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Recent Progress in Congenital Diarrheal Disorders

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Abstract Congenital diarrheal disorders (CDD) are a group of rare enteropathies related to specific genetic defects. Infants with these disorders have chronic diarrhea, frequently requiring parenteral nutrition support. Etiologies and prognoses are variable. We propose a new classification of CDD into four groups, taking into account the specific etiology and genetic defect: 1) defects in digestion, absorption, and transport of nutrients and electrolytes; 2) disorders of enterocyte differentiation and polarization; 3) defects of enteroendocrine cell differentiation; and 4) dysregulation of the intestinal immune response. The present review focuses on the recent advances made in understanding the pathophysiology of CDD that could potentially improve the clinical approach to these conditions.

Keywords Congenital chloride-losing diarrhea · Congenital sodium diarrhea · Microvillous inclusion disease · Tufting enteropathy · Enteric anendocrinosis · Immune dysregulation · Polyendocrinopathy · Enteropathy · X-linked syndrome

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Introduction

Congenital diarrheal disorders (CDD) are a group of rare enteropathies related to specific genetic defects, which are generally inherited as autosomal recessive traits [1, 2••]. Frequently, infants with these disorders present, early in life, with chronic diarrhea of sufficient severity to require parenteral nutrition support. Milder forms with subtle clinical signs that remain undiagnosed until adulthood, when patients develop complications, are also possible for some conditions [3••]. Despite similar clinical presentations, the causes, management, and prognosis of the various CDD are very different. The number of conditions included in the CDD group has gradually increased over the years, and many new genes have been identified and functionally related to CDD. We suggest a new classification of CDD in relation to the genetic defects, taking into account diagnostic approach and therapeutic management [3••]. This classification includes four groups of CDD: 1) defects in digestion, absorption, and transport of nutrients and electrolytes; 2) disorders of enterocyte differentiation and polarization; 3) defects of enteroendocrine cell differentiation; and 4) dysregulation of intestinal immune response.

The exact incidence of these disorders remains to be established and differs widely among populations and geographic areas [4, 5]. In a recent nationwide Italian study, reviewing 5,801 cases, we estimated an occurrence rate of CDD of 1 per 2,000 hospitalized newborns. A study from the Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition (SIGENP) estimated that altered modulation of the intestinal immune response and altered enterocyte differentiation and polarization are the most common causes of CDD [6].

From Genes to Future Strategies for Effective Clinical Management

The main diseases included in the CDD group are presented here, subdivided according to the pathogenetic mechanisms. Tables 1 and 2 provide summary information about the molecular bases of all conditions presenting with congenital diarrhea of osmotic or nonosmotic nature.

Digestion, Absorption, and Transport of Nutrients and Electrolytes

Congenital Lactase Deficiency

Lactose, a disaccharide unique to mammalian milk, is hydrolyzed into the monosaccharides glucose and galactose at the brush border of small intestinal enterocytes on the villous tip by the enzyme lactase, a β-D-galactosidase known as lactase phlorizin hydrolase (LPH) (EC 3.2.1.23) [7]. Although LPH has structural analogies with other α-glycosidases of the brush-border membrane, it has a distinctive neutral β-glycosidic activity. Evidence indicates the presence of two independent active sites in the lactase molecule, one responsible for LPH activity and the other associated with the phlorizin hydrolase activity. The physiologic significance of the phlorizin hydrolase activity is unknown; however, it has been proposed that the active site could be involved in the hydrolysis of the glycosidic moiety of glycosylceramides and vitamins. LPH is synthesized as a polypeptide of about 220 kDa, which follows a complex intracellular processing route involving heavy glycosylation with N-linked and O-linked carbohydrates, with intermediate forms displaying a molecular weight of up to 280 kDa, and one or two proteolytic steps that render a final mature product with a molecular weight of about 160 kDa. Congenital lactase deficiency (CLD [MIM 223000]), a severe form of lactase deficiency in which lactase activity is very low or absent in the intestinal wall from birth [7], seems to be more common than previously assumed. Mutations in the lactase gene (*LCT* [GeneID: 3938]) were recently identified to underlie CLD in Finnish families [8]. Of the five mutations, *Y1390X* was the founder mutation present in 90% of the disease alleles. In addition, four other family-specific mutations, *S1666fsX1722*, *S218fsX224*, *G1363S*, and *Q268H* were identified in the

