Advances in Evaluation of Chronic Diarrhea in Infants

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Abstract

Diarrhea is common in infants (children less than 2 years of age), usually acute, and, if chronic, commonly caused by allergies and occasionally by infectious agents. Congenital diarrheas and enteropathies (CODEs) are rare causes of devastating chronic diarrhea in infants. Evaluation of CODEs is a lengthy process and infrequently leads to a clear diagnosis. However, genomic analyses and the development of model systems have increased our understanding of CODE pathogenesis. With these advances, a new diagnostic approach is needed. We propose a revised approach to determine causes of diarrhea in infants, including CODEs, based on stool analysis, histologic features, responses to dietary modifications, and genetic tests. After exclusion of common causes of diarrhea in infants, the evaluation proceeds through analyses of stool characteristics (watery, fatty, or bloody) and histologic features, such as the villus to crypt ratio in

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intestinal biopsies. Infants with CODEs resulting from defects in digestion, absorption, transport of nutrients and electrolytes, or enteroendocrine cell development or function have normal villi to crypt ratios; defects in enterocyte structure or immune-mediated conditions result in an abnormal villus to crypt ratios and morphology. Whole-exome and genome sequencing in the early stages of evaluation can reduce the time required for a definitive diagnosis of CODEs, or lead to identification of new variants associated with these enteropathies. The functional effects of gene mutations can be analyzed in model systems such as enteroids or induced pluripotent stem cells and are facilitated by recent advances in gene editing procedures. Characterization and investigation of new CODE disorders will improve management of patients and advance our understanding of epithelial cells and other cells in the intestinal mucosa.

Keywords

Pediatric; Gastrointestinal Disorder; Inherited; Detection

Diarrhea during early infancy (children less than 2 years of age) is relatively common, generally mild, and often self-limited. Most frequently, this form of diarrhea in infancy is of allergic or infectious origin, and usually not associated with any significant long-term sequelae. In contrast, anatomical disorders, such as gastroschisis, necrotizing enterocolitis (NEC), or acute volvulus can cause short-bowel syndrome (SBS) and long-lasting diarrhea after surgical resection.[1] Another class of persistent and severe diarrhea presenting in the first weeks of life results from monogenic disorders and is termed *congenital diarrhea and enteropathies* (CODEs). CODEs are typically associated with feeding intolerance and malabsorption. Both CODE and SBS require significant dietary and therapeutic interventions, including specialized formulas or parenteral nutrition (PN) to sustain appropriate growth, electrolyte, and nutrient balance.

The availability of genetic diagnostic testing before 2008 was limited, and infants with many of the non-surgical severe diarrheal disorders often carried the catch-all diagnosis of "chronic diarrhea of unknown etiology." These infants followed a diagnostic odyssey and clinical course associated with high levels of morbidity and prolonged expensive hospitalizations. In recent years, our understanding of the underlying genetic basis of these disorders, as with other rare Mendelian diseases, has been revolutionized by the availability of next-generation sequencing. These technologies have enabled the elucidation of the genetic basis of an increasing number of monogenic disorders causing CODEs, and most so far involve intestinal epithelial and/or immune function. However, these disorders are rare and clear diagnostic and therapeutic pathways for the care of these patients have not been established.

Here, we outline a prioritized approach for the diagnosis and evaluation of diarrhea in infants. The approach recognizes recent advances in next-generation sequencing, stem cell biology, gene editing, and other aspects of the rapidly evolving field of precision medicine.

Causes of Diarrhea During Early Infancy

Diarrhea is typically defined as acute for diarrhea lasting less than 2 weeks, or chronic when diarrhea persists for more than 2 weeks. In middle-and low-income countries, the term *persistent diarrhea* is also used to denote diarrhea lasting more than 2 weeks after infection or in relation to environmental enteric dysfunction.[2,3] Historically, the nomenclature for chronic diarrhea in infancy has included terms such as *intractable or protracted diarrhea of infancy.*[4] Our understanding and classification of chronic diarrheas in infants has evolved and the disease may be more helpfully divided into either acquired diarrheas or CODEs.

Acquired diarrheas can develop postnatally or can have their origin in utero, with varying severity, comorbid conditions, and underlying pathogeneses. Postnatally acquired disorders are most often infectious due to common enteric viral, bacterial, or less commonly, parasitic pathogens, or caused by allergic disorders induced by exposure to food allergens, such as cow's milk protein. Infectious causes often lead to acute symptoms, but in some cases can result in chronic diarrhea in immunodeficient patients or in persistent diarrhea due to a malabsorptive enteropathy, also termed *post-infectious enteropathy.*[5] NEC, an important postnatally acquired disorder, is almost always seen in premature infants and is thought to result from gut ischemia and the influx of pro-inflammatory lymphocytes secondary to abnormal gut microbial responses via Toll-like receptor 4 signaling.[6–8] The disease can lead to SBS after gut resection. Other acquired diarrheas leading to surgical SBS include in utero anatomic abnormalities, such as atresias and gastroschisis.

CODEs are less common but are often extremely severe, and represent unique diagnostic and management challenges. Most CODEs are monogenic in nature and can be broadly divided into genetic variants directly affecting the intestinal epithelium or variants affecting the immune system that secondarily cause severe impairment of epithelial function.

Diarrhea Terminology

Previous classifications have often divided diarrhea into osmotic and secretory, but these terms can be misleading. Therefore, some new terms are proposed.[9]

Osmotic diarrhea.—The term *osmotic diarrhea* has been used traditionally to refer to diarrhea resulting from unabsorbed solutes or nutrients; however, all diarrhea involves osmotic forces. Therefore, we prefer to use the more precise term, *diet-induced diarrhea.* Diet-induced diarrhea is characterized by an elevated stool osmotic gap (>100 mOsm). Examples include glucose or disaccharide malabsorption.

Secretory diarrhea.—The term *secretory diarrhea* is also imprecise. The term describes the underlying pathophysiology of the diarrheas caused by active ion secretion into the intestine, but it does not describe the watery high-salt diarrheas caused by defects in intestinal sodium absorption (eg, as seen in the congenital sodium diarrheas and in some viral infections). Neither can the term *secretory* be used to describe all the diarrheas with a low stool osmotic gap (<50 mOsm) (Table 1), because a low stool osmotic gap typically results from a combination of enhanced anion-driven fluid secretion and loss of Na+-driven

fluid absorption. We prefer to use the term *electrolyte-transport-related diarrhea.* Examples include congenital chloride or sodium diarrheas.

Mixed diarrhea.—Lastly, diarrhea that is obviously neither secretory nor osmotic, or has an intermediate stool osmotic gap (50–100 mOsm), has been referred to as "mixed." Intermediate values for the stool osmotic gap occur frequently and are generally caused by a combination of diet-induced diarrhea and electrolyte transport-related diarrhea resulting from different dietary intakes at the time of testing.

Evaluation and Diagnosis of Acquired Diarrhea in Infants

The general evaluation and diagnostic approach to infants with diarrhea is outlined in Figure 1. Diarrhea in infancy is difficult to define based on stool frequency or consistency, as the normal range for these parameters can vary greatly. A more reliable measure is stool weight or volume, with diarrhea defined as a stool volume of >20 g/ kg/d. From a practical standpoint, the presence of diarrhea can be inferred by the deviation from the daily stool pattern and by the level of dehydration and severity of electrolyte abnormalities.

Evaluation

Diarrhea presenting early or immediately postnatally in term or premature infants should prompt evaluation for congenital enteropathies, NEC, or anatomical abnormalities (Figure 1). For less-severe diarrhea presenting later in infancy, the initial workup should focus on investigating common acquired etiologies, such as infections, cow's milk protein allergy, or food protein-induced enterocolitis syndrome (Figure 1).

In both high-income and middle-/low-income countries, viral pathogens are the most likely agents responsible for infectious diarrhea in infants. Rotavirus, cytomegalovirus, adenovirus, and norovirus should all be considered. Less common in high-income countries but a significant factor globally are enteric bacterial pathogens, such as *Salmonella enterica, Shigella spp, Campylobacter jejuni,* and pathogenic *Escherichia coli.*[2,10] Persistent viral diarrheas, or diarrhea after administration of the rotavirus vaccine, may be the first sign of primary immunodeficiency or related to autoimmune enteropathies, such as immunodysregulation pol-yendocrinopathy, enteropathy X-linked.[11] Bloody diarrhea with no evidence of infection should be followed by either a change to extensively hydrolyzed formula and/or a maternal dairy exclusion diet for a potential diagnosis of cow's milk protein colitis.[12] Food protein-induced enterocolitis syndrome, a primarily T-cell-mediated inflammatory disorder triggered by a variety of food antigens, can present in infancy with acute-onset diarrhea, usually after dietary introduction of solid foods.[13,14]

Evaluation of severe early-onset diarrhea by radiographic small-bowel follow-through studies should be used to identify intestinal malrotation with intermittent volvulus, or congenital short gut. Hirschsprung's disease can rarely present with diarrhea, and should also be considered. Obstructive symptoms without an identifiable lead point are typically associated with chronic intestinal pseudo-obstruction. NEC can present with watery or bloody diarrhea, usually in association with prematurity, abdominal distension, feeding intolerance, and temperature instability. Initial evaluation and management involves serial

abdominal films to investigate the presence of bowel wall gas, cessation of enteral feeding, and antibiotic administration.[6,15]

Evaluation of Suspected Monogenic Diarrhea

Evaluation

Increased suspicion for CODE can be gleaned from the history and clinical course. Important information to gather includes prenatal history and testing; age at onset of symptoms; nature of symptoms; extraintestinal manifestations; nutrition and diet history; and a full family history, including any evidence of consanguinity and ethnicity (Figure 1). Specific populations have relatively high incidence rates of congenital enteropathies, such as the Finnish, Ashkenazi Jews, Navajo Native American, and those originating from the Arab Gulf regions.[16–19]

In patients undergoing evaluation after 1 month of age, the age of onset of the diarrhea is a key historical detail, with very-early-onset increasing the chance of CODE. Infectious and immune-related conditions generally have a symptom-free postnatal period of at least a few weeks before clinical symptoms are apparent. It should be noted that, in some conditions, the volume of diarrhea may be so severe that less-experienced parents and health care providers may confuse diarrhea for urine, delaying recognition and appropriate consultation.

