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 activity 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. **(Include complete information on diagnosis and methods used in the determination of diagnosis, such as evaluation of the patient's case history and 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.)**

In my clinical judgement, a small bowel biopsy is unwarranted in this case. Performing an endoscopic evaluation to obtain small bowel biopsies requires anesthesia, which carries unnecessary risks.

Endoscopic biopsy of the small bowel with disaccharidase levels has been used to diagnose CSID when other noninvasive and more cost-effective diagnostic tools were not available. Endoscopic biopsy of the small bowel with disaccharidase assay is invasive, expensive, and may

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not be feasible for all patients, due to the patient's health condition or logistical limitations at the clinic (biopsy samples require freezing and shipment using dry ice, etc.). In addition, the disaccharidase assay, although claimed as a diagnostic "gold standard," has several limitations:

1. Representative sampling is dependent on the anatomical distribution of brush border enzymes, which varies.[4]
2. Even with repeated biopsies from the same sampling location, measures of sucrase activity may vary by almost 30%.[5]
3. Depending on the gene mutation, the sampled sucrase enzyme could be transported to the wrong intracellular location.[6] If the sucrase enzyme is expressed on the basal-lateral side of the epithelial or sequestered rather than expressed in the brush border membrane of the intestine, the enzyme would not be available for digestive activity. Because disaccharidase assays homogenize biopsy samples and expose all enzymes in the homogenate to the substrate, the assay is not able to differentiate between effective and ineffective enzyme activity.

In contrast, combining the ^{13}C-sucrose breath test (^{13}C-SBT) with a careful history of symptoms has shown success in identifying patients with sucrase deficiency.[3]

Treatment Description and Rationale

Sucraid® is approved by the U.S. Food & Drug Administration and is indicated for the treatment of sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID). Please see attachments.

I have chosen to treat **(Patient's name)** with Sucraid® based on the history previously stated, and because it is the indicated medical treatment for CSID. I believe the patient's prognosis without Sucraid® is___. However, with Sucraid®, the prognosis is__. In summary, Sucraid® is medically necessary in this case and should be covered and/or reimbursed. 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. doi:10.1097/00005176-199507000-00001
2. Treem WR. Clinical aspects and treatment of congenital sucrase-isomaltase deficiency. *J Pediatr Gastroenterol Nutr*. 2012;55(suppl 2):S7-13. doi:10.1097/01.mpg.0000421401.57633.9

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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. doi:10.1097/mpg.0b013e318180cd09
4. Triadou N, Bataille J, Schmitz J. Longitudinal study of the human intestinal brush border membrane proteins. Distribution of the main disaccharidases and peptidases. *Gastroenterology*. 1983;85(6):1326-32.
5. Quezada-Calvillo R, Robayo-Torres CC, Ao Z, et al. Luminal substrate ''brake'' on mucosal maltase-glucoamylase activity regulates total rate of starch digestion to glucose. *J Pediatr Gastroenterol Nutr*. 2007;45(1):32-43. doi:10.1097/MPG.0b013e31804216fc
6. Gericke B, Amiri M, Scott CR, Naim HY. Molecular pathogenicity of novel sucrase-isomaltase mutations found in congenital sucrase-Isomaltase deficiency patients. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(3):817-26. doi:10.1016/j.bbadis.2016.017

Indication

Sucraid® (sacrosidase) Oral Solution is indicated for the treatment of sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID), in adult and pediatric patients 5 months of age and older.

Important Safety Information for Sucraid® (sacrosidase) Oral Solution

- Do not prescribe Sucraid® to patients known to be hypersensitive to yeast, yeast products, papain, or glycerin (glycerol).
- 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.
- 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, 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; single-use containers can be removed from refrigeration and stored at 59°F-77°F (15°C-25°C) for up to 3 days (72 hours). Refer to Instructions for Use for full information on how to take Sucraid®.

QL60103

Prescribing Information

Sucraid® (sacrosidase) Oral Solution:

DESCRIPTION

Sacrosidase is an enzyme with the chemical name of β,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 Da for the glycosylated monomer (range 66,000- 116,000 Da). Reports also suggest that the protein exists in solution as a monomer, dimer, tetramer, and octomer ranging from 100,000 Da to 800,000 Da. It has an isoelectric point (pI) of 4.5.

Sucraid®(sacrosidase) Oral Solution is an oral enzyme replacement therapy.

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.

Sucraid may contain small amounts of papain (see WARNINGS). 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 glycerin, water, citric acid, and sodium hydroxide 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 (see DOSAGE AND ADMINISTRATION, Administration Instructions).

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, and a moderate decrease in maltase activity.

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, diarrhea, 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.

CLINICAL STUDIES

A two-phase (dose response preceded by a breath hydrogen phase) double-blind, multi-site, crossover trial was conducted in 28 pediatric patients (approximately 5 months to 12 years of age) 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 pediatric patients up to 3 years of age, 86% became asymptomatic. In pediatric patients over 3 years of age, 77% became asymptomatic. Thus, the therapeutic response did not differ significantly according to pediatric 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, pediatric patients showed a marked decrease in breath hydrogen output when they received sacrosidase in comparison to placebo.

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

INDICATIONS AND USAGE

Sucraid®(sacrosidase) Oral Solution is indicated for the treatment of sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID), in adult and pediatric patients 5 months of age and older.

CONTRAINDICATIONS

Sucraid is contraindicated in patients known to be hypersensitive to yeast, yeast products, glycerin (glycerol), or papain (see WARNINGS).

WARNINGS

Severe Hypersensitivity Reactions

Severe hypersensitivity reactions, including wheezing, rash, and pruritis, have been reported with administration of Sucraid. Sucraid contains papain, which is associated with hypersensitivity reactions (see DESCRIPTION).

A pediatric patient in the clinical trials experienced a hypersensitivity reaction of severe wheezing that required hospitalization. Postmarketing cases of cutaneous hypersensitivity reactions have also been reported.

Instruct patients or caregivers to stop Sucraid and seek medical attention if symptoms suggestive of a hypersensitivity reaction occur. Sucraid is contraindicated in patients who have had a known hypersensitivity reaction (see CONTRAINDICATIONS).

PRECAUTIONS

Increased Blood Glucose Concentrations in Patients with Diabetes Mellitus

Sucraid enables the products of sucrose hydrolysis, glucose and fructose, to be absorbed and may increase blood glucose concentrations. Monitor blood glucose concentrations and adjust the diet accordingly for patients with diabetes mellitus.

Dietary Starch Restriction

Sucraid does not replace isomaltase. Therefore, patients may still experience symptoms of CSID while taking Sucraid. Consider dietary starch restriction in addition to Sucraid, especially in patients in whom symptoms are not adequately controlled by Sucraid.

Information for Patients

See Patient Package Insert and the Instructions for Use.

Drug Interactions

Fruit Juice

The acidity in fruit juice may reduce the enzyme activity in Sucraid. Administration of Sucraid with liquids other than water, milk, or infant formula has not been studied and is not recommended (see DOSAGE AND ADMINISTRATION, Administration Instructions).

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

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

The safety and effectiveness of Sucraid for the treatment of sucrase deficiency, which is part of congenital sucrase-isomaltase deficiency (CSID), have been established in pediatric patients aged 5 months and older. Use of Sucraid for this indication is supported by evidence from adequate and well-controlled studies in pediatric patients (see CLINICAL STUDIES and ADVERSE REACTIONS).

Geriatric Use

Clinical trials of Sucraid did not include patients 65 years of age and older to determine if they respond differently from younger adult patients.

ADVERSE REACTIONS

The following adverse reactions associated with the use of sacrosidase were identified in clinical studies or postmarketing reports. Because some of these reactions were reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

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

Hypersensitivity reactions (wheezing, rash, and pruritis) have been reported (see WARNINGS).

DOSAGE AND ADMINISTRATION

Important Administration Information

- Administer Sucraid with each meal or snack.
- Mix Sucraid with cold or room temperature water, milk or infant formula prior to administration. Administration of Sucraid in liquids other than water, milk, or infant formula has not been studied and is not recommended. Do not mix or consume Sucraid with fruit juice.
- Do not warm or heat the water, milk, or infant formula before or after addition of Sucraid.
- Administer half of the dose at the beginning of the meal or snack and the other half of the dose during the meal or snack.

Recommended Dosage

The recommended dosage is:

- *Patients weighing 15 kg and less:* 8,500 International Units (1 mL) administered orally with each meal or snack.
- *Patients weighing more than 15 kg:* 17,000 International Units (2 mL) administered orally with each meal or snack.

Preparation and Administration Instructions for Patients Weighing 15 kg or Less

Multiple-Dose Bottle:

1. Using the measuring scoop provided, add 1 scoop of Sucraid (1 mL) to 60 mL of cold or room temperature water, milk, or infant formula.
2. Stir to mix well.
3. Administer half of the mixed Sucraid solution (30 mL) at the beginning of the meal or snack and the other half of the mixed solution (30 mL) during the meal or snack.
4. Do not save any of the mixed Sucraid solution for later use.
5. Rinse the measuring scoop with water.

Single-Use Container:

1. Empty the entire contents of the single-use container (2 mL) in 120 mL of cold or room temperature water, milk, or infant formula.
2. Stir to mix well.
3. Divide the mixed Sucraid solution into two separate 60 mL portions. The first portion (60 mL) is for immediate use.
   - Administer half of the first portion (30 mL) of the mixed Sucraid solution at the beginning of the meal or snack and the other half of the first portion (30 mL) of the mixed Sucraid solution during the meal or snack.
4. Store the second portion of the mixed Sucraid solution (60 mL) at 2°C to 8°C (36°F to 46°F) for up to 24 hours for administration with the next meal or snack.
   - Discard the mixed Sucraid solution if not used within 24 hours.

Preparation and Administration Instructions for Patients Weighing More than 15 kg

Multiple-Dose Bottle:

1. Using the measuring scoop provided, add 2 scoops of Sucraid (2 mL) to 120 mL of cold or room temperature water, milk, or infant formula.
2. Stir to mix well.
3. Administer half of the mixed Sucraid solution (60 mL) at the beginning of the meal or snack and the other half of the mixed Sucraid solution (60 mL) during the meal or snack.
4. Do not save any of the mixed Sucraid solution for later use.
5. Rinse the measuring scoop with water.

Single-Use Container:

1. Empty the entire contents of the single-use container (2 mL) in 120 mL of cold or room temperature water, milk, or infant formula.
2. Stir to mix well.
3. Administer half of the mixed Sucraid solution (60 mL) at the beginning of the meal or snack and the other half of the mixed solution during the meal or snack (60 mL).
4. Do not save any of the mixed Sucraid solution for later use.

HOW SUPPLIED

118 mL Multiple-Dose Bottle

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

NDC# 67871-111-04 (2 x 118 mL multiple-dose bottles)

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

2 mL Single-Use Container

Sucraid (sacrosidase) Oral Solution is available in 2 mL, single-use containers that are packaged into a foil pouch. Each 2 mL single-use container contains 17,000 International Units of sacrosidase.
Each foil pouch holds a card of 5 containers. Five pouches are then packaged in a box (25 containers). Six boxes are further packaged in a carton (150 containers).

NDC# 67871-111-07 (150 x 2 mL single-use containers)

Store under refrigeration, 2°C to 8°C (36°F to 46°F). Protect from light. Single-use container can be removed from refrigeration and stored at 15°C to 25°C (59°F to 77°F) for up to 3 days (72 hours).

Manufactured by:
QOL Medical, LLC Vero Beach, FL 32963
U.S. License No. 2195

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

Rev <08/24>

Patient Information

SUCRAID® (Su-kreid) (sacrosidase) Oral Solution

What is SUCRAID?

SUCRAID is a prescription medicine for the treatment of people who were born with a lack of (deficiency) sucrase, which is part of congenital sucrase-isomaltase deficiency (CSID). It is not known if SUCRAID is safe and effective in children under 5 months of age.

Do not take or give your child SUCRAID if you or your child:

- are allergic to yeast, yeast products, glycerin (glycerol), or papain. See the end of this Patient Information leaflet for a complete list of ingredients in SUCRAID.

Before you take or give your child SUCRAID, tell your healthcare provider about all of your medical conditions, including if you or your child:

- have diabetes. SUCRAID can interact with the food in your diet and may change your blood sugar levels. Your healthcare provider will tell you if your diet or diabetes medicines need to be changed.
- are pregnant or plan to become pregnant. It is not known if SUCRAID will harm your unborn baby.
- are breastfeeding or plan to breastfeed. You and your healthcare provider should decide if you will take SUCRAID while breastfeeding.

Tell your healthcare provider about all the medicines you take, including prescription and over-the-counter medicines, vitamins, and herbal supplements.

How should I take or give SUCRAID?

- **See the detailed Instructions for Use that come with this Patient Information leaflet for instructions about the right way to take or give SUCRAID.**
- SUCRAID should be taken or given exactly as prescribed by your healthcare provider. Do not change the dose of SUCRAID without talking to your healthcare provider.
- SUCRAID comes in a 118-mL multiple-dose bottle or a 2-mL single-use container. Your healthcare provider will decide which type of SUCRAID is best for you to use.
- The dose of SUCRAID depends on body weight. Your healthcare provider will tell you how much SUCRAID you should take or give your child.
  - The dose for a child 33 pounds (15 kg) or less is 1 mL or 28 drops of SUCRAID in 2 ounces of water, milk, or infant formula.
  - The dose for a child or adult more than 33 pounds (15 kg) is 2 mL or 56 drops of SUCRAID in 4 ounces of water, milk, or infant formula.
- SUCRAID can only be dissolved in cold or room temperature water, milk, or infant formula. **Do not** put SUCRAID in warm or hot liquids.
  - **Do not** mix SUCRAID with fruit juice. **Do not** take or give SUCRAID with fruit juice.
  - **Do not** warm or heat the mixed solution before taking or giving SUCRAID.
- Measure your dose or your child's dose of SUCRAID using the measuring scoop that comes with the SUCRAID bottle. **Do not** use a kitchen teaspoon or other measuring device.
- SUCRAID should be taken or given with each meal or snack. Half of the SUCRAID dose should be taken at the beginning of each meal or snack. Take or give the remaining SUCRAID dose during the meal or snack.
- Rinse the measuring scoop with water after each use.
- SUCRAID does not break down some sugars found in foods that have starch, such as wheat, rice, and potatoes. Your healthcare provider may tell you to avoid eating foods with starch.

What are the possible side effects of SUCRAID?

SUCRAID may cause serious side effects, including:

- **severe allergic reactions.** Severe allergic reactions have happened in some people taking SUCRAID. Tell your healthcare provider right away or go to the nearest emergency room if you have any of the following symptoms:
  - difficulty breathing
  - wheezing
  - rash
  - swelling of the face, lips, mouth, or tongue

Your healthcare provider may need to monitor you or your child carefully when first starting treatment with SUCRAID.

The most common side effects of SUCRAID include:

• stomach (abdominal) pain	• vomiting	• nausea
• diarrhea	• constipation	• problems sleeping
• headache	• nervousness	• dehydration

These are not all of the possible side effects of SUCRAID. **Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.**

How should I store SUCRAID?

- **SUCRAID 118 mL multiple-dose bottle**
  - Store in the refrigerator between 36°F to 46°F (2°C to 8°C).
  - Throw away after 4 weeks of first opening the multiple-dose bottle.
  - Protect from heat and light.
- **SUCRAID 2-mL single-use container**
  - Store in the refrigerator between 36°F to 46°F (2°C to 8°C)
  - After removing from the refrigerator, the 2-mL single-use container can be stored between 59°F to 77°F (15°C to 25°C) for up to 3 days (72 hours).
  - Protect from heat and light.
- **Keep SUCRAID and all medicines out of the reach of children.**

General information about the safe and effective use of SUCRAID.

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet. **Do not** use SUCRAID for a condition for which it was not prescribed. **Do not** give SUCRAID to other people, even if they have the same symptoms that you have. It may harm them. You can ask your pharmacist or healthcare provider for information about SUCRAID that is written for health professionals.

What are the ingredients in SUCRAID?

Active ingredient: sacrosidase
Inactive ingredients: Citric acid, glycerol, sodium hydroxide, and water.

Manufactured by:
QOL Medical, LLC Vero Beach, FL 32963
U.S. License No. 2195
For more information, go to www.Sucraid.com or call 1-866-469-3773.

This Patient Package Insert has been approved by the U.S. Food and Drug Administration

Revised: August 2024

Instructions for Use

SUCRAID® (Su-kreid) (sacrosidase) oral solution:
118 mL Multiple-Dose Bottle

Read this Instructions for Use before you start taking or giving SUCRAID to a child, and each time you get a refill. There may be new information. This information does not take the place of talking to your healthcare provider about your or your child's medical condition or treatment.

