ORIGINAL ARTICLE

Familial Diarrhea Syndrome Caused by an Activating *GUCY2C* Mutation

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ABSTRACT

BACKGROUND

Familial diarrhea disorders are, in most cases, severe and caused by recessive mutations. We describe the cause of a novel dominant disease in 32 members of a Norwegian family. The affected members have chronic diarrhea that is of early onset, is relatively mild, and is associated with increased susceptibility to inflammatory bowel disease, small-bowel obstruction, and esophagitis.

METHODS

We used linkage analysis, based on arrays with single-nucleotide polymorphisms, to identify a candidate region on chromosome 12 and then sequenced *GUCY2C*, encoding guanylate cyclase C (GC-C), an intestinal receptor for bacterial heat-stable enterotoxins. We performed exome sequencing of the entire candidate region from three affected family members, to exclude the possibility that mutations in genes other than *GUCY2C* could cause or contribute to susceptibility to the disease. We carried out functional studies of mutant GC-C using HEK293T cells.

RESULTS

We identified a heterozygous missense mutation (c.2519G→T) in *GUCY2C* in all affected family members and observed no other rare variants in the exons of genes in the candidate region. Exposure of the mutant receptor to its ligands resulted in markedly increased production of cyclic guanosine monophosphate (cGMP). This may cause hyperactivation of the cystic fibrosis transmembrane regulator (CFTR), leading to increased chloride and water secretion from the enterocytes, and may thus explain the chronic diarrhea in the affected family members.

CONCLUSIONS

Increased GC-C signaling disturbs normal bowel function and appears to have a proinflammatory effect, either through increased chloride secretion or additional effects of elevated cellular cGMP. Further investigation of the relevance of genetic variants affecting the GC-C–CFTR pathway to conditions such as Crohn's disease is warranted. (Funded by Helse Vest [Western Norway Regional Health Authority] and the Department of Science and Technology, Government of India.)

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Chronic diarrhea is a health problem that poses challenges with respect to both diagnosis and treatment. The irritable bowel syndrome affects 15 to 20% of adults and is a common cause of diarrhea.[1] Other causes include inflammatory bowel disease, infections, paraneoplastic hormones, celiac disease, malabsorption syndromes, and bacterial overgrowth in the small intestine.[2] In addition to organic causes, psychological factors have an important effect on bowel function.[1]

Recent studies have focused on the importance of genetic factors in the development of chronic diarrhea[3-5] because genetic factors can provide insight into the pathophysiology of intestinal diseases and point to new treatments. The irritable bowel syndrome aggregates strongly in families,[6] and the genetic predisposition to Crohn's disease, particularly ileitis,[7] is well documented,[3] but no major genes causing these disorders have been found. Both diseases are considered to be multifactorial, and the causes include genetic susceptibility variants and environmental factors. Rare inherited forms of chronic diarrhea have been reported[4]; nearly all of these are severe, autosomal recessive, single-gene diseases that are manifested in the newborn period.

There is a fine balance between intestinal absorption and secretion of water and electrolytes.[2,8,9] The secretory capacity of the small intestine is substantial, as evidenced by the potentially life-threatening secretory diarrhea that results from enterotoxigenic *Escherichia coli* and *Vibrio cholerae* infections.[9] Heat-stable enterotoxins from *E. coli* bind to intestinal guanylate cyclase C (GC-C) receptors, resulting in elevated levels of cellular cyclic guanosine monophosphate (cGMP). cGMP elicits a signaling cascade involving protein kinases and the cystic fibrosis transmembrane conductance regulator (CFTR) (Fig. 1), ultimately causing increased chloride secretion through CFTR. This creates an osmotic drive that results in the secretion of sodium ions, and hence water, into the intestinal lumen (Fig. 1).[2,9,10]

We evaluated a large family (Fig. 2A) with a dominantly inherited, fully penetrant syndrome of chronic diarrhea and dysmotility. Other conditions present in some members of this family included Crohn's disease, small-bowel obstruction, and esophagitis with or without esophageal hernia. We performed whole-genome single-nucleotide-polymorphism (SNP)–based linkage analysis, as well as exome sequencing, and identified a heterozygous activating mutation in *GUCY2C*, encoding GC-C. We thus established a genetic cause for this novel inherited disease.

METHODS

PARTICIPANTS

We studied 32 affected persons from three branches of the same family (Fig. 2A), as well as 14 unaffected family members. We obtained written informed consent from all the participants in the study. The affected family members were examined by a gastroenterologist and completed questionnaires (Rome II and ad hoc forms) regarding bowel symptoms. The study was approved by the regional ethics committee of Western Norway.