Table 1 Molecular basis of congenital diarrheal diseases determined by osmotic mechanisms[a]

Disease	Gene	Function
Defects in digestion, absorption, and transport of nutrients and electrolytes		
Congenital lactase deficiency	LCT	Lactase-phlorizin hydrolase activity
Sucrase-isomaltase deficiency	EC 3.2.1.48	Isomaltase-sucrase
Maltase-glucoamylase deficiency	MGAM	Maltase-glucoamylase activity
Glucose-galactose malabsorption	SGLT1	Na^+/glucose cotransporter
Fructose malabsorption	GLUT5	Fructose transporter
Fanconi-Bickel syndrome	GLUT2	Basolateral glucose transporter
Cystic fibrosis	CFTR	cAMP-dependent Cl^- channel
Acrodermatitis enteropathica	SLC39A4	Zn^{2+} transporter
Congenital chloride-losing diarrhea	SLC26A3	Cl^-/base exchanger
Congenital sodium diarrhea	SPINT2	Serine-protease inhibitor
Lysinuric protein intolerance	SLC7A7	AA basolateral transport
Congenital bile acid diarrhea	ABAT	Ileal Na^+/bile salt transporter
Shwachman-Diamond syndrome	SBDS	RNA metabolism
Enterokinase deficiency	PRSS7	Proenterokinase
Trypsinogen deficiency	PRSS1	Trypsinogen synthesis
Pancreatic lipase deficiency	PNLIP	Hydrolyzes triglycerides to fatty acids
Abetalipoproteinemia	MTP	Transfer lipids to apolipoprotein B
Hypobetalipoproteinemia	APOB	Apolipoprotein that forms chylomicrons
Chylomicron retention disease	SAR1B	Intracellular chylomicron trafficking
Congenital bile acid malabsorption	SLC10A2	Bile salt transport
Defects of enteroendocrine cell differentiation		
Enteric anendocrinosis	NEUROG3	Enteroendocrine cell fate determination
Enteric dysendocrinosis	Unknown	Enteroendocrine cell function
Proprotein convertase 1 deficiency	PCSK1	Prohormone processing

[a] Diarrhea is considered osmotic if luminal substances are responsible for the induction of fluid secretion: diarrhea ends with fasting. The fecal ion gap > 100 mOsm suggest this mechanism. Fecal ion gap is calculated by a formula: fecal osmolality (290 mOsm) – 2 × {[Na^+]fecal+[K^+]fecal}. In the case of congenital chloride-losing diarrhea, a low ion gap is due to high fecal chloride loss

cAMP cyclic adenosine monophosphate

Table 2 Molecular basis of congenital diarrheal diseases determined by nonosmotic mechanisms[a]

Disease	Gene	Function
Defects of enterocyte differentiation and polarization		
Microvillous inclusion disease	*EpCAM*	Intracellular protein trafficking
Congenital tufting enteropathy	Unknown	Cell-cell interaction
Syndromic diarrhea	Unknown	Unknown
Defects of modulation of intestinal immune response		
IPEX syndrome	*FOXP3*	Transcription factor
IPEX-like syndrome	Unknown	Unknown
Immunodeficiency-associated autoimmune enteropathy	Unknown	Unknown
Autoimmune polyglandular syndrome-1 (APS-1)	*AIRE*	Regulation gene transcription
Autoimmune enteropathy with colitis-generalized autoimmune gut disorder (GAGD)	Unknown	Unknown

[a] Diarrhea is considered nonosmotic if endogenous substances induce fluid secretion: diarrhea persists even when the patient is fasting (fecal ion gap < 100 mOsm).

IPEX immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome

Finnish population. Very recently, four novel mutations in the *LCT* gene were identified [9]. The mutations are quite evenly distributed, covering both the pro region and the mature LPH. The location of mutation in *LCT* does not seem to affect the severity of diarrhea, and all mutations lead to a severe osmotic diarrhea, severe dehydration, and weight loss. The phenotype of CLD is in striking contrast with the phenotype of adult-type hypolactasia, associated with the normal down-regulation of lactase activity and producing mild or absent symptoms. It is noteworthy that in these two conditions, the values of lactase activity are partially overlapping, in spite of their having different underlying molecular mechanisms [7]. However, symptoms are much more severe in CLD.