Important information you need to know before taking or giving SUCRAID:

- Your healthcare provider will decide the right dose of SUCRAID for you or your child. **Do not** change the dose of SUCRAID without talking to your healthcare provider.
- The dose of SUCRAID depends on body weight. Your healthcare provider will tell you how much SUCRAID you should take or give your child.
  - The dose for a child 33 pounds (15 kg) or less is 1 mL or 28 drops of SUCRAID in 2 ounces of water, milk, or infant formula.
  - The dose for a child or adult more than 33 pounds (15 kg) is 2 mL or 56 drops of SUCRAID in 4 ounces of water, milk, or infant formula.
- SUCRAID can only be dissolved with cold or room temperature water, milk, or infant formula. **Do not** put SUCRAID in warm or hot liquids. **Do not** dissolve SUCRAID with fruit juice. **Do not** take or give SUCRAID with fruit juice.
- **Do not** warm or heat the mixed solution before taking or giving SUCRAID.
- Measure your dose or your child's dose of SUCRAID using the measuring scoop that comes with the SUCRAID bottle. **Do not** use a kitchen teaspoon or other measuring device.
- SUCRAID should be taken or given with each meal or snack. Half of the SUCRAID dose should be taken or given at the beginning of each meal or snack.Take or give the remaining SUCRAID dose during the meal or snack.
- **Do not** use the SUCRAID multiple-dose bottle if the seal has been damaged.Contact your pharmacist or healthcare provider if you cannot use the SUCRAID multiple-dose bottle.

Supplies needed to take or give SUCRAID:

- SUCRAID 118 mL multiple-dose bottle
- 1 measuring scoop (included in SUCRAID carton)
- 2 to 4 ounces of cold or room temperature water, milk, or infant formula (not included)
- Meal or snack (not included)

How to take or give SUCRAID:

Step 1:Check the expiration date on the SUCRAID bottle. **Do not** use SUCRAID after the expiration date on the bottle has passed.

Step 2: Write down the date the bottle is first opened in the space provided on the bottle label.

Step 3: Each bottle of SUCRAID has a plastic screw cap that covers a dropper dispensing tip. Remove the plastic screw cap by twisting it to the left.

Step 4: Use the measuring scoop that comes in your SUCRAID carton to measure your or your child's prescribed dose. See **Figure 1**. Reseal the bottle after each use by replacing and twisting the plastic screw cap to the right until tight.

Figure 1

Step 5: Mix your or your child's prescribed dose in 2 ounces or 4 ounces of cold or room temperature water, milk, or infant formula as instructed by your healthcare provider. **See Figure 2.**

Figure 2

Step 6: Take or give half of the mixed solution at the beginning of each meal or snack. Take or give the remaining mixed solution during the meal or snack.

Step 7: Rinse the measuring scoop with water after each use.

Throwing away (disposal of) SUCRAID:

- Throw away (discard) the SUCRAID multiple-dose bottle and any remaining medicine in your household trash 4 weeks after first opening.

How should I store SUCRAID?

- Store the SUCRAID multiple-dose bottle in the refrigerator between 36°F to 46°F (2°C to 8°C).
- Protect SUCRAID from heat and light.

Keep SUCRAID and all medicines out of the reach of children.

Manufactured by:
QOL Medical, LLC Vero Beach, FL 32963
U.S. License No. 2195

For more information, go to www.sucraid.com or call 1-866-469-3773.

This Instructions for Use has been approved by the U.S. Food and Drug Administration.
Issued: May 2022

Instructions for Use

Sucraid® (Su-kreid) (sacrosidase) Oral Solution:
2-mL Single-Use Container

Read this Instructions for Use before you start taking or giving Sucraid to a child, and each time you get a refill. There may be new information. This information does not take the place of talking to your healthcare provider about your or your child's medical condition or treatment.

Important information you need to know before taking or giving Sucraid:

- The 2-mL single-use container is for children and adults.
- Sucraid is supplied in 2-mL single-use containers in a foil pouch. Each foil pouch holds 5 single-use containers. **Each container is one 2 mL Sucraid dose.**
- Your healthcare provider will decide the right dose of Sucraid for you or your child. **Do not** change the dose of Sucraid without talking to your healthcare provider.
- Sucraid can only be dissolved with cold or room temperature water, milk, or infant formula. **Do not** put Sucraid in warm or hot liquids. **Do not** dissolve Sucraid with fruit juice. **Do not** give or take Sucraid with fruit juice.
- **Do not** warm or heat the mixed solution before taking or giving Sucraid.
- Sucraid should be taken or given with each meal or snack. Half of the Sucraid dose should be taken at the beginning of each meal or snack. Take or give the remaining Sucraid dose during the meal or snack.
- **Do not** use the Sucraid single-use container if the seal has been damaged. Contact your pharmacist or healthcare provider if you cannot use the Sucraid single-use container.

Supplies needed to take or give Sucraid:

- 1 Sucraid 2-mL container
- 4 ounces of cold or room temperature water, milk, or infant formula (not included)
- Meal or snack (not included)
- Spoon to mix (not included)

How to take or give Sucraid:

Step 1: Check the expiration date on the Sucraid foil pouch. **Do not** use Sucraid if it is past the expiration date. Remove 1 Sucraid 2-mL container from a foil pouch.

Step 2: Twist the cap to the left to remove it from the container. **See Figure 1.**

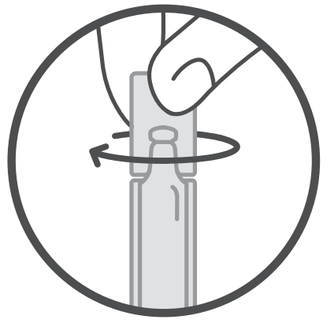

Figure 1

Step 3: Squeeze all the Sucraid solution in the container into 4 ounces of cold or room temperature water, milk, or infant formula. **See Figure 2.**

Figure 2

Step 4: Mix your or your child's prescribed dose in 4 ounces of cold or room temperature water, milk, or infant formula. **See Figure 3.**

Figure 3

Step 5: For patients weighing more than 33 pounds (15 kilograms):
- The entire 4 ounces of mixed solution will be taken or given during each meal or snack. Take or give half of the mixed solution (2 ounces) at the beginning of the meal or snack and take or give the other half of the mixed solution (2 ounces) during the meal or snack.

For patients weighing 33 pounds (15 kilograms) or less:
- Divide the 4-ounce mixed solution into two separate 2-ounce portions.
- Take or give half of the first portion (1 ounce) at the beginning of the meal or snack and take or give the other half of the first portion (1 ounce) during the meal or snack.
- Store the second portion (2 ounces) in the refrigerator at 36°F to 46°F (2°C to 8°C) for the next meal or snack. Take or give half of the second portion (1 ounce) at the beginning of the next meal or snack and take or give the other half of the second portion (1 ounce) during the meal or snack.
- Throw away the second portion (2 ounces) if you do not use it within 24 hours.

Throwing away (disposal of) Sucraid:

- Throw away expired or empty Sucraid containers in your household trash.

How should I store Sucraid?

- Store the Sucraid single-use container in the refrigerator between 36°F to 46°F (2°C to 8°C).
- The Sucraid single-use container may be stored between 59°F to 77°F (15°C to 25°C) for up to 3 days.
- Protect Sucraid from heat and light.

Keep Sucraid and all medicines out of the reach of children.

Manufactured by:
QOL Medical, LLC Vero Beach, FL 32963
U.S. License No. 2195

For more information, go to www.Sucraid.com or call 1-866-469-3773.

This Instructions for Use has been approved by the U.S. Food and Drug Administration.
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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

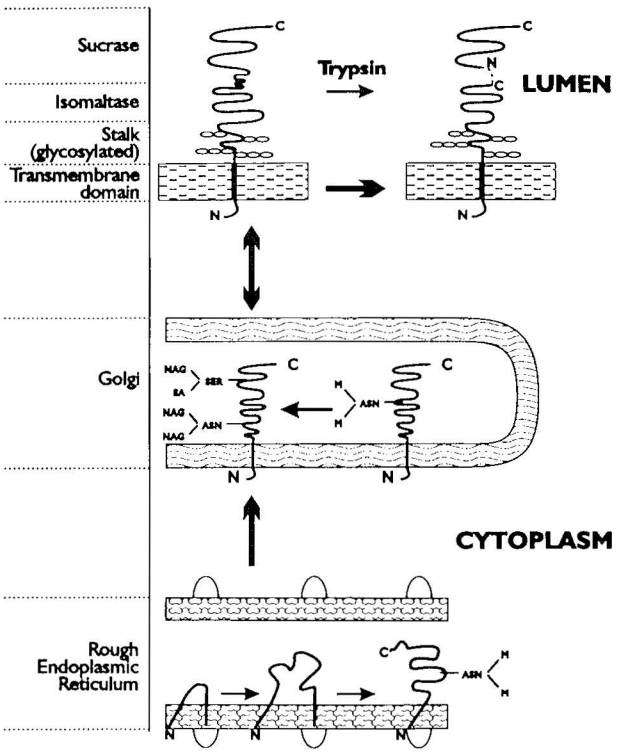

FIG. 1. Cotransitional modification and posttranslational processing of sucrase isomaltase (SI) in the enterocyte organelles and intestinal lumen. SI is synthesized as a long polypeptide chain carrying two similar but not identical active sites (pro–sucrase-isomaltase). The pro-SI is inserted into the 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 pro-

ceeds 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 con-

sumed 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 hydro-

gen 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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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) α-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 reticulum 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 completely 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

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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 α-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 symptoms.

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% and 13% 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 specifically 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), including 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 predilection 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)
Failure to thrive	39 (60)
Nausea/vomiting	22 (34)
Irritable bowel syndrome	12 (18)

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

	Sucrase (n = 36)	Isomaltase (palatinase) (n = 35)	Maltase (n = 36)	Lactase (n = 36)
Mean	2.3	1.9	41.5	30.5
Standard deviation	4.4	5.8	34.7	19.2
Median	0	0	29.2	27.6
Minimum	0	0	10.9	5.2
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 include the sucrose breath hydrogen study and differential urinary disaccharides (36,37). Although relatively easy to accomplish, the sucrose 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 of the 2-g/kg oral sucrose load given to patients with 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 and young 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% $\pm$ 21% compared with 146% $\pm$ 45% in 10 age-matched controls. A cutoff of 79% CGO yields 100% sensitivity and specificity for CSID. Although the test 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 β-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-hydrolyzing activity (8500 μmol glucose formed per minute per milliliter) (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

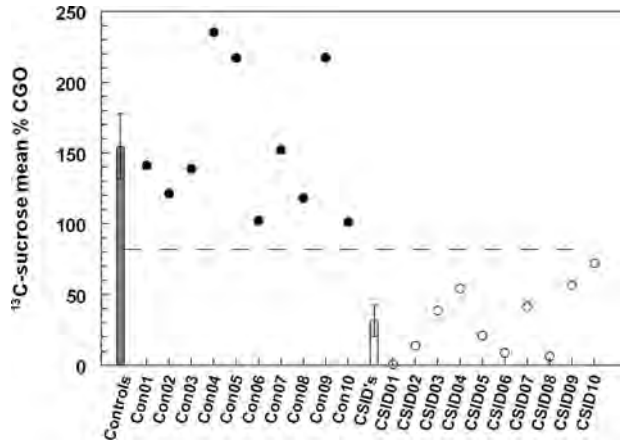

FIGURE 1. Data summary from studies of the utility of a ^{13}C-sucrose breath test to diagnose CSID.

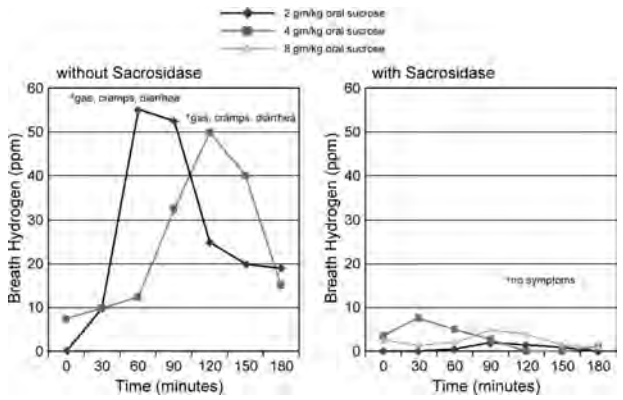

FIGURE 2. Results of sucrose breath hydrogen studies on the first child with CSID treated with sacrosidase under an orphan drug grant from the Food and Drug Administration.

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 $1.75–2.5\,g \cdot kg^{-1}\ day^{-1}$) and starch-containing ($5.2–5.8\,g \cdot kg^{-1}\ day^{-1}$) 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

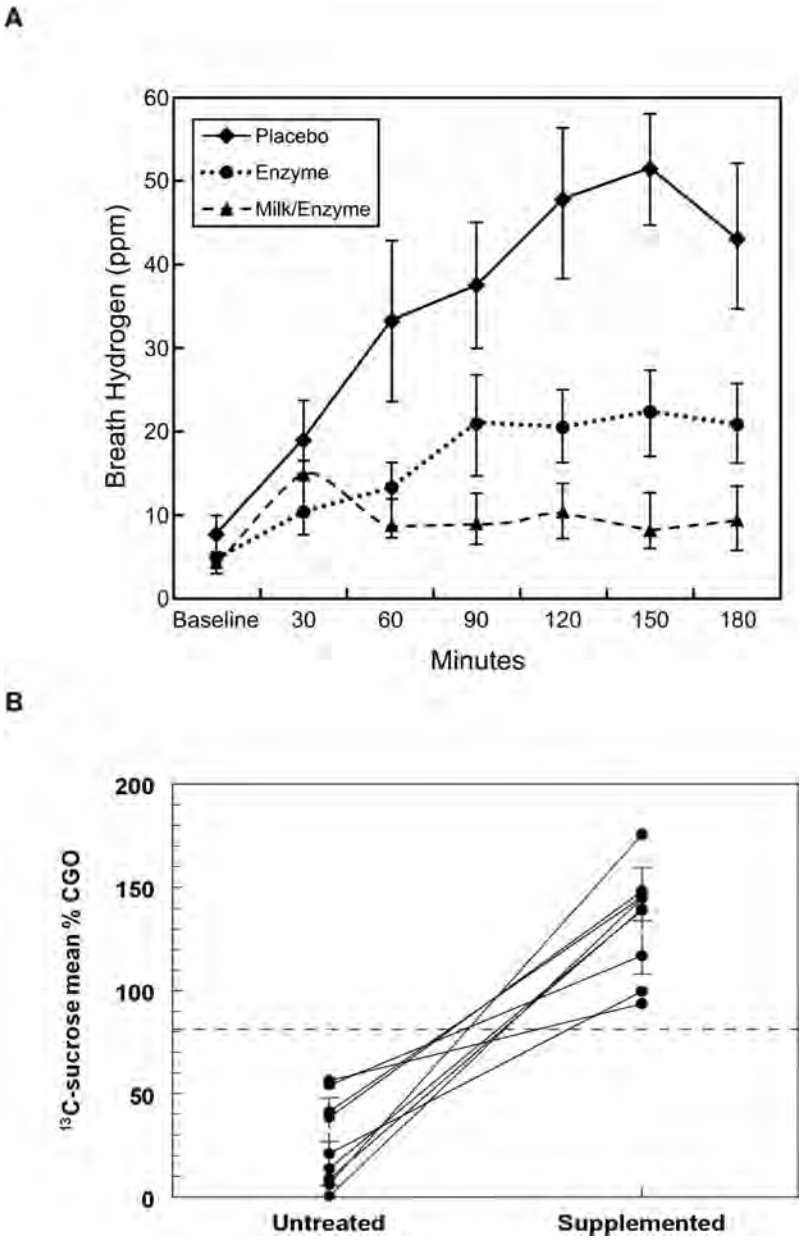

FIGURE 3. A, Excessive breath hydrogen production blocked by the double-blind administration of sacrosidase compared with placebo and was further reduced by consuming milk before sucrose ingestion. B, A study of the ^{13}C-sucrose breath test with and without sacrosidase administration confirmed these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

TABLE 3. Persistence of symptoms in 65 patients with 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 postmarketing 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 were not 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 α-glutamyl transpeptidase, are expressed in many tissues, including the intestine and the kidney (3,4). The

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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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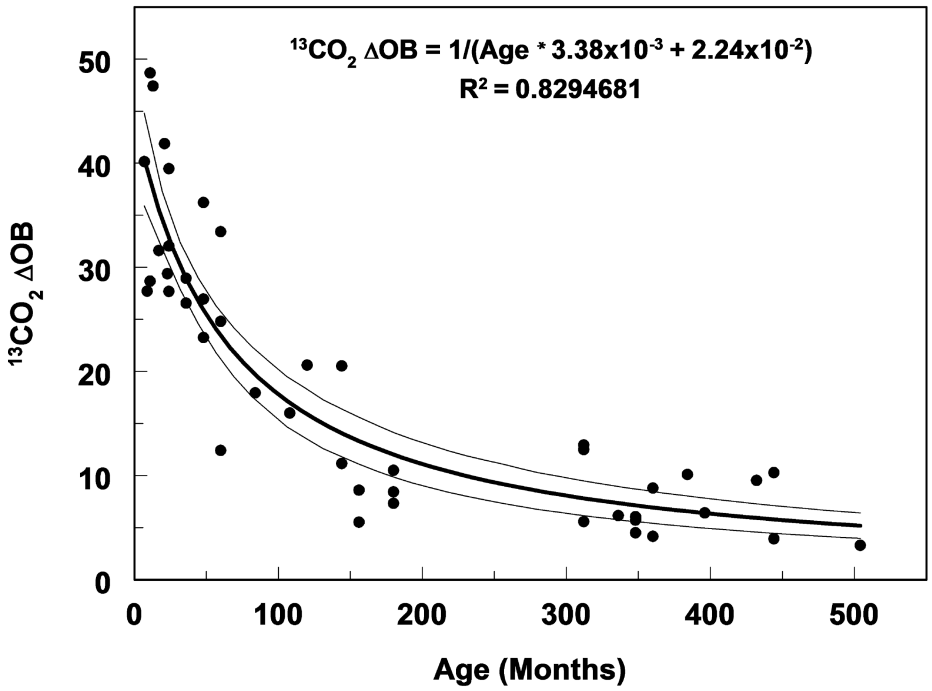

Figure 1. Effects of age on oral glucose breath test CO_2 enrichment

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.

