NOTICE TO PHYSICIAN

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(Sample) Letter of Medical Necessity

Insurer
(Address)

Re: **(Patient's name)**
Date of Birth:
Policy Number: **(Patient's ID number)**

Dear **(Insurer's contact's name and title)**:

I am writing to request prior authorization to initiate Sucraid® (sacrosidase) Oral Solution for (**Name of patient)**. This letter provides evidence that this enzyme replacement therapy is medically necessary for **(His/her)** care and that it is an accepted treatment for Congenital Sucrase-Isomaltase Deficiency (CSID). CSID is a rare genetic disorder that affects a patient's ability to digest certain sugars due to absent or low levels of two digestive enzymes, sucrase and isomaltase. These enzymes are involved in the digestion of sugar and starch. Untreated patients with CSID experience gastrointestinal symptoms such as diarrhea, gas, bloating, abdominal pain, and, in infants and young children, slow growth.[1,2]

The following sections provide detailed information about the patient's medical history, a description of the treatment, and the reasons for using Sucraid® in this case.

Patient History and Diagnosis

On **(Date),** I diagnosed **(Patient name)** with CSID (ICD10 Code E74.31). **(Include complete information on diagnosis and methods used in the determination of diagnosis, such as evaluation of the patient's case history, a disaccharidase assay test using a small bowel biopsy taken during an esophagogastroduodenoscopy [EGD] procedure or a sucrose breath test [hydrogen methane or ^{13}C].[3] Also list previous therapies that have been tried and failed [e.g., nutritional counseling, dietary adjustments] and what factors led to the discontinuation of these therapies.)**

Treatment Description and Rationale

Please note that your plan's claim denial was based on an incorrect prior authorization rule requiring sucrase activity <10 U/g for CSID diagnosis (please see Attachment 3). The impression that "data/publications demonstrated no clinical efficacy of Sucraid® for enzyme levels above 10 U/g" is incorrect. The normal sucrase activity from a small intestinal biopsy is

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54 µmol/min/g. A CSID diagnosis is confirmed with a sucrase level ≤**25 µmol/min/g**, although CSID associated symptoms have been observed in patients whose sucrase levels are **<35 µmol/min/g.**

Sucraid® is approved by the U.S. Food and Drug Administration and indicated for the treatment of genetically determined sucrase deficiency, which is part of CSID. Please see Attachments 1-3. In my clinical opinion, **(Patient name)** should receive this therapy for the following reasons:

- Suffers chronic abdominal pain, diarrhea, and bloating
- Unable to digest foods containing sucrose
- A positive breath test or low sucrase activity by small intestinal biopsy

Please feel free to contact me if you require additional information.

Sincerely,
(Physician's name)

[1]Treem WR. Congenital Sucrase-Isomaltase Deficiency. *J Pediatr Gastroenterol Nutr.* 1995;21(1):1-14.
[2]Treem WR. Clinical Aspects and Treatment of Congenital Sucrase-Isomaltase Deficiency. *J Pediatr Gastroenterol Nutr*. 2012;55 (suppl 2):S7-13.
[3]Robayo-Torres CC, Opekun AR, Quezada-Calvillo R, et al. ^{13}C-Breath Tests for Sucrose Digestion in Congenital Sucrase Isomaltase Deficient and Sacrosidase Supplemented Patients. *J Pediatr Gastroenterol Nutr*. 2009;48(4):412-8.

Indication

Sucraid® (sacrosidase) Oral Solution is an enzyme replacement therapy for the treatment of genetically determined sucrase deficiency, which is part of Congenital Sucrase-Isomaltase Deficiency (CSID).

Important Safety Information for Sucraid® (sacrosidase) Oral Solution

- Sucraid® may cause a serious allergic reaction. Patients should stop taking Sucraid® and get emergency help immediately if any of the following side effects occur: difficulty breathing, wheezing, or swelling of the face. Care should be taken when administering initial doses of Sucraid® to observe any signs of acute hypersensitivity reaction.
- Do not use Sucraid® with patients known to be hypersensitive to yeast, yeast products, papain, or glycerin (glycerol).
- Although Sucraid® provides replacement therapy for the deficient sucrase, it does not provide specific replacement therapy for the deficient isomaltase.
- Adverse reactions as a result of taking Sucraid® may include worse abdominal pain,

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vomiting, nausea, diarrhea, constipation, difficulty sleeping, headache, nervousness, and dehydration.

- Before prescribing Sucraid® to diabetic patients, the physician should consider that Sucraid® will enable sucrose hydrolysis and the absorption of those hydrolysis products, glucose and fructose.
- The effects of Sucraid® have not been evaluated in patients with secondary (acquired) disaccharidase deficiency.
- DO NOT HEAT SOLUTIONS CONTAINING SUCRAID®. Do not put Sucraid® in warm or hot fluids. Do not reconstitute or consume Sucraid® with fruit juice since the acidity of the juice may reduce the enzyme activity of Sucraid®. Half of the reconstituted Sucraid® should be taken at the beginning of the meal or snack and the other half during the meal or snack.
- Sucraid® should be refrigerated at 36°F-46°F (2°C-8°C) and should be protected from heat and light.

Sucraid® (sacrosidase) Oral Solution:

DESCRIPTION

Sucraid® (sacrosidase) Oral Solution is an enzyme replacement therapy for the treatment of genetically determined sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID).

CHEMISTRY

Sucraid is a pale yellow to colorless, clear solution with a pleasant sweet taste. Each milliliter (mL) of Sucraid contains 8,500 International Units (I.U.) of the enzyme sacrosidase, the active ingredient. The chemical name of this enzyme is ß,D-fructofuranoside fructohydrolase. The enzyme is derived from baker's yeast (*Saccharomyces cerevisiae*).

It has been reported that the primary amino acid structure of this protein consists of 513 amino acids with an apparent molecular weight of 100,000 g/mole for the glycosylated monomer (range 66,000-116,000 g/mole). Reports also suggest that the protein exists in solution as a monomer, dimer, tetramer, and octomer ranging from 100,000 g/mole to 800,000 g/mole. It has an isoelectric point (pI) of 4.5.

Sucraid may contain small amounts of papain. Papain is known to cause allergic reactions in some people. Papain is a protein-cleaving enzyme that is introduced in the manufacturing process to digest the cell wall of the yeast and may not be completely removed during subsequent process steps.

Sucraid contains sacrosidase in a vehicle comprised of glycerol (50% wt/wt), water, and citric acid to maintain the pH at 4.0 to 4.7. Glycerol (glycerin) in the amount consumed in the recommended doses of Sucraid has no expected toxicity.

This enzyme preparation is fully soluble with water, milk, and infant formula. DO NOT HEAT SOLUTIONS CONTAINING SUCRAID. Do not put Sucraid in warm or hot liquids.

CLINICAL PHARMACOLOGY

Congenital sucrase-isomaltase deficiency (CSID) is a chronic, autosomal recessive, inherited, phenotypically heterogeneous disease with very variable enzyme activity. CSID is usually characterized by a complete or almost complete lack of endogenous sucrase activity, a very marked reduction in isomaltase activity, a moderate decrease in maltase activity, and normal lactase levels.

Sucrase is naturally produced in the brush border of the small intestine, primarily the distal duodenum and jejunum. Sucrase hydrolyzes the disaccharide sucrose into its component monosaccharides, glucose and fructose. Isomaltase breaks down disaccharides from starch into simple sugars. Sucraid does not contain isomaltase.

In the absence of endogenous human sucrase, as in CSID, sucrose is not metabolized. Unhydrolyzed sucrose and starch are not absorbed from the intestine and their presence in the intestinal lumen may lead to osmotic retention of water. This may result in loose stools.

Unabsorbed sucrose in the colon is fermented by bacterial flora to produce increased amounts of hydrogen, methane, and water. As a consequence, excessive gas, bloating, abdominal cramps, nausea, and vomiting may occur.

Chronic malabsorption of disaccharides may result in malnutrition. Undiagnosed/untreated CSID patients often fail to thrive and fall behind in their expected growth and development curves. Previously, the treatment of CSID has required the continual use of a strict sucrose-free diet.

CSID is often difficult to diagnose. Approximately 4% to 10% of pediatric patients with chronic diarrhea of unknown origin have CSID. Measurement of expired breath hydrogen under controlled conditions following a sucrose challenge (a measurement of excess hydrogen excreted in exhalation) in CSID patients has shown levels as great as 6 times that in normal subjects.

A generally accepted clinical definition of CSID is a condition characterized by the following: stool pH < 6, an increase in breath hydrogen of > 10 ppm when challenged with sucrose after fasting and a negative lactose breath test. However, because of the difficulties in diagnosing CSID, it may be warranted to conduct a short therapeutic trial (e.g., one week) to assess response in patients suspected of having CSID.

CLINICAL STUDIES

A two-phase (dose response preceded by a breath hydrogen phase) double-blind, multi-site, crossover trial was conducted in 28 patients (aged 4 months to 11.5 years) with confirmed CSID. During the dose response phase, the patients were challenged with an ordinary sucrose-containing diet while receiving each of four doses of sacrosidase: full strength (9000 I.U./mL) and three dilutions (1:10 [900 I.U./mL], 1:100 [90 I.U./mL], and 1:1000 [9 I.U./mL]) in random order for a period of 10 days. Patients who weighed no more than 15 kg received 1 mL per meal; those weighing more than 15 kg received 2 mL per meal. The dose did not vary with age or sucrose intake. A dose-response relationship was shown between the two higher and the two lower doses. The two higher doses of sacrosidase were associated with significantly fewer total stools and higher proportions of patients having lower total symptom scores, the primary efficacy end-points. In addition, higher doses of sacrosidase were associated with a significantly greater number of hard and formed stools as well as with fewer watery and soft stools, the secondary efficacy end-points.

Analysis of the overall symptomatic response as a function of age indicated that in CSID patients up to 3 years of age, 86% became asymptomatic. In patients over 3 years of age, 77% became asymptomatic. Thus, the therapeutic response did not differ significantly according to age.

A second study of similar design and execution as the first used 4 different dilutions of sacrosidase: 1:100 (90 I.U./mL), 1:1000 (9 I.U./mL), 1:10,000 (0.9 I.U./mL), and 1:100,000 (0.09 I.U./mL). There were inconsistent results with regards to the primary efficacy parameters.

In both trials, however, patients showed a marked decrease in breath hydrogen output when they received sacrosidase in comparison to placebo.

INDICATIONS AND USAGE

Sucraid® (sacrosidase) Oral Solution is indicated as oral replacement therapy of the genetically determined sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID).

CONTRAINDICATIONS

Patients known to be hypersensitive to yeast, yeast products, glycerin (glycerol), or papain.

WARNINGS

Severe wheezing, 90 minutes after a second dose of sacrosidase, necessitated admission into the ICU for a 4-year-old boy. The wheezing was probably caused by sacrosidase. He had asthma and was being treated with steroids. A skin test for sacrosidase was positive.

Other serious events have not been linked to Sucraid.

PRECAUTIONS

Care should be taken to administer initial doses of Sucraid near (within a few minutes of travel) a facility where acute hypersensitivity reactions can be adequately treated. Alternatively, the patient may be tested for hypersensitivity to Sucraid through skin abrasion testing. Should symptoms of hypersensitivity appear, discontinue medication and initiate symptomatic and supportive therapy.

Skin testing as a rechallenge has been used to verify hypersensitivity in one asthmatic child who displayed

PATIENT PACKAGE INSERT

0110-0219

INFORMATION FOR PATIENTS

Sucraid® (sacrosidase) Oral Solution

Please read this leaflet carefully before you take Sucraid® (sacrosidase) Oral Solution or give Sucraid to a child. Please do not throw away this leaflet. You may need to read it again at a later date. This leaflet does not contain all the information on Sucraid. For further information or advice, ask your doctor or pharmacist.

BEFORE TAKING SUCRAID

WARNING: Sucraid may cause a serious allergic reaction. If you notice any swelling or have difficulty breathing, get emergency help right away. Before taking your first and second doses, be sure that there are health professionals nearby (within a few minutes of travel) just in case there is an allergic reaction.

INFORMATION ABOUT YOUR MEDICINE

The name of your medicine is Sucraid® (sacrosidase) Oral Solution. It can be obtained only with a prescription from your doctor.

The purpose of your medicine:

Sucraid is an enzyme replacement therapy for the treatment of the genetically determined sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID). CSID is a condition where your body lacks the enzymes needed to break down and absorb sucrose (table sugar) and other sugars from starch.

The symptoms of CSID often include frequent watery diarrhea, abdominal pain, bloating, and gas. In many cases, the symptoms of CSID are similar to other medical problems. Only your doctor can make a definite diagnosis of CSID.

ATTACHMENT 1

Sucraid can help improve the breakdown and absorption of sucrose (table sugar) from the intestine and can help relieve the gastrointestinal symptoms of CSID.

Sucraid does not break down some sugars resulting from the digestion of starch. Therefore, you may need to restrict the amount of starch in your diet. Your doctor will tell you if you should restrict the amount of starch in your diet.

Discuss the following important information with your doctor before you begin to take Sucraid:

Tell your doctor if you are allergic to, have ever had a reaction to, or have ever had difficulty taking yeast, yeast products, papain, or glycerin (glycerol).

Tell your doctor if you have diabetes. With Sucraid, sucrose (table sugar) can be absorbed from your diet and your blood glucose levels may change. Your doctor will tell you if your diet or diabetes medicines need to be changed.

Side effects to watch for:

Some patients may have worse abdominal pain, vomiting, nausea, or diarrhea. Constipation, difficulty sleeping, headache, nervousness, and dehydration have also occurred. Other side effects may also occur. If you notice these or any other side effects during treatment with Sucraid, check with your doctor.

Stop taking Sucraid and get emergency help immediately if any of the following side effects occur: difficulty breathing, wheezing, or swelling of the face.

How to take your medicine:

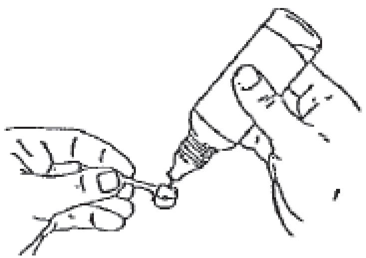
Each bottle of Sucraid is supplied with a plastic screw cap which covers a dropper dispensing tip. Remove the outer cap and measure out the required dose. Reseal the bottle after each use by replacing and twisting the cap until tight.

Write down the date the sealed bottle is first opened in the space provided on the bottle label. Always throw away the bottle four weeks after first opening it because Sucraid contains no preservatives. For the same reason, you should rinse the measuring scoop with water after each time you finish using it.

To get the full benefits of this medicine, it is very important to take Sucraid as your doctor has prescribed. The usual dosage is 1 to 2 milliliters (mL) with each meal or snack: 1mL = 1 full measuring scoop (28 drops from the bottle tip) and 2 mL = 2 full measuring scoops (56 drops from the bottle tip).

Measure your dose with the measuring scoop provided (see Figure 1). Do not use a kitchen teaspoon or other measuring device since it will not measure an accurate dose.

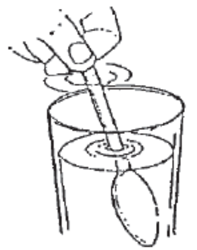
Figure 1. Measure dose with measuring scoop.



Mix your dose in 2 to 4 ounces of water, milk, or infant formula (see Figure 2). Sucraid should not be dissolved in or taken with fruit juice.

NEVER HEAT SUCRAID OR PUT IT IN WARM OR HOT BEVERAGES OR INFANT FORMULA. Heating Sucraid causes it to lose its effectiveness. The beverage or infant formula should be taken cold or at room temperature.

Figure 2. Mix dose in beverage or infant formula.



It is recommended that approximately half of your dosage be taken at the beginning of each meal or snack and the remainder of your dosage be taken during the meal or snack.

Storing your medicine:

Sucraid is available in 4 fluid ounce (118 mL) see-through plastic bottles, packaged two bottles per box. A 1 mL measuring scoop is provided with each bottle. Always store Sucraid in a refrigerator at 36°F - 46°F (2°C - 8°C). Protect Sucraid from heat and light.

If your bottle of Sucraid has expired (the expiration date is printed on the bottle label), throw it away.

Keep this medicine in a safe place in your refrigerator where children cannot reach it.

QOL Medical, LLC
Vero Beach, FL 32963

www.sucraid.net
For questions call 1-866-469-3773

Rev 02/19
Part No. 0110

wheezing after oral sacrosidase.

GENERAL

Although Sucraid provides replacement therapy for the deficient sucrase, it does not provide specific replacement therapy for the deficient isomaltase. Therefore, restricting starch in the diet may still be necessary to reduce symptoms as much as possible. The need for dietary starch restriction for patients using Sucraid should be evaluated in each patient.

It may sometimes be clinically inappropriate, difficult, or inconvenient to perform a small bowel biopsy or breath hydrogen test to make a definitive diagnosis of CSID. If the diagnosis is in doubt, it may be warranted to conduct a short therapeutic trial (e.g., one week) with Sucraid to assess response in a patient suspected of sucrase deficiency.

The effects of Sucraid have not been evaluated in patients with secondary (acquired) disaccharidase deficiencies.

INFORMATION FOR PATIENTS

See Patient Package Insert. Patients should be instructed to discard bottles of Sucraid 4 weeks after opening due to the potential for bacterial growth. For the same reason, patients should be advised to rinse the measuring scoop with water after each use.

Sucraid is fully soluble with water, milk, and infant formula, but it is important to note that this product is **sensitive to heat.** Sucraid should not be reconstituted or consumed with fruit juice, since its acidity may reduce the enzyme activity.

USE IN DIABETICS

The use of Sucraid will enable the products of sucrose hydrolysis, glucose and fructose, to be absorbed. This fact must be carefully considered in planning the diet of diabetic CSID patients using Sucraid.

LABORATORY TESTS

The definitive test for diagnosis of CSID is the measurement of intestinal disaccharidases following small bowel biopsy.

Other tests used alone may be inaccurate: for example, the breath hydrogen test (high incidence of false negatives) or oral sucrose tolerance test (high incidence of false positives). Differential urinary disaccharide testing has been reported to show good agreement with small intestinal biopsy for diagnosis of CSID.

DRUG INTERACTIONS

Neither drug-drug nor drug-food interactions are expected or have been reported with the use of Sucraid. However, Sucraid should not be reconstituted or consumed with fruit juice, since its acidity may reduce the enzyme activity.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long-term studies in animals with Sucraid have not been performed to evaluate the carcinogenic potential. Studies to evaluate the effect of Sucraid on fertility or its mutagenic potential have not been performed.

PREGNANCY

Teratogenic effects. Pregnancy Category C. Animal reproduction studies have not been conducted with Sucraid. Sucraid is not expected to cause fetal harm when administered to a pregnant woman or to affect reproductive capacity. Sucraid should be given to a pregnant woman only if clearly needed.

NURSING MOTHERS

The Sucraid enzyme is broken down in the stomach and intestines, and the component amino acids and peptides are then absorbed as nutrients.

