Europe PMC Funders Group
Author Manuscript
IUBMB Life. Author manuscript; available in PMC 2021 August 11.

Published in final edited form as:
IUBMB Life. 2020 June 01; 72(6): 1145–1159. doi:10.1002/iub.2283.

Mutational landscape of receptor guanylyl cyclase C: Functional analysis and disease-related mutations

Avipsa Bose, **Sanghita Banerjee**, **Sandhya S. Visweswariah**
Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bengaluru, India

Abstract

Guanylyl cyclase C (GC-C) is the receptor for the heat-stable enterotoxin, which causes diarrhea, and the endogenous ligands, guanylin and uroguanylin. GC-C is predominantly expressed in the intestinal epithelium and regulates fluid and ion secretion in the gut. The receptor has a complex domain organization, and in the absence of structural information, mutational analysis provides clues to mechanisms of regulation of this protein. Here, we review the mutational landscape of this receptor that reveals regulatory features critical for its activity. We also summarize the available information on mutations in GC-C that have been reported in humans and contribute to severe gastrointestinal abnormalities. Since GC-C is also expressed in extra-intestinal tissues, it is likely that mutations thus far reported in humans may also affect other organ systems, warranting a close observation of these patients in future.

Keywords

cGMP; guanylyl cyclase C; human mutation; meconium ileus; secretory diarrhea

1 Introduction

One of the most distinguishing features of living organisms is their ability to sense the external environment and respond accordingly. At the cellular level, this translation of external stimuli into regulatory outcomes is mediated by complex signal transduction pathways. Second messengers serve to transmit signals from receptors to effector molecules in response to diverse stimuli. Guanosine 3',5'-cyclic monophosphate (cGMP) was first discovered in rat urine in 1963,[1] following which several studies demonstrated its relevance as a second messenger. cGMP levels are maintained inside the cell by conversion of GTP to cGMP by guanylyl cyclases and hydrolysis of cGMP to GMP by cyclic-nucleotide phosphodiesterases. Humans express seven membrane-spanning forms of guanylyl cyclases (GC)—GC-A, GC-B, (GC-C), GC-D, GC-E, GC-F, and GC-G, and the focus of this

Correspondence to: Sandhya S. Visweswariah.

Correspondence: Sandhya S. Visweswariah, Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bengaluru, India, sandhya@iisc.ac.in.

Conflict of Interest
All authors declare no conflict of interest.

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

review will be on GC-C, the target for bacterial heat-stable enterotoxin[2] and gastrointestinal peptides, guanylin[3] and uroguanylin.[4]

The membrane spanning or receptor guanylyl cyclases (rGCs) harbor an extracellular ligand-binding, transmembrane, kinase-homology, linker, and catalytic domains (Figure 1). GC-C is expressed mostly on the apical surface of intestinal cells[5–8] but also in the kidney, airway epithelium, perinatal liver, stomach, brain, adrenal glands, and reproductive tissues.[9,10] GC-C is activated by the peptide hormones guanylin and uroguanylin, and the heat-stable enterotoxin (ST) produced by Enterotoxigenic *Escherichia coli*.[8,11] GC-C regulates colonic epithelial cell proliferation,[12–14] and activation of GC-C leads to diarrhea.[15,16] This review provides an overview of the domain organization of this receptor, mutational analysis that has been performed to understand mechanisms of its regulation, along with a discussion on naturally occurring mutations in GC-C which cause disease.

2 GC-C: Signaling Events

GC-C is activated by binding of guanylin, uroguanylin or the ST peptide to the extracellular domain (ECD). Ligand binding results in the production of cGMP which activates cGMP-dependent protein kinases (PKG), cyclic-nucleotide-gated (CNG) channels, and cGMP-regulated cyclic-nucleotide phosphodiesterases[11] (Figure 2). In intestinal cells, protein kinase G II (PKGII) phosphory-lates the cystic fibrosis transmembrane conductance regulator (CFTR) leading to increased efflux of chloride ions from intestinal cells,[17] and greater surface CFTR expression.[18] Cyclic GMP-mediated activation of PKGII also results in phosphorylation of NHE3, thereby inhibiting NHE3 activity.[19] Activation of GC-C causes cell cytostasis in a cGMP-dependent manner via activation of p21 and influx of calcium.[14,20] More extensive reviews of signaling events downstream of GC-C are available.[8,11]

3 Regulation of GC-C: Multiple Domains, Multiple Means for Allosteric Control of Guanylyl Cyclase Activity

Analogous to other rGCs, the primary sequence of GC-C reveals a multidomain architecture (Figure 1). Residues 1–23 comprise a putative signal peptide that directs the receptor to the cell surface (http://www.cbs.dtu.dk/services/SignalP/). This is followed by the ECD (residues 24–430). Hydropathy plots predict the presence of a single transmembrane domain encompassing residues 431–454. The intracellular region has a short juxta-membrane domain (residues 455–489) which is followed by a kinase-homology domain (KHD) (residues 490–735), the linker region (residues 736–810), the guanylyl cyclase domain (GCD) (residues 811–1,010), and a C-terminal domain (CTD) (residues 1,011–1,073).

Binding of ligands to the ECD must result in dramatic conformational changes along the receptor, ultimately activating the GCD to produce more cGMP. No structural information is available for any region of GC-C from any species. Apart from the GCD, for which recent structural information has provided insight on residues involved in catalytic activity and changes that occur on activation,[21] no other domain in GC-C has more than 25% sequence identity with any protein for which structural information is available. Therefore, while homology modeling may provide clues to the overall fold that a domain may adopt, such

approaches will not provide exact explanations as to why certain mutations bring about changes in the activity of GC-C.

4 Extracellular Domain

The rGCs bind to a diverse set of ligands via the ECD; therefore, to accommodate binding of such dissimilar ligands, there is poor sequence conservation across the ECD amongst members of receptor GCs.[22] The ECD of GC-C has only 15–20% sequence identity with that of GC-A and retinal GCs. The ECDs of mammalian GC-Cs show 70–75% sequence identity (Figure 3a); however, GC-Cs from other species show lower sequence identity (37–46%; Figure 3a). This may indicate that the endogenous ligands for GC-C in fish, amphibians, reptiles, and birds could differ significantly from mammalian guanylin and uroguanylin peptides. The ECD of human GC-C has the highest affinity for ST peptide (Kd ~ 0.1 nM), followed by uroguanylin (Kd ~ 1 nM) and guanylin (Kd ~ 10 nM).[23]

4.1 Mutational analysis

Wada et al. generated a series of mutations in the ECD of porcine GC-C to understand the residues involved in ligand binding. Porcine GC-C shares 85% sequence identity to human GC-C. Replacing Arg136, Asp347, and Asn348 with Gly, Ala, and Ser, respectively, resulted in loss of ST binding.[24] Hasegawa et al. identified a stretch of residues in the ECD of porcine GC-C responsible for ligand binding.[25] Using a photoaffinity-labeled analog of ST, they identified a region spanning residues SPTFIWK (residues 410–416) in human GC-C (Figure 3a) that was critical for ST binding. This region in the ECD lies close to the transmembrane domain toward the extracellular surface. Site-directed mutagenesis of residues in this region individually, except for Ser, markedly reduced cGMP production by mutant receptors.[25]

Multiple sequence alignments reveal a significant degree of conservation of these amino acids (Figure 3a). For example, the Pro and Trp residues in the SPTFIWK motif are conserved from fishes till mammals. Thr is more variable, being replaced by Asn (frog, mouse, and rat), Asp (fowl), or Ser (turtle). Phe is conserved except in the frog, where it is replaced by Leu. Ile is replaced by a Thr in human, a Pro in fish, and Arg in the turtle.

Structural analysis of the ECD of GC-A revealed that the C-terminal region is folded into a β-sheet structure.[26] Sequence alignment of the ECD of GC-A and GC-C showed that there exists 23% sequence similarity from residue Met341 to Gln407 of GC-C. This region in GC-C was overexpressed in *E. coli* and bound ST with an IC_{50} of ~10^{-8} M,[27] lower than that of the full-length receptor.[23] This microdomain is also predicted to harbor a β-sheet domain between residue Met356 and Pro383, as is seen in the crystal structure of ECD of GC-A.[26]

GC-C activity is regulated by post-translational modifications. In mammalian intestinal cells or upon heterologous expression in HEK293 cells, two differentially glycosylated forms of GC-C are observed.[28–30] The 145 kDa form contains sialic acid and galactose and is found on the plasma membrane, whereas, the 130 kDa form is rich in mannose residues and is primarily present within the endoplasmic reticulum.[29,30] Deglycosylation of GC-C following

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

PNGase F treatment of the membrane-associated receptor resulted in a receptor that retained ST-binding ability, possessed guanylyl cyclase activity, but showed no ligand-mediated increase in cGMP production.[30]

There are 10 putative N-linked glycosylation sites present in the ECD of human GC-C (Asn32, Asn75, Asn79, Asn195, Asn284, Asn307, Asn313, Asn345, Asn357, and Asn402).[30] Extensive mutational analysis revealed that glycosylation at Asn75 and Asn79 is necessary for proper folding of the receptor to adopt a conformation that is suitable for ligand binding.[30] Glycosylation at Asn345 and Asn402 is not required for ligand binding per se, but glycosylation at these sites allows efficient ligand-mediated signal transduction to the catalytic domain. Asn75 is replaced by Asp (fish), Thr (pig), or Lys (fowl). Asn79 is replaced by Lys (fish) and Thr (frog). Interestingly, in porcine GC-C, glycosylation at Asn195, Asn284, and Asn402 are needed for ligand binding.[28] Even though these residues are conserved in human GC-C, none of them were found to be necessary for ligand binding.[30] These observations demonstrate that orthologs can adopt various folding trajectories during the trafficking from the endoplasmic reticulum/Golgi complex to the plasma membrane, and glycosylation at specific sites is critical to attain a biologically active receptor.