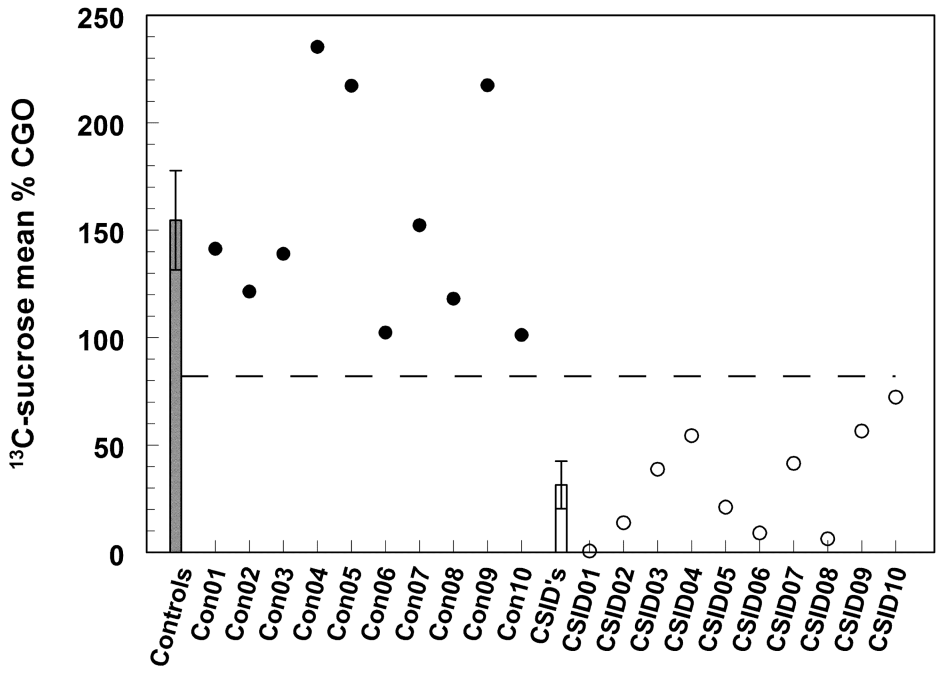

Figure 2. Effects of CSID on oral sucrose breath test mean % CGO

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).

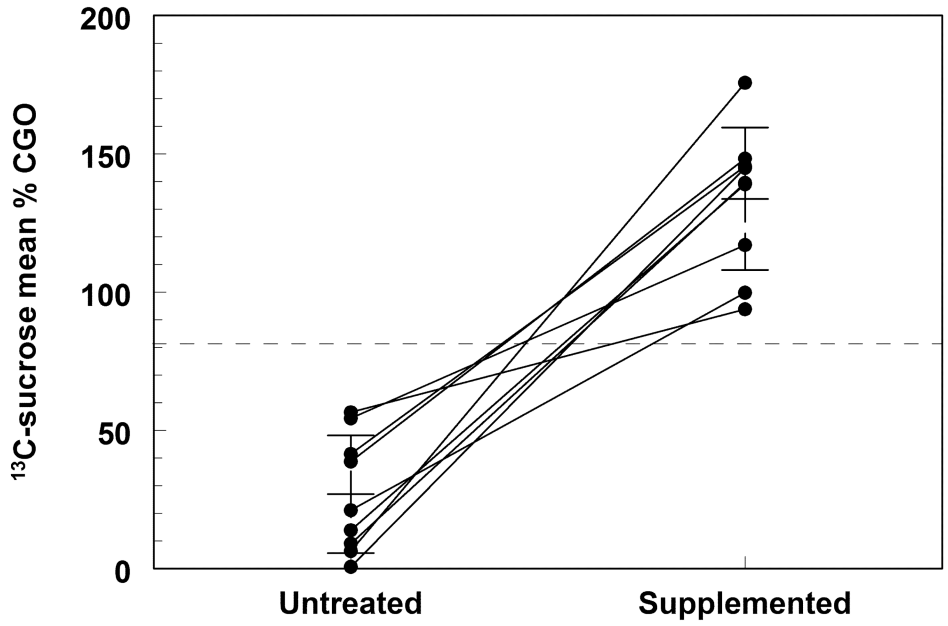

Figure 3. Effects of oral sacrosidase supplementation of CSID patients on sucrose breath test mean % CGO

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

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

Longitudinal Study of the Human Intestinal Brush Border Membrane Proteins

Distribution of the Main Disaccharidases and Peptidases

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The longitudinal distribution of the main brush border membrane hydrolases was studied in six entire human small intestines, one of which was found to be lactase-deficient. Sucrase and lactase activities were found to be highest in the jejunum, whereas glucoamylase activity rose steadily and reached its highest activity near the ileocecal valve. Maltase activity distribution was intermediate between that of sucrase and of glucoamylase. Neutral aminopeptidase, acid aminopeptidase and dipeptidyl peptidase IV activities tended to increase toward the end of the small bowel, the latter two activities rising more than the first one. Furthermore, the protein compositions of the brush border membrane in the jejunum and in the ileum were compared after electrophoresis on polyacrylamide gels and crossed-immunoelectrophoresis; protein patterns were found to be similar along the gut, and enzyme-specific activities varied in parallel with the amounts of their corresponding proteins. In the lactase-deficient intestine, the protein band corresponding to lactase was not visible. Maximal digestive capacity was thus localized in the jejunum only for disaccharides, and in the ileum for the more complex substrates, oligosaccharides, and peptides; this finding suggests that the ileum may play a greater role in their terminal digestion than is usually admitted.

Terminal digestion of proteins and carbohydrates and subsequent absorption of their hydrolysis products occur at the enterocyte–luminal border by means of numerous peptide and saccharide hydrolases and transport systems. Our knowledge concerning their distribution along the gut remains surprisingly fragmentary in humans, taking into account the important pathophysiologic implications of this localization. Among the disaccharidases, maltase, sucrase (EC 3.2.1.48), and lactase (EC 3.2.1.23) activities have been repeatedly measured and found to be higher in the proximal jejunum (1–4). However, serial longitudinal studies have not always reached the terminal ileum, where activities have often been obtained at the time of ileostomy or by retrograde biopsy through the ileostomy (2–4). Furthermore, glucoamylase (EC 3.2.1.20) activity distribution has never been studied and has only been inferred indirectly from maltase levels. On the other hand, the distribution of the more recently known brush border peptidases is poorly documented; their activities appear to be similar or higher in the ileum than in the jejunum, at least for neutral and acid aminopeptidases (NAP, EC 3.4.11.2 and AAP, EC 3.4.11.7), dipeptidyl peptidase IV (DPP IV, EC 3.3.14.x), and a carboxypeptidase (EC 3.4.12.x) (4,5).

Data regarding the human brush border membrane protein composition are even fewer. Jejunal and ileal microvillus membrane proteins have been compared only twice, after sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (6) and more recently after crossed-immunoelectrophoresis (4). The patterns were considered to be qualitatively different in the first study, yet were found to be very similar in the second one.

Finally, our knowledge of the distribution of these proteins, as our knowledge of the main brush border hydrolase activities, derives from very heteroge-

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Table 1. Data Concerning the Patients

Patient	Sex	Age (yr)	Blood group	Cause of death	Duration of coma (days)	Length of intestine (cm)
K	F	5	O Rh−	Intracranial hypertension	5	228
L	M	6	O Rh+	Polytraumatism	1	347
B	M	22	A Rh+	Suicide	1	376
D	F	17	A Rh+	Polytraumatism	1	280
H	F	62	A Rh+	Intracranial hemorrhage	21	289
S	F	31	O Rh−	Polytraumatism	3	315

− = negative Rh factor. + = positive Rh factor.

neous material: biopsy specimens, surgical or autopsy samples, serial studies, or pooled measures obtained at a given site but from different patients (2–4). It thus seemed worthwhile to undertake again the study of enzyme activities and brush border membrane protein patterns along entire small intestines in order to obtain a more complete and accurate idea of the distributions of most of the physiologically relevant hydrolases and a better comparative description of the jejunum and ileum in humans.

Material and Methods

Small Intestines

Six entire small intestines from organ donors were obtained within 15 min after death, with the agreement of the France Transplant Association. Relevant data concerning the patients are presented in Table 1. Intestines were quickly washed with cold saline and stored at −80°C until use. After thawing, 5-cm-long samples were removed at 50-cm intervals along the bowel and were used for the biochemical analysis reported here. In the case of patient S, the small intestine was cut into four equal pieces and assays were performed on each piece. Because intestines varied in length (Table 1), distances along the gut were expressed as percentages of the distance between the angle of Treitz and the ileocecal valve (taken as 100%). When comparing proximal jejunal to distal ileal activities, two measures at both ends were pooled: for the jejunum, the samples removed nearest to 0% and 25% of total gut length were chosen; for the ileum, those nearest to 75% and 100% were chosen. For the intestine of patient S, only one measure at each site was available.

Enzymatic Assays and Protein Determination

Segments used in this study were opened along their entire length; their mucosa was scraped and homogenized in ice-cold 0.05 M mannitol–2 mM Tris, pH 7.4. All of the following operations were performed at 4°C. Disaccharidases were assayed according to Dahlqvist (7), and glucoamylase was assayed by the method of Schlegel-Haueter et al. (8) using soluble starch (Merck Darmstadt, FRG) as substrate. Peptidase activities were measured according to Andria et al. (9) with 0.2 mM leucine β-naphthylamide (Sigma Chemical Company, St. Louis, Mo.), α-glutamic β-naphthylamide (Bachem, Liestal, Switzerland), and glycyl-L-prolyl β-naphthylamide (Bachem) as substrates for neutral aminopeptidase (NAP), acid aminopeptidase (AAP), and dipeptidyl peptidase IV (DPP), respectively. Incubations were performed in 0.05 M potassium phosphate, pH 7.2, (NAP); 0.05 M Tris-HCl–10 mM $CaCl_2$, pH 8, (AAP); or 0.05 M Tris-HCl, pH 8, (DPP) for 30 min at 37°C, and the amount of β-naphthylamide liberated was measured by diazotation (10). All specific activities were expressed as micromoles per minute per gram protein (IU/g protein). Proteins were estimated according to the method of Lowry et al. (11) with crystalline bovine serum albumin as a standard.

Polyacrylamide Gel Electrophoresis

Brush border membranes were obtained as previously described (12), the final ultracentrifugation usually being omitted. Proteins were then solubilized with 2% SDS and separated by electrophoresis, either on 11% polyacrylamide gels using a multiphasic buffer system (6), or on 5%–15% gradient polyacrylamide gels using the buffer system of Laemmli (13). In the latter case, samples were boiled for 3 min in the presence of 25 mM dithiotreitol before running. Gels were stained with Coomassie Blue or with the Schiff reagent.

Crossed-Immunoelectrophoresis

Brush border proteins were solubilized with papain (0.3 U/mg membrane protein in 0.01 M potassium phosphate, pH 6.8, 0.005 M cystein–HCl, 0.003 M EDTA) for 60 min at 37°C, and recovered in the supernatant after a 1-h centrifugation at 35,000 g. The percentages of solubilized activity were similar for all tested enzymes in the jejunum as well as in the ileum and were never lower than 85%. Crossed-immunoelectrophoresis was performed as described by Weeke (14) and adapted to brush border proteins (15). The antihuman brush border antiserum was obtained in New Zealand rabbits injected subcutaneously with three 1-mg doses of antigen at 10-day intervals and bled 7 days after the last injection. The brush border antigen used was the papain supernatant fraction of the brush border membrane, enriched 12 to 20 times in the main hydrolase activities when compared with the homogenate. The immunoglobulin G (IgG) fraction was isolated

by ammonium sulfate fractionation and ion exchange chromatography (16). On histological sections of human intestinal mucosa, the peroxydase-labeled IgG fraction stained conspicuously only the enterocyte brush border regions, indicating a high brush border specificity. Glass plates were layered with 1% agarose (Bio-Rad Laboratories, Richmond, Calif.) in 0.037 M Tris–0.1 M glycine buffer, pH 8.7. Electrophoresis was run for 1.5 h at 200 V in the first dimension and at 55 V overnight in the second dimension. Parallel gels were either stained for protein with Coomassie Blue, or specially stained for enzyme activities. For peptidase activities, each gel was covered with 15 ml of the respective buffer containing 10 mg of the appropriate substrate and with 20 mg Fast Garnett GBC salt (Sigma); enzymes appeared as red bands. For disaccharidase activities, the following reaction mixture was used: 15 ml of 0.05 M Na^+ phosphate, pH 6.0, with 100 mg of their respective substrates, 15 mg MTT tetrazolium bromide (Sigma), 15 mg phenazine methosulfate (Sigma), and 5 mg glucose oxidase (Sigma); enzymes appeared as black bands.

Results

Enzyme Activities

Levels of enzyme activities were similar from 1 patient to another except for patient H, whose activities were consistently higher. On the contrary, 1 patient, B, appeared to be lactase-deficient, lactase activities never being higher than 7 IU/g protein.

Lactase and sucrase activities increased from pylorus to jejunum, reaching a maximum at 25% of gut length, decreased from 50% to 70% of gut length, and then remained stable. Glucoamylase, on the contrary, increased regularly from the pylorus to the ileocecal valve. Maltase activity distribution reflected its dual origin, with only a discrete decrease in the ileum (Figure 1). Sucrase and lactase activities were 1.6 and 2.1 times higher, respectively, in the jejunum (proximal 25% of gut length) than in the ileum (distal 25%); maltase activity was rather constant [jejunal to ileal ratio (J/I) = 1.1], whereas glucoamylase activity was higher in the ileum (J/I = 0.6) (Table 2).

In general, peptidase activities increased from the duodenum to the terminal ileum (Figure 2). NAP activity rose only slightly, however, (J/I = 0.8), whereas AAP and DPP IV increased steeply, their activities in the ileum being two times higher than in the jejunum (J/I = 0.4 and 0.5, respectively) (Table 2).

