

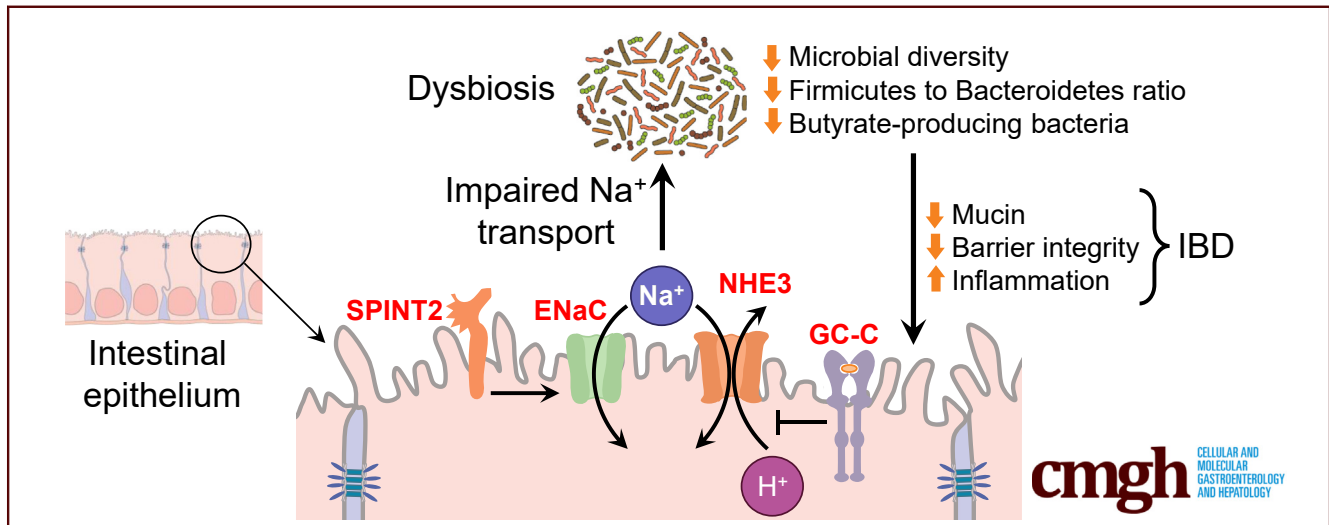
REVIEW

Impaired Intestinal Sodium Transport in Inflammatory Bowel Disease: From the Passenger to the Driver's Seat



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SUMMARY

We synthesized genetic and cellular evidence to construct a model proposing that impaired intestinal sodium transport can act as an upstream pathogenic factor in inflammatory bowel disease. The model predicted that strategies designed to correct disturbed electrolyte homeostasis carry high therapeutic potential.

Although impaired intestinal sodium transport has been described for decades as a ubiquitous feature of inflammatory bowel disease (IBD), whether and how it plays a pivotal role in the ailment has remained uncertain. Our identification of dominant mutations in receptor guanylyl cyclase 2C as a cause of IBD-associated familial diarrhea syndrome brought a shift in the way we envision impaired sodium transport. Is this just a passive collateral effect resulting from intestinal inflammation, or is it a crucial regulator of IBD pathogenesis? This review summarizes the mutational spectrum and underlying mechanisms of monogenic IBD associated with congenital sodium diarrhea. We constructed a model proposing that impaired sodium transport is an upstream pathogenic factor in IBD. The review also synthesized emerging insights from microbiome and animal studies to suggest how sodium malabsorption can serve as a unifying mediator of downstream pathophysiology. Further investigations into the mechanisms underlying salt and water transport in the intestine will provide newer approaches for understanding

the ion-microbiome-immune cross-talk that serves as a driver of IBD. Model systems, such as patient-derived enteroids or induced pluripotent stem cell models, are warranted to unravel the role of individual genes regulating sodium transport and to develop more effective epithelial rescue and repair therapies. (*Cell Mol Gastroenterol Hepatol* 2021;12:277-292; <https://doi.org/10.1016/j.jcmgh.2021.03.005>)

Keywords: Congenital Sodium Diarrhea; Inflammatory Bowel Disease; Receptor Guanylyl Cyclase C; Sodium-Hydrogen Exchanger 3; *SPINT2*; Microbiome.

Congenital diarrhea and enteropathies are rare heterogeneous genetic disorders characterized by persistent and often severe diarrhea in the first few months of life, commonly associated with dehydration, electrolyte imbalances, feeding intolerance, and growth failure.¹ In 1985, Holmberg and Perheentupa² and Booth et al³ independently published reports of 2 patients presenting with a new subtype of congenital diarrhea and enteropathies, known as congenital sodium diarrhea (CSD), characterized by watery diarrhea and a high content of sodium in the stools. Clinically defining features of CSD, such as alkaline fecal pH and metabolic acidosis, distinguished this disease from congenital chloride diarrhea. CSD also is referred to as distal intestinal acidosis, and the primary pathology was defined to involve a defect in Na⁺/H⁺ exchange.^{2,3} Since then, approximately 50 cases have been reported worldwide.⁴

Although progress has been made in understanding immune processes that lead to tissue damage in late stages of inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), mechanisms for causal and early pathogenic factors and drivers remain elusive.⁵ Pioneering intestinal salt and water absorption studies using perfusion experiments showed that water is passively absorbed in the intestine and sodium and water absorption are linked directly.⁶ Impaired sodium absorption is one of the earliest pathologies documented in patients with IBD. As early as 1937, substantial loss of sodium and base via the intestinal tract was reported in patients with UC, indicating a defect in Na^+/H^+ exchange and a mechanistic overlap with CSD.⁷ Subsequently, several independent investigators have documented impaired sodium absorption in IBD^{8,9} and in microscopic colitis, a poorly understood type of IBD, which is almost as common as CD and UC.^{10,11} Is there a link between impaired sodium absorption in the gut and IBD? Until recently, the answer to this question could simply be that sodium malabsorption is a secondary consequence of mucosal inflammation and release of cytokines, which may mediate the intractable diarrhea seen in some patients with IBD.^{11–15} More recent studies, however, have indicated that changes in the ionic milieu in the intestine appears to be a pivotal mechanism for inducing gut inflammation and colitis. For example, high dietary salt intake can activate effector immune cells in the gut, thereby influencing the development and/or course of IBD.^{16,17} Therefore, increased salinity of the intestinal milieu and concomitant changes in the microbiome would exacerbate the pathologies caused by impaired sodium transport (Figure 1A).

In 2012, we identified a dominant mutation in *GUCY2C* (which encodes the receptor guanylyl cyclase C [GC-C], which regulates fluid and ion transport in the gut) as the cause of congenital familial diarrhea syndrome associated with CD. This finding brought a conceptual shift toward the importance of electrolyte homeostasis in IBD pathogenesis.¹⁸ Recent years have seen significant progress in our understanding of the genetic heterogeneity of CSD and monogenic IBD, with mutations identified in additional genes that encode proteins that regulate intestinal sodium absorption, including *SLC9A3*, which encodes Na^+/H^+ exchanger 3 (NHE3),¹⁹ and *SPINT2*, which encodes a Kunitz-type serine protease inhibitor.⁴ Nevertheless, for an estimated approximately 40% of all cases of CSD, the underlying genetic pathogenesis remains unknown.⁴ Given the variable penetrance of the IBD-like phenotype in CSD, there is a likely role for modifier genes and gene–environment interactions. However, the identification of the genes regulating intestinal sodium transport as being linked to IBD-like intestinal inflammation establish that impaired intestinal sodium transport may occur as a primary event in IBD, upstream of mucosal inflammation, and changes in the microbiome (Figure 1A and B). Importantly, apart from offering therapeutic options for affected patients, understanding the rare monogenic causes of CSD could provide data regarding the mechanisms underlying salt and water


transport and complement the functional understanding of IBD pathogenesis.