4.2 Disease-causing mutations

Given the fact that this domain is responsible for ligand binding and subsequent activation of GC-C, polymorphisms in this domain may lead to inactivation of the receptor and impact gut health (Figure 3a; Table 1). Patients from an extended Israeli/ Bedouin family harbor a homozygous mutation of Asp387 to Gly due to a transition of A>G at base 1160[31] (Figure 3a; Table 1). Newborns presented with meconium ileus (MI), which is a blockage in the ileum of the new-born, caused by thick meconium. The mutation in GC-C, when tested following heterologous expression, resulted in reduced cGMP production on ST addition.[31] This poorer activity of the receptor in the gut could have resulted in reduced activity of CFTR, and therefore lower fluid secretion into the gut lumen. Indeed, almost 20% of cystic fibrosis patients (that possess inactivating mutations in *CFTR*) present with MI.[35] Another mutation has been reported in the ECD and converts Leu137 to Ser due to a transition of T>C at position 410 (Figure 3a; Table 1). The patient had duodenal atresia, asplenia, abnormal biliary tract morphology, and reduced number of intrahepatic bile ducts, but no information is available on the activity of GC-C harboring this mutation (https://www.ncbi.nlm.nih.gov/clinvar/variation/375380/).

There is also a mutation reported recently where a transition of C>T at base position 893 results in a conversion of Thr 298 to Met (Figure 3a; Table 1). The patient was reported to suffer from MI, suggesting this mutation could be inactivating (https://www.ncbi.nlm.nih.gov/clinvar/variation/714778/).

5 Kinase-Homology Domain

GC-C contains a stretch of residues between the juxta-membrane and GCDs which shares sequence similarity to protein tyrosine kinases.[36] This domain, referred to as the KHD, extends in GC-C from residue 490 to 735 (Figure 1). The KHD plays a crucial role in

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

modulating the response of most receptor GCs to their ligands. For example, binding of adenosine triphosphate (ATP) to the KHDs of GC-A and GC-B, following ligand-activation, allosterically activates the GC domain by decreasing the Km for guanosine triphosphate (GTP).[37–39] GC-C is activated by its ligands in vitro in the absence of ATP.[36] However, ATP and nonhydrolyzable analogs of ATP enhance the activation of GC-C by its ligands,[40–42] and ATP can bind to the KHD of GC-C when expressed as an isolated domain along with the linker region.[36,43]

Many structural features may be conserved between Src kinases and KHD of GC-C,[44] including Lys516 which was shown to be important for binding ATP in GC-C, and which interacts with the β-phosphate of ATP in kinases of known structure.[36,43,45] Interestingly, the KHD lacks the HRD motif, critical for catalysis by protein kinases,[36] and instead contains the HGR motif. Sequence alignment indicates that the His and Gly are highly conserved across the KHD of all rGCs (Figure 3b), with Arg being replaced by either Ser or Asn in some members.[46] We have recently reviewed functional and structural aspects of the KHD of GC-C in the context of other receptor GCs, kinases, and pseudokinases, to which the reader is referred.[45]

5.1 Mutational analysis

Mutant $hGCC_{K516A}$ showed no change in affinity for the ST peptide, but ST-mediated intracellular cGMP accumulation was significantly reduced.[36] In vitro studies, with membranes prepared from cells expressing either wild type or K516A receptor, showed that the mutant receptor produced higher levels of cGMP in the absence of ligand but little increase in cGMP production on ST stimulation.[36] A monoclonal antibody, raised against an epitope within the KHD of GC-C, was sensitive to the presence of ATP; that is, it failed to bind to GC-C in the presence of ATP.[43] The epitope is present from residue 517–568, suggesting that Lys516 and the epitope of the monoclonal antibody are present in a region which stays “open” in the absence of ATP, allowing the antibody to bind to its epitope. However, upon ATP binding a conformational change occurs that sequesters the epitope denying access to the antibody. Since ATP is also known to alter the oligomeric states of GC-C,[36] ATP may also induce the orientation of hydrophobic surfaces resulting in a shift in its oligomeric status.

5.2 Disease-causing mutations

Since the KHD plays a critical role in regulation of GC-C, it is not surprising that a number of disease-causing mutations lie in this domain and mis-regulate GC-C activity (Table 1). Children in a Lebanese family presenting with MI harbored a change at Ala670 to Thr due to a transition of G>A at base 2008 (Figure 3b; Table 1).[33] We predict that this mutation would be pathogenic and result in inactivation of GC-C, in agreement with the MI seen in this patient. Ala670 is conserved across species except in fishes, where it is replaced with Ser.

We have characterized a hyperactivating mutation in the KHD found in a child with congenital secretory diarrhea (CSD) (Figure 3b; Table 1). The female child was French/Algerian and presented with a large abdomen due to intestinal dilation after delivery. The

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

child needed parenteral fluids for 2 years and apart from experiencing frequent bouts of diarrhea, could lead a normal life. Whole exome sequencing revealed a transition of A>G at 1519 position in GC-C that arose de novo (both her parents did not harbor the mutation) leading to substitution of Lys507 with Glu.[16] The mutant receptor showed elevated basal activity (i.e., in the absence of ligand) and also produced higher levels of cGMP on ligand stimulation. In the gut, this mutation would have generated higher levels of cGMP on activation by guanylin and uroguanylin, resulting in supraphysiological activation of CFTR and more profound inhibition of NHE3. Indeed, fecal sodium levels in this child were high (110 mmol/L as opposed to 25–50 mmol/L in normal individuals).[16]

A mutation was reported recently in the KHD where a transversion of G>C at 2155 position changes Glu719 to Gln (Figure 3b; Table 1; https://www.ncbi.nlm.nih.gov/clinvar/variation/802827/). The patient presented with MI, suggesting inactivation of GC-C as a result of this mutation. The amino acid change probably leads to a critical conformational change in the KHD which inhibits the relay of the signal following binding of guanylin/uroguanylin from ECD to the catalytic domain, resulting in low cGMP production.

6 Linker Region

A stretch of ~70 amino acid residues (from 736–810 in GC-C) between the KHD and the GCD is called the linker region. The linker region in many guanylyl cyclases is highly conserved in terms of sequence as well as length[46] (Figure 3c). This domain is present in the NO-activated soluble guanylyl cyclases, for which structural information has recently become available.[21] The linker, or “transducer module” in soluble guanylyl cyclase adopts a coiled-coil structure, as we had predicted earlier in GC-C, based on sequence analysis.[47] In the inactive state, the transducer region adopts a bent conformation, and the GCD adopts a structure that is incompatible with substrate binding. Following NO binding to the HNOX domain, there are extensive conformational changes that occur in the transducer domain, permitting it to fold into a long continuous coiled-coil structure. These structural rearrangements now induce alterations in the catalytic domain such that it opens out to accommodate binding of GTP and Mg^{2+}.[21] Given the conservation in sequence between the linker region in soluble guanylyl cyclase and GC-C, we could anticipate similar structural alterations in the linker in GC-C that reposition the catalytic domain into a more active conformation on ligand binding.

6.1 Mutational analysis

We have performed proline scanning mutagenesis in GC-C from residues Tyr760 to Tyr786 which would result in disruption of the helical structure of the linker domain.[47] Some mutants resulted in inactivation of GC-C (e.g., L760P, D762P, L864P, L764P, L773P, and E780P). A number of mutations (Q769P, Y771P, S772P, N774P, and H777P) resulted in receptors that showed high levels of basal cGMP in HEK293T cells, whereas other mutations (L768P, L770P, L775P, and L785P) showed a more modest increase in basal activity. Except for the L770P mutant receptor, none of the other constitutively active mutant receptors could be further stimulated by ST.[47] No mutations altered trafficking of the receptor to the cell surface or ligand-binding properties.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Receptor GCs show high guanylyl cyclase activity in the presence of nonionic detergents, which can be reduced in the presence of ATP.[36] Interestingly, many of the linker mutations lost the ability to show ATP-mediated inhibition of guanylyl cyclase activity in the presence of detergents, suggesting that the linker module is also involved in transmitting conformational changes in the KHD to the GCD. Therefore, in a manner similar to that seen in soluble GCs, the linker region is a key transducer module that mediates the allosteric activation of GC-C.

