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The Effect of Pathogenic Tumor Suppressor Gene Variants on COVID-19: A Report of Three Cases with Varied Severity and Outcomes

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Abstract: The COVID-19 pandemic has created unique challenges for people with comorbidities, including hereditary diseases and cancer cancers. Several studies have reported a link between the presence of disease-causing genetic variants and the outcome of the COVID-19 infection. In this study, we used clinical exome sequencing in a cohort of 840 COVID-19 patients to identify pathogenic and likely pathogenic genetic variants present in these individuals. While we did not identify any statistically significant differences in the overall burden of pathogenic variants between different patient groups, we discovered three known pathogenic alleles associated with hereditary cancer syndromes, including a frameshift mutation in *MSH6* and two missense mutations in *TP53*. The patients carrying these mutations presented with different severity of the disease and outcome. Thus, a 58-year old male subject with an *MSH6* mutation developed a severe form of COVID-19 that resulted in death, even though the patient had few pre-existing conditions and no evidence of malignant tumors. On the other hand, two female subjects carrying pathogenic *TP53* variants successfully recovered from the disease despite suffering from various forms of cancer. Our results highlight the importance of personalized approaches to the diagnosis, management and treatment of COVID-19 in patients with specific genetic mutations. Further studies are needed to elucidate the complex relationship between these mutations and COVID-19.

Keywords: COVID-19; SARS-CoV-2; oncology; genetic variants; NGS; severity; genetic associations; exome

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1. Introduction

At the end of 2019, a new strain of coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an outbreak of pneumonia in Wuhan, China, that was later named COVID-19 [1]. The outbreak quickly turned into a pandemic; as of December 2023, over 700 million of COVID-19 cases were confirmed, and nearly 7 million people died from the disease (World Health Organization, https://covid19.who.int/, accessed 03 December 2022).

The COVID-19 pandemic has ushered in an era of unprecedented healthcare challenges, testing the limits of our understanding of both viral pathogenesis and patient

responses to infection. Amid this global crisis, individuals with underlying comorbidities represent a particularly vulnerable subgroup. This is especially true for patients with oncological diseases. Their unique clinical characteristics, treatment regimens, and immune profiles have rendered the intersection of cancer and COVID-19 a field of paramount importance, meriting careful scrutiny and analysis [2].

The COVID-19 pandemic has revealed a complex web of interactions between the SARS-CoV-2 virus and various comorbidities, among which oncology stands as a significant risk factor for severe disease and poorer clinical outcomes. Within the cancer population, the genetic landscape further complicates the clinical trajectory, as mutations in cancer-related genes can introduce unique challenges and nuances to the course of COVID-19. To date, several reports have examined the presence of pathogenic genetic variants in patients with COVID-19, with some of these studies reporting disease-causing variants in tumor suppressor genes [3]

In this report, we present three clinical cases selected from a cohort of 840 COVID-19 patients, all of whom bore mutations in the tumor suppressor genes. These case studies illuminate the intricate interplay between COVID-19 severity, clinical outcomes, and the genetic underpinnings of oncological conditions, providing valuable insights into the multifaceted nature of the pandemic's impact on cancer patients. By examining these three individual cases, we aim to describe the complexities in the management of COVID-19 in patients with cancer, particularly in those with mutations in critical tumor suppressor genes. This study not only sheds light on the heterogeneity of COVID-19 manifestations, but also highlights the importance of a personalized approach to diagnosis, management, and treatment decisions in this unique clinical context. Through comprehensive analysis of these cases, we aim to further our understanding of the complex relationship between COVID-19 and cancer

2. Materials and Methods

2.1. Study Design and Inclusion Criteria

The research employed an observational clinical trial design, involving the analysis of 840 medical records from COVID-19 patients treated at St. Petersburg State Budgetary Institution of Healthcare City Hospital 40 (City Hospital 40, St. Petersburg) between Apr. 18, 2020, and Nov. 21, 2020. These patients tested positive for SARS-CoV-2 RNA through polymerase chain reaction (PCR) amplification of nucleic acids from clinical material. They presented various clinical manifestations and symptoms.

