Gitelman’s and Bartter’s Syndromes: From Genetics to the Molecular Basis of Hypertension and More

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Abstract

**Background:** Gitelman’s and Bartter’s syndromes (GS/BS) are rare genetic tubulopathies characterized by electrolyte imbalance and activation of the renin-angiotensin-aldosterone system (RAAS). These syndromes have intriguing biochemical and hormonal abnormalities that lead them to be protected from hypertension and cardiovascular and renal remodeling. **Summary:** In this review, we explore the biochemical/molecular mechanisms induced by the activation of the RAAS and its counterregulatory arm which is particularly activated in GS/BS patients, in the context of blood pressure regulation. In addition, we report our findings in the context of the COVID-19 pandemic where we observed GS/BS subjects being protected from infection. **Key Messages:** The intracellular pathways induced by Ang II, starting from induction of oxidative stress and vasoconstriction, are crucial for the progression toward cardiovascular-renal remodeling and might be useful targets in order to reduce/halt the progression of Ang II/oxidative stress-induced cardiovascular-renal morbidity in several diseases.

Introduction

In the sixties of the last century, Bartter et al. [1] and Gitelman et al. [2] independently observed different cases of patients with electrolyte imbalances, metabolic alkalosis, and salt wasting. These subjects had activation of the renin-angiotensin-aldosterone system (RAAS) and excess of mineralocorticoids yet were normotensive or even had hypotension. Subsequent investigations have made it clear that these syndromes are caused by impaired sodium reabsorption in the thick ascending limb and in the distal convoluted tubule (DCT) due to mutations affecting ion channels and cotransporter in those regions [3–8]. The clinical characteristics and the severity of biochemical abnormalities reflect in fact gene-specific patterns; hence, the etiology of both syndromes is determined by mutations in genes that encode for cotransporters or channels involved in the trafficking of electrolytes in the nephrons. These alterations concur to depict a clinical picture composed of hypokalemia, metabolic alkalosis, hypercalciuria or normocalciuria (Bartter’s syndrome [BS]) or hypocalciuria (Gitelman’s syndrome [GS]), muscle weakness, polyuria, failure to thrive, polydipsia, and other syndrome-specific complications. Nevertheless, in most cases, the rapid recognition of the disease and the treatment permit acceptable conditions of life. We took
advantage of our cohort of GS/BS patients to investigate the reasons why notwithstanding the excessive activation of the RAAS and the increase of angiotensin II (Ang II) and aldosterone, the affected subjects display normo- or even hypotension and why they do not exhibit cardiovascular-renal remodeling. Our studies demonstrated that GS/BS have an altered Ang II signaling downstream the binding of Ang II with its receptor (AT1R) such that they have an endogenous blockade of Ang II signaling [9]. Therefore, due to the fundamental/critical action of Ang II, GS/BS patients may be a useful means to understand the pathophysiology of diseases involving Ang II signaling. From this perspective, in fact, GS/BS patients serving as a human model of altered Ang II signaling for our investigations into other diseases in which Ang II plays a key role, such as hypertension, chronic kidney disease (CKD), end-stage kidney disease (ESKD), Fabry disease, and even the COVID-19 pandemic, might not only provide insights into Ang II signaling in these diseases but also eventually give the chance for the identification of novel potential targets of therapy.

