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Guanylate cyclase-C receptor activation: unexpected biology

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Abbreviations:

ADHD: Attention Deficit Hyperactivity Disorder

cGMP: cyclic-guanosine-3',5'-monophosphate

Cl^-: chloride

CRD: Colorectal distension

C-IBS: Constipation-predominant Irritable Bowel Syndrome

CFTR: cystic fibrosis transmembrane regulator

DSS: dextran sodium sulfate

GC-C: Guanylate cyclase-C

GTP: guanosine triphosphate

HCO_3^-: bicarbonate

IBD: Inflammatory Bowel Disease

IBS: Irritable Bowel Syndrome

PKG: cGMP-dependent protein kinase

STa: Heat-stable enterotoxin type A

TNBS: Trinitrobenzenesulfonic acid

-/-: gene knockout

+/-: heterozygous

+/+: wild-type

Abstract:

Guanylate cyclase-C (GC-C) is a transmembrane receptor activated by bacterial heat-stable enterotoxins and by the endogenous hormones guanylin and uroguanylin. GC-C plays key roles in the regulation of intestinal fluid and electrolyte homeostasis. This is highlighted by several recently identified human mutations in *GUCY2C*, the gene encoding GC-C, which leads to the respective gain or loss of function of GC-C, resulting in profound effects on gastrointestinal function. However, a wealth of recent studies indicates GC-C signalling extends to a multitude of diverse additional functions. Recent pre-clinical and clinical studies demonstrate a novel first-in-class GC-C activating peptide, Linaclotide, provides effective relief from constipation and abdominal pain in patients with chronic constipation and constipation-predominant Irritable Bowel Syndrome. Accumulating evidence also suggests GC-C plays protective roles in mucosal barrier function, tissue injury and inflammation, whilst GC-C signalling is a key regulator of intestinal cell proliferation and apoptosis. Finally, recently identified extra-intestinal GC-C signalling pathways make novel contributions to the regulation of food intake and symptoms associated with Attention Deficit Hyperactivity Disorder. Consequently, these findings provide GC-C expression and its associated mutations as potential diagnostic markers for disease. They also provide current and future therapeutic potential for GC-C signalling within and outside the gastrointestinal tract.

Introduction:

Guanylate cyclase-C (GC-C) is one of seven mammalian transmembrane guanylate cyclase receptors. It is predominantly expressed on epithelial cells within the gastrointestinal tract, where it was initially identified as the receptor for the bacterial heat-stable enterotoxin, STa, which is produced by a variety of enteric pathogens, including *Escherichia coli*, *Citrobacter freundii*, *Vibrio cholerae*, *Vibrio mimicus*, and *Yersinia enterocolitica* [1]. STa acts as a super agonist of GC-C and triggers a signalling cascade that eventually leads to large and uncontrolled release of electrolytes and water into the intestinal lumen, resulting in secretory diarrhea and dehydration. In very severe cases, without adequate treatment, this mechanism is responsible for over half a million deaths per year, mostly of children in developing countries [2-4]. This is the disturbing, pathological extreme of GC-C signalling. However, GC-C does have endogenous ligands, the hormones guanylin and uroguanylin. Uroguanylin is a 19 amino acid peptide synthesized primarily in the duodenum and proximal small intestine, whilst guanylin is a 15 amino acid peptide primarily synthesized in the distal ileum and proximal colon [5,6]. Both are evolutionarily conserved across species, which strongly indicates they have unique physiological roles [7]. Secretion of these peptides into the intestinal lumen results in autocrine and paracrine GC-C activation and the subsequent conversion of guanosine triphosphate (GTP) to cyclic-guanosine-3',5'-monophosphate (cGMP). This leads to intracellular cGMP accumulation, PKGII-dependent phosphorylation of the cystic fibrosis transmembrane regulator (CFTR) and increased chloride (Cl^-) secretion into the intestinal lumen. Release of Cl^- and bicarbonate (HCO_3^-) through the CFTR is accompanied by inhibition of Na^+/H^+ exchanger, the net effect of which is to elevate extracellular Na^+ and Cl^- and drive fluid accumulation in the lumen [8] (Figure 1). Although this is the same mechanism by which STa causes secretory diarrhea, uroguanylin and guanylin have respective affinities for GC-C ten- and one hundred-times lower than that of STa [4]. Furthermore, unlike STa these endogenous peptides undergo rapid proteolytic processing following secretion [1]. As such this physiological

activation of GC-C regulates intestinal fluid homeostasis, preventing dehydration and intestinal obstruction. In addition to this key function recent advances indicate GC-C signalling has a multitude of additional and unexpected functions, which are diverse and occur within and outside of the gastrointestinal tract. These recent findings have provided current human therapeutics, potentially useful leads in the development of new therapeutics and insights into disease. The review of this unexpected biology starts with the recent discovery of human familial gene mutations, which result in aberrant GC-C activity.