In accordance with the International and Russian Recommendations for the Prevention, Diagnosis and Treatment of New Coronavirus Infection (COVID-19), all patients were divided in three groups of comparable age ([4]; Ministry of Health of the Russian Federation.2020). The three groups corresponded to patients with a mild (49 patients, 5.8%), moderately severe (436, 51.9%), and severe (or extremely severe) (355, 42.2%) course of disease. The criteria for a mild course were considered to be body temperature below 38°C, cough, weakness, sore throat, and the absence of criteria for moderate and severe courses. The criteria for a moderate course are fever, temperature above 38°C, respiratory rate over 22/min, dyspnea, pneumonia (exposed to CT of the lungs), and SpO2 $<$ 95%. Clinical and radiological criteria for severe course were respiratory rate more than 30/min, SpO2 $\leq$ 93%, PaO2/FiO2 $\leq$ 300 mmHg, progression of changes in the lungs typical for COVID-19 pneumonia according to CT data, including an increase in the prevalence of revealed changes by more than 25%, as well as the appearance of signs of other pathological conditions, changes in the level of consciousness, unstable hemodynamics (systolic blood pressure less than 90 mmHg or diastolic blood pressure less than 60 mmHg, urine output less than 20 ml/h), and qSOFA $>$ 2 points. The criteria for an extremely severe course were signs of ARF with the need for respiratory support (invasive ventilation), septic shock, and multiple organ failure.

2.2. Exome sequencing and variant calling

The details regarding exome sequencing are given in our previous work [5].

Paired-end sequencing reads were aligned onto a b37 human reference genome assembly using the BWA MEM aligner [6]. Variants were called using the DeepVariant variant caller [7]. Ensembl Variant Effect Predictor (VEP) [8] was used to annotate the variants with gene names, transcript information, variant type (e.g., missense, nonsense, frameshift, etc.), population frequencies and clinical significance of a variant. The annotated data were used for variant interpretation.

For subsequent statistical comparison, an additional cohort genotyping of the selected variant sites was performed using the Genome Analysis ToolKit (GATK) v. 4.3 [9]. After cohort genotyping, the identified variants were primary filtered according to the following thresholds based on GATK metrics: 1) DP>10, 2) GQ>20

2.3. Variant interpretation

To aid the interpretation of the variants identified during our analysis, we focused our attention on known and expected pathogenic and likely pathogenic variants according to the American College of Medical Genetics and Genomics (ACMG) criteria [10]. Interpretation of pathogenic effects of variants was restricted to a predefined set of genes that are potentially related to the COVID-19 pathology. The list of genes is compiled based on probability that variations in these genes could explain at least part of the severity of COVID-19. There are genes involved in cilia and mucociliary clearance, DNA-repair, immune response, complement system, blood clotting, cell-cycle control, vessel endothelium.

2.4. Statistical analysis of the prevalence of variants in patient subgroups

To test for the potential effects of the identified variants on the disease severity and outcome, the total number of occurrences of each variant was calculated for each subgroup (two subgroups based on outcome (death or recovery), and three subgroups based on severity (mild, moderate, severe)). In addition to variant-level analysis, variant counts were aggregated to the level of individual genes, and the number of individuals in each subgroup carrying selected variants was calculated for each gene. Similarly, variant counts were aggregated up to the level of gene groups (DNA repair and cell proliferation, blood clotting, cardiac and vascular function, ciliopathy genes, immune system genes, mucous-related genes, and other genes).

Statistical testing was then conducted for individual variants, genes, and gene groups. In each case, the number of individuals carrying a particular variant or any variant in a gene/gene group was compared between the patient subgroups using Fisher's exact test. p-values were corrected using the Benjamini-Hochberg FDR method.

3. Results

3.1. Identification of pathogenic genetic variants in COVID-19 patients

Given multiple previous reports about the impact of pathogenic genetic variants on the severity and outcome of COVID-19, we set off to test such an effect using a cohort of 840 patients with varying degree of severity of the disease and clinical outcomes (see Methods for description of the study sample) (Shcherback et al., 2022). To do so, we performed variant calling using previously generated exome sequencing data [5]. We next filtered this set of variants, retaining pathogenic and likely pathogenic variants (according to the American College of Medical Genetics and Genomics (ACMG) guidelines [10]) in several groups of genes linked to Mendelian disease (see methods). Such a filtering yielded as many as 221 variants in 120 genes which were then subjected to manual curation and validation.