**Gitelman’s Syndrome**

GS’s (OMIM® #263800) typical clinical features are salt craving, muscle weakness, fatigue, and cramps with electrolyte imbalance associated with the side effects of treatment with thiazide diuretics that target the DCT. The cloning and characterization of the gene SLC12A3 encoding for the sodium chloride cotransporter NCC showed that several mutations can be detected in GS patients, most of them impairing NCC function with ensuing sodium wasting and the activation of adaptive mechanisms such as the aldosterone-driven increased excretion of K⁺ in exchange for Na⁺ and the flux-dependent K⁺ secretion along the collecting duct [10]. Specifically, in the early portion of the DCT, the transport of Na⁺ is electrogenic for the concomitant release of Cl⁻ through the NCC, while in the late portion of the tubule, the activation of the epithelial sodium channel in order to restore the water balance leaves a negative charge in the lumen. As a counterpart, to restore the neutral charge, the renal outer medullary potassium channel (ROMK) drives out K⁺, inducing hypokalemia. Besides hypokalemia, hypocaliuria is another feature of GS [11]. Experimental evidence in the animal model of GS showed that these animals’ lack of Ca²⁺ (TRPV5) channels in the early DCT – further supporting the evidence that impaired function of the NCC – leads to major structural remodeling of the DCT [12]. Calcium imbalance is associated with arthropathy with calcium pyrophosphate crystal deposition or chondrocalcinosis [13]. Other clinical symptoms of GS are dizziness and prolongation of the QT interval on electrocardiogram (due to the depolarization and repolarization of the ventricles) that causes arrhythmia. Onset time of the symptoms can vary but mostly appear in the early adulthood as less severe, while in young patients causes growth retardation, pubertal delay, and short stature [11]. The suspect for GS comprises the following biochemical anomalies: hypokalemia (<3.5 mmol/L) and renal potassium wasting as spot urine sample with the potassium/creatinine ratio >2.0 mmol/mol (>18 mmol/g); metabolic alkalosis; hypomagnesemia <0.7 mmol/L (<1.70 mg/dL) and renal magnesium wasting (fractional excretion of magnesium >4%); hypocaliuria as a spot urine calcium-creatinine ratio <0.2 mmol/mmol (0.07 mg/mg) for adults; fractional excretion of chloride >0.5%; normal or low blood pressure; and the absence of morphological and functional renal abnormalities [11].

**Bartter’s Syndrome**

Clinical BS manifestations are muscle weakness, anorexia, polydipsia, polyuria, failure to thrive, and growth retardation with salt wasting and high plasma levels of prostaglandins. Biochemical explanation can be related to the impaired tubuloglomerular feedback when chloride is not reabsorbed in the macula densa, and the ensuing activation of cyclooxygenases promotes prostaglandins-induced renin release and aldosterone production in order to recover normal intravascular fluid balance. Other consequences of impaired sodium reabsorption are hypercalciuria and progressive medullary nephrocalcinosis and the reduced capacity to concentrate urine [14]. BS generally differs from GS because of the very early onset time: it presents mostly early in the childhood with severe failure to thrive and mental or growth retardation, but an antenatal form can also be observed by refractory polyhydramnios, leading to premature delivery [14]. Despite considerable phenotypic overlap, mutations in different genes result in 5 different BS types. Mutations in the NKCC2 cotransporter have classified BS type 1 (OMIM® #601678), with typical Na⁺ and K⁺ waste and systemic alterations from fetal gestation onwards. The clinical manifestations are severe salt-wasting hypokalemic metabolic alkalosis, hypercalciuria, and high levels of prostaglandins in urine associated with progressive medullary nephrocalcinosis. Further, the excess of the prostaglandin E₂ leads to fever, hypocalcemia, hypotension, and severe dehydration [14]. Other subtypes of BS are caused by mutations in genes that regulate the activity of NKCC2. The KCNJ1 (Kir1.1) encodes for the apical ATP-
sensitive K+ channels ROMK that recycles K+ from the cell back to the lumen. A nonfunctional ROMK identifies BS type 2 (OMIM® #241200): the falling luminal K+ shuts down NKCC2 activity and induces salt wasting with transient hyperkalemia associated with a pseudo hypoaldosteronism type 1 phenotype in the antenatal form [3]. BS type 3 (OMIM® #607364) is caused by mutations in the chloride channel CLCNKb and is characterized by salt wasting and hypovolemia [5]. A necessary β-subunit for CLCNKb activity is barttin encoded by the BSND gene. Mutations affecting the barthtin interacting motifs, the dimerization sites, and the selectivity filter result in severe phenotype contrary to the milder or even undetected form caused by mutations in the alpha-helices [15]. The consequences of barttin alteration and its functionality reflect in the NKCC2 activity for the accumulation of chloride which induces salt wasting. Mutations in the barthtin identify BS type 4a (OMIM® #602522). The barttin protein is required for the basolateral location of the CLCNKb isoform as well as for the location of the CLCNKa isoform. Digenic mutations might inactivate all the 4 alleles of both CLCNKb and CLCNKa, resulting in a different BS subtype named BS type 4b with nerve deafness and defect in the sensory-neural transduction of sound, especially for the high presence of CLCNKa in the inner ear [6]. Finally, mutations in the MAGED2 gene identify a severe antenatal form of BS which resolves spontaneously during the first weeks to months of life, and it is now identified as BS type 5 (OMIM® #300971), which originally was referred to patients with alterations of the Ca2+-sensing receptor with autosomal dominant hypocalcemia (OMIM® #601198) [7, 16].