Mutations in GUCY2C, the gene encoding GC-C, are associated with altered gastrointestinal motility.

Two very recent studies have demonstrated several distinct mutations in *GUCY2C* are associated with profound resultant effects on gastrointestinal motility (Table 1). The first study described a novel autosomal dominant 'gain of function' disease in 32 members of a Norwegian family who had a family history of chronic diarrhea that started in infancy. The severity of symptoms could be linked to specific sides of the family tree, with two branches having more severe phenotypes and more hospitalisations due to a variety of gastrointestinal problems ••[9]. By isolating genomic DNA from the affected family members sequence analysis identified a heterozygous base substitution, c.2519G→T, in exon 22, with the replacement of the amino acid serine in codon 840 with isoleucine. This was a key finding for two reasons, first the amino acid Ser840 is highly conserved among mammalian and non-mammalian GC-C proteins. Second, the substitution is located in the catalytic domain of GC-C and as such the authors hypothesized that it may subsequently alter the guanylate cyclase activity of this mutant GC-C receptor and alter the subsequent production of cGMP. In order to investigate, the authors expressed the normal non-mutant GC-C and the mutant GC-C (S840I) in HEK cells for comparison. The authors found that basal GC-C enzyme activity, and basal cellular cGMP levels were similar between normal and

mutant GC-C. However, when mutant GC-C cells were treated with STa, uroguanylin and guanylin they produced significantly more cGMP than cells expressing the normal non-mutant GC-C. Moreover, the mutant GC-C cells displayed a significant leftward shift in their EC50's to uroguanylin, but not STa. Functionally this is a crucial finding as this suggests uroguanylin acts more potently on the mutant receptor and physiological levels of endogenous uroguanylin in the intestine could result in abnormally elevated levels of cGMP in epithelial cells expressing the mutant receptor. As such this 'gain of function' GC-C mutation could explain this familial chronic diarrhea syndrome.

The importance of GC-C to overall motility is further highlighted by the recent identification of separate *GUCY2C* homozygous autosomal-recessive mutations in two unrelated Bedouin families, where loss of GC-C function is associated with meconium ileus ••[10]. The first mutation identified was a single homozygous mutation, c.1160A>G, leading to a p(Asp387Gly) amino acid substitution in the encoded protein. This mutation is within one of the essential GC-C extracellular agonist binding domain regions. Like the previous study the mutation was transfected into a cell line where STa-mediated activation of GC-C cells expressing the mutant protein produced 60% less cGMP than normal GC-C expressing cells. The second mutation in an unrelated family was a c.2270dupA insertion mutation resulting in a premature stop codon, which fully abolishes the guanylate cyclase catalytic domain of GC-C, thereby preventing cGMP production.

The identification of these 3 different mutations may provide additional benefit to understanding the aetiologies of various gastrointestinal diseases. Intriguingly, the Norwegian family affected by the gain of function mutation also had increased susceptibilities for Irritable Bowel Syndrome (IBS), Inflammatory Bowel Disease (IBD), small-bowel obstruction and oesophagitis later in life. Correspondingly, it would be of great interest to screen patients with these disorders to determine

if similar or indeed newly identified *GUCY2C* mutations are an underlying cause of the symptoms associated with IBS, IBD and oesophagitis. In particular as different subtypes of IBS patients display either diarrhea, constipation or can alternate between the two, then these mutations may be informative in understanding why these differences in gut function are apparent.

As the two 'loss of function' *GUCY2C* mutations were found in Bedouin families who live in the desert, the authors speculated these 'loss of function' mutations might have led to an evolutionary advantage against infectious diarrhea. This has some credence as both GC-C gene knockout (-/-) and heterozygous (+/-) mutant mice display lower mortality rates of their wild-type (+/+) counterparts in response to severe STa induced secretory diarrhea [10-12]. Based on these findings one approach has been to develop compounds that block these deleterious effects of STa, resulting in several pyridopyrimidine derivatives as potential lead compounds for the treatment of diarrhea [13]. The alternate approach has utilized GC-C induced secretion as a novel therapeutic target, Linaclotide, for the treatment of constipation-predominant IBS and chronic constipation [14-16].

