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Congenital Sucrase-Isomaltase Deficiency: Heterogeneity of Inheritance, Trafficking, and Function of an Intestinal Enzyme Complex

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Brush border membranes are the largest exposed surfaces in tissues. They constitute the interface between the "milieu exterieur" and the "milieu interieur" of the body in a variety of organs such as the gastrointestinal tract and bile canaliculi, where hydrolytic, absorptive, and secretory processes take place. The intestinal mucosa is the exclusive site for nutrient metabolism and subsequent uptake of the generated products, such as monosaccharides and amino acids. The hydrolysis and absorption of micronutrients are achieved by the concerted action of hydrolases and transporters that are predominantly located in the brush border membranes (BBMs) (1).

The hydrolases are divided into 2 major families, the peptidases and the disaccharidases (2). The peptidases, such as aminopeptidases N (CD13), A, and W, carboxypeptidases P and M, dipeptidylpeptidase IV, or α-glutamyl transpeptidase, are expressed in many tissues, including the intestine and the kidney (3,4). The

expression of many of the disaccharidases, by contrast, is limited to the intestinal BBM. Prominent members of this family are the enzymes sucrase-isomaltase (SI) (5), maltase-glucoamylase (6), and lactase-phlorizin hydrolase (7). SI and maltase-glucoamylase hydrolyze α-glycosidically linked starch, glycogen, sucrose, and maltose. The generated monosaccharides are eventually transported across the BBM of epithelial cells into the cell interior. Another major brush border disaccharidase is lactase-phlorizin hydrolase, which cleaves β-glycosidic linkage in lactose, the main carbohydrate in mammalian milk that constitutes the primary diet source for newborns (8). Several other glycosidases with similar enzymatic specificities toward cleaving α- or β-glycosidic linkages exert their function in intracellular compartments, such as the lysosomes. Examples of this group of carbohydrases are lysosomal α-glucosidase (9), β-glucosidase (10), and α-galactosidase (11) or glycosidases of the glycogen catabolism.

Pathological conditions, most notably malabsorption of disaccharides and the subsequent symptoms, are associated with the absence of these enzymes in the intestinal lumen. Examples of these disorders are congenital SI deficiency (CSID), adult-type hypolactasia, congenital lactase deficiency, secondary lactase deficiency, and maltase-glucoamylase deficiency. This review focuses on the structural and biosynthetic features of SI and the molecular basis of sugar malabsorption in CSID. The diversity and heterogeneity of this disease is reflected in the existence of several mutant phenotypes of SI that vary in their posttranslational processing, cellular localization, and function. The pathogenetic mechanisms of CSID are unique for intestinal malabsorption disorders and have implications for the pathobiology of the intestinal mucosa. Unraveling the molecular basis of this disease revealed novel mechanisms of protein trafficking and polarized sorting.

PATHOPHYSIOLOGY, CLINICAL FEATURES, AND DIAGNOSIS OF CSID

CSID is an autosomal recessive intestinal disorder that was first described by Weijers et al in 1960 (12). It arises from mutations in the intestinal brush border enzyme complex SI. CSID occurs in 0.2% of individuals of European descent (13) and approximately 5% in indigenous Greenlanders (14). Heterozygotes with normal small intestinal morphology and with sucrase activity level below the lower limit for the normal population represent approximately 2% to 9% of Americans of European descent (15,16).

SI comprises 2 activities: sucrase that hydrolyzes α-1,2- and α-1,4-glucosidic bonds and isomaltase that cleaves α-1,6 linkages. The sucrase activity overlaps with that of intestinal maltase-glucoamylase, which digests α-1,4-glucosidic linkages of the end and intermediary product of α-amylolysis of starches such as maltose, maltotriose and low- and high-molecular-weight branched dextrins.

Patients with CSID experience vomiting, osmotic diarrhea, mild steatorrhea, chronic diarrhea, and crying spells upon ingestion of sugars (17). Occasionally dehydration, failure to thrive, developmental retardation, and muscular hypotonia were observed, which were compatible with broad clinical heterogeneity (13,18). CSID also has been reported to be associated with nephrocalcinosis, renal calculi, metabolic acidosis, and hypercalcemia (19,20). The clinical heterogeneity is supported by the findings that diverse mutant phenotypes of SI are responsible for the onset of CSID.

