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ORIGINAL ARTICLE

Mutant Neurogenin-3 in Congenital Malabsorptive Diarrhea

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ABSTRACT

BACKGROUND

Neurogenin-3 (NEUROG3) is expressed in endocrine progenitor cells and is required for endocrine-cell development in the pancreas and intestine. The NEUROG3 gene (*NEUROG3*) is therefore a candidate for the cause of a newly discovered autosomal recessive disorder characterized by generalized malabsorption and a paucity of enteroendocrine cells.

METHODS

We screened genomic DNA from three unrelated patients with sparse enteroendocrine cells for mutations of *NEUROG3*. We then tested the ability of the observed mutations to alter NEUROG3 function, using in vitro and in vivo assays.

RESULTS

The patients had few intestinal enteroendocrine cells positive for chromogranin A, but they had normal numbers of Paneth's, goblet, and absorptive cells. We identified two homozygous mutations in *NEUROG3*, both of which rendered the NEUROG3 protein unable to activate *NEUROD1*, a downstream target of *NEUROG3*, and compromised the ability of NEUROG3 to bind to an E-box element in the *NEUROD1* promoter. The injection of wild-type but not mutant *NEUROG3* messenger RNA into xenopus embryos induced NEUROD1 expression.

CONCLUSIONS

A newly discovered disorder characterized by malabsorptive diarrhea and a lack of intestinal enteroendocrine cells is caused by loss-of-function mutations in *NEUROG3*.

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Patients with congenital diarrhea generally present within the first weeks after birth with severe, life-threatening watery diarrhea that can be classified as either secretory or malabsorptive in nature.[1] Routine clinical evaluation, including intestinal biopsy, may be used to further categorize the diarrhea according to the severity of the inflammation and to assess the integrity of the crypt–villus axis and the architecture of the epithelial layer. On the basis of both clinical and pathological characteristics, various specialized formulas may be used to minimize the diarrheal symptoms, although some children require extended periods of intravenous nutrition to sustain normal growth and development.

Many patients with congenital diarrhea in early infancy have a selective defect in one of a variety of transporters and enzymes that have nonredundant roles in the processes of nutrient assimilation and ion absorption.[2] In contrast, generalized malabsorptive disorders involving simple nutrients (such as monosaccharides and amino acids) result from a nonselective decline in the capacity to absorb nutrients, which is due to either a decrease in the small-bowel surface area or mucosal inflammation.[1] Thus far, defects in the structure and function of absorptive and inflammatory cells of the gut have been associated with diarrhea and the alteration of ion and nutrient absorption and secretion.

Studies involving mice have shown that two basic helix–loop–helix transcription factors — mouse atonal homologue 1 (encoded by *Math1*) and Neurogenin-3 (Neurog3, encoded by *Neurog3*) — influence the fates of enteroendocrine, Paneth's, goblet, and enterocyte cells.[3-6] *Neurog3*$^{-/-}$ mice lack endocrine cells in the pancreas and intestine, which is presumably why they die of diabetes during the first several days of life.[3] These and other findings suggest that Neurog3 is a key transcription factor that controls the fate of endocrine cells in both the pancreas and intestine.[3-6] Despite its clear role in the development of endocrine cells, no disease-causing mutation of *NEUROG3* (or of the genes encoding the closely related NEUROG1 and NEUROG2) has to our knowledge been described in humans.[7] We studied three boys with severe generalized congenital malabsorptive diarrhea to determine the clinical characteristics and molecular basis of this disorder in humans.

METHODS

PATIENTS

We identified three boys with similar clinical characteristics and obtained informed, written consent from the legal guardians of each patient. The study protocol was approved by the institutional review board of the University of California at Los Angeles. Pertinent medical and pathology records were obtained and summarized for each patient.

PATHOLOGICAL ANALYSIS OF THE INTESTINE

We analyzed formalin-fixed, paraffin-embedded biopsy samples of intestinal tissue using immunohistochemical techniques and stained them with hematoxylin and eosin and periodic acid–Schiff. We carried out immunohistochemical assays for chromogranin A (Dako), synaptophysin (Dako), lysozyme (Dako), serotonin (Zymed), gastrin (Dako), somatostatin (Dako), and vasoactive intestinal polypeptide (Biogenics) with standard techniques and using commercially available antibodies, according to the manufacturers' instructions. Archival samples of small bowel were used as control samples. Electron microscopy was used to examine biopsy samples of the small-intestinal mucosa from each patient. Information on DNA sequencing, cloning, site-directed mutagenesis, messenger RNA (mRNA) and protein synthesis, and analysis in xenopus embryos can be found in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

