Communication

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SIT1 transporter as a potential novel target in treatment of COVID-19

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Abstract: Studies published earlier this year demonstrated the association of the solute carrier *SLC6A20* gene with the risk and severity of COVID-19. The *SLC6A20* protein product (Sodium-dependent Imino Transporter 1 (SIT1)) is involved in the transport of amino acids, including glycine. Here we summarized the results of recent studies demonstrating the interaction of SIT1 with the ACE2 receptor for SARS-CoV-2 as well as an observed association of *SLC6A20* with the risk and traits of Type 2 diabetes (T2D). Recently, it was also proposed that *SLC6A20* represents the novel regulator of glycine levels and that glycine has beneficial effects against the proinflammatory cytokine secretion induced by SARS-CoV-2 infection. Ivermectin, as a partial agonist of glycine-gated chloride channels, was also recently suggested to interfere with the COVID-19 cytokine storm by inducing the activation of glycine receptors. Furthermore, plasma glycine levels are found to be decreased in diabetic patients. Thus, further clinical trials are warranted to confirm the potential favorable effects of targeting the SIT1 transporter and glycine levels in the treatment of COVID-19, particularly for the severe case of disease associated with hyperglycemia, inflammation, and T2D. These findings suggest that SIT1 may potentially represent one of the missing pieces in the complex puzzle observed between these two pandemic diseases and the potential novel target for their efficient treatment.

Keywords: *SLC6A20*; SARS-CoV-2; Diabetes; Glycine; Ivermectin.

Introduction

In search of potential genetic factors associated with the development of the coronavirus disease 2019 (COVID-19), studies published earlier this year identified the solute carrier *SLC6A20* as one of the few genes associated with the risk and severity of COVID-19 [1,2]. The protein product of *SLC6A20* is Sodium-dependent Imino Transporter 1 (System IMINO transporter (SIT1)), which is involved in the transport of amino acids, including glycine [3] and proline [4]. Interestingly, the SIT1 transporter is reported to co-express with the angiotensin-converting enzyme 2 (ACE2) receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the human small intestine [5,6]. It is also demonstrated that SIT1 heterodimerizes with ACE2, which appears to be required for formation of quaternary structures able to function as binding sites for SARS-CoV-2 spike glycoproteins [7]. Thus, these findings indicate that the interaction of the SIT1 transporter with the ACE2 receptor is required for viral entry and the consequent development of SARS-Cov-2 infection. The modes of SIT1 and ACE2 interaction, particularly in the case of severe COVID-19 associated with hyperglycemia and other traits of Type 2 diabetes (T2D), are still not well understood and the potential mechanisms are discussed in the chapters below.

Association of SIT1 and COVID-19

In addition to the recent reports of the association of the solute carrier *SLC6A20* gene with the risk and severity of COVID-19 [1,2], the results of the genome-wide association study (GWAS) demonstrated that the *SLC6A20* gene, located in an intronic region of chromosome 3, locus 3p21.31, with the minor rs11385942(A) allele, yielded the strongest association signal across the genome for COVID-19 respiratory failure [8] (Table 1). Furthermore, it was reported that *SLC6A20* is predominantly expressed in alveolar type 2 cells and that changes in the *SLC6A20* expression in these cells appear to impact the severity of the infection of the lungs with SARS-CoV-2 [9].

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Table 1: Relevant publications dealing with association of *SLC6A20*/SIT1 with COVID-19 and/or ACE2 receptor for SARS-CoV-2.

SIT1 association with COVID-19 and ACE2 receptor	Type of Study	References
Risk and severity of COVID-19	GWAS	(1, 2)
COVID-19 respiratory failure	GWAS	(8)
SIT1 co-localize with ACE2 receptor in human small intestine; ACE2 expression promotes SIT1 levels	<i>In vitro</i> (human small intestine enterocytes)	(6)
SIT1 co-express with ACE2 in intestine	<i>In vivo</i> animal (ACE2-null mice)	(10)