DETECTION OF MUTATIONS

Genomic DNA was purified from blood with the use of the QIAsymphony system (Qiagen). Whole-genome genotyping of SNPs was performed with the use of GeneChip Human Mapping 250K NspI array (Affymetrix). All exons and flanking intron sequences of *GUCY2C* (National Center for Biotechnology Information [NCBI] reference sequence NM_004963.3), were amplified and sequenced by means of standard methods. Exome sequencing was performed with the use of HiSeq (Illumina) (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org).

SITE-DIRECTED MUTAGENESIS

We generated mutant complementary DNA (cDNA) containing the c.2519G→T [p.Ser840Ile] as described previously.[11] Nonmutant and mutant GC-C cDNAs were cloned into the mammalian expression vector pcDNA3 (Invitrogen), and the respective proteins (nonmutant GC-C and mutant GC-CS840I) were transiently expressed in HEK293T cells.[11]

CHARACTERIZATION OF MUTANT PROTEIN GC-CS840I

We measured ligand-stimulated GC-C activity in intact cells 72 hours after transfection, after the addition of varying concentrations of heat-stable enterotoxin (STa) or uroguanylin (for 15 minutes) or guanylin (for 60 minutes).[11] For in vitro guanylate cyclase assays, membranes were prepared from transfected cells.[11] Details are provided in the Supplementary Appendix.

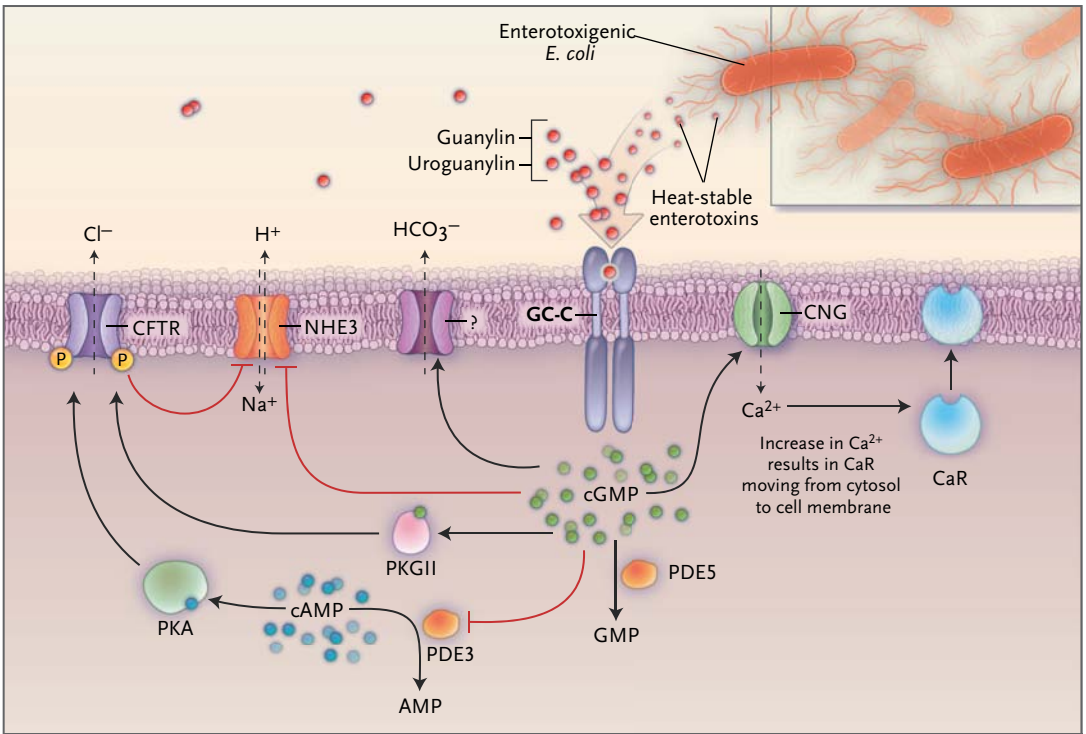


Figure 1. Signaling Mechanisms Mediated by Guanylate Cyclase C (GC-C).