RESULTS

OVERVIEW OF CLINICAL HISTORIES

The patients presented during the first several weeks of life with vomiting, diarrhea, dehydration, and a severe hyperchloremic metabolic acidosis after the ingestion of standard cow's-milk–based formula (Table 1 and the Supplementary Appendix). They were readmitted on several occasions during the first month of life when it became clear that they all had chronic unremitting diarrhea that was malabsorptive in nature. Comprehensive evaluations for allergic, immunologic, infectious, and metabolic causes of diarrhea were all negative. The pancreatic exocrine function was normal in all three patients. The radiographic evaluation of Patients 1 and 2 showed that the length and orientation of the small bowel were normal. Biopsy of the stomach and esophagus revealed

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Patient 1	Patient 2	Patient 3
Age	35 mo	8 yr	9 yr
Sex	M	M	M
Birth weight — g	2530	2720	2334
Hyperchloremic metabolic acidosis	Yes	Yes	Yes
Cessation of diarrhea while receiving nothing by mouth	Yes	Yes	Yes
Viral, parasitic, and bacterial pathogens	None	None	None
Sweat chloride test and test for CF DNA	Normal	Normal	Normal (CF DNA ND)
Mitochondrial DNA	Normal	Normal	ND
Lactate and pyruvate	Normal	Normal	ND
Serum amino acids	Normal	Normal	Normal
Urine organic acids	Normal	Normal	Normal
Cholesterol and triglyceride panel	Normal	Normal	Normal
Apolipoprotein profile	Normal	Normal	ND
Zinc, ammonia, and creatine kinase	Normal	Normal	ND
HIV-1 or HIV-2 antibody	Negative	Negative	Negative
T-cell and B-cell subgroups	Normal	Normal	Normal
Stool			
Trypsin activity	Maximum (+4)	ND	ND
Reducing substances	Maximum (+2)	Maximum (+2)	Maximum (+2)
Alpha-1-antitrypsin activity	Normal	Mild elevation	ND
Mucosal lactase, sucrase, and maltase activity	Normal	Normal	Normal
Upper gastrointestinal and small-bowel follow-through	Normal	Normal	ND
Secretin stimulation test (pancreatic enzymes)	ND	Normal	Normal

normal histologic features and biopsy of the duodenum and sigmoid colon revealed histologic features initially interpreted as normal. The activity of various mucosal disaccharidases was within the normal range.

The volume of diarrhea in all three patients was in the range of 60 to 120 ml per kilogram of body weight per day (normal stool volume, less than 20). The diarrhea ceased during periods of fasting. Water was well tolerated, but the administration of a glucose-based oral rehydration solution led to diarrhea. Two patients continued to have diarrhea while being fed a carbohydrate-free cow's-milk–based formula, and the addition of either fructose or glucose exacerbated the severity of the diarrhea. Several amino acid–based formulas, including one without carbohydrates, were administered but did not lead to resolution of the diarrheal symptoms in two patients. Patient 1 even had diarrhea when long- or medium-chain triglycerides or amino acids (typically used for parenteral nutrition) were added to the drinking water.

All three children were eventually discharged home with the means of delivering and receiving parenteral nutrition and limited enteral feeding and were readmitted on several occasions because of sepsis related to an infection from the central venous catheter. Parenteral nutrition was discontinued in the third patient because of the severity and frequency of such sepsis. Severe cholestatic liver disease and portal hypertension developed in the index patient (Patient 1), and by two years of age the patient underwent a native subtotal enterectomy and total hepatectomy and received an orthotopic en bloc liver–intestine transplant. He

Table 1. (Continued.)

Characteristic	Patient 1	Patient 2	Patient 3
Response to various nutrients†			
Water	Normal	Normal	Normal
Water + MCT	Diarrhea	ND	ND
Water + LCT	Diarrhea	ND	ND
Water + amino acids	Diarrhea	ND	ND
Glucose-based oral rehydration solution	Diarrhea	Diarrhea	Diarrhea
Carbohydrate-free formula	Diarrhea	Diarrhea	? Decreased
Carbohydrate-free formula + fructose	Diarrhea	Diarrhea	? Decreased
Carbohydrate-free formula + glucose	Diarrhea	Diarrhea	Diarrhea
Amino acid–based formulas	Diarrhea	Diarrhea	Diarrhea
Carbohydrate-free, amino acid–based formula	Diarrhea	ND	ND
Cow's-milk–based formula	Diarrhea	Diarrhea	Diarrhea
Soy-protein–based formula	Diarrhea	Diarrhea	Diarrhea
Response to drug management (during receipt of cow's-milk–based formula)			
Pancreatic enzymes	Diarrhea	Diarrhea	Diarrhea
Loperamide	Diarrhea	Diarrhea	ND
Cholestyramine	Diarrhea	Diarrhea	Diarrhea
Clinical course			
Received home PN (duration)	Yes (24 mo)	Yes (23 mo)	Yes (2 mo)
Currently receiving PN	No‡	No	No
Current diarrhea	No‡	Yes	Yes
Small-bowel transplantation	Yes	No	No

* CF denotes cystic fibrosis, ND not done, HIV human immunodeficiency virus, MCT medium-chain triglycerides, LCT long-chain triglycerides, and PN parenteral nutrition.
† Diarrhea is defined as diarrhea with a volume of more than 20 ml per kilogram of body weight per day.
‡ Patient died of sepsis when manuscript was under review.

did well, without diarrhea and while being fed enterally, for nearly a year but then died unexpectedly of sepsis. The second patient continued to receive parenteral nutrition at home until the age of two years, and since then, he has been receiving supplemental oral vitamins and electrolytes. Patients 2 and 3 continue to have large-volume loose stools daily, and their weights are either at or below the 5th percentile for age. Type 1 diabetes developed in both patients when they were 8 years old.