Sucrase-Isomaltase Deficiency

Intestinal digestion of sucrose requires hydrolysis to glucose and fructose, which in mammalian species is performed by the sucrase enzyme activity (EC 3.2.1.48) of the sucrase-isomaltase (SI) complex [10]. The gene coding for the human SI complex is located on chromosome 3, producing a protein with a predicted size of about 210 kDa. The mature SI is a complex composed of two subunits: sucrase and isomaltase. The molecule is synthesized as a single polypeptide with a molecular weight of 240–260 kDa. The precursor molecule is subjected to complex intracellular glycosylation, with the addition of N-linked and O-linked carbohydrates, but without proteolysis. After glycosylation, the fully active pro-enzyme is transported and inserted in the apical membrane of the enterocytes via its N-terminus. In the apical membrane of enterocytes, SI is subjected to extracellular processing by pancreatic proteolytic enzymes present in the intestinal lumen that cleave the complex, generating free S and membrane-bound I subunits. The cleaved molecules remain associated with each other through noncovalent interactions. Congenital sucrase-isomaltase deficiency usually becomes apparent after an infant is weaned and starts to consume fruits, juices, and grains. After ingestion of sucrose or maltose, an affected child typically will experience stomach cramps, bloating, excess gas production, and diarrhea. These digestive problems can lead to failure to gain weight and grow at the expected rate (failure to thrive) and malnutrition. Most affected children are better able to tolerate sucrose and maltose as they get older.

Maltase-Glucoamylase Deficiency

Maltose (O-α-D-glucopyrinosyl-[1→4]-α-D-glucopyranoside) and longer glucose oligomers are the principal products of amylase digestion of starches. The limited amylolytic activities of salivary and pancreatic amylases cause the accumulation of glucose oligomers with 2–20 residues, without significant release of free glucose monomers. The enzyme MGAM, which shows a high α–1-4 glycohydrolytic activity, provides the activity necessary to degrade these oligomers efficiently [11]. In contrast to the endoglucanase α–1-4 activity of amylases, MGAM is an exoglucanase that cleaves the terminal α–1-4 glycosidic bond at the nonreducing end of amyloses and glucose oligomers (EC 3.2.1.20). Although in human beings MGAM contributes only about 20% of total intestinal maltase activity (EC 3.2.1.3), the averaged Michaelis constant (K_m) of MGAM for the hydrolysis of glucose oligomers is about 75 times lower than SI complex. MGAM activity is therefore an efficient in vivo producer of free glucose and is limited only by branching at α–1-6 glucosidic bonds. Thus, MGAM accounts for the bulk of the

glucogenic activity resulting from the digestion of glucose oligomers generated by amylase activities. The combined activities of MGAM and SI are not rate-limiting to starch oligomer digestion and glucose absorption. The human *MGA* deficiency gene is located on the chromosome 7 [11]. The encoded protein contains 1,857 amino acid residues that undergo extensive N and O glycosylation, which renders a mature protein with a molecular weight of about 335 kDa inserted into the plasma membrane by its N-terminal end. In contrast to SI and LPH, no intracellular or extracellular proteolytic processing has been documented in human *MGAM*. The genomic and complementary DNA sequences for human *MGAM* have revealed the presence of at least two different subunits in the mature protein, which display high homology to the respective subunits of the SI complex and contain one potential active site each. These similarities have led to the suggestion that *MGAM* and SI are related proteins arising by duplication of an ancestral gene. Because of the relatively recent description of the *MGAM* gene and its complementary DNA, no studies on the hormonal or genetic regulation of its messenger RNA (mRNA) expression exist. In addition, in most studies, no attempts to differentiate *MGAM* maltase and amylolytic activities from those of SI complex have been performed, hampering an accurate evaluation of the effects of feeding and hormone stimuli on the synthesis of the *MGAM* protein. These limitations are particularly important in human beings, in whom *MGAM* contributes to a small fraction of the maltase activity [10]. The effects deduced from the differential changes of sucrase and maltase activities have suggested that *MGAM* expression and activity often follow that of its complementary enzyme, SI. The symptoms of *MGAM* are poorly defined. *MGAM* deficiency may cause chronic diarrhea in young children. Although the osmolar force of starch is less than that of sucrose or lactose because of its larger molecular weight, starch-elimination diets relieved diarrhea in the reported cases.

