A Potential Treatment of Congenital Sodium Diarrhea in Patients With Activating *GUCY2C* Mutations

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INTRODUCTION: **Gain-of-function mutations in guanylyl cyclase C (GCC) result in persistent diarrhea with perinatal onset. We investigated a specific GCC inhibitor, SSP2518, for its potential to treat this disorder.**

METHODS: **We investigated the effect of SSP2518 on GCC-mediated intracellular cyclic guanosine monophosphate (cGMP) levels and on GCC-mediated chloride secretion in intestinal organoids from 3 patients with distinct activating GCC mutations and from controls, with and without stimulation of GCC with heat-stable enterotoxin.**

RESULTS: **Patient-derived organoids had significantly higher basal cGMP levels than control organoids, which were lowered by SSP2518 to levels found in control organoids. In addition, SSP2518 significantly reduced cGMP levels and chloride secretion in patient-derived and control organoids ($P < 0.05$ for all comparisons) after heat-stable enterotoxin stimulation.**

DISCUSSION: **We reported in this study that the GCC inhibitor SSP2518 normalizes cGMP levels in intestinal organoids derived from patients with GCC gain-of-function mutations and markedly reduces cystic fibrosis transmembrane conductance regulator–dependent chloride secretion, the driver of persistent diarrhea.**

SUPPLEMENTARY MATERIAL accompanies this paper at https://links.lww.com/CTG/A719

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INTRODUCTION

Guanylyl cyclase C (GCC) is a receptor enzyme in the apical membrane of enterocytes. Its extracellular receptor domain binds guanylin, uroguanylin, the heat-stable *Escherichia coli* enterotoxin heat stable enterotoxin (ST) (1–3), and the synthetic peptide linaclotide (4). GCC ligand binding increases the intracellular cyclic guanosine monophosphate (cGMP) level, which triggers protein kinase–mediated activation of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel and inhibition of the sodium-proton exchanger NHE3. This dual action promotes the retention of salt and water in the gut lumen (2,5), and abnormally high GCC activity leads to diarrhea.

One type of congenital enteropathy, the classical form of congenital sodium diarrhea (CSD), is caused by gain-of-function mutations in *GUCY2C*, encoding GCC (6–8), or by loss-of-function mutations in NHE3 (9). Such GCC mutations result in polyhydramnios and severe diarrhea from birth onward, generally requiring long-term parenteral nutrition (PN) (7). Given the severe complications of long-term PN, new treatment options are warranted. Two different types of specific GCC inhibitors were developed so far, an N-2-(propylamino)-6-phenylpyrimidin-4-one–substituted piperidine (SSP2518) (10) and a pyridopyrimidine derivative (BPIPP) (11).

MATERIAL AND METHODS

Patients and study design

Three patients with GCC-related diarrhea participated in this study. All human material was obtained with informed consent and in accordance with local ethical requirements. The study adhered to the principles set out in the Declaration of Helsinki.

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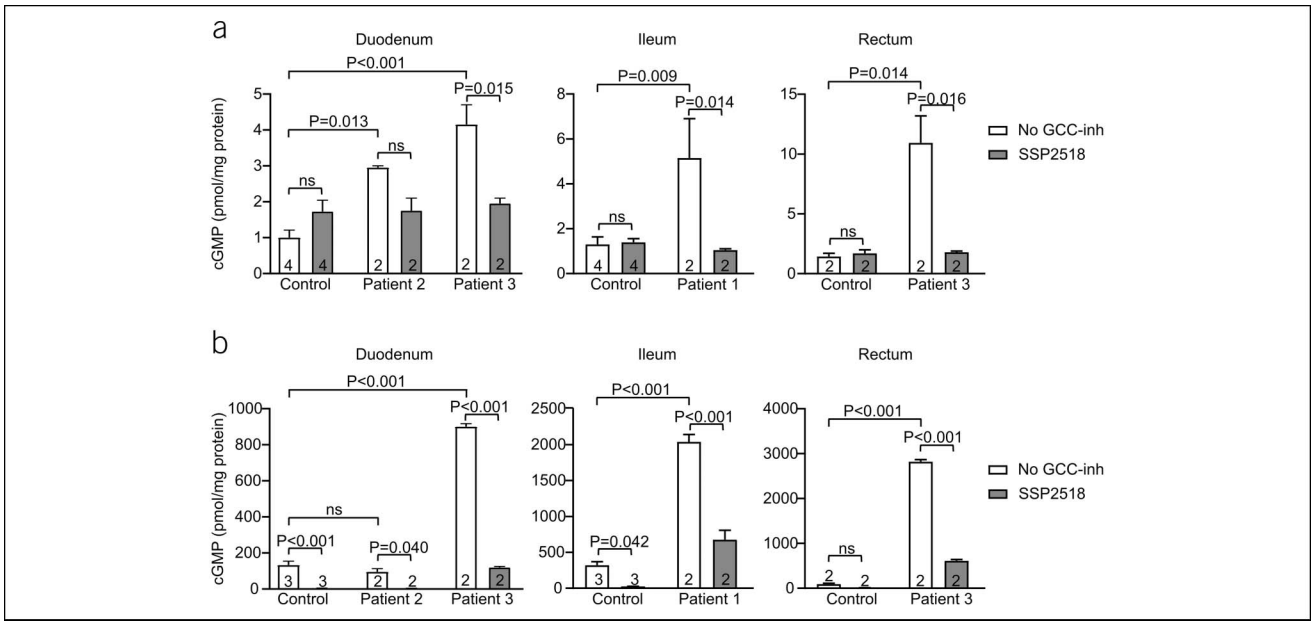