Patterns of Brush Border Membrane Proteins

Patterns of SDS-solubilized brush border membrane proteins from the jejunum and the ileum, separated as indicated in Methods, were compared

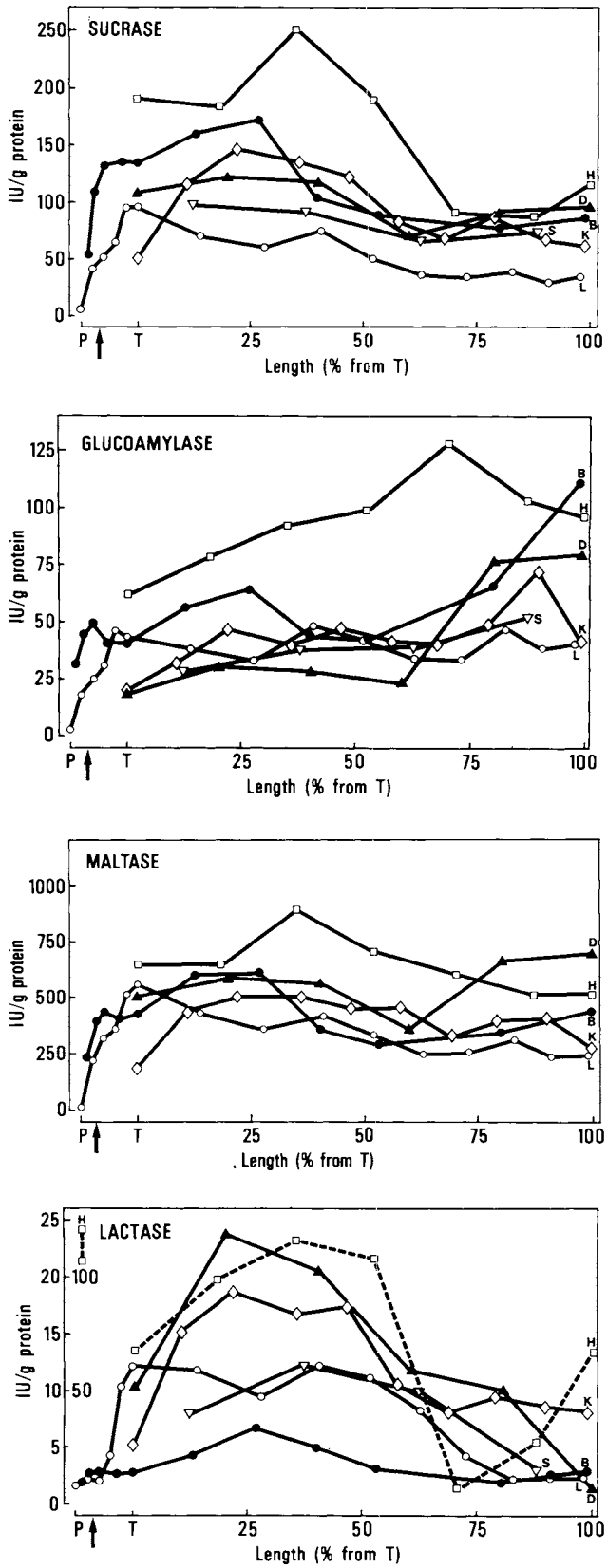

Figure 1. Distribution of sucrase, glucoamylase, maltase, and lactase activities along six entire human small intestines. Subject B was lactase-deficient. *Arrow* indicates biliopancreatic duct orifice. *P* and *T* stand for pylorus and Treitz.

Table 2. Disaccharidase and Peptidase Specific Activities (in IU/g Protein) in Homogenates of Jejunum and Ileum

	Sucrase-isomaltase	Glucoamylase	Maltase	Lactase	Neutral aminopeptidase	Acid aminopeptidase	Dipeptidyl peptidase IV
Jejunum[a]	122 ± 17[b]	40 ± 7	505 ± 49	28 ± 12	72 ± 14	16 ± 5	16 ± 4
Ileum[a]	76 ± 9	69 ± 9	440 ± 71	13 ± 7	96 ± 19	45 ± 19	34 ± 10
Jejunum/ileum	1.60	0.58	1.15	2.15	0.75	0.35	0.47

[a] Defined in Material and Methods. [b] Mean ± SEM of one measurement for intestine S and averages of pairs of measurements for the other intestines. Activities are significantly different in ileum as compared with jejunum for sucrase-isomaltase ($p < 0.01$), glucoamylase ($p < 0.02$), and lactase ($p < 0.05$).

on slab gels. On 11% polyacrylamide gels, bands corresponding to glucoamylase (G), lactase (L), and the sucrase-isomaltase complex (SI) have already been characterized (17,18). On 5%–15% gradient polyacrylamide gels, bands corresponding to G and to SI and its subunits (S) were identified by comparison with the purified enzymes (19). Four other bands were tentatively identified (Figure 3). The lactase band was recognized because it was lacking in patient B who was lactase-deficient. Dipeptidyl peptidase IV, acid aminopeptidase, and neutral aminopeptidase were identified by comparison with the migration patterns of partially purified enzymes (20). With both types of gels, jejunal and ileal patterns were very similar. The intensities of the main hydrolases were slightly different, the sucrase-isomaltase band being more intense in the jejunum, whereas glucoamylase, DPP IV, and acid aminopeptidase bands were more prominent in the ileum. Furthermore, two additional bands were visible in the ileum as compared with the jejunum: one, between the glucoamylase and lactase bands, was stained strongly with the Schiff reagent; the other was found among the low-molecular-weight bands (Figure 3).

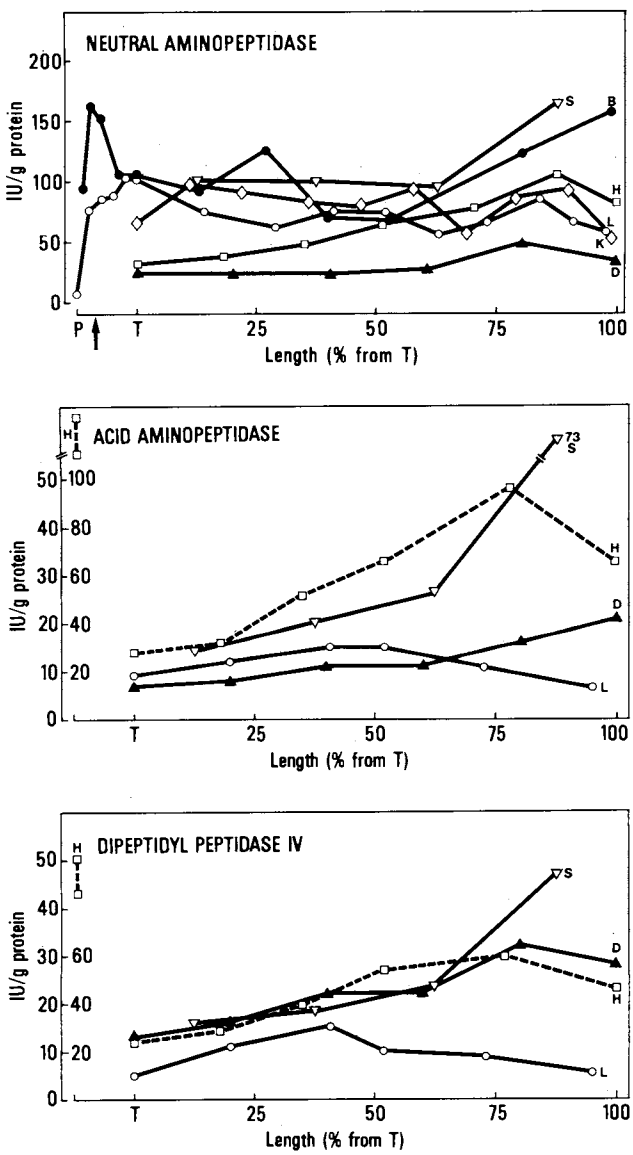

Figure 2. Distribution of neutral aminopeptidase, acid aminopeptidase, and dipeptidyl peptidase IV activities along entire human small intestines; the first activity was measured in six intestines, the other two activities in four intestines. *P* and *T* stand for pylorus and Treitz.

Crossed-immunoelectrophoresis of papain-solubilized brush border membrane proteins allowed the visualization of 8–10 peaks; 6 of these peaks were characterized as hydrolases that were still active after their separation, namely sucrase-isomaltase, glucoamylase, lactase, neutral and acid aminopeptidases, and DPP IV (Figure 4). The results obtained were in agreement with those obtained by polyacrylamide gel electrophoresis. The areas under the peaks of sucrase and lactase were greater in the jejunum than in the ileum, whereas the opposite was true for glucoamylase. In the case of patient B, a small peak of residual lactase, migrating at its usual place, was seen; it had both lactase and phlorizin hydrolase activities. Neutral aminopeptidase and, to a greater extent, AAP and DPP IV peaks were greater in the ileum than in the jejunum. It also appeared that DPP IV had a lower electrophoretic mobility in the ileum than in the jejunum.

Discussion

Our study confirms and extends previous knowledge of the distribution of the main brush border membrane hydrolases along the human small

intestine. The distribution of sucrase, maltase, and lactase activities is in accordance with earlier studies (1–4) that showed higher activities of these enzymes in the proximal intestine; in addition, these activities do not decrease until the ileocecal valve, as might have been inferred from the studies in which distal measures were not done (2,3), but instead retain a stable activity over the distal ileum. Such a plateau has been described in the case of sucrase (1,4). Our study represents the first determination of glucoamylase activity, which was found to increase gradually from the jejunum to the terminal ileum. This finding corroborates the recent immunoelectrophoretic demonstration that the amount of maltase (glucoamylase) is greater in the ileum than in the jejunum (4) and explains why maltase activity, shared by sucrase-isomaltase and glucoamylase, has an intermediate distribution. The results obtained for the three peptidase activities are in agreement with the only available study on peptidase distribution, which shows that these activities increase along the small intestine parallel with the amounts of their respective immunoreactive proteins (4). As reported in the latter work, and in another short report (5), AAP and DPP IV jejunoileal gradients of activity were steeper than the gradient of NAP. The longitudinal distributions of AAP, DDP IV, and glucoamylase are thus very similar.

On both polyacrylamide gel electrophoresis after SDS solubilization (6) and crossed-immunoelectrophoresis after papain solubilization (4), the most abundant brush border membrane (glyco)proteins have been found to have a high molecular weight (6), and to correspond to the main hydrolases (15,17). With both techniques we found that the distribution of the main brush border membrane (glyco)proteins along the human small intestine parallels the distribution of the corresponding enzyme activities. This appears more clearly on crossed-immunoelectrophoresis than on polyacrylamide gels, because the former method allows a much better quantification of the solubilized proteins (15). With this technique, our results are in accordance with those of Skovbjerg (4). Conversely, the results that we obtained on polyacrylamide gels are at variance with those of the only comparable published study in which differences between jejunum and ileum were emphasized (6). However, in the latter case, compared pieces of intestine originated from different patients, which may have lead to artifactual differences.

The general picture of the digestive function of the small intestine that emerges from the present work is different from that drawn from most earlier studies based on sucrase, maltase, and lactase activity distribution (2,3) and on in vivo perfusion studies (21), which tended to emphasize the role of the proximal

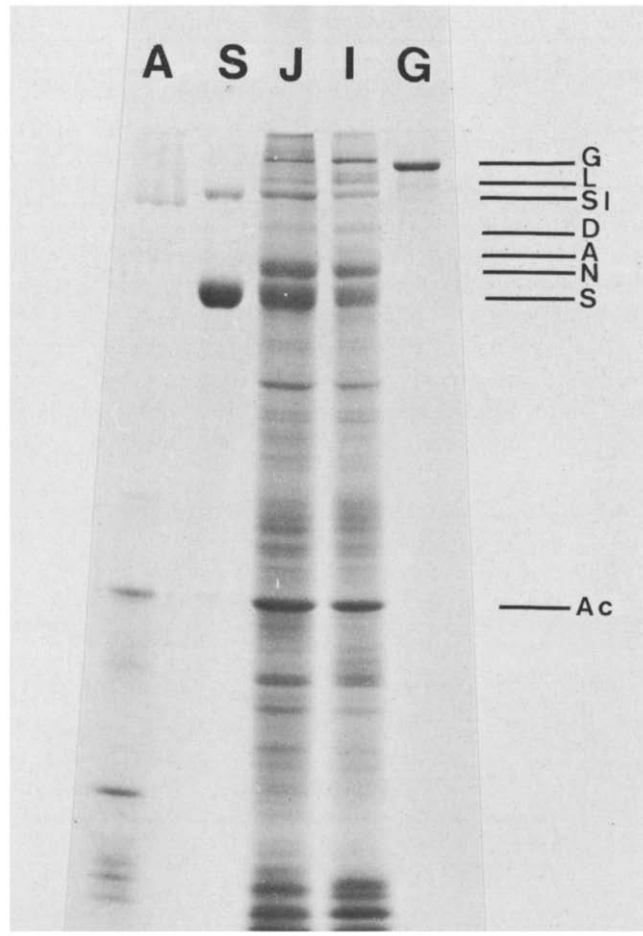

Figure 3. Sodium dodecyl sulfate solubilized microvillus membrane proteins separated after electrophoresis on a 5%–15% gradient polyacrylamide slab gel. From left to right, lanes correspond to actomyosin (*A*), pure sucrase-isomaltase (*SI*), a jejunal sample (*J*), an ileal sample (*I*), and pure glucoamylase (*G*). Bands corresponding to glucoamylase (*G*), lactase (*L*), the sucrase-isomaltase complex (SI) and its subunits (S), dipeptidyl peptidase IV (*D*), acid aminopeptidase (*A*), neutral aminopeptidase (*N*), and actin (*Ac*) are indicated.

intestine. In fact, higher hydrolytic activities are located in the jejunum only for natural disaccharides, activities for oligosaccharides and peptides being higher in the ileum. The distal small intestine might thus play a significant role in the terminal digestion of these more complex substrates. However, presence of these substrates in significant amounts in the ileum is probably not directly responsible for the high ileal activities splitting these substrates since neither disaccharidase-specific nor peptidase-specific activities are significantly increased in the ileum after a major proximal intestinal resection (22,23) or after a jejunoileal bypass (24). The same studies also rule out a significant role for the pancreatic-biliary flow in the maintenance of these gradients, even though pancreatic proteases

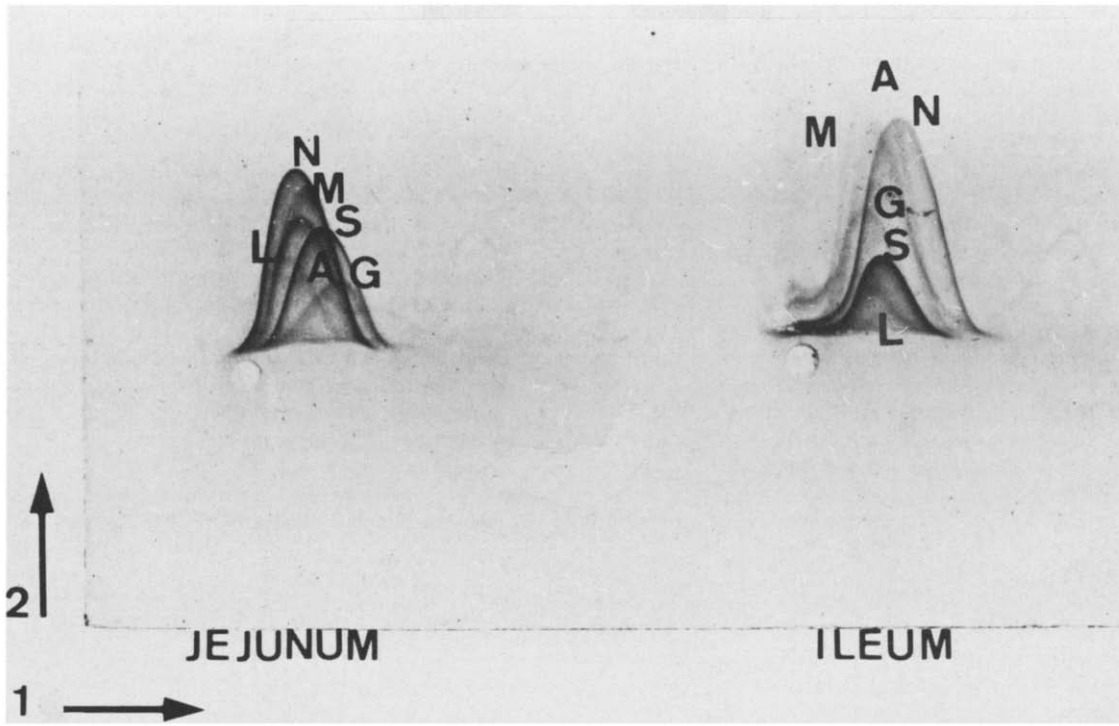

Figure 4. Papain-solubilized microvillus membrane proteins separated by crossed-immunoelectrophoresis. Patterns are from patient D at 20% and 80% of gut length. Sucrase (*S*), glucoamylase (*M*), lactase (*L*), neutral aminopeptidase (*N*), acid aminopeptidase (*A*), and dipeptidyl peptidase IV (*G*) peaks are clearly visible. Ratios of the areas under the peaks in jejunum as compared with ileum for *S* (J/I = 1.7), *M* (0.7), *L* (2.6), *N* (0.7), *A* (0.5), and *G* (0.5) are in agreement with those that can be calculated from the different activities at the same sites.

are known to play a role in the final processing of microvillus enzymes (25). It is more likely that their longitudinal distribution is the consequence of a long-term phylogenic adaptation. Evidence favoring this proposition comes from experiments in the rat in which lactase and sucrase distributions were shown to be genetically programmed (26), and from measures done in human fetuses where the same gradients of activity already exist (27).

