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GENETIC VARIATIONS IN THE COAGULATION PATHWAY AND ELEVATED D-DIMER LEVELS IN COVID-19 PATIENTS

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Abstract

The rapid spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has profoundly affected routine life and healthcare services globally, with COVID-19 cases varying from mild to severe, including fatal consequences. Indications range from pyrexia, tussis, and respiratory difficulties to immobility and thoracic pain. In extreme cases, patients exhibited multi-organ dysfunction, respiratory failure, and widespread thrombosis. Elevated D-dimer levels, a biomarker for hemostasis, are correlated with genetic variations in the coagulation pathway, increasing the risk of thrombotic events. This review underscores the correlation between these genetic variations and elevated D-dimer levels among people with COVID-19, Uncovering pivotal elements in thrombogenesis. Clarifying this relationship may lead to novel strategies for preventing and managing thrombotic issues in COVID-19 patients.

Keywords: Genetic Variations, Coagulation Pathway, Elevated D-Dimer Levels, COVID-19, Thrombogenesis.

INTRODUCTION

Covid-19:

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus, alternatively termed coronavirus, disseminated extensively in the last quarter of 2019, sparking a global health crisis that worsened in 2021-2022. Regimes worldwide enacted comprehensive lockdown measures to counteract the outbreak [1]. The Coronaviridae family encompasses a wide range of ribonucleic acid (RNA) viruses, commonly known as coronaviruses, exhibiting diverse properties, have exerted a profound influence on human well-being, inducing a diverse range of the COVID-19 pandemic, along with earlier respiratory infections like Severe Acute Respiratory Syndrome Coronavirus (SARS) and middle East respiratory syndrome (MERS), has underscored the need for enhanced preparedness and response measures. COVID-19, a beta coronavirus subtype, was discovered in animal hosts like swine, avian species, and coronaviruses are broadly categorized into four subtypes: beta, alpha, delta and gamma [2]. The virions exhibits spherical configuration, measuring 60-140 nanometers in diameter, with a central nucleus comprising RNA and phosphorylated nucleocapsid, encircled defined by the presence of three critical proteins: E, S, and M, which form the envelope, spike, and membrane components [3]. The elevated pace of genetic evolution in the COVID-19 viral genome engenders variations in S protein structure, resulting in disparate virus strains with distinct infection severity and host responses, influencing symptoms and incubation durations [4]. Recent investigations indicate that Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) primarily disseminates through proximity to an infected individual (less than 1 meter) or via respiratory droplets transmitted during tussis/sternutation [5, 6]. Additionally, touching contaminated surfaces and then directly contacting one's oculi, nares, and oral orifice can also enable transmission [5].

The presence of COVID-19 condition in animals (mammals) is typified by a gamut of symptoms, ranging from asymptomatic to severe, with varying gradations of severity [7,8]. Common manifestations include hyperthermia, bronchitis, lassitude, and loss of gustatory or olfactory faculty. However, severe manifestations observed in COVID-19 patients include respiratory distress, immobility, and thoracic anguish [9]. Patients with underlying chronic comorbidities are more likely to experience severe infections, which can lead to fatal outcomes. Notably, COVID-19 is associated with a high risk of severe outcomes, principally due to pneumonitis, dyspnea [10, 11]. As of January 2023, the world health organization (WHO) reported 6.817 million fatalities since the pandemic's onset [12]. The mortality level has varied throughout the pandemic, peaking at 9.6% in April 2020 and decreasing to 8% in January 2023 [12]. This trend was particularly pronounced in patients with cardiovascular disease (CVD), who face a fourfold greater vulnerability of mortality [13]. Most COVID-19-related deaths involved pre-existing conditions like atrial arrhythmia, venous thromboembolism, irregular function of heart [14], suggesting a higher risk of cardiovascular complications post-infection. Additional research revealed, COVID-19 can disrupt blood coagulation, leading to cardiovascular complications like cardiac lesion, irregular heartbeat, and venous thromboembolism [15].