Several factors contribute to the development and extent of symptoms in patients with CSID: residual enzymatic activities of sucrase and isomaltase, amount of fed carbohydrate (in association with other foods), gastric emptying, small-bowel transit, degree of fermentation of unabsorbed carbohydrates by colonic bacteria, and absorption of the colon. Furthermore, the CSID symptoms also

depend on the patient's age. Symptoms, and particularly starch tolerance, spontaneously improve with age. Onset of symptoms in adulthood with diagnosis up to age 59 years has been reported (19,21,22).

The diagnosis of CSID is often delayed or perhaps missed because the symptoms are erroneously recognized as being related to diseases such as cystic fibrosis and celiac disease or to other causes of recurrent diarrhea and food allergy. A major step in diagnosing CSID is to recognize the complaints and clinical features of the patients in relation to age-dependent alterations and composition of nutrition. An increase in blood glucose of <20 mg/dL after a 2.0-g/kg sucrose load as well as an increase in breath hydrogen are compatible with sucrose intolerance (23–25).

The diagnosis of CSID requires the determination of SI activity in mucosa with normal histology. Enterocytes of patients with CSID lack the sucrase activity of the enzyme SI, whereas the isomaltase activity can vary from absent to practically normal. The disaccharidase activities in the duodenum are reduced by almost 40% as compared with the proximal jejunum (25,26). In some SI-deficient patients, the activity of maltase-glucoamylase is reduced, as is the isomaltase activity (27). The activities of other brush border disaccharidases such as lactase-phlorizin hydrolase and maltase-glucoamylase and peptidases such as aminopeptidase N, dipeptidyl peptidase IV, and meprin are usually within the normal range.

THERAPY OF CSID

Lifelong sucrose restriction is an effective therapeutic option for patients with CSID. The degree of restriction, however, depends on the individual complaints of a patient because patients with CSID show variable tolerances toward sucrose. Sucrose concentrations between 3 and 6 g/100 g in nutrients (onion, honey, soybean flour) are considered to be high. In many cases of CSID the isomaltase activity is also affected. Therefore, the diet of patients with CSID should also exclude starch and glucose polymers, such as wheat and potatoes. *Saccharomyces cerevisiae* possesses sucrase activity and a low isomaltase and maltase activity and can be used in CSID therapy. The use of lyophilized preparations of *S cerevisiae* reduced hydrogen excretion by 70%, with loss or reduction of clinical symptoms in CSID (28,29). Sacrosidase or invertase (Sucraid), a liquid preparation produced from *S cerevisiae,* has been used successfully in the treatment of patients with CSID (30).

STRUCTURAL FEATURES AND TRAFFICKING OF SI

SI is a type II integral membrane glycoprotein that is exclusively expressed in the small intestinal microvillus membrane and is responsible for the terminal digestion of dietary sucrose and starch. The glycoprotein comprises 2 subunits that are highly homologous and are thought to be derived from the same ancestral gene (31,32). These 2 subunits are associated with each other by strong noncovalent, ionic interactions (33). SI is synthesized in the rough endoplasmic reticulum (ER) as a single-chain mannose-rich precursor comprising both subunits (pro-SI$_h$, 210 kDa) (5) (Fig. 1). It is transported to the Golgi apparatus at a relatively slow rate and does not form homodimers before ER exit (5,34). The strong homologies between the 2 main domains suggest that quasidimers or pseudodimers are formed, which are presumably sufficient for the acquisition of transport competence from the ER to the Golgi apparatus and further to the cell surface. After modification of the N-linked glycans and O-glycosylation in the Golgi apparatus, SI is sorted to the apical membrane with high fidelity. In fact, 90% to 95% of the de novo synthesized protein is transported to the apical membrane. In the apical membrane pro-SI is cleaved in situ by
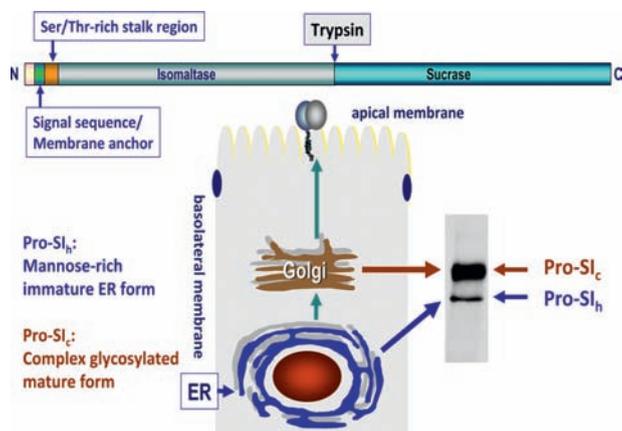
FIGURE 1. Pro-sucrase-isomaltase (1827 amino acids).