The current therapy for GS and BS is the correction and improvement of electrolyte abnormalities, with supplementation of sodium chloride, potassium chloride, and fluids based on the severity of the disease. For most BS patients in case of severe hypokalemia unsolvable with dietary supplementation, the use of potassium-sparing diuretics, renin-angiotensin system blockers, or nonsteroidal anti-inflammatory drugs has been proposed [14]. GS is usually managed by free sodium chloride intake together with oral magnesium and potassium supplements [11]. The use of potassium-sparing diuretics and renin-angiotensin system inhibitors has been reported occasionally in GS; however, evidence supporting the efficacy, safety, and tolerability of these options either in GS or in BS is limited, and for their nature, they might aggravate renal sodium wasting and increase the risk of symptomatic hypovolemia [11].

**Insights on Angiotensin II Signaling from GS/BS**

The RAAS activity mediates most of the pathophysiological effects related to vascular remodeling, including peripheral resistance, inflammation, cellular growth, and fibrosis [17, 18]. In healthy individuals, vasoconstriction is a physiological response to physical activity and circadian rhythms and is driven through Ang II activity [19]. However, Ang II signaling might be overactivated in nonphysiological conditions with consequences on both circadian rhythms and systolic/diastolic blood pressure. The altered equilibrium of electrolytes in the thick ascending limb and the DCT of BS and GS, respectively, induces sodium wasting and activation of counterregulatory systems in the nephrons. These include a reaction of the baroreceptors which, once detected volume depletion, induces release of renin and expression of aldosterone and finally vascular contraction. In healthy individuals, a chronic stimulation of these systems induces sympathetic overactivity, accentuating arteriolar vasoconstriction, influencing oxygen delivery, and inducing hypertension. Sustained increased blood pressure reflects vascular remodeling: in endothelium and vascular smooth muscle cells (VSMCs), responsive intracellular mechanisms are activated with ensuing short-term and long-term consequences. The primary outcome of the short-term is vasoconstriction, but in the long term, cellular growth and fibrosis prevail, causing hypertrophy in several districts of the body including the kidneys and the heart. We observed that GS/BS patients’ chronic hypotension is not associated with either sympathetic or parasympathetic activities [20] and does not display any of these hypertrophic outcomes, and somehow, those intracellular pathways are blunted (as shown in Fig. 1). Specifically, while in physiological conditions, Ang II binds to its angiotensin II-receptors type 1 to promote the activation of the Gq proteins and the ensuing intracellular events such as the release of the messengers inositol triphosphate (IP3) and Ca2+, the generation of superoxide (O2•−), and the activation of protein kinase C (PKC) and RhoA/Rho kinase pathway; in GS/BS patients, those messengers are less or even not released [9]. Compared to healthy subjects, GS/BS patients have in fact reduced gene expression of the α subunit of Gq and blunted downstream events that promote Ca2+ release and PKC activation. In addition, since PKC is a negative regulator of the endothelial nitric oxide synthase (eNOS), which is part of a counterregulatory intracellular system deputed to promote vasodilation, in our studies, we detected a generalized reduction of PKC activity [9]. This latter matched with the significantly in-
increased eNOS expression and increased urinary excretion of NO metabolites. Increased eNOS and NO-mediated vasodilation are crucial for the response of endothelium to increased flow in the brachial artery. Consistently, in comparison to hypertensive patients and also to normotensive healthy subjects, GS/BS display increased flow-mediated dilation [21].