Assessing the Severity of COVID-19: Prognostic Factors and Indicators:

The severity of symptoms is determined by a complex interplay of variables, including ancestral heritage, seniority, sexual orientation, pre-existing health conditions, and personal habits

Ancestral Heritage:

A comprehensive analysis of fatalities across all countries affected by the pandemic revealed disparate mortality rates attributed to various demographic factors, including ancestral heritage. Ethnic groups exhibited disparate mortality rates [16]. Africans experienced a markedly higher mortality rate [17, 18], while Hispanics had an elevated risk compared to non-Hispanic Caucasians. Individuals from the Middle Eastern and North African regions may be exposed to increased hazards [19]. Socioeconomic status had a profound impact on outcomes, with affluent nations reporting a 5.0% case fatality rate and economically disadvantaged nations reporting 2.8% [20].

Age:

As per the Centre for Disease Control and Prevention states that COVID-19 fatalities are most prevalent among individuals aged 85 and above (1,130/100,000), followed by those between 65 and 84 (460/100,000), and substantially lower for those aged 55 to 64 (140/100,000). While the data indicate a clear inverse correlation between age and mortality ratio, it's essential to note that severe morbidity and mortality can still occur in younger individuals, particularly those with pre-existing medical comorbidities [21].

Sexual Orientation:

Investigations indicate a substantial disparity in COVID-19 fatalities between genders, with men exhibiting a 1.9-fold increased risk of succumbing to the illness compared to women [22, 23]. Similarly, scientific observation in Jordan found a significant augmentation in mortality rates among males compared to females [24]. The underlying factors contributing to this disparity may include differences in immunological responses, pre-existing medical comorbidities, and behavioral patterns

such as tobacco usage. Further investigation is necessary to unravel the connection between gender and COVID-19 fatalities and to minimize the vulnerabilities of male patients [25].

Understanding the Immunological Aspects of Covid – 19:

The Initial Barrier: Innate Immunity's Role in Combating Covid-19:

COVID-19 binds to (Angiotensin converting enzyme 2 (ACE2) receptors on alveolar cells, triggering a physiological response and cellular reactions [26, 27]. The viral invasion activates the innate immune system's rapid counterattack, detecting viral pathogen-associated molecular patterns (PAMPs) through endosomal pattern recognition receptors (PRRs) like toll-like receptors. Recognition of the virus initiates a signaling pathway that activates the expression of transcriptional regulators like interferon regulatory factors (IRFs) nuclear factor Kappa B (NF- Kappa B) [28]. Resulting in the production of interferons and pro-inflammatory mediators, which triggers a robust antiviral defense, inhibiting viral spread and inducing cellular suicide [28-30]. An unrestrained immune response can culminate in unbridled inflammation, leading to organ deterioration. A sluggish interferon type 1 (IFN-I) response and unchecked viral proliferation trigger an overzealous interferon type 1 (IFN-I) reaction, aggravating inflammation and disease intensity. Interferons coordinate antiviral resistance, curbing replication and inducing cellular self-destruction to arrest viral spread [31].

Immunothrombosis is a disrupted process where monocytes detecting damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs) trigger tissue factor production, initiating coagulation [32]. Neutrophils release traps, entrapping pathogens and triggering clotting [33]. The intricate interplay between immune and coagulation pathways in severe COVID-19 cases culminates in a complex pathophysiology [33]. NETs interacting with TF precipitates the extrinsic coagulation cascade, resulting in extensive hypercoagulability [33]. Factor XII activation instigates the intrinsic pathway. In COVID-19, NETs-platelet conglomerations aggravate disease severity [1, 34, 35].

Adaptive YImmunity:

Adaptive immunity plays a vital role in viral elimination in COVID-19, with B-cells and cytotoxic T-cells executing crucial functions [36]. However, lymphopenia precipitates a depletion of clusters of differentiation 4 (CD4+) T-cells, clusters of differentiation 8 (CD8+) T-cells, and B-cells, possibly attributable to diminished interferon type I (IFN-I) production or direct T-cell invasion [37,38]. Lymphopenia may also arise from lymphocyte annihilation, pulmonary sequestration, and disrupted bone marrow hematogenesis [39-41]. COVID-19 directly ravages lymphoid organs, leading to splenic atrophy and structural disintegration [42-44].