GC-C expressed on the surface of enterocytes serves as the receptor for the endogenous ligands uroguanylin and guanylin or for heat-stable enterotoxins produced by enterotoxigenic *Escherichia coli*. Guanylin-family hormones are synthesized in the intestine and released both luminally and into the circulation. Uroguanylin exerts a natriuretic effect in the kidney. Ligand binding to GC-C increases intracellular levels of cyclic guanosine monophosphate (cGMP). The cGMP activates cGMP-dependent protein kinase II (PKGII) and inhibits the activity of a cyclic AMP (cAMP) phosphodiesterase, PDE3, thereby cross-activating cAMP–dependent protein kinase (PKA). PKGII and PKA phosphorylate the cystic fibrosis transmembrane conductance regulator (CFTR), increasing its chloride-secreting activity. In addition, cGMP enhances duodenal bicarbonate secretion through an unknown channel in a CFTR-dependent manner. These processes are involved in the maintenance of fluid and ion homeostasis. The cGMP also directly activates cyclic nucleotide gated channels (CNGs), leading to Ca^{2+} influx. Elevated intracellular Ca^{2+} levels activate calcium-sensing receptors (CaRs), promoting cell differentiation and migration. GC-C signaling is terminated by hydrolysis of cGMP to GMP by a cGMP-dependent phosphodiesterase, PDE5.[10] NHE3 denotes sodium–hydrogen exchanger 3.

HETERODIMERIZATION AND INTERACTION OF GC-C

To monitor heterodimerization, plasmids harboring either nonmutant GC-C fused at the C-terminal to glutathione S-transferase[11] or mutant GC-CS840I were mixed in varying ratios and cotransfected. Seventy-two hours after transfection, solubilized membrane protein was incubated with glutathione beads for 1 hour and then washed, and protein bound to the beads was detected by Western blot analysis (for details, see the Supplementary Appendix).

STATISTICAL ANALYSIS

Linkage analysis was used to determine the candidate region for the mutation in the family. Multipoint parametric linkage analysis and haplotyping were performed with the use of the Allegro program, version 2, on a set of 45,000 SNPs pruned for strong local linkage disequilibrium. Details of the analysis are provided in the Supplementary Appendix.

RESULTS

PARTICIPANTS

We studied 32 affected family members (14 females and 18 males), with a mean age of 44 years (range, 2 to 89), and examined their medical histories. Family branch A came to our attention first, when the 88-year-old index member (A-IV1) was admit-

ted to the hospital owing to dehydration (Fig. 2A, and Table 1 in the Supplementary Appendix). The affected family members in branch A typically had a history of chronic diarrhea that started in infancy and was fairly constant over the years but that tended to subside by middle age in some persons. The results of colonoscopic examinations of affected family members in branch A were essentially normal (Table 1 in the Supplementary Appendix). Across all branches, the affected family members had an average of 3.6 stools per day (range, 0.3 to 20.0); the stools typically had a watery or loose consistency and were accompanied by meteorism and in some cases abdominal pain. The pattern of inheritance was autosomal dominant, with some variation in expression. Four of the 32 cases (in family members C-VI2, B-VI1, B-VI3, and C-VII3) had previously been diagnosed as the irritable bowel syndrome, although they did not strictly meet the Rome II criteria.[12] However, 5 other cases (in family members A-IV1, A-V3, A-VI6, B-VII4, and B-VII5) did meet these criteria.

Some members of family branches B and C had more severe phenotypes (Table 1 in the Supplementary Appendix). Ten family members underwent laparotomy for suspected bowel obstruction; in eight of them (including family member B-IV1, who is not included in Table 1 in the Supplementary Appendix), obstruction resulting from volvulus, adhesional bands, or ileal inflammation was confirmed. Two family members with bowel obstruction had anatomical variants in the ileocecal region, such as a partly nonfixated ascending colon and slits in the ileal mesenterium. Three family members with bowel obstruction (B-V2, B-V3, and C-VI3) underwent a second operation to resolve adhesions, and family members C-VI3 and B-IV1 were described as having adhesional-band obstruction, even on the basis of the first laparotomy. Five family members with bowel obstruction (B-V2, B-V3, B-VI5, B-VI7, and B-VI9) eventually underwent resection of the terminal ileum and in some cases also the cecum; four of the five had verified or suspected Crohn's disease (Table 1 in the Supplementary Appendix). In addition, Crohn's disease was diagnosed in family members B-VI8 and C-VI2, and this diagnosis was suspected in family member C-V1. Family member A-VI6 received a diagnosis of possible eosinophilic enteritis.

Eight family members had been hospitalized for dehydration, metabolic acidosis, and electrolyte disturbances when they were newborns (Table 1 in the Supplementary Appendix). They were found to have hyponatremia, hypokalemia, and in some cases also hypomagnesemia and hypocalcemia, accompanied by abdominal distention and dilatation of the small intestine. None had a confirmed infectious disease. Hirschsprung's disease was ruled out in three of these family members, including B-VII1, who at 2 years of age had periods of constipation alternating with diarrhea. Seven other family members were hospitalized for dehydration at various times later in life, usually with infectious gastroenteritis, which, according to some of the family members, was followed by a prolonged period of recovery (Table 2 in the Supplementary Appendix). Several other conditions that members of this family had may be associated with this inherited condition (Table 2 in the Supplementary Appendix), including urolithiasis (in four family members), vitamin B12 deficiency (in six) and esophagitis with or without esophageal hernia (in five). We observed no evidence of behavioral disturbances[13] or a tendency toward obesity or excessive leanness.[14] Most of the family members reported food sensitivity, and several limit their intake of fruits, vegetables, and sweets.