Previous studies indicated the functional interaction of SIT1 with key players involved in the SARS-CoV-2 infection, including its ACE2 receptor [6,10]. The SIT1 transporter and ACE2 receptor were reported to co-localize in the human small intestine [5,6]. The authors showed that ACE2 expression promotes SIT1 levels, its localization to plasma membrane and its function in amino acid transport [6]. Furthermore, it was demonstrated that ACE2 protein heterodimerizes with *SLC6A20*/SIT1 and its close relative *SLC6A19*, encoding the Broad neutral Amino acid Transporter 1 (BOAT1), which seems to enable these transporters to function as binding sites for SARS-CoV-2 spike glycoproteins [7]. Thus, these findings suggest that in addition to targeting the ACE2 receptor as recently suggested [11], SIT1 may also represent a potential novel target for COVID-19 treatment.

Type 2 diabetes puzzling interconnection, whose roles and regulation are still not completely understood.

It was recommended that patients with diabetes and COVID-19 should be closely monitored and adequately treated to prevent or minimize the development of the potential deleterious effects associated with the co-presence of these diseases [29]. Interestingly, recent studies showed that metformin treatment of Type 2 diabetic patients was associated with reduced mortality for COVID-19 [30–32]. Different mechanisms are implicated so far among protective roles of metformin, including an improvement of insulin resistance [33], regulation of the blood glucose levels [34], regulation of the renin-angiotensin-aldosterone system (RAAS) [35] and ACE2 receptor [34] that is a part of the RAAS [36], and decreasing levels of circulating cytokines [21,37].

COVID-19 and Type 2 diabetes

The evidence has been accumulating to demonstrate that patients with severe COVID-19 are more likely to have a history of diabetes, hypertension, and/or cardiovascular disease [12–14]. The diabetic patients are exposed to an enhanced risk of serious complications, such as cardiac arrest [15,16], neurological disorders [17,18], and venous thromboembolism [19], as well as to an increased severity of this devastating disease [20]. A chronic low-grade inflammation and a more rapid ageing of the immune system have been suggested as the potential mechanisms associated with the higher susceptibility to serious COVID-19 outcomes in T2D patients [21,22]. Furthermore, it was shown that patients with a more severe progression of diabetes seem to have a worse prognosis of COVID-19 as compared to diabetic patients with a milder stage of disease [23]. In addition, it was reported that uncontrolled hyperglycemia augments the risk of poor prognosis [24] and may be a strong predictor of mortality and adverse outcomes in COVID-19 patients [25–28]. Figure 1 illustrates the potential components/mechanisms having a role in COVID-19 and

Association of SIT1 and Type 2 Diabetes

Strikingly, Ling et al. demonstrated a highly significant association of the rs13062383 variant of the *SLC6A20* gene with Type 2 diabetes in Caucasian and Chinese population [38] (Table 2). *SLC6A20* has been reported to regulate the RAAS and water/salt reabsorption [38,39]. RAAS antagonists are known to reduce the risk of diabetes development [40,41] and improve glycemic control in diabetic patients [42], suggesting that the potential targeting of *SLC6A20*/SIT1 may perhaps produce beneficial effects in T2D patients by affecting the RAAS. Interestingly, the pharmacological targeting of *SLC6A19*/BOAT1 transporter, which is a close relative of *SLC6A20*/SIT1 transporter, has been recently suggested as a target in treatment of Type 2 diabetes [43]. Although decreased expression of *SLC6A20* mRNA levels is reported in the kidneys of nonhuman primate and mouse models of T2D [44], further studies are pertinent to analyze the expression profile and regulation of SIT1 in diabetic patients.

Table 2: Relevant publications dealing with association of *SLC6A20*/SIT1 with Type 2 Diabetes (T2D).