6.2 Disease-causing mutations

As is clear from the earlier discussion, the linker regulates the conformational changes of the GC-C intricately, both in the absence as well as presence of its ligands. Therefore, mutations in this region may lead to dramatic alteration in its activity. Two dominant de novo mutations were reported recently in patients with CSD[16] (Table 1). In one case, a transversion of G>C at 2376 position resulted in the formation of a mutant receptor where Arg792 was replaced with Ser. In the second case, a transition of T>C at 2324 position led to a substitution of Leu775 with Pro (Figure 3c; Table 1). Both the residues are conserved from fish to mammals (Figure 3c) and the mutant receptors showed elevated basal activity. Remarkably, the R792S produced almost 100-fold higher cGMP basally.[16] Upon ligand stimulation, the R792S receptor was further stimulated and demonstrated markedly enhanced cGMP levels compared with the wild type receptor, with a reduced EC50. The male patient harboring this mutation was on total parenteral nutrition, showed markedly higher plasma and ileal sodium levels, and suffered from recurrent pseudo-obstructions and colitis and required partial small bowel resection. This clearly indicates the dramatic and deleterious effects of elevated cGMP in the human gut, and the critical role the linker region has in restricting nonligand-mediated activation of GC-C.

Another homozygous mutation in *GUCY2C* (c.2270dupA) in a patient results in an insertion of A (Figure 3c; Table 1). Due to this frame-shift mutation, Asn757 is changed to Lys and a premature stop codon forms immediately after two amino acids from the site of insertion, leading to complete deletion of the GCD. This mutation would therefore result in no cGMP production on ligand binding, thereby causing poor activation of CFTR eventually leading to MI,[31] as seen in this patient.

7 Guanylyl Cyclase Domain

Guanylyl cyclases belong to the Class III nucleotide cyclase family which include adenylyl cyclase, soluble guanylyl cyclase, and rGC.[46] The nucleotidyl cyclase domain is highly conserved across both receptor and soluble GCs (~80%) and bears similarities with adenylyl cyclase.[48] In GC-C, this domain is present toward the C-terminus and extends from residue 811–1,010 (Figure 1). Mammalian adenylyl cyclases and soluble guanylyl cyclases possess two independent cyclase domains that are either present in a single polypeptide chain,[49] or in two independent proteins that come together to form heterodimers,[21] creating an active site at the dimer interface. Receptor GCs are therefore homodimers which may bind two substrate molecules per dimer.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Crystal structures of guanylyl cyclases Cya2 from cyanobacterium *Syncheocystis PCC6803* and CYG12 from the green algae *Chlamydomonas reinhardtii* reveal the presence of a head-to-tail homodimer where the two monomers form a wreath-like arrangement.[50,51] Conserved Asp residues (Asp448 and Asp492 in Cya2) are oriented toward each other to form the binding site for two divalent cations required for catalytic activity.[50] Residues that impart substrate specificity to guanylyl cyclases (Glu and Cys)[46] are conserved in GC-C (Figure 3d).

7.1 Disease-causing mutations

Two activating mutations and a number of presumably inactivating mutations in the GCD have been identified in patients over the years (Figure 3d; Table 1). The first mutation ever identified in GC-C and associated with human disease, was reported in a Norwegian family presenting with familial diarrhea.[15] This mutation arose due to a transversion of G>T at 2519 position within the GCD, which resulted in conversion of Ser840 to Ile (Figure 3d; Table 1). Patients passed multiple watery stools in a day, some required ileal resection, and some were treated for Crohn's Disease. This residue is highly conserved from fish to mammals and the mutation resulted in GC-C being more responsive to ligand stimulation in terms of cGMP production, with no change in affinity of the receptor for ST.[15] This would have resulted in elevated levels of cGMP in the gut as a result of activation of GC-C by uroguanylin and guanylin, causing greater Cl^- secretion and resulting in diarrhea.

A second activating mutation was identified recently in a child with CSD, and resulted in a conversion of Asn850 to Asp[16] (Figure 3d; Table 1). This female patient can currently survive on oral nutrition but showed elevated sodium levels in the ileum and the feces. This mutation again led to a receptor with marginally higher basal activity and hyperactivation by its ligands.[16]

A mutation associated with MI was reported in a Lebanese family. Due to a transition from T>C at 2782 position, Cys928 is converted to Arg[31] (Figure 3d; Table 1). We predict this mutation to be inactivating based on the condition reported in the child.

Another patient showed poor feeding and failed to pass meconium by the second day of his life. Imaging of the abdomen revealed distended bowel loops and absence of air in the rectum. Ileal resection also did not restore normal bowel functions even by day 14 post-surgery. Obstructed bowel resection and diversion ileostomy was performed which enabled him to pass stool. A prenatal SNP microarray and karyotyping had been performed via amniocentesis because of detection of congenital anomalies on prenatal ultrasound scan. This led to the identification of two mutations in *GUCY2C* (paternally inherited 2575A>G and maternally derived 2864_2865delCCinsTA) which converts Ile859 to Val and Ser995 to Leu[34] (Figure 3d; Table 1). These mutations may have led to inactivation of the GCD, again resulting in poor cGMP production, low CFTR activity and the disease phenotype observed in the infant.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

8 C-Terminal Domain

GC-C contains a short CTD of approximately 60 residues[52] (Figure 1). The CTD may play a role in allowing tight association of receptor GCs with the cytoskeleton as revealed by the requirement of harsher detergent extraction procedures to release GC-C from the membrane fraction of intestinal cells.[34] The CTD aids in sorting of GC-C to the apical surface of polarized epithelial cells.[53] Moreover, deletion of the CTD leads to complete loss of ligand-mediated activation.[54] This domain interacts with IKEPP (intestinal and kidney enriched PDZ protein) which is expressed on the apical surface of intestinal cells. Association of IKEPP with GC-C via the CTD inhibits ST-mediated cyclase activity.[54]

9 Extra-Intestinal Roles of GC-C

GC-C has high expression in the human gut during the neonatal period, but by 1–2 months of age, the expression level reduces drastically and stays stable throughout life.[55,56] GC-C expression can be detected in the liver of new-born rats but was undetectable in the adult.[57] Liver damage by hepatic resection or CCl_4^--induced injury led to an increase in GC-C levels in the liver during regeneration.[58,59] In accordance with a role for GC-C during liver regeneration, CCl_4^--induced liver injury led to greater mortality in GC-C knock-out mice and the extent of hepatic injury was more severe and long-lasting.[60]

Another emerging paradigm in GC-C signaling is its role in the endocrine gut-brain axis in regulating feeding, metabolic homeostasis, and body weight. *GUCY2C* transcripts are seen in the cell bodies of neurons present in the ventral premammillary nucleus (PMV) in hypothalamus and ventral tegmental area (VTA) and substantia nigra (SN) of mid-brain.[61] In PMV, GC-C is mostly produced by neurons expressing anorexigenic leptin receptors (LepR), whereas in the VTA/SN, GC-C is seen in neurons expressing tyrosine hydroxylase. After ingestion of food, prouroguanylin is secreted into the circulation,[62–64] and is processed into mature uroguanylin by hypothalamus.[65] This would result in activation of GC-C and the increased accumulation of cGMP results in proopiomelanocortin and c-Fos expression, which imparts the sense of satiety and reduces feeding.[66]

However, a contrasting report suggests that administering GC-C agonists in the central nervous system did not reduce food intake significantly in rats.[67] Moreover, GC-C knock-out mice in this study showed normal body weight, adiposity, and glucose tolerance, while uroguanylin null mice showed a small but significant gain in body weight, adiposity, and glucose intolerance. Therefore, this report suggests the presence of a GC-C independent pathway regulating energy homeostasis and diabetes.

GC-C may also regulate dopamine release in the midbrain by cGMP-dependent protein kinase.[68,69] In presynaptic terminals, cGMP mediates opening of CNG channels which leads to Ca^{2+} entry and presynaptic depolarization, resulting in release of action-potential independent neurotransmitters.[70,71] cGMP also strengthens existing synapses and mediates synaptogenesis by presynaptic and postsynaptic phosphorylation of VASP[72] via PKGII. This reorganizes the actin cytoskeleton and potentiates accumulation of synaptophysin and

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

glutamate receptor (GluR1-AMPA receptor) in the presynaptic and postsynaptic neurons, respectively.[73,74]

A transcriptome analysis in the mesenteric fat of rats resistant to high-fat diet (HFD)-induced obesity showed very high levels of GC-C and guanylin expression in macrophages,[75] and transgenic rats overexpressing GC-C and guanylin in macrophages were found to be resistant to HFD-induced obesity. It was also observed that co-culturing adipocytes with macrophages expressing guanylin and GC-C markedly reduced lipid accumulation. While PKG levels decrease in white adipose tissue in HFD-induced obese mice,[76] transgenic rats overexpressing guanylin and GC-C showed an increase in PKG levels after HFD. After a HFD, guanylin and GC-C may elevate cGMP levels in adipose tissue-associated macrophages, resulting in activation of PKG and phosphorylation of VASP. This reduces HFD-induced inflammation by decreasing the production of inflammatory factors such as TNF-α, Cox-2, iNOS, and MCP-1.