luminal pancreatic proteases to its 2 active subunits, sucrase and isomaltase (33). On its way to the apical membrane, SI associates with cholesterol- and sphingolipid-enriched membrane microdomains (lipid rafts), which act as platforms to warrant an efficient sorting of SI (35). Interestingly, the association of SI with lipid rafts substantially increases the activity of sucrase and isomaltase by a factor of almost 3-fold (36).

MOLECULAR BASIS OF CSID

Conformational modifications of membrane and secretory proteins commence during their translocation across the ER membrane and continue in the ER lumen (Fig. 1). Cotranslational glycosylation (37), intermolecular or intramolecular disulfide bond formation (38), and subunit assembly or oligomerization are examples of early modifications directly implicated in the protein maturation events, and are rate limiting along the exocytic pathway (39). Further posttranslational modifications in the Golgi apparatus, such as acquisition of a complex type of N-linked glycans and O-glycosylation (37), also affect protein trafficking to the cell

surface and secretion into the external milieu and polarized protein sorting in epithelial cells.

The dissection of molecular mechanisms required for efficient cellular transport of membrane and secretory proteins to the cell surface has greatly benefited from molecular analyses of genetic diseases directly associated with misfolded proteins and impaired protein targeting. Examples of these diseases are cystic fibrosis (CFTR protein) (40), familial hypercholesterolemia (low-density lipoprotein receptor) (41), Wilson disease (a P-type adenosine triphosphatase) (42), nephrogenic diabetes insipidus (aquaporin 2) (43), Liddell's syndrome (amilroide-sensitive epithelial Na^+ channel) (44,45), and CSID (intestinal SI) (13).

Early research on the molecular basis of CSID has suggested the presence of an enzymatically inactive SI in CSID (46,47). Hauri et al demonstrated a trafficking defect and an intracellular accumulation of SI in the *trans*-Golgi as an underlying cause of CSID (48).

In a multicenter collaborative study, biopsy specimens from patients with CSID were analyzed at the biochemical and cellular levels (49). The study defined CSID as a heterogeneous carbohydrate malabsorption disorder that exists in several phenotypes relevant to altered trafficking, cellular localization, or function of SI (Table 1). This study further proposed that the CSID phenotypes are elicited by different mutations in the coding region of the SI gene. In fact, the first successful cloning and characterization of a cDNA-encoding SI from a biopsy sample of a patient with CSID led to the identification of a point mutation that is responsible for the impaired intracellular transport behavior of SI (50). In the meantime, the mutations and their consequences on the trafficking and function of SI in 7 different phenotypes of CSID have been identified (Table 1, Figs. 2 and 3) (48,49,51). A survey of the features of the individual phenotypes follows.

Phenotypes of SI in CSID

Intracellular Arrest of SI in the ER Characterizes Phenotype I

Here, SI revealed characteristics of a misfolded, immature, and enzymatically inactive SI that is not capable of passing through the quality control machinery in the ER and is blocked there as a mannose-rich glycosylated protein that is ultimately degraded (51).