Embracing GC-C mediated secretion as a treatment for C-IBS and chronic constipation.

Constipation predominant IBS (C-IBS) and chronic constipation are highly prevalent functional gastrointestinal disorders in the Western Population. C-IBS is characterized by symptoms of recurring chronic abdominal pain, discomfort and bloating in association with constipation, infrequent bowel movements [17,18]. Chronic constipation symptoms include abdominal pain, discomfort, hard stool, infrequent bowel movements, straining during defecation, and a feeling of incomplete evacuation [16,19,20]. Linaclotide is a first-in-class, orally administered 14-amino acid peptide that is structurally similar to both uroguanylin and guanylin [21]. Pre-clinical studies demonstrate that Linaclotide activates GC-C on intestinal epithelial cells, leading to increased

intraluminal cGMP accumulation [21, ••22], and increased intestinal fluid secretion [21, ••22]. All of these effects are GC-C dependent, as they are lost in GC-C -/- mice [21]. Importantly, orally administered Linaclotide dose-dependently accelerates gastrointestinal transit [21, ••22]. Pharmacokinetic studies in rats and mice show very low oral bioavailability (0.10%) of Linaclotide [21, ••22], indicating its activity is restricted to the gastrointestinal tract. Overall, these pre-clinical studies indicate Linaclotide displays key qualities for the treatment of constipation in C-IBS and chronic constipation [23-27]. Correspondingly, in the clinic a Phase I study of healthy volunteers demonstrated a single dose of Linaclotide increased stool frequency, decreased stool consistency and the time to first bowel movement [28]. These findings were extended to C-IBS in a double-blind, placebo-controlled, randomized, multiple repeat-dose study in 36 female C-IBS patients orally administered either Linaclotide (100 and 1000 μg) or placebo once daily for 5 days [23]. Overall, versus placebo, Linaclotide (1000 μg) increased stool frequency, consistency, ease of passage and the time to first bowel movement [23]. Importantly, this study also indicated no safety issues associated with Linaclotide use. This was followed up by a 2 week randomized, double-blind, placebo-controlled pilot study in 42 patients with chronic constipation [24]. Linaclotide (100, 300, or 1,000 μg) produced dose-dependent increases in weekly complete spontaneous bowel movement frequency, stool consistency scores, straining scores and provided reduced constipation severity. However, a key additional benefit was also identified, a significant improvement in abdominal discomfort [24]. Similar beneficial effects on abdominal discomfort were also observed in a larger, longer term study in 310 patients with chronic constipation undergoing a randomized, double-blind, parallel-group, placebo-controlled, dose-range finding study. In this study patients were administered oral Linaclotide (75, 150, 300, or 600 μg) or placebo once daily for 4 weeks •[26], where Linaclotide also significantly improved the weekly rate of complete spontaneous bowel movements, stool consistency, straining, bloating, and quality of

life [26]. Furthermore, in a Phase IIb clinical trial with very similar study parameters, but this time in 420 C-IBS patients, oral Linaclotide (75, 150, 300, or 600 µg) once daily for 12 weeks showed similar efficacy of improvement including frequency of spontaneous bowel movements and complete spontaneous bowel movements, severity of straining, and stool consistency ••[25]. However, this study also showed Linaclotide significantly reduced abdominal pain, as well as discomfort and bloating ••[25]. This is a key finding as pain is a defining symptom of IBS. Finally, and most recently Linaclotide significantly reduced bowel and abdominal symptoms in patients with chronic constipation in 2 major randomized, 12-week, multicenter, double-blind, parallel-group, placebo-controlled, dual-dose trials involving a total of 2379 patients with chronic constipation. A key aspect of this study was a 4-week randomized withdrawal period, whereby patients were either maintained on Linaclotide/placebo or swapped from Linaclotide to placebo, or vice versa. The patients who continued to take Linaclotide and those who switched from placebo to Linaclotide had sustained increases in complete spontaneous bowel movements during the withdrawal period. These effects were similar to the levels reported during the treatment period. In contrast, the patients who switched from Linaclotide to placebo had a decreased rate of complete spontaneous bowel movements, which was similar to the rates in the placebo groups during the treatment period ••[27]. Overall, these clinical studies suggest Linaclotide is an apparently safe and effect treatment for constipation, discomfort and abdominal pain in chronic constipation and C-IBS patients, and has just received USA (FDA) and European (CHMP) approval. The most common symptom reported in these clinical studies relating to Linaclotide use was dose-related diarrhea [19,23-27], which is not surprising given the secretory mechanisms induced by GC-C activation.