PEDIATRIC USE

Sucraid has been used in patients as young as 5 months of age. Evidence in one controlled trial in primarily pediatric patients shows that Sucraid is safe and effective for the treatment of the genetically acquired sucrase deficiency, which is part of CSID.

ADVERSE REACTIONS

Adverse experiences with Sucraid in clinical trials were generally minor and were frequently associated with the underlying disease.

In clinical studies of up to 54 months duration, physicians treated a total of 52 patients with Sucraid. The adverse experiences and respective number of patients reporting each event (in parenthesis) were as follows: abdominal pain (4), vomiting (3), nausea (2), diarrhea (2), constipation (2), insomnia (1), headache (1), nervousness (1), and dehydration (1).

Note: diarrhea and abdominal pain can be a part of the clinical presentation of the **genetically determined sucrase deficiency,** which is part of congenital sucrase-isomaltase deficiency (CSID).

One asthmatic child experienced a serious hypersensitivity reaction (wheezing) probably related to sacrosidase (see Warnings). The event resulted in withdrawal of the patient from the trial but resolved with no sequelae.

OVERDOSAGE

Overdosage with Sucraid has not been reported.

DOSAGE AND ADMINISTRATION

The recommended dosage is 1 or 2 mL (8,500 to 17,000 I.U.) or 1 or 2 full measuring scoops (each full measuring scoop equals 1 mL; 28 drops from the Sucraid container tip equals 1 mL) taken orally with each meal or snack diluted with 2 to 4 ounces (60 to 120 mL) of water, milk, or infant formula. The beverage or infant formula should be served cold or at room temperature. The beverage or infant formula should not be warmed or heated before or after addition of Sucraid because heating is likely to decrease potency. Sucraid should not be reconstituted or consumed with fruit juice since its acidity may reduce the enzyme activity.

It is recommended that approximately half of the dosage be taken at the beginning of the meal or snack and the remainder be taken during the meal or snack.

The recommended dosage is as follows:

1 mL (8,500 I.U.) (one full measuring scoop or 28 drops) per meal or snack for patients up to 15 kg in body weight.

2 mL (17,000 I.U.) (two full measuring scoops or 56 drops) per meal or snack for patients over 15 kg in body weight.

Dosage may be measured with the 1 mL measuring scoop (provided) or by drop count method (1 mL equals 28 drops from the Sucraid container tip).

HOW SUPPLIED

Sucraid® (sacrosidase) Oral Solution is available in 118 mL (4 fluid ounces) translucent plastic bottles, packaged two bottles per box. Each mL of solution contains 8,500 International Units (I.U.) of sacrosidase. A 1 mL measuring scoop is provided with each bottle. A full measuring scoop is 1 mL.

Store in a refrigerator at 2°- 8° C (36°- 46°F). Discard four weeks after first opening due to the potential for bacterial growth. Protect from heat and light.

Rx only.

Distributed by:
QOL Medical, LLC
Vero Beach, FL 32963

To order, or for any questions, call 1-866-469-3773
www.sucraid.net

NDC# 67871-111-04

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Clinical Aspects and Treatment of Congenital Sucrase-Isomaltase Deficiency

William R. Treem

Congenital sucrase-isomaltase deficiency (CSID) was first described by Weijers and colleagues in 1960 and has subsequently been defined as an inherited deficiency in the ability to hydrolyze sucrose, maltose, short 1–4 linked glucose oligomers, branched (1–6 linked) *a*-limit dextrins, and starch (1). Exposure to these nutrients provokes osmotic diarrhea with pain, bloating, and abdominal distention; rapid small bowel transit and malabsorption of other nutrients; excessive bacterial fermentation of malabsorbed carbohydrate with colonic gas production and acidification of the stools; and at times, chronic malnutrition and failure to thrive (2). After the sucrase-isomaltase (SI) gene was identified on chromosome 3 (3q25–26) and was cloned in 1992 by Chantret and colleagues, more than 25 mutations in the gene responsible for the synthesis of SI have been discovered (3–6). These mutations result in a variety of defects in the folding of the synthesized propeptide chain; the initial high mannose and then complex glycosylation; the sequential export from the endoplasmic reticu- lum to the Golgi apparatus, and eventually to the apical membrane; the anchoring of the N-terminal aspect of the isomaltase subunit in the enterocyte microvillus membrane; and the normal architecture of the sucrase and isomaltase catalytic sites, which are independent of each other and can be affected separately, leading to isolated deficiencies (5,6). The intracellular phenotypic heterogeneity is reflected in a range of enzymatic capability ranging from comple- tely absent sucrase activity to low but present residual activity and from completely absent isomaltase activity to normal activity. Because SI is responsible for approximately 60% to 80% of the maltase activity in the brush border of the enterocyte, maltase activity is also significantly reduced in almost all cases.

In addition to the degree of enzyme deficiency, the appearance of overt clinical manifestations of CSID is partially determined by the amount of sugar and starch being consumed. Approximately 60% of the total calories consumed in the average diet in the United States originate from carbohydrates, with 30% of carbohydrate calories deriving from sucrose (7). The typical adult consumes about 150 lb of sugar per year and 65 lb of sucrose. The influence of the dietary consumption of sucrose is best illustrated by the natural history of CSID in Greenland, where approximately 5% to 10% of Greenland Eskimos are affected (8). Before the introduction of a Western diet in the middle part of the last century provoked by the settlement of Greenland by northern Europeans from Denmark and other European countries, CSID was unknown among the indigenous population, who consumed a fish-and-marine mammal–based diet, relatively high in fat and protein and low in carbohydrates and sucrose. A marked increase in diarrhea and other gastrointestinal symptoms in the indigenous population led to studies in the 1970s that delineated the prevalence of CSID. The early introduction of sucrose and starch in the form of baby juices, baby food fruits and certain vegetables, and sucrose- and maltodextrin-containing infant formulas also plays a role in the timing of clinical manifestations of CSID.

Other hormonal and dietary factors and micronutrients also influence small intestinal sucrase activity. Unlike lactase activity

From the Established Products Division, Johnson and Johnson Pharmaceutical Research and Development, Titusville, New Jersey.

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that is unresponsive to lactose consumption, sucrase activity is inducible by a high-sucrose, high-carbohydrate diet and reduced by a high-protein, low-carbohydrate diet (9). Both thyroxine and corticosteroids induce the expression of SI on the brush border of the enterocyte (10). In animal models, dietary-induced iron deficiency results in decreased small-bowel disaccharidase activity, with lactase affected more than SI (11). This appears to be the result of decreased gene expression caused by overexpression of PDX-1, a repressor of the lactase and sucrase promoter regions. PDX-1 overexpression can be reversed with restoration of a normal iron-containing diet and replenishment of iron stores. Naturally occurring phytochemicals in the diet (eg, cinnamon extract, onions, garlic, certain spices, mushrooms, chamomile tea) can act as inhibitors of amylase and intestinal *a*-glucosidases, thus influencing luminal sucrase activity (12). In patients with CSID and mutations allowing some residual SI activity, these hormonal and dietary factors may influence the onset and severity of symp- toms.

PREVALENCE OF CSID

The actual prevalence of CSID is still a matter of debate. Substantial progress in cloning disease-causing mutations has opened the possibility of conducting large-scale population-based screening. In a recent study by Scott and colleagues, all 48 exons of the 100-kb SI gene on chromosome 3 were sequenced in 31 biopsy-proven patients with CSID and 55 different mutations were identified, with at least 1 of the 4 most common mutations found on 32 (59%) of the affected alleles (4). If one assumes the Hardy-Weinberg equilibrium for mutations in the population, then there is an 83% probability that an individual with severe clinical manifestations of CSID will have at least 1 of these 4 mutations. The results of this study raise the possibility in the near future of a genetic screening test both for population prevalence studies and to aid in the diagnosis of new cases. With the availability of DNA harvesting from buccal mucosa, the feasibility of genetic testing in young infants and children increases substantially. Studies are in progress to determine whether genetic testing also can be done on intestinal epithelial biopsy specimens opening the possibility of simultaneously determining disaccharidase levels and genetic mutations for CSID.

Clinical studies of relatively homogenous selected populations have yielded high rates of CSID, ranging from 5% to 10% in Greenland Eskimos, 3% to 7% in Canadian native peoples, and about 3% in Alaskans of native ancestry (13,14); however, estimates of the prevalence of CSID in other North American and European populations generally range from 1 in 500 to 1 in 2000 among non-Hispanic whites, with a lower prevalence in African Americans and whites of Hispanic descent. These studies evolved from older studies of intestinal disaccharidase levels in adult patients undergoing endoscopy for gastrointestinal symptoms (15,16). The estimates have shown low levels of sucrase activity >1 standard deviation (SD) below the mean in mucosal biopsy specimens from 2% to 9% of patients, even in the absence of overt mucosal injury. If one assumes that some of these patients represent heterozygotes for CSID, then the prevalence quoted above seems plausible; however, the diagnosis of CSID is rarely made even in infants and young children, suggesting the possibility that the phenotype of CSID may be much broader and more variable than previously thought and that a large proportion of affected adult and pediatric patients are not being tested and diagnosed.

This hypothesis receives support from the analysis of recently released whole exome sequence data (Exome Variant Server, *http://evs.gs.washington.edu/EVS*). Belmont and colleagues at the Children's Nutrition Research Center at the Baylor College of Medicine reviewed the SI gene sequence data in a population of approximately 3500 North American white adults ascertained as controls or with atherosclerosis and no known bias for gastrointestinal disease. These data showed 271 rare missense variants with an aggregate allelic frequency of 0.03864. Based on this allele frequency, and assuming that the alleles segregate independently, Hardy-Weinberg proportions were used to estimate the frequency of homozygotes and compound heterozygotes for rare alleles. Although it is not known whether all of these variants result in decreased enzyme activity, the large number of variants could be consistent, with an estimated frequency of 1:670 affected patients and 7% carriers in this population (personal communication, Dr John Belmont, February 28, 2012; public data at the Exome Variant Server).

There are several pieces of clinical evidence that support the view that CSID is more prevalent than previously believed. Studies of disaccharidase levels from intestinal biopsy specimens sent to 2 pediatric reference laboratories have shown surprisingly frequent results for a pattern suggesting CSID. In 2 studies of almost 1000 biopsies each, sucrase deficiency was defined as >1 SD below the mean activity level in 1 study and <10% of the mean in another (17,18). As defined, sucrase deficiency was found in 11% and13% of biopsy specimens in the 2 studies. Included were specimens with isolated sucrase or SI deficiency only (1.0% and 1.1%, respectively), SI and maltase-glucoamylase (MGAM) deficiency only (3.0% and 2.4%, respectively), and pandisaccharidase deficiency (5.8% in both studies). Pandisaccharidase deficiency was more likely accounted for by acquired diffuse intestinal villous injury. Although correlation with histology was not provided, the surprisingly high numbers of isolated SI and combined SI-MGAM deficiencies without lactase deficiency suggest that specific genetically determined enzyme deficiencies may be playing a role.

Although small intestinal disaccharidases are most often investigated in the clinical setting of diarrhea in infants and young children, the role of disaccharidase deficiencies and specifi- cally SI deficiency in other gastrointestinal syndromes also has been entertained. Small series of patients with CSID have revealed a subgroup of adolescents and even adults who present with dyspepsia, gas, and /or irritable bowel syndrome (IBS) rather than the classic presentation of watery diarrhea, failure to thrive, diaper rash, irritability, and acidic stools in infancy (2,19,20). Karnsakul and colleagues studied 44 children and adolescents with dyspepsia, only 4 of whom had intermittent diarrhea (21). Patients underwent endoscopy with small bowel biopsies and disaccharidases and one-third had low sucrase activity (>1 SD from the mean), includ- ing 4 of 44 with isolated low sucrase activity, and 11 of 44 with sucrase and pandisaccharidase deficiency, but no significant villous atrophy. In addition, in preliminary follow-up studies of families with index cases of CSID uncovered in a child, parents with a long- term diagnosis of IBS were subsequently identified as having CSID (22).

After the sequencing of all of the exons of the CSID gene, most patients with CSID studied by Scott and colleagues have been found to be homozygous or compound heterozygotes for disease-causing mutations (4). Kerry and Townley showed that the parents of 4 children with CSID had intestinal sucrase activity below the lower limits of normal and a sucrase:lactase ratio <0.8, both consistent with the heterozygous state and supporting an autosomal recessive pattern of inheritance (23); however, 3 patients in Scott and colleagues' study who presented with classical symptoms and biopsy-proven absent sucrase activity with absent or low isomaltase activity, and 2 others with milder decreases in both enzymes, appeared to be heterozygote carriers with a mutation on 1 allele and a wild-type gene on the other. These small studies lend credence to the hypothesis that CSID is more prevalent than previously thought; manifests with milder phenotypes that may even omit diarrhea as a prominent symptom; and may be transmitted in ways other than strict autosomal recessive inheritance. The combination of the ''heterozygous'' state with other genetic and/or dietary and nutritional interactions may provoke gastrointestinal symptoms in certain patients.

PRESENTATION AND NATURAL HISTORY OF CSID

The classical presentation of CSID is severe watery diarrhea, failure to gain weight, irritability, and diaper rash in a 9- to 18-month-old infant who has been exposed to sucrose and starch in the form of baby juices, baby food fruits, teething biscuits, crackers, and other starches. Factors that contribute to the predilec- tion for a presentation during infancy include the shorter length of the colon and a decreased capacity for colonic reabsorption of fluid and electrolytes, more rapid small intestinal transit, a high carbohydrate diet, and the ontogeny of amylase activity that does not reach ''adult'' levels until the second year of life (24); however, clinical studies during the last 20 years and a retrospective review of 65 patients with CSID have revealed a variety of presentations that defy the conventional view (5,22,25,26). Table 1 describes the symptoms at presentation in these 65 patients. Although most have presented with the classic symptoms, a significant minority have only been diagnosed between 2 to 8 years old after normal growth and a previous diagnosis of chronic nonspecific diarrhea of childhood (''toddler's diarrhea''), or even later during adolescence or young adulthood carrying a diagnosis of diarrhea-predominant IBS. Up to one-third have had vomiting as a prominent symptom, suggesting again that dyspepsia, gas, bloating, and even reflux-like symptoms may predominate in some patients. Other anecdotal reports have mentioned hypercalcemia and nephrocalcinosis in infants with CSID, and even renal calculi in 2 adults with CSID (27,28).

In a follow-up study of 65 patients with CSID who responded to a questionnaire after being identified by a record of prescriptions for enzyme replacement therapy, 53 of 65 reported the onset of symptoms before 1 year of age, 7 between 1 and 10 years old, and 5 after 10 years of age (22); however, the age at which a diagnosis was made was shifted to the right, with only 17 of 65 diagnosed in the first year, 30 between 1 and 5 years, 10 between 5 and 10 years, and 8 after 10 years of age. The potential reasons for this delay in diagnosis include a mistaken diagnosis of protein intolerance in infancy with multiple formula changes and the elimination of glucose oligomers (maltodextrin) that are partially hydrolyzed by sucrase in favor of glucose monomers in amino acid–based formulas (29). A diagnosis of food allergy often also leads to the elimination juices and baby foods that may have a high sucrose load, further masking the true underlying cause of diarrhea in patients with CSID. Later in childhood, a diagnosis of chronic nonspecific diarrhea often will result in a lower carbohydrate, higher fat diet, and the elimination of all juices with improvement in symptoms of patients with CSID (30). Older children and adolescents with CSID and diarrhea-predominant IBS may learn which foods trigger their symptoms and avoid those foods, thus masking their true diagnosis. In addition, chronic carbohydrate malabsorption may act as a prebiotic stimulus to colonic bacterial growth, creating a significant increase in the capacity to ferment and salvage malabsorbed carbohydrate, and stimulate colonic short-chain fatty acid synthesis and sodium and fluid reabsorption by the colonocyte (31). Colonic bacterial flora ''adaptation'' may thus contribute to a decrease in diarrhea symptoms over time in some patients with CSID.

DIAGNOSIS OF CSID

At present, the gold standard for the diagnosis of CSID remains small intestinal biopsy specimens assayed for lactase, sucrase, isomaltase (palatinase), and maltase activity. In general, the criteria applied to make the diagnosis of CSID include normal small bowel morphology in the presence of absent or markedly reduced sucrase activity, isomaltase activity varying from 0 to full activity, reduced maltase activity, and normal lactase activity, or in the setting of reduced lactase, a sucrase:lactase ratio of <1.0. Table 2 summarizes the disaccharidase activities in 36 patients with CSID; all were included in 2 pivotal clinical trials included as part of the new drug application (NDA) for sacrosidase submitted to the Food and Drug Administration (FDA; NDA 20-772/S-011, 1998). Sucrase activity was absent in 24 of 36 (66%) patients, and in all but 3, activity was less than the third percentile of 977 values in ''controls,'' which consisted of unselected small bowel biopsies from children with diarrhea and other gastrointestinal symptoms (18). All sucrase activity values in patients with CSID were <10th percentile of controls. Almost two-thirds (23/35) had absent palatinase (isomaltase) activity, and all but 2 were <10th percentile, with 1 of those in the normal range and 1 with elevated activity. Maltase activity was variable. No patient had absent activity, but the mean equaled 41.5 U/g protein and the majority (25/36, 69%) exhibited reductions >2 standard deviations from the mean in controls. All but 2 patients demonstrated <10% of control activity. Two patients exhibited normal activity. There was no clear correlation between absent or residual sucrase activity with the spectrum of decreased maltase activity. Because the brush border enzyme MGAM is responsible for at least 20% of maltase activity, those patients with low maltase activity may be examples of combined deficiencies of SI and MGAM (32,33). Elevated lactase enzyme activity levels were found in 3 of our patients and have been found in a small minority of patients with CSID in most studies to date.