Serum Testing

Initial testing should include a complete blood count, serum electrolytes, inflammatory markers, liver function tests, immunoglobulin levels, a lipid panel (triglycerides, cholesterol), fat-soluble vitamins, a coagulation profile, and zinc level. Additional immunologic investigation should be considered if there is suspicion of immune dysfunction, such as specific T-and B-cell subset analysis.

Stool Testing

Accurate stool testing is a key component of the diagnostic evaluation for congenital diarrheas. Collection of stool samples can be challenging due to the presence of mixed urine and stool in the diapers of neonates, and by rapid loss of stool water content due to absorption into the diaper material. Use of a urine catheter for a few days may allow for accurate stool sampling and volume measurement. Ideally, fresh stool samples obtained immediately after excretion should be tested (Table 1). A key component in the evaluation of stool testing is an exact quantification of dietary intake at the time of testing.

Diagnostic Algorithm for Suspected Monogenic Diarrheas and Enteropathies

The workup for CODE is initiated after the exclusion of acquired diarrhea. As an initial diagnostic evaluation, it is helpful to grossly characterize the stool appearance in suspected CODE (Figures 1 and 2). Although sometimes difficult, most stools can be broadly differentiated into 3 main categories: watery, fatty, and bloody stools, and this allows for clear prioritization of initial testing. Watery stool is characterized by a high liquid content—often with very little form—that can be mistaken for urine. Fatty stools are usually foul-smelling, can have a bulky or "fluffy" appearance, are pale in color, and/or are spot fecal fat-

positive. Bloody stools contain gross blood mixed in with stools. The presence of large volumes of bright red blood or melena should trigger evaluation of vascular or anatomic gastrointestinal bleeding as well as infection.

It should be noted that genetic testing (see section Genomic Testing) can occur in parallel or early in the diagnostic algorithm, especially if there are clear factors to suspect a monogenic diarrheal disease, such as significant consanguinity; a family history of gastrointestinal disease in infancy; and clinical indicators, such as diarrhea severity and neonatal onset.

Evaluation of Watery Diarrhea

After documentation of diarrheal output with normal feeding, evaluation should include a no-feeding (nil per os) trial of at least 24 hours, with assessment of stool output and electrolytes (Figure 2, *top*). If diarrheal volume is unchanged or is minimally changed after fasting, this points to electrolyte-transport-related diarrhea. Significant improvement in diarrheal output after termination of enteral feeds points to diet-induced diarrhea. Subsequent evaluation should aim to elucidate whether a specific nutrient is malabsorbed or if the patient has a generalized malabsorptive diarrhea. A feeding trial with carbohydrate-free (Ross CarbohydrateFree; Abbott, Macquarie Park, NSW, Australia) or fructose-based formula (Galactomin-19; Nutricia, Wiltshire, UK) leading to significant improvement, along with reduced stool pH and elevated stool-reducing substances with carbohydrate-containing formula, supports the diagnosis of carbohydrate malabsorption. Elucidating the specific abnormality of carbohydrate assimilation can be performed with specific monosaccharide (glucose and fructose), disaccharide (sucrose, lactose, and maltose) dietary challenges and assessed by changes in stool volume and/or breath hydrogen testing.[20] Disaccharidase activity assays for lactase, sucrase, maltase, and palatinase performed on proximal small bowel biopsies may be helpful to diagnose disaccharidase defi-ciency.[21] However, these enzymatic assays are often unreliable due to poor sampling or in the setting of inflammation or villus atrophy due to secondary disaccharidase deficiency.

If there is no clear evidence of a selective carbohydrate malabsorption, diagnostic evaluation should begin with esophagoduodenoscopy and flexible sigmoidoscopy with biopsies for histologic analysis; if not contraindicated by the clinical status of the infant.[22] Biopsies should include samples for both routine histology and electron microscopy, as well as for measuring mucosal disaccharidase activity.

Early endoscopy and biopsy differentiating normal from an abnormal villus to crypt ratio,[23] and/or an inflammatory predominance allows for significant streamlining and prioritization of the evaluation, planning for dietary interventions, and initiation of genetic testing.

Parallel assessment for a possible protein-losing enteropathy is important, as it may be indicative of a compromised epithelial barrier, suggesting autoimmune enteropathy, or newly described monogenic disorders, such as DGAT1 and CD55 deficiency. The presence of elevated levels of α1-antitrypsin in the stool and low serum albumin, IgG, and lymphopenia are consistent with protein-losing enteropathy.[24,25]

Evaluation of Fatty Diarrhea

Evaluation of fatty diarrhea is by spot fecal fat testing, including neutral and split fat and, if available, quantitatively by 72-hour stool fat collection (Figure 2). Stool elas-tase is a useful initial test, as it can help distinguish between conditions resulting from pancreatic insufficiency and those caused by intestinal fat malabsorption, although fecal elas-tase can often be falsely low (false positive) with highvolume diarrhea. The presence of fat-laden enterocytes in histologic sections along with serum lipid abnormalities can point to disorders of fat transport and metabolism, such as chylomicron retention disease and abetalipoproteinemia. Pancreatic insufficiency is confirmed by the responsiveness of diarrheal symptoms to enzyme replacement therapy.[26]

Evaluation of Bloody Diarrhea

The presence of gross blood implies significant colitis and further evaluation should include stool inflammatory markers and endoscopy (Figure 2). The presence of inflammatory changes in histologic sections should precipitate further investigation of infantile very-early-onset inflammatory bowel disease, autoimmune enteropathy, or primary immunodeficiency. It should be noted that not all forms of immune-dysregulation-related enteropathies are associated with bloody stools.[27]

Endoscopy/Histology

Initial evaluation of H&E-stained sections should focus on overall intestinal epithelial architecture, namely villus to crypt ratio, abundance of the epithelial cell type and structure, and the immune cell composition in the lamina propria and intraepithelial compartments.[28]

- Normal villus/crypt architecture: Suggests conditions with defective digestion, absorption and transport of nutrients and electrolytes, or defects of enter-oendocrine cells.

- Abnormal villus/crypt architecture: Villi are generally short or flattened and may be associated with either crypt hyper-or hypoplasia, and may be seen in defects of enterocyte structure, vesicular trafficking, differentiation, and immune-mediated conditions. Specifically, villus blunting and apoptosis at the crypt base is suggestive in most cases of an immune-mediated disorder. Epithelial crowding and disorganization at the top of the villus is found in disorders of adhesion, such as congenital tufting enteropathy (CTE), and is associated with crypt hyperplasia.

- The relative abundance and distribution of cells in the stem cell-based compartment and differentiated cells of the epithelium can be appreciated by H&E and by cell-type-specific staining. Selective depletion of differentiated cell types may be associated with autoimmune enteropathy and endocrinopathies.

- Abnormal abundance or absence of mononuclear cells in the lamina propria and intra-epithelial compartments suggest an immune-mediated disorder. A dominance of eosinophilic infiltrates within the mucosa-associated with villus blunting may be an indicator of eosinophilic gastroenteritis.

Immunohistochemical Assessments

Immunohistochemical staining of differentiated cell populations may be helpful to confirm H&E findings and/or a specific diagnosis (Figure 3, Supplementary Table 1). Staining may be limited by the small amount of available tissue acquired during endoscopy. The assessment includes staining for enteroendocrine (chromogranin/synaptophy-sin), Paneth (lysozyme), and goblet cells (periodic acid-Schiff). Identification of proliferating cells can be done using Ki67, proliferating cell nuclear antigen, or pHisH3. Immune cell types can be initially assessed using specific surface markers for B cells (CD20), T cells (CD3/CD4/ CD8), macrophages (PU.1/CD68/F4/80), and plasma cells (CD138); however, identification of specific immune cell subsets requires more extensive and specialized staining.

If histologic assessment suggests abnormal epithelial architecture, initial immunohistochemical staining in all cases should include CD10/villin (microvillus inclusion disease [MVID]), periodic acid-Schiff (DGAT1), MOC31 (CTE), and frozen-section staining with Oil Red O if lipid trafficking disorders are under consideration.[29,30]

Further evaluation includes electron microscopy to assess the presence and relative size and location of the microvilli and to identify intracellular microvillus inclusions or abnormal vesicular structures suggestive of disorders of intracellular trafficking.

Immunolocalization of specific transporter, structural, or intracellular proteins, such as (DGAT1, PCSK1, and others) may provide increased diagnostic validation. Figure 3 depicts the typical histology of MVID, CTE (epithelial cell adhesion molecule [EPCAM]), abetalipoproteinemia, and autoimmune enteropathy (immune-dysregulation-related enteropathies). All studies, however, should be performed in concert with genetic analyses.

Genomic Testing

Advances in next-generation sequencing technologies promise to shorten the diagnostic odyssey for many CODE patients. Although the clinical diagnostic algorithm provides a framework for evaluation and prioritized testing, in many cases where the diagnosis of CODE is highly suspected but the specific etiology is not identified or requires confirmation, either targeted genetic testing (Sanger sequencing) or whole-exome sequencing can identify the genetic cause and allow for appropriate early treatment.