How does Linaclotide provide abdominal pain relief in chronic constipation and C-IBS?

Although the mechanism underlying Linaclotide's ability to improve frequency of bowel movements is clearly related to a pro-secretory action, a key question relates to how it relieves abdominal pain and discomfort. Is this simply an outcome of reduced constipation or is it an independent process? A recent study suggests in the case of C-IBS, where visceral hyperalgesia is a hypothesised pathophysiological mechanism, that Linaclotide has anti-nociceptive properties independent of constipation relief. In this pre-clinical study orally administered Linaclotide (0.01, 0.03, 0.3, 3 or 30 $\mu g/kg^{-1}$) had no effect on baseline visceromotor responses, the frequency of abdominal contractions in response to increased colorectal distension (CRD) pressures •[29]. However, Linaclotide significantly decreased colonic hypersensitivity in separate models of non-inflammatory and inflammatory visceral pain •[29]. The remarkable aspect of this finding is that these models displayed different types of hypersensitivity, and Linaclotide reversed each one of them. For example, in an acute trinitrobenzenesulfonic acid (TNBS) induced colitis model Linaclotide reversed the hypersensitivity which was only present at low CRD pressures. In a water avoidance stress model Linaclotide reversed the hypersensitivity which was evident at only high CRD pressures, whilst in an acute restraint stress model Linaclotide reversed the hypersensitivity evident throughout a range of CRD pressures •[29]. The mechanisms underlying these anti-nociceptive effects remain unclear, as altered bowel function or smooth muscle contractility were not contributing factors. However, recent preliminary *in vitro* studies potentially suggest that Linaclotide and its downstream mediator cGMP can inhibit the mechanical responsiveness of colonic nociceptors [30,31]. This could potentially provide an underlying mechanism for the anti-nociceptive effects previously observed during *in vivo* animal studies and the analgesia reported in human Phase II and III clinical trials. However, a significant amount of additional investigation is required to definitively determine the precise mechanisms and effectors involved in this process [15] and if it translates to analgesia in humans.

GC-C -/- mice display reduced satiety and increased food intake.

Several recent studies have identified cGMP-dependent protein kinase (PKG) pathways as key regulators of feeding behaviour and satiation in invertebrates [32,33]. In mammals GC-C has recently been identified as a key cGMP signalling component controlling appetite, and in the process identifying uroguanylin as a new satiety hormone [34, ••35]. This study found several key findings. First, GC-C -/- mice displayed accelerated growth, with increased body mass compared with their +/+ counterparts. Interestingly, this increase was observed on a multitude of diets, including a standard low calorie, a moderate calorie (mainly carbohydrates) and a high calorie diet (mainly fats). These mice had increased adiposity, via visceral and subcutaneous fat, and displayed an amplified metabolic syndrome associated with diet-induced obesity, including cardiac hyper-trophy, hyper-insulinemia hyper-leptinemia, and impaired glycemic control ••[35]. These mice also exhibited hyperphagia and diminished satiation, which was amplified by fasting, but independent of caloric intake. Interestingly, intravenous administration of the GC-C agonist STa induced dose-dependent satiation in +/+ mice, but not GC-C -/- mice. Somewhat surprisingly, oral administration of STa, and therefore activation of intestinal GC-C, had no effect on satiation in +/+ mice [35]. In order to determine the mechanism of action of these effects, which appeared to be not attributable to intestinal GC-C, the authors performed a search for GC-C in extra-intestinal tissues and found mRNA and protein expression in the hypothalamus. The authors found administration of GC-C agonists directly into the third ventricle of the brain reduced feeding, indicating a central mechanism for this effect. Having found this pathway the authors wondered how it was activated endogenously. Although the hypothalamus is devoid of uroguanylin and guanylin, nutrient consumption induces endocrine secretion of prouroguanylin and proguanylin from the intestine into the circulation. Once in the hypothalamus, proteolytic hydrolysis converts prouroguanylin into its active peptide, uroguanylin, which induces satiation ••[35]. Crucially, this nutrient-induced satiation could not be induced by guanylin, but could be blocked by

administration of prouroguanylin-neutralizing antibodies. Overall, these findings present a novel therapeutic development strategy for the treatment of obesity [34]. Although these findings point towards a central mechanism of GC-C signalling in food intake, finding little effect of orally administered STa on food intake over a 12 hour period, these animals are likely to have also suffered from STa induced diarrhea. Diarrhea alone causes dehydration, malaise and may cause decreased food intake over the course of several days [36], so overall interpretation maybe confounded. Given the prevalent role of intestinal GC-C and the increased appreciation of peripheral mechanisms in the regulation of food intake [37,38], then future studies may also indentify additional peripheral GC-C mechanisms regulating food intake (Figure 2).

