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2	Article Sub- Title		
3	Article Copyright - Year	**Springer Science + Business Media New York 2017 (This will be the copyright line in the final PDF)**	
4	Journal Name	Current Pediatrics Reports	
5		Family Name	**Canani**
6		Particle	
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88		e-mail	
89	Schedule	Received	
90		Revised	
91		Accepted	

92 Abstract

Purpose of Review: We highlight new entities of congenital diarrheal disorders (CDDs) and progresses in understanding of functionally related genes, opening new diagnostic and therapeutic perspectives.

Recent Findings: The more significant advances have been made in field of pathogenesis, encouraging a better understanding not only of these rare diseases but also of more common pathogenetic mechanisms.

Summary: CDDs represent an evolving group of rare chronic

		enteropathies with a typical onset early in the life. Usually, severe chronic diarrhea is the main clinical manifestation, but in other cases, diarrhea is only a component of a more complex systemic disease. The number of conditions has gradually increased, and many new genes have been indentified and functionally related to CDDs, opening new diagnostic and therapeutic perspectives. Advances in molecular analysis procedures have modified the diagnostic approach in CDDs, leading to a reduction in invasive and expensive procedures.
93	Keywords separated by ' - '	Chronic diarrhea - Genes - Molecular analysis - Mutations - Children
94	Foot note information	This article is part of the Topical Collection on *Gastrology*

JrnlID 40124_ArtID 136_Proof# 1 - 31/05/2017

Curr Pediatr Rep
DOI 10.1007/s40124-017-0136-5

GASTROLOGY (S KHAN, SECTION EDITOR)

New Insights and Perspectives in Congenital Diarrheal Disorders

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Abstract

Purpose of Review We highlight new entities of congenital diarrheal disorders (CDDs) and progresses in understanding of functionally related genes, opening new diagnostic and therapeutic perspectives.

Recent Findings The more significant advances have been made in field of pathogenesis, encouraging a better understanding not only of these rare diseases but also of more common pathogenetic mechanisms.

Summary CDDs represent an evolving group of rare chronic enteropathies with a typical onset early in the life. Usually, severe chronic diarrhea is the main clinical manifestation, but in other cases, diarrhea is only a component of a more complex systemic disease. The number of conditions has gradually increased, and many new genes have been indentified and functionally related to CDDs, opening new diagnostic and therapeutic perspectives. Advances in molecular analysis procedures have modified the diagnostic approach in CDDs, leading to a reduction in invasive and expensive procedures.

Keywords Chronic diarrhea · Genes · Molecular analysis · Mutations · Children

Introduction

Congenital diarrheal disorders (CDDs) are a group of rare hereditary enteropathies, characterized by a typical onset during the first days of life [1••]. Although, most of these diseases present similar clinical features, the causes, the management, and prognosis of various forms of CDDs are very different. For most of these conditions, a severe chronic diarrhea is the primary clinical manifestation; more rarely, diarrhea is only one component of a multiorgan more complex picture. In most cases, an appropriate therapy should be initiated immediately in order to prevent dehydration and serious short- and long-term complications [1••]. There are also milder forms of CDDs, with a less severe clinical picture, diagnosed in later ages, typically due to mutations that less severely impair the residual activity of the disease-protein. To date, genes responsible for disease are known in most cases of CDDs. Therefore, molecular analysis has assumed a key role in the diagnostic approach to a patient suspected of CDDs and, in some cases, in the prediction of the outcome of the disease (genotype–phenotype correlation). Evolving knowledge of the pathogenesis of CDDs suggests the utility of a classification system based on the main pathogenetic mechanism, which could help the approach to these patients (Fig. 1). This classification comprises four groups of disorders:

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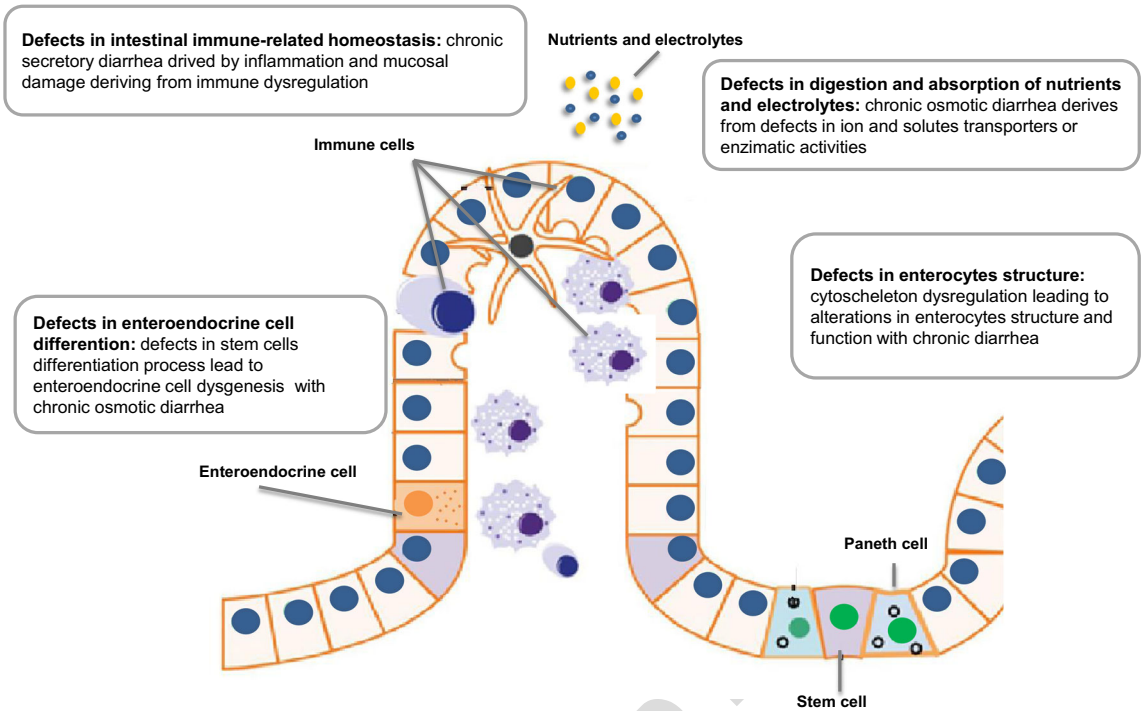