TABLE 1. Naturally occurring phenotypes of congenital sucrase-isomaltase deficiency

Phenotype	Cellular localization	Molecular forms	Enzymatic activity	Reference
I	ER	Mannose-rich 210-kDa pro-SI	Completely inactive	(48,50–52)
II	ER, ER-Golgi intermediate compartment and *cis*-Golgi	Predominant mannose-rich 210-kDa pro-SI and partial complex 245-kDa pro-SI	Completely inactive	(49–51)
III	Brush border membrane	Mannose-rich 210-kDa pro-SI and complex 245-kDa pro-SI	Completely inactive	(50)
IV	Random on apical and basolateral membranes	Mannose-rich 210-kDa pro-SI and complex 245-kDa pro-SI	Active sucrase and isomaltase	(51,56)
V	Intracellular cleavage, degradation of sucrase, isomaltase is correctly located at the apical membrane	Mannose-rich 210-kDa pro-SI, complex 245-kDa pro-SI and 150-kDa isomaltase	Active isomaltase and absent sucrase activity	(51)
VI	Intracellular cleavage, enzyme secreted	Mannose-rich 210-kDa pro-SI and mannose-rich 207-kDa cleaved pro-SI and complex glycosylated 240-kDa cleaved pro-SI	Active sucrase and isomaltase	(58)
VII	ER, random cell surface distribution at the apical and basolateral membranes	Predominant mannose-rich 210-kDa pro-SI and partial complex 245-kDa pro-SI	Decreased sucrase activity and absent isomaltase	(59)

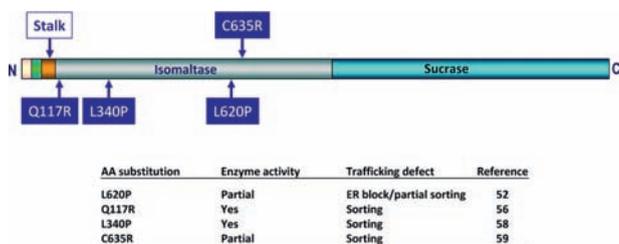ER = endoplasmic reticulum; SI = sucrase-isomaltase.

FIGURE 2. Isomaltase-based mutations in CSID.

This phenotype is the predominant one among most of the CSID phenotypes. Another CSID case with properties similar to those of phenotype I has been analyzed at the molecular and cellular levels (52). Although biosynthetic labelings of an intestinal biopsy specimen and immunoelectron microscopy revealed predominant localization of SI in the ER and thus are similar to phenotype I, a partial conversion of the SI protein to a complex glycosylated mature form suggests a classification of this case as a subtype of phenotype I. The SI cDNA in this phenotype revealed a point mutation that results in an exchange of a leucine by a proline at position 620 (L620P) of the isomaltase subunit (Fig. 2). Meanwhile, a number of other mutations have been identified that were assessed at the protein, cellular, and functional levels and have been shown to generate phenotype I. These are the V577G in isomaltase (Fig. 2) and the G1073D, C1229Y, and F1745C mutations in the sucrase subunit (Fig. 3) (53).

SI Is Blocked in the cis-Golgi Compartment and the ER/cis-Golgi Intermediate Compartment (ERGIC) in Phenotype II

