol* 2018;34:137–162. doi:10.1146/annurev-cellbio-100617-062802, PMID:30110556.
- [62] Zhao M, Zhang M, Yang Z, Zhou Z, Huang J, Zhao B. Role of E3 ubiquitin ligases and deubiquitinating enzymes in SARS-CoV-2 infection. *Front Cell Infect Microbiol* 2023;13:1217383. doi:10.3389/fcimb.2023.1217383, PMID:37360529.
- [63] Che Y, Jiang D, Zhang Y, Zhang J, Xu T, Sun Y, *et al.* Elevated ubiquitination contributes to protective immunity against severe SARS-CoV-2 infection. *Clin Transl Med* 2022;12(12):e1103. doi:10.1002/ctm2.1103, PMID:36447039.
- [64] Kueck T, Bloyet LM, Cassella E, Zang T, Schmidt F, Brusic V, *et al.* Vesicular Stomatitis Virus Transcription Is Inhibited by TRIM69 in the Interferon-Induced Antiviral State. *J Virol* 2019;93(24):e01372–19. doi:10.1128/JVI.01372-19, PMID:31578292.
- [65] Bagga T, Tulsian NK, Mok YK, Kini RM, Sivaraman J. Mapping of molecular interactions between human E3 ligase TRIM69 and Dengue virus NS3 protease using hydrogen-deuterium exchange mass spectrometry. *Cell Mol Life Sci* 2022;79(5):233. doi:10.1007/s00018-022-04245-x, PMID:35397701.
- [66] Han Y, Li R, Gao J, Miao S, Wang L. Characterisation of human RING finger protein TRIM69, a novel testis E3 ubiquitin ligase and its subcellular localisation. *Biochem Biophys Res Commun* 2012;429(1-2):6–11. doi:10.1016/j.bbr.2012.10.109, PMID:23131556.
- [67] Rihn SJ, Aziz MA, Stewart DG, Hughes J, Turnbull ML, Varela M, *et al.* TRIM69 Inhibits Vesicular Stomatitis Indiana Virus. *J Virol* 2019;93(20):e00951–19. doi:10.1128/JVI.00951-19, PMID:31375575.
- [68] Balasubramaniam M, Ayyadevara S, Shmookler Reis RJ. Structural insights into pro-aggregation effects of C. elegans CRAM-1 and its human ortholog SERF2. *Sci Rep* 2018;8(1):14891. doi:10.1038/s41598-018-33143-1, PMID:30291272.
- [69] Dick TP, Bangia N, Peaper DR, Cresswell P. Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. *Immunity* 2002;16(1):87–98. doi:10.1016/s1074-7613(02)00263-7, PMID:11825568.
- [70] Peaper DR, Wearsch PA, Cresswell P. Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. *EMBO J* 2005;24(20):3613–3623. doi:10.1038/sj.emboj.7600814, PMID:16193070.
- [71] Müller IK, Winter C, Thomas C, Spaapen RM, Trowitzsch S, Tampé R. Structure of an MHC I-tapasin-ERp57 editing complex defines chaperone promiscuity. *Nat Commun* 2022;13(1):5383. doi:10.1038/s41467-022-32841-9, PMID:36104323.
- [72] Domnick A, Winter C, Sušac L, Hennecke L, Hensen M, Zitzmann N, *et al.* Molecular basis of MHC I quality control in the peptide loading complex. *Nat Commun* 2022;13(1):4701. doi:10.1038/s41467-022-32384-z, PMID:35948544.
- [73] Mutze K, Vierkotten S, Milosevic J, Eickelberg O, Königshoff M. Eno1ase 1 (ENO1) and protein disulfide-isomerase associated 3 (PDIA3) regulate Wnt/ $\beta$ -catenin-driven trans-differentiation of murine alveolar epithelial cells. *Dis Model Mech* 2015;8(8):877–890. doi:10.1242/dmm.019117, PMID:26035385.
- [74] Yang CL, Wang FX, Luo JH, Rong SJ, Lu WY, Chen QJ, *et al.* PDIA3 orchestrates effector T cell program by serving as a chaperone to facilitate the non-canonical nuclear import of STAT1 and PKM2. *Mol Ther* 2024;32(8):2778–2797. doi:10.1016/j.ymthe.2024.05.038, PMID:38822524.
- [75] Yudhawati R, Shimizu K. PGE2 Produced by Exogenous MSCs Promotes Immunoregulation in ARDS Induced by Highly Pathogenic Influenza A through Activation of the Wnt- $\beta$ -Catenin Signaling Pathway. *Int J Mol Sci* 2023;24(8):7299. doi:10.3390/ijms24087299, PMID:37108459.