Fig. 1 The improving knowledge about the pathogenesis of congenital diarrheal disorders has inspired a new classification that could help the diagnostic and therapeutic approach to these conditions

I. Defects in digestion and absorption of nutrients and electrolytes
II. Defects in enterocyte structure
III. Defects in enteroendocrine cell differentiation
IV. Defects in intestinal immune-related homeostasis.

We review new CDD entities and advances understanding of functionally related genes that are opening new diagnostic and therapeutic perspectives, underlining the crucial role of molecular analysis.

Defects in Absorption and Transport of Nutrients and Electrolytes

Most of the CDDs belong to this group. These diseases derive from a defect in one of the main mechanisms of digestion or transport that leads to severe diarrhea and subsequent dehydration and weight loss early after birth. Specifically, the defect can be charged to brush border membrane, membrane carriers, pancreatic enzymes, lipid transport and metabolism, ribosomal proteins, and mitochondrial DNA [2••] (Table 1). The alteration of digestion or absorption of carbohydrates, proteins, and electrolytes results in osmotic diarrhea. Fecal pH <5, ion gap >50, and molecular analysis confirm the diagnosis. The prototypes of this group are glucose–galactose malabsorption and congenital chloride diarrhea [1••], but new conditions have been described. No histological or ultrastructural defects are generally observed in these patients at gut level [1••].

Familial Diarrhea Syndrome

Main Clinical Features This condition has been described in 32 members of a Norwegian family, and is characterized by early-onset chronic diarrhea and meteorism. In a subset of patients, abdominal pain and dysmotility have been described as main features [4•]. Patients have an increased risk to develop Crohn's disease and intestinal obstruction resulting from volvulus, adhesional bands, and/or ileal inflammation [5].

Genotype All affected members have an activating heterozygous missense mutation (p.Ser840Ile) in the GUCY2C gene, which encodes for the intestinal guanylate cyclase receptor for uroguanylin, guanylin, and heat-stable enterotoxins [4•]. Activation of the guanylate cyclase C (GC-C) receptor increases cellular levels of cyclic guanosine monophosphate (cGMP), leading to phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) channel [5]. The efflux of Cl^- and water into the intestinal lumen, with reduced sodium ion (Na+) absorption owing to inhibition of the Na^+–H^+ exchanger 3 (NHE3) [5], leads to a severe chronic secretory diarrhea. GC-C signaling also has implications for renal electrolyte homeostasis, intestinal cell proliferation, apoptosis, intestinal barrier function, and inflammation [5].

Q3 t1.1 **Table 1** Main features of congenital diarrheal disorders: genetics and epidemiology