The phenotype II of CSID is characterized by an intracellular block of SI in the ER, the ERGIC, and the cis-Golgi (49,50). As in phenotype I, the enzymatic activities of sucrase and isomaltase are below detection limit. This phenotype was the first in which a mutation in the SI gene has been identified and resulted in a substitution of a glutamine by a proline in the sucrase subunit of SI at amino acid 1098 (Q1098P) (50). In this phenotype, the mutant SI (Q1098P) retains its mannose-rich form and is ultimately degraded during prolonged chase time points. In confocal analyses, a YFP-tagged chimera of mutant SI (Q1098P) is predominantly located in the ER and in the Golgi. The Q1098P exchange confers temperature sensitivity on mutant SI (54). In fact, correct folding, full enzymatic activity, and competent intracellular transport can be partially restored by expression of the mutant SI (Q1098P) at the permissive temperature of 20°C instead of 37°C. Restoring function and normal trafficking and function is associated with several
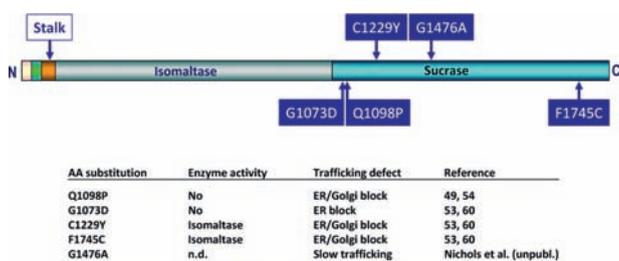
cycles of anterograde and retrograde steps between the ER and the Golgi, implicating the molecular chaperones calnexin and BiP, the immunoglobulin heavy chain-binding protein (54). This SI phenotype is the first of its kind in an intestinal disease that implicates a temperature-sensitive mutation. The Q1098P substitution is located in a region of the sucrase subunit (Fig. 3) that shares striking similarities with the isomaltase subunit and other functionally related enzymes, such as human lysosomal α-glucosidase and glucoamylase of the yeast *Schwanniomyces occidentalis* (32). Introduction of this mutation into the homologous region of lysosomal α-glucosidase elicited similar cellular and biochemical characteristics to phenotype II of SI. A likely proposal is that Q1098P is a part of a motif within SI that leads to a retention of SI in the cis-Golgi within the framework of a quality-control machinery that operates in the intermediate compartment or cis-Golgi (54). A potential phenylalanine-rich motif, F(1093)-X-F(1095)-X-X-Q-F(1099) (X could be any amino acid), that flanks the Q1098 residue has been suggested to be implicated in sensing the folding and subsequent trafficking of SI from the ER to the Golgi (55). The phenylalanine cluster in this motif is most likely required for shielding a folding determinant in the extracellular domain of SI, whereby substitution of a Q by a P at residue 1098 of sucrase disrupts this determinant and elicits retention of SI(Q1098P) in ERGIC and cis-Golgi in phenotype II of CSID (Table 1).

Normal Trafficking, but Absent Enzymatic Activities in Phenotype III

SI is transported to the cell surface with similar kinetics as the wild-type protein. It is correctly folded because it reacts efficiently with different epitope-specific monoclonal antibodies and is correctly sorted to the apical membrane (Table 1) (49). These criteria are adequate to propose that gross structural alterations did not occur in this specific phenotype of CSID. The defect in this phenotype correlates with the catalytic site of sucrase that is not active, whereas the isomaltase subunit expresses normal activity. Although further information about the location of the putative mutation in this phenotype is lacking, it is reasonable to assume that the mutation is located immediately or in the immediate vicinity of the catalytic domain of sucrase (31).

Random Delivery of SI to the Apical and the Basolateral Membranes Characterize Phenotype IV

SI is targeted with high fidelity to the BBM in intestinal epithelial cells (95%) (35), where it exerts its digestive function (Table 1). Impaired trafficking of SI would, therefore, be associated with malabsorption because of reduced levels of the enzyme in the BBM. Analysis of 2 cases of CSID using immunoelectron microscopy demonstrated an altered distribution of SI from an exclusive apical to a random localization at the apical and basolateral membranes (51,56). This phenotype is elicited by the amino acid substitution of glutamine to arginine at residue 117 (Q117R) in the isomaltase subunit (Fig. 2) that is located in close proximity to the O-glycosylated stalk domain that is implicated in the sorting of SI. In wild-type SI, the stalk domain itself is directly involved in targeting the SI molecule to the apical membrane through an interaction of its O-glycosylated carbohydrate content with a putative lectin receptor that recruits SI to detergent-insoluble

FIGURE 3. Sucrase-based mutations in CSID.

cholesterol/sphingolipid-rich lipid microdomains (lipid rafts) (35). It is likely, therefore, that the Q117R mutation generates a misfolded determinant near the stalk region leading to an inadequate recognition of the O-glycosylated stalk in SI by such a putative lectin-like sorting receptor or an O-glycan receptor. This CSID phenotype provides an exquisite model to be used in resolving the identity of this putative receptor.

Intracellular Proteolytic Cleavage of SI at 2 Different Sites in Phenotypes V and VI

Human SI is transported to the BBM as a single-chain polypeptide, pro-SI, that is cleaved in the intestinal lumen by pancreatic trypsin to isomaltase and sucrase (5). In phenotype V, the pro-SI precursor is intracellularly cleaved in the *trans*-Golgi network (TGN), whereby the sucrase subunit is degraded and the isomaltase subunit is properly transported per se to the apical membrane (51). This phenotype of CSID provided the first indication that isomaltase contains all of the necessary information required for apical transport of SI (Fig. 2). Later, this hypothesis was experimentally verified and the signals for apical sorting were identified in the O-glycosylated stalk region and the membrane anchoring domain; both domains are located in the isomaltase subunit (35,57).