Gut microbiota play a crucial role in protecting against diarrheal infection and causing inflammation in chronic intestinal diseases.^{20,21} A paradigm has emerged that intestinal fluid and ion transport is a strong determinant of a stable eubiotic gut microbiome, and any disturbances therein may disrupt microbial homeostasis and foster host-damaging mucosal inflammatory responses.^{22–25} To develop new effective curative strategies for IBD, an understanding of the mechanism of how changes in electrolyte homeostasis result in the breakdown of intestinal immunotolerance is needed. We review recent insights into the genetic pathogenesis of CSD, together with their association with chronic intestinal inflammation, to establish a pathogenic model of IBD that focuses on impaired intestinal sodium transport and changes in the ionic milieu. We propose that sodium malabsorption, at least in part, acts as an upstream pathogenic factor and a mechanistic driver by which many downstream pathophysiological effects, including changes in microbiota, inflammation, tissue damage, and diarrhea, can be mediated.

The Role of Guanylyl Cyclase C and Implications for IBD

Guanylate cyclase 2C (encoded by *GUCY2C*; Online Mendelian Inheritance in Man [OMIM] 601330;DIAR6) is a transmembrane receptor expressed in intestinal epithelial cells. GC-C catalyzes the formation of guanosine 3',5'-cyclic monophosphate (cGMP) from guanosine triphosphate.²⁶ Increased mucosal cGMP is a long-recognized but underappreciated pathologic feature of IBD.²⁷ There has recently been a resurgence of interest in cGMP signaling in IBD as a result of the discovery of activating mutations in GC-C, in which patients present with susceptibility to IBD development.^{18,28} Indeed, recent studies have reported mutations in GC-C as the most common cause of monogenic epithelial IBD.²⁹ GC-C is a multidomain protein consisting of an extracellular ligand-binding domain, a transmembrane domain, a juxtamembrane domain, a kinase homology domain (KHD), a linker region, a guanylyl cyclase domain (GCD), and a C-terminal domain.²⁶ GC-C is also expressed in extraintestinal tissues, such as brain and adipose tissue, but its role in these tissues is not clearly understood.^{26,30} Ligands of GC-C

Abbreviations used in this paper: CD, Crohn's disease; CFTR, cystic fibrosis transmembrane conductance regulator; cGMP, guanosine 3',5'-cyclic monophosphate; CSD, congenital sodium diarrhea; ENaC, epithelial Na^+ channels; GC-C, guanylyl cyclase C; GCD, guanylyl cyclase domain; IBD, inflammatory bowel disease; KHD, kinase homology domain; NHE3, Na^+/H^+ exchanger 3; PKGII, guanosine 3',5'-cyclic monophosphate-dependent protein kinase II; SCS, syndromic congenital sodium diarrhea; SPINT2, serine peptidase inhibitor, Kunitz type 2; UC, ulcerative colitis.

 Most current article

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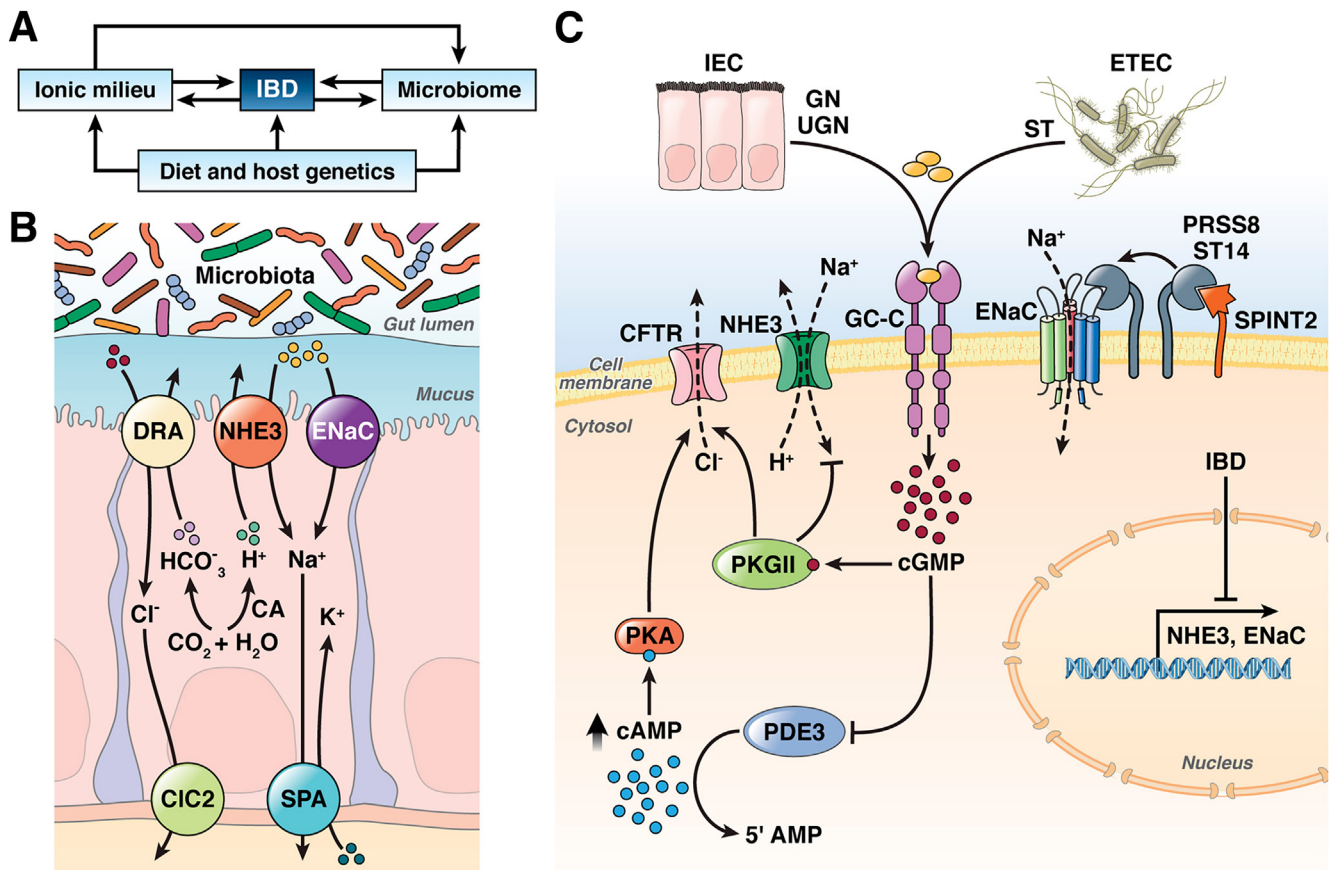