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Luminal Substrate ''Brake'' on Mucosal Maltase-glucoamylase Activity Regulates Total Rate of Starch Digestion to Glucose

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ABSTRACT

Background: Starches are the major source of dietary glucose in weaned children and adults. However, small intestine α-glucogenesis by starch digestion is poorly understood due to substrate structural and chemical complexity, as well as the multiplicity of participating enzymes. Our objective was dissection of luminal and mucosal α-glucosidase activities participating in digestion of the soluble starch product maltodextrin (MDx).

Patients and Methods: Immunoprecipitated assays were performed on biopsy specimens and isolated enterocytes with MDx substrate.

Results: Mucosal sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM) contributed 85% of total in vitro α-glucogenesis. Recombinant human pancreatic α-amylase alone contributed <15% of in vitro α-glucogenesis; however, α-amylase strongly amplified the mucosal α-glucogenic activities by preprocessing of starch to short glucose oligomer substrates. At low glucose oligomer concentrations, MGAM was 10 times more active than SI, but at higher concentrations it experienced substrate inhibition whereas SI was not affected. The in vitro results indicated that MGAM activity is inhibited by α-amylase digested starch product ''brake'' and contributes only 20% of mucosal α-glucogenic activity. SI contributes most of the α-glucogenic activity at higher oligomer substrate concentrations.

Conclusions: MGAM primes and SI activity sustains and constrains prandial α-glucogenesis from starch oligomers at approximately 5% of the uninhibited rate. This coupled mucosal mechanism may contribute to highly efficient glucogenesis from low-starch diets and play a role in meeting the high requirement for glucose during children's brain maturation. The brake could play a constraining role on rates of glucose production from higher-starch diets consumed by an older population at risk for degenerative metabolic disorders. ***JPGN 45:32–43, 2007.*** **Key Words: α-Amylase—α-Limit dextrin—Maltodextrin—Maltase-glucoamylase—Sucrase-isomaltase.** © 2007 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

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INTRODUCTION

Carbohydrates play a major role in the human energy equation. Digestion to monosaccharides is a requirement for small intestinal food carbohydrate assimilation and utilization. Here we examine the mucosal mechanisms that digest starches into absorbable glucose. Digestible plant carbohydrates exist in 2 vastly contrasting glucoside forms: sucrose and starches. Sucrose is the energy

transport molecule in most plants; it is freely soluble and is digested to glucose and fructose by a small intestinal disaccharidase enzyme complex named sucrase-isomaltase (SI). Sucrose monosaccharide products are rapidly assimilated and metabolized. Whereas sucrose contains a single glucose, starches often contain >100,000 glucose units. Starch consists of complex glucose polymers generally found in semicrystalline storage structures of reproductive plant tissues. These characteristics mean that starch must undergo extensive modification before assimilation in the gut.

In humans, the digestion of starches to glucose requires a consortium of 6 known enzyme activities. The luminal phase of starch digestion uses the related salivary and pancreatic α-amylases whose α-1,4 endoglucosidase activities result in production of soluble glucose oligomers, but little free glucose (1). Exhaustive α-amylase digestion produces a reproducible pattern of glucose oligomers called α-limit dextrin (LDx) consisting primarily of maltose (G2) and maltotriose (G3), as well as related α-1,6 branched products. The final mucosal phase of starch digestion is required for a substantial production of free glucose from LDx. Four small intestinal mucosal α-1,4 exoglucosidases active at the nonreducing ends of glucose oligomers, usually assayed as maltases, were identified by early investigators (2). Two "maltase" activities were associated with the sucrase and isomaltase activities of SI described previously. Two other "maltase" activities, not associated with any other identifiable activities, were called maltase-glucoamylase (MGAM) (3). Subsequent investigations (4–7) revealed that these 4 maltases share α-glucogenic activities for all α-1,4 glucoside substrates from G2 to G7 in length. Thus, the activities are better described as α-glucosidases than maltases. As a consequence of the shared series of substrates, there is no single substrate that can be used to identify MGAM. In contrast, sucrose, isomaltose, and palatinose are specific substrates for SI. The sucrase (S) subunit of SI is the only endogenous human intestinal enzyme displaying specific activity against the α-1,2 glucosidic link of sucrose; in addition the isomaltase (I) subunit hydrolyzes the α-1,6 D-glucosidic branching linkages of starch oligosaccharides, as well as isomaltose and palatinose. The last disaccharide is commonly used as substrate to assay isomaltase subunit activity. The multiplicity of maltase activities led Dahlqvist (8) to predict that "maltose intolerance cannot occur unless 4 or 5 enzymes are absent simultaneously, and will be combined with intolerance to sucrose and isomaltose."

Early investigations suggested that 60% of in vitro mucosal maltase activity was contributed by SI with the remainder by MGAM (2,3,9–13). Both SI and MGAM belong to the glucohydrolase family 31 and their respective amino acid sequences show an overall homology of 59%. There is complete conservation of the catalytic residues within all 4 subunits of the 2 enzymes. These enzymes are anchored to the luminal surface of the apical enterocyte membrane by hydrophobic N-terminal binding domains and extend away from this surface through long O-glycosylated stalks (14,15).

There has been recent progress in understanding the beneficial and degenerative roles of α-glucosidases in human nutrition and clinical medicine. The first focus on starch digestion to glucose meets the unique requirement of the human brain for almost exclusive use of glucose as a source of energy (16–20). This dependence on glucose oxidation constitutes a unique linkage between brain function and food starches. In children, the rate of brain glucose oxidation is 3 times greater than in adults (21). Brain oxidation of glucose accounts for most of the increased basal energy expenditure in children (19–21). It has been suggested that a "selfish" brain is the center of the homeostatic universe by controlling appetite and glucose allocation to and from supporting tissues to meet glucose requirements for brain oxidation (17). With this background, we report the range and reproducibility of variations in mucosal α-glucosidase enzyme activities found in clinical duodenal biopsies from children.

A second focus on digestion of food starches is driven by concerns about increasing rates of degenerative diseases in adults, diabetes, cardiovascular disease, and obesity, which may be due to lifetime rates of α-glucogenesis from contemporary high-starch diets. This has led to a classification of food starches according to the observed blood glucose response to feeding through a "glycemic index." Notably, approximately two thirds of the prandial blood glucose concentration is accounted for by the α-glucogenic activities of the small intestine (22). One approach taken in reducing the α-glucogenic activities has been treatment with α-glucosidase inhibitors. The most studied of these is acarbose, a pseudomalto-tetrose resistant to α-glucosidases that reduces the prandial increase of blood glucose after starch feedings (1,23,24). This may be beneficial in the prevention and treatment of type II diabetes and cardiovascular disease (22). Furthermore, colonic digestion of malabsorbed carbohydrates, through formation of short fatty acids that generate less energy, may aid in the prevention of obesity (25).

Given the importance of starch digestion to short-term energy requirements and to long-term health, there is a critical need to understand the mechanistic details of this process. In this respect, considerable progress has been made in the study of the luminal phase α-1,4 endoglucosidase activities contributed by human α-amylases (1,26–29). In contrast, studies of the mechanisms of human mucosal α-1,4 exoglucosidase activities have been limited. To gain a better understanding of the overall process of starch digestion, the work described herein seeks to determine the total starch digestive capacity of the small intestinal mucosa using a well-characterized solubilized starch oligosaccharide preparation called

maltodextrin (MDx) as substrate; in addition, we describe some of the features of the synergism observed between the complementary activities of α-amylase, SI, and MGAM in their luminal and mucosal digestive roles.

PATIENTS AND METHODS

Human Small Intestinal Mucosal Collection, Homogenization, and Lysis

Surplus duodenal biopsy homogenates from selected clinical assays, all with activities greater than the 10th percentile, were pooled and frozen at −70°C in the Gastrointestinal Laboratory at the State University of New York. Transplant organ donor jejunum was collected from children in compliance with approval H-1614 from the Baylor College of Medicine institutional review board. The donor enterocytes were harvested by scraping the jejunal surface with a glass slide, they were then concentrated by centrifugation, and the pellet was frozen at −70°C. These donor enterocytes were used for immunizing mice to produce the monoclonal antibodies (mAbs) (40) and as preparations used in the present studies. Frozen pooled biopsy or donor enterocytes were homogenized in phosphate-buffered saline (PBS) solution. The homogenates were lysed with a sodium deoxycholic acid and Nonidet P 40 solution by vortexing and are referred to as ''Lys'' in these experiments. Although there was a 4-fold enrichment of enzyme activities in donor homogenates, there was no difference in relative enzyme activities or peptide concentrations in the biopsy homogenates (not shown).

Immunoprecipitations of MGAM and SI Activities

Two pooled sets of mAbs (IP-mAb) recognizing undenatured epitopes of MGAM (HBB 3/41, HBB 4/46/5/1, HBB 4/102/1/1) or SI (HIS 3/190, HIS 3/42/1/2, HSI 1/691/79) (30,40–42) were used for immunoprecipitation (IP) of the respective activities. The antibodies and enzyme IP methods have been described (30,41,42). Homogenates were lysed with a 10% sodium deoxycholic acid and 10% Nonidet P 40 solution by vortexing for 30 minutes at 4°C. Lys was centrifuged for 30 minutes at 100,000*g* and 1 mL of the supernatant was precleaned with 50 μL of a 50% slurry of protein A beads in PBS solution on a rotating wheel for 1 hour. After removal of beads by centrifugation, 3 sequential IP steps were performed on the supernatant adding 10 μL of mAb bound to 100 μL of protein A beads in PBS solution and rotated for 3 hours. The first 2 steps (IP 1 and 2) were performed using IP-mAbs against MGAM and the third was performed using IP-mAbs against SI. At each step, the enzyme-mAb-bead complexes were recovered by centrifugation and washed twice with PBS solution. Recovered materials of IP 1 and 2 (MGAM-mAbs beads) were pooled. Enzyme activities were measured in IP materials as well as in Lys using MDx, maltose G2, sucrose, and palatinose as substrates.

Aliquots of the Lys, MGAM-IP, and SI-IP were subjected to denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis. Western blots were obtained from the gels and detected with a mixture of α-mAbs LAMA1/207/140/12, LAMA 1/77/6/2/1, and LAMA 1/127 specific for denatured epitopes of MGAM, or a mixture of α-mAbs HSI-14, HSI 4/34, and HBB 3/56/4/1 (30,41,42) specific for denatured epitopes of SI. The blot images were developed with a luminescent secondary Ab against mouse mAbs. The intensity of the luminescent signal obtained for SI-IP and MGAM-IP was recorded on film for 1- and 30-second exposures and, after developing, measured as optical density on the exposed film.

Kinetic Glucogenesis Assays

Real-time glucose release from starch-derived oligosaccharides was measured by a modification of the 96-well plate tris-glucose oxidase (TGO) assay described previously (43). Phosphate glucose oxidase developing reagent (190 μL), consisting of 15 U/mL of glucose oxidase (Sigma G0543; Sigma Chemical, St Louis, MO), 0.75 U/mL of horseradish peroxidase (Sigma P8250), 0.2% Triton X100, and 50 μg/mL of *O*-dianosidine-HCl (Sigma D3252) dissolved in 10 mmol/L phosphate buffer at pH 6.8 containing 150 mmol/L NaCl (PBS solution) was placed in each well. Then, 10 μL of substrate solution at 5 mg/mL dissolved in PBS solution was added to each assay well (240 μg/mL final concentration). These mixtures were incubated for 10 minutes at 37°C for temperature equilibration and then 10 μL of enzyme preparations were added to the wells. The optical density at 450 nm was measured in a SpectraMax190 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at 37°C at 2-minute intervals, with a 3-second shaking period before each reading. Recombinant human pancreatic α-amylase (rhpAmy2) (28,29,44) was added in a volume of 10 μL to obtain a final concentration of 1.8 μg/mL. Frozen donor enterocytes, homogenized in 100 μL of PBS solution, were used to prepare Lys (as described earlier), which was diluted at a 1:10 ratio and then 10 μL of the obtained solution was added to appropriate wells. All of the measurements were performed in triplicate. Blanks for substrate and enzyme preparations and glucose concentration standard curves were included in each assay. Rates of reaction under steady-state conditions were calculated by linear regression during the interval of 10 to 30 minutes of reaction. Data were analyzed by analysis of variance using the general linear model with time as a covariate and enzyme mixture and/or substrate as classifying factors. Differences were determined by pairwise comparisons by the simultaneous test of Tukey.

Kinetics of Enzyme Activities

The hydrolytic activities present in Lys, MGAM-IP, and SI-IP were measured using concentrations ranging from 3.125 to 100 mmol/L for G2 and from 1.25 to 20 mg/mL for MDx. The mixtures were incubated at 37°C for 60 minutes and then immersed in a boiling water bath for 5 minutes. The glucose concentration was quantified using Sigma infinity glucose reagent. Apparent V_{max}, K_m, and, when necessary, K_i values were calculated for each activity using nonlinear regression with the Marquardt-Levenberg algorithm and models were adjusted to a single substrate Michaelis-Menten or to substrate inhibition kinetics. Individual contributions of MGAM and SI to total Lys activities were calculated by nonlinear regression as described earlier with a 2-enzyme Michaelis-Menten model with or without substrate inhibition.

Maltodextrin and α-limit Dextrin Characterizations

The food-grade MDx used (Polycose; Ross Laboratories, Columbus, OH) was manufactured by partial fungal amylase α-1,4-digestion of cornstarch (45). The global digestion of MDx was assayed by the Englyst test of starch digestion (22). The glucose oligomer composition of MDx was analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Voyager 6270; Applied Biosystems, Foster City, CA) (46) before and after a 15-hour α-1,4-digestion period with porcine pancreatic α-amylase (30 U/100 mg Polycose, Type VI-B; Sigma). The data of mass fraction and molecular weight (MW) for each oligomer obtained by MALDI-TOF analysis were used to calculate the average MW of MDx, by summation of the products of the fractional mass of each oligomer multiplied by its respective MW. To determine the number of α-1,6 branched points, MDx was also analyzed by debranching with isoamylase (Megazyme International Ireland, Bray, Ireland) for 24 hours (31,46). Reagent grades of maltose (G2), maltotriose (G3), maltotetraose (G4) maltopentose (G5), sucrose, palatinose, whole starch, amylopectin, and amylase substrates were purchased from Sigma (St Louis, MO).

Clinical Biopsy Collection

A total of 977 unselected duodenal biopsy homogenates, obtained as part of the clinical evaluation of patients (47) from January 1 through June 20, 2002, were used for combined sucrase, maltase, palatinase, and α-glucosidase assays. Frozen biopsy specimens were received from a national distribution of endoscopists whose instructions were to "place intestinal biopsy in a small, tightly capped tube, store frozen, and ship on dry ice." Other than the patient's age, no clinical information was provided. Ages were a mean of 10 ± 5 years. Replicate biopsies for variance analyses were obtained at time of endoscopy for clinical indications with signed informed consents approved by the institutional review boards at Baylor College of Medicine (H-1320) and Women and Children's Hospital at the State University of New York at Buffalo (DB 817). The mean coefficient of variation percentage for replicate biopsy activities was computed by patient (22 subjects) from 4 adjacent endoscopic biopsy specimens obtained from visualized adjacent circumferential sites in the duodenum distal to the ampulla. Each biopsy was individually homogenized and its enzyme activities assayed.

Clinical Biopsy Assays

Clinical assays were performed in the Gastrointestinal Laboratory at the State University of New York at Buffalo (47), which is CLIA- and CAP-certified and licensed by the state of New York. Duodenal biopsies were obtained by endoscopy, snap-frozen, and then shipped on dry ice to the laboratory. All of them were received in the frozen state. The sucrase, maltase, and palatinase assays were those described by Dahlqvist (10) at 16 mmol/L concentrations. The α-glucosidase assay was a modification described by Kernsakul (30) with 20 mg/mL MDx used as substrate. Activity was reported as international enzyme units (U/g protein).