HISTOPATHOLOGICAL ANALYSIS OF THE INTESTINE

We obtained small-bowel–biopsy samples from the index patient at various ages; these samples consistently revealed a normal villus structure and no pathologic infiltration of inflammatory cells (Fig. 1). Extensive evaluation of the small- and large-bowel mucosa after enterectomy showed profound dysgenesis of the enteroendocrine cells (compare Fig. 1D with Fig. 1E). The appearance of the remaining mucosa, including the Paneth's and goblet cells, was normal (Fig. 1A, 1B, and 1C). Staining for chromogranin A revealed only one enteroendocrine cell (which was aberrant in shape) in the more than 350 small-bowel crypts examined (Fig. 1D) and none in the colon. In contrast, five or six enteroendocrine cells were present per crypt in normal mucosa (Fig. 1E). The lack of cells was also observed in biopsy specimens obtained from the index patient 18 months before enterectomy. Staining of small-bowel–biopsy samples for chromogranin A also revealed a dramatic depletion of enteroendocrine cells in Patients 2 and 3. Only

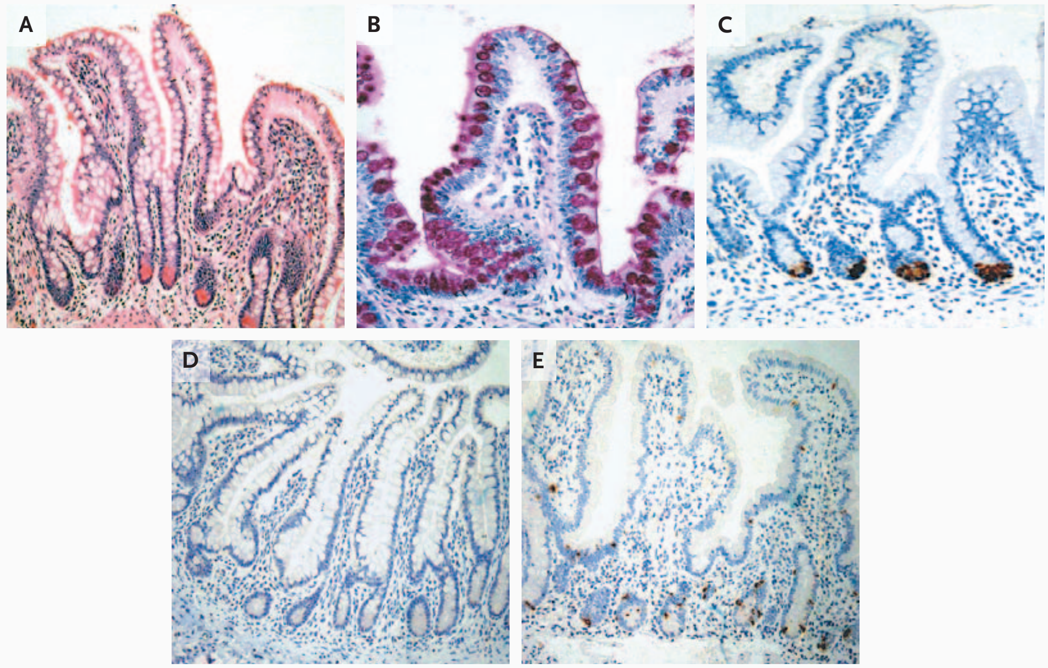


Figure 1. Sample of Small Bowel with Sparse Enteroendocrine Cells from Patient 1.

The crypt and villus architecture was normal (hematoxylin and eosin, Panel A), as were goblet cells (periodic acid–Schiff, Panel B) and Paneth's cells (antilysozyme, Panel C), but there was a deficiency of enteroendocrine cells (antichromogranin, Panel D), as compared with the number in a control sample (antichromogranin, Panel E).

1 cell was identified in 100 crypts in the duodenum, and 8 were identified in 100 crypts in the jejunum.