Figure 1. Defective Na^+ absorption as a unifying mechanism underpinning CSD and IBD. (A) As outlined in the flow diagram, the composition of the gut microbiome is dictated primarily by intestinal ionic milieu along with other factors including diet and host genetics. Similar to impaired intestinal sodium transport, high dietary salt intake also may increase the salinity of the intestinal ionic milieu and contribute to changes in the microbiome. The pathophysiology of IBD is thought to be related to a disruption of microbial homeostasis that fosters host-damaging mucosal inflammatory responses. In addition, electrolyte homeostasis can directly influence the activation of immune cells in the intestine and the risk of IBD. (B) Electroneutral Na^+ absorption is mediated by NHE3 and is coupled to Cl^- absorption through $\text{Cl}^-/\text{HCO}_3^-$ exchangers (eg, down-regulated in adenoma/DRA or SLC26A3). Carbonic anhydrase II (CA) plays a key role in the generation of H^+ and HCO_3^- ions from metabolic CO_2 for the regulation of NaCl absorption. Electrogenic Na^+ absorption occurs via amiloride-sensitive and aldosterone-responsive ENaC. Na^+/K^+ -adenosine triphosphatase (SPA) and ClC_2 chloride channel mediate basolateral NaCl extrusion. Impairment of both sodium absorption mechanisms and changes in microbiota have been documented in IBD patients. (C) In IBD, mucosal inflammation and released cytokines could down-regulate expression/activity of proteins involved in intestinal Na^+ absorption associated with CSD, namely NHE3 and ENaC, underpinning the mechanistic and phenotypic overlap between CSD and IBD. The activities of NHE3 and ENaC, in turn, are regulated by GC-C and SPINT2 proteins, respectively. GC-C is activated by binding of guanylin (GN), uroguanylin (UGN), or heat-stable enterotoxin (ST) produced by enterotoxigenic *Escherichia coli* (ETEC), leading to the generation of cGMP. Increase of intracellular cGMP results in the activation of cGMP-dependent PKGII and inhibition of adenosine 3',5'-cyclic monophosphate (cAMP)-specific phosphodiesterase (PDE3), which cross-activates adenosine 3',5'-cyclic monophosphate-dependent protein kinase (PKA). PKGII-mediated phosphorylation and inhibition of NHE3 results in reduced intestinal Na^+ absorption. In addition, PKGII and PKA activate the CFTR ion channel, leading to increased intestinal Cl^- secretion. Defective NHE3-mediated electroneutral Na^+ absorption underlies CSD as a result of activating mutations in GC-C and inactivating mutations in NHE3. The serine proteases matriptase (ST14, suppressor of tumorigenicity 14 protein) and prostasin (PRSS8, serine protease 8), both of which are regulated by SPINT2, are involved in the activation of ENaC. Matriptase/prostasin overactivation due to inactivating mutations in SPINT2 could adversely affect ENaC-mediated electrogenic Na^+ absorption and cause sodium diarrhea.

include endogenous hormones, guanylin and uroguanylin, and heat-stable enterotoxin produced by enterotoxigenic *Escherichia coli*. Binding of ligands to GC-C catalyzes the production of cGMP, which results in the activation of cGMP-dependent protein kinase II (PKGII) and inhibition of adenosine 3',5'-cyclic monophosphate-specific phosphodiesterase, which, in turn, cross-activates adenosine 3',5'-cyclic

monophosphate-dependent protein kinase. PKGII and adenosine 3',5'-cyclic monophosphate-dependent protein kinase phosphorylate and activate the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, leading to increased chloride and water secretion. PKGII-mediated phosphorylation and inhibition of NHE3 results in reduced intestinal sodium absorption (Figure 1C).^{26,30}

Indeed, cGMP-mediated NHE3 inhibition may underlie attenuation of NHE3 transport activity reported in some patients with UC, despite its preserved expression and cellular localization.³¹

Studies in genetically modified mouse models have shown that GC-C/cGMP signaling is essential for the mediation of heat-stable enterotoxin-induced diarrhea, as well as protection against enteric pathogens and the maintenance of microbiota homeostasis.^{32,33} As a testament to the central role at the intersection of the ion-microbiome-immune axis, mutations in *GUCY2C* have been linked to 2 extreme phenotypes within the continuum of intestinal fluid and electrolyte balance. Homozygous and compound heterozygous mutations that inactivate or truncate GC-C cause meconium ileus as a result of reduced intestinal fluid and ion secretion. In contrast, increased cGMP production as a result of activating mutations in GC-C results in congenital sodium diarrhea and IBD (Figure 2A and Table 1).^{18,28,30} The underlying mechanism in these cases may be the reduction of Na⁺ absorption resulting from GC-C/cGMP-mediated inhibition of NHE3 activity, along with an increase in chloride secretion via activation of CFTR. The majority of patients with CSD, resulting from *GUCY2C* mutations, present to the clinic with intrauterine onset of diarrhea, as indicated by maternal polyhydramnios. Massive abdominal distension resulting from fluid-distended intestinal loops and failure to pass meconium was seen at birth. Patients needed total parenteral nutrition to treat dehydration and electrolyte imbalances, and ileostomata to relieve intestinal obstruction. Massive sodium loss of up to 33 mmol/kg/d was seen in these patients. Complications included severe dehydration, electrolyte imbalance, sepsis, ileus, volvulus, severe colitis, and IBD-like pathology.²⁸

A number of mutations have been reported in *GUCY2C* that are associated with CSD and comorbid IBD (Tables 1 and 2, Figure 2A).³⁰ A summary of clinical features present in patients with IBD, and their location and properties of the mutations, are presented later.

The linker region, also known as the helical domain, is an integral structural element of nucleotidyl cyclases and has co-evolved with the type III cyclase fold.^{34,35} Remarkable conservation of the linker region was noted (Figure 2B). Combining modeling and proline-scanning mutagenesis studies, we previously defined the regulatory role of the GC-C linker region, which could serve as a signal transducer from the extracellular ligand-binding domain via the KHD to the catalytic domain, to regulate its transition from the inactive to an active conformation.³⁴ Intriguingly, one of the proline mutations created in this study was discovered years later, as naturally occurring in a male child, delivered at 34 weeks' gestation to a Dutch family with no known close consanguinity. Whole-exome sequencing showed a heterozygous de novo L775P mutation in this patient, who had CSD, severe early onset IBD, recurrent pseudo-obstruction, and chronic arthritis at 4 years of age (Figure 2A and B).²⁸

Notably, although in vitro assays showed increased basal cGMP levels in all reported CSD mutations in GC-C, the L775P and another linker region CSD-mutation R792S

showed the highest basal cGMP (up to ~100-fold) compared with wild type.²⁸ Ligand-mediated activation also was enhanced significantly in R792S mutant– but not in L775P mutant–expressing cells, wherein ligand binding did not further increase abnormally high basal activity.^{28,34} It is noteworthy that these linker region mutations were seen in severely affected patients, both of whom had premature delivery (<37 weeks of gestation) and required total parenteral nutrition.²⁸ Thus, by examining genotype-phenotype correlations, we could infer that mutation localization (linker region vs other protein domains) could be correlated with the effect on enzymatic function and the severity of disease phenotypes.

The GCD is the catalytic domain of GC-C. It consists of a conserved class III cyclase domain fold where cGMP synthesis occurs (Figure 2C).^{26,30} In 2012, we reported a novel pathologic mutation in GC-C in 32 individuals (14 females and 18 males) from a Norwegian family with relatively mild, chronic secretory diarrhea and dysmotility (familial diarrhea syndrome) (Diarrhea 6; OMIM 614616). Linkage analysis and sequencing of *GUCY2C* detected a heterozygous, dominantly inherited, c.2519G→T (p.S840I) mutation in all the affected members, more than 25% of whom were diagnosed with Crohn's disease (Figure 2A and C).¹⁸ Notably, in vitro assays showed increased ligand-stimulated cGMP production in the S840I mutant when compared with the wild-type receptor, albeit to a lesser extent than the N850D mutation that was associated with severe CSD and septic complications. In addition, among these 2 GCD mutants, only the S850I mutant showed no increase in basal activity of the receptor that may be correlated with relatively milder diarrheal phenotype and delayed onset of IBD.^{18,28}

The KHD or the pseudo-kinase domain of GC-C plays an important role in ligand-mediated receptor activation and cGMP production.^{26,30,36} We previously showed that KHD in GC-C has a functional adenosine triphosphate-binding site and adenosine triphosphate binding triggers a conformational change, resulting in allosteric modulation of the receptor activity.³⁷ Two mutations in KHD associated with IBD have been reported in the literature.²⁹ An F525L mutation was detected in a Caucasian girl with bloody loose stools, chronic pancolitis, and focal duodenitis requiring colectomy and ileoanal anastomosis (Figure 2A).²⁹ A CD-associated mutation (G549S) was detected in a Caucasian boy with a positive family history of IBD who presented with abdominal pain and chronic stricturing ileocolitis that required surgical resection (Figure 2A).²⁹ Based on the autosomal-dominant mode of inheritance of the F525L and G549S mutations,²⁹ similar to the CSD-mutation K507E in the KHD,²⁸ these substitutions are likely to result in a gain-of-function phenotype. As discussed earlier, early onset of IBD is a well-documented comorbidity associated with CSD with GC-C mutations.²⁸ Thus, although not explicitly documented, it is likely that CSD and chronic inflammation also may underlie the pediatric onset of IBD phenotype seen in patients with these mutations.