In order to match dietary salt intake with urinary salt excretion, there needs to exist a "gut-renal axis" mediated by "intestinal natriuretic factors." Several studies have suggested the role of guanylin and uroguanylin as intestinal peptides responsible for establishing this entero-renal axis.[77–80] GC-C mRNA expression levels were found to be relatively high in the collecting tubule, proximal convoluted tubule and medullary thick ascending limb. Much lower levels of GC-C mRNA were found in cortical thick ascending limb, distal convoluted tubule and glomeruli.[81] Guanylin, uroguanylin and ST showed increased natriuresis, kaliuresis, and diuresis with increased urinary cGMP levels in a time and dose-dependent manner. Surprisingly, the effects of these ligands were not affected in a GC-C null mouse, implying the presence of other receptors of guanylin/uroguanylin in the kidney.[82]

It is conceivable that given the extra-intestinal expression of GC-C, mutations in the receptor occurring in humans may also affect pathways and phenotypes present in the liver, nervous system, and perhaps the kidney. However, no reports mention abnormalities in these organs in patients harboring mutations in GC-C. This perhaps is because the major phenotypes seen in these patients relate to gut physiology, as would be expected since GC-C is expressed at high levels in the intestine.

10 Conclusions

GC-C has recently been recognized as being responsible for some instances of heritable gastrointestinal disease in humans, with the identification of a number of mutations in GC-C that are associated with early onset diarrheal disease and MI. Inspection of mutations in *GUCY2C* reported in dbSNP (https://www.ncbi.nlm.nih.gov/snp/) suggest that additional alterations are likely to be deleterious (Table 2). Variations are found along the length of the receptor, and individuals in which a stop codon is present are likely to have reduced levels of expression of GC-C in the gut. What is lacking at present is a deeper understanding of why nonsynonymous variants cause mis-regulation of GC-C and this can only be obtained by structural analysis. Without this information, we will be hindered in identifying antagonists for this receptor, or allosteric regulators that could bind to the pseudokinase/catalytic domain to regulate its activity and perhaps allow therapeutic intervention. Much remains to be learnt

from increased understanding of the signaling outputs consequent to GC-C activation. The availability of molecules that regulate GC-C[83] should allow investigations using transgenic mouse models as well as intestinal organoids from patients with disease.

Acknowledgments

S. S. V. is a recipient of the J. C. Bose National Fellowship from the Department of Science and Technology, Government of India (SB/S2/JCB-18/2013), and is a Margdarshi Fellow supported by the Wellcome Trust DBT India Alliance (IA/M/16/1/502606). Support from the Department of Biotechnology, Government of India (BT/PR15216/COE/34/02/2017), and funding from the DBT-IISc Partnership Program Phase-II (BT/PR27952/INF/22/212/2018/21.01.2019) are acknowledged.

Funding information

Department of Biotechnology, Ministry of Science and Technology, Grant/Award Numbers: BT/PR15216/COE/34/02/2017, BT/PR27952/INF/22/212/2018/21.01.2019; Department of Science and Technology, Government of India, Grant/Award Number: SB/S2/JCB-18/2013; Wellcome Trust-DBT India Alliance, Grant/Award Number: IA/M/16/1/502606

Abbreviations

CFTR	cystic fibrosis transmembrane conductance regulator
CNG	cyclic-nucleotide-gated
CSD	congenital secretory diarrhea
CTD	C-terminal domain
ECD	extracellular domain
GC-C	guanylyl cyclase C
GCD	guanylyl cyclase domain
KHD	kinase-homology domain
MI	meconium ileus
ST	heat-stable enterotoxin

References

1. Ashman DF, Lipton R, Melicow MM, Price TD. Isolation of adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate from rat urine. Biochem Biophys Res Commun. 1963; 11 :330–334. [PubMed: 13965190]
2. Schulz S, Green CK, Yuen PS, Garbers DL. Guanylyl cyclase is a heat-stable enterotoxin receptor. Cell. 1990; 63 :941–948. [PubMed: 1701694]
3. Currie MG, Fok KF, Kato J, et al. Guanylin: An endogenous activator of intestinal guanylate cyclase. Proc Natl Acad Sci U S A. 1992; 89 :947–951. [PubMed: 1346555]
4. Hamra FK, Forte LR, Eber SL, et al. Uroguanylin: Structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. Proc Natl Acad Sci USA. 1993; 90 :10464–10468. [PubMed: 7902563]
5. de Jonge HR. Properties of guanylate cyclase and levels of cyclic GMP in rat small intestinal villous and crypt cells. FEBS Lett. 1975; 55 :143–152. [PubMed: 237789]

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

6. De Jonge HR. The localization of guanylate cyclase in rat small intestinal epithelium. FEBS Lett. 1975; 53 :237–242. [PubMed: 237786]
7. Swenson ES, Mann EA, Jump ML, Witte DP, Giannella RA. The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine. Biochem Biophys Res Commun. 1996; 225 :1009–1014. [PubMed: 8780725]
8. Arshad N, Visweswariah SS. The multiple and enigmatic roles of guanylyl cyclase C in intestinal homeostasis. FEBS Lett. 2012; 586 :2835–2840. [PubMed: 22819815]
9. Forte LR, Krause WJ, Freeman RH. *Escherichia coli* enterotoxin receptors: Localization in opossum kidney, intestine, and testis. Am J Physiol. 1989; 257 :F874–F881. [PubMed: 2556042]
10. Jaleel M, London RM, Eber SL, Forte LR, Visweswariah SS. Expression of the receptor guanylyl cyclase C and its ligands in reproductive tissues of the rat: A potential role for a novel signaling pathway in the epididymis. Biol Reprod. 2002; 67 :1975–1980. [PubMed: 12444076]
11. Prasad H, Shenoy AR, Visweswariah SS. Cyclic nucleotides, gut physiology and inflammation. FEBS J. 2019; doi: 10.1111/febs.15198
12. Birbe R, Palazzo JP, Walters R, Weinberg D, Schulz S, Waldman SA. Guanylyl cyclase C is a marker of intestinal metaplasia, dysplasia, and adenocarcinoma of the gastrointestinal tract. Hum Pathol. 2005; 36 :170–179. [PubMed: 15754294]
13. Li P, Lin JE, Chervoneva I, Schulz S, Waldman SA, Pitari GM. Homeostatic control of the crypt-villus axis by the bacterial enterotoxin receptor guanylyl cyclase C restricts the proliferating compartment in intestine. Am J Pathol. 2007; 171 :1847–1858. [PubMed: 17974601]
14. Basu N, Saha S, Khan I, Ramachandra SG, Visweswariah SS. Intestinal cell proliferation and senescence are regulated by receptor guanylyl cyclase C and p21. J Biol Chem. 2014; 289 :581–593. [PubMed: 24217248]
15. Fiskerstrand T, Arshad N, Haukanes BI, et al. Familial diarrhea syndrome caused by an activating GUCY2C mutation. N Engl J Med. 2012; 366 :1586–1595. [PubMed: 22436048]
16. Muller T, Rasool I, Heinz-Erian P, et al. Congenital secretory diarrhoea caused by activating germline mutations in GUCY2C. Gut. 2016; 65 :1306–1313. [PubMed: 25994218]
17. Lin M, Nairn AC, Guggino SE. cGMP-dependent protein kinase regulation of a chloride channel in T84 cells. Am J Physiol. 1992; 262 :C1304–C1312. [PubMed: 1317106]
18. Golin-Bisello F, Bradbury N, Ameen N. STa and cGMP stimulate CFTR translocation to the surface of villus enterocytes in rat jejunum and is regulated by protein kinase G. Am J Physiol Cell Physiol. 2005; 289 :C708–C716. [PubMed: 15872007]
19. Chen T, Kocinsky HS, Cha B, et al. Cyclic GMP kinase II (cGKII) inhibits NHE3 by altering its trafficking and phosphorylating NHE3 at three required sites: Identification of a multifunctional phosphorylation site. J Biol Chem. 2015; 290 :1952–1965. [PubMed: 25480791]
20. Pitari GM, Lin JE, Shah FJ, et al. Enterotoxin preconditioning restores calcium-sensing receptor-mediated cytostasis in colon cancer cells. Carcinogenesis. 2008; 29 :1601–1607. [PubMed: 18566015]
21. Kang Y, Liu R, Wu JX, Chen L. Structural insights into the mechanism of human soluble guanylate cyclase. Nature. 2019; 574 :206–210. [PubMed: 31514202]
22. Potter LR. Guanylyl cyclase structure, function and regulation. Cell Signal. 2011; 23 :1921–1926. [PubMed: 21914472]
23. Visweswariah SS, Ramachandran V, Ramamohan S, Das G, Ramachandran J. Characterization and partial purification of the human receptor for the heat-stable enterotoxin. Eur J Biochem. 1994; 219 :727–736. [PubMed: 7906648]
24. Wada A, Hirayama T, Kitaura H, et al. Identification of ligand recognition sites in heat-stable enterotoxin receptor, membrane-associated guanylyl cyclase C by site-directed mutational analysis. Infect Immun. 1996; 64 :5144–5150. [PubMed: 8945558]
25. Hasegawa M, Hidaka Y, Matsumoto Y, Sanni T, Shimonishi Y. Determination of the binding site on the extracellular domain of guanylyl cyclase C to heat-stable enterotoxin. J Biol Chem. 1999; 274 :31713–31718. [PubMed: 10531382]
26. van den Akker F, Zhang X, Miyagi M, Huo X, Misono KS, Yee VC. Structure of the dimerized hormone-binding domain of a guanylyl-cyclase-coupled receptor. Nature. 2000; 406 :101–104. [PubMed: 10894551]