The lack of the general endocrine marker synaptophysin also indicated an extreme deficiency of endocrine cells in the three patients. Only 1 cell in 350 crypts was positive for serotonin. No cells containing gastrin or somatostatin were identified in more than 300 crypts, and as expected, vasoactive intestinal polypeptide stained only nerves (data not shown). The results of electron microscopy indicated that the patients had normal brush borders, microvilli, intracellular mitochondria, and tight junctions. We observed an occasional lipid-laden enterocyte in the upper third of the villus, an abnormal finding previously described in patients with abetalipoproteinemia and hypoproteinemia.[1]

SEQUENCING OF *NEUROG3*

We hypothesized that the enteroendocrine-cell dysgenesis was caused by a null mutation of *NEUROG3*. We therefore sequenced *NEUROG3* in DNA from blood samples from the index patient and observed a homozygous missense change predicted to result in the substitution of a serine residue for an arginine residue at position 107 (R107S) (Fig. 2). The other two patients had a homozygous missense change predicted to result in the substitution of a leucine residue for an arginine residue at position 93 (R93L) (Fig. 2). Position 107 is in the first helix of the protein, which is critical to the activation of downstream genes. Position 93 is in the DNA-binding or basic domain (i.e., the region that binds the promoters of genes regulated by NEUROG3), just upstream of the first helix (Fig. 2B). We did not observe these mutations in specimens from 100 ethnically matched control persons (data not shown). The arginine residues at positions 93 and 107 are conserved in all known neurogenin proteins across a wide range of species, including *Caenorhabditis elegans* (Fig. 2B). We hypothesize that the missense mutations result in loss of function of the protein because of their critical location and the extent to which the affected residues are conserved across species.

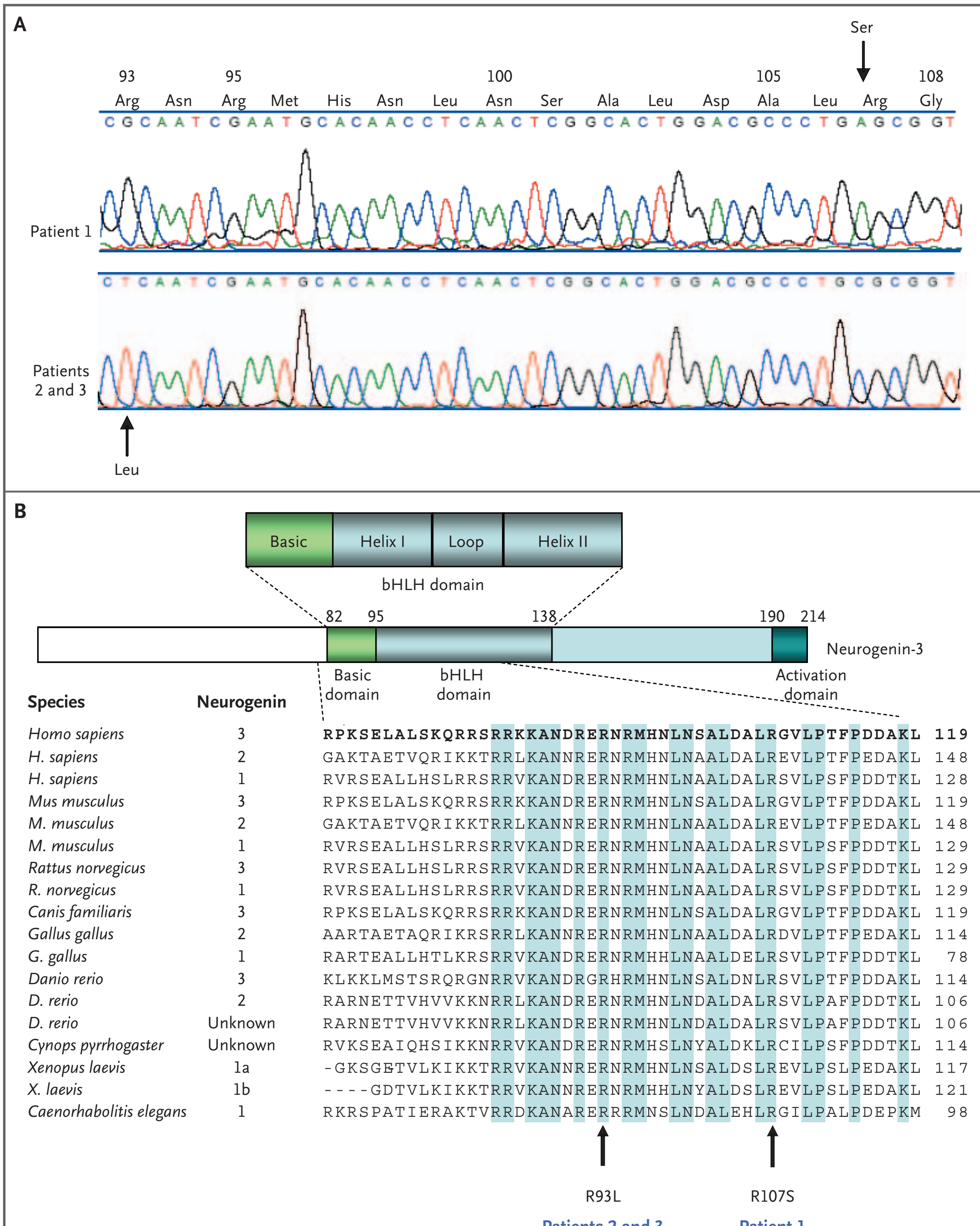


Figure 2. **Sequence of *NEUROG3* (Panel A) and Structure of the Protein (Panel B).**