This evidence had made it clear that deep investigations into Ang II signaling might help in understanding what is or are the pivotal mechanisms to be targeted to reduce the sequelae of oxidative stress in the kidneys. To this aim, in the following paragraphs of this review, GS/BS patients’ intracellular pathways linked to Ang II signaling will be compared to those of hypertensive, CKD, and ESKD in dialysis patients.

**Ang II Signaling: The Binding with AT1R**

The VSMCs are components of the blood vessels, influenced by mechanical and biochemical signals, and are physically bound to the extracellular matrix. Their intracellular pathways induce variation in the subcellular structures such as microtubules, actin, and intermediate filaments; therefore, any stimulus affecting those cells reflects into rearrangements of cytoskeletal actin filament in order to modulate vascular diameter. The binding of Ang II with its AT1R in VSMCs induces the coupling of phospholipase Cβ1, mediated by Gaq/11βγ and Gaq/12βγ proteins, and the subsequent activation of PLCγ. This mechanism triggers the other intracellular messengers IP3 and Ca2+, diacylglycerol, and PKC. The release of Ca2+ is crucial for the sensitivity of cytoskeletal filaments and for the contraction of the VSMCs.

Regulator of G Protein Signaling

The unique abnormally low vascular reactivity of GS/BS patients does not rely on alteration in numbers of AT1R or on the affinity of Ang II peptide itself, as it has been demonstrated in those patients compared to healthy controls [9]. However, already in the very immediate downstream signaling of Ang II, which is controlled by
the regulator of G protein signaling-2 (RGS2), crucial for GTP hydrolysis of the Ga subunit. GS/BS patients present a peculiar trait. RGS2 inhibits the Ga binding to effectors such as PLC and inhibits the GPCR-mediated vasoconstrictor signaling pathways. As it has been observed in animal models, the stimulus with Ang II in RGS2 knock-out mice resulted in a larger increase in blood pressure compared to wild-type animals, further demonstrating the role of RGS2 in the transduction of vasoconstrictor signals [22]. GS/BS patients show increased RGS2 expression that may explain their downregulation of Gq protein signaling and the reduced peripheral resistance and vascular hyporeactivity [23]. Experiments on RGS2 isolated from fibroblasts of GS/BS patients showed that increased RGS2 desensitizes Ang II-GPCR signaling, determining reduced IP₃ and diacylglycerol and ensuing less intracellular Ca²⁺ release and PKC activity [24, 25]. In addition, this latter is a stimulus for the upregulation of eNOS and for the increase of the vasorelaxant NO (which in turn is negatively regulated by PKC) [23, 26, 27]. RGS2 is also involved in the long-term Ang II signaling through other intracellular pathways leading to cardiac alteration. In vitro models of cardiac fibroblasts demonstrated that RGS2 upregulates in response to Ang II stimulus as a mechanism to halt cell proliferation and fibrosis [28].