GC-C signalling regulates epithelial cell proliferation/apoptosis and tumour susceptibility.

The intestinal epithelium rapidly regenerates, undergoing cycles of proliferation, migration, differentiation and apoptosis. These cycles maintain the integrity of the crypt-surface axis [39]. Interestingly, uroguanylin, guanylin and GC-C also regulate intestinal regenerative homeostasis by inhibiting cell proliferation and co-ordinating metabolic transitions along the crypt-villus axis [39, •40]. GC-C has been identified as a tumour suppressor that controls proliferation and metabolism of intestinal epithelial cells by inactivating a key signalling molecule, AKT ••[41]. The importance of this role is highlighted by the observation that guanylin and uroguanylin are the most commonly lost gene products in colorectal cancer cells, and their loss occurs in the earliest stages along the neoplastic continuum. Their loss can be detected as early as dysplastic crypts, hyperplastic polyps and adenomas [39,•40,42]. In these cancer cells, without its endogenous ligands, GC-C undergoes over-expression [39-43] and as such GC-C has been suggested as a selective marker for metastatic colorectal tumours in human extra-intestinal tissues [44]. By contrast, activation of GC-C signalling reverses the tumorigenic phenotype of human colon cancer cells by regulating proliferation and metabolism. Similarly, exogenous uroguanylin application decreases tumorigenisis in mouse

models of intestinal carcinogenesis, whilst GC-C -/- mice are more susceptible to colon cancer induced by either carcinogens or mutations [39-43,45]. Importantly, GC-C signalling actually disrupts metastasis via inhibition of matrix metalloproteinase-9 (MMP-9), which is produced by colorectal cancer cells, and is a critical determinant of metastatic disease progression [46]. Based on these findings GC-C signalling has been described as tumour suppressing, by co-ordinating proliferative homeostasis, and silencing of GC-C signalling, through the loss of endogenous hormones initiates transformation [39]. Colorectal cancer is one of the leading causes of tumour-related morbidity and mortality worldwide [40]. As a loss of GC-C signalling drives tumorigenisis this suggests administration of current or newly developed GC-C agonists could counteract the loss of endogenous hormones for anti-colorectal cancer therapy [41].

GC-C signalling regulates intestinal barrier function, injury and inflammation.

Intestinal barrier function is closely regulated by tight junction proteins, which regulate intestinal permeability, thereby controlling access to the submucosa and beyond •[47]. Recent studies have shown GC-C signalling plays a protective role in this process. GC-C -/- and uroguanylin -/- mice both display increased paracelluar permeability in small intestine •[48], whilst GC-C -/- mice are also predisposed to lipopolysaccharide challenge-induced intestinal injury •[48]. Similarly, the GC-C signalling pathway protects intestinal epithelial cells from acute radiation-induced apoptosis • [49]. By contrast, the contribution of GC-C signalling to inflammatory responses appears more contentious. Studies show down-regulation of uroguanylin and guanylin in biopsies from Ulcerative Colitis patients [50] and in a *Citrobacter rodentium* murine model of colitis [51], suggesting loss of GC-C signalling leads to a susceptibility for inflammatory damage. However, GC-C -/- mice, and to a lesser extent uroguanylin -/- mice, display less severe dextran sodium sulfate (DSS) induced colitis [52]. Given the roles for GC-C signalling described above, this seems

counterintuitive. However, GC-C -/- mice were found to have minimal production of RELMβ, which is expressed in goblet cells and is important at inducing TNF-α production in DSS mediated inflammatory injury. Furthermore, although RELMβ -/- mice are resistant to innate immune driven DSS colitis, they are actually more susceptible to TNBS-induced T-cell colitis [53]. Therefore, GC-C signalling may have differential effects on the development of intestinal inflammation, which are dependent on the disease or disease model and the underlying immune response mechanism employed.