t1.2 Disease	Gene			Protein	Inheritance and incidence
t1.3	Name	OMIM number	Position		
t1.4 Defects in absorption and ransport of nutrients and electrolytes					
t1.5 Congenital chloride diarrhea	SLC26A3	126650	7q31.1	Cl^-/base exchanger	AR, sporadic; common in some ethnic groups
t1.6 Congenital sodium diarrhea[a]	SLC29A3	182307	5p15.33	Na^+–H^+ exchanger	AR, <1:1,000,000
t1.7 Congenital lactase deficiency	LCT	603202	2q21.3	Lactase–phlorizin hydrolase	AR, 1:60,000 in Finland; lower in other ethnic groups
t1.8 Sucrase–isomaltase deficiency	SI	609845	3q26.1	Isomaltase–sucrase	AR, 1:5000; higher in Greenland, Alaska, and Canada
t1.9 Maltase–glucoamylase deficiency	MGAM	154360	7q34	Maltase–glucoamylase	Only few cases described
t1.10 Glucose–galactose malabsorption	SLC5A1	182380	22q13.1	Na^+/glucose cotransporter	AR, a few hundred cases described
t1.11 Fanconi–Bickel syndrome	SLC2A2	138160	3q26.2	Basolateral glucose transporter	AR, rare
t1.12 Acrodermatitis enteropathica	SLC39A4	607059	8q24.3	Zn^{2+} transporter	AR, 1:500.000
t1.13 Lysinuric protein intolerance	SLC7A7	603593	14q11.2	Cationic amino acid transporter	AR, approximately 1:60,000 in Finland and Japan; rare in other ethnic groups
t1.14 Primary bile acid diarrhea	SLC10A2	601295	13q33.1	Ileal Na^+/bile salt transporter	AR
t1.15	FGF-19	603891	11q13.3	Bile acids negative feedback	Only few cases described
t1.16 Enterokinase deficiency	TMPRSS15	606635	21q21.1	Proenterokinase	AR
t1.17 Abetalipoproteinemia	MTTP	157147	4q23	Microsomal triglyceride transfer protein	AR, about 100 cases described; higher frequency among Ashkenazi
t1.18 Hypobetalipoproteinemia	Apo B	107730	2p24.1	Apolipoprotein B 100/48	Autosomal codominant
t1.19 Chylomicron retention disease	SAR1B	607690	5q31.1	Intracellular chylomicron trafficking	AR, about 40 cases described
t1.20 Familial diarrhea syndrome	GUCY2C	601330	12p13.1-p12.3	Receptor for heat-stable enterotoxins	Described in 32 members of a Norwegian family
t1.21 Diarrhea-associated DGAT1 mutation	DGAT1	604900	8q24.3	Diacylglycerol acyltransferases	One family has been reported
t1.22 Defects in enterocyte structure					
t1.23 Microvillous inclusion disease	MYO5B	606540	18q21.1	Myosin VB	AR; rare; highest frequency mong Navajo
t1.24	STX3	600876	11q12.1	Syntaxin3	AR; two patients described
t1.25 Congenital tufting enteropathy[b]	EPCAM	185535	2p21	Protein for cell–cell interaction	AR; 1:50–100.000; higher among Arabians
t1.26	SPINT2	605124	19q13.2	Serine protease inhibitor	<1/1,000,000
t1.27 Trichohepatoenteric syndrome (syndromic diarrhea)	TTC37	614589	5q15	Component of the SKI complex	AR; <1/1,000,000
t1.28	SKIV2L	600478	6p21.33	Helicase	AR; <1/1,000,000

t1.29 **Table 1** (continued)

	Disease	Gene			Protein	Inheritance and incidence
t1.30		Name	OMIM number	Position		
t1.31	Defects in enteroendocrine cell differentiation					
t1.32	Enteric anendocrinosis	NEUROG3	604882	10q22.1	Transcriptional regulator	AR; few cases described
t1.33	X-linked lissencephaly and MR	ARX	300382	Xp21.3	Homeodomain transcription factors	X-linked
t1.34	Proprotein convertase 1/3 deficiency	PCSK1	162150	5q15	Neuroendocrine convertase	AR; <1/1,000,000
t1.35	Mitchell–Riley Syndrome	RFX6	612659	6q22.1	Transcription factors	AR
t1.36	Defects in intestinal immune-related homeostasis					
t1.37	Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome	FOXP3	300292	Xp11.23	Transcription factor	X-linked
t1.38	IPEX-like disorders	CD25	147730	10p15.1	Interleukin-2 receptor, alpha chain	AR
t1.39		STAT5b	604260	17q21.2	Transcriptional regulator	AR
t1.40		STAT-1	600555	2q32.2	Transcriptional regulator	AD, loss/gain of function
t1.41		ITCH	606409	20q11.22	Ubiquitin protein ligase	AR (one family)
t1.42		LRBA	606453	4q31.3	Protein involving in apoptosis	AR, three family described
t1.43		MALT-1	604860	18q21.32	Protein involving in NF-κB activation	AR (one family)
t1.44	Early onset enteropathy with colitis	IL-10 IL-10Rα IL-10Rβ	124092 146933 123889	1q32.1 11q23.3 21q23.3	Cytokine or cytokine receptors	AR

[a] Analysis of the intestinal brush border membrane of affected patients revealed that the condition is caused by a SLC9A3 loss-of-function mutations. SLC9A3 encodes Na^+/H^+ antiporter 3 (NHE3), the major intestinal brush border Na^+/H^+ exchanger. A syndromic form of CSD is characterized by the presence of choanal and intestinal atresias as well as recurrent corneal erosions. Small bowel histology frequently detects an epithelial "tufting" dysplasia. It is autosomal recessively inherited and associated to SPINT2 mutations [3]

[b] Congenital tufting enteropathy associated to EPCAM mutation is characterized by only intestinal involvement, while mutation in SPINT2 leads to a syndromic form with dysmorphic features, wooly hair, small birth weight and immune deficiency, and diarrhea with high sodium content in the stools

Treatment Total parenteral nutrition is the only therapeutic option at the moment [4•, 5].

DGAT1-Deficiency-Related Diarrhea

Main Clinical Features Protein-losing enteropathy (PLE) is a clinical disorder of protein loss from the gastrointestinal system that results in hypoproteinemia and malnutrition.

