Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to Editors

Further comment on articles pertaining to: "Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19"

Dear Editor-in-Chief Mehar S. Manku:

I have been intrigued with the article "Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19" and the follow-up article "COMMENT ON AN ARTICLE: Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19," both published in the October 2020 issue of *Medical Hypotheses.* Each of these articles validates the medical literature research I have conducted on the links between homocysteine (Hcy) and COVID-19 severity. I hope to further add value to this conversation by extrapolating this research.

Recent studies have found a correlate between higher Hcy levels and higher COVID-19 mortality rates. In October 2020, researchers in Italy noted vasculitic damage in seriously ill patients and suspected Hcy played a contributing role. After initially observing a small cohort of only 40 patients, they conducted a study of Hcy levels in 313 patients and definitively identified Hcy as a predictive marker for COVID-19 mortality risk [1,2]. In addition, Hcy has been associated with many conditions which share comorbidity with COVID-19, including cardiovascular disease, diabetes, and stroke [3,4]. Hcy is found in higher quantities in men, Hcy levels increase with age, and high Hcy is also associated with poor nutrition/low folate which may correlate to socioeconomic disparities for COVID-19 fatalities within the United States [5]. Decreasing homocysteic acid, a homocysteine metabolite, has been shown to decrease serum C-reactive protein in Kawasaki Disease—a disease similar to Multisystem Inflammatory Syndrome in Children (MIS-C) [6]. High Hcy has been found in first-episode schizophrenia [7]; the *MTHFR* C677T polymorphism that contributes to Hcy imbalances has also been associated with schizophrenia [8]; and schizophrenia was recently linked to an increased risk of death in COVID-19 [9]. Given these numerous correlates, it is relevant to explore how Hcy may be impacting COVID-19 severity.

Three Hcy-related GPCRs have repeatedly surfaced in studies on SARS-CoV-2; namely, AT_1R, B_2, and CXCR6. Hcy interference with these GPCRs may be contributing to COVID-19 severity in two primary ways. Firstly, Hcy can act as an alternate agonist to AT_1R at c289s [10], which could exacerbate the renin-angiotensin system (RAS) imbalances induced by SARS-CoV-2 interactions with angiotensin-converting enzyme 2 (ACE2). A recent paper hypothesized that SARS-CoV-2 may be disrupting GPCR signaling and dysregulating adenylyl cyclase [11]. Because AT_1-B_2 receptor heterodimer activity is known to enhance Gi/o and Gq/11 activation, SARS-CoV-2 GPCR signaling disruption may be attributed to potential heterodimeric overexpression in severe COVID-19 as well as to potential heterodimeric hypersensitivity to c289s Hcy agonism. Secondly, Hcy upregulation of CXCL16 [12] in conjunction with SARS-CoV-2 possible binding to CXCL16 and chemotactic disabling [13] could be problematic in those with a chromosome 3 locus that exhibits lower CXCR6 expression [14].

SARS-CoV/-2, homocysteine, AT_1R and B_2

Because ACE2 is used by SARS-CoV-2 for viral entry, the subsequent disruption of the RAS in COVID-19 remains a focal point of study. ACE2 normally converts angiotensin II (AngII) to Ang 1–7, but viral usurpation of ACE2 may leave AngII bioavailable to act instead as an agonist in the AT_1R-NADPH oxidase pathway [15]. The byproduct of this pathway is superoxide ($O_2^{\bullet-}$), a reactive oxygen species (ROS) that interacts with NO to produce peroxinitrite ($ONOO^-$) [16]. Studies done on SARS-CoV have shown that NO reduces viral load, indicating that a decrease in NO may lead to an increase in viral load [17]. Hcy also has been shown to enrich the AT_1R-NADPH oxidase pathway in two ways: via the N- and S-homocysteinylation of ACE [18] and via separate activation of AT_1R through c289s [10]. This c289s Hcy AT_1R agonism may be relevant to the results obtained from a supercomputer analysis of bronchoalveolar lavage fluid (BALF) in COVID-19 which found that AGTR1, the gene that codes for AT_1R, was upregulated 430 fold [19]. The supercomputer analysis, however, discovered that two other GPCRs, B_1 and B_2, were also upregulated 2945 fold and 207 fold respectively. In addition, it found that ACE2 was upregulated 199 fold while ACE was downregulated -8 fold. The co-occurring upregulation of angiotensin- and bradykinin-related GPCRs/GPCR genes in BAL COVID-19 is perplexing, given that these systems involved in vasoconstriction and vasodilation normally counterbalance one another. Furthermore, because ACE cleaves AngI into AngII, downregulation of ACE normally results in a subsequent downregulation of AngII. With AngII being the primary agonist for AT_1R, it is unclear as to why, if ACE is downregulated -8 fold, AGTR1 is upregulated 430 fold in BALF COVID samples. Hcy as an alternate agonist to AT_1R should therefore be considered as potentiating this outcome.