GC-C -/- mice display attention deficits and hyperactive behaviour.

A recent study not only described GC-C expression in neurons located within the midbrain, but also showed that a lack of GC-C signalling results in hyperactivity and attention deficits in GC-C -/- mice ••[54]. In addition to finding mRNA and protein expression for GC-C on intestinal mucosal cells, these authors also identified GC-C expression in the soma and dendrites of dopaminergic neurons in midbrain ventral tegmental area and substantia nigra compacta of mice ••[54,55]. Activation of GC-C signalling normally potentiates the excitatory responses mediated by group 1 metabotropic glutamate receptors (mGluRs) and muscarinic acetylcholine receptors (mAChRs) on midbrain dopamine neurons, via a PKG mechanism. However, this effect was lost in GC-C -/- mice. Correspondingly, these mice also displayed increased locomotor activity and increased times spent investigating newly introduced stimuli. These are key observations as it suggests GC-C -/- mice have reduced habituation responses, which are associated with impaired attention in both animals and humans. Therefore, many of the behavioural phenotypes of GC-C -/- mice mimic the predominant symptoms associated with human Attention Deficit Hyperactivity Disorder (ADHD). Given the increasingly apparent association between diet and ADHD [56,57], and the recently identified role for GC-C signalling in the brain driving food intake (see above) [54], it is tempting to speculate that a lack of prouroguanlyin release in response to a poor diet may be a contributing

factor to the symptoms of ADHD, via reduced GC-C mediated modulation of midbrain dopamine neurons. Therefore an improved diet or the use of a specific GC-C agonist to target these pathways may be of particular therapeutic benefit in treating symptoms associated with ADHD.

Conclusions:

This is an exciting time for the field of GC-C signalling. Over the last few years GC-C has progressed from a being a receptor located in the intestine responsible for secretory diarrhea to one that underlies a series of physiological functions within the body. In most instances it is the loss of GC-C signalling, either through the loss of the endogenous hormones, or by altered function of GC-C and its downstream mediators, which leads to deleterious effects, disease development and disease progression. Correspondingly, this opens up key avenues for therapeutic intervention. The current clinical GC-C therapy, Linaclotide, provides a novel treatment for relieving constipation and abdominal pain in chronic constipation and C-IBS patients. These breakthroughs pave the way for additional targeting of GC-C, potentially leading to future treatment options for controlling appetite, regulating intestinal barrier function, whilst preventing tumorigenesis and symptoms of ADHD. Finally, the recently identified human mutations in the gene encoding GC-C provide potential diagnostic markers for sufferers of chronic constipation and diarrhea.

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Recommended reading:

• of special interest:
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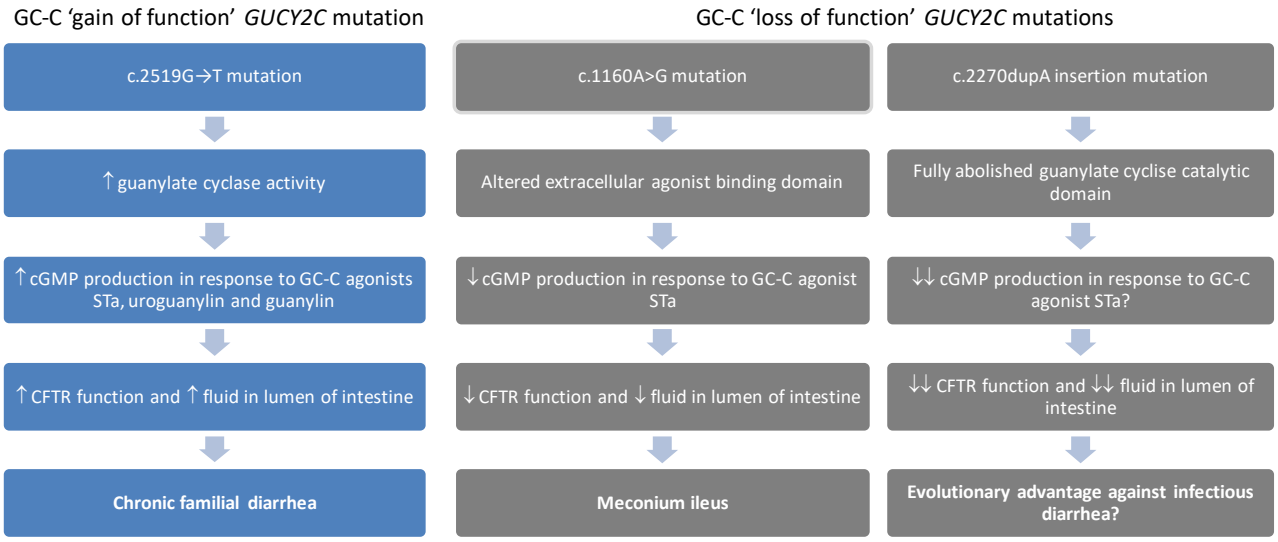