Panel A shows the *NEUROG3* sequence in Patient 1 (top) and in Patients 2 and 3 (bottom). The human *NEUROG3* gene (region encoding amino acids 93 to 108) is shown, and the specific mutations are indicated by the arrows. Patient 1 had a homozygous mutation resulting in the substitution of serine for arginine in residue 107 (R107S), whereas Patients 2 and 3 had a homozygous mutation resulting in the substitution of leucine for arginine in residue 93 (R93L). Panel B shows the structure of NEUROG3, including the basic helix–loop–helix (bHLH) domain. The conservation of various residues within this domain is shown for all members of the neurogenin family in a wide variety of species. Unknown means that the specific type of neurogenin is unknown. Dashes represent amino acids unrelated to the neurogenin family. The numbers to the right of the sequences are the residue numbers for the last amino acid listed for each species.

TRANSACTIVATION OF THE *NEUROD1* PROMOTER

When bound to another transcription factor, E47, NEUROG3 activates the transcription of the *NEUROD1* gene.[8] More specifically, the NEUROG3–E47 heterodimer binds two well-characterized E-box DNA elements (called E-box 1 and E-box 3) that lie in the promoter region of *NEUROD1*; such binding is critical to the activation of NEUROD1 by the heterodimer.[8] A reporter vector composed of the three E-boxes in the promoter region of *NEUROD1* followed by the luciferase gene was co-transfected, together with a wild-type *NEUROG3* expression vector, into HeLa cells. The resulting promoter activity was five times that of the negative control (Fig. 3A). We observed insignificant promoter activity, however, when we replaced the wild-type NEUROG3 expression vector with a vector encoding either the R93L or R107S NEUROG3 mutant. A Western blot of transfected, flag-tagged NEUROG3 revealed approximately equal amounts of the wild-type and mutant forms (Fig. 3B). We therefore concluded that the mutations render NEUROG3 incapable of inducing *NEUROD1* gene expression in this transient-transfection assay.