POTENTIALLY CAUSATIVE MUTATION

Linkage analysis of samples from 11 affected members and 14 healthy members of family branch A revealed only one significant shared region in the affected members, on the short arm of chromosome 12 (12p), with a maximum LOD score of 5.1 (Fig. 1 in the Supplementary Appendix). A haplotype spanning approximately 2.9 megabases (Mb) (base pairs 14,466,726 to 17,410,570 from the start of 12p) showed complete cosegregation with the disease in the family. The region contained 28 putative protein-coding genes (Fig. 2B). Among these, *GUCY2C* (Fig. 2B) was the best candidate, because it encodes GC-C, an intestinal transmembrane receptor with known function in heat-stable enterotoxin–mediated diarrhea.[10] Sequence analysis identified a heterozygous base substitution, c.2519G→T, in exon 22 (Fig. 2C), predicting the replacement of the amino acid serine in codon 840 with isoleucine (p.Ser840Ile). Whole-exome sequencing in 3 persons (1 from each family branch) did not identify any other rare coding variant (Table 3 in the Supplementary Appendix). Using Sanger sequencing, we found the *GUCY2C* c.2519G→T mis-

Figure 2 (facing page). Identification of a Pathogenic Mutation in a Large Family with Chronic Diarrhea.
Panel A shows the pedigree of the family. All affected males (black squares) except B-IV1 and C-IV1 and all affected females (black circles) except A-IV5 and B-IV3 were investigated. The inheritance pattern is autosomal dominant, and linkage analysis was performed on samples from 14 healthy members and 11 affected members of branch A. The numbers of the generations are shown in Roman numerals to the left. Family members in each generation are designated by Arabic numbers from left to right, starting on 1 within each branch (with numbers shown only for the first and last person within each branch). Information about disease status in the affected members in generations I, II, and III was limited; therefore these persons are shown as unaffected (white squares or circles). Slashes denote deceased family members. Panel B shows the map of chromosome 12 and the 2.9-Mb region identified by linkage analysis (www.ensembl.org). The region on the short arm of chromosome 12 (p13.1 to p12.3) extends from base pair 14,466,726 to base pair 17,410,570 from the start of the short arm (according to NCBI Build 36.3). It contains 28 putative protein-coding genes, including *GUCY2C* (red circle), which is known to be the receptor for the *E. coli* heat-stable enterotoxin. Panel C shows the heterozygous missense mutation (c.2519G→T, arrow) in exon 22 in *GUCY2C*, which was found in all affected family members. This base change predicts the replacement of the amino acid serine in codon 840 with isoleucine [p.Ser840Ile] in the GC-C protein. Panel D shows the domain organization of GC-C. The GC-C protein contains an extracellular domain that binds its ligands. A single transmembrane domain is followed by a juxtamembrane domain and a kinase homology domain that bears sequence similarity to protein kinases. This is followed by a linker region and finally the C-terminal guanylate cyclase domain. Ligand binding to the extracellular domain results in activation of the catalytic domain, leading to increased cGMP production. GC-C forms oligomers in cells. A homodimer is shown; the catalytic domains of guanylate cyclases are functional only when in the context of a dimer. Highlighted in the red box is the position of S840 (red sphere). Numbers indicate amino acid positions of human GC-C, which includes a signal sequence predicted to be 23 amino acids long. The modeling of the various domains was performed as described previously.[10]