Hypercytokinemia:

COVID-19 patients exhibited markedly elevated levels of pro-inflammatory mediators in their bloodstream [45], culminating in severe inflammatory perturbations in pulmonary tissue [26]. Computed tomography scans revealed extensive lung damage and hemorrhaging [37, 46]. Interleukin-6 (L-6) concentrations demonstrated a strong correlation with COVID-19 mortality, precipitating acute respiratory distress syndrome [47, 48]. The binding of COVID-19 to ACE2 receptors activated the nuclear factor kappa B (NF-κB) pathway, triggering the production of IL-6 [26]. In critical cases, lung

scans revealed an appreciable increase in Chemokine receptor 6 (CCR6+) T helper 17 (Th17) cells and enhanced cytotoxicity of Clusters of differentiation 8 (CD8+) T cells, while Interleukin-10 (IL-10) levels remained steadfast. However, NK cells and Clusters of differentiation 8 (CD8+) T cells exhibited diminished functionality. Furthermore, resulting the devastation among Clusters of differentiation 169 (CD169+) macrophages, which imperils both lymphoid and splenic tissues. Clusters of differentiation 169 (CD169+) macrophages also contribute to elevated Fas levels, triggering activation-induced cell death of Interleukin-6 (IL-6) and Interleukin-10 (IL-10) [46].

Blood Coagulation: A Hematological Process:

Hemostasis refers to the intricate mechanism of blood coagulation that ensues in response to vascular or tissue trauma, leading to the formation of blood clots. This process is accompanied by various clinical manifestations and laboratory evidence, which increase the susceptibility to cardiovascular disease (CVD) [49]. Figure 1 illustrates that fibrinogen and D-dimer serve as vital indicators for diagnosing cardiovascular disease (CVD), with the specific biomarker dependent on the disease's manifestation. When the body experiences internal injury, the coagulation process is activated, transforming fibrinogen converted into fibrin through the enzymatic action of thrombin. The enzyme plasmin counteracts clot formation by dissociating fibrin into fibrin degradation products (FDPs), including D-dimer. Fibrinogen, a protein secreted by the hepatic cells, plays a vital role in regulating blood clotting [50]. Low fibrinogen levels in the blood can indicate aberrant clotting activity, suggesting excessive clot formation [51]. Fibrinogen measurement is essential for detecting and managing bleeding and clotting risks associated with cardiovascular diseases, such as systemic thrombosis and vascular pathology [52].

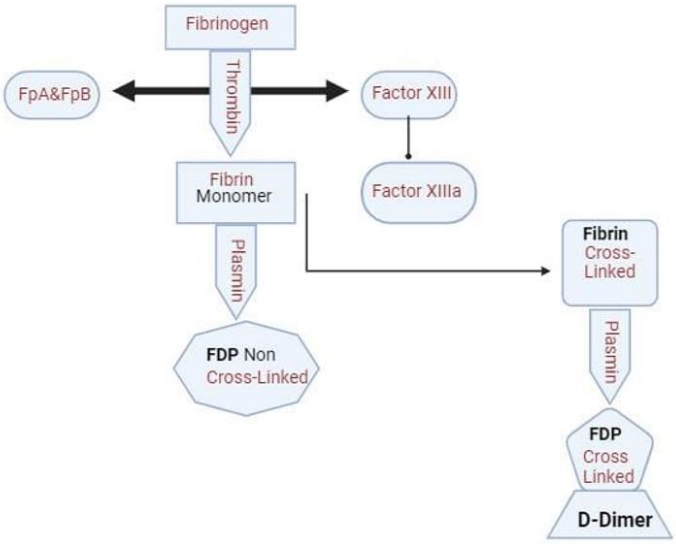
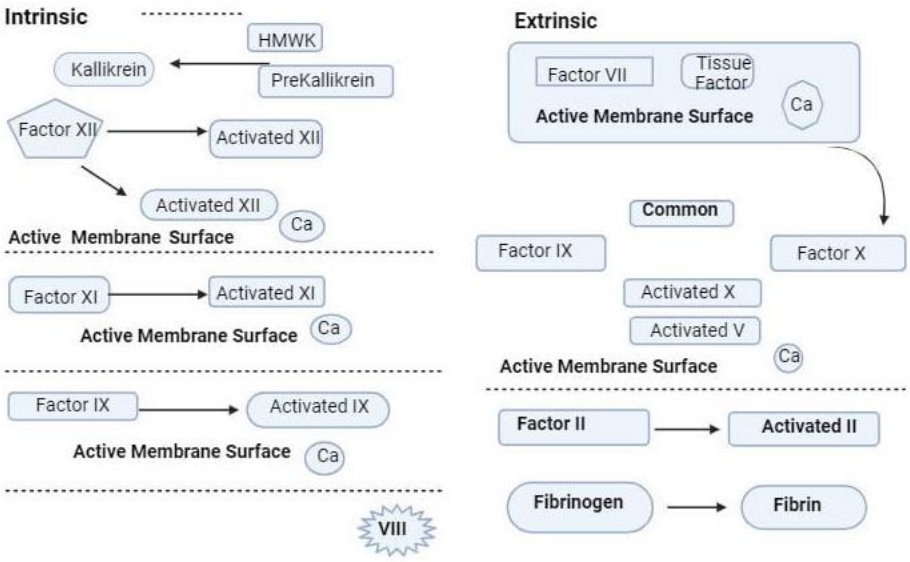