Nitric Oxide System

We observed with great interest that the role of the NO system in the pathophysiology of other diseases with renal involvement may vary depending on the surrounding environment. In this regard, in essential hypertension, the increased oxidative stress disrupts the counterbalancing antioxidant systems and in particular, pushes the eNOS to its uncoupling to produce O₂⁻• and peroxynitrite rather than NO [29]. Contrary to GS/BS patients, hypertensive subjects have reduced bioavailability of NO as well as reduced endogenous antioxidant defenses such as superoxide dismutase and glutathione peroxidase, alongside increased oxidative stress [30, 31]. The concomitant presence of free radicals and inactivation of compensatory systems favor NO sequestration to further produce other reactive oxygen species (ROS). Those damaging mechanisms are present also in CKD and ESKD patients where the increased plasma levels of oxidative-stress-related proteins reduce NO bioavailability [32]. In addition, kidney transplant recipients frequently develop posttransplant hypertension, but in response, their endogenous eNOS and NO levels are elevated. These patients undergo endothelial dysfunction and general inflammation and activation of other intracellular pathways targeted for cardiovascular remodeling [33]. Therefore, it seems that the increased reaction of the NO system in transplanted patients might serve as a further supply for peroxynitrite production rather than to fight against oxidative damage. Indeed, the immunosuppressive treatment of transplanted patients with calcineurin inhibitors induces ROS production and TGFβ which further regulate ROS activity and downregulate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and others, prompting even more endothelial remodeling and vasoconstriction [33, 34].

RhoA and Rho Kinase System

The binding of Ang II with its receptors triggers Rho guanine nucleotide exchange factors (GEFs) such as p63RhoGEF and p115RhoGEF via the Ga subunit. These proteins are upstream regulators of monomeric G protein RhoA and its effector Rho kinase (ROCK system) that are held to promote in the short term vasoconstriction and in the long term vascular remodeling [35]. In GS/BS patients, the gene and protein expression of p63RhoGEF and p115RhoGEF are reduced and are associated with increased guanine nucleotide dissociation inhibitor (RhoGDI) which in turn inhibits dissociation of GDP from GDI maintaining RhoA inactive [9, 36]. In addition, the downstream ROCK pathway is also reduced as demonstrated by the lower expression of ROCK’s target observed in mononuclear cells of GS/BS patients [9, 37]. The function of this system relies on the signaling cascade that phosphorylates the myosin phosphatase target subunit 1 (MYPT-1) in order to inhibit the myosin light-chain phosphatase so that the MLC kinase activity increases and the actomyosin-based contractility persists. The inhibition of myosin light chain phosphatase contributes to stress fiber formation, Ca²⁺ sensitization of the VSMCs, and contraction [38]. It is therefore clear that the activation of this system might be crucial also in hypertension. The evaluation of p63RhoGEF, p115RhoGEF, and ROCK showed increased protein expression in hypertensive patients compared to GS/BS patients. These later have a blunted ROCK system even compared with healthy subjects, providing one more piece of evidence for their reduced peripheral resistance and normal/reduced blood pressure [37]. The downstream targets of the ROCK system are also mitogen-activated protein kinases such as extracellular signal-regulated kinases 1/2 which control nuclear transcription factors for cellular proliferation. These factors strongly influence cardiac remodeling. It has been observed that contrarily to GS/BS patients, which do not display cardiac remodeling, stage 3–4 CKD
patients and ESRD patients under dialysis develop left ventricular hypertrophy. In a study on these patients, we observed that those who displayed left ventricular hypertrophy had remarkably increased phosphorylation of MYPT-1, which positively correlated with LV mass [39]. Subsequent experiments ex vivo on leukocytes isolated from stage 3–4 CKD and dialysis patients demonstrated that the inhibition of ROCK with fasudil (a ROCK’s specific inhibitor) dose-dependently decreased MYPT-1 phosphorylation. Taken together, this evidence confirms that regulation of ROCK in these patients might be useful to prevent cardiovascular-renal remodeling.