Figure 1. Elevated basal and stimulated cGMP levels in patient-derived organoids are reversed by SSP2518. cGMP levels in patient-derived and control organoid monolayers, in the absence or presence of GCC inhibitor SSP2518, without (**a**) or after (**b**) apical ST stimulation (mean ± SE; number of technical replicates as indicated within/above bars). cGMP, cyclic guanosine monophosphate; GCC, guanylyl cyclase C; ns, no statistically significant difference; ST, heat stable enterotoxin.

Organoid cultures and generation of organoid monolayers

Organoid cultures were generated from patient and control biopsy specimens as described (12), and monolayers were generated from Matrigel-embedded (3D) organoid cultures as described (13); details are provided in Supplemental Materials and Methods (http://links.lww.com/CTG/A719).

cGMP levels in monolayers from intestinal organoids and assessment of chloride secretion across organoid monolayers

We investigated the effect of SSP2518 on GCC-mediated intracellular cGMP levels and on GCC-mediated chloride secretion in intestinal organoids with activating GCC mutations and from controls, with and without stimulation of GCC with ST; CFTR-mediated chloride secretion in organoid-derived monolayers was recorded in Ussing chambers. The detailed experimental designs and statistical methods are provided in Supplemental Materials and Methods (http://links.lww.com/CTG/A719).

RESULTS

CSD patients harbor *de novo GUCY2C* variants

Clinical details of patients 1–3 (P1–P3) are provided in Supplemental Materials and Methods (http://links.lww.com/CTG/A719). P1 is heterozygous for a novel *GUCY2C* variant, c.2485C>T (p.Thr783Ile). P2 and P3 are heterozygous for *GUCY2C* variants c.2324T>C (p.Leu775Pro) and c.2376 G>C (p.Arg792Ser) (7). All 3 *GUCY2C* variants occurred *de novo* in patients.

Patient-derived organoids display abnormally elevated intracellular cGMP levels, which are reversed by SSP2518

All patient-derived organoids had significantly higher basal cGMP levels than control organoids. SSP2518 reduced basal cGMP levels in patient organoids to levels in control organoids (Figure 1a).

ST stimulation increased cGMP levels significantly in all patient-derived and control organoids ($P < 0.05$ for all comparisons). However, in organoids derived from P1 and P3, ST increased cGMP to levels significantly above those found in ST-stimulated control organoids (Figure 1b). The increase in cGMP in organoids derived from P2 with the p.Leu775Pro mutation was similar to that seen in control organoids. SSP2518 significantly decreased cGMP levels in all patient-derived organoids and in control organoids derived from ileum and duodenum (Figure 1b).

Patient-derived organoids displayed abnormally elevated chloride secretion, which were reversed by SSP2518

ST concentration dependently increased chloride secretion in ileal organoids from P1 and control 1, but more markedly in the patient (Figure 2a). A plateau in the Isc response in the patient organoids was reached at an ST concentration ≥0.1 μmol/L, which corresponded to 94% ± 4% (n = 3) of the Isc response elicited by forskolin/IBMX, a combination of cAMP agonists that triggers near-maximal CFTR activation in intestinal tissue. This suggests that the intracellular cGMP levels attained are at or above the threshold for maximal stimulation of the enzymes targeted by cGMP and involved in CFTR activation.

The effect of GCC inhibition by SSP2518 (3 μmol/L) on the Isc response elicited by ST (0.3 μmol/L) was studied in ileal organoids (control 1 and P1) and duodenal organoids (P2, P3). Inhibition was substantial in all patient organoids (Figure 2b). These experiments also showed that the ST/GCC/cGMP/CFTR pathway for chloride secretion seemed less active in duodenal compared with ileal organoids (Figure 2b).