After the initial selection of candidate variants, we first performed a series of statistical tests to evaluate the effect of the carrier status (i.e., presence of at least one pathogenic allele in the patient) on the course of COVID-19 illness. To this end, we compared the incidence of the selected variants in individuals with different outcomes (i.e., death and recovery) and

severity of the COVID-19 illness (mild, moderate, and severe (see Methods)). The statistical analysis did not identify any differences in the proportion of carriers of pathogenic genetic variants between the groups. Similar negative results were obtained when each gene was considered separately, or when genes were grouped together according to the associated disorders. These results indicate that the pathogenic allele carrier status does not directly influence the course of COVID-19.

Despite the absence of a significant effect of pathogenic variant presence on COVID-19 illness, we noticed that several patients in our dataset carried known pathogenic variants in genes linked to autosomal dominant diseases. To our surprise, this subset included three individuals with variants in the MSH6 and TP53 tumor suppressor genes which have been previously implicated in the pathogenesis of COVID-19 [11,12]. The course of illness in these donors had notable features, which shall be detailed below.

3.2. Case presentation in individuals with pathogenic tumor suppressor gene variants

Clinical features of the subjects bearing the identified variants, including the major parameters of the COVID-19 disease course, are summarized in Table 1.

Table 1. Clinical features of the three COVID-19 patients with pathogenic variants in *MSH6* and *TP53* genes.

Parameter	Patient #1	Patient #2	Patient #3
Age	58	54	70
Sex	male	female	female
COVID-19 severity	severe	severe	moderate
COVID-19 outcome	death	recovery	recovery
Charlson-Comorbidity-Index (CCI)	2	5	10
NEWS† upon admission	12	11	11
PSI‡ upon admission	188 (class V)	50 (class II)	90 (class III)
Variant identified (rsID)	rs267608058	rs11540652	rs148924904
Gene	*MSH6*	*TP53*	*TP53*
Variant consequence	frameshift	missense	missense
Nucleotide change	NM_000179.3:c.2150_2153del	NM_000546.6:c.743G>A	NM_000546.6:c.488A>G
Protein change	NP_000170.1:p.Val717fs	NP_000537.3:p.Arg248Gln	NP_000537.3:p.Tyr163Cys

† — National Early Warning Score (NEWS 2); ‡ — Pneumonia Severity Index.

3.2.1. A pathogenic *MSH6* variants in patient #1

The first subject was a 58-year-old male patient that was undergoing treatment for preexisting coxarthrosis at a medical institution. The patient had few pre existing conditions, including mild hypertension and gastritis. The total Charlson-Comorbidity-Index (CCI) value of 2 indicated a relatively low risk.

At 13.04.2020 the patient presented with first symptoms of COVID-19 in a form of fever (body temperature = 38.4 C). The next day, a bilateral pneumonia was identified during a CT scan, with progressive respiratory failure developing since the fourth day of disease course. The patient was put on artificial ventilation and transferred to a specialized COVID-19 facility.

Upon admission to the COVID-19 facility, the patient had a mild fever (37.4 °C), blood pressure of 140/80, and a heart rate of 100 BPM. Artificial ventilation was performed in the following modes: SIMV PEEP 18 Vt 500 FiO2 o,1%, followed by BiPAP with the following parameters: Pins 35 mbar, PEEP 18 mbar, FiO2 0,9%, f 16, Vt- 400-500 ml, SpO2 91%. Auscultation was decreased across all lung fields. The patient presented with no peripheral oedema, normal urination and bowel function.

On the seventh day, the CT scan showed signs of inflammation of the lung parenchyma, with a high probability of a viral etiology. Multiple ground glass opacity areas combined with diffuse reticular changes with predominant localization in several lung fields. Subtotal ($>$ 75% of area) and total lung damage was observed for right and left lungs of the patient, respectively.