The Nox System

In the long term, the intracellular signaling induced by Ang II influences the oxidative state and is related to hypertension, atherosclerosis, and cardiovascular and renal remodeling [17, 40]. The most relevant sources of ROS are NAD(P)H oxidases, xanthine oxidases, P-450 monooxygenases, lipooxygenases, and cyclooxygenases. In particular, one of those enzymes is triggered by Ang II: the NAD(P)H oxidases, also known as Nox, which catalyze the production of O$_2^•$ from oxygen and NAD(P)H [41]. Noxs are characterized by a catalytic subunit and a little p22 phox subunit that is fundamental for the formation of the complex heterodimeric cytochrome b558. The association between p22 phox and Nox induces cascades of phosphorylation for cytosolic subunits that can translocate to the membrane and promote conformational changes in both the enzyme and the cellular membrane [41]. The presence of p22$^{\text{phox}}$ allows electron transfer to molecular oxygen that, once it becomes O$_2^•$, spread the production of free radicals and other ROS to the lipids membranes and has a trickle-down effect on other districts [42]. In GS/BS patients, the response of Noxs to Ang II is reduced. Stimulation of monocytes isolated from these patients with Ang II showed in fact a decreased activation of oxidative mechanisms in terms of decreased p22$^{\text{phox}}$ gene expression and TGFβ [43]. This evidence represents the mirror image of what has been observed in essential hypertensive patients that are known to have high oxidative stress status (see Table 1). Treating hypertensive patients with AT1R blockers their endogenous production of p22$^{\text{phox}}$ decreases alongside the phosphorylation state of extracellular signal-regulated kinases 1/2 [44]. Consistently, in dialysis patients, the use of vitamin E-coated dialyzers or the use of hemodiafiltration with online regeneration of ultrafiltrate or even the treatment with green tea supplements significantly reduced the expression of p22$^{\text{phox}}$ and oxidative stress state, further confirming the importance of this intracellular pathway in the progression of oxidative-stress-mediated cardiovascular-renal remodeling [45–48].

In addition, we have also observed that GS/BS have reduced susceptibility of the low-density lipoprotein (LDL) to oxidation [49]. Oxidated LDL is deeply involved in the progression of atherosclerotic lesions both in the cardiovascular and renal system, and the increased endogenous NO levels in GS/BS participate to protect LDL from oxidation. Again, treatments aimed at reducing the

### Table 1. Comparison of angiotensin II short- and long-term signaling between GS/BS and hypertension and end-stage kidney disease (ESKD)

| Ang II short- and long-term signaling effects | GS/BS | Hypertension | ESKD | References |
|-----------------------------------------------|-------|--------------|------|------------|
| Intracellular Ca$^{2+}$ release                | Reduced | Increased | Increased | [18]          |
| Intracellular IP3 level                        | Reduced | Increased | Increased | [18]          |
| PKC expression and activity                    | Reduced | Increased | Increased | [18]          |
| NO system                                      | Upregulated | Downregulated | Downregulated | [19]          |
| eNOS expression                                | Increased | Reduced | Reduced | [19]          |
| NO-dependent relaxation                        | Increased | Reduced | Reduced | [19]          |
| $G_{sat}$ expression                            | Increased | Increased | Not evaluated | [18]          |
| RGS2 expression                                | Increased | Reduced | Not evaluated | [18]          |
| ROCK activity (MYPT-1)                         | Reduced | Increased | Increased | [26, 28]      |
| ERK 1/2 expression                             | Reduced | Increased | Increased | [30, 35]      |
| Oxidative stress                               | Reduced | Increased | Increased | [34, 37, 40, 43, 48] |
| Inflammatory state                             | Reduced | Increased | Increased | [18]          |
| Cardiovascular-renal target organ damage       | Lack | Present | Present | [18, 48]      |

NO, nitric oxide; ERK1/2, extracellular signal-regulated kinases 1/2.
And More (Posttransplant Hypertension, Fabry Disease, and COVID-19)