Recent studies of the SI gene in symptomatic patients with intestinal disaccharidase deficiency have identified compound

TABLE 1. Presenting symptoms in 65 patients with CSID (22)

Symptom	No. patients (%)
Diarrhea	62 (95)
Bloating/gas	55 (85)
Abdominal pain	43 (66)
Irritability	43 (66)
Diaper rash	40 (62)

TABLE 2. Intestinal biopsy disaccharidase activities in 36 patients with CSID (U/g protein) (42,43)

	Sucrase	Isomaltase (palatinase)	Maltase	Lactase
	(n ¼ 36)	(n ¼ 35)	(n ¼ 36)	(n ¼ 36)
Mean	2.3	1.9	41.5	30.5
Standard deviation	4.4	5.8	34.7	19.2

Failure to thrive	39 (60)	Median	0	0	29.2	27.6
Nausea/vomiting	22 (34)	Minimum	0	0	10.9	5.2
Irritable bowel syndrome	12 (18)	Maximum	15.4	33.3	166.7	101.5

heterozygotes with less severely reduced sucrase and isomaltase and even what appears to be true heterozygotes with 1 normal allele and what appears to be a more severe mutation on the other allele (4–6,34). One patient in the cohort studies by Scott et al appeared to have normal wild-type genes on both alleles with moderately reduced sucrase activity and symptoms provoked by sucrose consumption, which suggested acquired sucrase deficiency even in the presence of normal small intestinal morphology (4). Other causes of false-positive results come from biopsies taken in the proximal duodenum, where disaccharidase levels are often only approximately two-thirds of the levels found in the proximal jejunum (35). In addition, mishandling of biopsy specimens resulting in inadequate rapidity of freezing and premature thawing can result in a diffuse reduction in disaccharidase activity. Studies of replicate intestinal biopsy disaccharidase assays have demonstrated a coefficient of variation of 27%, stressing the variability of the assay (18). This variation emphasizes the role of clinical judgment in making the diagnosis of CSID from mucosal disaccharidase assay values. Other less invasive methods of diagnosis includethe sucrose breath hydrogen study and differential urinary disaccharides (36,37). Although relatively easy to accomplish, thesucrose breath hydrogen study is compromised by significant contamination from both false-positives (secondary sucrase deficiency from villous injury, dumping syndrome, and bacterial overgrowth) and false- negatives (nonhydrogen producers, antibiotic interference, delayed gastric emptying). Also, this test can provoke severe symptoms as a result ofthe 2-g/kg oral sucrose load given to patientswith CSID. The differential urinary disaccharide test examines the ratio of urinary sucrose:lactulose, which should approach 1.0 in patients with CSID; however for accurate results, this test relies on obtaining an accurate 10-hour urine collection that is difficult in many infants andyoung children and the presence of normal intestinal permeability.

Figure 1 summarizes data from studies of the utility of a ^{13}C-sucrose breath test to diagnose CSID (38). This test requires the administration of a small dose of uniformly labeled ^{13}C-sucrose mixed in unlabeled maltodextrin in water as a carrier and the subsequent collection of $^{13}CO_2$-enriched breath samples every 15 minutes for 2 hours. The separate administration of ^{13}C-glucose mixed in maltodextrin and collection of $^{13}CO_2$ allows ^{13}C-sucrose hydrolysis and digestion to be expressed as a coefficient of glucose oxidation (CGO). As Figure 1 shows, the mean percent CGO of ^{13}C-sucrose in 10 patients with CSID is 25% ± 21% compared with 146% ± 45% in 10 age-matched controls. A cutoff of 79% CGO yields 100% sensitivity and specificity for CSID. Although thetest requires 2 breath tests and infrared spectrophotometry, it has several advantages: it is noninvasive, has excellent sensitivity and specificity, and avoids provocation of gastrointestinal symptoms because of an excessive sucrose load.

TREATMENT OF CSID

Previous follow-up studies of children with CSID treated with sucrose- and starch- restricted diets have demonstrated that only 10% of patients remain consistently asymptomatic, and 60% to75% still experience diarrhea, gas, and/or abdominal pain, with a lower proportion (20%) complaining of nausea. Only approximately half of these children are compliant with the prescribed diet (39,40). Harms and colleagues described the amelioration of both hydrogen production and gastrointestinal symptoms in 8 children with CSID treated with Baker's yeast (*Saccharomyces cerevisiae*) cakes before a sucrose breath hydrogen test (41). *S cerevisiae* contains a *b*-fructofuranoside fructohydralase with sucrase but not maltase or isomaltase activity. By using specific growing conditions to promote increased enzyme activity and belt drying to preserve this activity, the food industry has for many years been using this enzyme to convert sugarcane (sucrose) to molasses and keep the centers of cream-filled candies liquid. Preclinical studies on a liquid preparation derived from the *S cerevisiae* (sacrosidase) grown under these conditions showed that 1 mL of this preparation contained approximately 8500 U of sucrose-hydro- lyzing activity (8500 *m*mol glucose formed per minute per milli- liter) (42). Sacrosidase was free of lactase, isomaltase, or maltase activity; rich in mannose glycosylation; maintained stable activity with refrigeration; and did not lose significant activity with a pH down to 1.0. Incubation of the enzyme with pepsin at or near the pH optimal for pepsin activity (1.5), however, produced a rapid loss of activity. Preincubation of the pepsin with bovine serum albumin provided a decoy for the pepsin and allowed preservation of sacrosidase activity even at a pH of 1.5.

Figure 2 shows the results of sucrose breath hydrogen studies on the first child with CSID treated with sacrosidase under an orphan drug grant from the FDA. Two breath tests with 2 and 4 g/kg sucrose loads produced a marked rise in breath hydrogen and gastrointestinal symptoms; however, breath tests accompanied by sacrosidase treatment prevented the rise in breath hydrogen and the symptoms. Subsequent pivotal trials in >40 subjects between the ages of 5 months and 29 years were conducted, with the diagnosis of CSID based on chronic watery diarrhea with an acid pH, a tissue

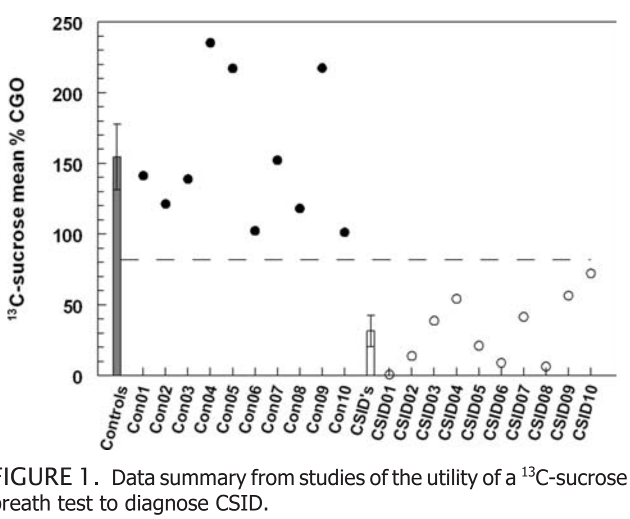

FIGURE 1. Data summary from studies of the utility of a ^{13}C-sucrose breath test to diagnose CSID.

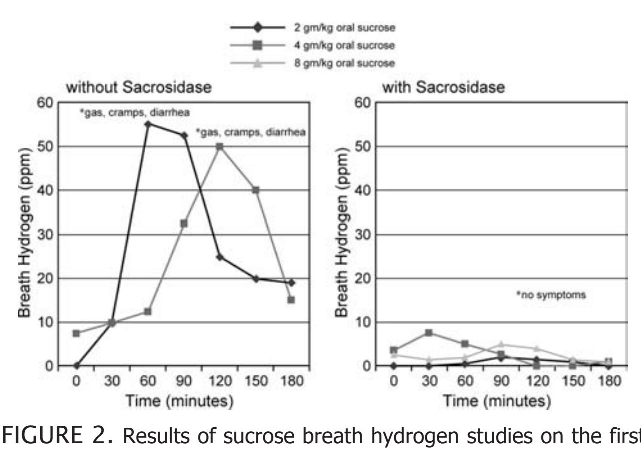

FIGURE 2. Results of sucrose breath hydrogen studies on the first child with CSID treated with sacrosidase under an orphan drug grant from the Food and Drug Administration.

sucrase activity level of <10% of the mean of controls, a normal lactase level, and a normal lactose breath hydrogen test (42,43). These multicenter, double-blind, randomized studies used 3 increasing dilutions of sacrosidase and an undiluted form in 4 arms given to each subject in random order during a 10-day period in which time the subjects consumed a normal sucrose-containing (approximately 1.75–2.5 g · kg^{-1} day^{-1}) and starch-containing (5.2–5.8 g · kg^{-1} day^{-1}) diet. Two breath hydrogen studies (with and without sacrosidase) were performed in the first study and 3 (with and without sacrosidase and with sacrosidase plus cow's milk acting as a pepsin decoy) in the second pivotal study.

The results of these studies can be summarized as follows. All dilutions of sacrosidase reduced symptoms of sucrose malabsorption provoked by both the breath tests and the period of unrestricted diet; the undiluted preparation most significantly reduced watery stools, gas, cramps, and bloating. Full-strength (undiluted) sacrosidase normalized these symptoms and the stool frequency in comparison with the baseline period of a sucrose-free, starch-restricted diet and no sacrosidase treatment. Full-strength sacrosidase resulted in 81% of patients, consuming an unrestricted diet, remaining asymptomatic, compared with 78% untreated during the baseline, diet-restricted period. Excessive breath hydrogen production was blocked by the double-blind administration of sacrosidase compared with placebo and was further reduced by consuming milk before sucrose ingestion (Fig. 3A). A study of the ^{13}C-sucrose breath test with and without sacrosidase administration

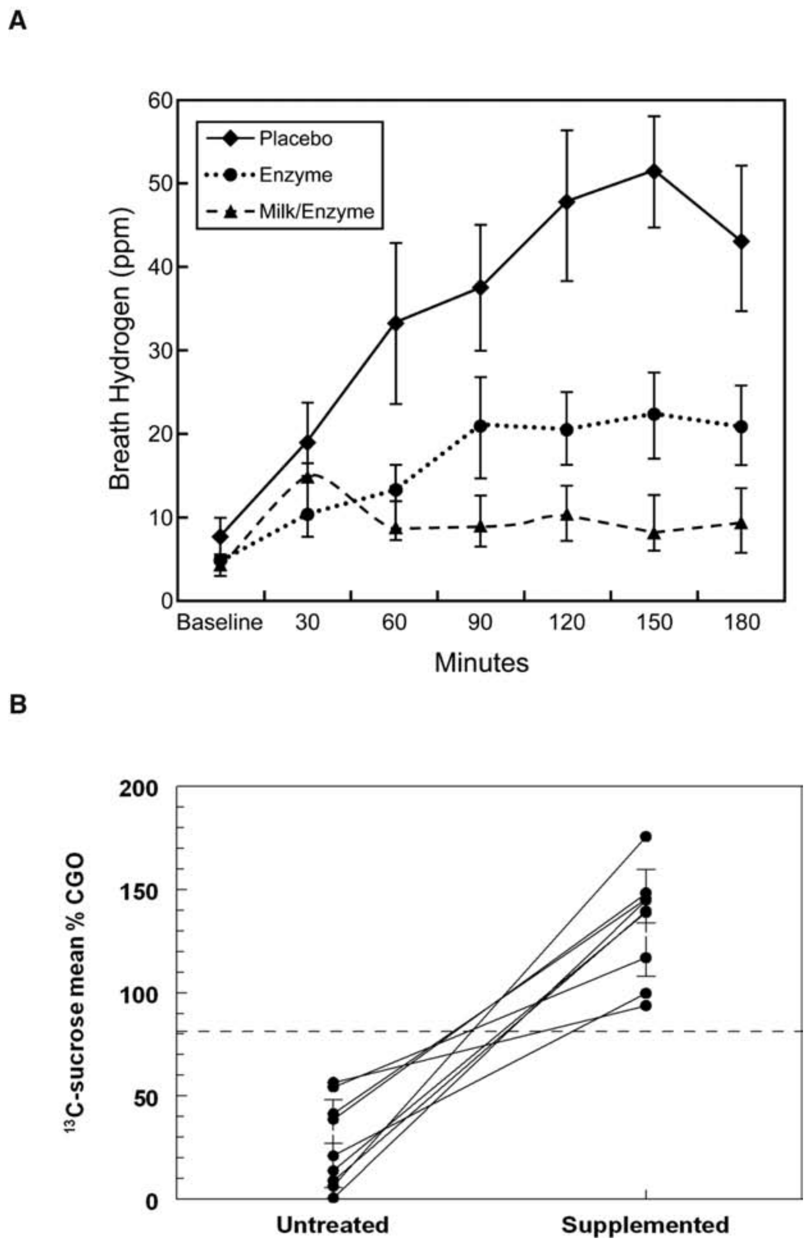

FIGURE 3. A, Excessive breath hydrogen production blocked by the double-blind administration of sacrosidase compared with placebo and was further reduced by consuming milk before sucrose ingestion. B, A study of the ^{13}C-sucrose breath test with and without sacrosidase administration confirmed these results and shows that all of the subjects had normalized CGO with therapy.

TABLE 3. Persistence of symptoms in 65 patients wit h CSID treated with Sucraid (22)

Symptom frequency	Diarrhea, %	Bloating/gas, %	Nausea/vomiting, %	Abdominal pain, %
0 times per week	46	43	96	91
1 time per week	28	18	4	9
2–3 times per week	12	13	0	0
>3 times per week	14	26	0	0

confirmed these results and shows that all of the subjects had normalized CGO with therapy (Fig. 3B) (37). Adverse events were limited to unrelated episodes of vomiting, pallor, and dehydration, each in a single subject, and a possibly related event of wheezing in a young child with known asthma, who was later found to have a positive skin test for sacrosidase (43). This incident led to the recommendation on the label to perform skin tests on patients with asthma before sacrosidase is administered. No other patients have been described with this adverse effect. These studies resulted in the submission of an NDA to the FDA and approval of Sucraid (sacrosidase) as treatment for CSID in 1998. Treatment was covered by Medicaid, after which private insurance coverage was approved. Recommendations for dosing on the label suggest using 1 mL with meals or snacks for patients <15 kg and 2 mL with meals or snacks for those >15 kg. Doses are to be split, with half the dose given at the onset of a meal and the other half midway through, when the intragastric environment is buffered to a higher pH and pepsin may be partially decoyed by other proteins.

A preliminary post marketing surveillance study was conducted involving 229 patients with CSID who received prescriptions for Sucraid (sacrosidase) between 2004 and 2009. Results are summarized in a published abstract and in the proceedings of this symposium (22). Sixty-nine of 229 questionnaires were returned from 60 of 69 patients in 27 states in the United States and from 9 patients in 4 other countries. Included were 39 male patients and 66 of 69 patients younger than 18 years old. Sixty-five patients continued taking Sucraid; 2 had abandoned it because of lack of efficacy and 2 because of its cost. The median duration of therapy was 3 years and one-third had been treated continuously for >5 years. Nine of 65 (14%) patients were exceeding the maximum recommended dose per meal (2 mL) to try to control symptoms. Either a normal diet or a mild sucrose- and starch-restricted diet was consumed by 41 of 65 (65%) patients, but in 27%, strict sucrose restriction with either mild or strict starch restriction was necessary to maintain acceptable suppression of symptoms, even while taking Sucraid. Table 3 summarizes symptoms while patients are being treated with Sucraid. The majority (59/65, 92%) had <3 bowel movements per day, and 74% experienced either no diarrhea or diarrhea once per week, 12% had diarrhea 2 to 3 times per week, and 14% had diarrhea >3 times per week. In 74%, bloating occurred <3 times per week. Abdominal pain and nausea/vomiting werenot seen in any patients >1 time per week and were completely absent in >90% of patients. The most common adverse events reported included constipation in 6 of 65, headaches in 5 of 65, and sleep disturbances in 8 of 65. None of these events resulted in discontinuing Sucraid.

CONCLUSIONS

Both clinical studies and molecular/genetic investigations suggest that CSID is a more common disease than previously believed and that genetically modified small intestinal SI digestion accounts for a broad spectrum of clinical phenotypes, including some potentially hidden in large cohorts of patients with IBS, chronic nonspecific diarrhea, and perhaps even dyspepsia (44). The advent of noninvasive breath tests with excellent sensitivity and specificity and genetic tests of relatively common mutations in the CSID gene hold out the promise of more accurate population prevalence studies and diagnosis of less classic cases, even in adults who are believed to have lifelong functional bowel disorders. The recent approval of an enzyme replacement therapy has allowed liberalization of the previously mandatory sucrose restrictive diet and restored a more normal lifestyle, particularly to infants and young children exposed to a high carbohydrate diet (45). Further modifications of this therapy with the possible additions of enzymes geared to supplement higher maltase and glycoamylase activity may be in the offing to help patients cope with the continued problem of starch malabsorption. Research has demonstrated that additional amylase activity amplifies the effect of SI and MGAM on starch digestion and offers another potential addition to enzyme replacement therapy (18,46).

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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 *a*-glutamyl transpeptidase, are expressed in many tissues, including the intestine and the kidney (3,4). The

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Reference Intervals for Intestinal Disaccharidase Activities Determined from a Non-Reference Population

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Background: Cutoff activities for diagnosing disaccharidase deficiencies are historical and are difficult to verify from a reference population. The objectives of this study were to validate the utility of histor- ical disaccharidase cutoffs using data from clinical samples and to evaluate the demographics of individuals for whom intestinal disaccharidase testing was performed.

Methods: Results from 14,827 disaccharidase test samples were extracted from the laboratory information system. Data were analyzed by the Hoffman method to calculate a reference interval for each enzyme, and the lower limits were compared to historical cutoffs. The observed frequencies of disaccharidase deficiencies were determined using historic and calculated cutoffs.

Results: The median patient age of the entire data set was 13 years(range<1–88 years), and 45% were male. The cutoffs for lactase, maltase, palatinase, and sucrase were determined to be 10, 100, 9, and 25 U/g protein, respectively. Applying these cutoffs to the data set, 61% had no enzyme deficiencies, 35% were lactase deficient, 11% were maltase deficient, 13% were palatinase deficient, and 13% were sucrase deficient. Pandisaccharidase deficiency was present in 8%.

Conclusions: Disaccharidase testing is most commonly performed in patients <18 years. Lactase deficiency is the most frequently observed single-disaccharidase deficiency. The historical cutoffs for maltase and sucrase were validated. To align with calculated reference intervals, the palatinase cutoff should increase from 5 to 9 U/g protein, and the lactase cutoff should decrease from 15 to 10 U/g protein.

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[3] **Nonstandard abbreviations:** LIS, laboratory information system.

IMPACT STATEMENT

A deficiency of one or more intestinal disaccharidase enzymes can result in carbohydrate maldigestion. The measurement of their activities in small bowel mucosa is considered to be the gold standard test for a deficiency diagnosis. Establishing reference intervals for disaccharidase activities is challenging because biopsy of the small bowel is invasive; thus, reference samples are not available for testing. We applied the Hoffman method to a large cohort of clinical results to validate historic deficiency cutoffs. Results indicate historic cutoffs for maltase and sucrase were valid, but changes are recommended for lactase and palatinase. Use of appropriate cutoffs is important to identify individuals with disaccharidase deficiency.

Dietary disaccharides are important sources of exogenous glucose. The small intestine is impermeable to disaccharides, requiring enzymatic hydrolysis of disaccharides into monosaccharides. This hydrolysis is catalyzed by disaccharidase enzymes, which are localized to the brush-border membrane of the small intestine. Decreased or absent activity of one or more of the disaccharidase enzymes can result in carbohydrate maldigestion, which is commonly characterized by abdominal symptoms including cramping, flatulence, and diarrhea (*1*, *2*).