Genotype Patients with mutations in DGAT1 (which encodes acyl CoA/diacylglycerol acyltransferases 1) present aspects of both PLE and CDD [6]. DGATs catalyze the final step of triglyceride synthesis. Animal models lacking DGAT1 show delayed fat absorption with more fat reaching the distal gut. However, how DGAT1 deficiency causes diarrhea and protein losing enteropathy is still unknown, but it is possible that excess diacylglycerols or fatty acids could have a toxic role, maybe acting as bioactive signaling lipids or via a detergent-like action [7•]. Several differences, however, are apparent among the reported cases and regard the serum lipid profile, including triglycerides, the presence of digital clubbing and the onset of diarrhea. These phenotypic differences may reflect genotypic differences [7•].

Treatment Total parenteral nutrition is the only therapeutic option at the moment [7•].

Defects in Enterocyte Structure

This group of CDDs includes microvillus inclusion disease (MVID), congenital tufting enteropathy (CTE), more recently trichohepatoenteric syndrome (THE) has added to the group (Table 1). During the last years, mutations in genes, involved

in intestinal epithelial physiology, have been associated with different CDDs, opening new perspectives in understanding the pathogenetic mechanism and in the clinical approach [1••]. Parenteral nutrition and intestinal transplantation are the only therapeutic strategies available at the moment; they have reduced the morbility and mortality rates of these diseases [1••].

Microvillus Inclusion Disease

Main Clinical Features Loss of apical microvilli and formation of microvillus inclusion in the cytoplasm of enterocytes are the main hallmarks of MVID [8•]. These alterations lead to persistent diarrhea, nutrient malabsorption, and failure to thrive. In most cases (95%), symptoms develop within days after birth, but there is a late-onset variant, which presents 2–3 months postnatally. Extra intestinal symptoms could be intrahepatic cholestasis and renal Fanconi syndrome. Some individuals with MVID present less-severe digestive symptoms for reasons that are not clear [9••]. Intestinal biopsy is the most important method to diagnose this disease. This will display: features of villus atrophy, microvillus atrophy, and the redistribution of CD10 and periodic acid Schiff (PAS)-stained material from the brush border to intracellular sites in the enterocytes. A definitive diagnosis includes analysis by electron microscopy (EM) for microvillus inclusions in the cytoplasm of enterocytes. It is interesting that microvillus inclusions are also present in rectal biopsies, facilitating diagnosis if a duodenal biopsy is not available [9••].

Genotype Loss of function mutations in the actin motor myosin Vb (MYO5B) is responsible for most cases of MVID. Recently, mutations in the SNARE fusion protein syntaxin 3 (STX3) and STXBP2 were reported in the milder MVID variant [10]. MYO5B encodes the actin-based motor protein myosin Vb, which consists of an N-terminal actin binding motor domain and a C-terminal tail domain that includes the cargobinding domain. The myosin Vb cargobinding domain binds selectively to small Rab GTPases, among which RAB11A and RAB8A. MYO5b, in concert with RAB11A and RAB8A associated with apical recycling endosomes (AREs), in polarized epithelial cells controls the activity of the small GTPase CDC42, and it modulates intestinal epithelial cells polarity, apical trafficking, and microvilli growth [10]. At the basis of MVID's pathogenesis, it was demonstrated an uncoupling of myosin Vb from RAB11A and RAB8A, caused from the mutation of myosin Vb. Two other mutations involved in MVID interested STX, which encodes the transmembrane protein syntaxin-3, or STXBP2, which encodes Munc18-2 [11]. In enterocytes, syntaxin-3 is localized at the apical cell-surface domain, where it, in concert with SNAP23 and Munc18-2, has the function of mediating the fusion of transport vesicles with the apical plasma membrane. The mutation STX3, responsible for MVID, leads to depletion of the syntaxin-3 or the expression of a syntaxin-3 protein that lacks its transmembrane domain, with the loss of its function. STXBP2 mutations abolish the interaction of Munc18-2 with syntaxin proteins [11].

THE

Main Clinical Features Trichohepatoenteric syndrome, also called syndromic diarrhea, is a rare life-limiting autosomal recessive bowel disorder [12]. Main symptoms are chronic diarrhea, facial dysmorphism, trichothiodystrophy associated or not with liver disease, hepatomegaly, siderosis, congenital cardiac defects, and platelet anomalies [12]. Affected people are susceptible to infection, because they might fail to produce antibodies upon vaccination, or present with low immunoglobulin levels. In 50% of all cases, it is possible to find mild intellectual deficiency. Clinical dates and via biopsies of the small intestine are useful to make diagnosis. The biopsies display the typical histological and ultrastuctural defects: villus atrophy and variable immune cell infiltration of the thin layer of loose connective tissue that lies beneath the epithelium [12].

Genotype Recent studies have shown that the mutations involved in this disease are associated with TTC37 or SKIV2L [13]. The gene's products of both TTC37 and SKIV2L are human homologs of components of the yeast Ski complex, which is linked with exosome-mediated degradation of aberrant messenger RNA (mRNA) and associated with transcriptionally active genes. TTC37 (also called Thespin) encodes the tetratricopeptide repeat protein 37 and it is expressed in many tissues like vascular endothelium, lung and intestine, but not in the liver. In enterocytes with TTC37 mutations, the brush-border-associated NHE-2 and NHE-3, aquaporin 7, the Na^+/I^- symporter, and the H^+/K^+-ATPase show reduced expression or mislocalization to the apical cytoplasm, with different patterns of mislocalization relative to their normal pattern [12]. In conclusion, the loss of TTC37 results in the defective trafficking and/or decreased expression of apical transport proteins, including aquaporin 7.