RESULTS

Characterizations of MDx and LDx Substrates

We performed analyses of the digestibility and composition of the MDx substrate used in our experiments. As determined by in vitro starch digestibility, MDx was 88% digested to glucose at 20 minutes and 95% digested at 2 hours. The MALDI-TOF analyses allowed the detection of a wide distribution of molecules with discrete peaks with MW corresponding to oligomers from G3 up to G20 glucose residues, whereas higher size molecules with up to G60 glucose residues were observed only as a flat signal above the background (Fig. 1). The most abundant oligomers were G6 followed by G7, whereas small quantities of G4, G5, and G8, and an even smaller amount of G3, were observed (Fig. 1A). Although the MALDI-TOF analysis did not quantify the amount of

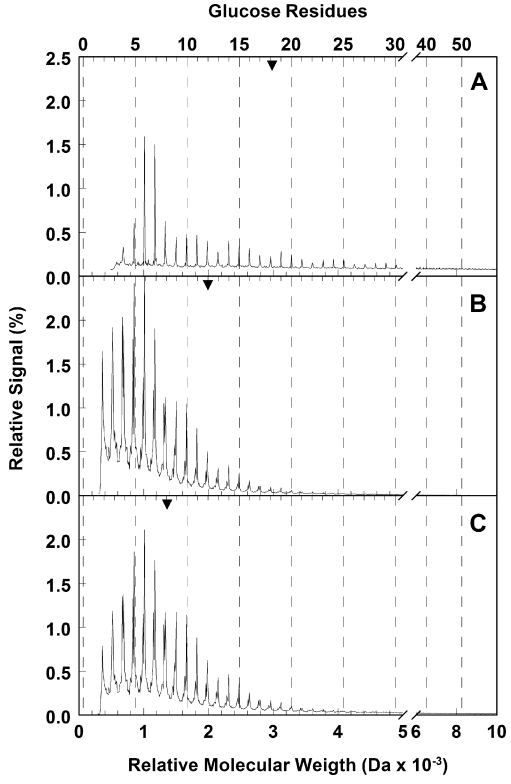

FIG. 1. Analysis by MALDI-TOF of starch α-amylase hydrolysis products. MALDI-TOF mass spectral analyses of food-grade MDx (A), MDx after exhaustive α-1,4 digestion with α-amylase (B), and MDx after exhaustive α-1,6 digestion with isoamylase (C) are shown. The relative abundance in percent (ie, ordinate) of the cumulative signal of intervals of 10 Da was calculated in relation to the total signal recorded for each sample. The molecular weight (bottom abscissa) in Da ($\times 10^{-3}$) and the corresponding number of glucose residues (top abscissa) are indicated. Triangles indicate the average molecular weight of the analytical samples calculated by summation of the products of the fractional mass of each oligomer multiplied by its respective MW.

free glucose; using the TGO colorimetric technique we found a small content of free glucose in the MDx (3.8%). Exhaustive α-1,4-digestion of MDx with porcine pancreatic α-amylase resulted in substantial reductions of oligosaccharide lengths (Fig. 1B); the resulting pattern corresponded to that typically reported from direct analyses of LDx found in prandial luminal fluids. Exhaustive α-1,6-digestion of MDx with fungal isoamylase also caused a substantial shift in the MALDI-TOF pattern (Fig. 1B and 1C). Oligomers smaller than 10 glucose residues, including G2 and G3, were observed in relative large proportion and accounted for >75% of the fully digested MDx. The isoamylase treatment revealed that MDx has 13.4 mol% α-1,6-glucose bonds and 86.6 mol% α-1,4-glucose bonds, which are available for hydrolysis by α-glucosidase activity. Only a marginal increment in the proportion of free glucose was detected with the TGO technique after amylase or isoamylase digestion. Although G6 continued to predominate in MDx and LDx, the distribution of oligomer lengths was shifted from larger than G10 to smaller than G10 (ie, Fig. 1A–C). Debranching showed that MDx has a large amount of short branched linear chains compared with the native amylopectin molecule.

Substrate Specificities of MGAM-IP and SI-IP

To determine the relative contributions of MGAM and SI to maltase and α-glucosidase in pooled biopsy and donor enterocyte homogenates, these activities were measured in lysate preparations (ie, Lys) as well as in MGAM-IP and SI-IP (Table 1; replicates, n = 4). Twenty percent of maltase activity present in Lys was IP by mAb against MGAM, whereas 80% was IP by the mAb against SI. With respect to MDx substrate, 22% of the activity in Lys was IP with mAb against MGAM and 78% by mAb against SI. Ninety-nine percent of the sucrase and 95% of isomaltase activities in Lys were IP by SI mAbs (Table 1).

Purity and Concentrations of MGAM-IP and SI-IP Peptides

Western blot analysis of MGAM-IP and SI-IP displayed intense bands only with the homologous set of antibodies (Fig. 2), indicating a high degree of purity. The blot resolved with the MGAM mAbs required a 30-second longer exposure than the blot resolved with the SI mAbs. Good efficiency of SI-IP and MGAM-IP was supported by the small amounts of supernatant activities (<11%) found after IP (Table 1). Despite known limitations of Western blot analysis as a quantitative tool, we attempted an evaluation of the relative proportion of SI and MGAM by measuring the respective optical density signal obtained from the x-ray films. The apparent ratio of the total SI to MGAM signals was >20:1.

TABLE 1. *Distribution of α-glucosidase activities in human intestinal mucosa homogenate and immunoprecipitated enzymes (4 replicates)*

	Substrate (%)			
Fraction	Maltodextrin	Maltose	Sucrose	Palatinose
Lys (total homogenate)	100	100	100	100
MGAM-IP	20	18	1	2
SI-IP	70	72	99	95
Supernatant after IP	10	11	0	3

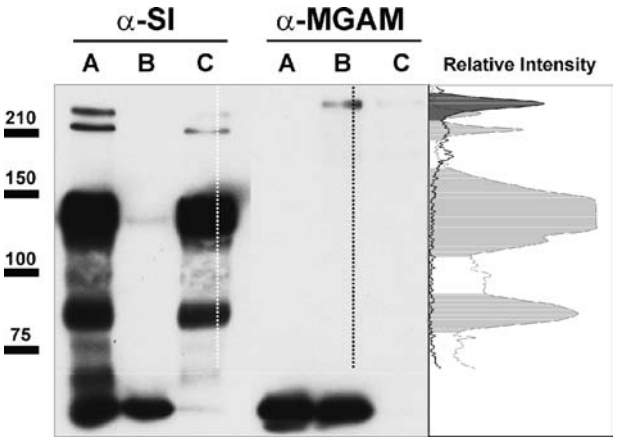

FIG. 2. Western blot demonstrating purity and relative abundance of mucosal IP activities. Typical Western blots of immunoprecipitated SI-IP (A), MGAM-IP (B), and whole duodenal homogenate (Lys, C). Protein bands were developed with 2 sets of 3 α-mAbs each specific for denatured SI (α-SI) or for denatured MGAM (α-MGAM) by chemoluminescence. The left blot (α-SI) was exposed to film for 1 s and the right (α-MGAM) for 30 s. Within each α-blot, the individual lanes, left to right, are loaded with IP-mAb sets effective for SI activity (A), MGAM activity (B) precipitations, or whole duodenal homogenate (C, Lys; see fractionation of activities in Table 1 for IP efficiency). The optical densities of protein bands obtained from α-SI of the homogenate C are indicated by a white dotted line and concentrated bands from MGAM-IP are indicated by a gray dotted line. The areas corresponding to the main protein bands detected for the SI-IP (light gray plot and shading) and MGAM-IP (dark gray plot and shading) were quantified to determine the ratio of SI-IP and MGAM-IP total peptides from the same homogenate. The apparent peptide ratio of unconcentrated SI in lane C of α-SI to MGAM-IP in B of α-MGAM was >20:1.

Enzyme Kinetics of Lys, MGAM-IP, and SI-IP

Assays of clinical biopsy homogenates were performed at substrate concentrations of 16 mmol/L for disaccharides and 20 mg/mL for MDx. Because enzymatic activity is not a linear function of substrate concentration, we wanted to determine the relationship between the enzyme kinetic properties of Lys and the MGAM-IP and SI-IP fractions. Plots for α-glucogenesis vs substrate concentrations are shown in Figure 3. Apparent K_m for maltase activity of SI-IP calculated by nonlinear regression was 35.8 ± 2.0 mmol/L ($r^2 = 0.999$),

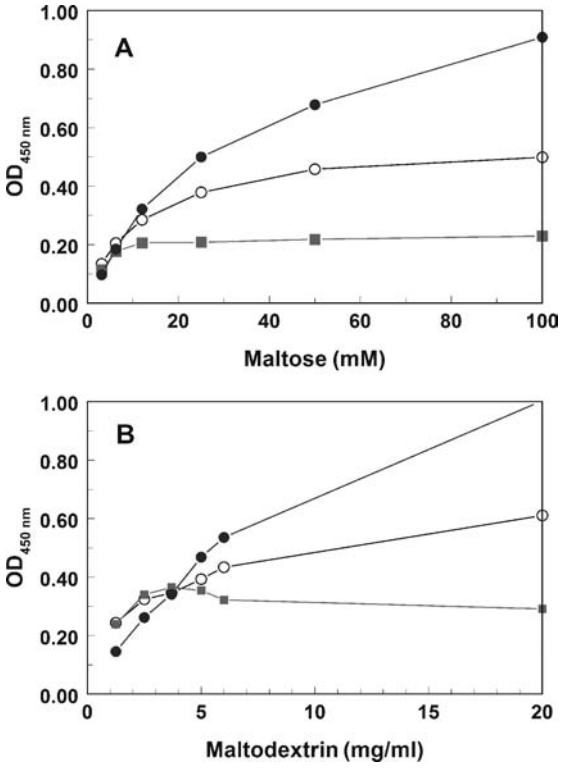

FIG. 3. Enzyme kinetics of mucosal Lys, SI-IP, and MGAM-IP. Dependence of the α-glucogenic activity of Lys (extracted human duodenal mucosa; open circles), immunoprecipitated SI-IP (filled circles), or immunoprecipitated MGAM-IP (squares) on the concentration of maltose (A) or MDx (B) as substrates. The ordinate is expressed as OD450, visualized by real-time phosphate glucose oxidase reactions (see V_{max} calculations in text).

significantly higher than that reported previously (4,5,7) for the same enzyme purified by biochemical methods. The apparent K_m for maltase activity of MGAM was 2.7 ± 0.3 mmol/L ($r^2 = 0.996$), which is comparable to the values reported by others (5–7). As expected, the apparent K_m for Lys of 10.7 ± 0.4 mmol/L ($r^2 = 0.999$) was intermediate between the K_m observed for each of the immunoisolated enzymes (Fig. 3A; Table 2).

Because the rate of maltase activity of Lys (v_{Lys}) results from the addition of the respective activities of SI and MGAM, it follows that $Vm_{Lys} = Vm_{SI} + Vm_{MGAM}$. Thus, the rate of reaction v_{Lys} at any substrate concentration expressed as a fraction of Vm_{Lys} can be represented as follows:

$$\frac{v_{Lys}}{Vm_{Lys}} = (f_{SI}) * \frac{S}{Km_{SI} + S} + (1 - f_{SI}) * \frac{S}{Km_{MGAM} + S}$$

where Km_{SI} and Km_{MGAM} are the respective apparent Michaelis constants measured in the purified enzyme preparations, and f_{SI} is the fraction of Vm_{Lys} contributed by SI, and 1-f_{SI} corresponds to that contributed by MGAM. The 3-dimensional plot of v_{Lys}/Vm_{Lys} vs S and f_{SI} (relative contribution of SI) is shown in Fig. 4A. Using nonlinear regression, the value of f_{SI} for Lys was calculated using our experimental data. The value for f_{SI} computed to be 0.6 ± 0.03 ($r^2 = 0.96$). The experimental data and the respective calculated kinetics are also plotted in Figure 4A (white line and dots). Although the calculated relative contributions of SI to maltase activity (0.6 fraction or 60%) were lower than that observed in the IP experiments (0.8 fraction or 80%), the value is in good agreement with that reported previously (5–7).

Using MDx as substrate and SI-IP, we found an activity displaying typical Michaelis-Menten kinetics with apparent K_m value of 13.4 ± 0.6 mg/mL ($r^2 = 0.999$), significantly lower than that observed for maltase (Fig. 3B; Table 2). In the case of MGAM-IP, we found a deviation from the normal Michaelis-Menten kinetics because a strong substrate inhibitory effect was observed with a value of apparent K_m of 1.1 ± 0.3 mg/mL and a calculated Ki_S of 29.3 ± 7.8 mmol/L ($r^2 = 0.996$). This value of K_m is substantially lower than those reported previously (5–7) for oligomers G2 to G7, suggesting the presence of components in MDx displaying strong noncompetitive inhibition on MGAM. Because this inhibition causes a significant decrease in the rate of hydrolysis, we have named this effect the luminal "maltodextrin brake" on MGAM α-glucogenic activity. Despite the substrate inhibitory effect on MGAM, the kinetics for Lys showed a typical Michaelis-Menten behavior with K_m value of 3.0 ± 0.4 mg/mL ($r^2 = 0.994$), corroborating that the in vitro contribution of MGAM to α-glucogenic activity of Lys is small.

TABLE 2. *K_m and K_i for α-glucogenic activities of human intestinal mucosa homogenate and immunoprecipitated SI and MGAM*

	Maltose (G2)		MDx			
	$K_m \pm$ SE		$K_m \pm$ SE		$K_i \pm$ SE	
Fraction	mmol/L	mg/mL	mmol/L*	mg/mL	mmol/L*	mg/mL
Lys (total)	10.7 ± 0.4	3.7 ± 0.1	1.0 ± 0.1	3.0 ± 0.4	ND	ND
SI-IP	35.8 ± 2.0	12.2 ± 0.7	4.5 ± 0.2	13.4 ± 0.6	ND	ND
MGAM-IP	2.7 ± 0.3	0.9 ± 0.1	0.4 ± 0.1	1.1 ± 0.3	9.8 ± 2.6	29.3 ± 7.8

ND indicates no detected substrate inhibitory effect.
* MW for MDx of 2981 Da corresponds to an 18.3 glucose residues oligomer (DP18).

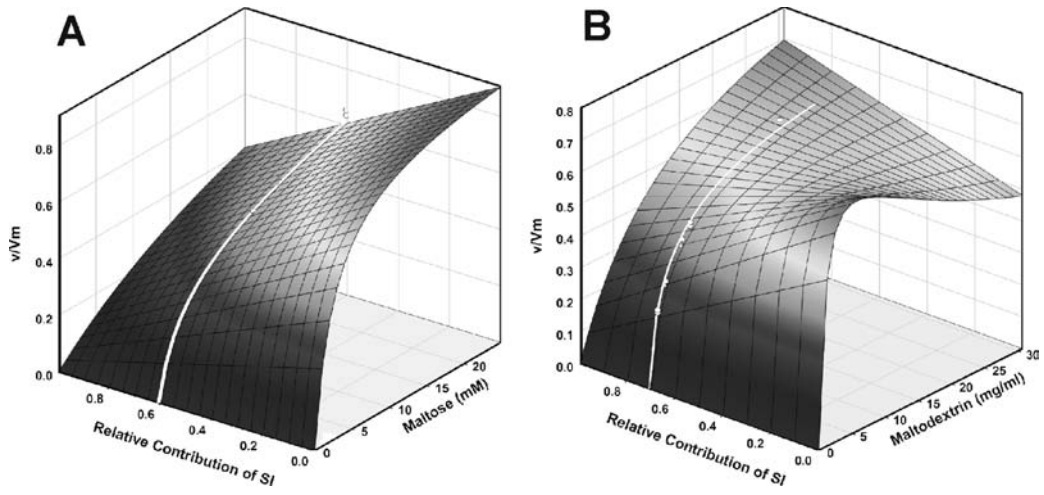

FIG. 4. Mucosal α-glucogenic activities as function of maltose and MDx concentrations. Three-dimensional plots of the predicted relative intestinal mucosal α-glucogenic activity (v/V_m) as a function of the relative contribution of SI (fraction of 1) vs MGAM (1 – fraction of SI) and of the concentration of maltose (G2; A) or maltodextrin (MDx; B) using a model of pure Michaelian kinetics with maltose as substrate for both enzymes, or a model of Michaelian kinetics for SI plus substrate inhibition for MGAM with MDx as substrate. White dots indicate experimental values obtained with intestinal mucosa Lys (some points are hidden by the 3-dimensional surface). Plots with the predicted values of relative contribution of SI in intestinal mucosa extracts obtained with the experimentally derived K_m and K_i for the respective enzyme and substrate are shown as white lines.

