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Mutant Neurogenin-3 in Congenital Malabsorptive Diarrhea

Jiafang Wang, B.S., Galen Cortina, M.D., Ph.D., S. Vincent Wu, Ph.D., Robert Tran, M.D., Jang-Hyeon Cho, Ph.D., Ming-Jer Tsai, Ph.D., Travis J. Bailey, Ph.D., Milan Jamrich, Ph.D., Marvin E. Ament, M.D., William R. Treem, M.D., Ivor D. Hill, M.D., Jorge H. Vargas, M.D., George Gershman, M.D., Douglas G. Farmer, M.D., Laurie Reyen, M.N., and Martín G. Martín, M.D.

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.
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. 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. 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. 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.

Overview of Clinical Histories

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.
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. Baseline Characteristics of the Patients.

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

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* 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.

Table 1. (Continued.)

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

---

* 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.
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 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 (anticromogranin, Panel D), as compared with the number in a control sample (anticromogranin, Panel E).
**NEUROG3 Mutations and Malabsorptive Diarrhea**

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.

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**Figure 2.** Sequence of NEUROG3 (Panel A) and Structure of the Protein (Panel B).
TRANSACTIVATION OF THE NEUROD1 PROMOTER

When bound to another transcription factor, E47, NEUROG3 activates the transcription of the NEUROD1 gene. 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. 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 E47 and NEUROG3 (both 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.

IN VIVO ACTIVATION OF NEUROD1

The ectopic injection of NEUROG3 RNA into xenopus embryos induces the expression of NeuroD1 in neurons. 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...
of the Notch receptor. 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. 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. 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 binding of the Notch receptor by one of its ligands.)

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. 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. This family of enzymes is located in enteroendocrine and other endocrine cells and converts inactive peptide hormone precursors into their mature functional forms.

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. 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. 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. They may lie downstream of transcription factors activated by Neurog3: NeuroD1, Pdx1, or both. 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. 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.

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) or 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).
How does an absence of enteroeendocrine 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. 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.

A paucity of enteroeendocrine cells might lead to generalized malabsorption if it decreased intestinal transit time. 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. 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 Neuro3−/− 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. 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). 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.

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 Neuro3 has a nonredundant role as a proendocrine master switch in the pancreas as well as the gut. 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. No mutations of NEUROG3 have been identified in patients with MODY; 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.

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**REFERENCES**


8. Huang HP, Liu M, El Hodiri HM, Chu


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