INTERACTION BETWEEN NEUROG3 AND THE *NEUROD1* PROMOTER

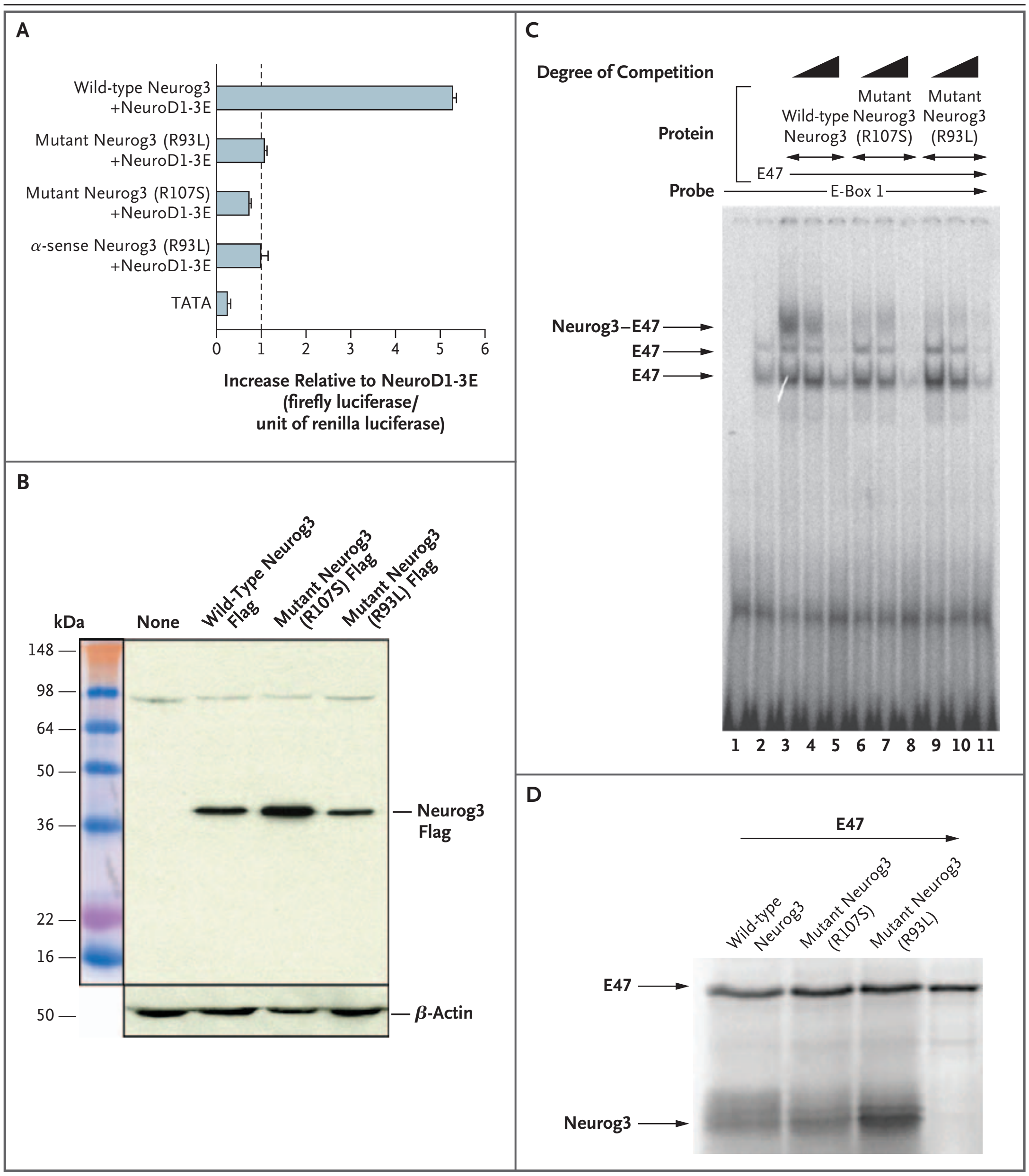
To clarify the mechanism by which the mutations of *NEUROG3* prevented the activation of the *NEUROD1* promoter, we assessed the ability of each mutant protein to interact in vitro with an E-box in the *NEUROD1* promoter. The E47 homodimer bound to the E-box 1 DNA element in a specific manner (lane 2 in Fig. 3C). Wild-type NEUROG3, on the other hand, failed to bind DNA in the absence of E47 (data not shown), but the NEUROG3–E47 heterodimer formed an abundant and specific complex, indicating robust binding between the heterodimer and the DNA element (lanes 3, 4, and 5 in Fig. 3C). In contrast, mutant NEUROG3 (encoded by constructs containing the R93L or R107S mutation) generated less signal than the wild-type NEUROG3, indicating weaker binding (lanes 6 through 11 in Fig. 3C). Approximately equal amounts of E47 and NEUROG3 (both wild-type and mutant forms) were used in this analysis (Fig. 3D). These data suggest that the failure of mutant NEUROG3 to augment the *NEUROD1* promoter activity is due, at least in part, to its compromised ability to bind the *NEUROD1* promoter.

Figure 3 (facing page). In Vitro Effects of NEUROG3 on the *NeuroD1* Promoter.

The clone containing the *NeuroD1* promoter (*NeuroD1-3E*) was cotransfected into HeLa cells with either wild-type or mutant (R107S or R93L) NEUROG3 expression vectors. Panel A shows the resulting activity of firefly luciferase per unit of renilla luciferase, relative to that of the negative control: *NeuroD1-3E* cotransfected with the expression vector containing the mutant NEUROG3 (R93L) cloned in the antisense orientation. The TATA vector is the proximal promoter region of NeuroD1 that lacks the E-boxes. Values are means ±SE of six replicates from three different experiments. Panel B shows the results of Western blotting, with anti-flag M2 and anti–β-actin antibodies, of the whole-cell lysate from HeLa cells into which flag-tagged wild-type and mutant NEUROG3 vectors had been transiently transfected 24 hours earlier. "None" represents cells into which NEUROG3 was not introduced. β-Actin served as an internal control for the equal loading of protein. Panel C shows the binding of a *NeuroD1* promoter element to NEUROG3–E47. An electrophoretic mobility shift assay was performed with the use of a recombinant protein (E47 or NEUROG3–E47) and a labeled duplex oligonucleotide (NeuroD1 E-box 1). Recombinant E47 alone (lane 2), with wild-type NEUROG3 (lanes 3, 4, and 5), or with mutant NEUROG3 — R107S (lanes 6, 7, and 8) or R93L (lanes 9, 10, and 11) — bound to the oligonucleotide duplexes. The formation of complexes with the labeled oligonucleotide duplexes lessened proportionally in the presence of 10 and 100 times the baseline amount of unlabeled oligonucleotide duplexes (increasing levels indicated by the increasing slope of the black triangles). Panel D shows the results of the in vitro translation of E47 and NEUROG3 (wild-type and mutant forms) labeled with [^{35}S]methionine and visualized on a 12 percent acrylamide gel to confirm their relative sizes and abundances.