The other mutation identified in the THE regard SKIV2L, which encodes SKI2 homolog, superkiller viralicidic activity 2-like protein, which might be involved in antiviral activity by blocking translation of poly (A)-deficient mRNAs. The mechanism, which is the base of the disease, is associated with loss of function a cytoplasmic-exosome cofactor involved in various mRNA decay pathways and required for normal cell growth [12].

Congenital Tufting Enteropathy

Main Clinical Features Typical is the presence of epithelial tufts that can be localized from the duodenum to the large

intestine [1••]. Patients affected by this condition have persistent diarrhea that presents immediately or just after birth, despite bowel rest, and total parenteral nutrition.

A subset of individuals with CTE displays a syndromic form of the disease that includes other signs and symptoms like dysmorphic features, wooly hair, punctate keratitis, atresias, reduced body size, and immune deficiency [14]. Histological features of CTE reveal various degrees of villus atrophy, basement membrane abnormalities, disorganization of enterocytes and focal crowding at the villus tips, resembling tufts.

Genotype In CTE, the absence of epithelial cell adhesion molecule (EPCAM) in enterocytes is considered the most important diagnostic marker [15•]. EPCAM, expressed along the basolateral membranes, is a multifunctional transmembrane glycoprotein, and it has an important role in cell–cell adhesion, proliferation, and differentiation. In this disease, EPCAM protein levels in the intestine are decreased and all CTE-associated EPCAM mutations lead to loss of cell-surface EPCAM, either because of impaired plasma membrane targeting or because of truncation of the protein, both of which result in its secretion [15•].

Recent studies have demonstrated that in the EPCAM KO mouse intestine, E-cadherin, and beta-catenin, two adherens junction-associated proteins are also mislocalized, leading to disorganized transition from crypts to villi [15•]. Recently, it has been demonstrated that a second group of CTE individuals is characterized by mutations in SPINT2 [14]. SPINT2 encodes the transmembrane protein Kunitz-type 2 serine-protease inhibitor which is involved in epithelial regeneration, in the Nf-Kb and TGF-beta signaling pathways. The inhibition of trypsin-family serine peptidases, which are encoded by SPINT2, abolishes the stimulation of apical Na^+ transport by nonvoltage-gated sodium channel-1-alpha (SCNN1A) in polarized intestinal epithelial cells, which could contribute to secretory diarrhea. It is possible that such a mechanism is the basis of the syndromic form of congenital sodium diarrhea that is associated with SPINT2 mutations [14].

Defects in Enteroendocrine Cell Differentiation

Abnormal enteroendocrine cell development or function and congenital malabsorptive diarrhea, associated or not with other systemic endocrine abnormalities, are the main features of this group of extremely rare forms of CDDs. Various null mouse models of each of these genes are associated with early postnatal mortality and occasionally diarrhea [1••]. Genes involved in this group are as follows: neurogenin-3 (NEUROG3), regulatory factor X-6 (RFX6), aristaless-related homeobox (ARX), and proprotein convertase subtilisin/kexin type 1 (PCSK1) (Table 1). Parenteral nutrition and intestinal transplant are the only two therapeutic options in these patients [1••, 16–19].

Enteric Anendocrinosis and Mitchell–Riley Syndrome

Main Clinical Features and Genotype NEUROG3 controls the fate of enteric cells in both the pancreas and the intestine. Biallelic mutations in NEUROG3 are known to cause a rare but well-defined clinical syndrome characterized by severe malabsorptive diarrhea from early life and mild nonketotic diabetes with a variable age of onset [16–18]. Few reports suggest that NEUROG3 may influence pancreatic exocrine function, possibly through its activation of NEUROD1 [20]. Homozygous mutations of RFX6 are associated with a complex clinical phenotype characterized by duodenal atresia, biliary abnormalities, neonatal diabetes mellitus, and malabsorptive diarrhea (Mitchell–Riley Syndrome) [20]. RFX6 is a winged helix transcription factor that is downstream of NEUROG3, and it is required for islet cell development and for enteroendocrine cells function [21•]. Mutation of RFX6 is associated with normal enteroendocrine cells number. The intestinal atresia associated with RFX6 mutations is probably related to a not yet fully characterized role in early gut endoderm. Furthermore, while murine studies suggest that RFX6 is exclusively expressed in enteroendocrine K-cells that express gastric inhibitory polypeptide and others hormones, it remains uncertain if this subset of cells are depleted in humans with RFX6 deficiency [22].