The main dietary disaccharides are lactose, maltose, and sucrose. The disaccharidase activities that are of clinical interest are lactase, sucrase, maltase, and isomaltase (more commonly referred to as palatinase). The disaccharidases responsible for hydrolysis of these sugars are the enzyme complexes lactase-phlorizin (EC 3.2.1.108), sucrase-isomaltase (EC 3.2.1.48), and maltase-glucoamylase (EC 3.2.1.20) (*1*). As enzyme complexes, they contain more than one active site and may display specificity for more than one substrate. Because of overlapping activities of the disaccharidase enzyme complexes, some measured activities, such as that of maltase, represent the aggregate activity of multiple enzyme complexes (maltase-glycoamlyase and sucrose-isomaltase). Additionally, deficiency of a single enzyme (sucrase-isomaltase) often results in decreased activities of maltase, sucrase, and palatinase.

Primary (hereditary) disaccharidase deficiencies are rare and include congenital sucrase-isomaltase deficiency, an autosomal recessive disease caused by a mutation in the sucrase-isomaltase gene; glucose-galactose malabsorption, due to a glucose transporter deficiency; and starch malabsorption, due to glucoamylase deficiency. Acquired deficiencies are much more common and are associated with injury to the intestinal mucosa. Such injuries may be due to anatomic anomalies or autoimmune disorders that affect the gastrointestinal tract. Deficiencies may also be transient due to extended antibiotic or other drug uses.

Establishing reference intervals for disaccharidase activity is challenging because these tests involve obtaining a biopsy of the small bowel mucosa and reference samples are not available for testing. Laboratories that perform disaccharidase testing have often adopted historical cutoff activities that are used to identify a disaccharidase deficiency (lactase, <15 U/g protein; maltase, <100 U/g protein; palatinase, <5 U/g protein; and sucrase, <25 U/g protein). The source of these cutoffs is not clear. The utilization of patient test results to establish a reference interval was originally described by Hoffman in 1963 (*3*) and has been shown to be accurate and reproducible (*4*, *5*). The primary objective of this study was to retrospectively validate these historic cutoffs using the Hoffman method for reference interval determination. The secondary objective of this study was to evaluate the demographics and disaccharidase activities of individuals for whom disaccharidase testing was clinically performed.

METHODS

Data set

The results of all samples for which disaccharidase activity testing was performed at ARUP Laboratories between November 2009 and May 2013 were extracted from the laboratory information system (LIS)[3] and deidentified (n = 14,886 samples). Results were excluded if they were determined at a laboratory other than ARUP (n = 7), identified as an "LIS test" sample (n = 11), were associated with an absurd patient age of >110 years (n = 5), or failed to produce any reportable result (n = 36). The results from the remaining 14,827 samples were included in subsequent analyses. The University of Utah Institutional Review Board approved this project and its protocols.

Disaccharidase activity determination

The method used in our laboratory to determine disaccharidase activities has been described (*6*). Briefly, biopsies of small intestine mucosa were homogenized in saline and the homogenate incubated with each of four substrates (lactose, maltose, palatinose, and sucrose) to liberate glucose. After stopping the reactions by heat inactivation, the glucose concentration was determined using an automated chemistry analyzer. One unit (U) of disaccharidase activity was calculated as the quantity of enzyme that will hydrolyze 1 μmol disaccharide substrate per minute at 37 °C. Because of inherent variations in specimen size, the disaccharidase activity is determined relative to the protein concentration of the homogenate that was determined using a Lowry protein assay (*7*).

Determination of reference intervals

The reference intervals for disaccharidase activities were determined using the Hoffman method (*3*, *4*). All enzyme activity results of 0 U/g protein were excluded from this analysis only (n = 408, 24, and 20 for lactase, palatinase, and sucrase, respectively). Enzyme activities were log-transformed before determining cumulative frequency distributions. Presumed outliers were not excluded from the data set because their influence on the cumulative distribution frequency was minimal and their exclusion did not make the analysis more scientifically or methodologically robust. The linear portion of the cumulative frequency distribution for each enzyme was visually estimated and used for linear regression analysis. The reference interval (RI) was calculated as $RI_{lower} = 2.5(m) + b$ and $RI_{upper} = 97.5(m) + b$, where m is the slope and b is the y intercept of the linear regression. The calculated lower limit of the reference interval was used as the calculated cutoff activity. The cutoff ratio, defined as the ratio of the historical cutoff to the calculated cutoff, was determined for each enzyme. Microsoft Excel version 14.0.7166.5000 and GraphPad Prism version 5.0f software were used for all data analyses.

RESULTS

Disaccharidase reference intervals

The frequency of the log of enzyme activity for maltase, palatinase, and sucrase displayed unimodal distributions, while lactase showed a bimodal distribution (Fig. 1). This pattern was reflected in the cumulative frequency distributions of each enzyme, with an inflection point observed in the plot for log of lactase activity (Fig. 2). The linear portion of the cumulative frequency for each enzyme was used for linear regression analysis, and the upper and lower limits of the reference interval were calculated, as well as the cutoff ratio (Table 1). The cutoff ratios for maltase and sucrase were

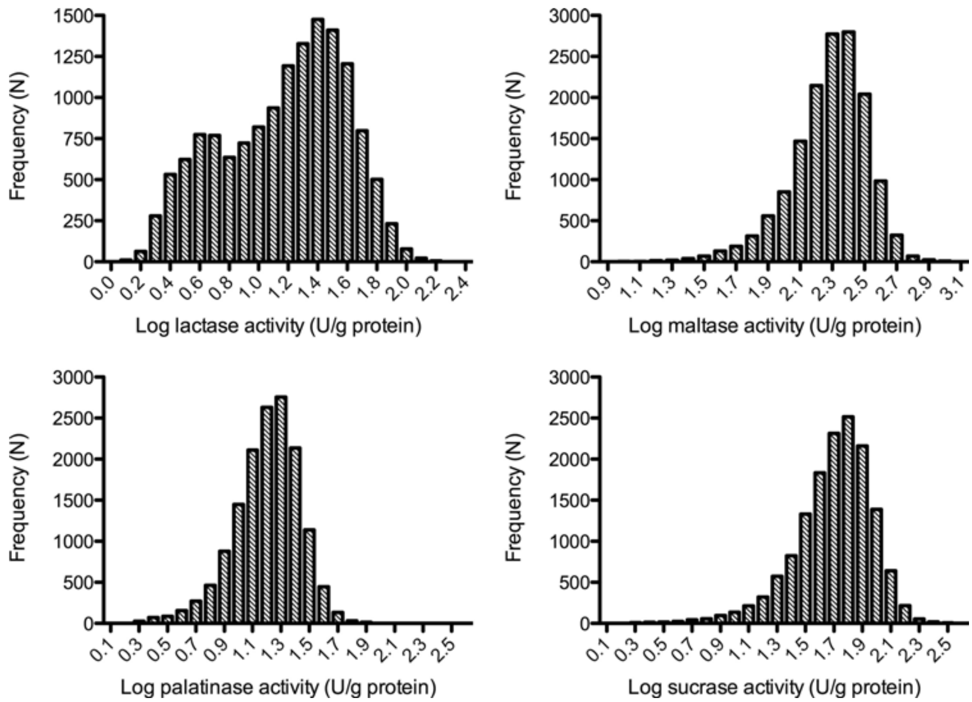

Fig. 1. Frequency distributions of log-transformed disaccharidase enzyme activities.
Lactase displays a bimodal distribution of activities; maltase, palatinase, and sucrase display unimodal distributions.

close to 1 (0.95 and 0.96, respectively), indicating close agreement between the historical and calculated cutoff values. The cutoff ratios for lactase (*3*) and palatinase (0.56) indicate discrepancies between the historical and calculated cutoffs, with the calculated lactase cutoff being 3 times lower than the historical cutoff and the calculated palati- nase cutoff being almost 2 times higher than the historical cutoff.

The bimodal distribution of lactase results and the presence of an inflection point in the cumula- tive frequency of log activity (Fig. 2) suggested that two distinct patient populations were represented in the data set: a lactase-deficient and a lactase- sufficient population. The inflection point of the cumulative frequency distribution corresponds to a lactase activity of approximately 10 U/g protein, and this activity was used as a proposed cutoff to distinguish lactase sufficiency from deficiency.

Spearman correlations between disaccharidase activities greater than or equal to the modified cutoffs and patient age were −0.04 ($P < 0.0001$), −0.02 ($P = 0.05$), −0.08 ($P = 0.01$), and 0.007 ($P = 0.42$) for lactase, maltase, sucrase, and palatinase, respectively. On average, enzyme activities were approximately 5% lower in males compared to females.

Disaccharidase deficiencies by historical and proposed cutoffs

The disaccharidase activity results of all 14,827 patient samples in the data set were analyzed to determine the observed frequency of each disaccharidase deficiency, both as a single-enzyme deficiency and also in combination with other disaccharidase deficiencies, based on the historic and proposed cutoffs for enzyme deficiency (Table 2). The median patient age of the entire sample set was 13 years (range 0 – 88 years), and 82% of the

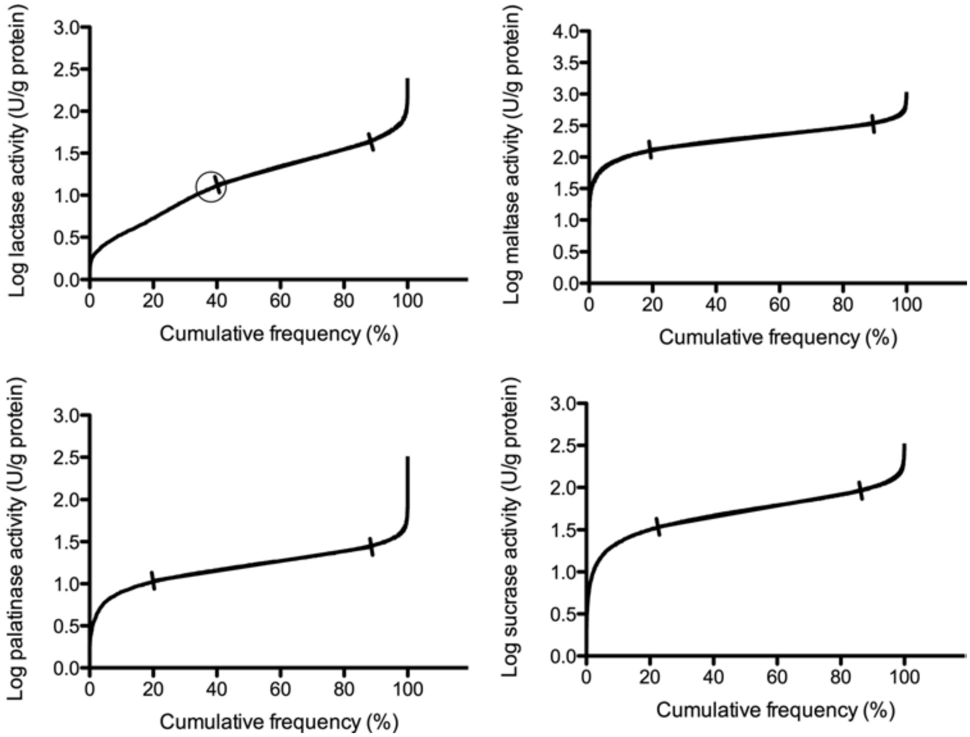

Fig. 2. Cumulative frequency curves of log-transformed enzyme activities.
The brackets identify the region of each curve that was visually estimated to be the linear portion of the distribution and used for linear regression analysis. The circle in the lactase cumulative frequency curve highlights the inflection point of this data set, corresponding to a lactase activity of 10 U/g protein.

samples were obtained from a pediatric (<18 years) population; 57.3% of the samples were collected from females. Using the historic cutoffs, 52.2% of the samples were not deficient in any disaccharidase, and this result increased significantly to 61.1% using the proposed cutoffs ($P < 0.0001$). The most commonly observed enzyme deficiency was lactase only, which was 34.6% using

Table 1. Comparison of historical and proposed cut offs used to determine disaccharidase deficiency.[a]

	Regression equation	Calculated lower limit, U/g protein	Calculated upper limit, U/g protein	Historical cutoff, U/g protein	Cutoff ratio, historical/calculated
Lactase	$y = 0.011x + 0.69$	5[b]	55	15	3
Maltase	$y = 0.0059x + 2.0$	105	380	100	0.95
Palatinase	$y = 0.0059x + 0.92$	9	32	5	0.56
Sucrase	$y = 0.066x + 1.4$	26	110	25	0.96

[a] Linear regression equations were used to determine the proposed lower and upper limits of the reference intervals; the r^2 for all regression equations was >0.99. The cutoff ratio was determined by dividing the historical cutoff for enzyme deficiency by the proposed cutoff (lower limit of the reference interval).
[b] Because the calculated cutoff of 5 U/g protein is likely skewed because of the bimodal distribution of lactase results, a proposed cutoff of 10 U/g protein was determined from Fig. 2.

Table 2. Demographics and disaccharidase activities of the population used for determination of enzyme deficiency cutoffs.[a]

			Historic cutoff						Calculated cutoff			
			15	100	5	25			10	100	9	25
		Median	Median activity, U/g protein					Median	Median activity, U/g protein			
Disaccharidase deficiency	N, (%)	age, years	Lactase	Maltase	Palatinase	Sucrase	N, %	age, years	Lactase	Maltase	Palatinase	Sucrase
All results	14 827 (100)	13	16.6	203.9	16.7	54.1	14 827 (100)	13	16.6	203.9	16.7	54.1
No deficiencies	7747 (52.2)	12	29.8	244.9	20	66.9	9062 (61.1)	12	26.7	234.6	19.2	63.7
Lactase only	5133 (34.6)	14	6.2	181.6	14.8	47.1	3408 (23)	14	4.4	195.5	15.9	51
Lactase, maltase, and sucrase	1057 (7.1)	14	5.3	78.1	7.2	18.2	163 (1.1)	14	5.1	82.3	10	19.5
Lactase, maltase, palatinase, and sucrase	445 (3)	14	3	47.9	3.6	9.4	1167 (7.9)	14	3.8	65.7	5.8	14.4
Lactase and sucrase	186 (1.3)	13	6.5	106.8	8.5	23.1	54 (0.4)	14	5.3	111.1	10.1	23.1
Maltase and sucrase	86 (0.6)	12	18.3	87.7	7.7	20.6	83 (0.6)	12	13.7	86	10.6	20.6
Lactase and maltase	65 (0.4)	13	7.2	95.7	8.8	26.1	20 (0.1)	14	6.3	96.1	10.8	26.5
Sucrase only	64 (0.4)	11.5	20.6	109.1	8.2	23.1	39 (0.3)	10	14.8	110.1	10.9	22.4
Maltase, palatinase, and sucrase	17 (0.1)	7	24.1	75.6	3.8	15.1	192 (1.3)	13	13.7	84.8	6.9	19.6
Maltase only	16 (0.1)	12.5	20	93.8	10.2	27.3	22 (0.1)	10.5	14.1	93.8	10.5	26.7
Lactase, palatinase, and sucrase	6 (0)	15	2.5	108.7	3.9	22.4	92 (0.6)	14	4.4	104.6	7.7	23.1
Lactase and palatinase	3 (0)	10	3.4	137.7	3.8	33.2	234 (1.6)	14	4.2	115.2	8.2	28.5
Palatinase only	1 (0)	12	28.5	178.1	4.7	38	180 (1.2)	13	16.8	120.4	8.2	28.8
Palatinase and sucrase	1 (0)	25	38.5	103.4	4.8	24.1	72 (0.5)	11.5	17.4	106.3	7.4	23.5
Maltase and palatinase	0 (0)	NA[b]	NA	NA	NA	NA	12 (0.1)	11	13.7	97.5	8.1	26
Lactase, maltase, and palatinase	0 (0)	NA	NA	NA	NA	NA	27 (0.2)	14	5.1	94.9	7.9	25.8
All lactase deficiencies	6895 (46.5)	14	5.6	152.9	12.8	39	5156 (34.8)	14	4.3	149	12.5	37.5
All sucrase deficiencies	1862 (12.6)	14	5	77	6.7	17.5	1862 (12.6)	14	5	77	6.7	17.5
All maltase deficiencies	1686 (11.4)	14	4.7	73.4	6.6	16.6	1686 (11.4)	14	4.7	73.4	6.6	16.6
All palatinase deficiencies	473 (3.2)	14	3.2	49.6	3.7	9.5	1976 (13.3)	14	5.1	82.6	6.8	19

[a] Data are shown for each possible combination, single or multiple, of disaccharidase deficiency, as well as combined data for each enzyme deficiency without consideration for coexisting disaccharidase deficiencies, using both the historic cutoffs and the proposed cutoffs of lactase and palatinase deficiency of 10 and 9 U/g protein, respectively.

[b] NA, not applicable.

the historic cutoff. As expected, this decreased significantly to 23% with use of the proposed cutoff ($P < 0.0001$).

Overall, with or without additional enzyme deficiencies, lactase deficiency was most common using historic and proposed cutoffs (46.5% vs 34.8%, respectively). Likewise, using the historic cutoff, a palatinase deficiency was least common and occurred in only 3.2% of samples, but this increased to 13.3% using the proposed cutoff. This increase brought the frequency of palatinase deficiency close to that observed for sucrase and maltase (12.6% and 11.4%, respectively), which were unchanged by use of the proposed cutoffs.

A deficiency of all 4 enzymes (pandisaccharidase deficiency) was not common using the historic cutoffs (3.0%), but this result increased significantly to 7.9% using the proposed cutoffs ($P < 0.0001$). The increased prevalence of pandisaccharidase deficiency was identical to that reported in a separate study that also used a lactase cutoff of 10 U/g protein and a palatinase cutoff of 11 U/g protein to identify deficiencies (*8*).

DISCUSSION

Determining reference intervals is frequently challenging, especially when dealing with analytes measured in specimens that are difficult or invasive to obtain. Such is the case for the intestinal disaccharidases. However, the measurement of their activities is considered to be the definitive biochemical test for the diagnosis of disaccharidase deficiencies. The analytical method developed by Dahlqvist in 1964 (*9*) is widely considered to be the gold standard test and continues to be used, albeit with some technological modifications, by clinical laboratories. Interpreting the test results is aided by availability of well-defined reference intervals, yet these are impossible to obtain from a true reference population due to the invasive nature of the specimen collection. The source of the current cutoffs used by the majority of laboratories is not clear, and to our knowledge an investigation into the suitability of these historic cutoffs has not been conducted. The current analysis illustrates one approach for retrospective validation of reference intervals when it is difficult or impossible to obtain reference population samples.