In selective populations with a high prevalence of known specific genomic variants, or when the diagnostic evaluation is strongly suggestive of a specific disorder, such as the characteristic epithelial tufts seen on biopsy in *EPCAM* mutations, Sanger sequencing should be considered for rapid diagnosis and treatment. For example, there are a number of relatively common founder CODE gene mutations, including Mexican/Arab: *EPCAM* (c. 491+1G>A and c.498insC),[18,31] Ashkenazi Jews: *DGAT1* (IVS8+2 T>C);[32] Navajo: *MYO5B* (p.Pro660Leu),[33] and Finns: *SLC26A3* (p.Val317del).[34]

In cases of a suspected CODE, where the diagnosis based on clinical evaluation is unclear, it is now standard of care to perform whole-exome sequencing to identify a possible causative genetic mutation.[35–38] It should always be considered, however, that whole-exome sequencing may not detect genetic defects in genes with poor coverage, large insertions and

deletions, and mutations in regulatory and splice or intronic regions. Therefore, in certain cases with a high likelihood of a monogenic disorder, whole-genome sequencing, or RNA sequencing should also be considered. Microarray comparative genomic hybridization is a rapid and frequently used method to assess significant copy number variation and changes in whole chromosomes, and discovery of large deletions (>200 kb) and duplications.[39]

Classification and Pathophysiology of Diarrhea in Infants: Acquired Diarrheal Disorders

SBS occurs more frequently in premature neonates (25/ 100,000 live births) and is associated with either anatomical intestinal defects or NEC. Many of these neonates will have malabsorption early on and be dependent on PN to sustain normal growth and development.[1,40]

Disorders such as gastroschisis[41] may result in diarrhea by various mechanisms, including impaired motility,[42] surgical formation of ostomies that shorten bowel length, or bacterial overgrowth. Patients with gastroschisis can also acquire SBS as a result of volvulus that can occur before or after birth.

Intestinal atresia results from defects in intestinal development early in gestation due to vascular anomalies or inherited defects in luminal development.[43] Atresia can be single, with reasonable complement of distal bowel, or there can be multiple atresias, with very limited bowel length. Next-generation sequencing has resulted in the identification of several severe inherited forms of intestinal atresia, including *TTC7A* and *RFX6* (discussed Disorders of Epithelial Trafficking and Polarity).[44,45]

NEC is predominantly an illness that affects preterm infants who are initiating enteral feeding. The pathogenesis is not clearly defined, but includes vascular abnormalities, bacterial dysbiosis, and abnormal immune responses, with the final common pathway being intestinal ischemia.[7] This can vary in severity, but uncommonly can include gangrene of nearly the entire small and large intestines. Diagnosis currently relies on careful clinical observation and plain films. Early biomarkers have been investigated, but are not yet used in routine clinical practice.[46] As with the aforementioned conditions, diarrhea and nonselective malabsorption result from SBS.

Monogenic Diarrheal Disorders

Monogenic diarrheal disorders can be broadly classified into 5 major categories (Figure 4, Table 2) reflective of a common pathophysiology, although there remains overlap among a number of these categories.[47] Epithelial cell defects are the hallmark of the first 4 CODE categories and range from defects in epithelial transporters, enzymes, and metabolism to defects in epithelial trafficking and polarity and enteroendocrine cell dysfunction. The clinical presentation is almost always within the first several months of life and is associated with high-volume watery diarrhea.

The fifth category encompasses monogenic disorders that cause dysfunction of the immune system, which result in a wide spectrum of both intestinal and extraintestinal manifestations. This broad array of monogenic entities includes genetic defects also classified as infantile-onset inflammatory bowel disease (children less than 2 years of age), autoimmune enteropathy, or primary immunodeficiency.[48] For a detailed description for the more common specific disorders, please see the Supplementary Material.

Disorders of Epithelial Nutrient/Electrolyte Transport

Alterations in epithelial transport proteins represent some of the most prevalent and well-known of the congenital diarrheas (Figure 4*A*). These encompass variants of pure electrolyte transporters, such as the Cl/HCO$_3^-$exchanger DRA *(SLC26A3),* which result in congenital chloride diarrhea[34]; the Na$^+$/H$^+$ exchanger NHE3 (SLC9A3), which results in congenital sodium diarrhea; and electrolyte-nutrient co-transporters, such as SGLT1 (SLC5A1),[49] which result in loss of both sodium and glucose absorption. Alterations in regulatory proteins, such as the guanylin receptor GC-C *(GUCY2C)*[50] or other transporters, such as those mediating intestinal sodium-coupled bile salt re-uptake *(SLC10A2),*[51] or primary excessive bile acid production, can induce secondary electrolyte transport defects in epithelial cells and excessive fluid loss.[52] These disorders generally exhibit a structurally intact epithelium and brush border with a normal villus to crypt ratio. Excessive stool chloride levels are found in congenital chloride diarrhea *(SLC26A3)*[53] whereas high stool sodium levels are found in congenital sodium diarrhea *(SLC9A3, GUCY2C, SPINT2).* Both of these disorders usually present immediately at birth with polyhydramnios often present in utero. In contrast, glucose-galactose malabsorption exhibits a diet-induced dehydrating diarrhea initially evident after initiation of feeding that ceases after institution of a glucose-galactose free diet.

Disorders of Epithelial Enzymes and Metabolism

Alterations in a number of important enzymes involved in both nutrient absorption as well as epithelial cell metabolism result in severe diarrhea (Figure 4*B*). Defects in brush-border enzymes involved in carbohydrate digestion, such as lactase and sucrose-isomaltase, result in a diet-induced diarrhea with onset after intake of carbohydrate-containing formula or food. These include the relatively common lactose intolerance caused by reduced function of *LPH,*[54,55] which also may be acquired after gastroenteritis or due to prematurity, vs the very rare cases of total loss-of-function mutations in *LPH*[56] or in the gene for sucrase-isomaltase (SI).[57] Bi-allelic mutations of *SI* result in a loss of sucrose or isomaltase, or both enzyme activities, and will result in diarrhea on a diet containing sucrose and/or starch, isomaltose, and maltose. Disorders of these brush-border enzymes exhibit grossly normal intestinal histology after biopsy.

A more recently described CODE characterized by an electrolyte transport-related diarrhea, emesis, protein-losing enteropathy, and growth failure induced by enteral intake of lipids was found to be due to a loss-of-function mutation in *DGAT1,* which is involved in cellular triglyceride formation.[32] Initial studies have indicated some loss in brush-border microvillus structure in patient biopsies, although it is unclear whether this persists in the absence of enteral lipids.

Other disorders of fat transport or metabolism result from mutations in proteins involved in fat absorption across the epithelium, such as microsomal triglyceride transfer protein resulting in abetalipoproteinmeia, apolipoprotein B resulting in hypobetalipoproteinemia, or chylomicron retention disease *(SAR1B)*. These disorders classically show lipid-laden vacuoles within enterocytes (Figure 3D).

Disorders of Epithelial Trafficking and Polarity

A number of disorders of epithelial trafficking and polarity lead to early-onset diarrhea, usually appearing in the first months of life. All disorders carry an autosomal recessive inheritance and have been described in various ethnic groups. The diagnosis is based on typical pathologic findings and/or extraintestinal manifestations, followed by confirmation of the diagnosis via genetic testing. The two most well-described disorders are MVID and CTE.

MVID results from mutations in the cytoskeletal motor protein Myosin 5b *(MYO5B)*, which results in defective apical membrane recycling in intestinal epithelial cells.[33,58] This results in the pathognomic structural abnormalities of the epithelial apical membrane, including loss of micro-villi[59] leading to abnormal CD10 and villin staining of intestinal biopsies and intracellular microvillus inclusions seen in electron micrographs (Figure 3E-H).[59] Patients present with profuse dehydrating diarrhea in the absence of enteral intake but worsened with feeding. A similar but milder phenotype to *MYO5B* mutations can be also found after loss of the trafficking protein syntaxin 3 *(STX3)*.[61]

CTE results from loss of function in the epithelial signaling and adhesion protein EPCAM and causes a severe sodium-losing diarrhea that usually presents from birth to 3 months of life.[31,62] The diarrhea in CTE does occur in the absence of feeding, however, it is generally much worse with enteral intake. The classical findings on biopsy include the presence of pathognomic surface epitheial "tufts" seen on H&E stains of biopsies, as well as the lack of EPCAM (MOC31) immunostaining (Figure 3*I-L*). "t

Other disorders involving epithelial structural defects include recently described mutations in the gene *TTC7A*,[44] which leads to loss of the apicobasal polarity of the enter-ocyte, crypt-base apoptosis, crypt and villus atrophy and chronic inflammation, and *TTC37* mutations,[63] which result in mild to severe villus atrophy and variable inflammatory infiltrate on biopsy.

Disorders of Enteroendocrine Cell Function

Disorders classified as enteric endocrinopathies result from either a loss of proper enteroendocrine cell (EEC) fate or generalized defects in processing of gut hormones. Collectively, these disorders result in a generalized mal-absorptive diarrhea that requires PN for the first several years of life, although diarrheal symptoms persist perhaps indefinitely. Each disorder is also associated with a unique set of systemic endocrinopathies that allow for anticipatory guidance of physicians and families alike.

The first disorder described involving enteroendocrine dysfunction resulted from bi-allelic loss-of-function mutations of *Neurogenin3*, a basic helix-loop-helix transcription factor, required for enteroendocrine and β-cell develop-ment.[64,65] Patients present with a primarily

diet-induced diarrhea that is not specific to any single nutrient and their intestinal biopsies reveal a normal crypt to villus ratio, with selective loss of all types of EECs.

Another EEC cell disorder was found to result from loss-of-function mutations of *PCSK1,* coding for prohormone convertase (PC1/3), a protease that is required for the biosynthetic processing of hormone precursors into their fully functional forms.[66] Infants present with diarrhea that is phenotypically similar to *NEUROG3* mutations but associated with a wider range of systemic endocrinopathies, including adrenal insufficiency, hypothyroidism, and diabetes insipidus-like picture.