Figure 1: Fibrinogen Degradation [86]

Figure 2 illustrates the two blood clotting (coagulation) pathways: the extrinsic route (occurring in peripheral tissues) and the intrinsic mechanism (operating within the vascular network). Each protein in these cascades plays a vital role in the process of clot dissolution, and any minute alteration due to genetic variation can significantly influence the entire process, rendering the individual prone to cardiovascular disease (CVD), potentially leading to severe morbidity in exceptional cases [53].



FII: prothrombin	TF: tissue factor
FIIa: thrombin	PC: protein c
FV: factor V	TM: thrombomodulin
FVa: activated factor V	K: kallikrein
FVII: factor VII	GLa: glutamic acid
FVIIa: activated factor VII	HMWK: high molecular weight kininogen
FVIII: factor VIII	HMWK: high molecular weight kininogen
FVIIIa: activated factor VIII	TFPI: Tissue factor pathway inhibitor
FIX: factor IX	aPC: activated protein C
FIXa: activated factor IX	PK: prekallikrein
FX: factor X	FXa: activated factor X
FXI: factor XI	FXIa: activated factor XI
FXII: factor XII	Fg: fibrinogen
Fb: fibrin	AT: antithrombin
BK: bradykinin	HSPG: heparan sulfate proteoglycans
PAI: plasminogen activator inhibitor	

Figure 2: Overview of the Coagulation Cascade, Highlighting Both the Extrinsic and Intrinsic Pathways, And The Impact of Genetic Variations on D-dimer Levels in COVID-19 Patients [86]

D-dimer:

The dismantling of cross-linked fibrin culminates in the formation of D-dimer, a minute fragment generated when plasmin dismantles fibrin clots [54]. Enhanced D-dimer concentrations in COVID-19 patients signify hypercoagulability, leading to intensified fibrin formation and a heightened risk of venous thromboembolic events (VTE) [55, 56, 57]. Elevated D-dimer levels are associated with increased morbidity, intensive care unit (ICU) admission, and mortality, rendering monitoring essential [58].

D-dimer Dysfunction: The Impact of Genetic Mutations:

The typical blood clotting sequence can be perturbed if genetic modifications occur in the genetic material responsible for encoding blood clotting proteins. The fibrinogen gene, comprising three subunits, is situated on chromosome 4q28-q31 and spans approximately 50 kilobases. Genome-wide association analyses have identified single nucleotide variants (SNVs) in the fibrinogen factor v, alpha, gamma, as well as F2, F11, MTHFR, F8 and KNG1 that significantly influence D-dimer concentrations [59].

Three noteworthy single nucleotide variants (SNVs) associated with elevated D-dimer concentrations are listed in Table 1. Additionally, other SNVs like AC093117.1 and Z99572.1 have been linked to type 2 diabetes mellitus and end-stage renal disease, respectively, and influence blood clotting factors II and V.