In summary, CSD mediated by mutations in *GUCY2C* has advanced our understanding of the complex

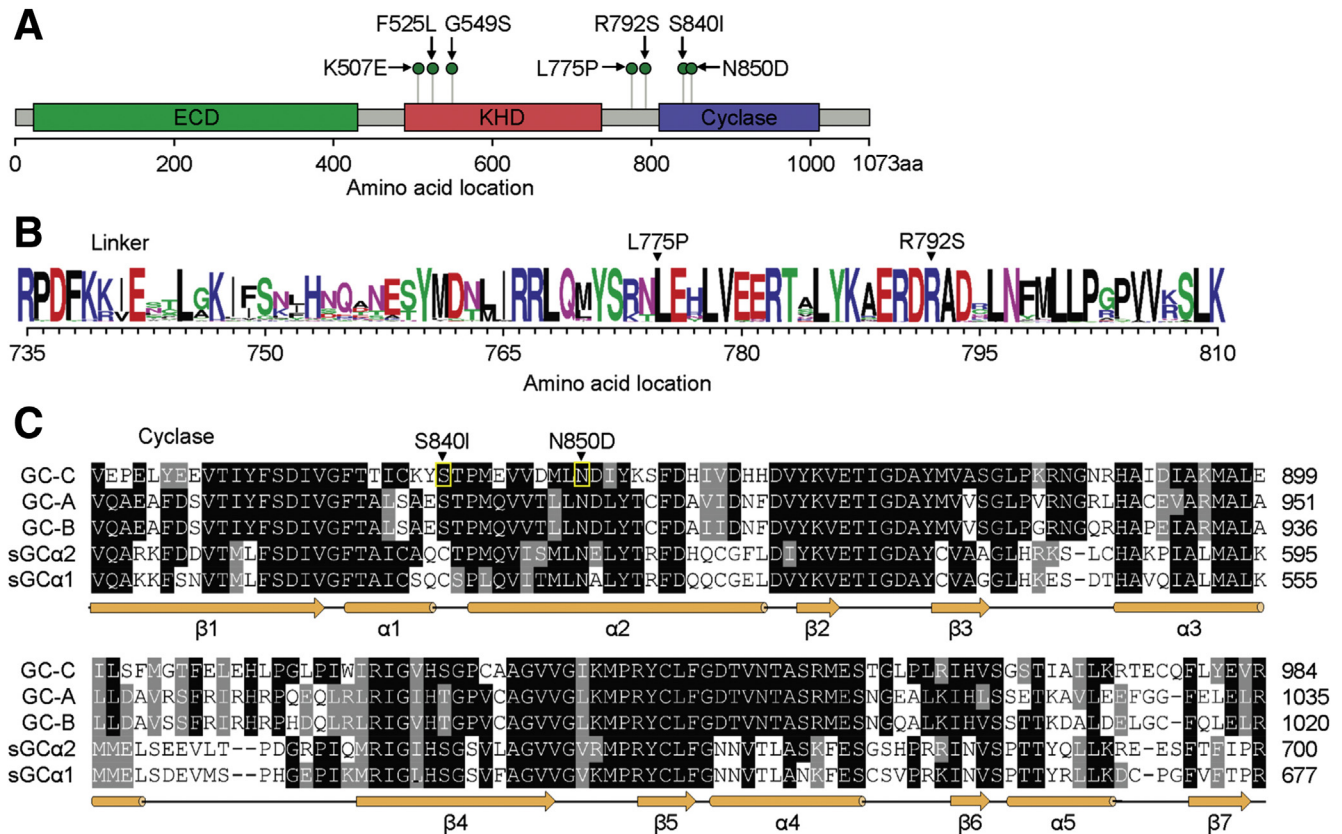


Figure 2. Mutational spectrum of GC-C in CSD. (A) Lollipop representation of CSD-associated mutations in GC-C as listed in Table 1. The x-axis indicates amino acid locations of GC-C. (B) Sequence logo showing high conservation of the linker region, including that of 2 CSD-associated mutations. (C) Alignment of the guanylyl cyclase core domain of human GC-C, GC-A, GC-B, sGCα1, and sGCα2 represented in Boxshade. The positions of activating mutations in GC-C are boxed. Secondary structural elements are represented as follows: cylinders, α -helices; arrows, β -sheets; lines, loops. ECD, extracellular ligand-binding domain.

structure–function relationship of GC-C and its physiological role in the management of salt and water homeostasis. A mutation in *GUCY2C* is a distinct clinical entity that needs to be distinguished from other causes of congenital diarrhea. It should be considered in infants with unknown diarrhea and severe electrolyte disturbances, especially when a dominant mechanism is suspected, and may result in potentially serious complications such as intestinal obstruction, severe dehydration, and sepsis. Importantly, enhanced GC-C signaling, as in the case of CSD-associated mutations, appears to have a proinflammatory effect that is associated with increased susceptibility to IBD. This could either be mediated by changes in electrolyte homeostasis or additional effects of increased cellular cGMP, such as cellular senescence or inflammasome activation.^{23,38}

Contrary to expectations, reduced expression of GC-C and its ligands has been reported in IBD patients and a model of experimental colitis.³⁹ Thus, we speculate that GC-C may also play a role in an adaptive mechanism of epithelial preservation under inflammatory conditions. For example, epithelial tight junctions are compromised in gastrointestinal disease, and this barrier loss is associated with a reduction in occludin levels present at tight junctions. However, occludin knockout mice are normal,

and interestingly show reduced severity to DSS-induced colitis owing to blockage of apoptotic pathways in knockout epithelia.⁴⁰ Similarly, the reduction in expression of occludin in biopsy specimens from CD and UC patients was correlated with a reduction in caspase-3 expression, thus limiting epithelial damage in the context of IBD.⁴⁰ GC-C knockout mice also are resistant to DSS-induced colitis,⁴¹ pointing to a distinct role for GC-C in repair of the intestinal epithelia under conditions of stress.

Mutations in NHE3 and Links With Inflammatory Diarrhea

NHE3 (OMIM 182307; DIAR8), encoded by the *SLC9A3* gene, is the primary intestinal brush-border Na^+/H^+ exchanger involved in the regulation of electroneutral Na^+ absorption, intracellular pH, cell volume, and intracellular signaling networks (Figure 1B and C).^{42–45} Na^+/H^+ exchangers have extraordinarily high transport rates of approximately 1500 ions/s, implying that even small changes in expression and/or activity may lead to significant changes in ionic and acid–base homeostasis.⁴⁶ NHE3 is organized into a membrane-embedded transport domain

Table 1. Survey of Studies Documenting Genetic Variants in GUCY2C, NHE3, and SPINT2 in Congenital Sodium Diarrhea