Other EEC-related disorders include mutations in *RFX6,*[67] a transcription factor that functions both up-and downstream of Neurogenin3 and mutations in ARX, a ho-meobox transcription factor that results in selective reduction of GLP-1 and cholecystokinin-expressing EECs.[68,69]

Immune Dysregulation-Associated Enteropathies

Recent genetics studies have rapidly increased the number of monogenic disorders, including FOXP3,[70,71] *ICOS,*[72] *IL10R,*[73] *TRIM22,*[74] and *ARPC1B,*[38] which cause dysregulation of the immune system and subsequently inflammation and enteropathy in the intestine. Immune-mediated intestinal disorders present with a wide variety of manifestations, but all have bloody or watery diarrhea, and are frequently associated with systemic disease and multi-organ involvement. Many of these disorders have been classified as infantile-onset inflammatory bowel disease, and have features characteristic of later-onset inflammatory bowel disease, such as bloody diarrhea, colitis, perianal disease, and ulceration, for example, *IL10R.*[73,75] Others have predominantly villus atrophy without ulceration and present with profuse watery diarrhea and malnutrition, for example, ICOS and FOXP3, and have also been termed *autoimmune enteropathies.* Most of these diseases are curable with bone marrow transplantation.

One of the disorders that presents with primarily watery diarrhea without bleeding or perianal disease is immuno-dysregulation polyendocrinopathy, enteropathy X-linked syndrome, which results from mutations in the *FOXP3* gene.[70,71] The intestinal pathologic features vary from complete villus atrophy with apoptosis in a graft-vs-host appearance, to loss of goblet and Paneth cells, with mild inflammation. Similarly, mutations in the gene encoding *ICOS,*[72] a T-cell co-stimulatory protein, presents with watery predominantly diet-induced diarrhea starting after a few months of life with biopsy findings of villus atrophy, crypt apoptosis, and an inflammatory infiltrate (Figure 3A-C).

Functional Testing of Unknown Genetic Variants

Whole-exome/genome sequencing has led to a plethora of new or previously unconfirmed gene mutations associated with CODE. After confirmation of a variant in a novel gene, the next critical step is to confirm a plausible biological mechanism through functional assessment of gene function. This requires careful phenotyping of patient's cells, physiological assays of cellular function in both model systems and patient-derived tissue and cells. Hints at gene function may first be discerned from the clinical and morphologic

data acquired during the initial stages of diagnosis and treatment, and can direct initial tissue and cellular phenotyping.

Initial phenotyping can be carried out through characterization of key epithelial structural, transport, and cellular trafficking proteins in patient-derived tissue sections. However, complete analysis of immunostaining is often limited by the small amount of paraffin-embedded biopsy tissue available. This characterization has been facilitated by the advent of multiplex immunofluorescence imaging plat-forms,[76] where a single section can be used to stain with up to 50 antibodies through iterative staining and imaging procedures (Supplementary Figure 1A). These methods have the ability to provide a detailed phenotyping of structural defects and barrier function; epithelial cell polarity; patterns of epithelial, immune, and neural cell differentiation; and identification of immune cell subsets.

Recent advancements in deriving intestinal organoids from human induced pluripotent stem cells and isolating multipotent intestinal stem cells from human crypts has provided unprecedented opportunities to model CODE disorders in a dish.[77–80] Patient or CRISPR/Cas9-generated intestinal stem cells or induced pluripotent stem cells also provides opportunities to perform high-throughput assays with intestinal epithelium. Specifically, functional analysis of barrier function using live intestinal enteroids plated on filter supports in 2-dimensional formats can be achieved using electrical and biochemical measures of passive intercellular and transcellular transport of small solutes (ions and membrane impermeant small solutes). Both 2-and 3-dimensional enteroid cultures can be used for assessment of active (energy-dependent) trans-epithelial ion and solute transport using fluorescence ratio-imaging, enteroid swelling, and in some cases electrical approaches (Supplementary Figure 1B).

Many monogenic causes of the CODE primarily affect epithelial structure and the organization of plasma and intracellular membrane compartments. These genes affect membrane trafficking, which can be effectively and comprehensively assessed in enteroids and cells using assays for IgG intracellular transport by the Fc-receptor, FcRn, and transferrin transport by the rapidly recycling transferrin recep-tor.[81] Both probes assess function for all endosomal compartments adapted to and serving the polarized epithelial cell phenotype (Supplementary Figure 1C).

Discussion and Conclusions

Infants with CODE pose a clinical challenge that requires a structured diagnostic approach to allow early and correct diagnosis. The relatively uniform presentation of early severe diarrhea with food intolerance and the large number of potential etiologies often makes it difficult to prioritize investigations. The updated algorithm in this article harnesses the recent progress in the understanding of the pathogenesis of these disorders, along with advances in genetic testing and immunohistochemistry techniques. The algorithm suggests an initial exclusion of common causes for diarrhea in infancy, followed by early endoscopy for tissue diagnosis, focusing on the crypt to villus ratio as a simple first-line diagnostic tool. Specific immunohistochemistry staining in addition to the traditional H&E analysis further enhances the diagnostic process. The new algorithm also employs early genetic testing, highlighting

the fact that the vast majority of the CODEs with sustained symptoms are monogenic disorders. Faster and accurate diagnosis of neonatal and infantile diarrheas should improve patient care, shorten length of stay, provide better prognostication to children and their families, and will provide clinical and pathological information for improved genotype and phenotype characterization and association.

Many of the epithelial-specific CODE disorders still require either life-long PN or allogenic intestinal transplantation.[82] Transplanted patients require life-long high-dose immunosuppression that is associated with significant risk for acute and chronic opportunistic infections, allograft rejection/loss, and subsequent malignancy. Cell-based therapies of CRISPR/Cas9-corrected intestinal stem cells and autologous intestinal epithelial transplantation may be a viable option in the coming years, but will require numerous advancements in several areas, including developing ablation, implantation, and engraftment protocols.[83]

Further research via multicenter collaborations is essential for both understanding the pathogenesis of CODE disorders and for developing new therapies targeted at these rare but severe diseases. Investigation of CODEs will likely enhance our understanding of the basic biology of the intestine greatly, and may provide insights into the pathogenesis and treatment of more common gastrointestinal diseases.

Disorders of Epithelial Nutrient/Electrolyte Transport

Congenital Chloride Diarrhea

Congenital chloride diarrhea is characterized by a chronic electrolyte transport-related diarrhea that starts in utero, commonly resulting in polyhydramnios in pregnancy and severe diarrhea at birth with concomitant hypochloremic hypokalemic metabolic alkalosis. A pathognomonic feature of the diarrhea is a high fecal chloride level (>90 mmol/L) that can be used as an initial diagnostic test.[53] Congenital chloride diarrhea is caused by mutations in the Cl^-/HCO_3^- exchanger DRA *(SLC26A3),* which is required for electroneutral sodium absorption (in conjunction with Na^+/H^+ exchange) particularly in the ileum and colon. SLC26A3 mutations, therefore, result in a loss of intestinal sodium-driven fluid absorption, with resulting profuse watery diarrhea. More than 50 different mutations have been reported with founder mutations in specific populations (eg, Finnish and Arab).[84,85] Management of congenital chloride diarrhea revolves around preventing dehydration and alkalosis through enteral fluid and electrolyte supplementation. Oral administration of butyrate was found to reduce diarrheal output, although subsequent studies have reported more equivocal results.[86,87]

Congenital Sodium Diarrhea

Congenital sodium diarrhea (CSD) is characterized by a severe electrolyte transport-related diarrhea that starts in utero, and infants are often born with dilated fluid-filled loops of intestine. In rare cases, infants can develop pseudo-obstruction-like features and volvulus due to dilated fluid-filled bowel. The diarrhea in CSD is characterized by a high fecal Na^+ content, and induces a metabolic acidosis. The genetic basis of CSD is heterogeneous,

currently with 3 major known genes involved that primarily impact Na^+ absorption in the intestine. Classical or non-syndromic CSD results from loss-of-function mutations in the Na/H$^+$ exchanger NHE3, which is critical for normal Na^+ and fluid absorption from the intestine, as well as normal acid-base homeostasis.[37] CSD also results from activating mutations in the guanylin receptor GC-C *(GUCY2C),* resulting in elevated cellular cyclic guanosine monophosphate levels, inhibition of NHE3 function, and stimulation of chloride secretion via CFTR chloride channels.[50] Lastly, a syndromic form of CSD *(SPINT2)* that overlaps in phenotype with CTE has been decribed.[88] Infants with CSD typically require intensive fluid, nutrient, and electrolyte support via PN during the first year of life. Classical CSD caused by *NHE3* or *GUCY2C* mutation appears to improve over time, with eventual weaning of PN. However, both NHE3 or *GUCY2C* variants appear to be associated with an increased risk of IBD in later life.[89]

Glucose-Galactose Malabsorption

Glucose and galactose are 2 monosaccharides transported across the apical membrane by the Na-dependent glucose/galactose cotransporter (SLC5A1). Bi-allelic loss-of-function mutations of SLC5A1 are associated with a selective form of malabsorption, glucose-galactose malabsorption, which has been described thoroughly.[49] While the majority of the missense mutations impaired the trafficking of the co-transporter to the plasma membrane, those that did reach the membrane provided important insight into the structure of the transporter. Nonetheless, these children present with severe dehydration with a metabolic acidosis, and a diarrhea that ceases on a glucose/galactose-free diet. The dietary carbohydrate is limited to fructose lifelong, which is transported across the brush border by GLUT5 *(SLC2A5).*