Other Defects of Enteroendocrine Cell Differentiation

Main Clinical Fetures and Genotype A complex clinical phenotype of X-linked mental retardation, seizures, lissencephaly, abnormal genitalia, and occasionally congenital diarrhea is due to mutations in the ARX gene (a prd-homeodomain transcription factor) [23, 24]. The ARX gene is a down-stream target of NEUROG3 and is expressed in a subgroup of enteroendocrine cells including those that express CCK, secretin, and glucagon [25]. More than 50% of patients described with loss-of-function ARX mutations present a polyalanine expansion that may be responsible for the highly variable neurologic and intestinal clinical phenotypes associated with this condition [26]. All active hormones produced by endocrine cells are processed by a specific Ca^{2+}-dependent serine endoprotease named prohormone convertase 1/3 (PC1/3). Homozygote loss-of-function mutations in PCSK1 gene encoding for PC1/3 have been associated with malabsorptive diarrhea and other endocrinopathies, including adrenal insufficiency, hypothyroidism, and hypogonadism [27]. PCSK1 is also expressed at hypothalamic level, producing various central orexigenic hormones that control appetite. Children presenting mutations of PCSK1 are extremely polyphagic [28].

In a cohort of children with this disorder (enteric dysendocrinosis) was found severe failure to thrive, and PN was required for the first several years of life [28]. These children also develop diabetes insipidus and growth hormone deficiency that distinguish PCSK1 deficiency from other enteric endocrinopathies. These findings suggest that enteric hormones may be particularly important to facilitate nutrient absorption during infancy when caloric requirement (per body weight) is at its highest [29].

Defects in Intestinal Immune-Related Homeostasis

IPEX Syndrome

Main Clinical Features Immunodysregulation, polyendocrinopathy, enteropathy, X-Linked (IPEX) syndrome is a monogenic autoimmune disease with early life onset that is considered the prototype of the defects in intestinal immune-related homeostasis. This rare syndrome is characterized by multiorgan autoimmunity, including severe diarrhea due to autoimmune enteropathy, chronic dermatitis, and endocrinopathy (type 1 diabetes mellitus, hypothyroidism). In more severe cases, symptoms onset starts in the immediate fetal period with hydrops [30]. In addition to the intestinal architectural changes due to dysregulation of Treg cells activity, patients with IPEX syndrome present serum autoantibodies to enterocyte antigens harmonin and villin that are uniquely found in IPEX and have high diagnostic value [31, 32]. IPEX syndrome is often fatal early in infancy; therefore, a prompt diagnosis is essential to start treatment as soon as possible, before tissue damage spreads to multiple organs [33].

Genotype IPEX is caused by loss of function mutations in the gene encoding the forkhead box P3 (FOXP3) on X-chromosome (Xp11.23) transcription factor for thymic-derived $CD4^+CD25^+$ regulatory T (Treg) cell (Table 1). These cells play a key role in the establishment and maintenance of immune tolerance [34]. Recently, a novel mutation in the FOXP3 gene is identified at Phe367 residue level. Phe367 is a key structural residue of the DNA-binding domain of FOXP because of its contribution to the dimeric state of FOXP3, and this mutation may have a disruptive effect on the interaction network whose integrity is essential for FOXP3 regulatory activity [34]. As a consequence, FOXP3mut Tregs normally differentiate in the thymus and can be detected in the peripheral blood and tissues of IPEX patients, but they are unable to suppress Teff cells. The early neonatal onset and severity of IPEX enteropathy suggests that likely the damage is established even during fetal life, independently from external environmental factors, such as nutrients and gut microbiota. Whole exome sequencing identified a novel nonsense mutation in the FOXP3 gene, c.1009C>T, which was inherited from the mother. FOXP3 is also transiently expressed by any activated T cells, in which it controls cell cycle and Th development [35••]. Functional data demonstrate that Treg cells isolated from IPEX patients are dysfunctional, as they cannot inhibit proliferation and cytokine production [33]. Indeed, IPEX patients manifest lymphoproliferation, skewing towards Th2 and increased frequency of peripheral IL-17 producing T cells, frequently found in autoimmune diseases. The increase in both Th2 and Th17 cells is involved in the pathogenesis of the disease and in tissues damage at different target organ levels. In addition to FOXP3 Treg cells, type 1 regulatory T (Tr1) cells represent nonthymic-derived Treg cells, able to provide immunoregulation. Tr1 cells maintain their function independently of FOXP3. In IPEX patients, the rapid development of immune disease after birth indicates that Tr1 cells are not sufficient by themselves to control autoimmunity [35••]. Lentiviral-mediated overexpression of wild-type FOXP3 successfully conveys stable regulatory function to FOXP3mut T cells, opening new therapeutic perspectives for IPEX patients [36].

IPEX-Like Syndromes

Main Clinical Features This group of CDDs includes an expanding spectrum of genetic defects that compromise T regulatory cell function that underlies human disorders of immune dysregulation and autoimmunity. Collectively, these disorders offer novel insights into pathways of peripheral tolerance and their disruption in autoimmunity. However, autoimmune symptoms phenotypically resembling IPEX often occur in the absence of detectable FOXP3 mutations; in fact, patients with clinical manifestations of IPEX have a normal Foxp3 gene. IPEX-like forms of autoimmune enteropathy manifestations have been associated with mutations in genes that are important for Treg maintenance, signaling, and expansion (Table 1) [37].