The relative contribution of SI and MGAM to the total α-glucogenic activity of Lys was also calculated by a similar procedure to that described earlier, with a modification to take into account the substrate inhibition experienced by MGAM:

$$\frac{v_{Lys}}{Vm_{Lys}} = (f_{SI}) * \frac{S}{Km_{SI} + S} + (1 - f_{SI}) * \frac{S}{Km_{MGAM} + S * (1 + \frac{S}{Ki_S})}$$

The 3-dimensional plot of v_{Lys}/Vm_{Lys} versus S and f_{SI} obtained in this case is shown in Figure 4B. In this case the combined Michaelis-Menten kinetics of SI with the substrate inhibition of MGAM generates a more complex topology with a saddle-shaped plot where the experimental values can be mapped to find the value of f_{SI} with the best correlation. The calculation of f_{SI} by nonlinear regression using our experimental data indicated a typical value of 0.7 ± 0.02 ($r^2 = 0.954$), which was in good agreement with that observed in the experiments using IP. The plot of the adjusted kinetics and the respective experimental values are also shown in Figure 4B (white line and dots).

Interactions of Luminal Mucosal α-Glucosidase Activities with α-Amylase

We measured glucogenesis from MDx substrate in a series of assays examining the interactive activities of rhpAmy2 with mixed α-glucosidase activities from donor enterocyte Lys. The assays demonstrated a 2-fold amplification of the MDx substrate α-glucogenic activity of Lys (Fig. 5A; Table 3). The amplification effect appeared to be caused by the transformation of large oligomers present in the MDx into short G2 and G3 oligomers caused by the activity of rhpAmy2 (Fig. 1). Two controls provide additional insights; the first was LDx, MDx fully digested with α-amylase (Fig. 1B), which completely blocked rhpAmy2 amplification of α-glucogenesis (Fig. 5B; Table 3). The second was an equimolar mixture of G2 to G5 linear glucose oligomers (100 μmol/L each, final concentration 240 μg/mL total) as synthetic substrate, which confirmed the amplification of α-glucogenic activities from MDx by rhpAmy2 (Fig. 5C; Table 3). The relatively low rate of glucose production by rhpAmy2 was not masked by transglycosylating reactions because the immediate transformation of free glucose into gluconic acid by phosphate glucose oxidase present in the reaction mixtures ensured a low concentration of free glucose during the course of the experiments.

Clinical Biopsy Activities

Mean and SD values of protein for the measured activities of MDx α-glucosidase, maltase, sucrase, and palatinase were 66.3 ± 27.9 U/g, 172.8 ± 67.2 U/g, 59.2 ± 28.5 U/g, and 13.2 ± 6.4 U/g, respectively (Fig. 6). No correlation between patient age and these enzyme activities was detected. The distribution of all of the activities differed from the normal distribution, displaying positive skewness and kurtosis but causing only minor differences between the values of the mean and

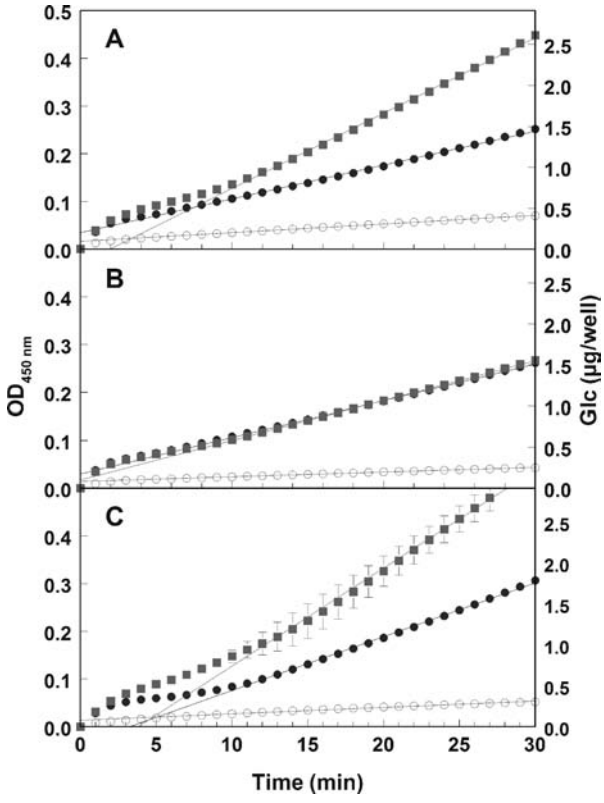

FIG. 5. α-Glucogenic hydrolysis of MDx and LDx by luminal and mucosal activities. Kinetic α-glucogenesis assay with rhpAmy2 (open circles), Lys (extract from human duodenal mucosa homogenate; filled circles), or rhpAmy2 plus Lys (squares) as source of enzymatic activity, and MDx (A), LDx (B), or an equimolar mixture of G2 to G5 (C) as substrates. SD bars of OD_{450} are shown. Rates of activity were calculated as a function of time by linear regression during the interval of 10 to 30 minutes of reaction and plotted for each enzymatic mixture (continuous lines). Slopes and relevant statistical values are listed in Table 3.

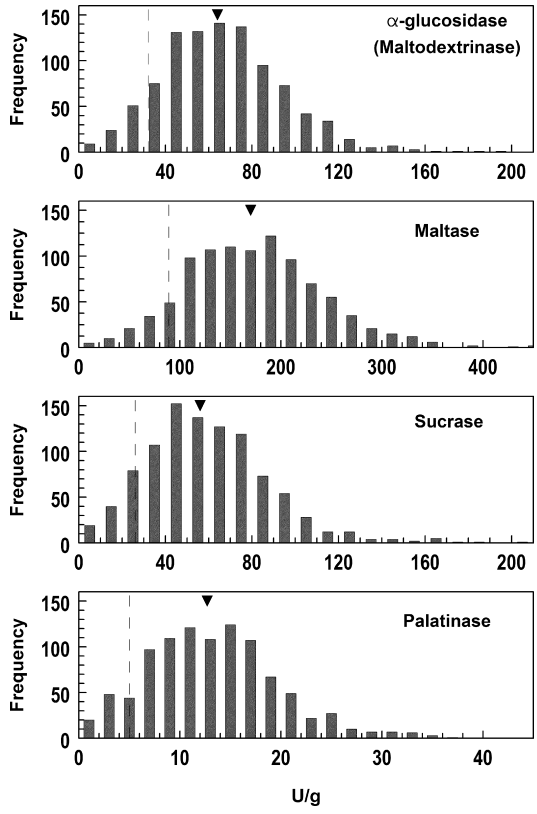

FIG. 6. Frequency histograms of childhood clinical duodenal enzyme activities. Frequency distributions of the activity of α-glucosidase (ie, maltodextrinase), maltase, sucrase, and palatinase in 977 unselected children's clinical duodenal biopsy homogenates. Median values (triangles) and 10th percentile values (vertical dashed lines) are indicated.

respective median (triangles in Fig. 6 and Table 4). No evidence of a binomial distribution was detected. Maltase, palatinase, and α-glucosidase displayed comparable values of coefficient of variation (18%–20%) for activities measured in 22 sets of repeated biopsies, whereas sucrase showed the highest variation with 27%. Despite this variation, a high correlation among sucrase, maltase, and α-glucosidase was observed (Pearson correlation coefficients: sucrase vs maltase, 0.922; maltase vs α-glucosidase, 0.907; sucrase vs α-glucosidase, 0.901), reflecting the dependence of these activities on the same enzyme protein elements.

Samples displaying low α-glucosidase activity were operationally defined as those with values at the 10th percentile or lower. This was 26, 5, 89, and 32 U/g of protein for sucrase, palatinase, maltase, and α-glucosidase, respectively (dashed vertical lines in Fig. 6). These

TABLE 3. *Effect of recombinant human pancreatic α-amylase on the rates of reaction of human intestinal mucosa*

	Substrate (µg/min ± SE)		
Enzyme mixture	G2–G5	MDx	LDx
rhpAmy2	0.0078 ± 0.0001	0.0106 ± 0.0004	0.0057 ± 0.0001
Lys (total)	0.0657 ± 0.0011	0.0413 ± 0.0004*	0.0445 ± 0.0005†
rhpAmy2 + Lys	0.1198 ± 0.0038	0.0920 ± 0.0010	0.0483 ± 0.0006

* Not different from Lys with LDx.

TABLE 4. *Descriptive statistics of intestinal α-glucosidase activities*

Activity	N	Mean	Median	SD	SE	Min	Max	1st quartile	3rd quartile	Skewness	Kurtosis
α-Glucosidase (maltodextrinase)	977	66.3	64.2	27.9	0.9	0	192.1	47.0	83.1	0.55	0.77
Maltase	977	172.8	169.8	67.2	2.2	6.2	450.7	125.3	216.1	0.38	0.43
Sucrase	977	59.2	56.0	28.4	0.9	1.2	207.2	39.9	75.7	0.82	1.68
Palatinase	977	13.2	12.7	6.4	0.2	0	37.7	8.6	17.2	0.54	0.44

values were consistent with those published or provided by reference laboratories (30). Using these 10th percentile values, we found that 67% (n = 657) of all of the analyzed clinical samples were above this level for all of the activities. Isolated low α-glucosidase was present in 1.3% of samples (n = 13). Isolated low sucrase was present in 1% (n = 10) and isolated low palatinase was observed in 2.5% (n = 25). Simultaneous low values of all 4 activities were found in 5.8% of samples (n = 57) (30). In addition, similar frequencies of low α-glucosidase activity (122 of 977) and low sucrase activity (125 of 977) were observed, with 93% of these patients displaying simultaneous low α-glucosidase and low sucrase activity.

DISCUSSION

Given the complexity and variability of food starches and starch products, we selected food-grade MDx as a standard substrate that is soluble, uniform in composition, and highly digestible for assays of starch digestion to free glucose (ie, α-glucogenesis). MDx is commonly used as a substitute for lactose in therapeutic infant formulas and is used throughout the food industry, in which it is described as corn syrup solids. Although the use of a single oligosaccharide, such as maltose, provides important biochemical information, pure oligosaccharides cannot assay starch digestion capacity. MDx used in these experiments consisted of a range of branched oligomers with a MW from 500 to >3000, which corresponds to a degree of dextrose (glucose) polymerization (DP) of 4 to >18 (DP18), close to that of a mean DP20 reported by the manufacturer. The prandial spectrum of luminal LDx maltosides that has been reported (31–35) (Fig. 1B) is different from the spectrum of maltosides in the MDx used in this study (Fig. 1A). MDx is an intermediate product between whole food starch and LDx after full hydrolysis with α-amylase. The use of MDx as assay substrate is justified by its generic representation of the vast variety of partially hydrolyzed starches and its persistent α-amylase susceptibility. Future studies will be needed to characterize the range of effects of variability of starch composition on the α-glucogenesis assays reported here.

The role of salivary and pancreatic amylases as processing enzymes that generate short and branched glucose oligomers in the digestion of starches is widely accepted. Our results have demonstrated that this picture is essentially correct. Under the assayed conditions, the 2-fold increment in the α-glucogenic rate of Lys appeared to be dependent on the generation of short glucose oligomers by rhpAmy2 because the same kinetic behavior was observed using an equimolecular mixture of G2 to G5 oligomers. In contrast, using LDx generated by extensive predigestion of MDx with α-amylase completely abrogated the synergistic effect. Although synergistic effects of α-amylases and α-glucosidases have been described for commercial enzymes used in industrial production of glucose syrups from starch (36–38), this is the first time that the synergistic behaviors of human pancreatic α-amylase, SI, and MGAM activities have been demonstrated to our knowledge.

The α-glucogenic capacity of human pancreatic and salivary α-amylase is controversial. rhpAmy2 has been shown to release free glucose from G2 to G5 linear glucose oligomers, leading to the belief that α-amylase is an important α-glucogenic enzyme for the digestion of starch. It was even proposed that in humans amylase performs most if not all of the digestion of starch to free glucose (35), whereas SI and MGAM may constitute backup systems for α-1,4 glucosidic and α-1,6 debranching activities. Our results clearly indicate that, under normal physiological conditions, rhpAmy2 is a poor contributor to glucogenesis from the digestion of starch. Its activity is most important in the transformation of large glucose oligomers into short ones, providing a synergistic effect on the α-glucogenic activity of SI and MGAM. We hypothesize that together, the 4 mucosal α-1,4 glucosidases and the synergistic effects of 2 α-amylases allow digestion of a wider range of food starches.

Based on its low K_m values against short glucose oligomers (G2–G7), MGAM has been regarded as the intestinal α-glucosidic enzyme with the highest glucogenic capacity (7). In this study we found K_m values of MGAM against maltose and MDx close to 1 order of magnitude smaller than those of SI, supporting this assumption. However, although this is correct from the point of view of catalytic efficiency (moles of glucose released per mole of enzyme), under physiological conditions this assumption may be incorrect, due on one hand to the relative small proportion of MGAM molecules in relation to those of SI present in the human intestinal mucosa, and on the other hand to the substrate

inhibition caused by the MDx brake. Additionally, the maltase activity measured at V_m conditions in the immunoprecipitated enzymes showed a ratio of between 4:1 and 5:1 for SI to MGAM fractions. However, the K_m of MGAM was approximately 10 times lower than for SI. Assuming that there is some degree of proportionality between the values of K_m and the catalytic constant (k_2 or k_{cat}) of each of the 2 enzymes, the data could suggest that catalytic SI molecules may be 40 to 50 times more abundant. This approximation would be in agreement with the aforementioned supposition derived from the SI:MGAM > 20:1 results of Western blot measurements.

In addition to substrate inhibition, using the average MW of MDx (2981 Da) derived from the MALDI-TOF analysis, the K_m values for MDx of Lys, SI-IP, and MGAM-IP were almost 10 times smaller than those observed with maltose. Thus, whereas the total maltase activity measured at any substrate concentration in intestinal mucosa homogenate results from additive individual activities of SI and MGAM, on a molar basis the α-glucosidase activity measured with MDx as substrate is higher than with maltose and includes the inhibitory component exerted on MGAM at MDx concentrations higher than 4 mg/mL (ie, the MDx brake on MGAM). Substrate inhibitory effect has been previously described for human MGAM using linear α-1,4 maltosides, particularly G3 and G4 (7). We have observed this substrate inhibition with the same concentrations of LDx substrate (not shown). Preliminary experiments (not reported here) using G3 and G4 as substrates confirmed the existence of strong substrate inhibition on MGAM-IP preparations, indicating that these may be components present in MDx and LDx that are responsible for the MDx brake inhibition. Other oligomers also may contribute to this inhibitory effect; however, extensive and more detailed studies would be required to determine the effects of the whole range of starch oligomers present in MDx and LDx on human MGAM.

Under physiological conditions, the actual effect of the MDx brake on MGAM activity would depend on the MDx and LDx concentration attained after a starch-containing meal. The prandial luminal concentration of total MDx derived from a starch-rich meal has been reported to peak at approximately 120 mmol/L of glucose equivalents after a meal (39). Therefore, a transition in the state of MGAM from high to low activity would occur in the course of ingestion of such a meal, and implies that MGAM will be subject to regulation by the MDx brake while SI becomes the default α-glucosidase. The functions of the levels of activity of the 2 complimentary α1-4-glucosidases are thus independent: the fast activity of MGAM primes the α-glucogenesis from MDx but is inhibited by prandial levels of luminal MDx and LDx; in contrast, SI provides a constant but steady α-glucogenesis, at approximately 5% of the uninhibited MGAM rate, derived from higher prandial levels of luminal glucose oligomers. As a consequence of this regulatory effect, the effective concentration of glucose available for absorption and transport into blood circulation and brain oxidation would increase in a slow and steady manner, dampening the potential adverse effects of a greater increase of blood glucose concentration with uninhibited MGAM activity.

Together, our observations indicate that MGAM has a substrate regulated role in the digestion of starch-rich diets yielding abundant MDx and LDx oligomers. This raises the question of what the physiological role of MGAM may be. High-starch diets are a part of the benefits of contemporary agriculture and modern food technology. The high activity of MGAM enzyme at low MDx and LDx oligomer concentrations suggests that its function is related to α-glucogenesis from starch-poor diets. MGAM may constitute a highly efficient mechanism for α-glucogenesis and brain oxidation from low-starch diets, perhaps as ingested by early *Homo sapiens* and as is persistent in primitive societies today. In children living under primitive conditions, efficient production of glucose from low-starch diets by MGAM could have a major impact on brain oxidation of glucose. In infants with developmental or nutritional reductions of amylase activity, MGAM activity likely would not be suppressed by the luminal MDx brake.