Glucose-Galactose Malabsorption

Glucose-galactose malabsorption (GGM) is a rare autosomal recessive disorder that causes severe life-threatening chronic diarrhea, because of a mutation in the gene *SGLT1* that codes for a transporter responsible for the tight coupling of two Na^+ ions and one glucose or galactose molecule across the membrane during one catalytic turnover [12••, 13•]. The basolateral Na-K-ATPase pump maintains the low intracellular Na^+ concentration, and this results in the absorption of Na^+, Cl^-, HCO_3^-, sugar, and water across the epithelium. In patients with GGM, more than 50 mutations in the *SGLT1* gene have been identified. The malabsorbed glucose and galactose and the derived short-chain fatty acids (SCFA) that reach the colon determine an osmotic diarrhea. All missense mutations that do not impair sugar transport, *Phe405Ser* and *His615Gln*, are not conserved and rarely occur in the general population. In contrast, the *Asn51Ser* variant occurs in the general population at a frequency of 4% [12••, 13•].

Fructose Malabsorption

Although the ability of *GLUT5* to transport fructose is well established, there remains insufficient evidence confirming that *GLUT5* represents the main fructose facilitative carrier in the intestine [14]. Recent studies have identified *GLUT7* as a high affinity glucose and fructose carrier on the brush border membrane of the enterocytes in the distal intestine. Recent studies also suggest a role of *GLUT2* in the absorption of large amounts of fructose across the enterocytes of rats [15]. Studies performed in rodents have confirmed that *GLUT5* expression is regulated in a tissue- and development-specific manner and is induced by dietary fructose [3••]. *GLUT5* is found at very low levels prior to weaning, and increases dramatically in weaned animals [3••]. However, an analysis of the age dependence of *GLUT5* levels has not been performed in humans.

Congenital Chloride-Losing Diarrhea

Congenital chloride-losing diarrhea (Online Mendelian Inheritance in Man [OMIM] 126650) is a typical clinical model of the CDD subgroup that is caused by altered absorption and transport of nutrients and electrolytes [16••]. The main clinical symptom is a lifetime watery diarrhea with high Cl^- content and low pH, causing dehydration and hypochloremic metabolic alkalosis. Congenital chloride-losing diarrhea (CCD) may be fatal, if not adequately treated. Long-term prognosis is generally favorable, but complications such as renal disease, hyperuricemia, inguinal hernias, spermatoceles, and male subfertility are possible. Individual variation in the clinical picture of congenital chloride-losing diarrhea is common, and some mild cases have been diagnosed in adulthood [16••]. This rare genetic disease is caused by mutations in the gene encoding the solute-linked carrier family 26-member A3 (*SLC26A3*) protein, which acts as a plasma membrane anion exchanger for Cl^- and HCO_3^-. The *SLC26A3* gene maps close to the *CFTR* gene (cystic fibrosis transmembrane conductance regulator) on chromosome 7 [16••]. In ethnic groups in which the disease is frequent, there is a single mutation: in Finland, the *p.V317del* mutation affects up to 90% of alleles; in Saudi Arabia and Kuwait, *p.G187X* is present in more than 90% of altered chromosomes; in Poland, 50% of alleles carry the *I675-676ins* mutation (official nomenclature: c.2022_2024dup–p.I675dup). In different ethnic groups, a wide genetic heterogeneity has been found [16••]. Little is known about the mechanism by which

these mutations undermine function. The C-terminal conserved domain, called "STAS," ensures the correct location of the *SLC26A3* protein on the apical membrane of enterocytes, where it interacts with the R-domain of the *CFTR* gene. Mutations in the STAS domain cause mistrafficking and cytosol retention of *SLC26A3* protein, leading to reduced levels of the protein at the plasma membrane. Treatment with the SCFA butyrate is able to increase expression of *SLC26A3*, thereby reducing fecal ion loss [3••].

Congenital Sodium Diarrhea

Congenital sodium diarrhea (CSD; OMIM 270420) is a rare disorder characterized by severe diarrhea consequent to sodium malabsorption, with consequent hyponatremia and metabolic acidosis [17]. High mortality due to electrolytic alterations has been reported in the few cases described so far. Studies based on the "candidate gene" approach failed to identify the disease-gene among the 6 known isoforms of sodium-proton exchangers (NHE 1–6). However, the gene of the syndromic form of CSD, namely *SPINT2*, was recently identified [2••, 3••]. The syndromic form of CSD is characterized by choanal or anal atresia, hypertelorism and corneal erosions, double kidney, cleft palate, and digital anomalies. No mutations have been identified in the *SPINT2* gene in patients bearing the classic form of the disease.

Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome (FBS; OMIM 227810) is a very rare autosomal recessive disorder that is characterized by carbohydrate malabsorption, tubular nephropathy, hepatomegaly, and glucose metabolism disorders. Homozygous mutations in the facilitative glucose transporter, *GLUT2*, have been identified in patients with FBS. Functional studies have not been performed to confirm that these mutations alter the functionality of *GLUT2* protein, and the mechanism by which mutation in the carrier *GLUT2* affects glucose metabolism is not well understood. Impaired hepatocyte transport and glucose-sensing mechanism by pancreatic β cells are a possible explanation. In the intestine, the presence of a *GLUT2*-independent pathway for glucose transport is plausible. In fact, severe osmotic diarrhea is generally not associated with FBS, and evidence from mice suggests that mutations in *GLUT2* do not particularly affect monosaccharide transport across the enterocyte [18].

Acrodermatitis Enteropathica

Primary acrodermatitis enteropathica (PAE; OMIM 201100) is a rare autosomal recessive disease of impaired intestinal absorption of zinc [19]. A candidate gene has been identified, which encodes for a member of the zinc- and iron-regulated transporter family of proteins (*ZIP4*). *ZIP4* is expressed on the apical membrane of epithelia in the intestine and kidney. Although patients with PAE have a reduced capacity to absorb luminal zinc, their responsiveness to oral zinc load raises the possibility that they retain a redundant, but less efficient, zinc transport mechanism in the intestine [19].

Lysinuric Protein Intolerance

Lysinuric protein intolerance (LPI; OMIM 222700) is transmitted in an autosomal recessive manner resulting from mutations in the *SL7A7* gene, which encodes for the cationic amino acid transporter y^+LAT1 [20]. The y^+LAT1 transporter is situated on the basolateral membrane of the enterocyte and renal tubules, and transports the cytoplasmic basic amino acids (lysine, arginine, ornithine) in exchange for sodium and neutral amino acids. More than 30 mutations have been identified in the *SLC7A7* gene. Founder mutations have been identified in Finnish, Japanese, and Italian patients. Ornithine and arginine are important urea cycle intermediates that fail to exit the epithelial cells of patients with LPI. Their relative deficiency, during a high-protein milk diet, results in urea cycle dysfunction that leads to hyperammonemia and subsequent alteration in mental status [20].

Shwachman-Diamond Syndrome

Shwachman-Diamond Syndrome (SDS; OMIM 260400) is a very rare autosomal recessive disorder (frequency of 1:76000) with clinical features that include hematological dysfunction, pancreatic exocrine insufficiency, and skeletal abnormalities [21]. Patients with SDS are significantly predisposed to the development of hematological abnormalities, including cytopenias of one or more lineages, myelodysplasia, and acute leukemia. SDS-associated mutations were recently described in a gene designated *SBDS* (Shwachman-Bodian-Diamond syndrome) that encodes a member of a highly conserved protein family of unknown function with orthologues in diverse species, including Archaea, plants, and eukaryotes but not Eubacteria [21]. Indirect evidence supports the hypothesis that the *SBDS* protein family may function in RNA metabolism. At least 90% of affected individuals carry one common conversion mutation, whereas 50% are compound heterozygotes with respect to the *K62X* and *C84Cfs* mutations. Strikingly, no homozygotes for the *K62X* mutation were identified, suggesting that this may be a lethal mutation. Alleles from affected individuals who do not have the common conversion mutations carry additional frameshift and missense mutations in the *SBDS* coding region [21].

Cystic Fibrosis

Cystic fibrosis is caused by mutations in the *CFTR* gene, which encodes a multifunctional protein that is expressed in many epithelial tissues [22]. *CFTR* has a pivotal role in regulating epithelial fluid balance. *CFTR* is a cyclic adenosine monophosphate (cAMP)-activated chloride channel that regulates other ion channels, notably the epithelial sodium channel ENaC. Cystic fibrosis is characterized functionally by the absence of a cAMP-regulated chloride secretory pathway. More than 95% of patients with cystic fibrosis fail to produce digestive enzymes in the pancreas, resulting in pancreatic insufficiency. Complications at the pancreatic level are responsible for chronic diarrhea observed in children with cystic fibrosis. Population studies suggest that most of the patients with pancreatic insufficiency were descendents from a single mutational event at the *CFTR* locus. More than a thousand mutations have been defined in the *CFTR* gene to date. These mutations are situated throughout the entire coding region of the gene and also the promoter region, although there are regions where mutations are more common, such as nucleotide binding domain and regulatory domain. Patients with pancreatic insufficiency are homozygous or compound heterozygous for two severe mutations [22].