IN VIVO ACTIVATION OF *NEUROD1*

The ectopic injection of *NEUROG3* RNA into xenopus embryos induces the expression of NeuroD1 in neurons.[8] We therefore tested the ability of mutant NEUROG3 to transactivate NeuroD1 production in vivo by injecting mouse *Neurog3* mRNA (wild-type or mutant) into xenopus embryos and then evaluating NeuroD1 levels (Fig. 4). The human and mouse orthologues of the protein showed a high degree of similarity (72 percent overall and 98 percent in the critical helix–loop–helix DNA-binding domain), and we were unable to detect significant differences in the extent to which they activated *NEUROD1*. Wild-type *Neurog3* mRNA induced ectopic NeuroD1-positive neurons in the injection side of the embryo (Fig. 4B). We observed



no such neurons in the sham-injected control or in the side that was not injected (Fig. 4A) and very few, if any, in the embryos injected with either of the two mutant forms of mRNA (Fig. 4D and 4F). These results suggest that the mutations attenuate NEUROG3 function in vivo.

DISCUSSION

Our findings indicate that disruption of the pathway that determines the differentiation of intestinal absorptive cells (enterocytes) and secretory cells (enteroendocrine, Paneth's and goblet cells) has profound effects on the capacity of the small intestine to absorb nutrients. Specifically, the clinical findings highlight a critical role of enteroendocrine cells in augmenting nutrient absorption. We have named the disorder exemplified by these cases "enteric anendocrinosis."

The fate of the embryonic or adult stem cell is determined by a series of molecular switches. The first switch is the lateral inhibition by a ligand

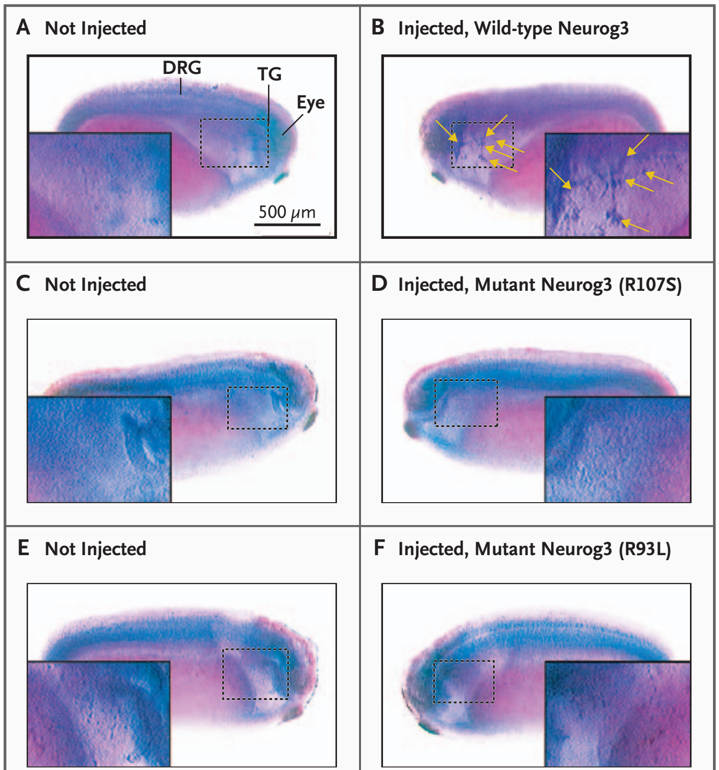


Figure 4. In Vivo Activation of NeuroD1 by NEUROG3.

Panel A shows the normal expression of NeuroD1 in the eye, trigeminal ganglia (TG), and dorsal-root ganglia (DRG) of a *Xenopus laevis* embryo. Shown are the side of each embryo that was injected (Panels B, D, and F) and not injected (Panels A, C, and E) with wild-type mouse Neurog3 (Panels A and B) or mutant mouse Neurog3 — R107S (Panels C and D) or R93L (Panels E and F). Some areas are magnified (insets), and some ectopic NeuroD1-positive neurons are marked by arrow (Panel B).

of the Notch receptor.[9,10] In the cells that express it, the ligand triggers a signaling cascade dominated by the sequential expression of numerous basic helix–loop–helix transcription factors, such as MATH1.[5,11,12] Cells expressing the Notch receptor, on the other hand, are induced (through the binding of the Notch receptor by one of its ligands) to inhibit this cascade of basic helix–loop–helix transcription factors — resulting in the exclusive development of absorptive cells.[9,11-13] This inhibition is mediated by a molecule called Hairy and Enhancer of Split (HES1): its expression is induced through the activation of the Notch receptor, and it inhibits the cascade of basic helix–loop–helix transcription factors by preventing the expression of MATH1.