The patient was given treatment according to the intensive care strategy, including gastroprotective, antibacterial, and symptomatic treatment. Despite intensive therapy, the patient's clinical course remained unfavorable. On the ninth day of the disease course, the patient died after suffering an effective cardiac arrest.

Exome sequencing in this patient identified a protein-truncating variant (PTV) rs267608058 in the exon 4 of 10 of the MSH6, specifically identified as NC_000002.11:g.48027269_48027272del (NM_000179.2:c.2150_2153del, NM_000179.2:p.Val717AlafsTer18). According to the joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC) [13] rs267608058 in MSH6 found in the patient is identified as pathogenic, which is supported by the 15 clinical interpretations previously reported in ClinVar [14]. Associations with Lynch Syndrome, endometrial carcinoma, breast cancer and hereditary cancer-predisposing syndrome are present among the condicions for rs267608058 [15–17]. Nevertheless, the patient did not have any symptoms of malignant tumors.

3.2.2. Known pathogenic *TP53* variants in patients #2 and #3

The second subject (patient #2) was a 54-year old female hospitalized into a COVID-19 facility for cough, shortness of breath, fever and fatigue. The patient was in a remission phase of an acute lymphoblastic leukemia after undergoing polychemotherapy twelve years prior to hospitalization. Other pre-existing conditions included stomach and duodenum ulcer (in remission phase), hemorrhoids, varicose veins of lower extremities, and a mild chronic anemia. The total CCI value was 5.

According to self-report, hospitalization occurred on the fourth day since the onset of the symptoms. The diagnosis of COVID-19 was confirmed by a positive SARS-CoV-2 PCR test on the first day of the disease course. Objective examination at admission showed normal body temperature (36.6) and blood pressure (110/60), and a heart rate of 100 BPM.

A CT scan upon admission showed a bilateral damage of the lung parenchyma, with a total damaged area of 26-49%. The scan showed round glass opacity areas with a crazy paving pattern of lung tissue damage. The patient had a respiratory rate of 25 breaths per minute, and an SpO2 of 66% (93% with additional oxygen supply (NHF 60l/min FiO2 80%). Normal auscultation was observed at all fields.

An intensive care strategy was used for treatment, olokizumab was administered together with the infusion of plasma with anti-SARS-CoV-2 antibodies. Respiratory function steadily improved; a CT scan on the 24th day since admission showed a decrease in the area of inflammatory tissue changes. The patient was discharged on the 25th day.

The third subject (patient #3) was a 70-year old female who was admitted to a self-isolation hospital unit after receiving a PCR-based COVID-19 diagnosis, two days after the onset of symptoms in a form of fever. The patient had multiple pre-existing conditions, including ischemic heart disease, aterosclerosis, pronounced hypertension, type 2 diabetes, diabetic kidney disease, basal cell skin cancer of the back, and a chronic lymphocytic leukosis (the total CCI value was 10).

The patient was transferred to a specialized COVID-19 medical facility three days after the initial hospitalization due to the progression of symptoms, high fever (up to 39 C), cough, and shortness of breath. Upon admission, the patient presented with a slightly elevated body temperature (36.8 C), normal blood pressure (130/80), and a heart rate of 88 BPM. No peripheral oedema, problems with bowel function and urination were detected. The patient received supplemental oxygen (50 l/min FiO2-50%), SpO2 90-91%.

A CT scan after admission to the COVID-19 facility showed progressive changes in the lungs, with bilateral damage to the lung tissue (more than 60% of the total area), with ground glass opacity areas and a crazy paving pattern.

The patient received intensive therapy with favipiravir, antibacterial therapy (azithromycin, sulperazon, levofloxacin), broncholytic and mucolytic medication, anticoagulants, gastroprotective therapy, and probiotics. The patient showed steady improvement of the condition; a control CT scan on the 25th day of the disease coursse showed improved lung

tissue condition (total tissue damage area - 49%). The patient was discharged on the 27th day due to stable condition.