Each of the cotransporters and channels in the different segments of the nephrons has a role in the physiology of fluid homeostasis and impacts the recruitment of hormones, salt retention, endothelial dysfunction, and the activation of the sympathetic system. We observed that the role of the NCC in GS patients has been useful also to understand the pathophysiology of posttransplant hypertension in kidney-transplanted patients. Those patients in fact use calcineurin inhibitors as immunosuppressive therapy, but the mechanisms through which those drugs operate are actually through their inhibitory effects on with-no-lysine kinases, glucocorticoid-regulated kinase 1, STE20/SPS1-related proline alanine-rich kinases, and oxidative stress-responsive protein type 1 kinase that are instead a switch-off for NCC activation [50]. However, Ang II still regulates sodium reabsorption in multiple ways along the nephron. Beside the stimulation of aldosterone release, which in turn induces Na⁺/K⁺-ATPase and epithelial sodium channel upregulation in the DCT and collecting duct, Ang II also stimulates the production of AVP that itself can modulate NCC function through STE20/SPS1-related proline alanine-rich kinase activation [33].

Investigations in GS/BS patients have given noteworthy insights to understand and assess the pathophysiology of oxidative-stress-related outcomes in another rare disease, the Fabry disease. It is an X-linked storage disorder with mutations affecting the lysosomal enzyme α-galactosidase, a gene which encodes for α-GalA (OMIM 301500) [51]. This disease results from the progressive accumulation of globotriaosylceramide, its deacetylated form (Lyso-globotriaosylceramide), and other glycosphingolipids in lysosomes due to insufficient activity of the enzyme. Fabry disease is a multifactorial disease with a strong renal involvement that is reflected in the long term as poor cardiac outcomes. The understanding of the pivotal role of Ang II signaling and oxidative stress in the involvement of cardiovascular-renal remodeling prompted us to assess the oxidative stress status in Fabry disease, which was found increased and not influenced by their specific enzyme replacement therapy, and to propose the possible positive effect of an adjunctive non-pharmacological treatment with an antioxidant on top of ERT to alter the progression of the disease [52, 53].

Finally, facing the pandemic emergency that has involved the whole planet in the last 2 years and monitoring GS/BS patients referring to our unit, we observed that they have been in some way protected against the SARS-CoV-2 infection. This was confirmed by three different surveys during the pandemic, the first one being performed on GS/BS patients of our cohort living in the hot-spot regions of the COVID-19 pandemic in Northern Italy during the first wave of the pandemic, and we found a complete absence of COVID-19 infection or symptoms in the very first wave of COVID-19 [54]. Two further surveys, the last during the third wave due to the more infective omicron variant, confirmed these findings with very few GS/BS patients infected and/or with very mild symptoms. This prompts us to investigate the role of angiotensin-converting enzyme 2 (ACE2) given that, due to the role of ACE2 as an entry point for the virus into the cell, patients under angiotensin receptor blockers or ACE inhibitors that are held to increase ACE2 levels might be more susceptible to the infection [55]. The increased levels of ACE2 are characteristics of GS/BS patients [9, 56], and it is also a very important pathway that drives Ang II to Ang 1–7 and to the counterregulatory axis of the RAAS [57]. GS/BS patients have a characteristic chronic metabolic alkalosis that may have altered the terminal glycosylation of ACE2 in the trans-Golgi network/endosome system. This latter may reduce the lysosomal acidic environment necessary for protein glycosylation process, including ACE2 glycosylation, thereby reducing the susceptibility of SARS-CoV-2 binding to ACE2 and resulting COVID-19 disease [54]. We have shown, in fact, that GS/BS patients have a reduced ACE2-glycosylated isof orm and reduced cathepsin-L activity, the latter being a protease necessary for the virus spreading, both correlated with patients’ metabolic alkalosis in terms of blood bicarbonate levels [58]. It is noteworthy that the protection from COVID-19 we have confirmed with our surveys in GS/BS patients and demonstrated to naturally occur in these patients has also the same mechanistic rationale, the reduction of cathepsin-L activity, on which is based the effect of Paxlovid, the new available drug to fight COVID-19 [59].

Conclusion

Altogether, the evidence coming from studies in GS/BS patients is useful to investigate the pathophysiology of other diseases with cardiovascular-renal involvement. The overactivity of the RAAS in GS/BS does not correlate with cardiovascular-renal outcomes in these patients,
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