Gene	Nucleotide change	Protein change	Location	CCS	dbSNP ID	ClinVar ID	Functional analysis	Phenotypes	References	
<i>GUCY2C</i>										
Missense	c.1519A>G	K507E	KHD	5			Gain	CSD	28	
	c.1575C>A	F525L	KHD	6	rs774522580			IBD	29	
	c.1645G>A	G549S	KHD	9	rs367698688			IBD	29	
	c.2324T>C	L775P	Linker	9			Gain	CSD, IBD	28	
	c.2376G>C	R792S	Linker	9			Gain	CSD	28	
	c.2519G>T	S840I	GCD	9	rs587776871	30176	Gain	FDS, IBD	18	
	c.2548A>G	N850D	GCD	9			Gain	CSD	28	
<i>SLC9A3</i>										
Missense	c.379G>A	A127T	TM2	8	rs1047334552		*	CSD	50	
	c.805G>A	A269T	TM6	9	rs869312807	224599	Loss	CSD, HA	50	
	c.932C>T	A311V	TM8	8	rs869312806	224595	Loss	CSD	50	
	c.1039G>A	E347K	TM9	9	rs766583286			CSD	92	
	c.1145G>A	R382Q	TM10	9	rs766076524	224597	Loss	CSD, GR	50	
	c.1153G>A	G385S	TM10	9	rs985101377			CSD, HA, IBD	50	
	c.1214A>G	D405G	TM11	8	rs1447447724			CSD	93	
	c.1814G>A	R605Q	CTD	6	rs146547322	547876		CSD	94	
	Splicing	c.1446+1G>A		Intron 8 ^a					CSD, IBD	50
	Small deletions	c.350_352delTCT	ΔF117	TM2	5	rs776026092	224596		CSD	50
c.963_964delGT		Y322Cfs*83	TM8/TM9	9				CSD	50	
	c.1745delC	S582Lfs*6	CTD	3	rs869320692	224598		CSD, GR	50	
Small insertion	c.782dupG	T262Hfs*144	TM6	5	rs869320759	224600		CSD	50	
Gross deletion	1.383Mb including entire gene							CSD, GR	50	
<i>SPINT2</i>										
Missense	c.442C>T	R148C	KD2	8	rs1279737892			SCSD, TE	61	
	c.443G>A	R148H	KD2	8	rs1353175955			SCSD	95	
	c.481T>G	F161V	KD2	9			Loss	SCSD	60	
	c.488A>G	Y163C	KD2	8	rs121908403	5205	Loss	SCSD, TE, 58, 60–66		
		c.502G>A	G168S	KD2	9	rs606231284	157607	Loss	SCSD, TE, 60, 61	
Start loss	c.1A>T	M1?	SP	7	rs121908404	5208	Loss	SCSD	58	
	c.2T>C	M1?	SP	7	rs1224770824			SCSD, TE	61	
Nonsense	c.247G>T	E83X	KD1	4	rs1191184688			SCSD, TE	61	
	c.447G>A	W149X	KD2	7	rs780880496	635284		SCSD, TE	60	
Splicing	c.337+2T>C		Intron 3 ^a		rs606231155	5206		SCSD	58	
	c.553+2T>A		Intron 5 ^a		rs112576957	5207		SCSD	58	
	c.593-1G>A		Intron 6 ^b		rs606231154	5204	Loss	SCSD	58	
Small insertion	c.166_167dupTA	N57Tfs*24	KD1	9				SCSD, ONC	63	
	c.172dupG	V58Gfs*3	KD1	5				SCSD, TE	61	

NOTE. The single letter codes denote amino acids in a protein sequence. Evolutionary conservation (ConSurf) scores for the mutated residues were calculated using a scale ranging from 1 (highly variable) to 9 (invariant). The A127T variant in NHE3 did not show functional defects in NHE-deficient fibroblasts and could well be a benign polymorphism. However, we cannot exclude cell-type-specific or subtle regulatory function (asterisk).

CCS, ConSurf conservation score; ClinVar, public archive of relationships among sequence variation and human phenotype; CTD, C-terminal domain; dbSNP, single nucleotide polymorphism database; del, deletion; dup, duplication; FDS, familial diarrhea syndrome; fs, frameshift; GR, growth retardation; HA, hyperaldosteronism; KD1, Kunitz-type domain 1; KD2, Kunitz-type domain 2; ONC, optic nerve coloboma; SP, signal peptide; TE, tufting enteropathy; TM, transmembrane segment; X, stop codon.

^aInvariant GT donor splice site.

^bInvariant AG acceptor splice site.

that performs the Na⁺/H⁺ exchange and a long cytoplasmic C-terminal domain that is involved in the regulation of transport kinetics and trafficking (Figure 3A).⁴³ Several lines of evidence suggest that NHE3 is the primary regulator of intestinal Na⁺ absorption and therefore is essential for

the determination of extracellular volume and acid-base homeostasis (Figure 1B and C).^{43–45}

Dysregulation of NHE3 expression and/or activity is associated with the pathogenesis of many gastrointestinal disorders.^{43–45} Increased NHE3 activity has been shown to

Table 2. Clinicopathologic Features of Patients With IBD Associated With Mutations in GC-C and NHE3

Gene	Variant	Ethnicity	Sex	Age at onset, y	Presenting feature	IBD type	Pertinent features on pathology	Treatment	Reference
GYCY2C	c.2324T>C (L775P)	Dutch	M	4	Abdominal distension	UC	Colitis, gastroduodenitis, arthritis	Partial small-bowel resection	28
	c.1575C>A (F525L)	Caucasian	F	15.1	Bloody loose stool	UC	Pancolitis, focal duodenitis	Colectomy and ileoanal anastomosis	29
	c.1645G>A (G549S)	Caucasian	M	12.3	Abdominal pain	CD	Ileocolitis, strictures	Surgical resection	29
	c.2519G>T (S840I)	Norwegian	M and F	22–65	Chronic diarrhea	CD	Ileal inflammation, colitis, and esophagitis in some cases	Resection of terminal ileum and cecum in some cases	18
SLC9A3	c.1446+1G>A	Turkish Kurd Caucasian	M	4	Abdominal distension	NS	Ileal ulcerations, inflammatory infiltration, villous atrophy	Ileocecal resection	50
	c.1153G>A (G385S)	Serbian Caucasian	M	16	Bloody loose stool	NS	Ileal granulomas, nodular lymphoid hyperplasia, ulceration in the rectum, sigmoid, and colon	Medical management	50

F, female; M, male; NS, not specified

increase sodium absorption in the intestine, as seen in the case of spontaneously hypertensive rats.⁴⁷ On the other hand, inhibition of NHE3, as seen in the case of heat-stable enterotoxin-mediated activation of GC-C, has been shown to reduce intestinal sodium absorption and cause diarrhea.²⁶ Consistent with these observations, the genetic disruption of NHE3 expression in mice resulted in modest diarrhea, alkaline pH of intestinal contents, mild metabolic acidosis, relatively low blood pressure, and significantly increased mortality in low-Na⁺ diets.^{48,49}

As summarized in Table 1, a spectrum of mutations that inactivate or truncate NHE3 is causally linked to CSD (Diarrhea 8; OMIM 618868) (Figure 3A and B, Table 1). In almost all reports, patients had a typical CSD presentation, including maternal polyhydramnios and prominent abdominal distension after birth owing to dilated intestinal fluid-filled loops. A subset of patients developed growth retardation, pointing to the critical role of fluid and ion homeostasis in normal development.⁵⁰ Three CSD-associated NHE3 variants tested, namely A269T, A311V, and R382Q, were scored as loss-of-function mutations in NHE-deficient PS120 fibroblasts and, therefore, could be causal to disease phenotype.⁵⁰ Inactivating mutations in NHE3 appear to phenocopy activating mutations in GC-C, or after stimulation of the receptor by the heat-stable enterotoxin (Figure 1C).

Reduced NHE3 expression/activity and attenuated sodium absorption have been recognized in patients with IBD; however, it has not been clear until recently whether the down-regulation of NHE3 plays a causal role or is merely a reflection of the disease.^{12,31} A significant shift in understanding was provided by the study of CSD by Janecke et al⁵⁰ that identified NHE3 mutations in 9 patients from 8 different families (Tables 1 and 2). One of the patients identified to harbor NHE3 mutations was a Turkish boy with a history of parental consanguinity. The individual developed IBD at 4 years of age and required ileocecal resection and a temporary ileostomy to relieve recurrent small-bowel obstruction. A homozygous splicing mutation (c.1446+1G>A) affecting the invariant guanine-thymine (GT) donor splice site of intron 8 was detected in this patient.⁵⁰ Of the remaining CSD patients in this cohort, a Serbian boy with no history of consanguinity and harboring a heterozygous G385S mutation in NHE3, developed IBD at 16 years of age.⁵⁰ Although functional characterization of this mutation remains to be performed, mutagenesis studies have shown that the equivalent Gly³⁰³ residue in the *E coli* Na⁺/H⁺ antiporter, NhaA, (Figure 3B) plays a critical role in mediating ion transport.⁵¹