Enterokinase Deficiency

Enterokinase deficiency seriously impairs protein absorption. Proteinase-activated receptor 2 is present at the apical and basolateral membrane of enterocytes; activation of this receptor by trypsin stimulates enterocytes to secrete eicosanoids, which act locally in the intestinal wall to regulate epithelial growth [23]. Therefore, in addition to its purely digestive role, enterokinase localization on the luminal surface of the duodenal villi possibly contributes to enterocyte growth by generating active trypsin on the cell surface. The human genetic locus appears to be close to the gene for β-amyloid precursor protein at band 21q.21.2 [23]. The human proenteropeptidase gene consists of 25 exons (24 introns) and it spans 88 kb. Mutations in the duodenase gene, resulting in defective activation of proenteropeptidase, may possibly lead to disease similar to enterokinase deficiency. Only 13 cases of primary enterokinase deficiency have been reported. Three additional patients were reported with a similar clinical picture, but with unmeasured intestinal enterokinase activity [23]. All mutations identified in the proenteropeptidase gene are null mutations that predict the absence of a correctly formed active site. Prognosis is good with adequate treatment. Pancreatic enzyme replacement is indicated in patients with intestinal enterokinase deficiency. Treatment of enterokinase deficiency involves no dietary restrictions or recommendations after starting proper pancreatic enzyme replacement therapy. Individualized dosing is based on age, body weight, and symptoms.

Chylomicron Retention Disease and Anderson Disease

Although several disorders are caused by genetic defects in vesicle-mediated trafficking, only chylomicron retention disease (CMRD; OMIM 246700) and Anderson disease (OMIM 607689) primarily involve the intestine [24, 25]. CMRD and Anderson disease are allelic and closely related autosomal recessive diseases that appear with severe lipid malabsorption, chronic diarrhea, failure to thrive, and hypocholesterolemia caused by hypobetalipoproteinemia. Microvacuolization of enterocytes caused by retention of chylomicrons and lipid vacuoles in the cytoplasm are typical features at histologic examination. The causative gene, *SARIB*, encodes a small GTPase associated with COPII vesicles involved in endoplasmic reticulum-to-Golgi transport and in chylomicron and low-density lipoprotein transport [3••, 24, 25].

Congenital Bile Acid Diarrhea

Because of a defect in the Na^+-dependent bile salt transporter (ASBT) coded by the *SLC10A2* gene [26], congenital bile acid diarrhea (PBAM; OMIM 601295) is a rare disorder transmitted in an autosomic recessive fashion and characterized by impaired bile acid reabsorption. Studies in mice also suggest the presence of a basolateral taurocholate transporter. Both proteins are required to required taurocholate reabsorption [26]. Other transporters, NTCP and BSET, are crucial for a physiologic bile acid cycle. Further studies are advocated to define the role of these proteins in the pathogenesis of the disease.

Enterocyte Differentiation and Polarization

Microvillous Inclusion Disease

Microvillous inclusion disease (MID; OMIM 251850) is a severe secretory diarrhea that usually starts shortly after birth [27•, 28]. A late-onset form (at 3–4 months of life) with a better outcome has been described. Pathology shows shortened microvilli and villous atrophy with an increased number of secretory granules within enterocytes and membrane-bound inclusions. It was recently demonstrated that *Rab8*, a small GTP-binding protein, and myosin Vb (*MYO5B*) are involved in the intracellular transport of proteins to the apical level of the intestinal epithelial cells

[2••, 27•]. A deficit of *Rab8* results in a pathological picture almost identical to that of MID in an animal model [2••]. Interestingly, although *Rab8* mRNA and protein were absent from one MID patient's biopsy specimen, no mutations were identified in the *Rab8* gene in patients with MID. On the other hand, mutations in the *MYO5B* gene that encoded for myosin Vb were recently found in nine families that included members affected by MID [3••, 27•]. Myosin Vb forms a complex with Rab protein and vesicles, and it is required for enterocyte polarization. Myosin Vb deficiency may block the apical traffic of intracellular vacuoles containing microvilli, thereby determining aggregation of apically bound vesicles. However, other genetic causes of MID are possible.