Europe PMC Funders Author Manuscripts

27. Hidaka Y, Matsumoto Y, Shimonishi Y. The micro domain responsible for ligand-binding of guanylyl cyclase C. FEBS Lett. 2002; 526 :58–62. [PubMed: 12208504]
28. Hasegawa M, Hidaka Y, Wada A, Hirayama T, Shimonishi Y. The relevance of N-linked glycosylation to the binding of a ligand to guanylate cyclase C. Eur J Biochem. 1999; 263 :338–346. [PubMed: 10406940]
29. Ghanekar Y, Chandrashaker A, Tatu U, Visweswariah SS. Glycosylation of the receptor guanylate cyclase C: Role in ligand binding and catalytic activity. Biochem J. 2004; 379 :653–663. [PubMed: 14748740]
30. Arshad N, Ballal S, Visweswariah SS. Site-specific N-linked gly-cosylation of receptor guanylyl cyclase C regulates ligand binding, ligand-mediated activation and interaction with vesicular integral membrane protein 36, VIP36. J Biol Chem. 2013; 288 :3907–3917. [PubMed: 23269669]
31. Romi H, Cohen I, Landau D, et al. Meconium ileus caused by mutations in GUCY2C, encoding the CFTR-activating guanylate cyclase 2C. Am J Hum Genet. 2012; 90 :893–899. [PubMed: 22521417]
32. Tal A, Carmi R, Chai-Am E, Zirkin H, Bar-Ziv J, Freud E. Familial meconium ileus with normal sweat electrolytes. Clin Pediatr (Phila). 1985; 24 :460–462. [PubMed: 4006357]
33. Smith A, Bulman DE, Goldsmith C, et al. Meconium ileus in a Lebanese family secondary to mutations in the GUCY2C gene. Eur J Hum Genet. 2015; 23 :990–992. [PubMed: 25370039]
34. Woods JD, Payton KSE, Sanchez-Lara PA, Au M, Simmons CF, Graham JM Jr. Non-cystic fibrosis-related meconium ileus: GUCY2C-associated disease discovered through rapid neonatal whole-exome sequencing. J Pediatr. 2019; 211 :207–210. [PubMed: 31079856]
35. Eggermont E. Gastrointestinal manifestations in cystic fibrosis. Eur J Gastroenterol Hepatol. 1996; 8 :731–738. [PubMed: 8864667]
36. Bhandari R, Srinivasan N, Mahaboobi M, Ghanekar Y, Suguna K, Visweswariah SS. Functional inactivation of the human guanylyl cyclase C receptor: Modeling and mutation of the protein kinase-like domain. Biochemistry. 2001; 40 :9196–9206. [PubMed: 11478887]
37. Chinkers M, Garbers DL. The protein kinase domain of the ANP receptor is required for signaling. Science. 1989; 245 :1392–1394. [PubMed: 2571188]
38. Chinkers M, Singh S, s Garbers DL, Adenine nucleotides are required for activation of rat atrial natriuretic peptide receptor/guanylyl cyclase expressed in a baculovirus system. J Biol Chem. 1991; 266 :4088–4093. [PubMed: 1671858]
39. Edmund AB, Walseth TF, Levinson NM, Potter LR. The pseudokinase domains of guanylyl cyclase-A and -B allosterically increase the affinity of their catalytic domains for substrate. Sci Signal. 2019; 12 eaau5378 [PubMed: 30696704]
40. Gazzano H, Wu HI, Waldman SA. Activation of particulate guanylate cyclase by *Escherichia coli* heat-stable enterotoxin is regulated by adenine nucleotides. Infect Immun. 1991; 59 :1552–1557. [PubMed: 1672303]
41. Vaandrager AB, van der Wiel E, de Jonge HR. Heat-stable enterotoxin activation of immunopurified guanylyl cyclase C. Modulation by adenine nucleotides. J Biol Chem. 1993; 268 :19598–19603. [PubMed: 8103520]
42. Deshmane SP, Parkinson SJ, Crupper SS, Robertson DC, Schulz S, Waldman SA. Cytoplasmic domains mediate the ligand-induced affinity shift of guanylyl cyclase C. Biochemistry. 1997; 36 :12921–12929. [PubMed: 9335551]
43. Jaleel M, Saha S, Shenoy AR, Visweswariah SS. The kinase homology domain of receptor guanylyl cyclase C: ATP binding and identification of an adenine nucleotide sensitive site. Biochemistry. 2006; 45 :1888–1898. [PubMed: 16460035]
44. Hanks SK, Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: Kinase (catalytic) domain structure and classification. FASEB J. 1995; 9 :576–596. [PubMed: 7768349]
45. Mishra V, Goel R, Visweswariah SS. The regulatory role of the kinase-homology domain in receptor guanylyl cyclases: Nothing ‘pseudo’ about it! Biochem Soc Trans. 2018; 46 :1729–1742. [PubMed: 30420416]
46. Biswas KH, Shenoy AR, Dutta A, Visweswariah SS. The evolution of guanylyl cyclases as multidomain proteins: Conserved features of kinase-cyclase domain fusions. J Mol Evol. 2009; 68 :587–602. [PubMed: 19495554]

Europe PMC Funders Author Manuscripts

47. Saha S, Biswas KH, Kondapalli C, Isloor N, Visweswariah SS. The linker region in receptor guanylyl cyclases is a key regulatory module: Mutational analysis of guanylyl cyclase C. J Biol Chem. 2009; 284 :27135–27145. [PubMed: 19648115]
48. Bassler J, Schultz JE, Lupas AN. Adenylate cyclases: Receivers, transducers, and generators of signals. Cell Signal. 2018; 46 :135–144. [PubMed: 29563061]
49. Krupinski J, Coussen F, Bakalyar HA, et al. Adenylyl cyclase amino acid sequence: Possible channel- or transporter-like structure. Science. 1989; 244 :1558–1564. [PubMed: 2472670]
50. Rauch A, Leipelt M, Russwurm M, Steegborn C. Crystal structure of the guanylyl cyclase Cya2. Proc Natl Acad Sci USA. 2008; 105 :15720–15725. [PubMed: 18840690]
51. Winger JA, Derbyshire ER, Lamers MH, Marletta MA, Kuriyan J. The crystal structure of the catalytic domain of a eukaryotic guanylate cyclase. BMC Struct Biol. 2008; 8 :42. [PubMed: 18842118]
52. Garbers DL, Chrisman TD, Wiegn P, et al. Membrane guanylyl cyclase receptors: An update. Trends Endocrinol Metab. 2006; 17 :251–258. [PubMed: 16815030]
53. Hodson CA, Ambrogi IG, Scott RO, Mohler PJ, Milgram SL. Polarized apical sorting of guanylyl cyclase C is specified by a cytosolic signal. Traffic. 2006; 7 :456–464. [PubMed: 16536743]
54. Scott RO, Thelin WR, Milgram SL. A novel PDZ protein regulates the activity of guanylyl cyclase C the heat-stable enterotoxin receptor. J Biol Chem. 2002; 277 :22934–22941. [PubMed: 11950846]
55. Guarino A, Cohen MB, Giannella RA. Small and large intestinal guanylate cyclase activity in children: Effect of age and stimulation by *Escherichia coli* heat-stable enterotoxin. Pediatr Res. 1987; 21 :551–555. [PubMed: 2885801]
56. Cohen MB, Guarino A, Shukla R, Giannella RA. Age-related differences in receptors for *Escherichia coli* heat-stable enterotoxin in the small and large intestine of children. Gastroenterology. 1988; 94 :367–373. [PubMed: 2891585]
57. Laney DW Jr, Mann EA, Dellon SC, Perkins DR, Giannella RA, Cohen MB. Novel sites for expression of an *Escherichia coli* heat-stable enterotoxin receptor in the developing rat. Am J Physiol. 1992; 263 :G816–G821. [PubMed: 1359797]
58. Laney DW Jr, Bezerra JA, Kosiba JL, Degen SJ, Cohen MB. Upregulation of *Escherichia coli* heat-stable enterotoxin receptor in regenerating rat liver. Am J Physiol. 1994; 266 :G899–G906. [PubMed: 7911278]
59. Scheving LA, Russell WE. Guanylyl cyclase C is up-regulated by nonparenchymal cells and hepatocytes in regenerating rat liver. Cancer Res. 1996; 56 :5186–5191. [PubMed: 8912855]
60. Giannella RA, Mann EA. *E. coli* heat-stable enterotoxin and guanylyl cyclase C: New functions and unsuspected actions. Trans Am Clin Climatol Assoc. 2003; 114 :67–85. discussion 85-66 [PubMed: 12813912]
61. Merlino DJ, Barton JR, Charsar BA, et al. Two distinct GUCY2C circuits with PMV (hypothalamic) and SN/VTA (midbrain) origin. Brain Struct Funct. 2019; 224 :2983–2999. [PubMed: 31485718]
62. Di Guglielmo MD, Tonb D, He Z, Adeyemi A, van Golen KL. Pilot study measuring the novel satiety hormone, pro-Uroguanylin, in adolescents with and without obesity. J Pediatr Gastroenterol Nutr. 2018; 66 :489–495. [PubMed: 29112082]
63. Folgueira C, Barja-Fernandez S, Gonzalez-Saenz P, et al. Circulating pro-uroguanylin levels in children and their relation to obesity, sex and puberty. Sci Rep. 2018; 8
64. Folgueira C, Barja-Fernandez S, Gonzalez-Saenz P, et al. Uroguanylin: A new actor in the energy balance movie. J Mol Endocrinol. 2018; 60 :R31–R38. [PubMed: 29203517]
65. Valentino MA, Lin JE, Snook AE, et al. A uroguanylin-GUCY2C endocrine axis regulates feeding in mice. J Clin Invest. 2011; 121 :3578–3588. [PubMed: 21865642]
66. Folgueira C, Beiroa D, Callon A, et al. Uroguanylin action in the brain reduces weight gain in obese mice via different efferent autonomic pathways. Diabetes. 2016; 65 :421–432. [PubMed: 26566631]
67. Begg DP, Steinbrecher KA, Mul JD, et al. Effect of guanylate cyclase-C activity on energy and glucose homeostasis. Diabetes. 2014; 63 :3798–3804. [PubMed: 24898144]