Table 1: An Explanation of the Primary Genes Influencing Fibrinogen and D – Dimer (DD)

Gene	Protein with coded	Roles	Related pathways	Location	Mutation SNP (ID)	Diseases
FGG	Fibrinogen (Gamma chain)	Augmented SELP production in stimulated platelets is facilitated by dependent of an ITGB3 process. Moreover, maternal (mother) fibrinogen essential for a fruitful gestation outcome. Fibrin aggregation is also connected to pathological conditions, where it functions as a bulwark against IFNG-modulated hemorrhage.	Cell adhesion molecule interactions with the cell membrane surface TLR 4 signaling activation	Sequence: NC_000004.12 Chromosome: 4 Size: 8,834 bases	D-Dimer: rs13109457 Fibrinogen: rs148685782, rs6056, rs7439150, rs76289367, rs768142	Fibrinogen deficiency syndrome, Hereditary Congenital fibrinogen disorder, inherited
F5	Factor (V)	Augment immune protection by synchronously engaging innate and T-cell-mediated immunity, promoting a unified and potent defense strategy. Triggering the activation of thrombin, activated protein c (apc) and factor X, thereby initiating a cascade of downstream effects.	Vesicular transport mechanisms and the coordinated formation of blood clot by the fibrinogen because of clotting factor.	Sequence: NC_000001.11 Chromosome: 1` Size: 74,680 base	D-Dimer: Rs6687813-A Rs6025	Hereditary factor V deficiency
		Transforms into a stable, insoluble fibrin aggregate via polymerization reactions. During the re-epithelialization	___	Hepatic secretion only occur Sequence: NC_000004.12	D-Dimer: rs13109457 Fibrinogen: rs6056 rs6050	Congenital fibrinogen disorder, inherited Fibrinogen deficiency

Fibrinogen alpha polymorphism (FGA)	Fibrinogen (Alpha chain)	process, provide stability to the wound site and direct cellular migration to facilitate tissue repair.		Chromosome: 4 Size: 7,620 bases		syndrome, Hereditary

Factor III:

"The F3 locus (thromboplastin/tissue factor) is connected to inherited thrombophilia, distinguished by a greater likelihood of blood coagulation [60]. And factor III, a vital component in the coagulation mechanism, is typically present in small amounts in sub endothelial tissue but becomes susceptible to blood contact following vascular harm" [61]. This exposure leads to the activation of factor VII (FVII) through complex formation with TF, resulting in the activation of factor X (FX) and the subsequent formation of the prothrombinase complex [62]. The prothrombinase complex catalyzes the conversion of prothrombin to thrombin, which executes multiple roles in the coagulation cascade [63]. Tissue factor triggers the coagulation cascade, leading to the formation of a stable fibrin clot [64, 65]. Mutations in the F3 gene impact D-dimer levels, influencing blood clotting and fibrin dissolution [66]. Research reveals elevated D-dimer levels (2.0 ng/ml) in individuals with F3 gene mutations compared to those without (1.5 ng/ml), with a p-value < 0.001 [67, 68].

Factor V Leiden (FVL):

Factor V is essential for blood coagulation, interacting with other factors to produce and fibrin and thrombin [69]. Activated protein C modulates factor V, preventing excessive clot formation [70, 71, 72]. The Factor V Leiden mutation enhances thrombosis susceptibility, and research found elevated D-dimer levels in individuals with this mutation, indicating a correlation between Factor V Leiden and venous thromboembolism risk [73, 74, 75].