Genotype A number of other gene defects that affect T regulatory cell function also give rise to IPEX-related phenotypes, including loss-of-function mutations in CD25, STAT5b, and ITCH. Recent progress includes the identification of gain-of-function mutations in STAT1 as a cause of an IPEX-like disease, emerging FOXP3 genotype/phenotype relationships in IPEX [38–41].The majority of IPEX-like patients, however, still lack a clear diagnosis. Mutations in the IL-2receptor alpha subunit (CD25) are responsible for early-onset enteropathy manifesting with severe diarrhea. These patients are more susceptible to early infections, like CMV infection. Probably, CD25 is essential not only for the Treg cells function but also to mount an appropriate immune response, concomitant with the immune-dysregulation. It is described that a patient

with clinical manifestations of IPEX had a normal Foxp3 gene, but who had CD25 deficiency due to autosomal recessive mutations in this gene. This patient exhibited defective IL-10 expression from CD4 lymphocytes, whereas a Foxp3-deficient patient expressed normal levels of IL-10. These data show that CD25 deficiency results in an IPEX-like syndrome and suggests that although Foxp3 is not required for normal IL-10 expression by human CD4 lymphocytes, CD25 expression is important [38]. Mutations in STAT5b, responsible for transactivation of the IL-2 signal from CD25 to FOXP3, have been described associated with reduced Treg cell number [37]. Children with STAT5b mutation have symptoms other than enteropathy that can help in establishing the diagnosis. Early-onset chronic/recurrent enteropathy is described in patients with either loss- or gain-of-function mutations in STAT1, which also impinges effective immunity [39]. Some patients with IPEX-like disorder, profound Treg cell deficiency, and a normal FOXP3 gene sequence were found to have a homozygous nonsense mutation in the LPS-responsive beige-like anchor (LRBA) gene, which was previously implicated as a cause of autoimmunity. In fact, some patients with LRBA deficiency manifest increased levels of autoantibodies against autologous antigens in association with a dramatic decreased numbers of circulating Treg cells [39]. The clinical features observed in patients with LRBA deficiency are heterogeneous with the age of presentation ranging from 2 months to 12 years. The most common features of the patients are chronic diarrhea, organomegaly, respiratory tract infections, and hypogammaglobulinemia [40]. Infections and progressive loss of T cells number and function, including Treg cells, is reported in patients with loss-of-function mutations, whereas Treg instability has been suggested as consequence of the gain-of-function variants, which is characterized by chronic mucocutaneous candidiasis [37]. Another mutation associated with inflammatory unbalance has been described in an extended family with recurrence of lymphoproliferation, inflammation, and dysmorphisms; this concerns ITCH gene, encoding a ubiquitin ligase implicated in several T-cell functions [37]. Whole exome sequencing performed in two affected children and their parents, have identified a homozygous missense mutation in MALT1 gene (mucosa associated lymphoid tissue lymphoma translocation 1), which inhibits protein expression [42]. NF-κB-dependent lymphocyte activation was resulted severely impaired and there was a drastic reduction in FOXP3 Treg accounting for the IPEX-like phenotype. Following identification of the mutation, both children received hematopoietic stem cell transplantation, which permitted full clinical recovery. Immunological controls at 6 and 12 months after transplantation showed normal NF-κB activation and correction of Treg frequency [42]. Mutation in IL-10 receptor alpha and beta is observed in patients with colitis early in life typically associated with skin perianal ulcers and strong local inflammation. This condition underlines the antiinflammatory action of IL10 and possibly peripheral development of Tr1 cells [41]. Fistula and abscesses can also be present, with recurrence, requiring multiple surgical interventions. Gene mutations in either the alpha or beta subunit of the IL-10 receptor (IL-10R1 and 2) abrogate response to IL-10, and this causes persistent colonic inflammation [41]. Currently, the most effective therapy to cure the disease is hematopoietic stem cell transplantation [41]. Although studies of Tr1 cells in these patients have not been directly performed, this disorder illustrates the essential role of IL-10 in controlling the intestinal homeostasis [43]. This confirms the nonredundancy of both regulatory pathways in the intestine and the importance of considering genetic screening in the presence of early-onset disease [43]. Indeed, hematopoietic stem cell transplantation could be a valid therapeutic option for several of not only these disorders but also novel gene therapy approaches using CRISPR/Cas9 or other technologies could be pursued. However, external factors, like nutrients or intestinal microbiota could influence the immune system, contribute to reduce intestinal inflammation, and induce tolerance that provide useful therapeutic insights for the benefit of patients with congenital defects [44].

Main Therapeutic Strategies for CDDs Deriving from Dysregulation of Intestinal Immune Response The current treatments available for IPEX and IPEX-like syndrome patients include supportive therapy, immunosuppressive therapy, and hematopoietic stem cell transplantation (HSCT) [36]. Positive long-term outcome for IPEX patients can be obtained with long term immunosuppressive treatment. However, studies demonstrate that immunosuppression does not cure the disease and can induce severe side effects, like osteoporosis, dyslipidemia secondary to corticosteroids, and also chronic renal dysfunction linked to cyclosporine or tacrolimus [45]. Early HSTC provides the best outcome, before organs are damaged by autoimmunity [46]. Gene correction of autologous stem cells will hopefully become an option for IPEX patients. Other therapeutic approaches, alternative to multiple immunosuppression, could also be envisaged, aiming to re-establish tolerance in a FOXP3-independent manner. For example, IPEX patient cells can secrete IL-10 and IL-10-dependent type 1 T regulatory (Tr1) cells, playing important role in peripheral regulation, which can be differentiated despite the presence of FOXP3mut. Interestingly, patients with FOXP3 mutations and late onset or unusual clinical presentation are frequently reported. Patients present nephritic-range proteinuria, microscopic hematuria and renal insufficiency. Renal biopsy demonstrates proliferative glomerulonephritis with immune complex deposition [47]. Whether this different phenotype is due to the presence of residual protein function or to the presence in some patients of more efficient FOXP3-

independent compensatory mechanisms of tolerance remains to be clarified.