The IBD phenotype associated with NHE3 mutations presents more definitive answers to questions of causality and provides a justification for additional studies to gain new insights into NHE3 function and its role in sodium and fluid homeostasis, mucosal epithelium integrity, and safeguarding against diverse colitogenic stimuli. To date, there are no therapeutic agents available in IBD therapy to specifically activate NHE3 and mitigate tissue-damaging processes associated with the disease. Importantly, data from several studies make a compelling case that NHE3^{-/-}

mice develop spontaneous, bacterial-mediated colitis, and IBD-like pathologies and show increased vulnerability to DSS-induced mucosal injury.^{52–55}

Electrogenic Sodium Absorption and IBD: Lessons Learned From Serine Peptidase Inhibitor, Kunitz Type 2 Mutations

Serine peptidase inhibitor, Kunitz type 2 (SPINT2) (OMIM 605124; DIAR3) is a transmembrane Kunitz-type serine protease inhibitor that is widely expressed in epithelial compartments.⁵⁶ SPINT2 is known to be involved in the regulation of the activities of several serine proteases, including matriptase (suppressor of tumorigenicity 14 protein [ST14]) and prostaticin (serine protease 8 [PRSS8]). These enzymes are important in cell surface proteolytic pathways that are required for epithelial function and protection against chronic inflammation that would predispose to IBD.^{56,57} Mutations in the *SPINT2* gene cause a syndromic form of congenital sodium diarrhea (SCSD) (Diarrhea 3; OMIM 270420), a rare autosomal-recessive disorder that occurs during early life, associated with intractable watery diarrhea, dehydration, multiple anatomic anomalies, failure to thrive, and dependence on parenteral nutrition. In a subset of patients, tufting enteropathy is seen in enteric biopsy specimens, characterized by epithelial dysplasia, villous atrophy, a compromised epithelial barrier, and severe intestinal insufficiency.^{4,58} Histopathologically, the telltale sign of tufting enteropathy is the presence of focal crowding of enterocytes at the tips of the villus, resembling tufts.^{4,56}

The mechanism underlying sodium diarrhea in SPINT2 mutations still is unclear. Serine proteases, matriptase and prostaticin, both of which are regulated by SPINT2, are known to be involved in the activation of epithelial Na⁺ channels (ENaC).⁵⁹ One potential mechanism would be that limited proteolysis under physiological conditions could activate ENaC. In contrast, uninhibited proteolysis resulting from matriptase/prostaticin overactivation as a result of mutations in SPINT2 could affect ENaC activity adversely, causing sodium diarrhea (Figure 1B and C).

Multiple genetic studies have uncovered several clinically relevant patient mutations in *SPINT2*. These span the gamut of missense and nonsense mutations, start loss mutations, small insertions, and splice site mutations (Figure 3C and D, Table 1). By far the most frequent and best-studied mutation in SCSD is the Y163C missense mutation in SPINT2. A total of 24 cases of 21 families with this mutation have been reported in the literature to date.^{58,60–65} Previous studies have shown chronic intestinal inflammation and increased levels of fecal calprotectin in SCSD patients with Y163C mutations.⁶⁰ Furthermore, the IBD Exomes Portal (Cambridge, MA) showed an association between Y163C, which accounts for approximately 70% of all SCSD cases, and UC in the non-Finnish European population with an odds ratio of 11.3 ($P < .05$). This would be in line with the findings of marked impairment of electrogenic Na⁺

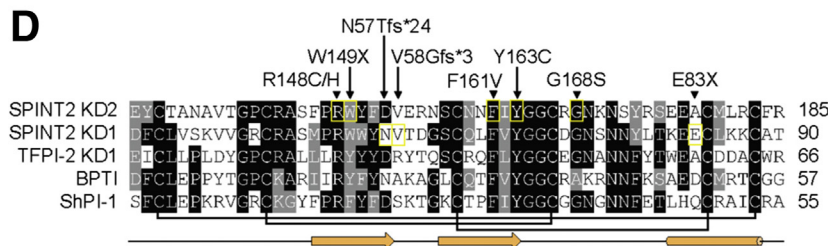
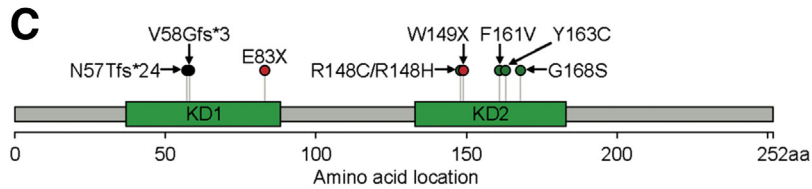
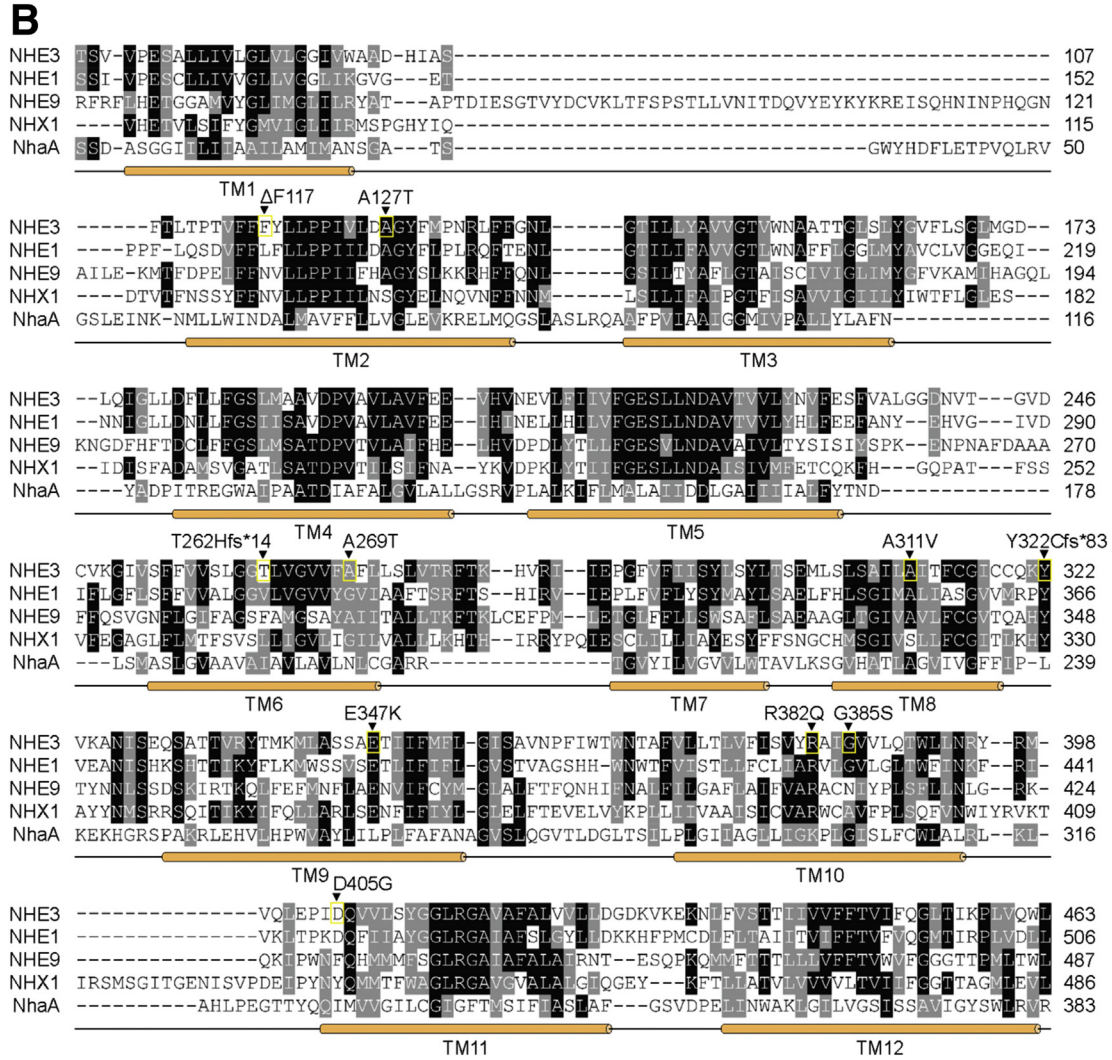
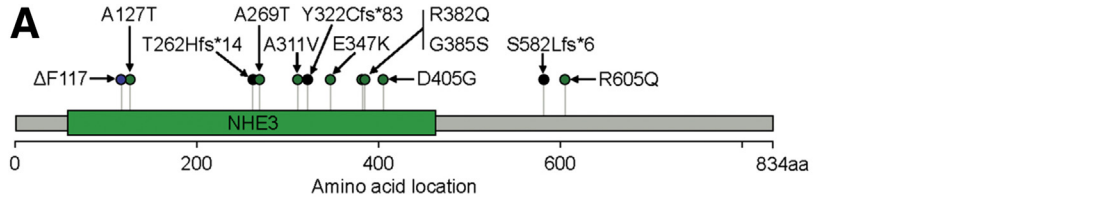
absorption in IBD.^{13,66,67} Three independent studies have functionally characterized the Y163C mutation, which results in a loss of function, and therefore could be causal to the disease phenotype.^{58,60,65} Experimental evidence, including crystallization studies, has established the critical role of the equivalent Tyr³⁵ residue (Figure 3D) in maintaining the functional conformation of the bovine pancreatic trypsin inhibitor, one of the well-characterized Kunitz-type inhibitors.⁶⁸