The Hoffman method for determining reference intervals was retrospectively applied to data generated during clinical testing for disaccharidase deficiencies. The lower limits of the calculated reference intervals for lactase, maltase, palatinase, and sucrase were 5, 105, 9, and 26 U/g protein, respectively. The calculated lower reference limits for maltase and sucrase were consistent with the historic cutoffs of 100 and 25 U/g protein, respectively, and the cutoff ratios (historic/calculated) were 0.95 and 0.96, respectively. These data indicate that the calculated lower reference limit is consistent with the historic cutoff used to define enzyme deficiency. In contrast, the lactase cutoff was lower and the palatinase cutoff was higher than the historic cutoffs of 15 and 5 U/g protein, respectively. The cutoff ratio for lactase was a factor of 3, indicating the historic cutoff is greater than the calculated lower limit of the reference interval (5 U/g protein). The historic cutoff for lactase deficiency may be inappropriately high, resulting in overdiagnosis of lactase deficiency (46.5% of the subjects in this study). If a cutoff of 5 U/g is used as the criteria for diagnosing lactase deficiency, this would result in an observed rate of lactase deficiency of 20.5% in this data set. Based on the bimodal distribution of results, this cutoff is likely inappropriately low and would result in missed diagnoses of lactose deficiency. Based on the inflection point of the data (Fig. 2), the proposed lactase cutoff of 10 U/g would result in an observed rate of lactase deficiency of 34.8%. Interestingly, the reference interval for lactase activity was originally reported as 9 –98 U/g protein (*10*) and, more recently, as 9 –91 U/g protein (*11*). Both of these reference intervals correspond to the proposed lower limit for lactase activity, based on the observed bimodal distribution in this data set.

The cutoff ratio for palatinase was 0.56, indicating that the historic cutoff of 5 U/g protein is less than the calculated lower reference limit of 9 U/g protein. This finding suggests that the historic cutoff for palatinase deficiency may be inappropriately low, resulting in an under-diagnosis of palatinase deficiency. Importantly, because sucrase-isomaltase is an enzyme complex, it is unusual to encounter samples that are sufficient for one enzyme but deficient for the other. Using the historic palatinase cutoff, the frequency of all palatinase deficiencies was 3.2% and was unexpectedly lower than the frequency of all sucrase deficiencies (12.6%). Using the proposed palatinase cutoff increased the frequency of all palatinase deficiencies to 13.3%.

The disaccharidase activities were minimally correlated to patient age and were only slightly lower in males compared to females. While reference intervals partitioned by age and/or sex could be considered, the differences between them would be too small to be of clinical usefulness. A similar conclusion has been reached by others (*11*).

One limitation of the current study is that the population included was from a retrospective data analysis. While the Hoffmann method for reference interval determination is designed for use with a non-reference population, a general assumption is that the data set to which this technique is applied reflects the general population. A previous study evaluated the Hoffman method by applying it to well-characterized laboratory tests such as creatinine and thyroid-stimulating hormone (*4*). The author reported that applying their calculated reference limits for creatinine and thyroid-stimulating hormone to their study data set, the percentage of results that fell outside of those limits correlated with the prevalence of chronic kidney disease and thyroid dysfunction, respectively (*4*). However, in the present study, close to 50% of the data set represented individuals having one or more disaccharidase deficiencies. Since the overall prevalence of disaccharidase deficiency in the population is thought to be low, this data set was skewed toward deficient individuals and not representative of the general population.

Another limitation of this study is the lack of clinical data accompanying the laboratory test results. While the data set appears skewed toward disaccharidase deficiency with respect to the expected prevalence in the population, no information on the ethnic demographics of this data set were available. For instance, the frequency of lactose in- tolerance, presumably caused by lactase defi- ciency, is known to vary throughout different populations and different regions of the world (*12*). Additionally, to truly validate the clinical utility of the proposed modifications to the activity cut- offs to classify deficiency, correlation with clinical symptoms is needed to determine the activity at which a clinical presentation consistent with en- zymatic deficiency is present. A study by Gupta et al. (*13*) reported on such an approach in 232 samples obtained from patients <18 years of age with and without diarrhea and evaluated for mu- cosal histology and disaccharidase activities. The geometric means of enzyme activities decreased with increasing severity of mucosal injury but were significantly lower only in individuals with diarrhea and moderate to severe histologic changes. Access to biopsy histology and patient symptoms in this study's data set would have been useful and might have altered the outcome of the Hoffman analysis.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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Invited Review

Congenital Sucrase-Isomaltase Deficiency

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Sucrase-isomaltase deficiency has received much less attention than lactase deficiency. Although much of the world's population is predisposed to become lactose-intolerant at an early age, the occurrence of sucrase-isomaltase deficiency, either as a result of an inherited condition or secondary to diffuse mucosal injury, is relatively rare. Recently, however, sucrase-isomaltase deficiency has been the focus of increased research activity; important new work has included the elucidation of molecular defects associated with the inherited form of sucrose malabsorption and the recent cloning of the human sucrase-isomaltase gene.

This paper will focus on congenital sucrase-isomaltase deficiency (CSID), including its epidemiology, clinical presentation, and natural history. Normal enzyme structure, synthesis, and processing will be reviewed in order to facilitate understanding of the molecular pathogenesis of CSID. Finally, newer aspects of treatment, including the demonstration of effective enzyme-replacement therapy, will be emphasized. The reader is referred to several excellent reviews for further details (1–3).

SUCRASE-ISOMALTASE: STRUCTURE, BIOSYNTHESIS, AND CONTROL OF ACTIVITY

Role in Digestion (Table 1)

Sucrase-isomaltase (SI) is one of four brush-border disaccharidases. Three of these, including SI, maltase-glucoamylase, and trehalase, are α-glucosidases involved in the digestion of sucrose and starch. After hydrolysis of starch by salivary and pancreatic α-amylases, the resulting products are α 1–4 linked maltose, maltotriose, and malto-oligosaccharides, α 1–6 linked branched dextrins (α-limit dextrins), and glucose. Sucrase hydrolyzes the α 1–4 linked glucose linkages of maltose and maltotriose and the glucose-frustose linkage of sucrose. Isomaltase is an α-glucosidase and cleaves the α 1–6 glucopyranosyl bonds of branched oligosaccharides (α-limit dextrins), the 1–6 linkages of isomaltase, as well as the 1–4 linkages of maltose. The SI complex also hydrolyzes α-glucosides with up to six glucose residues (4). The maltase-glucoamylase complex overlaps with SI activity by hydrolyzing α 1–4 glucose linkages of maltose, maltotriose, starch, glycogen, and other oligosaccharides from their nonreducing ends with maximal affinity for medium-sized polysaccharide chains with 6–10 glucose residues (5). Approximately 80% of the maltase activity is accounted for by SI and only 20% by the maltase-glucoamylase complex. The fourth brush-border disaccharide, lactase-phlorizin hydrolase, is a β-galactosidase that hydrolyzes the β 1–4 linkage of disaccharide but not of cellulose. SI activity is distributed along the whole length of the small intestine. The highest activity occurs in the jejunum, with 20–30% less activity proximal to the ligament of Treitz and distally in the ileum (6).

Structure

SI is a heterodimer complex composed of two similar but not identical subunits. Each subunit consists of a single glycosylated polypeptide chain with an apparent molecular weight in the 120–160 kDa range. Carbohydrate moieties account for ~15% of the molecular mass (7). Recent cloning of the SI cDNA has shown that the SI complex is synthesized as a single precursor of ~260 kDa starting from the N-terminus of isomaltase with ~1827 amino acid residues (3,8).

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TABLE 1. *Role of brush-border enzymes in digestion of disaccharides and starch*

Enzyme	Bond cleaved	Substrate	Products
Lactase	β-(1–4) galactosidase (β-glucosidase)	Lactose	Glucose, galactose
Sucrase	α-(1–4) glucosidase	Sucrose, maltose, maltotriose, α-limit dextrins with terminal α 1–4 links	Glucose, fructose malto-oligosaccharide with α 1–6 linkage
Glucoamylase	α-(1–4) glucosidase	Maltose, maltotriose malto-oligosaccharide (glucose polymers with maximal affinity for chains of 6–10 residues)	Glucose, malto-oligosaccharide with terminal α 1–6 linkage
Isomaltase (α-dextrinase)	α-(1–6) glucosidase	Maltose, isomaltose, α-limit dextrins (malto-oligosaccharide with terminal α 1–6 links)	Glucose, malto-oligosaccharides
Trehalase	α- and β-glucosidase (tested on renal trehalase)	Trehalose (found principally in mushrooms)	Glucose

The isomaltase subunit alone interacts with the enterocyte membrane directly via a highly hydrophobic segment at its N-terminal region (Fig. 1). This segment is 20 amino acid residues long and spans the lipid membrane bilayer only once. This domain functions both as a permanent membrane anchor and as a signal peptide that directs targeting to the endoplasmic reticulum (9). It is followed by a 22-residue serine/threonine-rich glycosylated stretch, which presumably forms the stalk on which the globular, catalytic domains are directed into the intestinal lumen (8). The active sites of both enzymes protrude out into the lumen. The sucrase subunit is more peripheral and does not interact with the hydrophobic core of the membrane at all.

After synthesis, glycosylation, and transport to the brush border, prosucrase-isomaltase is rapidly processed by pancreatic proteases, predominantly elastase in the rat and trypsin in humans (10). These proteases cleave the molecule, yielding isomaltase (~125 kDa) and sucrase (~140 kDa). The two subunits remain associated by noncovalent strong ionic interactions. Recent work with rat intestinal membrane vesicles suggests that the postinsertional processing of the prosucrase-isomaltase as well as the structural and functional relationships of the final subunits are much more complex than has been generally assumed. The enzyme, rather than being a simple dimer, may exist in two oligomeric forms consisting of combinations of the subunits strategically interrelated so that the sucrase catalytic site appears to sterically regulate the availability of the isomaltase site (11). A reduction in sucrase activity in rat brush-border membrane vesicles in response to increasing temperature leads to a reciprocal increase in isomaltase activity through recruitment of functional isomaltase catalytic sites.

The glycosylation of SI is similar to other disaccharidase complexes and includes two main steps (Fig. 1); the cotranslational acquisition of glucan units of a high mannose type at the endoplasmic reticulum and the subsequent trimming and complex glycosylation in the Golgi apparatus (12). This results in a mature SI that contains a large proportion of asparagine-linked oligosaccharides made up of sialic acid, galactosamine, N-acetyl galactosamine, and mannose, as well as mucin type O-glycosidic linkage characterized by a bond between an N-acetyl galactosamine residue and a serine or threonine residue on the polypeptide chain (13). The peptide sequence of human SI precursor contains 18 putative N-glycosylation sites (14). Knowledge of the N-glycosylation sites is particularly useful for the study of CSID, where the absence of expression of this enzyme is often associated with a block in its transport and with abnormalities in glycosylation.

Molecular Biology

The gene encoding human SI has been localized to the long arm of chromosome 3 (15,16). A comparison between the human enzyme and SI in the rabbit, rat, and pig shows a high degree of homology of both nucleotide and amino acid sequences in the N-terminal and active site regions (16). An optimal alignment of the two subunits reveals a high degree of homology between the isomaltase and sucrase portions (41% for amino acids and 52% at the DNA level), indicating that SI probably evolved by partial gene duplication (8). In addition, homology

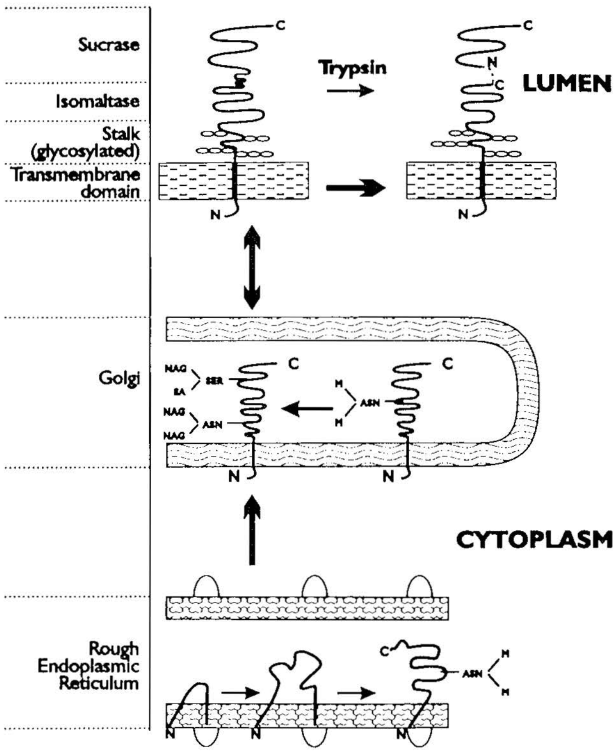

FIG. 1. Cotransitional modification and posttranslational processing of sucrase isomaltase (SI) in the enterocyte organelles and intestinal lumen. SI is synthesized as a long polypeptide chain carrying two similar but not identical active sites (pro–sucrase-isomaltase). The pro-SI is inserted into the rough endoplasmic reticulum (RER) via the same N-terminal hydrophobic region, acting as a targeting protein to the RER, which will later act as the anchor in the brush-border membrane. In the RER, the polypeptide elongates and is glycosylated at asparagine sites (ASN) with mannose (M) residues. The glycoprotein then migrates to the Golgi complex, where mannose residues are trimmed and complex glycosylation with N-acetyl galactosamine (NAG) and sialic acid (SA) residues at ASN and serine (SER) sites takes place. After complex glycosylation, the pro-SI is inserted into the enterocyte membrane, with the sucrase catalytic domain protruding furthest out into the lumen. Pro-SI is then rapidly processed by trypsin, yielding the two subunits of isomaltase and sucrase associated by noncovalent strong ionic interactions.

at the active site indicates that human SI, human lysosomal α-glucosidase, and yeast glucoamylase probably shared an ancestral gene and are only differentiated significantly at the N-terminal regions, accounting for the different biosynthetic pathways and cellular location of these enzymes (17). The SI complex is synthesized by small-intestinal epithelial cells with a noncleavable signal sequence that also contains the membrane anchoring domain. In contrast, the N-terminus of human lysosomal α-glucosidase comprises a signal peptide that is cleaved off, generating a soluble glycoprotein whose final destination is an intracellular organelle, the lysosome. Southern blotting, sequencing, and mRNA studies indicate that, in comparison with normal small intestine, the structure of the SI gene and its mRNA are unaltered in the two human colon cancer cell lines Caco-2 and HT-29 (14).

Northern blots of RNA extracted from subpopulations of rat and human intestinal epithelial cells that are isolated from villus and crypt compartments show that the cloned gene hybridizes to a 6.5 kb band predominantly in villus RNA (18). RNA probes have localized the greatest accumulation of SI mRNA to the nucleus of cells at the crypt-villus junction. Abundant mRNA is also seen in cells from the lower mid-villus region in both the nucleus and cytoplasm, with a disappearance of nuclear mRNA and a decline in cytoplasmic mRNA from the mid-villus to the tip (19).

Using full-length rabbit and partial human SI cDNA clones as probes, a good correlation has been demonstrated between the expression of SI at the levels of mRNA and protein. Thus, similar to other proteins expressed in enterocytes including liver fatty acid binding protein, cytochrome P450IIB1, and aminopeptidase N, SI is regulated at the level of increasing mRNA abundance as cells migrate from crypt to mid-villus (19). For these reasons, SI is considered a useful marker for enterocyte differentiation. The decrease in sucrase enzymatic activity in villus tip cells has been attributed to enzymatic degradation of the sucrase portion of the dimeric enzyme by luminal pancreatic proteases (20); however, a decrease in the steady-state levels of SI mRNA may also play a role secondary to either a decrease in transcription of the gene or more rapid degradation of cytoplasmic mRNA.

Control of Enzyme Activity (Table 2)

The regulation of oligosaccharidases is a dynamic process since their half-life is only 4–16 h; therefore, maintenance of activity at the brush border requires several cycles of synthesis and degradation during the life cycle of the human intestinal cell. Multiple factors modulate the activity of SI at the level of transcription, translation, glycosylation, and processing by luminal proteases. In addition, factors such as the age of the cell, its degree of differentiation along the villus, and proximal versus distal intestinal location play an important role in determining enzyme activity. Finally, dietary components and circulating hormones may alter the ac-

TABLE 2. *Control of sucrase-isomaltase activity at different levels and sites*

	Increased activity	Decreased activity
Transcription	Crypt-villus junction	Villus tip
Translation	Jejunum	Ileum
Glycosylation	Complex	Simple (high-mannose)
Pancreatic proteases	Pancreatic duct obstruction	↑ Pancreatic enzymes
Diet	High-sucrose, high-carbohydrate diet	Fasting, high-protein, low-carbohydrate diet
Hormones	Thyroxine, corticosteroids	

tivity of brush-border enzymes by varying their synthesis or degradation rate.

Both in rabbits and in humans, SI is most likely primarily controlled at the transcriptional level, since the enzyme activities have a high correlation coefficient with the level of SI mRNA (21). The fact that autoradiographic grains representing SI mRNA are first noted over nuclei in cells at the crypt-villus junction and only seen in the cytoplasm as these cells migrate into the mid-villus region further supports the hypothesis that transcription of the sucrase-isomaltase gene is activated (18). The cellular or extracellular factors that signal the nucleus to initiate SI gene transcription are largely unknown. Over 3000 base pairs of the 5′ flanking region of the gene are required for high-level expression. Recently, Traber et al. have shown the enterocyte-specific transcription of the gene in mice and humans is controlled by a 183 base pair promoter located immediately upstream of the transcriptional start site (22,23). This promoter contains at least three nuclear protein-binding sites that appear to bind intestine-specific nuclear protein complexes required for transcriptional activity. These protein complexes have not been fully characterized.

Levels of SI activity may be regulated posttranslationally as well as at the mRNA level. Based on results of differential immunohistochemical staining and immunoprecipitation studies, Beaulieu et al. concluded that SI protein is synthesized in both crypt and villus cells, but that there are differences in posttranslational processing of the protein (24). In the rat, there is a three- to fivefold greater activity of SI in the jejunum versus the ileum. Although no differences are found in SI mRNA abundance between the two sites, the relative rate of de novo synthesis of all forms of the enzyme is three to fivefold greater in the jejunum than the ileum, and a greater proportion of jejunal SI mRNA is associated with membrane-bound polyribosomes, suggesting greater translational efficiency (25).

These results indicate that along the longitudinal axis of the small intestine, SI expression is regulated by differences in translational mechanisms. In the rabbit, the in vitro biosynthesis of SI correlates well with the steady-state levels of its cognate mRNA all along the small intestine; however, the ratio of sucrase activity to SI mRNA is lower in the jejunum versus the ileum, again suggesting that variations in sucrase activity along the intestine are due both to transcriptional and posttranslational events (26).

Changes in glycosylation may be partially responsible for the posttranslational regulation of SI activity. After synthesis of a carbohydrate-free precursor in ribosomes bound to the membrane of endoplasmic reticulum, SI is conjugated to N-linked polymannose chains to form high-mannose glycoproteins. The high-mannose precursor is then transported from the rough endoplasmic reticulum to the Golgi complex, where the addition of complex O-linked oligosaccharide chain takes place, yielding the mature "complex" precursor. The high-mannose form has a substantially lower specific activity than the complex glycosylated form (27). High-mannose glycosylation seems to be essential for proper and timely polypeptide folding of the enzyme, allowing it to escape the endoplasmic reticulum. Fructose rapidly induces a block in the expression of SI and other brush-border membrane glycoproteins. The underlying mechanism involves abnormal high-mannose glycosylation and misfolding of the nascent polypeptide chains, thereby delaying exit from the endoplasmic reticulum and leading to degradation by rapid proteolytic breakdown (28,29). Changes in glucose metabolism may also inhibit the biosynthesis of SI both through a decrease in mRNA levels and an inhibitory effect on the conversion of the high-mannose to the complex glycosylated form. Glucose itself, monensin (when used in concentrations that induce increased glucose consumption), and forskolin through increased glycogenolysis via activation of adenylate cyclase all impair glycosylation of the enzyme (30,31).