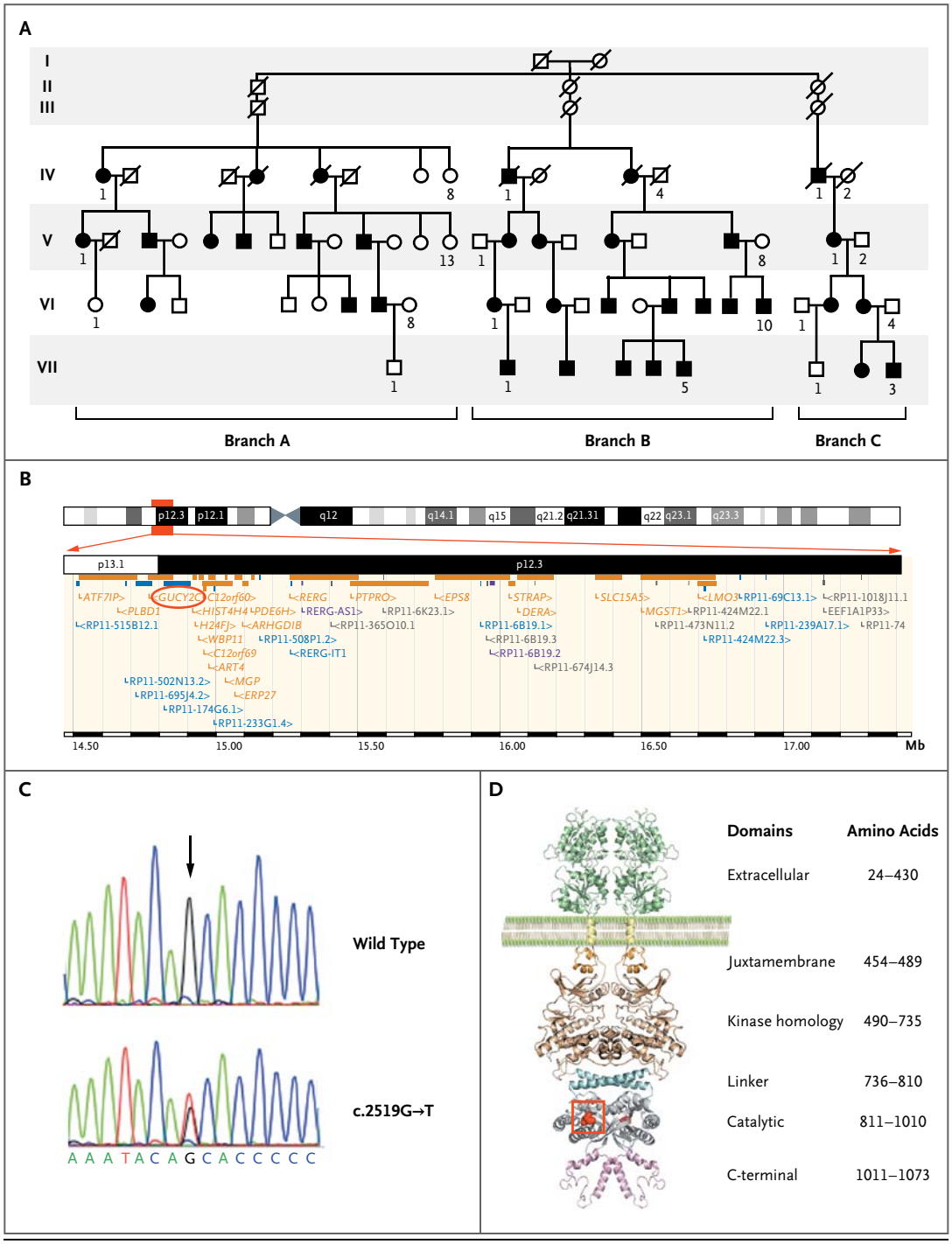
sense mutation to be present in all affected family members. We did not find this mutation in the NCBI human dbSNP database (build 132) or in 190 local healthy blood donors. None of the 14 unaffected family members we tested carried the mutation.

The amino acid Ser840 is highly conserved among mammalian GC-C proteins[15] and also in chicken and zebrafish (Fig. 2 in the Supplementary Appendix). The substitution is located in the catalytic domain (Fig. 2D), and we hypothesized that it may alter the guanylate cyclase activity of the mutant receptor.

FUNCTIONAL CHARACTERIZATION OF THE MUTATION

We performed biochemical experiments using intact transfected cells (Fig. 3, and Fig. 3 in the Supplementary Appendix) and membranes isolated from these transfected cells (Fig. 4 through 9 in the Supplementary Appendix), expressing either normal (nonmutant) or mutant (S840I) protein in equal amounts; the results of Western blot analysis are shown in Figure 3A, and in Figures 3 and 4 in the Supplementary Appendix. The basal GC-C enzyme activity and cellular cGMP levels (Fig. 3A, and Fig. 4 and 5 in the Supplementary Appendix) and affinities of ligands (heat-stable enterotoxin, uroguanylin, and guanylin) (Fig. 7 and 8 in the Supplementary Appendix) were similar in cells expressing the normal receptor and those expressing the mutant receptor. However, heat-stable enterotoxin (Fig. 3A and 3B, and Fig. 4 and 6 in the Supplementary Appendix), uroguanylin (Fig. 3C), and guanylin (Fig. 3 in the Supplementary Appendix) activated the mutant receptor to a greater extent than the nonmutant receptor. When cells were treated with 10^{-7} M heat-stable enterotoxin (Fig. 3B), 10^{-6} M uroguanylin (Fig. 3C), or 10^{-6} M guanylin (Fig. 3 in the Supplementary Appendix), cGMP production was increased by a factor of 7 to 8 (in the case of heat-stable enterotoxin), 5 (in the case of uroguanylin), or 3 to 4 (in the case of guanylin) with the mutant receptor, as compared with the nonmutant receptor. With respect to heat-stable enterotoxin, there was no significant difference between mutant and nonmutant receptors in the concentration required for half-maximal activation (EC_{50}) of the receptor (Fig. 3B). In contrast, with respect to uroguanylin, the EC_{50} of the mutant receptor was lower than that of the nonmutant receptor by a factor of about 5 to 6 (Fig. 3C), indicating that uroguanylin acts more potently on the mutant receptor. This suggests that the concentrations of uroguanylin present in the intestine could result in abnormally elevated levels of cellular cGMP in intestinal cells harboring the mutant receptor.