- [76] Kumar A, Souza De Lima D, Mark Z, Anathy V. Lung Epithelial Redox Chaperones in Human Coronavirus Propagation. *Am J Respir Crit Care Med* 2023;207:A5663. doi:10.1164/ajrccm-conference.2023.207.1\_MeetingAbstracts.A5663.
- [77] Chamberlain N, Anathy V. Pathological consequences of the unfolded protein response and downstream protein disulphide isomerases in pulmonary viral infection and disease. *J Biochem* 2020;167(2):173–184. doi:10.1093/jb/mvz101, PMID:31790139.
- [78] Chamberlain N, Korwin-Mihavics BR, Nakada EM, Bruno SR, Heppner DE, Chapman DG, *et al.* Lung epithelial protein disulfide isomerase A3 (PDIA3) plays an important role in influenza infection, inflammation, and airway mechanics. *Redox Biol* 2019;22:101129. doi:10.1016/j.redox.2019.101129, PMID:30735910.
- [79] Fink JK, Jones SM, Esposito C, Wilkowski J. Human microtubule-associated protein 1a (MAP1A) gene: genomic organization, cDNA sequence, and developmental- and tissue-specific expression. *Genomics* 1996;35(3):577–585. doi:10.1006/geno.1996.0400, PMID:8812494.
- [80] Fava F, Raynaud-Messina B, Leung-Tack J, Mazzolini L, Li M, Guillemot JC, *et al.* Human 76p: A new member of the gamma-tubulin-associated protein family. *J Cell Biol* 1999;147(4):857–868. doi:10.1083/jcb.147.4.857, PMID:10562286.
- [81] Han S, Zhang D, Guo Y, Fu Z, Guan G. Prevalence and Characteristics of STRC Gene Mutations (DFNB16): A Systematic Review and Meta-Analysis. *Front Genet* 2021;12:707845. doi:10.3389/fgene.2021.707845, PMID:34621290.
- [82] Karki P, Birukova AA. Microtubules as Major Regulators of Endothelial Function: Implication for Lung Injury. *Front Physiol* 2021;12:758313. doi:10.3389/fphys.2021.758313, PMID:34777018.
- [83] Aminpour M, Hameroff S, Tuszyński JA. How COVID-19 Hijacks the Cytoskeleton: Therapeutic Implications. *Life (Basel)* 2022;12(6):814. doi:10.3390/life12060814, PMID:35743845.
- [84] Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* 2020;20(6):355–362. doi:10.1038/s41577-020-0331-4, PMID:32376901.
- [85] Vanderbeke L, Van Mol P, Van Herck Y, De Smet F, Humblet-Baron S, Martinod K, *et al.* Monocyte-driven atypical cytokine storm and aberrant neutrophil activation as key mediators of COVID-19 disease severity. *Nat Commun* 2021;12(1):4117. doi:10.1038/s41467-021-24360-w, PMID:34226537.
- [86] Ahnach M, Zbiri S, Nejari S, Ousti F, Elkettani C. C-reactive protein as an early predictor of COVID-19 severity. *J Med Biochem* 2020;39(4):500–507. doi:10.5937/jomb0-27554, PMID:33312067.
- [87] Smilowitz NR, Kunichoff D, Garshick M, Shah B, Pillinger M, Hochman JS, *et al.* C-reactive protein and clinical outcomes in patients with COVID-19. *Eur Heart J* 2021;42(23):2270–2279. doi:10.1093/eurheartj/ehaa1103, PMID:33448289.
- [88] Wang L. C-reactive protein levels in the early stage of COVID-19. *Med Mal Infect* 2020;50(4):332–334. doi:10.1016/j.medmal.2020.03.007, PMID:32243911.
- [89] Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z. Plasma CRP level is positively associated with the severity of COVID-19. *Ann Clin Microbiol Antimicrob* 2020;19(1):18. doi:10.1186/s12941-020-00362-2, PMID:32414383.
- [90] Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. *Immunity* 2011;35(2):161–168. doi:10.1016/j.immuni.2011.07.010, PMID:21867926.
- [91] Malysheva A, Brüggemann A, Paschen A, Dittmer U. Cytotoxic CD4(+) T cells in chronic viral infections and cancer. *Front Immunol* 2023;14:1271236. doi:10.3389/fimmu.2023.1271236, PMID:37965314.
- [92] Alexaki VI, Hennecke H. The Role of Glucocorticoids in the Management of COVID-19. *Horm Metab Res* 2021;53(1):9–15. doi:10.1055/a-1300-2550, PMID:33207372.
- [93] Bruscoli S, Puzzovio PG, Zaimi M, Tiligada K, Levi-Schaffer F, Riccardi C. Glucocorticoids and COVID-19. *Pharmacol Res* 2022;185:106511. doi:10.1016/j.phrs.2022.106511, PMID:36243331.