Primary Bile Acid Diarrhea

Among the rarest causes of CODE is a selective inability to reabsorb bile salts in the intestinal portion of the enter-ohepatic circulation. A bi-allelic loss-of-function mutation of the sodium coupled bile salt re-uptake *(SLC10A2)* has been associated with watery diarrhea that stimulates colonic chloride secretion and can be managed using a bile acid seques-trant.[51] More recently bi-allelic loss-of-function mutations in the basolateral bile acid transporter OST-β *(SLC51B)* have been associated with diarrhea, severe fat-soluble vitamin deficiency, and features of cholestatic liver disease.[52]

In contrast to the diarrhea that is associated with excessive luminal bile acids, their deficiency is also associated with diarrhea and malabsorption of fat. Certainly, inherited and non-inherited conditions associated with cholestatic liver disease and surgical resection of the distal bowel may result in steatorrhea, and as many as 6 rare selective autosomal recessive congenital defects of bile acid synthesis have been described.[98]

Disorders of Epithelial Enzymes and Metabolism

Primary Lactase Deficiency

Lactose intolerance or hypolactasia is likely the most common inherited disorder that results in a gastrointestinal phenotype. Hypolactasia is an autosomal recessive age- dependent

mutation of the *LPH* gene that results in lactose-induced diarrhea after 5 years of age.[54,55] Indeed, lactase non-persistence results from 2 variants located far upstream of the *LPH* gene that impairs enhancer activity.[90] In contrast, primary lactase deficiency is exceedingly rare, and results from bi-allelic loss-of-function mutations of the *LPH* gene and leads to congenital diarrhea.[56] It should be noted that transient lactose intolerance is common in premature infants and older infants after acute gastroenteritis. Patients are managed on a lactose-free diet, and can be supplemented with oral lactase before the meal.

Sucrase-Isomaltase Deficiency

The *sucrase-isomaltase* gene *(SI)* encodes 2 subunits, sucrase and isomaltase, that form a heterodimer. Sucrase hydrolyzes sucrose, while isomaltase processes starch, isomaltose, and maltose. Bi-allelic mutations of *SI* result in a loss of either sucrase, isomaltase, or both enzyme activities, and will result in diarrhea while on such a diet.[57] Missense mutations have been shown to allow for either proper or improper targeting to the plasma membrane, with the latter resulting in a combined sucrase and isomaltase deficiency.

Diacylglyceroltransferase-1

A recently described CODE characterized by an electrolyte transport-related diarrhea, emesis, protein-losing enteropathy (PLE), and growth failure induced by enteral intake of lipids was found to be due to a loss of function mutation in *DGAT1,* which is involved in cellular triglyceride formation.[32] More recently, hypomorphic mutations with a less severe phenotype have been reported.[91] Management of *DGAT1* diarrhea relies on limiting enteral fat intake in conjunction with intravenous lipid administration to prevent essential fatty acid deficiency. The underlying mechanisms of how loss of *DGAT1* function leads to the clinical phenotype remain to be elucidated.

Disorders of Epithelial Trafficking and Polarity

Microvillus Inclusion Disease

Patients with MVID present with severe watery, electrolyte transport-related diarrhea and dehydration with failure to thrive beginning in the first days or weeks of life.[59] MVID results from inactivating mutations in myosin-Vb *(MYO5B),* a cytoskeletal motor protein, which regulates apical membrane recycling through interaction with Rab small GTPases.[33,58] MVID patients display pathognomonic microvillus inclusions by transmission electron microscopy in 10% of duodenal enterocytes.[60] Other pathological hallmarks of the disease include villus atrophy, abnormal subapical periodic acid-Schiff/CD10 staining in enterocytes, inclusion staining with antibodies against ezrin or villin, and diminution of brush-border microvillar markers, including ezrin, villin, phospho-ERM, NHE3, and DPP-IV.[92–94] Abnormal transmission electron microscopy findings and immunostaining suggest that the disease relates to impairments in Rab11A and Rab8A-dependent recycling and trafficking of critical transporters to the apical and potentially basolateral membrane in the small bowel.[92,95] Liver failure associated with MVID patients may be related to the effects of chronic PN. More recently a non-PN-related phenotype with normal GGT cholestasis was described in patients demonstrating MYO5B mutations with and without the MVID

intestinal phenotype.[63,96,97] Patients with MVID require PN support for life and can benefit from liver/in-testine transplantation if they develop PN-associated complications.

Other Microvillar Enteropathies

A truncation mutation of *STX3* and mutation in *STXBP2* elicit milder chronic diarrhea phenotypes.[98,99] Patients with *STXBP2* mutations also display hemophagocytic lympho-histiyocytosis (HLH) type 5, requiring bone marrow transplantation (BMT) to treat their HLH. However, BMT has no effect on the intestinal phenotype and PN is still required post-transplantation. Both of these mutations show short apical microvilli in enterocytes with the presence of microvilli on the lateral membrane surfaces. This phenotype resembles the pattern noted in mice with intestinally targeted deletion of Rab11a.[100]

Congenital Tufting Enteropathy

Children with CTE present with watery, sodium-losing diarrhea in the first weeks of life. Mutations in the *EPCAM* gene are the cause for the majority of CTE cases and are associated with the non-syndromic form of CTE that usually leads to isolated diarrhea.[31,62] EPCAM is localized at the basolateral membrane of the epithelial cell and plays a role in the regulation of cell adhesion and proliferation via Claudin-7.[101] The syndromic form of CTE is characterized by choanal atresia, rarely intestinal atresia, and chronic diar-rhea.[88] Mutations of the *SPINT2* gene that encodes a Kunitztype protease inhibitor have been described in syndromic CTE, as well as in cases of congenital sodium diarrhea, probably representing phenotypic diversity associated with the same mutation.[102] The pathological hallmark of CTE is focal epithelial tufts in the small and large bowel with villus atrophy. EPCAM staining is negative in cases of EPCAM mutation. Most CTE patients remain PN-dependent, but some have improved over time and can tolerate various amounts of enteral nutrition.

TTC7A Deficiency

TTC7A deficiency was recently described in children with early-onset severe abnormal electrolyte transport diarrhea and enterocolitis.[44] TTC7A is a TPR domain protein important in cell polarization and TTC7A deficiency leads to loss of the apicobasal polarity of the enterocyte, crypt-base apoptosis, crypt and villus atrophy, and chronic inflammation. The phenotype of TTC7A deficiency is diverse and ranges from severe enterocolitis to multiple intestinal atresia with recurrence of the atresia after surgical resection. Severe combined immunodeficiency with T- and B-cell lymphopenia and diminished function and hypo-gammaglobulinemia are integral parts of the disease. The prognosis is poor and almost all patients succumb during infancy. The role of BMT in this population is questionable, with limited improvement post-BMT and no effect on enterocyte structure and function.

Tricho-Hepato-Enteric Syndrome

Tricho-hepatic-enteric syndrome, also known as syndromic diarrhea, is a multisystemic disease with abnormal intestinal function and hair and facial dysmorphism in all patients, as well as liver disease in some. Woolly hair with trichorhexis nodosa on hair analysis supports the diagnosis. intrauterine growth restriction, abnormal T-cell function and antibody

production, short stature, developmental delay, abnormal platelet morphology, and cardiac defects are additional features of the disease. Small bowel biopsy demonstrates normal or mild to severe villus atrophy and variable infiltration of the lamina propria with mononuclear cells. Gastritis or colitis can appear in some cases. Tricho-hepatic-enteric syndrome is caused by mutations in the *TTC37* gene in 60% of the cases, while the remainder are associated with *SKIV2L* mutation.[106,107] Both gene products form parts of the human Ski complex that have an antiviral role. Prognosis is not only influenced by PN use and malabsorption, but also by the increased risk of infections and progressive liver disease.

PLVAP Deficiency

Mutations in the plasmalemma vesicle-associated protein (PLVAP) were recently found to result in a very severe form of PLE characterized by hypoproteinemia, hypo-albuminemia, and hypertriglyceridemia. Loss of PLVAP expression results in deletion of the diaphragms of endothelial fenestrae, leading to plasma protein extravasation and PLE, with early fatality at 5 months of age in the only case reported so far.[36]

Disorders of Enteroendocrine Cell Function

Enteric Anendocrinosis (NEUROG3)

The original disorder in this group, enteric anendocri-nosis, results from bi-allelic loss-of-function mutations of *Neurogenin3,* a basic helix-loop-helix transcription factor, which, in mice, is required for enteroendocrine and β-cell development.[64,65] Intestinal biopsies reveal a normal crypt to villus ratio, and a selective loss of all types of EECs. These children eventually develop an insulin-dependent diabetes beyond the third year of life, with an absence of autoantibodies. The lack of hyperglycemia during early infancy was originally attributed to a hypomorph missense mutation. However, subsequent cases with severe nonsense mutations and an absence of diabetes suggest that, unlike mice, other transcription factors may be sufficient to generate a b-cell mass that is sufficient to maintain normoglycemia into childhood in the absence of Neurogenin3. Recent studies indicate that mature enteroendocrine cells function as at least 1 of the quiescent stem cell populations that can dedifferentiate to active stem cells after injury, suggesting that children with this disorder may have a limited reserve capacity to replenish stem cells after stem cell injury.[108]

Enteric Dysendocrinosis (PCSK1)

Enteric dysendocrinosis is an autosomal recessive disorder resulting from loss-of-function mutations of *PCSK1,* coding for prohormone convertase (PC1/3), a protease that is required for the biosynthetic processing of hormone precursors into their fully functional forms.[66] The early clinical features mimic those of its anendocrinosis counterpart with generalized malabsorption that will require PN at least for the first several years, despite normal-appearing crypt-villus units. A distinguishing feature of children with impaired PC1/3 function is the early development of a broad group of systemic endocrinopathies, including adrenal insufficiency, hypothyroidism, and diabetes insipidus, among others; although, diabetes mellitus is not among them.[109] PC1/3 also processes neuropeptides in the hypothalamus that are required for energy homeostasis and control of appetite, including

pro-opiomelanocortin, and explains the biphasic nature of this condition. During early infancy, these children are malnourished, have severe diarrhea, and require PN, but subsequently develop a profound polyphagia and modest obesity, despite ongoing diarrhea. Pro-insulin is markedly elevated in these patients and can serve as a diagnostic test.

