Balkan Med J 2025;42:477-8

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Impact of Smoking on SARS-CoV-2 Infection and Outcomes, An Evidence from Enrichment Analysis of Genes Associated with COVID-19 Susceptibility and Outcomes

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During the coronavirus disease-2019 (COVID-19) pandemic, genome-wide association studies (GWAS) were extensively used to identify genes linked to disease outcomes, including susceptibility to infection, hospitalization, severity, and mortality. These studies have produced comprehensive lists of genes associated with different aspects of COVID-19.

In this study, enrichment analysis was performed on protein-coding genes (data retrieved on July 7, 2024) using the Enrichr platform (https://maayanlab.cloud/Enrichr/). Genes were included if at least one of their polymorphisms exhibited a significant association with COVID-19 outcomes. To account for multiple comparisons and minimize false-positive results, an adjusted *p-value* of ≤ 0.001 was considered statistically significant.

A total of 177 genes were associated with susceptibility, 91 with hospitalization, 67 with severity, and 22 with mortality (Supplementary Table S1). Notably, pathway enrichment analyses (Reactome, Kyoto Encyclopedia of Genes and Genomes, Gene Ontology) did not identify any significant biological pathways (Supplementary Table S2), contradicting our initial hypothesis.

Venn diagrams were created to examine the overlap in the genetic architecture of different COVID-19 outcomes (Supplementary Figure S1). However, minimal overlap was observed among the four gene sets (Supplementary Table S3). For instance, the gene sets associated with severity and mortality shared only 11 loci. The limited commonality across all categories highlights the substantial heterogeneity in COVID-19 progression and suggests that non-genetic factors play a predominant role in shaping disease trajectories. This reinforces the idea that the studied aspects of COVID-19 likely represent distinct multifactorial traits.

The GWAS Catalog 2023 database was used to assess the overlap between each of the four gene sets and other multifactorial traits. A key finding was the significant overlap between genes associated with COVID-19 susceptibility and outcomes and those linked to smoking initiation (Table 1). Smoking initiation is a binary trait, defined as ever versus never smoking,[1] and is strongly correlated with smoking behavior, as frequently described in the literature. Specifically, 39 of the 177 susceptibility genes, 22 of the 91 hospitalization genes, 19 of the 67 severity genes, and 8 of the 22 mortality genes overlapped with smoking-related loci (Table 1).

Epidemiological data collected during the COVID-19 pandemic identified multiple risk factors associated with different aspects of the disease, including chronic conditions such as diabetes mellitus, hypertension, and heart failure, as well as obesity and smoking.[2] Early research during the pandemic highlighted smoking as a significant risk factor, linking it to an increased likelihood of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, severe illness, hospitalization, and COVID-19-related mortality. Smoking has long been associated with a higher risk of respiratory infections, including COVID-19, Middle East respiratory syndrome, and influenza, and may contribute to worsened disease progression in affected individuals.[3-7] Additionally, evidence suggests that smoking initiation increases the risk of various cancers (e.g., colorectal, lung, breast),[8-10] 14 different cardiovascular diseases,[11,12] type 2 diabetes mellitus,[13] and adverse metabolic outcomes such as abdominal fat accumulation.[14] These findings highlight the broader public health impact of smoking beyond its association with respiratory infections.

In conclusion, this study identifies a significant genetic overlap between loci associated with SARS-CoV-2 infection outcomes and those linked to smoking initiation. While this aligns with the epidemiological evidence supporting smoking as a risk factor for COVID-19 susceptibility and severity, it also suggests the involvement of shared genetic mechanisms. This challenges the assumption that comorbidities function as independent risk factors and reinforces

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Received: January 25, 2025 **Accepted:** February 13, 2025 **Available Online Date:** 01.09.2025 • **DOI:** 10.4274/balkanmedj.galenos.2025.2025-1-132

Available at www.balkanmedicaljournal.org

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Cite this article as: Saadat M. Impact of Smoking on SARS-CoV-2 Infection and Outcomes, An Evidence from Enrichment Analysis of Genes Associated with COVID-19 Susceptibility and Outcomes. Balkan Med J.; 2025; 42(5):477-8.

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TABLE 1. Polymorphic Genes Associated with Susceptibility to, Hospitalization for, Severity of, and Death from COVID-19 That are Shared with the Smoking Initiation Trait.

Traits	Number of loci	Adjusted *p* value	OR	Combined score	Genes
Susceptibility	39	5.0e-10	4.52	124.49	*PRKN; RYR2; SETD2; GALNT13; MCTP1; EHMT1; CACNA1D; FRY; FHIT; CDH4; DACH1; GLRA3; HERC1; POLR2A; CDH22; DLEU1; MACROD2; ZNF385D; CSMD1; PRKG1; MAGI1; RBFOX1; SZT2; CA10; NEGR1; NUBPL; MAGI2; LSAMP; KSR2; PTPRD; OLFM1; KANSL1; TRAF3; ELMO1; KCNQ3; SSPN; PRKD1; CNTNAP5; SOX2-OT*
Hospitalization	22	2.3e-6	5.05	90.82	*ROBO2; CADPS2; RBFOX1; TENM3; CPNE4; CELF2; ULK4; HSF2BP; DCAF5; KALRN; SGCZ; DNM3; TAFA5; ERBB4; KCNMA1; SSPN; CAMTA1; WDR7; HIVEP2; ASIC2; ANKS1B; RAPGEF4*
Severity	19	1.3e-6	6.26	116.16	*GUCY1A2; CADM2; STK39; AGAP1; RORA; CDH8; EMCN; NFIA; RARB; SPOCK1; NCAM1; MAPT; GALNTL6; ASTN2; ZFPM2; BEND7; LRRC4C; PRKG1; CNTNAP5*
Death	8	9.7e-4	8.97	94.88	*RGS17; CADM2; IQSEC1; PRKCE; CTNNA3; NCAM1; LRRC4C; CNTNAP5*

COVID-19, coronavirus disease-2019, OR, odds ratio.

the critical role of smoking as a major contributor. These findings highlight the need to integrate genetic data with behavioral interventions, such as smoking cessation programs, in pandemic preparedness and personalized risk reduction strategies. Further Mendelian randomization analysis of the available data is necessary to validate this conclusion.

Supplementary: https://balkanmedicaljournal.org/img/files/BalkanMedJ-2025-1-132-supplement.pdf

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