In patients who are heterozygous for the mutation, nonmutant and mutant receptors may be co-



expressed in a single cell. We therefore engineered the coexpression of a nonmutant receptor (fused with glutathione *S*-transferase) and the mutant receptor in HEK293T cells. The mutant receptor along with the glutathione *S*-transferase–tagged nonmutant receptor was captured by glutathione beads (Fig. 9 in the Supplementary Appendix), indicating the formation of heterodimers. There was a significant increase in the production of cGMP when the two receptors were coexpressed (in a 1:1 ratio), as compared with the production of cGMP when the nonmutant receptor was ex-

Figure 3 (facing page). Functional Analyses of Mutant GC-C Protein in HEK293T Cells.

Panel A shows heat-stable enterotoxin (ST)–mediated cyclic GMP (cGMP) production by GC-C. HEK293T cells were transfected with plasmids that allowed expression of either nonmutant GC-C or mutant GC-C (S840I). Equivalent expression of nonmutant or mutant GC-C was observed on Western blot analysis with the use of a monoclonal antibody to GC-C (inset). GC-C migrates as two differentially glycosylated proteins of molecular weight 130 and 145 kD.[11] Cells were treated with medium alone or with ST (10^{-7} M) for 15 minutes, and intracellular cGMP levels were measured by radioimmunoassay. Data shown are from one experiment (two measurements), and the experiment was repeated twice. T bars indicate standard errors. Panel B shows the dose response with ST. Cells expressing either nonmutant GC-C or mutant GC-C (S840I) were treated for 15 minutes with varying concentrations of ST as indicated, and intracellular cGMP levels were measured. The values in the graph are the mean values of a representative experiment; I bars indicate standard errors. The experiment (two measurements) was repeated three times with similar findings, and the concentration required for half-maximal activation (EC_{50} nanomolar values) was calculated from six measurements. EC_{50} values did not differ significantly between nonmutant and mutant GC-C. Panel C shows the dose response with uroguanylin. Cells expressing either nonmutant GC-C or mutant GC-C (S840I) were treated for 15 minutes with varying concentrations of uroguanylin as indicated, and intracellular cGMP levels were measured. The values in the graph are the mean values in a representative experiment; I bars indicate standard errors. The experiment (two measurements) was repeated three times with similar findings, and the EC_{50} values were calculated from six measurements. EC_{50} (micromolar values) differed significantly between nonmutant and mutant GC-C ($P<0.001$). Panel D shows heterodimerization of nonmutant GC-C and mutant GC-C (S8401). Indicated ratios of plasmids expressing either nonmutant GC-C tagged with glutathione *S*-transferase (GST) or mutant GC-C were cotransfected in HEK293T cells. Nonmutant GC-C–GST migrates at a size of approximately 160 kD owing to the C-terminal fusion of GST, and mutant GC-C migrates at 130 and 145 kD. The relative expression of the two proteins was monitored by Western blot analysis with the use of a monoclonal antibody to GC-C. At 72 hours after transfection, cells were treated with ST (10^{-7} M) for 15 minutes, and intracellular cGMP was measured by radioimmunoassay. Data shown represent the means of duplicate measurements, and the experiments were repeated twice. T bars indicate standard errors.