The intestines of *Math1*$^{-/-}$ mice lack secretory cells, and the mice inexplicably die soon after birth. Neurog3 expression is abrogated in *Math1*-null mice, suggesting that *Neurog3* is a downstream target of Math1. Unlike their *Math1*-null counterparts, *Neurog3*-null mice have normal goblet and Paneth's cells and no islet cells in the pancreas, and like *Math1*$^{-/-}$ mice, they lack enteroendocrine cells.[3-6] Diarrhea has not been described in either strain of mouse. Although diabetes has been suggested as the cause of death of *Neurog3*-null mice, *Math1*-null mice presumably have normal pancreatic function, because *Math1* is not expressed in the pancreas.[5] Combining the data from mouse knockout studies and our findings, it appears that loss-of-function mutations of *NEUROG3* contribute to early death by causing a severe malabsorptive diarrhea.

The particular hormone factors and paracrine factors downstream of NEUROG3 that affect nutrient absorption have yet to be identified.[14] They may lie downstream of transcription factors activated by Neurog3: NeuroD1, Pdx1, or both.[15,16] Mice deficient in either NeuroD1 or Pdx1 lack a subgroup of enteroendocrine cells, but because they die shortly before or after birth, the relevance of a deficiency of a portion of enteroendocrine cells to early death is uncertain, and diarrhea has not been described in these mice. An assortment of null mice, with selective deficiencies of specific hormones secreted by the enteroendocrine cells, have been created.[14-18] Many of these mice have specific physiological phenotypes, but none have been described as having the malabsorption, malnutrition, or perinatal death that would mimic the clinical findings in our patients.

Two other findings, together with our results, indicate that enteroendocrine cells and their products have a profound role in the capacity of the small intestine to absorb simple nutrients. First, the transient depletion of enteroendocrine cells was reported in a patient with temporary malabsorption and the autoimmune polyglandular syndrome type I; the symptoms resolved after the spontaneous recovery of enteroendocrine cells.[19] Second, a mutation of the gene encoding the enzyme prohormone convertase 1 has been reported to result in malabsorption, obesity, hypoadrenalism, and elevated levels of prohormones with a proportionate depletion of mature hormones.[20] This family of enzymes is located in enteroendocrine and other endocrine cells and converts inactive peptide hormone precursors into their mature functional forms.[20,21]

How does an absence of enteroendocrine cells lead to malabsorptive diarrhea? Sensors that prime the intestine for the absorption of luminal nutrients have long been suggested to play a critical role in intestinal adaptation.[22] However, their precise identity, location, and function have remained elusive. Various nutrient, pH, and osmotic sensors are present in enteroendocrine cells and submucosal cholinergic neurons, but the intracellular signals of these sensors and their subsequent effector products are not well understood.[23-25]

A paucity of enteroendocrine cells might lead to generalized malabsorption if it decreased intestinal transit time.[1] Although we did not carry out gastric-emptying studies in our patients, the results of barium imaging of two of the cases suggested normal luminal transit. Cholinergic neurons clearly influence intestinal motility and can be influenced by effector products secreted by adjacent enteroendocrine cells.[26,27] However, the index patient was intolerant to simple amino acid solutions, suggesting that rapid transit by itself would not be likely to lead to such profound malabsorption.

Our patients and *Neurog3*$^{-/-}$ mice were found to have normal disaccharidase activities; the mice have normal targeting of lactase to the brush-border membrane, suggesting that the apical targeting of these enzymes is not defective.[6] Although NEUROG3 is not abundantly expressed in enterocytes, most genes that express proteins with important roles in nutrient assimilation are augmented by a hepatocyte nuclear factor (HNF1).[28] Since HNF1 is known to form a heterodimer with NEUROG3, the absorptive capacity of the enterocyte may be deleteriously affected in the absence of the NEUROG3–HNF1 complex.[29] The localization and abundance of brush-border enzymes and transporters in humans with a deficiency of NEUROG3 have yet to be analyzed. Alternatively, the presence of progenitor or mature enteroendocrine cells that express NEUROG3 could be important for the normal development of the absorptive cells.

The two eldest patients (Patients 2 and 3) have recently shown evidence of hyperglycemia that has yet to be thoroughly investigated. Mouse studies have shown that Neurog3 has a nonredundant role as a proendocrine master switch in the pancreas as well as the gut.[3-6] The absence of overt glucose intolerance, even with intravenous glucose administration, in our patients suggests that another putative, unidentified factor can at least partially compensate for the absence of functional NEUROG3 and stimulate the development of pancreatic islet cells. Six forms of maturity-onset diabetes of the young (MODY) have been identified and are attributable to heterozygous mutations of genes that critically affect the development and function of beta cells.[30] No mutations of *NEUROG3* have been identified in patients with MODY[31]; it would be interesting to evaluate whether our patients and their related obligate heterozygotes have evidence of considerable glucose intolerance, since such evidence would qualify *NEUROG3* as a candidate gene for MODY. These findings may also help uncover the cause of several other associated forms of diarrhea, including diarrhea-predominant irritable bowel syndrome and various inflammatory and other forms of congenital diarrhea.[32]

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