In both Patient #2 and Patient #3, we identified known pathogenic missense mutations, rs11540652 and rs148924904 in the TP53, respectively. The first TP53 mutation in exon 7 of 11 was identified as NC_000017.10:g.7577538C>T (NM_000546:c.743G>A, NM_000546:p.Arg248Gln), and the second in exon 5 of 11 as NC_000017.10:g.7578442T>C (NM_000546:c.488A>G, NM_000546:p.Tyr163Cys). The rs11540652 and rs148924904 variants for patient #2 and patient #3 in the TP53 gene have been reported 63 and 19 times in ClinVar, respectively [14]. Of the disease terms, hereditary cancer-predisposing syndrome and Li-Fraumeni syndrome and many other cancer conditions are found [18–20], which also allows it to be considered as "pathogenic" according to ClinGen, CGC and VICC recommendations [13]. Notably, both patients in our study had pre-existing cancer (either in remission or active), and both recovered from the disease despite suffering a severe form of the disease with high levels of lung tissue damage.

4. Discussion

In this study, we used a cohort of 840 COVID-19 patients to search for the presence of pathogenic variants in Mendelian disease genes that may affect the disease course or outcome. Despite several previous reports suggesting a possibly increased burden of such alleles in severe COVID-19 [3], our analysis did not identify any correlation between the presence of a pathogenic allele in the genotype and COVID-19 severity or outcome. Moreover, we identified several patients with known pathogenic variants in tumor suppressor genes (*MSH6* and *TP53*) that presented with different disease severity and outcomes. In light of these findings, it is important to consider the functions of these genes and their possible roles in COVID-19.

MSH6 is a crucial component of the DNA mismatch repair system, and alterations in this gene have been associated with an increased risk of various malignancies, including colorectal and endometrial cancers [21]. And rs267608058 has been previously mentioned as a “pathogenic” in accordance with these phenotypes. Despite this, MSH6 is a low-conserved gene (pLI = 0) and severe mutations have been previously described in large populations like Genome Aggregation Database (gnomAD). The rs267608058 affects the MutS domain (DNA mismatch repair ATPase MutS). The MutS domain is widely spread in almost all organisms, from bacteria to humans, and plays a key role in various DNA operations, such as DNA mismatch repair. MutS2 is thought to suppress homologous recombination by endonucleolytic resolution of early process intermediates [22]. In our case, this MSH6 frameshift mutation in MutS domain was observed in a subject that did not present with any symptoms of malignant tumors; however, the patient had a steadily progressing disease course that resulted in death despite the patient having few pre-existing conditions and an age below 60. Hence, we may hypothesize that the observed pathogenic variant may have played a certain role in defining the disease outcome.

The *TP53* gene encodes a critical tumor suppressor gene with a transcription factor function, which is moderately conserved (pLI = 0.53). Missense mutations in TP53 are well-documented in cancer research and are known to disrupt the normal function of the p53 protein. Both rs11540652 and rs148924904 affect the P53 (P53 DNA-binding domain). Additionally rs11540652 affects the DNA binding site. Despite this, in silico predictions indicate that neither rs11540652 nor rs148924904 affect important highly conserved regions of p53. These mutations can result in the accumulation of dysfunctional p53, impairing its ability to regulate cell cycle progression and DNA repair mechanisms. In the context of COVID-19, such mutations in *TP53* may further complicate the immune response and cellular processes involved in viral clearance, potentially influencing the severity and clinical outcomes of the infection. Both patients carrying pathogenic *TP53* variants in our study had history of blood cancer (acute lymphoblastic leukemia and chronic lymphocytic leukosis in patient #2 and #3, respectively). Moreover, one of the patients presented with multiple tumors. Nevertheless, both patients recovered from the disease, indicating that

while the mutations may have had a negative impact on the disease course, they do not have a dramatic effect on the patient's survival.

Taken together, our results provide additional insights into the role of pathogenic variants in COVID-19, and demonstrate variation in the effects of such variants in genes affecting cancer risk on the course of the COVID-19 disease and its outcome.

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Abbreviations

The following abbreviations are used in this manuscript:

COVID-19	Coronavirus disease 2019
WHO	World Health Organization
NEWS	National Early Warning Score
CCI	Charleson Comorbidity Index
PSI	Pneumonia Severity Index

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