SIT1 association with T2D risk and traits	Type of Study	References
<i>SLC6A20</i> rs13062383 SNP associated with increased T2D risk	Prospective, population-based study	(38)
Decreased expression of <i>SLC6A20</i> mRNA levels in T2D	Nonhuman primate model of T2D	(44)
SIT1 regulates glycine levels; (glycine levels are decreased in T2D (79-81)).	<i>In vivo</i> animal (<i>SLC6A20a</i> ^{-/-} knockout mouse)	(3)

Figure 1: Schematic diagram of the potential common components/mechanisms involved in pathogenesis of both, coronavirus disease 2019 (COVID-19) and Type 2 diabetes mellitus (T2D). COVID-19 may predispose the patients to difficult-to-treat hyperglycemia (51, 85) as well as to insulin resistance and impaired insulin secretion (86), which are known T2D traits; A chronic low-grade inflammation, increased levels of inflammatory cytokines, and a perturbed immune system response are also associated with both of these diseases (21, 22, 87, 88); Furthermore, infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) stimulates the production of reactive oxygen species (ROS) (20, 89), which is also an established factor associated with T2D (90), vascular endothelial damage, thromboembolism, and cardiovascular complications often present in serious COVID-19 (20); The modulation of the renin–angiotensin–aldosterone system (RAAS) has been associated with hypertension, inflammation, thrombosis, and myocarditis in COVID-19 patients (91); It is established that RAAS inhibition is beneficial in preventing new onset of T2D (92); In addition, decreased glycine levels are also associated with T2D (79-81). Glycine has demonstrated beneficial effects against the inflammatory cytokine secretion induced by SARS-CoV-2 infection (53).

As mentioned earlier, the SIT1 transporter and ACE2 receptor for SARS-CoV-2 were reported to co-localize and interact with each other *in vitro* [6]. Decreased kidney expression of ACE2 was also observed in patients with Type 2 diabetes [45,46], which was suggested to contribute to poor prognosis in COVID-19 patients [47]. Furthermore, ACE2 knockout mice were more prone to high-fat diet-induced pancreatic β -cell dysfunction and had impaired glucose tolerance/diabetes [48]. Since it was also shown

that the activation of the ACE2/(A1-7)/Mas axis can improve insulin resistance and increase glucose uptake in the liver [49] as well as regulate the insulin/Akt signaling pathway, ACE2 was proposed to be a novel drug target for treating insulin resistance [50]. Furthermore, SIT1 (*SLC6A20*) and ACE2 may potentially regulate the mechanistic link associated with the severity of COVID-19 in Type 2 diabetic subjects [20] and difficult-to-treat hyperglycemia cases in COVID-19 patients [26,51].

Regulation of glycine levels in COVID-19

In order to further clarify the function of the SIT1 protein, earlier this year Bae et al. developed the *SLC6A20a*^{-/-} knockout mouse model for SIT1 and reported that these knockout mice had a decreased survival rate [3]. Interestingly, the extracellular levels of brain glycine were strongly increased in the *SLC6A20a*^{-/-} mice brain and the antisense knockdown of *SLC6A20* expression also increased the glycine concentration in the brain [3], suggesting that *SLC6A20* is a novel regulator of glycine levels which may play an important role in COVID-19 development (Figure 2).

Interestingly, the plasma levels of glycine appear to be significantly decreased in ACE2 null mice [52], suggesting that the activation of ACE2 would increase plasma glycine levels. Recently, it was proposed that glycine itself should be used in the treatment of COVID-19 patients due to its anti-inflammatory, anti-oxidative, neurological functions and role in metabolic regulation [53]. It was suggested that glycine, by binding to its receptor GlyR, induces a chloride influx causing cellular membrane hyperpolarization that guards the cells from pyroptosis and a proinflammatory cytokine storm resulting from SARS-CoV-2 infection [53].

One of the drugs that could be potentially repositioned for COVID-19 treatment and used to affect the glycine levels is ivermectin (IVM), which is known as a partial agonist of glycine-gated chloride channels [54,55]. Recently, IVM

Figure 2: Schematic representation of a potential role of SLC6A20 protein product SIT1 in regulation of glycine levels as well as in treatment of COVID-19 and Type 2 diabetes (T2D). The interaction of SIT1 with the ACE2 receptor for SARS-CoV-2 (6) as well as with the risk and traits of T2D (38) were proposed; The SIT1 transporter is involved in the transport of amino acids, including glycine (3), which appears to have beneficial effects against the proinflammatory cytokine secretion induced by SARS-CoV-2 infection (53) and in prognosis of T2D (82); Ivermectin, as a partial agonist of glycine-gated chloride channels, was recently suggested to interfere with the COVID-19 cytokine storm by inducing the activation of glycine receptors (GlyR) (77).