Other Endocrinopathies (RFX6/ARX)

Mitchell-Riley syndrome is a complex clinical condition associated with intestinal atresia, malrotation, intrinsic and extrinsic biliary duct abnormalities, and typically neonatal diabetes among many others abnormalities.[67] This disorder results from a bi-allelic mutation of *RFX6*, a transcription factor that functions both up- and downstream of NEUROG3. These children have a malabsorptive diarrhea that was once attributed to the complex gastrointestinal malformations, but it mimics other enteric endocrinopathies and biopsies reveal EECs. Recent data suggest that RFX6 may also be required for production of components of the insulin secretion pathway and is therefore required for β-cell function, and may contribute to the early age at onset of diabetes.[110]

ARX is an X-linked gene that encodes a homeobox transcription factor whose loss-of-function mutations result in a complex of clinical features of lissencephaly (smooth cerebral cortex), seizure disorder, ambiguous genitalia, and malabsorptive diarrhea. Limited studies in mice and humans suggest a possible selective reduction of GLP-1 and cholecystokinin-expressing EECs.[68,69]

Immune Dysregulation-Associated Enteropathies

FOXP3

Mutations in the *FOXP3* gene are known to cause IPEX syndrome.[70,71] The gene is located on X chromosome and, therefore, males are affected. Typically, these patients present before 6 months of age with severe watery diarrhea that does not respond to nil per os, and PN is required.[111] The intestinal pathological features vary from complete villus atrophy with apoptosis in a graft-vs-host appearance, to loss of goblet and Paneth cells, and mild inflammation. *FOXP3* mutations should be considered in all male patients with persistent diarrhea, especially those with diabetes, severe eczema, and/or thyroiditis. Patients ultimately require BMT, but may be managed with sirolimus that attenuates effector T cells.[112]

X-Linked Inhibitor of Apoptosis

X-linked inhibitor of apoptosis is encoded by the *BIRC4* gene, initially described in patients with X-linked HLH syn-drome.[113] However, it is now known that X-linked inhibitor of apoptosis patients may present with intestinal disease that varies from villous atrophy to severe enterocolitis with perianal disease.[114] Unlike other CODE disorders, the onset of symptoms occurs from less than 6 months of age to up to 40 years of age. A high degree of suspicion is needed for patients with diarrhea and biochemical features of HLH.

LRBA/CTLA4

Recently, a new multisystemic disease with autoimmunity, primary immunodeficiency, and autoimmune enteropathy as the main feature was described and caused by 2 distinct genes. *CTLA4* mutations were identified as autosomal dominant with variable penetrance,[115] while *LRBA*[116] mutations cause a phenocopy due to autosomal recessive mutations. CTLA4 functions as an early checkpoint controlling T-cell response to antigens. LRBA is expressed on Rab11-positive recycling endosomes and appears to function in CTLA4 recycling.[117] Patients with CTLA4 and LRBA mutations have a variable age of presentation from the first days of life to adulthood. They may have severe villus atrophy with IPEX-like autoimmune features, including autoimmune enteropathy and common variable immune deficiency features. These patients may be treated with abatacept, a drug containing the extracellular domain of CTLA4. BMT has been shown to be a potential long-term therapy.

CD55 Deficiency (CHAPLE Syndrome)

CD55 deficiency with hyperactivation of complement, angiopathic thrombosis, and PLE (CHAPLE syndrome).[118] Most patients present in the first 2 years of life with PLE due to primary intestinal lymphangiectasia, abdominal pain, thromboembolic disease, recurrent infections, and various degrees of bowel inflammation. Mutations in the gene encoding *CD55,* a complement regulator, lead to hyperactivation of complement. Treatment with eculizumab, a complement inhibitor, leads to improvement in PLE symptoms.

ADAM17 Deficiency

ADAM17 is a protein with metalloprotease properties that cleaves and activates numerous membrane-bound precursors, including TNFα, EGF, and Notch. Bi-allelic mutations of *ADAM17* have been described in several patients with a severe skin rash; bloody/watery diarrhea; abnormal hair and disorganized eyelashes and eyebrows; and recurrent skin, nail, and enteric infections.[119,120] The disease appears in the first days of life, and 2 of the 3 patients described died during childhood. Small bowel biopsies show villus blunting, lengthening of crypts, and mononuclear cell infiltrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations used in this paper:

CODE	congenital diarrhea and enteropathy
CTE	congenital tufting enteropathy
EEC	enteroendocrine cell
EPCAM	epithelial cell adhesion molecule
MVID	microvillus inclusion disease
NEC	necrotizing enterocolitis

PN	parenteral nutrition
SBS	short-bowel syndrome

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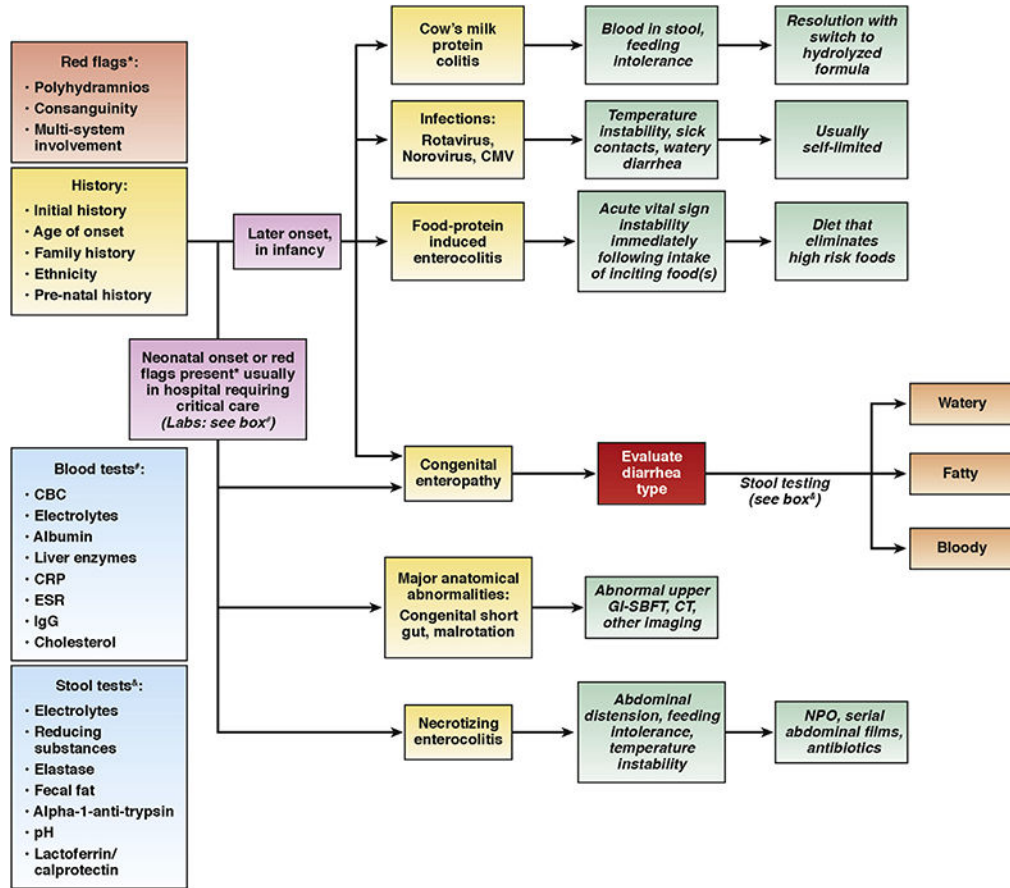
Figure 1.
Initial evaluation of neonate/infant with diarrhea. Initial evaluation of diarrhea in infants starting with key historical information, identification of red-flag findings, and key stool studies and blood tests. Infants with red-flag findings or that require critical intervention soon after birth will need evaluation for NEC and anatomical abnormalities, as well as further investigation for congenital diarrheas. Important common etiologies to investigate especially presenting beyond the neonatal period include cow's milk protein colitis, infections, food protein enterocolitis. CBC, complete blood count; CMV, cytomegalovirus; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GISBFT, gastrointestinal fluoroscopy with small bowel follow-through, NPO, nil per os.
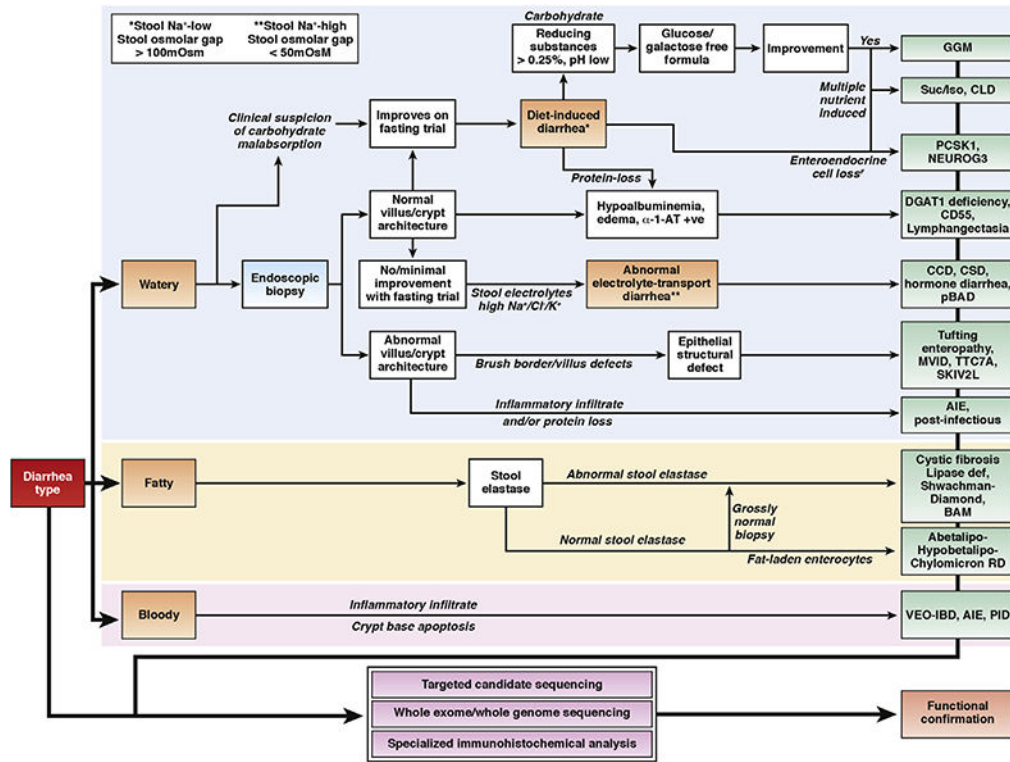
Figure 2.