After complex glycosylation in the Golgi body and transport to the microvillus membrane in vesicles, insertion and processing of SI to subunits proceeds via a complex series of cleavage steps mediated by pancreatic trypsin (32). The major cleavage site in humans is located between an arginine and isoleucine residue, yielding the sucrase subunit with isoleucine at its N-terminus. This is a trypsin-specific site that is not attacked by either elastase or chymotrypsin. Pancreatic proteases also participate in the luminal degradation of mature SI and appear to be at least partially responsible for the loss of sucrase activity in mature villus tip cells and in ileal enterocytes. Studies in animal models of pancreatic duct obstruction or bypass have demonstrated a decreased rate of degradation in duct-ligated animals, leading to increased SI activity and a disappearance of the usual proximal to distal gradient of sucrase activity in the small bowel (33–35).

Dietary factors and endogenous hormones are also important regulators of SI activity. SI is an inducible brush-border enzyme; both enzyme activities are increased by feeding a high-sucrose or high-carbohydrate diet and decreased by fasting (36). In rats, the mRNA levels of SI increase rapidly after sucrose force-feeding, and these changes correlate with the corresponding increase in enzyme synthesis, enzyme activity, and amounts of immunoreactive enzyme (37). This rapid increase in mRNA accumulation suggests that sucrose feeding induces an increase in transcription of the gene. Rats fed a high-protein, low-carbohydrate diet develop decreased sucrase activity. This effect appears to be at least partially a consequence of increased degradation of sucrase because it is correlated with marked increases in luminal trypsin activity and accumulation of isomaltase monomer, considered a degradation product of the enzyme (35).

Both thyroxine and glucocorticoids induce the precocious appearance of SI in the rat small intestine, mediated primarily by increases in the abundance of its mRNA (38,39). In humans, the SI complex is expressed in small intestine throughout gestation and in an identical form in the fetal colon between 12 and 30 weeks gestation. Before 30 weeks gestation, the enzyme is present only as the single polypeptide prosucrase-isomaltase; whereas after that time, two subunits are also present (40). Mature active SI is also expressed in adenocarcinoma of the colon and in the human colon carcinoma cell lines, Caco-2 and HT-29 (41). These cell lines have been particularly useful in studying enterocyte differentiation and the factors that regulate gene expression of human disaccharidases.

CONGENITAL SUCRASE-ISOMALTASE DEFICIENCY

Molecular Defect

There is abundant phenotypic variation in patients with CSID. All CSID patients lack sucrase, but some have only traces of isomaltase activity, others have reduced but significant isomaltase activity, and still others almost normal activity. The presence of residual isomaltase activity in many patients suggests that CSID is not the consequence of complete absence of SI gene expression. It appears that this phenotypic variation may be mirrored in genotypic heterogeneity. Although specific genetic mutations have not been identified as yet, different molecular defects documented in patients with CSID indicate abnormalities of intracellular processing (glycosylation and folding), intracellular transport, and homing and insertion of the enzyme into the brush-border membrane (Table 3).

It is well known that cellular mutations leading to amino acid substitutions may influence the processing and intracellular transport of glycoproteins (42,43). These point mutations may substantially affect the folding of peptide chains, leading to improper glycosylation. Normal glycosylation of disacharidases is necessary for the sorting of the enzymes to the brush-border membrane. Tunicamycin, an antibiotic that inhibits N-linked high-mannose glycosylation of proteins, greatly reduces the expression of disaccharidases in brush-border membranes of pig small intestine, leading to rapid intracellular degradation of newly synthesized enzyme (44). Monensin, which allows high-mannose glycosylation but interferes with complex glycosylation of dissacharides in the Golgi body, affects the further transport of the enzyme to the microvillus membrane.

As many as five different transport incompetent or functionally altered enzymes have been discovered in patients with CSID (45) (Table 3). The first molecular phenotype was described by Hauri et al. in 1985 in a 5-year-old girl with no sucrase but low residual intestinal isomaltase activity (46). Immunoelectron microscopy with monoclonal antibodies that reacted specifically with various forms of the prosucrase-isomaltase in biopsy samples from healthy subjects revealed that the enzyme was confined predominantly to the microvillus membrane of enterocytes and there was minimal labeling of the Golgi apparatus. In contrast, in the patient, immunoreactive SI was found almost exclusively in the

TABLE 3. *Molecular defects in patients with CSID*

	Molecular phenotype				
	I	II	III	IV	V
Location	Golgi	RER	Brush border	Brush border	RER, basolateral membrane
Form	High-mannose precursor	High-mannose and complex precursors	Mature enzyme (catalytically altered sucrase subunit)	Complex precursor (intracellular)	High-mannose precursor
Intracellular degradation products	Present	Present	Absent	Present (sucrase subunit)	?
Microvillus membrane	Absent	Absent	Present (both subunits)	Present (isomaltase subunit only)	Absent
Sucrase activity	0	0	0	0	0
Isomaltase activity	Low	0	Normal	Normal	0

RER, rough endoplasmic reticulum.
Adapted from Sterchi EE, Lentze MJ, Nail HY. Molecular aspects of disaccharidase deficiencies. *Baillieres Clin Gastroenterol* 1990;4:79–96; and from Fransen AM, Hauri HP, Ginsel LA. Naturally occurring mutations in intestinal sucrase-isomaltase provide evidence for the existence of an intracellular sorting signal in the isomaltase subunit. *J Cell Biol* 1991;115:45–57.

Golgi cisternae and associated vesicular structures, with no specific labeling in the microvillus membrane. Immunoprecipitation experiments revealed that the enzyme localized to the Golgi appeared to be the high-mannose form plus lower-molecular-weight degradation products. Subsequently, a second patient was reported with abundant synthesis of a high-mannose SI with arrest of further intracellular processing and failure of a mature glycoprotein form to reach the brush-border membrane (47).

There are several other human diseases associated with disordered intracellular processing of glycoproteins. The intrahepatic accumulation of abnormal glycoprotein in the piZZ phenotype of α-1-antitrypsin deficiency is related to a single amino acid substitution with subsequent failure to transport the high-mannose secretory product through the endoplasmic reticulum (48).

Further study at the subcellular and protein level of patients with CSID has revealed that the maturation and intracellular transport of the enzyme are blocked at different stages along with biosynthesis pathway (45). In a second molecular phenotype, a high-mannose form of the enzyme is incompletely trimmed and blocked not in the Golgi but in the endoplasmic reticulum. A third phenotype appears to be the result of a mutation affecting only the catalytic site of sucrase; the mature enzyme is found inserted into the brush-border membrane and isomaltase activity is relatively preserved (49). Study of a fourth phenotype reveals variants of pro–sucrase-isomaltase precursors that are converted from the high-mannose form to the mature complex glycosylated form at a slow rate. The enzyme undergoes intracellular cleavage to two subunits and the sucrase subunit is degraded, whereas the isomaltase subunit is normally transported to the brush border (50). In this patient, isomaltase activity was normal. Finally, a mutant phenotype has been recently described where the mannose-rich polypeptide precursor of the enzyme is normally synthesized but remains in the endoplasmic reticulum, does not undergo terminal glycosylation in the Golgi, and is missorted to the basolateral membrane rather than homing to its normal location in the brush-border membrane (50).

These last two naturally occurring mutations provide evidence that structural features in the isomaltase region of pro–sucrase-isomaltase act as an intracellular sorting signal, allowing for transport from the trans-Golgi network to the brush-border membrane (51). The nature of these structural features and of the intracellular elements that recognize them is not yet known.

There have been several cases of CSID in which no immunoreactive forms of sucrase-isomaltase were observed via immunoprecipitation or electron microscopy either on the brush border or intracellularly (45). These cases may represent a defect in transcriptional regulation of sucrase-isomaltase expression. Alternatively, the enzyme may be synthesized but improperly folded and hence not recognized by the specific monoclonal antibodies used to detect the protein.

Incidence

Congenital sucrase-isomaltase deficiency (CSID) is considered a rare autosomal recessively inherited disease, but it is likely that the prevalence has been underestimated (Table 4). Given the wide phenotypic variation and the probability that a variety of genetic mutations cause CSID of varying severity, it is likely that numerous patients suffering from chronic diarrhea remain undiagnosed. Previous studies have attempted to ascertain the number of heterozygote carriers in the general population based on measurements of sucrase enzyme activity in small-intestinal biopsy specimens. Heterozygotes are defined as those with a level of sucrase activity below the lower limit for the normal population, with ratios of sucrase:lactase activity of <0.9 and with normal small-bowel morphology. Using these criteria, Peterson and Herber estimated the incidence of heterozygotes to be 8.9% of the general population in the United States (52). Welsh et al. found a much lower incidence of ~2% heterozygotes in the Caucasian population (one in 2500 homozygotes according to the Hardy-Weinberg equation) and no case that satisfied these criteria among 53 African Americans tested (53). In Denmark, only one case of CSID was uncovered in over 2000 patients biopsied because of abdominal pain and diarrhea (54). The incidence appears to be much higher in Greenland, Alaskan, and Canadian Eskimos (54–56). In Greenlanders with diarrhea, the incidence of sucrose malabsorption is 10.5% (47). In the general population of Greenland, ~5% of those tested showed very low sucrase activity in small-bowel biopsies, and 12.5% had activity below the lower limit of the control population (2).

Numerous cases have been described of CSID among siblings and parents. Kerry and Townley biopsied parents of four children with CSID and found most of them to have sucrase activities below the lowest values in a control group. Seven of the eight parents had a sucrase:lactase ratio below 0.8 (57). From these data, it seems reasonable to assume that CSID is transmitted via autosomal recessive inheritance.

TABLE 4. *Prevalence of CSID in various populations*

Group	Percentage
Greenland Eskimos	2–10%
Native Alaskans	3.0%
Canadian native peoples	3.6–7.1%
Danes	<0.1%
North Americans	≤0.2%

Data compiled from references (2), (52–57).

The previous data on heterozygotes suggests that CSID may be more prevalent than previously believed. A small number of patients with intermittent or persistent diarrhea have been diagnosed in adult life (2,58). Because they have no family history and no history of growth failure or malabsorption, these patients have been assumed to suffer from irritable bowel syndrome.

Pathogenesis

Malabsorption of dietary disaccharides and starch in the proximal small intestine gives rise to an osmotic load that stimulates peristalsis in the ileum and colon. In response to the osmotic pressure difference between blood and lumen, water flows into the permeable jejunum and sodium moves into the lumen down its concentration gradient. The end-result is a large volume of intraluminal isotonic fluid with a normal sodium concentration held within the lumen because of the osmotic pressure generated by the malabsorbed carbohydrate solute. When the capacity of colonic bacteria to ferment malabsorbed carbohydrate and the ability of the colonocyte to absorb fluid and the resulting short-chain fatty acids is overwhelmed, diarrhea ensues.

Unabsorbed carbohydrates present in the distal small intestine have effects on distant gastrointestinal functions and the absorption of other nutrients as well (59). They inhibit gastric emptying and accelerate small-intestinal transit because of a decrease in water and sodium absorption. Accelerated duodenal and small-bowel transit may also contribute to the malabsorption of starch, fat, or even monosaccharides. Malabsorption of oligo- and monosaccharides may lead to disruption of the normal postprandial surge of hormones such as insulin, C-peptide, and gastric inhibitory peptide (60).

CSID is not invariably associated with severe diarrhea. Whether sugar or starch malabsorption produces symptoms depends not only on the residual enzyme activity, but also on additional factors such as the quantity of ingested carbohydrate, the rate of gastric emptying, the effect on small-bowel transit, the metabolic activity of colonic bacteria, and the absorptive capacity of the colon. For many of these parameters, the infant is at a disadvantage compared to the adult; this undoubtedly contributes to the increased severity of symptoms seen in many infants with CSID. In infants, the length of the small intestine is shorter and the reserve capacity of the colon to absorb excess luminal fluid is reduced compared to adults. Some infants may be consuming a high-carbohydrate diet in the form of juices, baby food fruits and vegetables, and cereals. In young infants with carbohydrate malabsorption, small-intestinal and colonic transit is likely to be more rapid, allowing less time for alternative paths of carbohydrate digestion, including the salvage of malabsorbed carbohydrate by colonic bacterial fermentation.

Compensatory mechanisms for starch digestion limit the diarrheagenic effects of starch malabsorption in patients with CSID. Isomaltase activity is often low but not necessarily absent in these patients. Most starch consumed by young patients has a low content of α-1–6 glucosyl bonds, and the residual isomaltase may be sufficient to hydrolyze these linkages. Glucoamylase activity is normal or increased and is still sufficient to ensure the adequate digestion of the α-1:4 bonds of amylopectin. In addition, the capacity of colonic bacteria to ferment starch is usually well developed in infants by 6 months of age (61,62).

Clinical Presentation

The clinical presentation of CSID is variable; in part, it depends on the introduction of sucrose into the diet. Breast-fed babies or infants consuming lactose-containing formulas will not manifest symptoms until they ingest juices, solid foods, or medications sweetened by sucrose. Baby cereals usually cause less severe symptoms because of the compensatory mechanisms for starch digestion.

Table 5 summarizes the presenting symptoms in 23 patients with CSID. There is an even sex distribution but an overwhelming predilection for Caucasians to be affected, with only one Hispanic patient and no African Americans diagnosed. In only two instances is there a family history, with two affected sisters and a father and son among the group studied. Chronic watery diarrhea and failure to thrive are common findings in infants and toddlers (63). Other nonspecific findings in this age group include abdominal distention, gassiness, colic, irritability, excoriated buttocks, diaper rash, and (at times) vomiting. Half the patients were diagnosed after the age of 5 years with long histories of chronic diarrhea and abdominal pain.

TABLE 5. *Presenting symptoms in 23 patients with CSID*

Symptoms	Frequency	Mean age at diagnosis (yr)
Chronic diarrhea and failure to thrive	7/23	2.0 ± 1.1
Chronic diarrhea with normal growth	9/23	5.6 ± 3.5
Irritable bowel syndrome, abdominal pain	7/23	15.4 ± 7.3

A minority of severely affected patients require hospitalization for diarrhea and dehydration, malnutrition, muscle wasting, and weakness (64). Often, the correct diagnosis is delayed while other causes of severe chronic diarrhea are entertained (65). These infants may be presumed to have cow's milk or soy protein allergy and often are subject to multiple formula changes. An improvement in symptoms while ingesting a casein-hydrolysate formula may be interpreted as support for this mistaken diagnosis when in truth it reflects the switch in carbohydrate to glucose polymers, which are more dependent on glucoamylase activity for intraluminal digestion. Other diagnoses often considered are cystic fibrosis, celiac disease, severe viral gastroenteritis, or other causes of intractable diarrhea. Support for these possibilities may come from the mild steatorrhea documented in some patients (2). This finding is thought to be due to rapid intestinal transit or chronic malnutrition with partial villus atrophy. Transient hypoglycemia, acidosis, dehydration, and lethargy may lead to consideration of inborn errors of metabolism.

A delay in the diagnosis may also be related to empiric institution of a low-sucrose diet by the parents. Some children attain relatively normal growth and manifest chronic symptoms of intermittent diarrhea, bloating, and abdominal cramps (Table 5). As toddlers, they may be considered to have chronic, nonspecific diarrhea of childhood (66) and are often not diagnosed until the age of 5 years. In older children, symptoms of crampy abdominal pain, gas, and intermittent diarrhea suggest irritable bowel syndrome. Institution of a diet for these conditions including the avoidance of fruit juices, soft drinks, and fructose- and sorbitol-containing beverages and fruits may actually ameliorate symptoms by simultaneously reducing the sucrose load in the diet.

In some societies, dietary habits may mask symptoms. Up until recently, Greenland Eskimos consumed low-carbohydrate, high-protein, high-fat diets. Only recently has the sugar content of their diet reached European levels (2,54,64). Of 20 Greenland Eskimos diagnosed by McNair et al. with CSID in 1972, seven were adults who denied any gastrointestinal symptoms, presumably as a result of their low-sucrose diet (67). In spite of the various ages and symptoms at presentation of patients with CSID shown in Table 5, there was no difference in the intestinal levels of sucrase-isomaltase or maltase activity measured from small-bowel biopsies in any of these groups.

CSID has been diagnosed in adult patients (58,68, 69). Many adults with CSID give a history of feeding difficulties during their infancy and intermittent symptoms since childhood (58,63). Occasionally, the symptoms appear as late as the time of puberty (69). In these patients, the underlying enzyme deficiency can be unmasked by an enteric infection. The symptoms that persist in adult life may be limited to some increase in bowel frequency and to abdominal distention and flatulence, especially at the end of the day, although episodic watery diarrhea associated with large sucrose intake still occurs. In a few patients, diarrhea has alternated with constipation, causing further confusion with irritable bowel syndrome. Some investigators have noted a tendency for spontaneous improvement of symptoms with age; in particular, the starch tolerance seems to improve (1). Possible explanations for these observations include self-regulation of the diet to limit sucrose ingestion and an adaptive increase in colonic salvage of carbohydrate through the stimulatory effects of chronic carbohydrate malabsorption on the fermentative activity of colonic flora.

Diagnostic Evaluation

Several diagnostic tests are available; each has its advantages and pitfalls. An excess of reducing substances (>0.5%) may be demonstrated in liquid stool from a patient with CSID provided the fecal sucrose is hydrolyzed by boiling with 0.1 N HCL. The pH of the stools in a patient with CSID classically should fall between 5.0 and 6.0. Both of these tests have a high degree of false-negative results (70). The presence of sucrose in fecal effluent can also be sensitively detected by paper chromatography.

Prior to the advent of hydrogen breath tests, oral sucrose tolerance tests were the mainstay of the noninvasive diagnosis of CSID. In children, a rise in blood glucose of >20 mg/dl after a 2.0 g/kg sucrose load is considered an indication of sucrose malabsorption. However, there is a high incidence of false-positive tests (flat sucrose tolerance curve) due to delayed gastric emptying, which can only be verified by intraduodenal instillation of the sucrose load (71).

Sucrose Breath Tests

Sucrose breath hydrogen tests have been extensively validated in children with sucrose malabsorption and normal controls (72). In normal sucrose-tolerant subjects given a 1.0–2.0 g/kg oral sucrose load (≤50 g), the change in breath hydrogen excretion over baseline is <10 parts per million. Two previous studies of children with CSID have shown an elevation of breath hydrogen >20 parts per million over baseline between 90 and 180 min after the ingestion of sucrose (72,73).