Europe PMC Funders Author Manuscripts

68. Gong R, Ding C, Hu J, et al. Role for the membrane receptor guanylyl cyclase-C in attention deficiency and hyperactive behavior. Science. 2011; 333 :1642–1646. [PubMed: 21835979]
69. Lee DK, Oh JH, Yang JH, et al. Protein kinase G linked to dopamine D3 receptors in the dorsal striatum controls dopamine release, DeltaFosB expression and locomotor activity after repeated cocaine administration. Neurosci Lett. 2013; 541 :120–125. [PubMed: 23428510]
70. Zufall F, Shepherd GM, Barnstable CJ. Cyclic nucleotide gated channels as regulators of CNS development and plasticity. Curr Opin Neurobiol. 1997; 7 :404–412. [PubMed: 9232810]
71. Barnstable CJ, Wei JY, Han MH. Modulation of synaptic function by cGMP and cGMP-gated cation channels. Neurochem Int. 2004; 45 :875–884. [PubMed: 15312982]
72. Trichet L, Sykes C, Plastino J. Relaxing the Actin cytoskeleton for adhesion and movement with Ena/VASP. J Cell Biol. 2008; 181 :19–25. [PubMed: 18378777]
73. Wang HG, Lu FM, Jin I, et al. Presynaptic and postsynaptic roles of NO cGK, and RhoA in long-lasting potentiation and aggregation of synaptic proteins. Neuron. 2005; 45 :389–403. [PubMed: 15694326]
74. Feil R, Kleppisch T. NO/cGMP-dependent modulation of synaptic transmission. Handb Exp Pharmacol. 2008; 184 :529–560.
75. Akieda-Asai S, Sugiyama M, Miyazawa T, et al. Involvement of guanylin and GC-C in rat mesenteric macrophages in resistance to a high-fat diet. J Lipid Res. 2013; 54 :85–96. [PubMed: 23081987]
76. Handa P, Tateya S, Rizzo NO, et al. Reduced vascular nitric oxide-cGMP signaling contributes to adipose tissue inflammation during high-fat feeding. Arterioscler Thromb Vasc Biol. 2011; 31 :2827–2835. [PubMed: 21903940]
77. Forte LR, Fan X, Hamra FK. Salt and water homeostasis: Uroguanylin is a circulating peptide hormone with natriuretic activity. Am J Kidney Dis. 1996; 28 :296–304. [PubMed: 8768930]
78. Carrithers SL, Hill MJ, Johnson BR, et al. Renal effects of uroguanylin and guanylin in vivo. Braz J Med Biol Res. 1999; 32 :1337–1344. [PubMed: 10559834]
79. Forte LR, London RM, Freeman RH, Krause WJ. Guanylin peptides: Renal actions mediated by cyclic GMP. Am J Physiol Renal Physiol. 2000; 278 :F180–F191. [PubMed: 10662722]
80. Forte LR, London RM, Krause WJ, Freeman RH. Mechanisms of guanylin action via cyclic GMP in the kidney. Annu Rev Physiol. 2000; 62 :673–695. [PubMed: 10845107]
81. Carrithers SL, Taylor B, Cai WY, et al. Guanylyl cyclase-C receptor mRNA distribution along the rat nephron. Regul Pept. 2000; 95 :65–74. [PubMed: 11062334]
82. Carrithers SL, Ott CE, Hill MJ, et al. Guanylin and uroguanylin induce natriuresis in mice lacking guanylyl cyclase-C receptor. Kidney Int. 2004; 65 :40–53. [PubMed: 14675035]
83. Waldman SA, Camilleri M. Guanylate cyclase-C as a therapeutic target in gastrointestinal disorders. Gut. 2018; 67 :1543–1552. [PubMed: 29563144]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

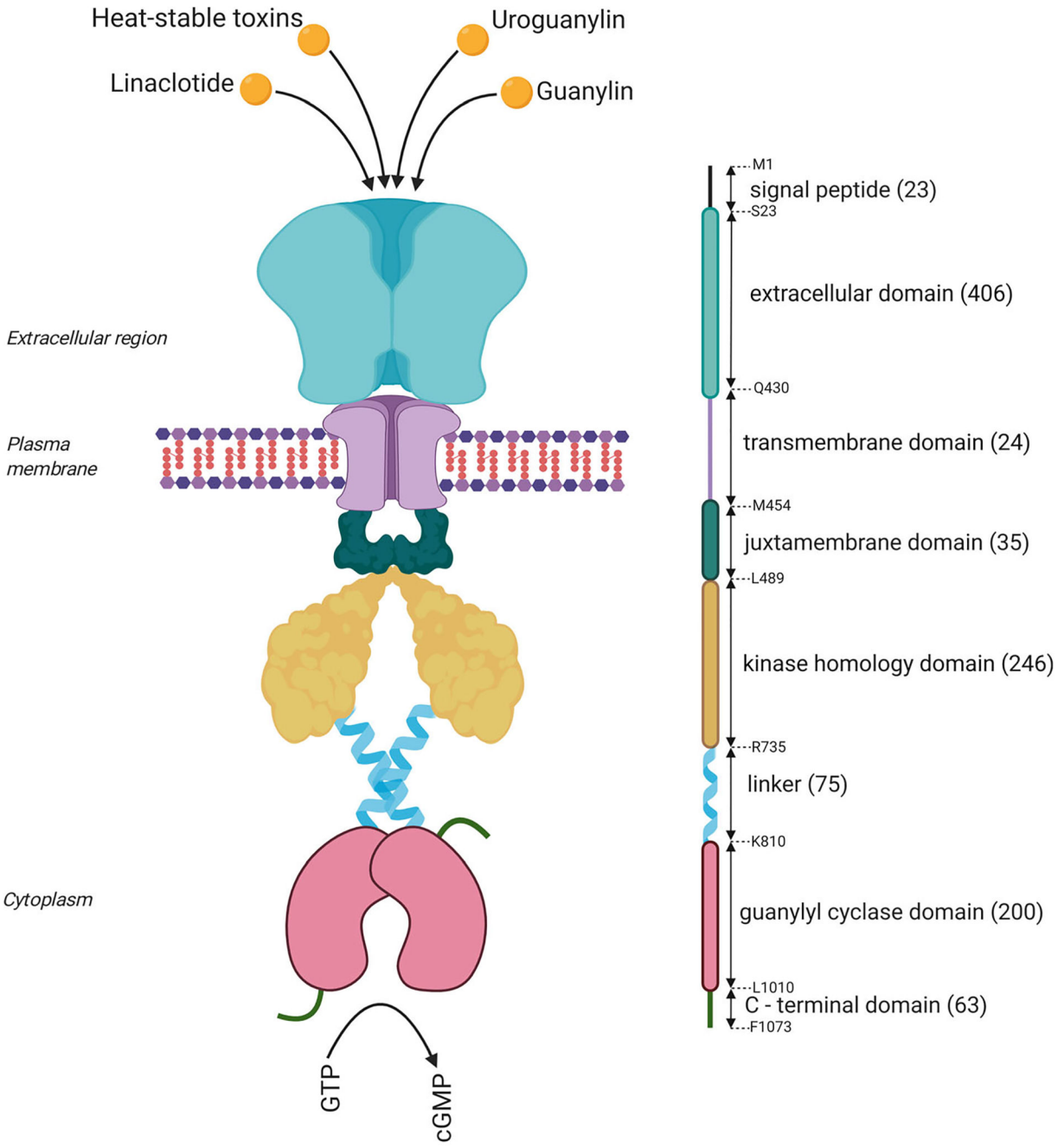

Figure 1.