Changes in electrolyte homeostasis in SCSD patients with *SPINT2* mutations may lead to subsequent initiation of an inappropriate microbiome immune response. Indeed, microbial overgrowth is a documented complication associated with SCSD.⁶⁰ However, a variety of factors complicate genotype–phenotype correlations. These include the relatively small number of mutations identified, high phenotypic heterogeneity associated with mutations, and the fact that patients with SCSD may develop disease-related complications such as dehydration and sepsis, which may require long periods of hospitalization, parental nutrition, and surgical interventions.

The Ion–Microbiome–Immune Axis and IBD Pathology

As discussed earlier, the impairment of both electro-neutral and electrogenic Na⁺ absorption and decreased expression/activity of NHE3 and ENaC has been reported in IBD patients (Figure 1B).^{12,13,31,67,69} Thus, evidence points to intestinal Na⁺ absorption defects as a unifying mechanism underpinning IBD. Given the growing consensus that IBD represents a system-level disruption of the mucosal immune system, and therefore the gut microbiota ecosystem, it is crucial to determine how dysregulation of Na⁺ absorption causes dysbiosis to shape the microbiome–immune interface.

Converging lines of evidence indicate that ion transport across intestinal epithelial cells is an emerging regulator for the establishment and maintenance of intestinal microbiota.^{22–25} The host exercises inside-out control over the microbiota by controlling the intestinal ion milieu. The microbiota, in turn, exerts outside-in influence on the host by shaping the immunity and modulating the microbiome–immune cross-talk.^{20–25} Indeed, studies in mouse models and human beings have shown that high salinity of the intestinal milieu depletes *Lactobacillus* species and induces proinflammatory T helper 17 (T_H17) polarization, known to play a role in IBD.^{16,70} Several studies have shown reduced NHE3 expression/activity owing to proinflammatory cytokines and *Clostridium difficile* toxin B production.^{14,71} The deletion of NHE3 exacerbated experimental colitis in mice and developed pathologies that resembled IBD.^{52–55} Consistent with these findings, mice with intestinal epithelial-specific NHE3 deletion showed impaired barrier function and intestinal epithelial cell apoptosis that are characteristic of IBD.¹⁹ Notably, the intestinal microbiome in NHE3^{-/-} mice had lower microbial diversity and a change in the *Firmicutes:Bacteroidetes* ratio in favor of inflammation-



associated *Bacteroidetes*, accompanied by a decrease in butyrate-producing bacteria.^{52,54,72} Interestingly, similar shifts in the microbiota and NHE3 deficiency also have been observed in human beings with IBD.^{12,73} Notably, a prominent overgrowth of a known colitogenic pathobiont of *Bacteroidetes* phylum and the main microbiota constituent, *Bacteroides thetaiotaomicron*, has been noted in the ileum of NHE3-deletion mice.^{72,74} Independent in vitro studies have confirmed the ability of high sodium levels and high pH correlating with the intestine of CSD patients to promote the growth of *B thetaiotaomicron*.^{72,75}

Evidence for the role of the ion-microbiome-immune axis in CSD and IBD was presented in a study of patients with familial diarrhea syndrome resulting from an activating mutation in GC-C.^{18,76} Analysis of intestinal microbiota in this cohort showed a substantial outgrowth of invasive *Enterobacteriaceae* and a pronounced loss of *Faecalibacterium prausnitzii* and *Bifidobacterium*.⁷⁶ The blooming of *Enterobacteriaceae* is a result of increased oxygenation of the intestinal lumen resulting from frequent bouts of inflammation.⁷⁷ *F prausnitzii* is a major butyrate producer and its loss often is seen as a marker of poor intestinal health.⁷³ Importantly, because *Bifidobacterium* species are known to metabolize tryptophan to indole metabolites, we speculate that GC-C activating mutations may influence the abundance of fecal tryptophan metabolites that act as aryl hydrocarbon receptor ligands and promote mucosal barrier function and reduce inflammatory responses.⁷⁸ Targeted alteration of the intestinal microbiota with *Bifidobacterium* in a probiotic formula or use of aryl hydrocarbon receptor agonists could be novel adjunct therapy approaches for IBD-like pathologies in patients with GC-C-activating mutations.

Recent studies have shown that high gut salinity is associated with enrichment of halophiles that have probesity and cancer-promoting properties.^{79,80} Future studies that aim to find specific halophilic bacteria in the intestinal microbiota that are under Darwinian selection in the increased fecal salinity environment seen in patients with CSD may yield novel pathobionts that may be important for mediating IBD pathology and could provide actionable therapeutic approaches.

Perspectives

Carrier State in CSD and the Risk of IBD

Heterozygous NHE3 mutations were found in 2 of 9 CSD patients documented by Janecke et al,⁵⁰ with 1 of them developing IBD in adolescence. However, no apparent differences in clinical presentation were seen when compared with patients with homozygous or compound heterozygous

NHE3 mutations.⁵⁰ Therefore, heterozygous NHE3 mutations could be etiologically important and plausibly have biological implications, as seen in heterozygous mutations in the related NHE9 (susceptibility to autism-16/AUTS16; OMIM 613410).⁸¹ It also is conceivable that the heterozygous frameshift mutation (Y322Cfs*83) reported in 1 patient may act in a dominant-negative manner that contributes to the development of CSD.⁵⁰ Importantly, a meta-analysis of 3 population cohorts from the IBD Exomes Portal (Cambridge, MA) showed significant associations of 4 missense variants in NHE3, namely P287T, G288S, A581S, and E684D, with IBD (odds ratio, >2), suggesting that the more prevalent heterozygous mutation status could be clinically meaningful.⁶⁶

A heterozygous SCSO carrier state was documented in a male patient with anal atresia who did not have diarrhea.⁸² A heterozygous splice site mutation c.593-1G>A in *SPINT2* was seen, which was reported previously as homozygous in 5 Austrian SCSO patients. Functional analysis of this mutation in CV-1 in Origin Simian-7 (COS-7) cells showed no detectable *SPINT2* protein.⁵⁸ As a result, homozygotes with this mutation would not have *SPINT2* activity, whereas heterozygotes are likely to show intermediate activity. Currently, because mutations in NHE3 and *SPINT2* are rare, the pathogenicity of heterozygous mutations and the risk of developing IBD is still debatable.