has become a subject of many controversies regarding its potential use for the treatment of COVID-19. Earlier this year it has been proposed that IVM can be used for this treatment [56,57] following the results of an *in vitro* study, which suggested that IVM may act against SARS-CoV-2 by blocking the nuclear import of viral proteins [57]. Since then, several recent systematic reviews [58–60] and other research reports [61,62] indicated the efficacy of ivermectin in the treatment and prevention of SARS-CoV-2 infection [58,63], while several other recent reports called for additional studies to clarify its use in COVID-19 treatment [64–67]. IVM has also demonstrated antiviral activity against other RNA and DNA viruses [68].

The proposed beneficial effect of IVM in the treatment of COVID-19 would accompany the enormous impact that this drug has already made in the treatment of a variety of parasitic infections that led to the Nobel Prize in 2015 and to its inclusion to the World Health Organization's Essential Medicines list [60,62,69]. IVM was found to be a highly effective, broad-spectrum, safe, and well tolerated drug [70], which, like other macrolide compounds, exhibited extremely wide diverse actions [71]. The inhibition of protease SARS-CoV-2 3CLPro activity that appears essential for viral replication [72,73] and the competitive binding of IVM with the viral S protein [74], were suggested as

additional potential mechanisms that inhibit viral binding to ACE2 receptors and prevent consequent infection. In addition, it has been also reported that IVM has anti-inflammatory properties [75,76], which can interfere with the COVID-19 cytokine storm by inducing the activation of glycine receptors on leukocytes and vascular endothelium [77]. The results of a recent study demonstrated a high sensitivity of neuronal glycine receptors to IVM [78]. Thus, in line with the diverse biological functions of IVM, it would be important to further study its effects on glycine levels and whether regulation of glycine concentration perhaps includes the potential inhibition/silencing of the SIT1/SLC6A20 that may contribute to the treatment of COVID-19.

Regulation of glycine levels in Type 2 Diabetes

Recent metabolomic and genetic studies confirmed a strong association of T2D with decreased glycine levels in human subjects [79–81]. Furthermore, additional studies reported that plasma glycine concentration were decreased in obese and diabetic patients, and that the improved insulin sensitivity led to increased plasma glycine levels [82]. It appears that hypoglycinemia occurs even during the prediabetic state, however the effects of decreased glycine levels on T2D development are still not completely understood [82]. In addition, insulin sensitizer therapy with the combination of pioglitazone and metformin increased plasma glycine concentration as compared to the placebo treatment [83]. Furthermore, it was shown that IVM treatment enhanced the gene expression of two subunits of the glycine receptor, significantly modulated the mRNA expression of key markers in adipogenesis and fatty acid metabolism, as well as indicated an improved insulin sensitivity *in vitro* [84].

Conclusions

Recent studies demonstrated the association of *SLC6A20* gene with the risk and severity of SARS-Cov-2 infection, where the preexisting hyperglycemia and diabetes diagnosis appear to be a strong predictor of mortality and adverse outcomes in COVID-19 patients. Previous reports also showed an association of *SLC6A20* with Type 2 diabetes development. Based on these findings, *SLC6A20*/SIT1 may represent a novel target in the treatment of COVID-19, particularly in severe cases associated with T2D traits.

Furthermore, it was also recently proposed that *SLC6A20* represents the novel regulator of glycine levels and that glycine has beneficial effects against the proinflammatory cytokine secretion induced by SARS-CoV-2 infection. It would be pertinent to further explore the possible mechanisms involved in the regulation of glycine levels, including the proposed inhibition of the SIT1 transporter and the activation of the glycine receptors by IVM or other potential drug candidates, which would result in increased levels of glycine. Hypoglycinemia has been found to be strongly associated with T2D, so it would be also important to analyze the glycine levels in COVID-19 patients, which seem not be studied yet. Therefore, additional clinical investigation is in order to confirm the potential favorable effects of targeting the SIT1 transporter and glycine levels for the efficient treatment of both, COVID-19 and Type 2 diabetes.

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