Considerations on the Diagnostic Approach and Molecular Analysis for CDD Patients

Diarrhea is relatively rare in the first weeks of life, but its early onset may be predictive of CDDs and requires hospitalization for an accurate diagnostic work up and therapeutic management. Early diagnosis is of paramount importance for the outcome of most CDDs. The diagnostic approach to CDDs is a multistep process based on different tools that include anamnestic and clinical data, laboratory and instrumental diagnostic tools, and pathology and molecular analysis. However, considering the complexity of the CDDs, many cases could have an atypical presentation that limited the application of a systematic approach. Positive familiar history of early-onset chronic diarrhea, polyhydramnios, and/or dilated bowel loops at ultrasound examination during pregnancy is highly suggestive of CDDs. In the vast majority of cases, the main clinical manifestation is chronic diarrhea [1••]. In the approach to a newborn or infant with suspected CDD, it is important to remember that also at this particular age, infections and food allergy are frequent causes of chronic diarrhea [48] and that these conditions together with malformations of gastrointestinal tract should be considered as primary hypothesis. In the last 10 years, many genes responsible for most CDDs have been identified (Table 1). The availability of DNA sequencing techniques has greatly ameliorated the diagnostic approach to these diseases. Molecular genetics has become helpful to obtain early and unequivocal diagnoses, allowing a rapid and targeted therapeutic strategies (Table 2) and reducing repetitive invasive and expensive procedures [1••]. Moreover, the identification of disease-causing mutations in the affected proband can help to reveal asymptomatic carriers and to offer counseling and future prenatal diagnosis [49].

Whether the type of mutation(s) can help about the severity of the clinical phenotype is still under discussion. In specific CDDs, such as congenital chloride diarrhea, the clinical expression is poorly related to the genotype and the presence of modifier genes might contribute to modulate the phenotype. However, genotype might predict response to therapy. In fact, it has been demonstrated that specific SLC26A3 mutations that retain at least some activity of the protein could predict a more remarkable effect of oral butyrate therapy [50]. More complex in vitro functional studies are needed to explain the effect of mutations of uncertain clinical significance, but such studies are rarely performed in a routine setting.

Conclusions

In recent years, much progress has been made on the understanding of the pathogenesis of these conditions, thanks to the development of 3D models derived from human stem cells, providing a new research perspective [1••]. The molecular diagnosis has further changed the scenario of the CDDs, opening the way for new therapeutic strategies such as the transplantation of hematopoietic stem cells [1••] and gene therapy endo-nucleases, including Talens or CRISPR/Cas9 [1••]. Long-term studies are necessary to provide other information about the prognosis of these conditions. Given the number of the CDDs, the complexity of the genotype–phenotype relationship and the need for a multidisciplinary counseling for family members are essential close collaboration between clinical and laboratory as part of an international network. Some examples are the Registry of patients with MVID [1••], the website Diarrheal Congenital Disorders [1••], and the

Table 2 Main therapeutic strategies for congenital diarrheal disorders

Defects in absorption and transport of nutrients and electrolytes	• Exclusion diet • Substitutive therapy • Parenteral nutrition
Defects in enterocyte structure	• Total parenteral nutrition • Antisecretory drugs • Intestinal transplantation
Defects in enteroendocrine cell differentiation	• Total parenteral nutrition • Hormone therapy • Intestinal transplantation
Defects in intestinal immune-related homeostasis	• Total parenteral nutrition • Hormone therapy • Immunosuppressive, immunomodulator drugs, biologics (corticosteroids, cyclosporine, azathioprine, 6-mercaptopurine, tacrolimus mycophenolate mofetil, sirolimus, infliximab, rituximab) • Bone marrow transplantation

consortium IPEX syndrome [1••], in order to provide quick access to analysis and other molecular diagnostic procedures for patients suspected of CDDs.

Compliance with Ethical Standards

Conflict of Interest Vincenza Pezzella, Giusi Grimaldi, Mariateresa Russo, Serena Mazza, Domenica Francesca Mariniello, Lorella Paparo, Ausilia Elce, Giuseppe Castaldo, and Roberto Berni Canani each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check and verify if the intended levels of the section titles were assigned correctly.
Q2. Please provide definition for “ITCH” in its first occurrence.
Q3. Table 1 has been restructured. Please check if the table was captured/presented correctly. Please note that the table footnote labels have been modified as well.
Q4. “Guanylate cyclase C” was provided as the definition for “GC-C.” Please check and change as necessary.
Q5. “Messenger RNA” was provided as the definition for “mRNA.” Please check and change as necessary.
Q6. Definition of “IPEX” has been modified. Please check if action taken is appropriate.