Table 1: Details the proposed mechanisms and outcomes of the respective 'gain of function' and 'loss of function' *GUCY2C* mutations that have been discovered in humans to date.

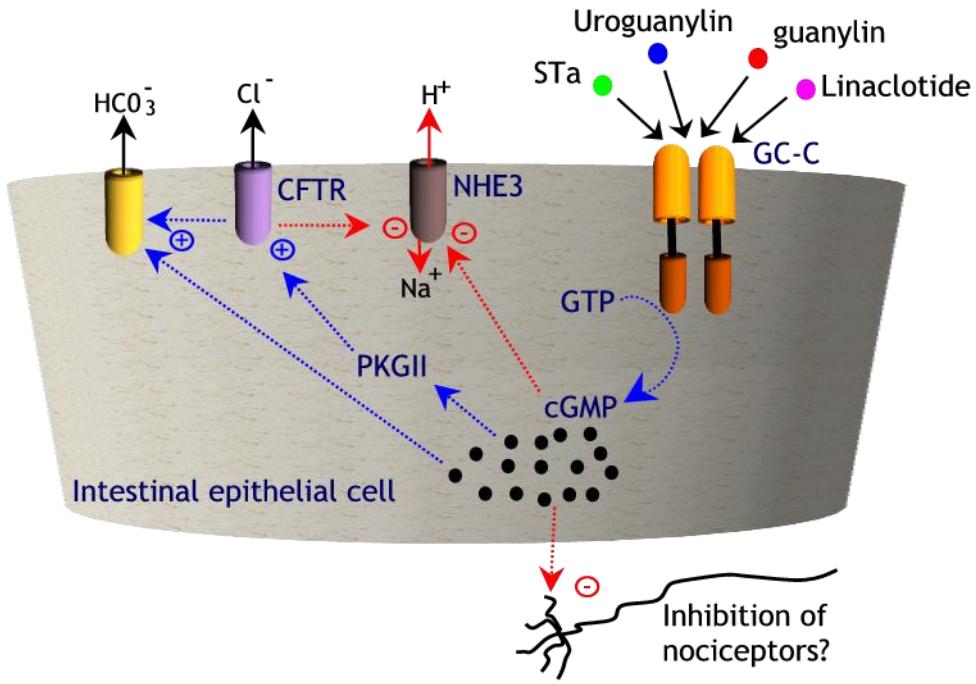

Figure 1: GC-C is expressed on the surface of intestinal epithelial cells where it is activated by the endogenous hormones uroguanylin and guanylin, which are synthesized in the intestine and secreted into the lumen and the circulation. GC-C is also the receptor for heat-stable enterotoxins (STa) produced by *E. coli* and the novel therapeutic peptide agonist, Linaclotide. Binding of these ligands to GC-C results in the conversion of guanosine triphosphate (GTP) to cyclic-guanosine-3',5'-monophosphate (cGMP). Increased levels of cGMP activate the cGMP-dependent protein kinase II (PKGII), which phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR), increasing chloride (Cl^-) secretion into the lumen. Bicarbonate secretion, through an as yet unidentified channel, also occurs in a CFTR-dependent manner. cGMP is also known to inhibit the sodium-hydrogen exchanger NHE3, thereby decreasing sodium absorption. These processes are involved in the maintenance of fluid and ion homeostasis. Recent clinical studies demonstrate that Linaclotide produces significant relief from abdominal pain in constipation-predominant IBS patients. Recent, pre-clinical studies suggest cGMP, or a related mechanism, may act to inhibit colonic nociceptors and thus potentially provide analgesia.