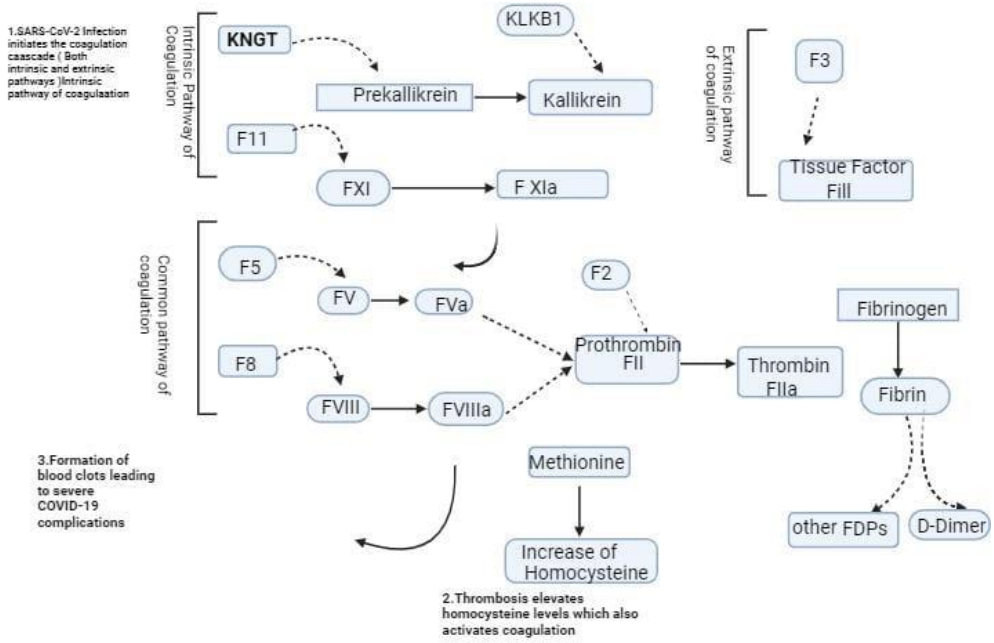
Fibrinogen alpha chain:

Fibrinogen, a vital glycoprotein comprising alpha, beta, and gamma chains, plays a pivotal role in blood coagulation. Recent research has concentrated on the alpha chain's involvement in the development of deep vein thrombosis (DVT), a condition characterized by the formation of blood clots in the legs' deep venous vessels [76]. "Vascular injury activates the coagulation cascade, leading to thrombin generation and fibrinogen transformation into fibrin monomers" [77,78]. Factor XIIIa facilitates polymerization and cross-linking, consolidating the clot [79, 80]. Genetic mutations in the fibrinogen alpha chain, such as the FGA gene, can enhance the risk of deep vein thrombosis [81]. Elevated alpha chain fibrinogen levels are associated with a higher risk of thromboembolic and deep vein thrombosis events [82].

The Impact of COVID-19 on Blood Clotting Mechanisms:

Researchers have unanimously observed elevated D-dimer levels in COVID-19 patients, with a noteworthy study of 191 patients disclosing remarkably higher levels in lethal cases [83]. This suggests potential D-dimer modifications resulting from COVID-19 infection, which may have significant consequences for patient outcomes.

As shown in figure 3, the liver responds to increased inflammatory agents, such as cytokines, by producing fibrinogen, leading to augmented D-dimer levels [84]. This serves as a reliable indicator of coagulation activity in COVID-19 patients, emphasizing the need to investigate the role of thrombotic processes in these cases. Further investigation is necessary to fully comprehend the relationship between D-dimer levels and COVID-19 severity, and to determine whether targeting D-dimer levels may be a useful therapeutic approach [85, 86].



1)	Infection with COVID-19 causes injuries in the endothelium
2)	Activation of coagulation pathway
3)	Extrinsic pathway, TF F3 is activated
4)	The common pathway is activated
5)	VIII and factor V are activated
6)	Thrombin converts fibrinogen to fibrin
7)	Fibrin is then broken down into D- dimer and other FDPs
8)	Genes that might be affected are FGG, FGA and MTHFR

Figure 3: Molecular Link between Key Coagulation Genes and Elevated D-Dimer Levels in COVID-19 [86]

CONCLUSION

COVID-19 has been found to exacerbate coagulopathy conditions through various mechanisms, including the activation of both the extrinsic and intrinsic pathways via factor III, factor V, and Fibrinogen Alpha/Fibrinogen gamma (FGA|FGG). Furthermore, genetic variations can lead to the anticipated surge in”-dimer levels, particularly in elderly individuals and those with age-related comorbidities. Additional investigation is necessary to decipher the complex relationships within the coagulation cascade and its interplay with COVID-19, paving the way for the development of innovative therapeutic approaches to alleviate the burden of this pandemic.

Author contributions:

All authors contributed to the study's design, data collection, analysis, and manuscript revisions, approved the final version, and accepted responsibility for the work.

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Conflicts of Interest:

The authors report no financial or any other conflicts of interest in this work.

Ethical Approvals:

This study does not involve experiments on animals or human subjects.

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