Cleavage of mutant SI in the ER occurs in phenotype VI (58), which is elicited by a point mutation in the isomaltase subunit that converts a leucine to proline at residue 340 (Table 1, Fig. 2) (L340P). Interestingly, cleaved SI is transported efficiently along the secretory pathway, processed in the Golgi apparatus, and ultimately secreted into the exterior milieu as an active enzyme. The pathogenetic mechanism underlying CSID here is elicited by the conversion of an integral membrane glycoprotein into a secreted protein that cannot exert its function in the BBM.

Altered Folding, Increased Turnover, and Partial Missorting Characterize Phenotype VII

Another mutation relevant to polarized sorting of SI to the apical membrane has been identified in CSID. This mutation, C635R, is located in the isomaltase subunit and has been shown to confer partial missorting of mutant SI to the basolateral membrane (59). It eliminates a disulfide bond and subsequently alters a protein determinant in isomaltase that is presumably important for fine tuning of the apical sorting signal of SI. Expression of mutant SI (C635R) in a mammalian cell line revealed an altered folding pattern with subsequent retarded intracellular transport, increased turnover rate, and an aberrant transport of mutant SI to the apical membrane (Fig. 2). Concomitant with the altered sorting pattern, the mode of association of mutant SI with the membrane is altered and the protein shifts from a partially soluble protein with Triton X-100 that is associated with lipid rafts (35) to a completely Triton X-100–soluble protein. The mutation has therefore affected an epitope implicated in the apical targeting fidelity of SI. Altogether, the combined effects of the C635R mutation on the turnover rate, function, polarized sorting, and detergent solubility of SI constitute a unique and novel pathomechanism of CSID.

COMPOUND HETEROGENOUS MUTATIONS

Although our initial knowledge of the molecular pathogenesis of CSID came from cases in which patients were homozygous for single mutations in the SI gene, a screen of a cohort of patients in Hungary with typical symptoms of disaccharide malabsorption has surprisingly suggested compound heterozygosity in several patients with CSID (60). Molecular analyses at the cellular and molecular levels of several of the newly discovered mutations confirmed that the single mutations on the individual alleles act in concert to elicit CSID (53). Here, 2 major groups of heterozygous mutations were characterized that resulted in the amino acid substitutions V577G and G1073D in 1 patient and C1229Y and F1745C in another. These individual mutations resulted in an intracellular block of SI in the ER (mutations V577G, G1073D, and F1745C) or in the Golgi apparatus (C1229Y). It is obvious, therefore, that each of the mutations per se could have elicited CSID if it occurred in a homozygous context. The locations of the individual mutations in various domains of sucrase or isomaltase raise the possibility that an altered folding of a particular domain of sucrase or isomaltase caused by an individual mutation could be compensated by the unaffected one of the protein product of the second allele in the same patient. If this is the case, then the consequence could be an interaction of the SI mutants of both alleles generating a new phenotype that differs at the functional and cell biological levels from the individual phenotypes. Such an interactive mechanism would modify the severity of the SI deficiency. This hypothetical concept was not supported experimentally, however, because coexpression of 2 mutants derived from 1 patient did not change the phenotype or function as compared with the individual mutants.