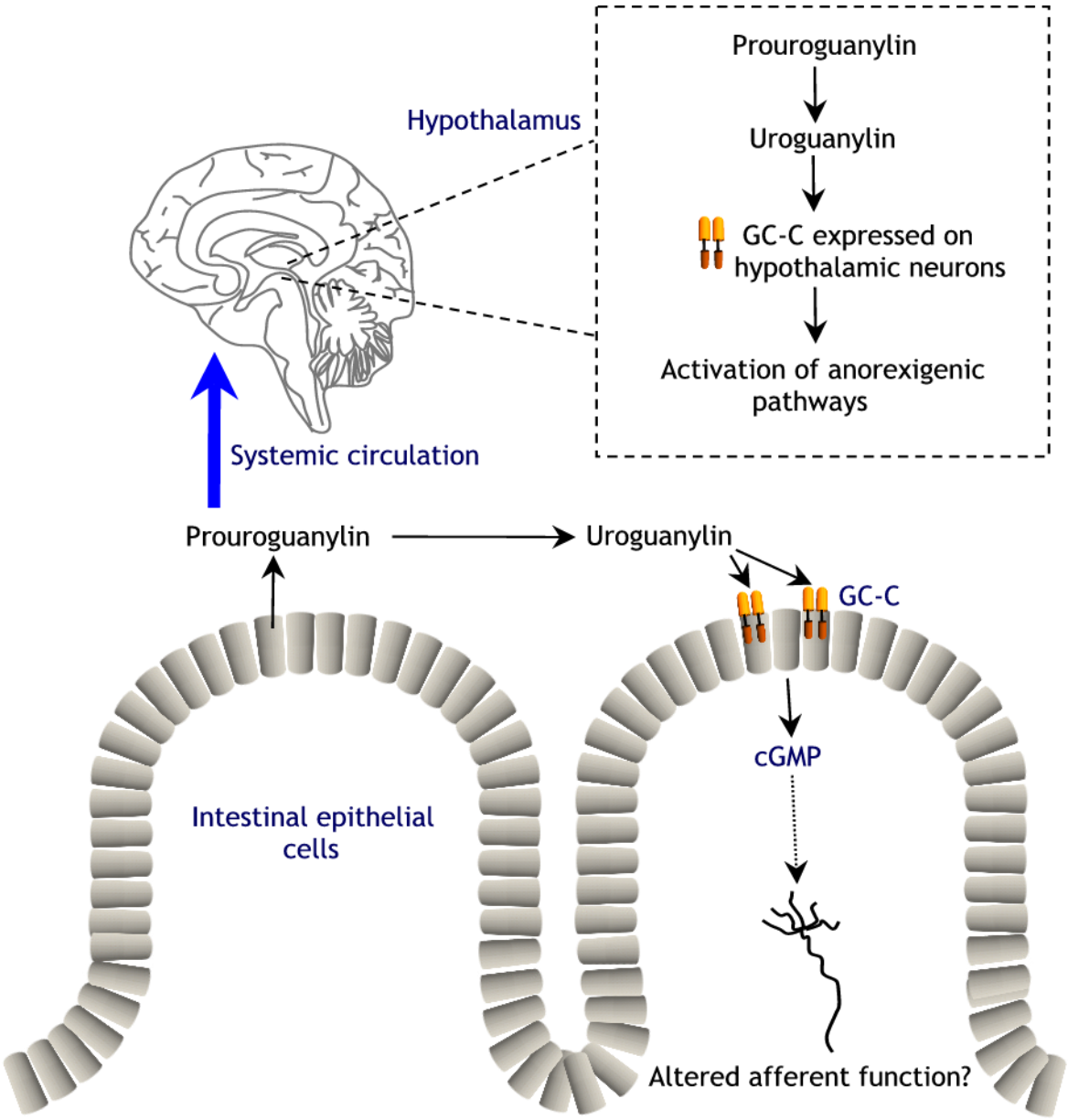

Figure 2: Mechanisms of uroguanylin/GC-C induced regulation of food intake in mice.
GC-C -/- mice display disrupted satiation, resulting in hyperphagia, obesity and metabolic syndrome. In this process nutrient intake induces intestinal prouroguanylin secretion into the lumen and systemic circulation. Prouroguanylin then undergoes proteolytic conversion to form uroguanylin, where in the brain it activates GC-C expressed on hypothalamic neurons, leading to the activation of anorexigenic pathways and decreased food intake.

Furthermore, as extrinsic afferent endings innervating the upper gastrointestinal tract are also important in regulating food intake, there may also be a local paracrine action of GC-C signalling in this process. In such a scenario uroguanylin induced activation of intestinal GC-C could alter afferent ending sensitivity leading to altered peripheral signalling to the CNS resulting in appetite suppression.