Intestinal Epithelial Dysplasia

Intestinal epithelial dysplasia is a rare autosomal recessive diarrheal disorder characterized by high morbidity and mortality [29•]. Mutations in the *EpCAM* gene are responsible for the disorder. The primary function of *EpCAM* is to mediate cell-cell interaction. *EpCAM* is known to recruit intracellular actin to the sites of homophilic contacts. *EpCAM* also co-localizes with E-cadherin in areas of cell-cell junctions and directly associates with the tight junction protein claudin-7 [2••, 29•]. The identification of these mutations will advance our understanding of this disorder and provide new strategies for its management.

Enteroendocrine Cell Differentiation

Enteric Anendocrinosis

Extensive evaluation of intestinal biopsies from patients with enteric anendocrinosis (OMIM 610370) has shown severe enteroendocrine cell dysgenesis associated with normal villus structure and crypt-villus axis, and absence of inflammation. The disorder is characterized by severe malabsorptive diarrhea and a lack of intestinal enteroendocrine cells caused by loss-of-function mutations in neurogenin-3 (*NEUROG3*) [30, 31]. Neurogenin-3 is a transcriptional factor that induces differentiation of stem cells located at the base of the crypt-villus axis into four functional cell types: epithelial cells, mucus-secreting goblet cells, antimicrobial Paneth cells, and hormone-secreting enteroendocrine cells. Because *NEUROG-3* is critical to the development of pancreatic islet cells, patients with enteric anendocrinosis develop clinical evidence of diabetes (without anti-islet antibodies) between 4 and 10 years of age. What remains unclear is the role that enteroendocrine cells have in facilitating the absorption of simple nutrients.

Modulation of the Intestinal Immune Response

Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX; OMIM 304930) is usually a fatal disorder unless treated with immunosuppressive therapy and/or bone marrow transplantation [32, 33••]. Patients with IPEX syndrome were found to harbor mutations in the *FOXP3* gene, which is the gene altered in scurfy mice, a mouse strain with severe autoimmunity and lymphoproliferation. The *FOXP3* gene encodes scurfin, a protein predominantly expressed in $CD4^+/CD25^+$ T cells that regulates T-cell activation (T-reg cells). Ablation of *FOXP3*-expressing cells in mice results in severe autoimmunity and lymphoproliferation. Additionally, loss of expression of interleukin (IL)-10 by *FOXP3*-expressing cells results in inflammation in the gut and lung. *FOXP3* coordinates the assembly of multiple transcriptional regulators into a complex. Structural and biochemical insights into these complex ensembles will increase our understanding of *FOXP3* function and facilitate the development of potential applications under disease conditions. A recently identified mutation within an upstream non-coding region of *FOXP3* results in a variant of IPEX syndrome that is associated with autoimmune and severe immunoallergic symptoms (ie, food allergy, hyper-IgE, atopic dermatitis, and hypereosinophilia). A syndrome related to IPEX was described in two patients with mutations in the IL-2 receptor α (*CD25*) gene. The *FOXP3* gene was found to be wild-type in these patients. In one patient, mutations in *CD25* resulted in defective secretion of IL-10 by lymphocytes. Because IL-10 is important in the down-regulation of inflammation, this finding suggests a possible mechanism by which mutations in *CD25* may phenocopy IPEX [32, 33••].

Conclusions

The recent progress made in understanding the genetics and pathophysiology of CDD could contribute to identification of novel strategies for the diagnosis and treatment of these conditions. Widespread use of efficient diagnostic tests may reveal a higher prevalence of the disorders classified within the CDD group than recognized currently. Additionally, molecular technology may help clinicians to better manage these disorders, particularly in early life, when severe diarrhea may be life-threatening, leading to massive dehydration and metabolic acidosis. Second-level approaches, including in vitro functional studies, could be useful to define the effect of a mutation and to confirm that

a novel variant is indeed disease-causing. Nevertheless, proteomic studies may predict the phenotype, and guide physicians in the clinical management of CDD. Close collaboration between clinicians and laboratory professionals appears crucial for current clinical management and future research in the field of CDD.

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- • Of importance
- •• Of major importance

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