Diagnostic algorithm for evaluation of CODEs. Abetalipo, abetalipoproteinemia; CLD, congenital lactase deficiency; CMP, cow's milk protein; DGAT1, diacylglyceroltransferase 1; GGM, glucose galactose malabsorption; Hypobetalipo, hypobetalipoproteinemia; NEUROG3, Neurogenin3 deficiency; pBAD, primary bile acid diarrhea; BAM, bile acid malabsorption; PCSK1, proprotein convertase kinase deficiency; RD, retention disease; Suc/Iso, sucrose-isomaltase deficiency; TTC7A, tetratricopeptide repeat domain 7A; VEOIBD, very-earlyonset inflammatory bowel disease.
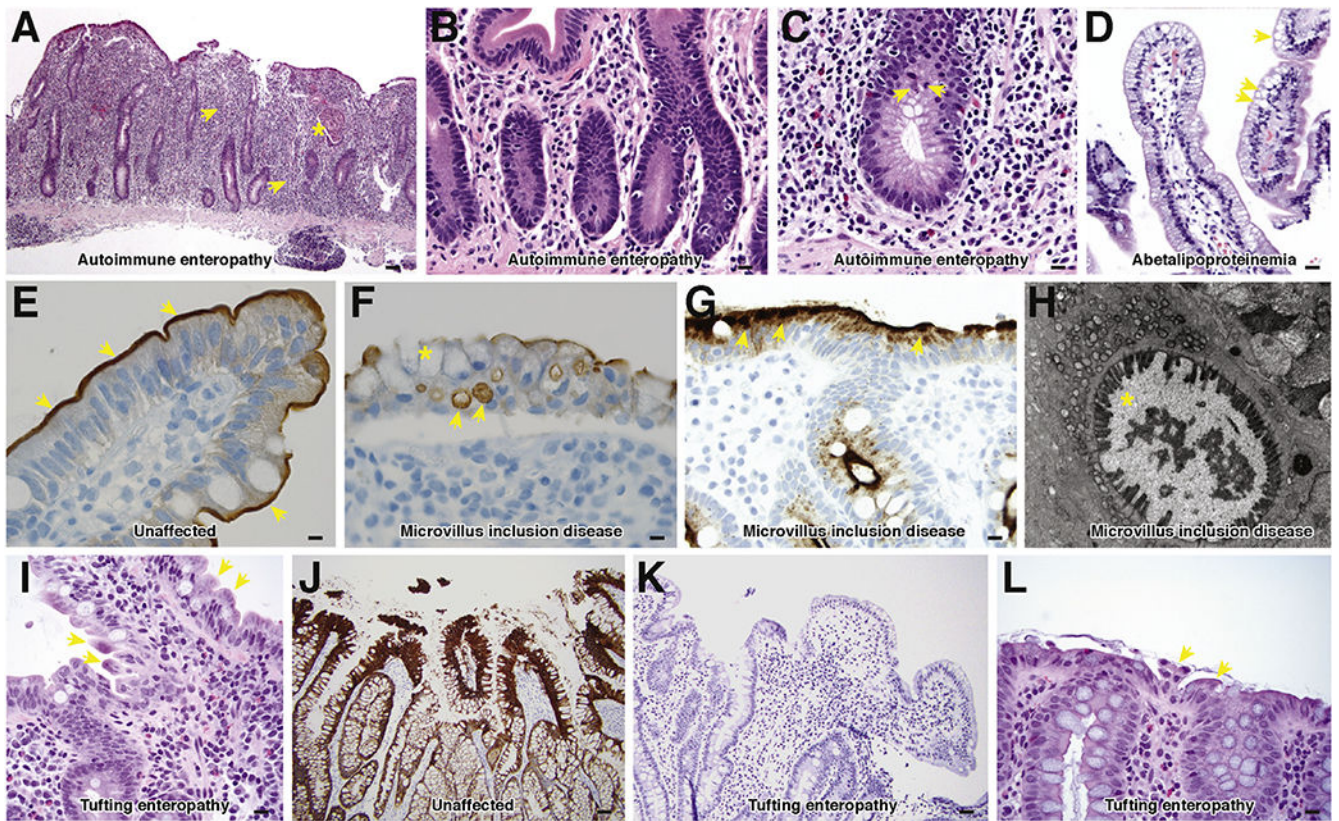
Figure 3.
CODE histology. (*A*) Autoimmune enteropathy (H&E) showing extensive villus atrophy, crypt hyperplasia, increased lamina propria inflammation (*arrows*), neutrophilic crypt abscess (*asterisk*). Scale bar = 50 μm. (*B*) Autoimmune enteropathy (H&E) showing crypt base apoptosis. Scale bar = 10 μm. (*C*) Autoimmune enteropathy (H&E) showing crypt intraepithelial lymphocytosis, absent Paneth cells and rare crypt epithelial apoptosis (*arrows*). Scale bar = 10 μm. (*D*) Abetalipoproteinemia (H&E) showing fat globules in enterocytes. Scale bar = 25 μm. (*E*) Normal villus stained with villin showing clear brush-border staining (*arrows*). Scale bar = 5 μm. (*F*) MVID patient with abnormal villin staining with villin signal present in intracellular microvillus inclusions (*arrows*) and relative lack of brush-border staining (*asterisk*). Scale bar = 5 μm. (*G*) MVID patient with abnormal intracellular CD10 staining (*arrows*). Scale bar = 10 μm. (*H*) Transmission electron micrograph showing characteristic microvillus inclusion in MVID. (*I*) Tufting enteropathy patient showing characteristic epithelial tufts in the small intestine (*arrows*). Scale bar = 25 μm. (*J*) Membranous MOC31 (EPCAM) staining in unaffected patient. Scale bar = 50 μm. (*K*) Complete loss of MOC31 (EPCAM) staining in tufting enteropathy patient. Scale bar = 50 μm. (*L*) Characteristic tufts also seen in the colon in tufting enteropathy (*arrows*). Scale bar = 25 μm.
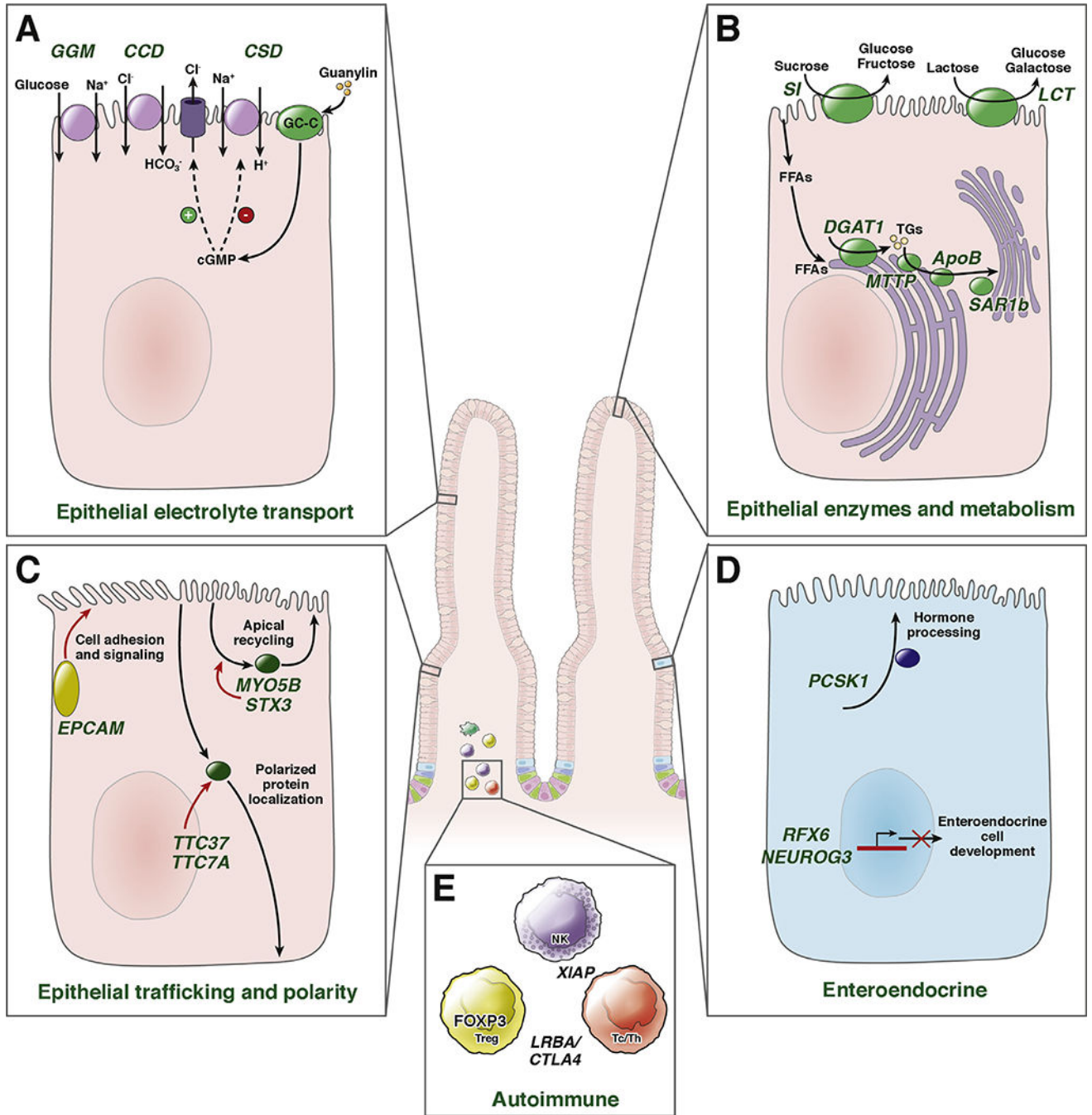
Figure 4.
Pathophysiology of CODEs: Illustration of major categories of CODEs with example disorders. (*A*) Disorders of epithelial transport: GGM, glucose galactose malabsorption affecting sodium glucose cotransporter *SLC5A1*; CCD, congenital chloride diarrhea affecting the Cl/HCO3 – exchanger DRA (*SLC26A3*); CSD, congenital sodium diarrhea affecting the Na/Hþ exchanger, NHE3 and the guanylin receptor GC-C (*GUCY2C*); cGMP, cyclic guanosine monophosphate. (*B*) Disorders of epithelial enzymes and metabolism: *SI*, sucrase-isomaltase deficiency; *LCT*, lactase deficiency; *DGAT1*, diacylglyceroltransferase 1