False negatives can occur with this test (74). Of 23 patients studied, we have documented that our two youngest patients with CSID (both 10 months of age) and one 10-year-old patient failed to show elevated breath hydrogen excretion over a 3-h period when given oral sucrose (2 g/kg sucrose up to 50 g). These patients appear to be non–hydrogen producers; this hypothesis can be confirmed by conducting a breath hydrogen test with a nonabsorbable carbohydrate substrate such as lactulose.

The prevalence of non–hydrogen producers has been estimated to be 2–20% of the general population (75–78). However, recent data have suggested that this figure is an overestimation and that most subjects will produce small amounts of hydrogen in response to malabsorbed carbohydrate if the test is extended beyond 3 h (79). A delay in gastric emptying of a concentrated sucrose load might prolong the transit of malabsorbed sucrose to the cecum in some patients with CSID. Another potential confounder is the acid milieu that may exist in the colon of patients with chronic sucrose and starch malabsorption. Reduction of colonic intraluminal pH secondary to chronic lactulose ingestion has been shown to significantly reduce the intracolonic production of hydrogen (80). A chronically low pH in the colon of patients with CSID may mask the expected rise in colonic hydrogen production and breath hydrogen excretion.

These potential pitfalls suggest that care must be taken in the interpretation of sucrose breath hydrogen tests in patients with potential CSID. First, it is important to monitor the symptoms and stool pattern of such patients for 24 h after the breath test is done. Patients who experience significant diarrhea and other symptoms in spite of "negative" sucrose breath hydrogen tests should be screened by other methods. Second, obtaining breath hydrogen determinations for up to 4 h after the ingestion of sucrose may enhance the sensitivity of the test. Third, insistence on a change in breath hydrogen excretion of >20 parts per million over baseline may exclude some patients with CSID, especially if the sucrose load ingested is <1 g/kg. Finally, an unrestricted diet prior to administration of the sucrose breath test may mask a positive test by lowering the intracolonic pH and limiting hydrogen production.

Differential Urinary Disaccharides

Following ingestion, a small fraction of intact disaccharide diffuses unmediated across the intestinal mucosa. The exact quantity is determined by absorptive area, permeability, rate of intestinal transit, and factors controlling intraluminal concentration, such as dilution and rate of hydrolysis. Because most absorbed disaccharides are completely and rapidly excreted into urine, the fraction of an ingested dose excreted in the urine is determined by the gastrointestinal factors described, provided renal function is normal. When lactulose, which resists mucosal hydrolysis, is ingested together with a hydrolyzable test disaccharide such as sucrose, correction for variables other than hydrolysis is obtained and the sucrose:lactulose ratio specifically indicates the corresponding mucosal sucrase activity. Active hydrolysis of sucrose or isomaltose results in calculated ratios >0.3, whereas the absence of SI produces ratios of these disaccharides to lactulose approaching one (81,82). In practice, this test of differential urinary disaccharide excretion consists of administering simultaneous lactulose, lactose, isomaltose, and sucrose after an overnight fast and then collecting urine for 10 h. After recording the urinary volume, an aliquot is analyzed by quantitative paper or thin-layer chromatography for the sugars tested.

Using this method, Maxton et al. have demonstrated excellent agreement between differential urinary disaccharide excretion and small-intestinal disaccharide determinations in patients with CSID (81,82). The addition of rhamnose to the test sugars allows the calculations of a urinary lactulose:rhamnose ratio, which has been shown to be a useful index of intestinal mucosal permeability (83). CSID is associated with normal mucosa and normal permeability. It can therefore be distinguished from disaccharidase deficiency secondary to diffuse small-intestinal disease, in which the lactulose-rhamnose permeability would be expected to be increased. This test appears to offer a noninvasive method of assessing the activity of multiple intestinal disaccharidases and mucosal permeability simultaneously.

Intestinal Disaccharidases

Measurement of intestinal disaccharidases has remained the gold standard for the diagnosis of CSID. A small-bowel biopsy obtained either with a capsule placed in the proximal jejunum or with the endoscope in the second or third portion of the duodenum will provide material not only for enzyme activity determinations but for histological examination as well. At least two biopsy specimens taken via a standard upper endoscope and three biopsy specimens taken with the pediatric upper endoscope should be obtained for disaccharidase determinations. The mucosa is usually normal histologically, but some patients with severe malnutrition may show mild partial villous atrophy.

In spite of the various ages and symptoms at presentation of the patients summarized in Table 5, sucrase activity is either completely or almost completely absent in 15 of 20 patients tested, isomaltase activity is markedly reduced in 14 of 20 tested, and maltase activity is reduced by 60–90% in 18 of 20 tested. Glucoamylase activity is usually normal, accounting for the residual measured maltase activity (5). In some cases, a reduction of the measured amount of glucoamylase activity has been observed (84). Lactase and alkaline phosphatase levels should be normal.

It is important to ascertain the location of small-bowel biopsy specimens when interpreting intestinal disaccharidase levels. Simultaneous biopsies of the proximal jejunum and the second portion of the duodenum in the patients with histologically normal mucosa and normal disaccharidases have shown a 30–40% reduction in lactase, sucrase, and maltase activity in the duodenum compared to the jejunum (85,86). This finding does not appear to be the result of a sampling error since it is in agreement with disaccharidase determinations in intestinal resection specimens (53,87). Most endoscopic small-bowel biopsy specimens are obtained from the duodenum; however, much of the published normative data for intestinal disaccharidases comes from tissue obtained from the jejunum with a Crosby capsule (88,89).

Sucrase-isomaltase deficiency is defined as the reduction of enzyme activities to levels lower than at least two standard deviations below the mean for biopsy specimens from normal patients with normal small-bowel histology. Combining the actual measured values of sucrase, isomaltase (palatinase), maltase, and lactase activities with the sucrase:lactase ratio can increase the diagnostic accuracy of the test for CSID. Provided the patient does not have primary lactase deficiency or secondary disaccharidase deficiency from partial or total villous atrophy, the normal sucrase:lactase ratio in adults is 1.9 + 0.2 (mean + SEM) when the biopsy specimen is obtained from the duodenum and 1.6 ± 0.2 when it is taken from the proximal jejunum (85). This ratio should decrease in children <3 years of age since among young children with normal small-bowel histology, lactase levels are generally increased compared to older children whereas sucrase activity remains constant (88). However, the ratio should never be <1.0 unless there is isolated decreased sucrase-isomaltase activity; it should actually increase in primary lactase deficiency or diffuse small-bowel injury and secondary disaccharidase deficiency, where lactase levels are usually more severely depressed than SI activity.

Treatment

Currently, the treatment of CSID consists of lifelong adherence to a strict sucrose-free diet. It is seldom necessary to make the diet starch-free as well except in infants, or in older children in whom the institution of a sucrose-free diet does not lead to prompt disappearance of symptoms. In this case, the starch content of the diet must be reduced with special attention to foods having a high amylopectin content, such as wheat and potatoes (2). Compliance with this diet is difficult, and there appears to be a high incidence of chronic gastrointestinal complaints, decreased weight for height, and decreased weight for age in patients with CSID followed after diagnosis (63,64,90). Neither sucrose nor fructose, both of which are known to stimulate sucrase and maltase activity when ingested by normal adults, have been shown to induce enzyme activity in patients with CSID. There is no evidence that deficient SI activity increases with age.

Enzyme substitution therapy has recently been applied to patients with CSID. A study of eight children with CSID showed that a small amount of lyophilized baker's yeast (*Saccharomyces cerevisiae*) eliminated or lessened symptoms of diarrhea, cramps, or bloating, and also lowered breath hydrogen when administered with an oral sucrose load (91). However, baker's yeast is not palatable in this form and is poorly accepted, especially by young children. As a by-product of the manufacture of belt-dried baker's yeast, a liquid preparation containing high concentrations of yeast-derived invertase (sucrase) is obtained. Invertase is a β-fructofuranosidase and cleaves only sucrose having no effect on maltooligosaccharides. In vitro, it is extremely potent, stable with refrigeration, and tasteless when mixed with water (92). It is also relatively resistent to changes in pH even at levels approximating the intragastric environment. Degradation by pepsin appears to be prevented by buffering intragastric pH and taking the enzyme with food to provide other potential protein substrates for pepsin activity (92).

Recently, 14 patients with CSID were treated with liquid yeast sucrase. Breath hydrogen excretion was significantly reduced in response to a sucrose load, and symptoms of diarrhea, abdominal pain, and gas were prevented or ameliorated in patients consuming a sucrose-containing diet. Improvement in symptoms correlated well with increasing concentrations of the enzyme supplement (92). These results suggest that liquid yeast sucrase may allow the consumption of a more normal diet by children with CSID and decrease the high incidence of chronic gastrointestinal complaints. Secondary sucrase deficiency caused by celiac disease, severe viral or parasitic gastrointestinal infections, the acquired immunodeficiency syndrome, or the short-bowel syndrome may also be amenable to treatment with liquid yeast sucrase.

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^{13}C-Breath Tests for Sucrose Digestion in Congenital Sucrase Isomaltase Deficient and Sacrosidase Supplemented Patients

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Abstract

Congenital sucrase-isomaltase deficiency (CSID) is characterized by absence or deficiency of the mucosal sucrase-isomaltase enzyme. Specific diagnosis requires upper gastrointestinal biopsy with evidence of low to absent sucrase enzyme activity and normal histology. The hydrogen breath test (BT) is useful but is not specific for confirmation of CSID. We investigated a more specific ^{13}C-sucrose labeled BT.

Objectives—were to determine if CSID can be detected with the ^{13}C-sucrose BT without duodenal biopsy sucrase assay and if the ^{13}C-sucrose BT can document restoration of sucrose digestion by CSID patients after oral supplementation with sacrosidase (Sucraid®).

Methods—Ten CSID patients were diagnosed by low biopsy sucrase activity. Ten controls were children who underwent endoscopy and biopsy because of dyspepsia or chronic diarrhea with

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normal mucosal enzymes activity and histology. Uniformly-labeled ^{13}C-glucose and ^{13}C-sucrose loads were orally administered. $^{13}CO_2$ breath enrichments were assayed using an infrared spectrophotometer. In CSID patients the ^{13}C-sucrose load was repeated adding Sucraid®. Sucrose digestion and oxidation were calculated as a mean % coefficient of glucose oxidation (% CGO) averaged between 30 and 90 minutes.

Results—Classification of patients by ^{13}C-sucrose BT % CGO agreed with biopsy sucrase activity. The breath test also documented the return to normal of sucrose digestion and oxidation after supplementation of CSID patients with Sucraid®.

Conclusion—^{13}C-sucrose BT is an accurate and specific non-invasive confirmatory test for CSID and for enzyme replacement management.

Keywords

^{13}C-breath test; glucose oxidation; congenital sucrase-isomaltase deficiency; sucrose digestion; sacrosidase supplementation

INTRODUCTION

Sucrose, also known as table sugar, is a disaccharide formed by glucose and fructose monosaccharide units. Sucrose is present in the human diet in fruits and is added to many prepared foods as refined beet or cane table sugar. Sucrase is the only brush border enzyme that digests sucrose. The membrane bound complex sucrase-isomaltase (SI) hydrolyzes disaccharide sucrose to free monosaccharides that are transported from the lumen by SGLT-1, GLUT-2, and GLUT-5 (2). A percentage of the absorbed glucose and fructose is quickly oxidized and exhaled as CO_2 and the remainder is metabolized or stored. SI has two maltase activities, which together with the two maltase activities of the maltase-glucoamylase (MGAM) complex, digest starch to free glucose. These four activities are better described as α-glucosidases. Approximately 60 to 80% of all mucosal α-glucosidase activity is accounted for by SI and the remainder of activity is due to MGAM (1). SI also has isomaltase and palatinase activities associated with the membrane bound isomaltase (I) portion of the enzyme complex.

Congenital sucrase-isomaltase deficiency (CSID) is an autosomal recessive intestinal disease caused by mutations of the SI gene (3–6). Duodenal mucosal histology is always normal. CSID patients have different phenotypes of enzymatic activities associated to SI, ranging from reductions of sucrase activity to total absence, as well as variable absence of isomaltase activity (7–10). Low sucrase activity leads to malabsorption of sucrose, resulting in dyspeptic-like symptoms such as diet-related chronic osmotic diarrhea and abdominal pain. Only rarely does CSID lead to failure to thrive (12). The severity of symptoms is related to the amount of sucrase activity and quantity of sucrose fed (11,12). A reduced maltase activity is expected to occur in patients with CSID because both subunits in the SI complex contribute to the total mucosal maltase activity (1). The low maltase activity can lead to malabsorption of starch products which may contribute to symptoms of dyspepsia and chronic abdominal pain (13). The prevalence of biopsy-assay proven CSID is 0.02% in individuals of European descent but is reported as high as 10% in indigenous Greenlanders (14). Frequency of heterozygous individuals carrying the CSID gene who have low but not deficient sucrase activity and normal small intestinal histology is reported to be from 2 to 9% in European Americans (7, 12). We found a frequency of isolated sucrase deficiency of 1% in our recent study of unselected clinically indicated duodenal biopsy enzyme assays (1)

Specific diagnosis of CSID presently requires duodenal biopsies with low to absent sucrase activity detected by enzyme assay and presence of normal histology to rule out secondary deficiency. (12, 13, 15). Multiple genotypes make it impossible to establish a single molecular test suitable for the diagnosis of all CSID (7). The technique for diagnosis of SI deficiency by intestinal biopsy and assay of mucosal hydrolysis of sucrose was first described forty years ago by Charlotte M. Anderson et. al. (16). Presently the principles for diagnosis of SI deficiencies remain the same but the development of less invasive and less complex techniques is needed. The simplest treatment for CSID is dietary sucrose and occasionally starch restriction. Enzyme supplementation with liquid yeast sacrosidase (sucrase) enzyme derived from *Saccharomyces cervisiae* relieves clinical symptoms and sucrose malabsorption in CSID patients. (17, 18, 19).

A hydrogen breath test (H_2 BT) for detecting carbohydrate malabsorption was introduced in the early 1970's creating the first clinical application for assessment of lactose malabsorption. The noninvasive nature of H_2 BT makes it particularly useful for application in pediatric clinical practice as an indirect test of carbohydrate malabsorption but it is not specific for the diagnosis of CSID (20). False-negative results may be obtained because of many factors affecting the H_2 production. The test requires absence of small bowel bacterial overgrowth and presence of colonic bacterial flora capable of fermenting proximally malabsorbed carbohydrate. There is great variability of fermentation by the colonic flora and no quantification of proximal carbohydrate malabsorption is possible. Failure to detect H_2 occurs in 2 to 40% of subjects. (21) A clinical problem arising from the H_2 BT is the large load of sucrose given to the patient. In CSID patients this load often precipitates severe symptoms of sucrose intolerance.

An evolution of the H_2 BT introduced in the early 1970's was the measurement of isotope-labeled CO_2 in breath using ^{13}C or ^{14}C (22). These tests depend on measurement of changes in isotope labeled breath CO_2 concentration; delta over baseline (ΔOB), detected by mass spectrometry or nuclear magnetic resonance (NMR) (23, 24). Isotope ratio ($^{13}C/^{12}C$) enrichment measured by mass spectrometry is the traditional method for BT and has high accuracy for low levels of enrichment (0.001 to 0.01 percent) (25–27). Most recently infrared mass-dispersion spectrophotometry has been introduced for breath $^{13}C/^{12}C$ isotope measurements and is clinically useful due to its simplicity and short turnaround time (28–30). Since the introduction of mass spectrometers for the detection of the stable isotope of ^{13}C in expired air the BT technique has been adapted for the study of malabsorption in the pediatric population with collection systems that are well-tolerated by infants and toddlers who can not actively cooperate (32, 33). The instruments required for measurement of ^{13}C-labeled CO_2 ($^{13}CO_2$) are less expensive now and naturally enriched and purified stable isotope labeled substrates are currently available (34, 35). The substrates most commonly used for $^{13}C/^{12}C$ BT include ^{13}C-labeled carbohydrates, starch, fatty acids, bile acids, amino acids and urea. Clinical applications include evaluation of the mucosal function, bacterial overgrowth, gastrointestinal motility, carbohydrate absorption, bile acid absorption, lipid absorption and lipase pancreatic activity, hepatic function, and protein absorption. (31). However the only test widely used in clinical practice is the ^{13}C urea BT for the diagnosis of *Helicobacter pylori* infection.

Since presently there are no practical and non-invasive methods for specific confirmation of SI deficiency conditions, we developed and validated a sucrose breath test for screening and confirmation of CSID using a novel non-invasive ^{13}C-sucrose labeled substrate. Our hypotheses were that primary sucrase deficiency can be confirmed using ^{13}C-sucrose breath test and that the effectiveness of sucrase replacement therapy can be evaluated by the same non-invasive method. The objectives of our investigation were to determine whether CSID can be detected with the ^{13}C-sucrose BT without duodenal biopsy sucrase assay and whether the ^{13}C-sucrose BT can document restoration of sucrose digestion in CSID patients after oral supplementation with yeast sucrase (Sucraid®).

METHODS

Clinical

After obtaining Institutional Review Board (IRB) approved informed consents under protocol H-10239, a total of 20 patients participated in this study. Ten CSID patients were diagnosed by intestinal enzyme activity determinations (5F: 5M, ages 1–15y) (Table 1). The CSID patients were recruited in three different ways: referral by Pediatric Gastroenterologists, direct self-referral by CSID families who called our study coordinator after reading an information letter about the study inserted in the Sucraid® package by QOL Medical Company; and families referred through the CSID website www.csidinfo.com. A control group of subjects was recruited from the Nutrition and Gastroenterology Service at Texas Children's Hospital (TCH). Ten controls (6F: 4M, ages 1–15 yrs) were patients who underwent endoscopy and biopsy because symptoms of dyspepsia or chronic diarrhea but with normal levels of mucosal enzymes measured according to the Dahlqvist method (36) and normal histology. The control group patients were participants of the IRB approved protocol H-1320 for recruiting children of both genders, 0–17 yrs with dyspepsia (ROME II criteria) and chronic diarrhea, pain or discomfort centered in the upper abdomen (37).

All CSID patients were biopsied and diagnosed by their primary GI physician before coming to the General Clinical Research Center (GCRC) at TCH for the BT study. In the control group the endoscopy procedures were performed for clinical indications by Pediatric Gastroenterologists at TCH. These biopsies were evaluated by the Pathology Department of TCH. Exclusion criteria for all subjects included villous atrophy on routine histology, fever, inability to cooperate with breath collections, failure to ingest the test ^{13}C-solution, diabetes, and chronic lung disease.

Biopsy enzyme assay and histology

The disaccharidase enzyme activity determinations for the control group and some of the CSID patients were done at the GI lab of Buffalo Women and Children's Hospital in N.Y (1). The remainder of the CSID patient's biopsies were assayed in other reference labs with the histology interpreted locally.