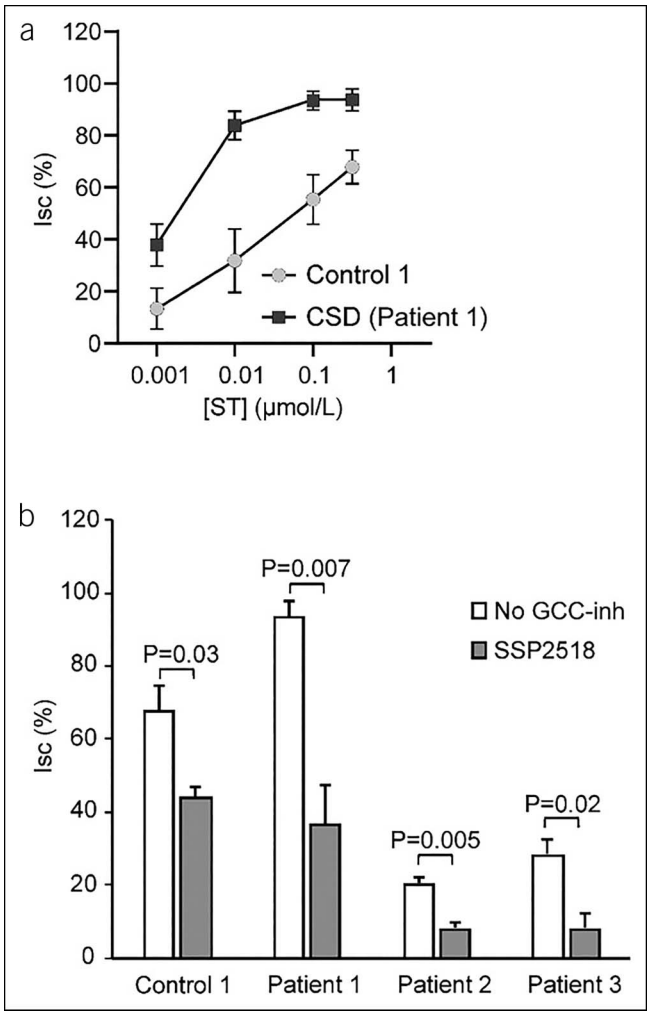

Figure 2. ST-dependent GCC-mediated chloride secretion across CSD organoid monolayers: (**a**) in ileum (control 1 and P1) decreases on SSP2518 treatment; (**b**) in ileum (P1 and control 1) or duodenum (P2, P3). Mean ± SE of 3 technical replicates. CSD, congenital sodium diarrhea; GCC, guanylyl cyclase C; ST, heat stable enterotoxin.

DISCUSSION

De novo GCC mutations that result in elevated constitutive GCC enzymatic activity and in abnormally increased sensitivity to stimulation by GCC ligands cause the severe and potentially lethal disease, CSD (7). Inherited GCC mutations that result in only increased ligand sensitivity cause a less severe diarrheal phenotype (6,8). We reported in this study a novel GCC mutation in a patient with PN-dependent CSD.

GCC has been identified as a potential target for treating diarrheal disorders, particularly the diarrhea caused by ST (travelers' diarrhea). Two different classes of compounds that specifically inhibit GCC were developed, BPIPP (11) and SSP2518 (10), but no approved GCC-targeting treatments exist (4). BPIPP was recently shown to reverse the effect of the GCC mutation p.Asp794Val *in vitro* (8). We showed in this study that SSP2518-mediated inhibition of GCC normalizes the basal and stimulated levels of cGMP and significantly reduces CFTR-dependent chloride secretion in organoids with distinct GCC mutations. The effect of SSP2518 on GCC activity was demonstrated in organoids derived from duodenum, ileum, and rectum alike. This suggests that rectal biopsies facilitate both the study of GCC mutations and their response to SSP2518. However, a limitation of our study is the small patient sample size.

SSP2518 potentially affects heart rythm characteristics (Ver Donck, oral communication) but might still be considered as treatment of CSD if patients have a normal cardiac ryhtm time before treatment and are monitored electrocardiographically (14).

CONFLICTS OF INTEREST

Guarantor of the article: Roderick H.J. Houwen, MD, PhD and Andreas R. Janecke, MD

Specific author contributions: H.R.d.J., R.H.J.H., A.R.J., and S.M.: conceptualization. A.H.M.v.V., M.J.C.B., and S.M.: organoid generation, processing, cGMP determination, and qRT-PCR. K.F.M.: transmembrane chloride measurements. T.R., M.B.B., A.B., B.K., and T.M.: involved in patient care and access to samples. All authors contributed to the revision of the manuscript.

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Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ Gain-of-function mutations in guanylyl cyclase C (GCC) cause an increase in enterocytic cyclic guanosine monophosphate (cGMP) levels.
- ✓ Increased enterocytic cGMP causes intestinal sodium loss and secretory diarrhea.

WHAT IS NEW HERE

- ✓ c.2485C>T (p.Thr783Ile) is a novel mutation in GCC, causing secretory diarrhea.
- ✓ Intestinal cGMP levels are normalized on SSP2518 treatment in organoids derived from patients with congenital sodium diarrhea.

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