Schematic representation of domain organization of receptor guanylyl cyclase C (GC-C). GC-C, expected to be a homodimeric transmembrane receptor, has seven conserved functional domains including[1] the extracellular domain that binds peptide ligands (heat-stable enterotoxins [ST], endogenous peptides like guanylin and uroguanylin, and the FDA approved ST analog, Linaclotide)[2]; a single transmembrane domain spanning the plasma membrane[3]; a short juxtamembrane domain[4]; the kinase-homology domain, which binds ATP[5]; a linker region that may facilitate catalytic subunit dimerization and regulates

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

catalytic activity[6]; the catalytic guanylyl cyclase domain, which mediates the conversion of GTP to cGMP; and[7] a carboxyl terminal tail (C-terminal domain) that modulates the guanylyl cyclase activity helps in cytoskeletal anchoring, and receptor internalization. The linear representation of the domain organization exhibits the domain boundaries with the single letter amino acid code at the particular position. The length of each domain has been mentioned within brackets. The figure has been created with Biorender. ATP, adenosine triphosphate; cGMP, guanosine 3',5'-cyclic monophosphate; FDA, Food and Drug Administration; GTP, guanosine triphosphate; ST, heat-stable enterotoxin

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

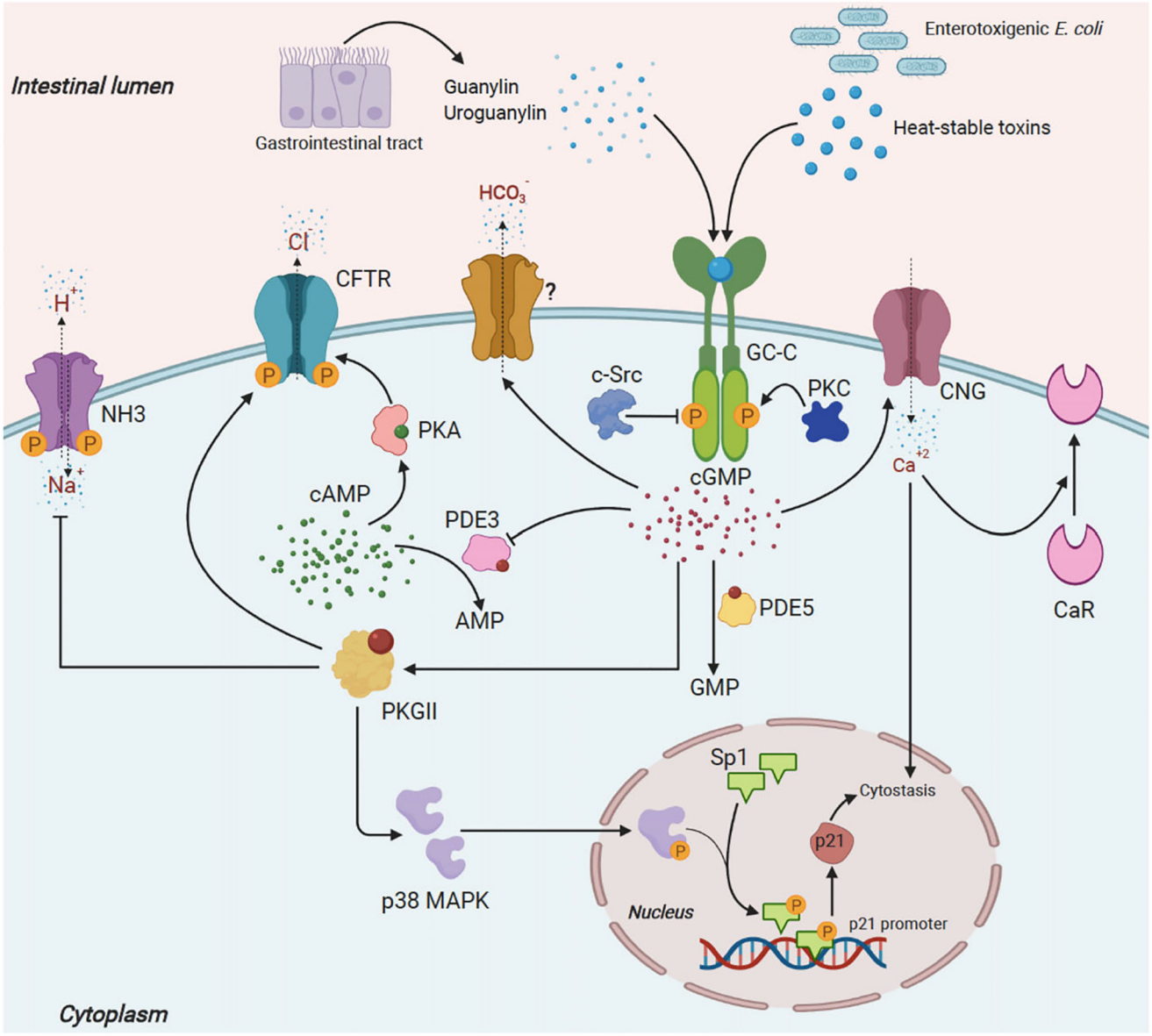

Figure 2.

GC-C signaling pathway in intestinal epithelial cells. GC-C is expressed on the surface of the enterocytes and acts as the receptor for uroguanylin and guanylin that are synthesized in the intestine and secreted into the lumen. GC-C is also activated by ST peptides produced by enterotoxigenic *E. coli.* Ligand-mediated activation of GC-C converts GTP to cGMP. Elevated levels of intracellular cGMP activates cGMP-dependent protein kinase II (PKGII) which in turn phosphorylates NHE3 thereby inhibiting its activity. Cyclic GMP inhibits the activity of the cAMP-phosphodiesterase PDE3, thereby cross-activating cAMP-dependent protein kinase A (PKA). PKGII and PKA phosphorylate the CFTR leading to chloride ion (Cl^-) efflux. Cyclic GMP also activates the secretion of bicarbonate ion (HCO_3^-) by an unidentified channel (marked "?" in the figure). These processes balance fluid-ion homeostasis in the intestine. Cyclic GMP also directly activates CNG channels leading to

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Ca^{2+} influx. Increased level of intracellular Ca^{2+} interacts with calcium-sensing receptors (CaRs), leading to cell differentiation and migration. Phosphorylation by protein kinase C (PKC) activates and phosphorylation by c-Src inhibits guanylyl cyclase activity of GC-C. PKGII on activation by cGMP activates p38 MAPK which phosphorylates the transcription factor Sp1 which in turn upregulates the transcription of p21 mRNA. Hydrolysis of cGMP to 5'GMP by the cGMP-specific phosphodiesterase, PDE5, can terminate GC-C signaling. The figure has been created with Biorender. cAMP, adenosine 3',5'-cyclic monophosphate; cGMP, guanosine 3',5'-cyclic monophosphate; CNG, cyclic-nucleotide-gated; GC-C, guanylyl cyclase C; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinase; ST, heat-stable enterotoxin