Breath tests

The $^{13}CO_2$ breath tests were done on 2 separate days for the control group and on 3 separate days for the CSID group at the GCRC at the TCH under protocol G-695. After overnight fasting, a 2.5 L reference breath sample was collected for comparison with the timed breath samples. Then 20 mg uniformly-labeled ^{13}C-glucose, (Isotec, Miamisberg, OH) was given using 10 gm unlabeled maltodextrins as carrier dissolved in water to a total volume of 100ml (Polycose ® from Ross Division of Abbot Laboratories). Starting 15 minutes after the ^{13}C-glucose load 0.25 L breath samples were collected every 15 minutes for 120 minutes. After finishing the BT the subject was fed and released from the GCRC. The second day the procedure was the same but ^{13}C-sucrose was used. On the third day CSID patients had a repeat ^{13}C-sucrose load with addition of 22 drops of Sucraid® (8,500 IU of sacrosidase, provided by QOL Medical, Mooresville, NC) to the load solution.

Breath $^{13}CO_2$ enrichment analysis

After ^{13}C-labeled substrate loads were administered, breath collections and measurement of $^{13}CO_2$ enrichments were performed every 15 min × 9 using a $^{13}CO_2$ infrared spectrophotometer (POCone®, Otsuka Electronics, Tokyo, Japan). At each time point the total CO_2 concentration exceeded 2% in the breath sample and was thus in the $^{13}CO_2$ analytical range of the instrument. The BT results were recorded as total breath CO_2 concentration expressed as glucose-ΔOB $^{13}CO_2$ or sucrose-ΔOB $^{13}CO_2$.

Calculations

Because of the age related variations of glucose oxidation to CO_2 described below, glucose-$\Delta OB^{13}CO_2$ was used as denominator to overcome the effect of age on sucrose-$\Delta OB^{13}CO_2$. ^{13}C-sucrose digestion and oxidation was expressed as a % coefficient of glucose oxidation (% CGO) as calculated from $\Delta OB\ ^{13}CO_2$ breath enrichments as follows:

$$\% \text{ CGO} = [\text{sucrose-}\Delta OB\ ^{13}CO_2 / \text{glucose-}\Delta OB\ ^{13}CO_2] \times 100$$

Since % CGO values were found relatively constant in the period of 30 to 90 minutes after the load these values were averaged for each individual. The individual subject mean % COG values were used to identify the lower reference limit of ^{13}C-sucrose BT for controls and used to compare ^{13}C-sucrose BT of CSID with duodenal sucrase activities (see below).

Statistical procedures

Agreement between duodenal sucrase activity and ^{13}C-sucrose BT mean % CGO was tested with receiver operation analysis (ROC) using the statistics software SPSS. Additional subjects were recruited from the families of CSID patients for replicate ^{13}C-glucose and ^{13}C-sucrose BT to evaluate the within subject variations (Table 2) and to test the effect of age on glucose-$\Delta OB^{13}CO_2$ (Figure 1). General linear modeling techniques were used to assess possible effects of group age distribution differences on CGO% values and the ability of the breath test to discriminate between normal and CSID subjects. Two tail t-tests were used to compare groups; p values < 0.05 were interpreted as significant.

RESULTS

Clinical Description of CSID patients

Patients from the CSID group were referred by Pediatric Gastroenterologists. Their duodenal biopsy enzyme assays are shown in Table 1. Clinical histories varied but all CSID patients had duodenal biopsy sucrase activities below 6.5; all had maltase activities below 115; and 9 of 10 had palatinase activities below 5 U/g protein. None had villous atrophy.

Clinical Description of control subjects

Ten controls were children biopsed for clinical indications by the Pediatric Gastroenterology service at TCH because of the complaint of dyspepsia. All controls had levels of duodenal biopsy disaccharidase enzyme activities well above the reference levels (Table 1). None had mucosal histologic abnormalities.

Glucose oxidation with age

% CGO was used to normalize the sucrose-$\Delta OB^{13}CO_2$. The effect of age in months on glucose-$\Delta OB\ ^{13}CO_2$ is shown in Figure 1. This analysis included 44 subjects by additional studies in CSID family members. 83% of the total variation of glucose-$\Delta OB\ ^{13}CO_2$ was accounted for by the subject's age. (Figure 1, R^2 83%).

Replicate ^{13}C-glucose and ^{13}C-sucrose BT

On replicate BT testing of the same subject, separated by 1–12 months, a mean % coefficient of variation (% CV) of 14% for the ^{13}C-glucose BT and 9% for ^{13}C-sucrose BT were observed (Table 2).

13C-sucrose oxidation in CSID and controls

In the control group an average of 146% ± 45.5 mean % CGO and for the CSID group an average of 25 ± 21mean % CGO were observed (p<0.001)(Figure 2). The lowest mean %

CGO obtained was 0.7% and the highest was 56.5% in the CSID patients (Table 1). Analysis controlling for differences in group age distribution found no relationship between % CGO and age or any effect of age on the above group averages. Therefore age did not effect the assessment of the BT ability to discriminate.

Clinical utility of ^{13}C-sucrose BT mean % CGO

ROC analysis of mucosal biopsy sucrase activity vs. ^{13}C-sucrose mean % CGO established a cut-off value for ^{13}C-sucrose BT mean % CGO of 79% which yielded 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both) for detection of low duodenal sucrase activity by ^{13}C-sucrose BT mean % CGO (Figure 2 and Figure 3).

Response of CSID patient's ^{13}C-sucrose BT to Sucraid® supplement

All CSID patients showed correction of sucrase deficiency with oral Sucraid® supplementation, responding to levels greater than their baseline ^{13}C-sucrose BT mean % CGO ($p = 0.001$) (Figure 3).

DISCUSSION

Duodenal Enzyme Activities

In this $^{13}CO_2$ BT study we included 10 CSID patients with biopsy proven sucrase deficiency and normal histology (Table 1). The $^{13}CO_2$ BT 9–14% coefficient of variation (CV%) of replicate BTs compares favorably with the 27 CV% of sucrase activity assayed reported in replicate duodenal biopsies (1). All CSID duodenal sucrase enzyme levels fell below the 10th % reference value (27 U /gp) in a range from 0 to 6.5 U/gp, and palatinase (isomaltase) levels were from 0 to 4.9 U/gp. Patient 7 had normal isomaltase activity (6.7 U/gp) (1). All CSID patients had low maltase activities. Patient 1 and patient 8, the only two with glucoamylase enzyme determinations, were below the10% reference value. For terminal starch digestion mucosal enzymes in the brush border are armed with 4 complimentary maltase activities, two from the SI complex and 2 from MGAM. SI accounts for 60–80% of the assayed maltase hydrolytic activity and the remainder is due to MGAM (1). From this we deduce that the CSID patients with mild reductions of maltase activities are retaining some hydrolytic activity from MGAM. In patient 7, where isomaltase was conserved, this also contributed to maintenance of maltase activity.

Glucose oxidation with age

Studies using combined gas chromatography-mass spectrometry (38) and neuroimaging techniques-positron emission tomography (PET) (39) have shown that fasting child endogenous glucose production and brain glucose oxidation are two-to-four fold greater than in the adult. In our study we confirmed that glucose oxidation was two to four times higher in children than adults (Figure 1). This may be due to the unique glucose needs for child brain development as reflected by our ^{13}C-glucose BT results in children. Central nervous system glucose consumption represents 60–80% of daily hepatic glucose output in the child, as it does in the adult (40), suggesting the importance of a good carbohydrate digestion and absorption in early child neurodevelopment. Because of the age dependence of glucose oxidation, % CGO is a necessary normalization for the digestion, absorption and oxidation of sucrose in children.

Gastric emptying

Using the ^{13}C-glucose BT we addressed the uniformity of liquid phase of gastric emptying for our study. We used 10% maltodextrin (Polycose ®) instead of water because maltodextrin made from corn is poorly isotopically enriched (0.2%) and provides a standard osmotic and energy matrix for the uniformly enriched ^{13}C-labeled tracer substrate. The same dose of maltodextrins was used for each loading test to increase the uniformity of gastric emptying and the small amount of ^{13}C in the maltodextrin was thus blanked out in % CGO. The maltodextrin serves to standardize caloric load to mimic a meal and provide a trigger for liquid gastric emptying (41)

Test of Hypothesis 1

One of our objectives was to compare the less invasive ^{13}C-sucrose BT with duodenal biopsy sucrase assays obtained by endoscopy. A very strong relationship was observed and ROC analysis indicated that a reference value of 79 % mean % CGO discriminated between CSID and control populations, as confirmed by duodenal sucrase activities, with 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both). This supports our first hypothesis that CSID can be confirmed with the ^{13}C-sucrose BT, however secondary sucrase deficiency cannot be excluded without clinical evaluation and biopsy.

Test of Hypothesis 2

We tested the ^{13}C-sucrose BT response to the enzyme supplement Sucraid® documenting a rise in mean % CGO for each CSID patient after the supplement to levels not different from controls ($P = 0.293$). The effectiveness of orally replacing sucrase was confirmed by the ^{13}C-sucrose BT. This response supports our second hypothesis that ^{13}C-sucrose BT quantitated the response of CSID patients to Sucraid® supplementation.

Non-invasive BT

One of the advantages of ^{13}C-sucrose BT which we and parents observed was that many CSID patients who had previous hydrogen BT experienced severe symptoms, passage of watery stools, bloating abdomen, and cramps from the 2 g/Kg sucrose load. We did not observe this symptomatic response in any CSID patient because the load of sucrose ingested was only 0.02 g for the ^{13}C-sucrose BT. As previously noted; the H_2 BT is not specific for sucrose malabsorption. With ^{13}C-sucrose BT we demonstrated a sensitivity and specificity of 100% (95% confidence interval 74% to 100% for both) in CSID patients and suggest that this diagnostic tool can be used as a non-invasive method for the confirmation and management of CSID.

SUMMARY

^{13}C-sucrose BT was evaluated as a non-invasive method for the confirmation of CSID. The results of sucrose digestion and oxidation were expressed as percentage of glucose oxidation (% CGO) and averaged between 30 and 90 minutes after the ^{13}C-substrate loads (mean % CGO). In controls and patients ^{13}C-sucrose BT mean % CGO agreed with duodenal sucrase enzyme activity determinations with 100% sensitivity and 100% specificity (95% confidence interval 74% to 100% for both). All CSID patients tested had ^{13}C-sucrose BT mean % CGO lower than 79%. Supplementation of CSID patients with sacrosidase enzyme corrected ^{13}C-sucrose BT mean % CGO to control levels.

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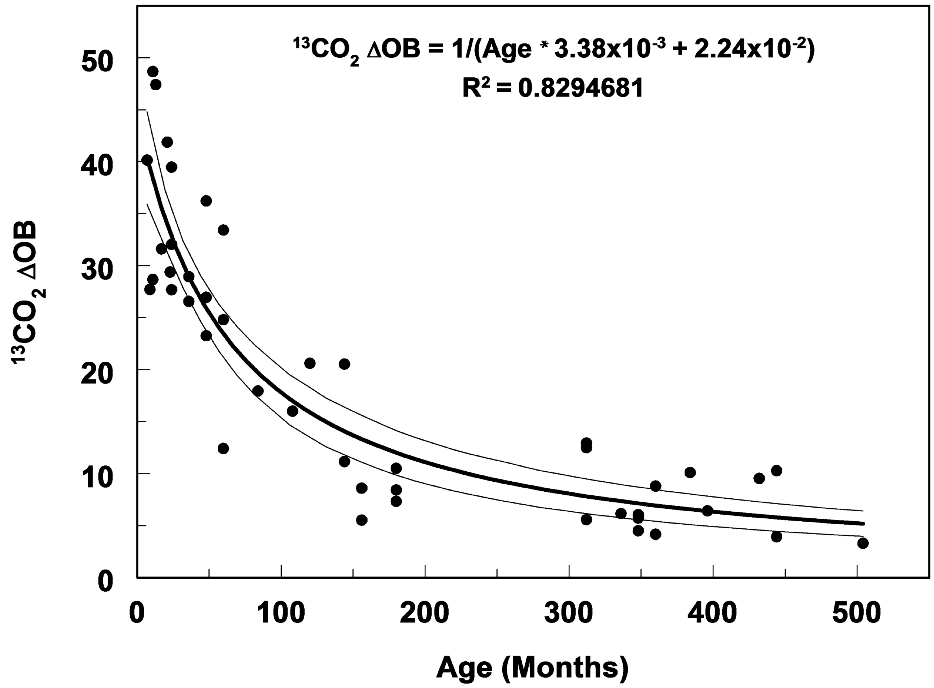

Figure 1. Effects of age on oral glucose breath test CO_2 enrichment

Effects of age in months on individual mean breath $^{13}CO_2$ ΔOB enrichments after a 20 mg ^{13}C-glucose load to controls, CSID patients and their family members. Breath enrichments of $^{13}CO_2$ ΔOB = 1/(Age * 3.38×10^{-3} + 2.24×10^{-2}); R^2 = 0.83, n = 44. Predicted mean $^{13}CO_2$ ΔOB is shown as heavy black line ± 95% CI thin lines.

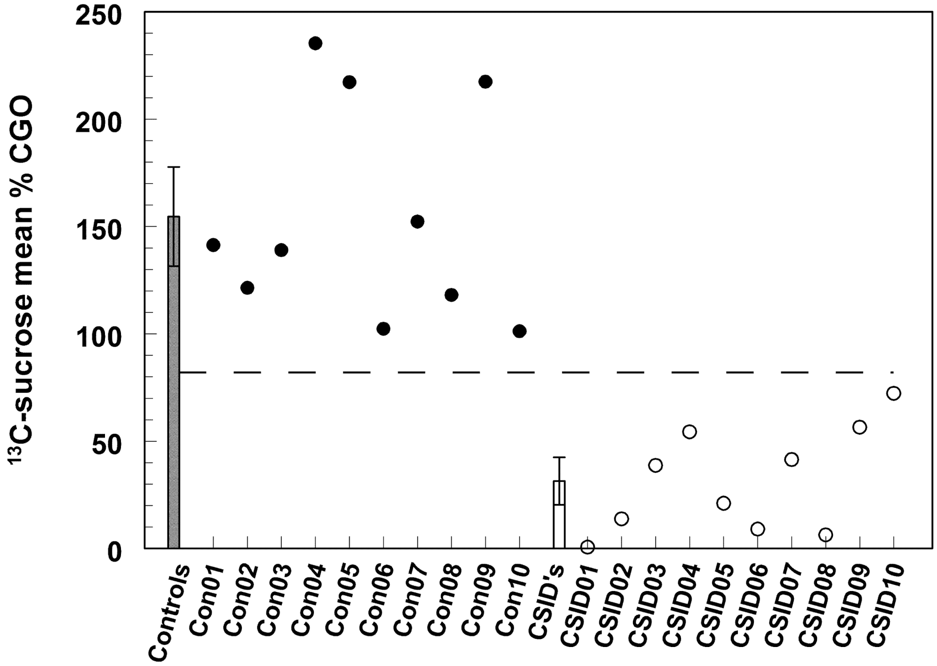

Figure 2. Effects of CSID on oral sucrose breath test mean % CGO

Mean % CGO of individual subjects after a 20 mg ^{13}C-sucrose BT load and group means of all control and CSID subjects. The solid bar depicts the group average ± SD of controls. Individual values are shown as filled circles. The open bar depicts the average ± SD of the CSID patients. Individual values are shown as open circles. The dashed line is the 79 % mean CGO reference value for discriminating between control and CSID subjects (see text).

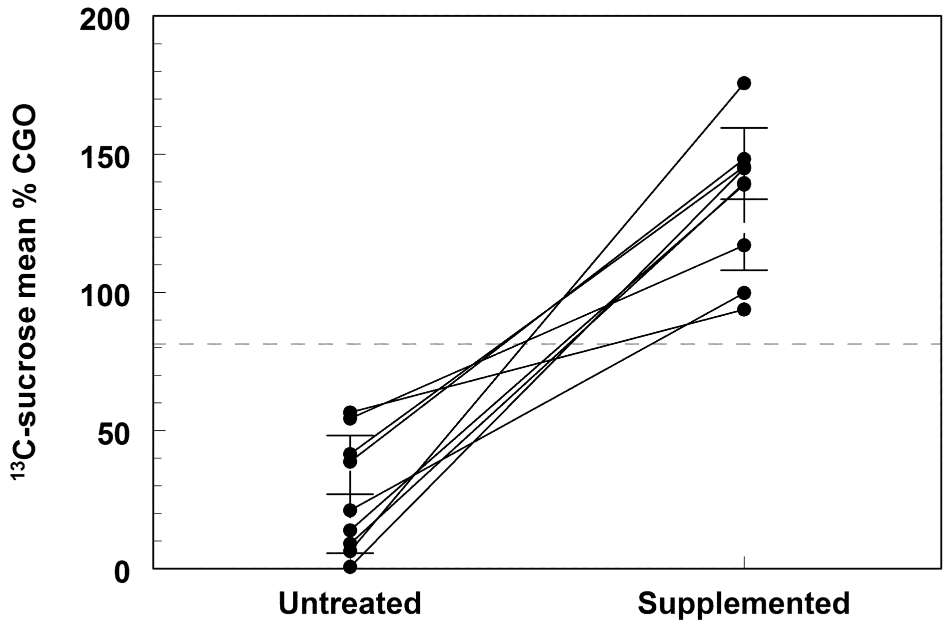

Figure 3. Effects of oral sacrosidase supplementation of CSID patients on sucrose breath test mean % CGO

Mean % CGO of individual CSID patients untreated (Left) and treated (Right) with 22 drops of oral sacrosidase supplement added to the sucrose load ($p = 0.001$, $n = 9$). The dashed line is the 79 % mean CGO reference value for discriminating between normal and untreated CSID subjects (see text).

Table 1

Duodenal biopsy disaccharidase activities (U g/protein)

Reference Values (1)		**6.5**	**26**	**89**	**5**	**32**
Patient	**Age**	**Lactase**	**Sucrase**	**Maltase**	**Palatinase**	**Glucoamylase**
CSID1	11m	91.5	0.3	28.1	2.3	5.4
CSID 2	15y	30.1	0	37.3	0	-
CSID 3	3y	43.1	0	39.4	0	-
CSID 4	2y	23.7	1.4	60.5	0	-
CSID 5	4y	126.4	3.6	93.9	1.8	-
CSID 6	4y	33.9	0.7	0	0.5	-
CSID 7	4y	23	0	0	6.7	-
CSID 8	23m	58.3	0	-	2	10.4
CSID 9	13m	53.3	6.5	50.9	4.9	-
CSID 10	11m	37.8	2.7	22.9	0	-
* Control	10 (2–15) yr	(23–126)	(35.5–96)	(115–268)	(5–16.5)	(95–110)

* Range of control group age and activities for each substrate

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Table 2

Within individual ^{13}C-glucose and ^{13}C-sucrose BT mean $^{13}CO_2$ ΔOB replicate variations (% CV) after 20 mg ^{13}C-substrate oral loads.

	13C-glucose BT % CV	13C-sucrose BT % CV
Average ± SD	13.5 ± 11.4	9.4 ± 7.1
Range	0–30	0–20
n	7	8