An AT_1-B_2 receptor heteordimer may also be involved in the AGTR1 and B_2 upregulation found in BAL COVID. As one study notes: "If AT_1-B_2 receptor heterodimers *in vivo* are hyperresponsive to AngII, as has been reported, then changes in the relative expression of the two receptors in disease may promote 'unbalanced' signaling by changing the ratio of homodimeric and heterodimeric receptors. For example, AT_1 receptor expression and RAS signaling activity are both up-regulated in experimental diabetes, which may pull a larger fraction of B_2 receptors into AT_1-B_2 heterodimers" [20]. It is possible, therefore, that severe COVID-19 cases may be impacted by imbalanced heterodimer expression. If so, Hcy may once again be a contributing player. While it has yet to be studied in conjunction with the AT_1-B_2 receptor heterodimer, Hcy could further complicate heterodimeric activity because, in addition to activation of AT_1R at c289s, Hcy also increases the expression of kallikrein [21] upstream of B_2 bradykinin agonism.

Furthermore, AT_1-B_2 receptor heterodimers enhance g-protein

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activation and have been found on the omental vessels of preeclamptic patients; likewise, elevated homocysteine levels have been listed as a predictive marker for preeclampsia [22]. It should be noted that AT_1-B_2 receptor heterodimer g-protein activation on the omental vessels of preeclamptic patients has been shown to resist ROS inactivation [23]. While this indicates that the relevance of Hcy to ROS production would bear no impact on heterodimeric g-proteins, it does not preclude the possibility that—just as this heterodimer is hypersensitive to AngII agonism—the concurrent prevalence of high Hcy levels and of the heterodimer in preeclamptic patients indicates the AT_1-B_2 receptor heterodimer may be similarly hypersensitive to c289s Hcy agonism.

Enhanced G-protein activation is also a prominent feature of *Pasteurella multocida*—an organism that produces similar symptomology in animals as SARS-CoV-2 produces in humans, including ground glass opacity, fibrinous pleuritis, pericarditis, splenic atrophy, renal impairment, and hepatic necrosis [24–26]. Both these organisms also have cys-his-asp catalytic triads [27,28]. *P. multocida* leaves g-proteins in an arrested state via deamidation of glutamine [26]. There is no immediate evidence of a similar glutamine deamidation by SARS-CoV-2; however, unbalanced AT_1-B_2 receptor heterodimeric and homodimeric expression could be enhancing Gi/o and Gq/11 activation [29], causing a similar cascade of events in severe COVID-19 as that which occurs with arrested g-proteins in *P. multocida*. All this suggests that further study of AT_1-B_2 receptor heterodimer expression in, potential heterodimer hyperresponsivity to Hcy agonism, and heterodimer-specific g-protein activation in severe COVID-19 is warranted.

SARS-CoV/-2, homocysteine, and reduced CXCR6 expression

Another GPCR that is impacted by Hcy and may be relevant to SARS-CoV-2 morbidity is C-X-C Motif Chemokine Receptor 6 (CXCR6)—a GPCR whose agonist is chemokine CXCL16. It is located at 3p21.31 (GCID: GC03P045982). Separate studies have shown CXCR6 functionality to be limited in SARS-CoV-2. The Genomewide Association Study of Severe Covid-19 with Respiratory Failure notes that the "risk allele GA of rs11385942 is associated with reduced expression of CXCR6" [14], and a recent study at the University of Edinburgh states that the chromosome 3 locus contained six genes, including CXCR6 that "could all be plausibly linked to COVID-19 pathophysiology on the basis of their known functions"; however, it also indicated that CXCR6 had "low ranks in our results" [30]. A third study compared this COVID-19 chromosome 3 locus to a Neanderthal haplotype and found direct relevance [31]. This is somewhat perplexing because CXCR6 deficiency has been shown to ameliorate "kidney injury, proteinuria, and kidney fibrosis" [32]. Additionally, CXCR6 knockout has been shown to "protect mice from LPS-induced lung injury" [33]. It seems, therefore, that reduced expression of CXCR6 should be conducive to better COVID-19 outcomes, not worse. It is possible, however, that Hcy could be a complicating factor. Hcy not only upregulates CXCL16, it also exacerbates CXCL16 ox-LDL scavenging receptor activity [12]. If there is a genetically-reduced expression of CXCR6 (such as with the Neanderthal haplotype) but an increased expression of Hcy-induced CXCL16, then ox-LDL scavenging and subsequent foam cell formation likely will be greater than it would be in those with a higher bioavailability of CXCR6 for CXCL16 binding. Additionally, a study on SARS-CoV found the virus and CXCL16 could interact with each other and hypothesized that the viral interaction with the chemokine inhibits the chemotactic function and subsequent immune response [13]. And, a recent study found that circulating CD8+CXCR6+T cells were "virtually absent in patients with severe COVID-19," but "peripheral levels of CXCL16 were significantly upregulated in severe COVID-19 patients" [34]. Consequently, this combination of genetically-reduced CXCR6 expression, Hcy upregulation of CXCL16, and SARS-CoV/CXCL16 chemotactic disabling, may be contributing to severe COVID-19 outcomes. Therefore, studying Hcy levels in tandem with the chromosome 3 locus/Neanderthal haplotype in severe COVID-19 should be considered.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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