deficiency, hypobetalipoproteinemia affecting apolipoprotein B (ApoB), abetalipoproteinemia affecting microsomal triglyceride transfer protein (*MTTP*) and chylomicron retention disease affecting *SAR1B*. (*C*) Disorders of epithelial trafficking and polarity: MVID affecting myosin 5b (*MYO5B*) and syntaxin 3 (*STX3*), Tufting enteropathy affecting EPCAM. *TTC7A*, tetratricopeptide repeat domain 7A. (*D*) Disorders of enteroendocrine cells: *PCSK1*, proprotein convertase kinase deficiency; *NEUROG3*, Neurogenin3 deficiency; *RFX6*, Mitchell-Riley syndrome. (*E*) Immune dysregulation–associated enteropathy: X-linked inhibitor of apoptosis (XIAP) affecting *BIRC4, FOXP3, CTLA4, LRBA* affecting T- and B-cell regulation/stimulation.

Table 1.

Stool Testing

Stool test	Notes
Electrolytes	This is a key test in evaluating the nature of the diarrhea and for calculation of the stool osmotic gap. High Na^+ or Cl^- is often reflective of alterations in intestinal ion transport.
	Stool osmotic gap calculation: $290 - 2 \times$ (Stool $Na^+ + K^+$):
	Stool osmotic gap >100 mOsm is defined as high; <50 mOsm is defined as low; and a gap between 50 and 100 mOsm is defined as intermediate.
Osmolality	In almost all cases, stool osmolality is iso-osmolar to serum (~290 mOsm) and, therefore, generally does not need to be sent. If there is a suspicion of improper collection (delay or contamination with urine/water), measurement of stool osmolality can provide objective evidence for this, as it will be relatively dilute or hypoosmolar to serum.
Reducing substances and pH	Reducing substances >0.5% indicates malabsorption of monosaccharides. Low pH (<5.3) results from an abundance of short-chain fatty acids that are the products of fermentation and is indicative of carbohydrate malabsorption. However, these must be interpreted in the context of the diet, as some amount of carbohydrate malabsorption is normal in neonates and related to ongoing development of the intestine.
α1-antitrypsin	Serum protein that is largely resistant to the action of intestinal proteases unless it is secreted in the stomach. Elevation in stool reflects intestinal protein loss (ie, protein-losing enteropathy).
Fat	Quantitative 72-h fecal fat collection is ideal to evaluate fat malabsorption, although it can be difficult in practice. It requires an accurate estimate of the daily enteral fat intake to calculate daily percentage absorption. Percentage fat absorption varies with age and is relatively low in infants, with 15%–20% loss in normal stools. Qualitative or spot fecal fat (neutral or split) is a helpful initial test. Elevation in neutral fat reflects increased mono-, di-, or triglyceride content in the stool and can suggest pancreatic insufficiency. Elevation in split fat reflects increased free fatty acids in stool and correlates with intestinal fat malabsorption
Elastase	Elastase is unchanged by intestinal proteases and, if low, can imply pancreatic insufficiency, but may be diluted and reduced in high-volume chronic diarrhea not associated with pancreatic insufficiency.
Occult blood	Microscopic blood can be evaluated in the stool, however, it should be noted that it is unhelpful as a discriminator of bowel inflammation vs cutaneous irritation caused by severe diarrhea. We therefore do not recommend its use in initial testing.
Inflammatory markers	Elevated lactoferrin or calprotectin are well-correlated with the presence of intestinal inflammation.
Infection studies	Standard assessment of enteric bacterial pathogens by culture and/or by polymerase chain reaction. Viral pathogens, such as rotavirus, cytomegalovirus, norovirus, and adenovirus, should be tested by polymerase chain reaction and/or enzyme-linked immunosorbent assay.

Table 2.

Classification of Diarrheas and Enteropathies in Infants

Variable	Gene Name	OMIM no.	Inheritance	Protein function
Epithelial nutrient/electrolyte transport				
Congenital chloride diarrhea	*SLC26A3*	126650	AR	Cl^-/HCO_3^-exchanger
Congenital sodium diarrhea	*SLC9A3*	616868	AR	Na^+/H^+ exchanger (NHE3)
Congenital sodium diarrhea	*GUCY2C*	601330	AD	Guanylate cyclase
Glucose-galactose malabsorption	*SLC5A1*	606824	AR	Na+−glucose cotransporter
Primary bile acid diarrhea	*SLC10A2*	601295	AR	Ileal bile salt transporters
	SLC51B			
Acrodermatitis enteropathica	*SLC39A4*	201100	AR	Zinc transporter
Epithelial enzymes and metabolism				
Congenital lactase deficiency	*LCT*	603202	AR	Disaccharidase
Sucrase-isomaltase deficiency	*SI*	609845	AR	Disaccharidase
Trehalase deficiency	*TREH*	612119	AR	Disaccharidase
Enterokinase deficiency	*TMPRSS15*	606635	AR	Pro-enterokinase
DGAT1 deficiency	*DGAT1*	604900	AR	Triglyceride synthesis
PLVAP deficiency	*PLVAP*	607647	AR	Endothelial fenestrae
Abetalipoproteinemia	*MTP*	157147	AR	Microsomal triglyceride transfer protein
Hypobetalipoproteinemia	*APOB*	107730	AR	Lipid absorption
	ANGPTL3	605019	AR	
Chylomicron retention disease	*SAR1B*	607690	AR	Intracellular chylomicron trafficking
Dyskeratosis congenita	*TERT*	613989	AR/AD	Maintenance of telomeres
Kabuki syndrome	*KMT2D*	147920	AD	Histone methyltransferase
Epithelial trafficking and polarity				
Microvillus inclusion disease	*MYO5B*	606540	AR	Cellular trafficking, polarity, and signaling
	STX3	600876	AR	
Tufting enteropathy	*EPCAM*	185535	AR	Cell-cell adhesion and signaling
Syndromic Na+ diarrhea	*SPINT2*	605124	AR	Serine protease inhibitor
Trichohepatoenteric syndrome 1	*TTC37*	614589	AR	Cell polarity and signaling
Trichohepatoenteric syndrome 2	*SKIV2L*	600478	AR	Helicase
Familial hemophagocytic lymphohistiocytosis 5	*STXBP2*	613101	AR	Syntaxin-binding protein
TTC7A deficiency	*TTC7A*	609332	AR	Protein transport and trafficking
Enteroendocrine cell dysfunction				
Enteric anendocrinosis	*NEUROG3*	604882	AR	Transcription factor-cell fate
X-linked lissencephaly and MR	*ARX*	300382	X-linked	Homeodomain transcription factors
Proprotein convertase 1/3 deficiency	*PCSK1*	162150	AR	Neuroendocrine convertase
Mitchell-Riley syndrome	*RFX6*	612659	AR	Transcription factor-cell fate
Immune dysregulation-associated enteropathy [a]				
IPEX	*FOXP3*	300292	X-linked	Regulatory T-cell
ICOS deficiency	*ICOS*	604558	AR	T-cell receptor

Variable	Gene		Inheritance	Protein function
	Name	OMIM no.		
ADAM17 deficiency	*ADAM17*	603639	AR	TNF-α-converting enzyme
EGFR deficiency	*EGFR*	616069	AR	EGF receptor
CD55 deficiency	*CD55*	125240	AR	Complement cascade inhibitor
CTLA4 deficiency	*CTLA4*	123890	AD	Costimulatory signaling
LRBA deficiency	*LRBA*	606453	AR	Endosomal trafficking regulator
XIAP	*BIRC4*	300079	X-linked	Inhibitor of apoptosis

AD, autosomal dominant; AR, autosomal recessive; MR, mental retardation; OMIM, Online Mendelian Inheritance in Man.

[a]For a detailed list of very-early-onset inflammatory bowel disease/autoimmune enteropathy genes refer to Uhlig et al.[75]