Potential Therapies in CSD and IBD

Congenital sodium diarrhea should be treated with a lifetime replacement of Na⁺ and other electrolyte fecal losses, and correction of fluid and acid-base imbalances. Although diarrheal disease resulting from activating mutations in GC-C is classified as CSD,^{1,28} an overlooked feature is CFTR-mediated substantial fecal loss of Cl⁻, which may even satisfy the criteria for a diagnosis of congenital chloride diarrhea (fecal Cl⁻, >90 mmol/L). However, metabolic acidosis is observed in these patients and stool pH is alkaline, in contrast to that found in classic congenital chloride diarrhea.^{28,83} The potential use of specific inhibitors of CFTR, such as CFTR(inh)-172, a thiazolidinedione derivative,⁸⁴ could be considered in these patients to minimize losses of Na⁺ and Cl⁻. Specific CFTR inhibitors also may help reduce severe recurrent diarrhea in IBD patients, which is mediated, at least in part, by cGMP-dependent CFTR activation under inflammatory conditions.^{18,85}

Therapeutic enhancement of electrogenic Na⁺ absorption via ENaC may help to compensate for NHE3 loss of function. Consistent with observations in NHE3^{-/-} mice, 2 of the 9 patients with CSD resulting from NHE3 mutations had

Figure 3. (See previous page). Mutational spectrum of NHE3 and SPINT2 in CSD. (A) Lollipop representation of CSD-associated mutations in NHE3 as listed in Table 1. The x-axis indicates amino acid locations of NHE3. (B) Alignment of the sequences of human NHE3, human NHE1, human NHE9, *Saccharomyces cerevisiae* Nhx1, and *E coli* NhaA represented in *boxshade*. Transmembrane segments based on NhaA are shown as *cylinders* and numbered.⁸¹ The positions of NHE3 variants are *boxed*. (C) Lollipop representation of syndromic CSD-associated mutations in SPINT2 as listed in Table 1. The x-axis indicates amino acid locations of SPINT2. (D) Alignment of the sequences of human SPINT2, human TFPI-2, bovine pancreatic trypsin inhibitor (BPTI), and ShPI-1 represented in *boxshade*. The positions of SPINT2 variants are *boxed*. Secondary structural elements are represented as follows: *cylinders*, α -helices; *arrows*, β -sheets; *lines*, loops. The 3 disulfide bonds stabilizing the KD structure are shown as *black lines* below the alignment.

hyperaldosteronism (Table 1), and we noted that both patients had the lowest levels of fecal Na^+ concentration in the cohort, and 1 of these patients had normal fecal Na^+ levels.^{48,50} Translating these observations into potential therapeutic options, perhaps involving the use of oral fludrocortisone, a synthetic mineralocorticoid, may be beneficial in CSD patients to minimize salt wasting. Another possible strategy is to target NHE3 because membrane transporter proteins are generally eminently druggable targets. Finally, nonabsorbable small molecules designed to target the active site and selectively inhibit SPINT2-targeted proteases, such as prostasin, can provide a new class of drugs for the treatment of SCSD-associated gastrointestinal pathologies, with limited systemic effects. In this context, it is essential to note that camostat mesylate, a synthetic serine protease inhibitor, was found to have a beneficial effect in UC patients.⁸⁶ Similar to CSD, approaches that aimed to improve Na^+ absorption discussed here may represent potential means of IBD therapy. In fact, glucocorticoids, when used to treat IBD, in addition to reducing inflammation, are thought to exert a therapeutic effect by enhancing Na^+ absorption by a mineralocorticoid-like effect.⁸⁷

Translational Insights With Learning Opportunities

A gut-centric approach to Na^+ management has recently gained prominence with efforts directed toward developing drugs to enhance excretion of Na^+ in feces.⁸⁸ Prominent Na^+ loss in stool in patients with CSD might lead to new therapies for managing sodium intake in many disease states, including hypertension, chronic kidney disease, and heart failure. Recently, selective pharmacologic inhibition of intestinal sodium absorption has been suggested as an effective method to manage systemic hypertension and lower the risk of cardiovascular mortality.⁸⁸ The inhibition of NHE3 with tenapanor decreased urinary excretion of Na^+ by 20 to 50 mmol/d, and resulted in a similar increase in fecal Na^+ loss.⁸⁸ Moreover, inhibition of NHE3 by targeting GC-C (Figure 1C) currently is possible with agents such as linaclotide and plecanatide, used in clinical practice for the treatment of constipation associated with irritable bowel syndrome.³⁰ Thus, the repositioning of Food and Drug Administration–approved GC-C agonists for potential clinical use to inhibit NHE3 and reduce intestinal Na^+ absorption could be an alternative strategy to prevent cardiorenal damage in patients with hypertension and chronic kidney disease. However, given the evidence presented earlier linking NHE3 deficiency with IBD in human beings and mouse models, it is crucial to determine how long-term NHE3 inhibition modulates the gastrointestinal intraluminal milieu and alters the microbiome.

Changes in expression of ligands also may lead to dysregulated GC-C activity, a phenomenon best studied in relation to colorectal cancer, in which expression of guanylin and uroguanylin frequently is silenced early in carcinogenesis. These observations have accounted for efforts to develop GC-C ligands for chemoprevention in colorectal

cancer.⁸⁹ There are early indications of possible efficacy with good tolerability of GC-C ligands that requires assessment in formal trials. The reproducible association of GC-C activating mutations with IBD strongly suggest that chronic mucosal inflammation is a pathology associated with stimulation of the GC-C/cGMP pathway. Therefore, chronic activation of the GC-C pathway by ligand treatment might activate cellular senescence and trigger IBD-like immunopathology.^{23,38} Thus, in-depth studies and understanding mechanism-based toxicities associated with GC-C agonism will be necessary for guiding future therapies for sodium management and colorectal cancer chemoprevention.

The development of gut-derived organoid culture systems has provided researchers with unparalleled opportunities to study functional aspects of biology of genes regulating sodium transport and disease pathophysiology. For example, the role of NHE3 in regulating the water/electrolyte homeostasis of the gut epithelium has been shown in mouse and human organoids.^{90,91} Patient-derived organoids and induced pluripotent stem cell models are resources for mechanistic studies and personalized medicine. Finally, whether driven by genes or by diet, high salinity of the intestinal milieu can lead to mucosal inflammation and release cytokines that could, in turn, down-regulate proteins involved in intestinal Na^+ absorption. These could include NHE3 and ENaC,^{13,14} thus creating a feedback loop between sodium transport and inflammation, which could be a critical component of the disease.

Conclusions

We believe that the genetics of IBD associated with CSD firmly establishes the importance of assigning impaired sodium transport as a central pathophysiological event, rather than a collateral effect, in a unified pathogenic model of IBD. Although there are still outstanding questions, the pathogenic model proposed here provides a rational basis for interventions that are directly designed to limit the luminal sodium levels in the intestine as a potential therapeutic approach to IBD. High dietary salt content has been shown to minimize the intestinal survival of *Lactobacillus* species, along with an increase in systemic $\text{T}_\text{H}17$ cells in human beings.¹⁶ It therefore is attractive to speculate that dietary sodium restriction might be beneficial in IBD patients, but clear evidence for this currently is lacking.

Comprehensive testing using whole-exome and whole-genome strategies may help to identify novel genes and pathways that are within the realm of CSD and pediatric IBD. Once the use of multigene panels becomes commonplace for clinical genetic testing, it is expected to change both the prevalence and treatment strategies for these diseases. We propose that the ion–microbiome–immune axis provides a protective mucosal firewall that reduces the susceptibility to unwanted inflammatory responses. The key challenge ahead will be to dissect epithelial-intrinsic functional pathways that control the complex cross-talk between intestinal salt and water transport, inflammation, and the

microbiome. Studies involving patient-derived tissues or enteroids and transgenic animals harboring disease variants will allow us to make substantial progress in understanding the underlying pathogenic mechanisms of CSD and IBD. This eventually may help to develop effective therapies and implement precision medicine.

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