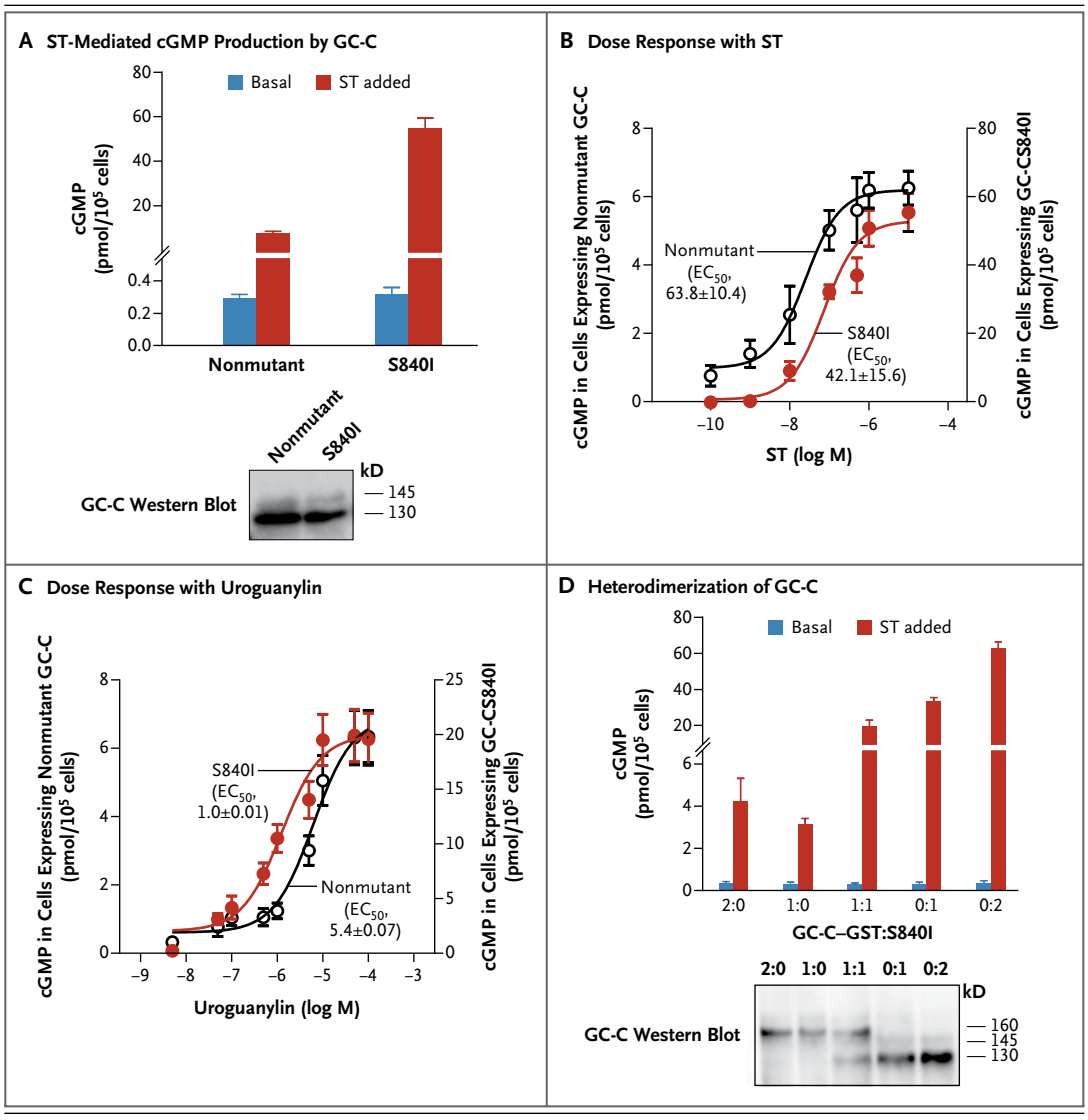
pressed alone (2:0 or 1:0 ratio) (Fig. 3D). This elevated activity was not as high as the activity observed with the mutant receptor alone (0:1 or 0:2 ratio), indicating that heterodimerization of the nonmutant and mutant receptors may blunt the ligand-mediated hyperactivation of the mutant receptor.

DISCUSSION

We describe a previously unrecognized disease characterized by familial diarrhea and define its genetic cause. The chronic diarrhea showed an inheritance pattern that was consistent with a dominant mutation in an autosomal gene, and we observed a heterozygous missense mutation, c.2519G→T [p.Ser840Ile], in *GUCY2C* in all affected family members (Fig. 2C and 2D). Subsequent functional analyses of the mutation in cell culture suggested that it effects a gain of function, in that it increases ligand-mediated activation of GC-C with subsequent intracellular accumulation of cGMP (Fig. 3). Persons with this mutation would therefore be prone to the production of comparatively high levels of cGMP in response to normal levels of endogenous ligands.