CLASSIFICATION OF THE MUTATIONS IN THE SI GENE BASED ON THEIR SUBUNIT LOCALIZATION AND TRAFFICKING RELEVANCE

The CSID phenotypes provide direct support of the notion that both subunits, the sucrase and isomaltase, are autonomously organized within the SI enzyme complex. Moreover, the distinct trafficking and functional alterations of SI in CSID relevant to the subunit location of the mutations are indicative of specific roles of each of the subunits in a particular trafficking mechanism. Thus, the mutations located in the isomaltase subunit, Q117R, L340P, and C635R (Fig. 3) elicit impaired trafficking of SI to the apical membrane compatible with an implication of this subunit with the polarized sorting mechanism of SI. This view is supported by phenotype V of CSID, in which isomaltase per se is correctly sorted to the apical membrane despite the complete degradation of sucrase in the Golgi (51). The mutation in this case has not been characterized, however, because of the lack of biological material from the patient. The locations of the mutations of Q117R and L340P in the vicinity of the stalk region in isomaltase lend particularly strong support to the notion that the O-glycosylated stalk region is the main key element required for the sorting fidelity of SI to the apical membrane. In fact, inhibition of O-glycosylation of the stalk region of SI by benzyl-*N*-acetyl-α-ᴅ-galactosaminide (benzyl-GalNAc) (35,57) leads to a random distribution of the protein on the apical and basolateral membranes. The sorting mechanism that uses these glycans is unknown; however, several lines of evidence have suggested that O-glycans function in the context of a signal-mediated mechanism characterized by an active recognition of the O-glycans via a lectin-like receptor that eventually recruits SI to cholesterol- and sphingolipid-enriched membrane microdomains or lipid rafts. The lipid rafts are trafficking platforms and major constituents of a subset of apical vesicular carriers that segregate in the TGN from another population of lipid rafts–free apical carriers (61). The nature of these putative lectin-like receptors is unknown; however, a group of galactose-binding animal lectins, galectin 3 and galectin 4, have been proposed to be implicated in the sorting of brush border proteins to the apical membrane (62,63). The precise mechanism of binding of these cytosolic proteins to proteins of the secretory pathway is still puzzling. Nevertheless, another member of this family, galectin

1, has been suggested to be exported making use of β-galactoside–containing surface molecules that would bind galectin and, thus, serve as its export receptor (64). The relevant sorting mutations in CSID are invaluable tools that can be used to entirely unravel the sorting mechanism of SI and its interacting partners.

The role of the sucrase subunit can be seen at the level of the ER to Golgi trafficking of SI. It acts perhaps as a shuttle that carries the isomaltase to the apical membrane. This is clearly demonstrated in the SI mutants containing the mutations C1229Y and F1745C (53) (Fig. 3) in which isomaltase persists as a correctly folded protein, whereas the mutations elicit malfolded conformation of sucrase. That a correctly folded and an enzymatically active isomaltase are not capable per se of being targeted to the apical membrane strongly suggests a chaperoning function of sucrase within SI through which an interaction between sucrase and isomaltase is required in the intracellular transport of SI. Other sucrase-based mutations such as Q1098P and G1073D support this view. An abolition of sucrase enzymatic activity reduces the activity of isomaltase by approximately 60%, suggesting a cooperative effect of intact sucrase and isomaltase in exerting optimal activities of the enzyme complex (53).

As mentioned above, several vesicular carriers bud from the TGN containing protein cargo that associates or does not associate with lipid rafts. The lipid rafts association of these proteins depends in turn on the specific apical targeting signals, which, unlike the basolateral signals, are diverse in their nature, structure, and location. These signals can be found in the O-glycosylated stalk domains of proteins (35,65,66) or in N-glycans (66,67), in membrane-anchoring domains (68), or in the cytosolic part of the apically sorted proteins (69–72).

SI associates with vesicular carriers that contain protein components such as annexin II, myosin Ia, and lymphocyte-associated α-kinase (73–75). Annexin II is particularly important because its downregulation not only leads to accumulation of SI in intracellular compartments of MDCK cells but also changes the morphology if downregulated in intestinal Caco-2 cells. Here, long-term downregulation of annexin II leads to dramatic alterations in the apical membrane, as revealed by flattened surface and substantial reduction in the number of microvilli (76).