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

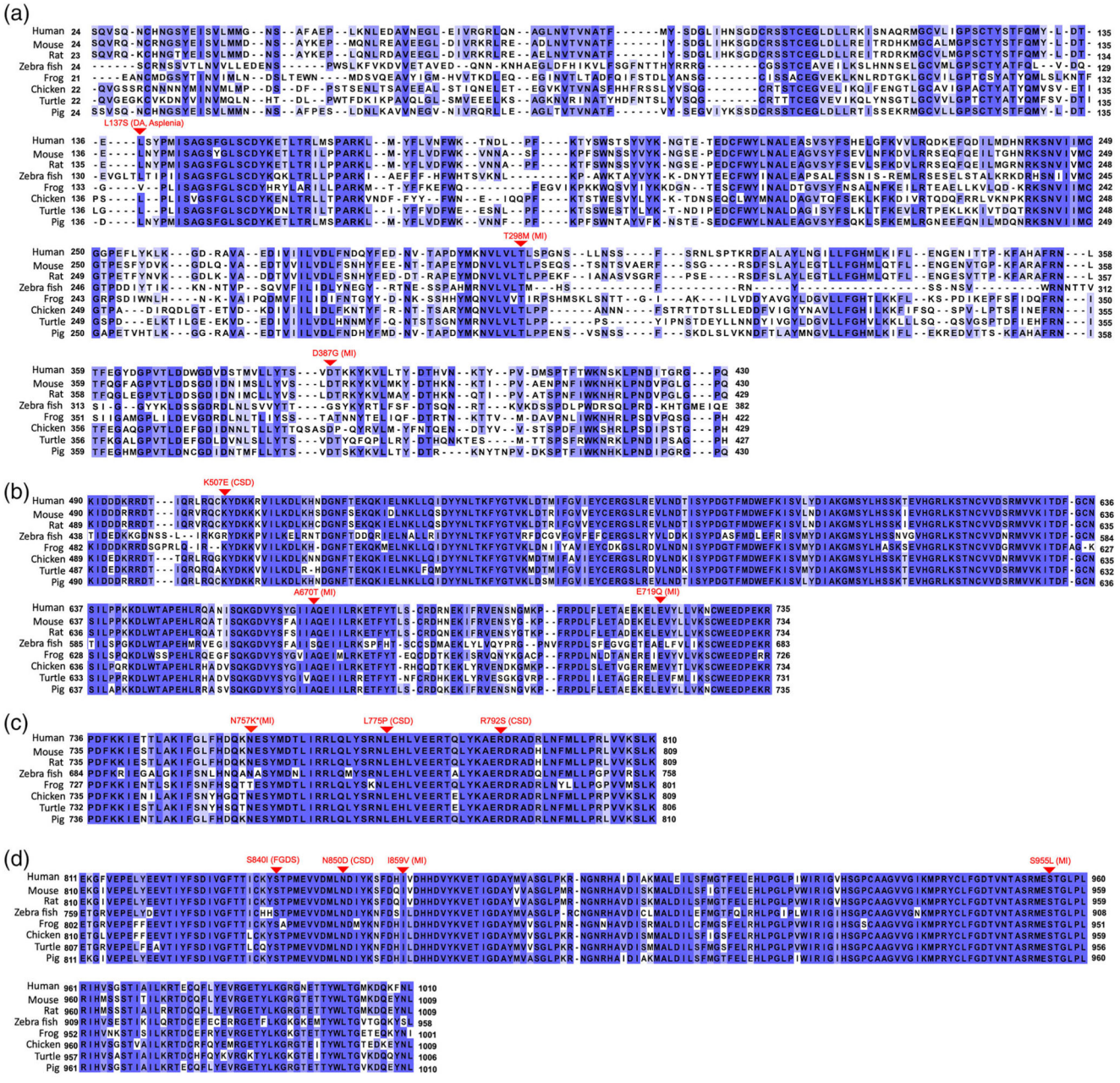

Figure 3. Alignment of receptor guanylyl cyclase C across species.

(a) Alignment of the extracellular domain (starting from residue 24 to 430 in human, ~406 amino acids). (b) Alignment of the KHD (starting from residue 490 to 735 in human, ~246 amino acids). (c) Alignment of the linker region (starting from residue 736 to 810 in human, ~75 amino acids). (d) Alignment of the GCD (starting from residue 811 to 1,010 in human, ~200 amino acids). Organisms selected are *Homo sapiens* (NP_004954.2), *Mus musculus* (NP_001120790.1), *Rattus norvegicus* (NP_037302.1), *Danio rerio* (XP_021329920.1), *Xenopus tropicalis* (XP_002934756.1), *Gallus gallus* (XP_416207.3), *Chelonia mydas* (XP_007064150.1), and *Sus scrofa* (NP_999270.1). Orthologs of GC-C were identified

using BLAST and aligned using Kalign. Residues are colored depending on the level of conservation across the eight species using Jalview v2.11. Residues marked with red triangles identify reported mutations in the amino acid in patients causing duodenal atresia (DA), asplenia, congenital secretory diarrhea (CSD), meconium ileus (MI), and familial *GUCY2C* diarrhea syndrome (FGDS). Mutation N757K* is a result of an insertion of one base pair, resulting in conversion of Asn to Lys and generation of a stop codon immediately after the Lys residue. GC-C, guanylyl cyclase C; GCD, guanylyl cyclase domain; KHD, kinase-homology domain

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

IUBMB Life. Author manuscript; available in PMC 2021 August 11.

Table 1

Clinical significance of genetic variations in *GUCY2C*

Protein change		Domain	Effect on function	Pathological condition(s)	Clinical significance	dbSNP ID	DNA change	References
Arg114Gln	R114Q	ECD		Not provided	Likely benign		c.341G>A	https://www.ncbi.nlm.nih.gov/clinvar/variation/774705/
Leu137Ser	L137S	ECD		Duodenal atresia; Asplenia; abnormal biliary tract morphology; reduced number of intrahepatic bile ducts	Pathogenic	rsl057519441	c.410T>C	https://www.ncbi.nlm.nih.gov/clinvar/variation/375380/
Thr156Ile	T156I	ECD		Not provided	Uncertain significance	rsl47745948	c.467C>T	https://www.ncbi.nlm.nih.gov/clinvar/variation/432841/
Glu210Lys	E210K	ECD		Not provided	Uncertain significance	rsl057520084	c.628G>A	https://www.ncbi.nlm.nih.gov/clinvar/variation/376900/
Thr298Met	T298M	ECD		Meconium ileus (submitted: October 22, 2019)	Likely benign[a]		c.893C>T	https://www.ncbi.nlm.nih.gov/clinvar/variation/714778/
Asp387Gly	D387G	ECD	Loss	Meconium ileus	Pathogenic	rs587776905	c,1160A>G	31 and 32
Lys507Glu	K507E	KHD	Gain	Congenital secretory diarrhea	Pathogenic		c,1519A>G	16
Ala670Thr	A670T	KHD	Loss	Meconium ileus	Pathogenic	rs587784572	c.2008G>A	33
Asn689Lys	N689K	KHD		Not specified	Uncertain significance	rsl38497004	c.2067T>G	https://www.ncbi.nlm.nih.gov/clinvar/variation/252641/
Glu696Ter	E696*	KHD		Not provided	Pathogenic	rsl057520701	c.2086G>T	https://www.ncbi.nlm.nih.gov/clinvar/variation/379706/
Glu719Gln	E719Q	KHD		Meconium ileus	Likely benign[a]			https://www.ncbi.nlm.nih.gov/clinvar/variation/802827/
Leu751Val	L751V	Linker		Not provided	Benign		c.2251C>G	https://www.ncbi.nlm.nih.gov/clinvar/variation/718878/
Asn757Lys[c] (frameshift mutation)[31] Pathogenic			c.2270dup		N757K	Linker		Meconium ileus
Arg773Gln	R773Q	Linker		Not provided	Uncertain significance	rsl057520052	c.2318G>A	https://www.ncbi.nlm.nih.gov/clinvar/variation/376780/
Leu775Pro	L775P	Linker	Gain	Congenital secretory diarrhea	Pathogenic		c.2324T>C	16
Gln784Lys	Q784K	Linker		Not provided	Likely benign		c.2350C>A	https://www.ncbi.nlm.nih.gov/clinvar/variation/738987/

Europe PMC Funders Author Manuscripts
Europe PMC Funders Author Manuscripts

Protein change		Domain	Effect on function	Pathological condition(s)	Clinical significance	dbSNP ID	DNA change	References
Arg792Ser	R792S	Linker	Gain	Congenital secretory diarrhea	Pathogenic		c.2376G>C	16
Ser840Ile	S840I	GCD	Gain	Familial *GUCY2C* diarrhea syndrome (diarrhea 6[b])	Pathogenic	rs587776871	c.2519G>T	15
Asn850Asp	N850D	GCD	Gain	Congenital secretory diarrhea	Pathogenic		c.2548A>G	16
Ile859Val	I859V	GCD		Meconium ileus	Uncertain significance Deleterious (SIFT) Probably damaging (PolyPhen)		c.2575A>G	34
Arg888Trp	R888W	GCD		Not provided	Likely benign		c.2662C>T	https://www.ncbi.nlm.nih.gov/clinvar/variation/745237/
Cys928Arg	C928R	GCD	Loss	Meconium ileus	Pathogenic	rs587784573	c.2782T>C	33
Ser955Leu	S955L	GCD		Meconium ileus	Uncertain Significance Deleterious (SIFT) Probably damaging (Polyphen)		c.2864_2865delCCinsTA	34
Val964Ala	V964A	GCD		Not provided	Benign		c.2891T>C	https://www.ncbi.nlm.nih.gov/clinvar/variation/775307/
Tyrl072Cys	Y1072C	C-TER		Not provided	Likely benign		c.3215A>G	https://www.ncbi.nlm.nih.gov/clinvar/variation/782874/

Abbreviations: C-TER, C-terminal domain; ECD, extracellular domain; GCD, guanylyl cyclase domain; KHD, kinase-homology domain.

[a]The clinical significance of the variation causing meconium ileus is denoted “likely benign” by ClinVar database. However, as the mutation is associated with disease, it is likely to be a pathogenic mutation.

[b]Familial *GUCY2C* diarrhea syndrome (FGDS), caused due to a mutation in *GUCY2C,* is marked as Diarrhea 6 in OMIN database.

[c]A frameshift mutation deleting the GCD of receptor GC-C.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 2

Deleterious variations mapped in *GUCY2C* reported in dbSNP (v2.0 Build 153)

Type of variation	Functional class of variation	Number of variation	Domain
Deletion	Stop codon gained	1	Linker
Deletion and insertion	Stop codon gained	1	Extracellular domain
Single nucleotide variation	Missense	11	Signal peptide
		266	Extracellular domain
		19	Transmembrane domain
		21	Juxtamembrane domain
		146	Kinase-homology domain
		41	Linker
		108	Guanylyl cyclase domain
		43	C-terminal
Single nucleotide variation	Stop codon gained	1	Signal peptide
		11	Extracellular domain
		1	Transmembrane domain
		2	Juxtamembrane domain
		12	Kinase-homology domain
		1	Linker
		6	Guanylyl cyclase domain
		1	C-terminal domain