The importance of GC-C signaling and CFTR function in infectious bowel disease is well established.[8-10,16] The binding of heat-stable enterotoxin to GC-C greatly increases the formation of intracellular cGMP, which activates protein kinase GII, leading to phosphorylation of the CFTR channel.[10] This results in efflux of Cl^- (or HCO_3^- in the duodenum) and water into the intestinal lumen (Fig. 1), as well as reduced sodium absorption through inhibition of the sodium–hydrogen exchanger 3 (NHE3).[16] The disease we describe here may thus represent a "knock-in" genetic model of enterotoxigenic *E. coli* infection, with increased signaling through cGMP explaining the diarrhea in the affected family members, who were particularly prone to diarrhea, dehydration, and electrolyte disturbances soon after birth (Table 1 in the Supplementary Appendix). This is consistent with the observation that GC-C in the intestine is most highly expressed in newborns and declines during the first months of life to the level found in adults.[17]

The symptoms in the family members we examined overlapped with those of common disorders such as the irritable bowel syndrome (particularly in nine family members) and Crohn's disease (which was confirmed or suspected in seven members). This familial diarrhea syndrome also appears to confer a predisposition in the affected family members to small-bowel obstruc-



tion (which has occurred in eight family members) and esophagitis or esophageal hernia (in five members). Thus, the spectrum of symptoms seen in our patients suggests that the increased level of cGMP present in cells harboring the c.2519G→T [p.Ser840Ile] mutation affects inflammation and motility in the gut.

cGMP signaling has been linked to the regulation of inflammation and cell proliferation.[18,19] It has been shown that mice deficient in *Gucy2c* have a reduced inflammatory response to a colitis-inducing agent, owing to reduced expression of proinflammatory molecules.[20] The possibility that increased cGMP signaling enhances the expression of proinflammatory cytokines should be tested, since that may explain the susceptibility to ileitis in our patients. An increased risk of inflammatory bowel disease is also seen in connection with other conditions that cause intestinal electrolyte disturbances. This increased risk is observed in mice that are deficient in *Nhe3*[21] and in patients with congenital chloride diarrhea.[22] Changes in electrolyte homeostasis and increased permeability[23,24] may cause a disturbance in intestinal barrier function, which was recently proposed as a primary contributor to the development of inflammatory bowel disease.[25,26] Disturbed intestinal barrier function may also be caused by chronic changes in tight-junction function or assembly (or both),[26] which are affected by GC-C signaling.[27]

The terminal ileum in the affected family members we examined appeared to be susceptible to

small-bowel obstruction. Eight of 33 family members underwent laparotomy owing to small-bowel obstruction (Table 1 in the Supplementary Appendix); some family members required a second or even third laparotomy owing to reobstruction, which is frequently related to the formation of adhesions. All the affected family members had meteorism, and dilatation of the small intestine was seen during quiescent phases in many of them (Table 1 in the Supplementary Appendix). The latter, which is also observed in children with congenital chloride diarrhea,[22] may result from increased fluid in the intestine, reduced tone of smooth muscle, or both. The ileocecal junction was not dilated on radiographs or at surgery, and it may represent a bottleneck for the increased fluid formation. Mechanical strain or abnormal mobility in the ileocecal region could increase the risk of recurrent colic and bowel obstruction.[28] The molecular mechanisms underlying the disturbed motility in the gut and the meteorism in these patients are not known.

Both guanylin and cGMP have a relaxing effect on smooth-muscle cells in the gastrointestinal tract.[29,30] Levels of cGMP are altered by several effectors in the intestine, including other natriuretic peptides and the strong muscle relaxant and secretory agent, vasoactive intestinal peptide,[31] suggesting the possibility of cross-talk between these signaling molecules and GC-C.[32,33] Such cross-talk is of interest in a wider perspective, because (pro)uroguanylin is also secreted into the bloodstream and affects renal electrolyte transport in humans,[34] as well as behavior[13] and satiety (as part of the gut–brain axis)[14] in animals. *GUCY2C* is expressed in the brain,[13,14] but we observed no symptoms consistent with an effect of the mutant protein on the central nervous system.

Our data are consistent with those obtained through study of the role of GC-C in intestinal hydration, meteorism, and bowel function.[18,20,35] Both low activity[35] and high activity of this receptor-cyclase may interfere with homeostasis in the intestine. To our knowledge, no common low-risk susceptibility variants in *GUCY2C* for either the irritable bowel syndrome or inflammatory bowel disease have been reported.[3,5] Forthcoming genomewide association studies that use next-generation sequencing may reveal rare susceptibility variants. Also, variants in any of the genes related to the GC-C–CFTR pathway probably contribute to overall signaling through GC-C, making the whole pathway an interesting target for the investigation of diarrhea or constipation of unknown cause.

In conclusion, we have identified an activating mutation in *GUCY2C* as the cause of a novel familial diarrhea syndrome, characterized by meteorism, abdominal pain, dysmotility, and inflammatory bowel disease. This finding highlights *GUCY2C* as a susceptibility gene for Crohn's disease, small-bowel obstruction, and functional gastrointestinal diseases such as the irritable bowel syndrome and points to the GC-C–CFTR pathway as a target for a further search of mechanisms underlying these conditions.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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