NOVEL CONCEPTS OF CSID INHERITANCE AND PREVALENCE

Compound heterozygosity has been demonstrated in several recessively inherited diseases such as cystic fibrosis, factor X deficiency, or familial Mediterranean fever (77–80), and CSID can now be added to this list as the first intestinal disaccharidase disorder with this pattern of inheritance. It is interesting to note that the normal activity levels of the disaccharidases SI, maltase-glucoamylase, and lactase-phlorizin hydrolase display a wide range (81,82), in which the maximum levels are more than 2-fold higher than the minimal normal levels. These activity levels may be explained by a genetic pattern of SI that correlates with 1 healthy allele for the ''low normal activity'' and 2 healthy alleles for the ''high normal activity.'' Consequently, it is likely that coding or noncoding regulatory mutations in these genes may be more common than initially thought and that the effects elicited by these mutations are compensated for by the wild-type alleles. Along these lines, it is likely that CSID does not arise as a consanguineous trait as was initially thought, whereby 2 alleles are defective, and it is likely to be more common instead.

CONCLUSIONS

Elucidation of pathomechanisms implicating impaired protein transport and altered intracellular processing in many diseases has greatly advanced our knowledge of the corresponding mechanisms and their protein and structural components. We presented cases of a mild intestinal brush border disease, CSID, through which several aspects of the trafficking between the ER and the Golgi apparatus, as well as the polarized apical sorting, have been defined and identified.

A novel quality-control mechanism has been proposed by phenotype II in which a temperature-sensitive mutant SI is synthesized and processed between the ER and *cis*-Golgi. It remains to be elucidated whether this quality-control machinery is limited to SI and structurally similar proteins or a ubiquitous network whose components have yet to be identified.

The sorting phenotypes IV to VI helped localize apical sorting signals of SI to O-glycosylation (stalk region) and the transmembrane domain and have unequivocally demonstrated that an active recognition of O-glycans, perhaps through a lectin-like receptor, is required for association of SI with lipid rafts. One main criterion of these sorting mechanisms is the association or non-association of the proteins with detergent-insoluble lipid microdomains (rafts) rich in cholesterol and glycosphingolipids.

Furthermore, proteins could be identified that were specifically involved in the peripheral distribution of the rafts-associated SI along actin filaments versus nonassociated lactase-phlorizin hydrolase, thus suggesting a mechanism in which annexin II interacts with the vesicle membrane and the cytoskeleton and lymphocyte-associated α-kinase activates the actin motor protein myosin Ia.

The pattern of inheritance of CSID is either based on single mutations on both alleles (homozygous pattern) or caused by compound heterozygosity, thus suggesting that CSID is a more common disease than initially thought. It is important to note that that all of the patients described in this review have essentially similar clinical symptoms, yet with different mutations in the sucrase or isomaltase coding regions. Nevertheless, a reliable correlation between the genotype/phenotype and variations in the clinical symptoms requires a higher sample number, to which none of the studies described so far had access. The progress in unraveling the molecular basis of CSID constitutes a strong asset for developing novel therapies in which protein components implicated in the onset of the disease could be targeted, rather than lifelong sucrose-prevention treatment. Along this line, it would be interesting to identify the genetics of CSID in the population of Greenland, where the incidence of CSID accounts for almost 5% of the entire population, and to determine whether CSID is elicited by novel mutations specific for this ethnic group. Finally, there are few reports on the onset of CSID in adults (21,83). In 1 case, however, the deficiency was the consequence of severe dietary carbohydrate restriction (21). Nevertheless, characterization of the genetic background of these rare cases and their comparison with the known mutations could shed light on the functionality of particular mutations in the context of long-term progression of carbohydrate malabsorption.

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Investigations of the Structures and Inhibitory Properties of Intestinal Maltase Glucoamylase and Sucrase Isomaltase

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Two enzyme complexes are largely responsible for the post-amylase metabolism of starch limit dextrins into monomeric glucose in the human small intestine, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI) (1). MGAM and SI each consist of 2 active enzyme domains or modules, with related structures but somewhat different enzymatic characteristics. Because there is some overlap in their substrate tolerances, the nomenclature used here is structurally defined: ntMGAM and ntSI for the respective N-terminal enzyme modules, and ctMGAM and ctSI for the C-terminal units.

All 4 MGAM and SI enzyme modules are classified into glycoside hydrolase family GH31 (Carbohydrate Active Enzymes Database, *http://www.cazy.org*) (2). This family is characterized by a number of enzymes with activities toward starch-derived structures. Interestingly, several GH31 enzymes are present in the proteome of gut-resident microbes, such as *Bacteroides thetaiotamicron*,

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