We found that α-glucogenesis, as assayed by hydrolysis of MDx by duodenal mucosal biopsy homogenates, has a population distribution with similar features to those observed with other disaccharides as substrate. These results indicated that similar sampling conditions existed for maltase, palatinase, and α-glucosidase, but differed for sucrase, which showed a greater variation of its activity in duodenal mucosa. In addition (and surprisingly), despite their common protein precursor, a relatively low degree of correlation was observed between sucrase and palatinase (Pearson correlation coefficient, 0.83), which probably reflected differences in extracellular processing of SI among individuals.

The assays of the activities associated with SI-IP and MGAM-IP showed that the strong correlation between disaccharidase activities and total α-glucosidase activities described earlier was not fortuitous. The results indicated that SI is the main contributor to the α-glucosidase activity in clinical biopsies when measured using MDx as a substrate at concentration of 20 mg/mL. This result was surprising because MGAM has been considered the major contributor to α-glucogenesis from starch digestion. Thus, the observed correlations of α-glucosidase and disaccharidase activities in mucosal biopsies were caused by the primary contribution of SI to total α-glucosidase, whereas MGAM contributed a substantial but significantly smaller proportion.

It was reported that α-amylase activity is present in human duodenal homogenates (32). Assays in the presence of ethylenediaminetetraacetic acid, which blocks

pancreatic α-amylase activity (35), revealed that in our experiments the contributions of α-amylase to Lys MDx α-glucogenesis were negligible (not shown).

In summary, in this work we report that mucosal MGAM and SI α-glucosidase activities contribute more than 85% of starch α-glucogenesis. MGAM has an intrinsically higher α-glucogenic activity than SI but is inhibited by mealtime concentrations of luminal glucose oligomers (ie, the MDx brake). Although SI has <5% of MGAM α-glucogenic activity, this enzyme is present in a mucosal concentration >20 times higher without experiencing inhibition by glucose oligomers. α-Amylase amplifies mucosal α-glucogenic activities approximately 2-fold by preprocessing of starch into soluble small glucose oligomer substrates. Duodenal α-glucosidase activity, as assayed with MDx substrate in clinical assays, mainly reflects SI activity. The precision of the MDx substrate assays was equal to other duodenal α-glucosidase assays. Finally, we speculate that highly active MGAM may be important to meet the oxidative needs of children's brain metabolism during meals containing low starch concentrations, whereas the slower SI may be a constraint to glucose-associated degenerative diseases in adults consuming high-starch diets.

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Molecular pathogenicity of novel sucrase-isomaltase mutations found in congenital sucrase-isomaltase deficiency patients

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ABSTRACT

Background & aims: Congenital sucrase-isomaltase deficiency (CSID) is a genetic disorder associated with mutations in the *sucrase-isomaltase* (*SI*) gene. The diagnosis of congenital diarrheal disorders like CSID is difficult due to unspecific symptoms and usually requires invasive biopsy sampling of the intestine. Sequencing of the *SI* gene and molecular analysis of the resulting potentially pathogenic SI protein variants may facilitate a diagnosis in the future. This study aimed to categorize SI mutations based on their functional consequences.
Methods: cDNAs encoding 13 SI mutants were expressed in COS-1 cells. The molecular pathogenicity of the resulting SI mutants was defined by analyzing their biosynthesis, cellular localization, structure and enzymatic functions.
Results: Three biosynthetic phenotypes for the novel SI mutations were identified. The first biosynthetic phenotype was defined by mutants that are intracellularly transported in a fashion similar to wild type SI and with normal, but varying, levels of enzymatic activity. The second biosynthetic phenotype was defined by mutants with delayed maturation and trafficking kinetics and reduced activity. The third group of mutants is entirely transport incompetent and functionally inactive.
Conclusions: The current study unraveled CSID as a multifaceted malabsorption disorder that comprises three major classes of functional and trafficking mutants of SI and established a gradient of mild to severe functional deficits in the enzymatic functions of the enzyme.
General significance: This novel concept and the existence of mild consequences in a number of SI mutants strongly propose that CSID is an underdiagnosed and a more common intestinal disease than currently known.

1. Introduction

About 70% of the human population is affected by some form of carbohydrate malabsorption [1]. Malabsorption of carbohydrates can result from genetic or environmental factors that inhibit the catalytic activity of intestinal disaccharidases. Sucrase-isomaltase (SI) [2], maltase-glucoamylase (MGAM) [3] and lactase-phlorizin hydrolase (LPH) [4] are the prominent intestinal disaccharidases, which contribute to the final step of carbohydrate breakdown. SI is predominantly expressed at the apical membrane of enterocytes. The isomaltase subunit of SI is the major enzyme for cleavage of branched α-1,6-linkages while the sucrase subunit of SI mainly hydrolyses α-1,2-glucosidic bonds of sucrose.

Functionally, the combined units account for 60% to 80% of maltose digestion [5–7]. The SI protein is translated, folded and N-glycosylated in the ER (SI_h, ~210 kDa) and transported from the ER to the Golgi apparatus where complex N- and O-glycosylation takes place. The mature protein (SI_c, ~245 kDa) is then transported *via* cholesterol- and sphingolipid-enriched membrane microdomains mainly to the apical cell surface [8].

Genetic defects of *SI* can lead to congenital sucrase-isomaltase deficiency (CSID) [9,10]. Patients with this disorder show a substantial reduction or absence of the sucrase and/or isomaltase activities linked to reduced digestive capacity of the small intestine in general [11]. The failure of digestion and impaired absorption of maldigested carbohydrates triggers osmotic diarrhea. The patients may also suffer from vomiting, flatulence or abdominal pain ranging from mild to severe [11,12]. Occasionally dehydration, failure to thrive, developmental retardation and muscular hypotonia have been observed [12,13]. The maldigestion of sugars can also affect the absorption of other nutrients and can influence the hormonal regulation of the intestinal function [12,14]. The chronic malabsorption of carbohydrates can lead to malnutrition in CSID patients [15]. Common symptoms of CSID with other congenital diarrheal

Abbreviations: CSID, congenital sucrase-isomaltase deficiency; SI, sucrase-isomaltase; SUC, sucrase; IM, isomaltase; endo H, *endo*-β-*N*-acetylglucosaminidase H.

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disorders like congenital lactase deficiency [16] and glucose-galactose malabsorption [17] or with secondary acquired intestinal disorders like inflammation and food allergies [18,19] complicate the diagnosis and therapy of CSID [20]. A univocal diagnosis of CSID mostly requires invasive biopsy sampling of the patients and subsequent analysis of disaccharidase activities and intestinal histology [11,21].

Mutations in the coding region of the *SI* gene are the major cause of CSID [22,23]. Genetically altered SI protein may exhibit perturbations in intracellular transport, polarized sorting and abnormal processing [24–26].

Population studies have estimated the frequency of CSID as 0.2% of individuals of European descents, 5% to 10% in indigenous Greenlanders and 3% to 7% of Inuits of Alaska and Canada [12,27–30]. Four common mutations, p.G1073D, p.V577G, p.F1745C and p.R1124X, are suggested to account for 83% of disease alleles in European descendants diagnosed with CSID. Gene sequencing and molecular analysis in patients with suspected CSID can help to identify pathogenic protein variants and aid to functionally categorize SI mutations. This may facilitate a diagnosis and subsequent earlier treatment of CSID.

Some of the naturally occurring SI mutants have been previously identified and characterized at the molecular level by our group [10,31–33].

In the present study we revealed the molecular pathogenesis in a selection of 13 novel SI mutations from patients with confirmed biopsy diagnosis of CSID and defined three biosynthetic phenotypes according to the molecular pathogenicity of the respective mutation. The analyzed mutations also included the R1124X mutation, which is described as one of the four most common mutations in European descents. The *in vitro* pathogenicity was determined based on the effect of the mutation on the SI functionality. Therefore we expressed cDNAs, encoding the different SI mutants, in a COS-1 cell system and analyzed the biosynthesis, structure and enzymatic activity of the SI protein variants.

2. Materials and methods

2.1. Materials and reagents

Tissue culture dishes were obtained from Sarstedt (Nüembrecht, Germany). Dulbecco's Modified Eagle's Medium (DMEM), methionine-free DMEM, penicillin, streptomycin, fetal calf serum, trypsin-EDTA for cell culture, protease inhibitors, DEAE-dextran, protein A-sepharose, trypsin, and Triton X-100 were purchased from Sigma-Aldrich (Deisenhofen, Germany). Acrylamide, TEMED, SDS, Tris, paraformaldehyde, dithiothreitol (DTT), polyvinyl difluoride (PVDF) membrane, ProLong Gold Antifade Reagent with DAPI, as well as sucrose and isomaltose were purchased from Carl Roth GmbH (Karlsruhe, Germany). [^{35}S]-methionine (>1000 Ci/mmol) was obtained from PerkinElmer (Waltham, Massachusetts). Glucose oxidase-peroxidase mono-reagent was obtained from Axiom GmbH (Bürstadt, Germany). Restriction enzymes, molecular weight standards for SDS-PAGE, Isis proofreading DNA polymerase and SuperSignal™ West Fermento maximum sensitivity western blot chemiluminescence substrate were obtained from Thermo Fisher Scientific GmbH (Schwerte, Germany). Endo-β-*N*-acetylglucosaminidase H (endo H) was acquired from Roche Diagnostics (Mannheim, Germany). Lubrol WX was purchased from MP Biomedicals (Eschwege, Germany). All other reagents were of superior analytic grade.

2.2. Immunochemical reagents

The monoclonal mouse anti-SI antibodies (mAb) hSI2, HBB1/691/79, HBB2/614/88, HBB2/219/20, and HBB3/705/60 [34,35] were kindly provided by Dr. H.P. Hauri and Dr. E.E. Sterchi (Bern, Switzerland). To recognize all conformations and glycoforms of the SI mutants a mixture of these antibodies was used for immunoprecipitation. Anti-calnexin antibody produced in rabbit was obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Secondary antibodies coupled to Alexa Fluor dyes were obtained from Invitrogen (Karlsruhe, Germany) and horseradish peroxidase conjugated secondary antibodies were from Thermo Fisher Scientific (Schwerte, Germany).

2.3. Recruitment of CSID patients and identification of mutations in the SI gene

A total of 31 probands with a confirmed biopsy diagnosis of CSID were recruited by different attempts, including a letter to gastroenterologists, booth and literature at gastroenterology meetings, package inserts in Sucraid boxes, and notices on patient listservs by families participating in the study. All patients had bowel biopsies with normal lactase but reduced or absent sucrase and/or isomaltase activities and a normal histology. DNA analysis was performed with patient consent and IRB approval. DNA for sequencing of the entire *SI* gene coding region was extracted from blood or saliva of the patients. DNA sequencing was performed by Sanger dideoxy sequencing [36] using an ABI Prism 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA). The sucrase-isomaltase exons and flanking introns were compared to the GeneBank number NM_001041.3 to detect nucleotide alterations [30].

2.4. Construction of cDNA clones

SI mutations were generated by site-directed mutagenesis PCR using a full length cDNA encoding SI in a pSG8 vector as template [37] and Isis proofreading polymerase for the PCR amplification. Sequences of single strand oligonucleotide pairs used for mutagenesis are presented in Table 1. For analytical digestion of the resulting clones particular restriction sites were introduced or deleted in the vicinity of the target site by silent mutations within the oligonucleotide pairs. SI mutations were finally validated by Sanger sequencing.

Table 1
Sequence of the forward oligonucleotides used for mutagenesis PCR of SI wild type. The reverse oligonucleotides have the reverse complementary sequence. The mutagenic base pair codons are underlined and the corresponding amino acids are listed below.

SI mutants	Oligonucleotide sequences used for mutagenesis (5′–3′)
W105C	CGTGGAATGACTCTCTTATTCCTGTTGCTTCTTCGTTGATAATCATG Cys
F139Y	CCTTCACCTACACTATACGGAAATGATATCAACAGTGTTC Tyr
Q307Y	GTTTTTTTAATGAATTCCAACGCAATGGAGATTTTTATCTATCCTACACCAAT AGTAAC Tyr
D536V	CACCGTTTACTCCTGATATCCTTGTCAAACTCATGTATTCCAA Val
S594P	CTCAACATTTGCTGGTCCTGGTCGACATGCTGCTCATTGG Pro
L741P	CTGAGTTTTTGTGGGGACCTGCATTACCTATTACTCCTGTTCTA Pro
Q930R	GAAACTTTAGTGTTCGATGGAATCAGATCTTCTCAGAAAATG Arg
W931R	GCAGATCTCAAGCTTAATCTCGGAAGAAACTTTAGTGTTCAACGGAATCAAA TTT Arg
W931X	CTTTAGTGTTCAATAGAATCAGATCTTCTCAGAAAATGA Stop
R1124X	GAACATACAGCATTTAAGTGATATCTGAACTGGAATAC Stop
C1531Y	TGTTTGGAATGTCATATACTGGGCAGATATCTATGGTTTTTTCAACAACTC Tyr
R1544C	GAATATCATCTCTGTACCTGTTGATGCAGCTGGGAGCATTTTATCCA Cys
T1606I	CATGAAATTCATGCTAATGGTGGCATTGTTATCCG Ile

2.5. Cell culture, transient transfection and biosynthetic labeling

COS-1 cells were grown in 100-mm culture dishes and maintained *in vitro* by serial passages at 37 °C in a humidified atmosphere with 5% CO_2. The cells were cultivated in DMEM containing 1 g/L glucose, 10% fetal calf serum (*v*/*v*), 100 U/mL penicillin and 100 μg/mL streptomycin. Transient transfection of the cells was performed by the DEAE-dextran method as described previously [38]. The COS-1 cells were used 48 h post-transfection for different analyses including biosynthetic labeling with [^{35}S]-methionine as described before [39].

2.6. Cell lysis and immunoprecipitation

Solubilization of the cells and immunoprecipitation of SI from the lysates has been essentially performed as described previously [2,40,41]. For endoglycosidase studies, the immunoprecipitants were subjected to *endo*-β-*N*-acetylglucosaminidase H (endo H) treatment as described before [2].

2.7. SDS-PAGE and protein detection

Cell lysates or immunoprecipitants of unlabeled samples were mixed with Laemmli buffer and DTT, cooked 5 min at 95 °C and resolved on 6% slab gels. After protein transfer to the PVDF membrane, immunedetection of SI was performed by either HBB2/614/88 (recognizing the sucrase subunit) or HBB3/705/60 (recognizing the isomaltase subunit) primary antibodies followed by HRP-conjugated secondary antibody incubation and washing steps after each incubation. The corresponding bands were detected by a ChemiDoc XRS System (Bio-Rad, Munich, Germany) after addition of chemiluminescent substrate. The radioactive labeled samples were similarly resolved on SDS-PAGE, dried on filter papers, exposed to phosphor plates and detected by a phosphor imager (Bio-Rad, Munich, Germany).

2.8. Isolation of detergent-resistant membrane microdomains

Transiently transfected COS-1 cells were solubilized at 4 °C for 4 h with 1% Lubrol WX or Triton X-100 in 10 mM Tris buffer pH 8.0 with 150 mM NaCl. Lipid rafts were isolated using sucrose discontinuous gradient as described before [42]. Fractions of 1 mL were collected from top and denoted 1 to 10. For SDS-PAGE analysis, a part from fractions 1 to 3, 4 to 7 and 8 to 10 was pooled separately and the proteins were extracted and precipitated using chloroform-methanol precipitation [43]. The protein pellet was dissolved in Laemmli buffer plus DTT, resolved on SDS-PAGE and immunoblotted for SI, flotillin-2 and Rho A proteins.

2.9. Enzyme activity measurements

Immunoprecipitants of SI were washed with PBS containing 0.5% Triton X-100 and split to two equal parts. One part was incubated with either sucrose (150 mM) or isomaltose (30 mM) substrate for 1 h at 37 °C to measure the enzyme activity. Activities were calculated based on the detection of the released glucose by adding glucose oxidase-peroxidase mono-reagent and measuring of 492 nm absorbance with a microplate reader. The second part of the immunoprecipitants was used for immunoblotting against SI. The intensity of the SI signal in each sample was quantified and normalized to the band intensity of the wild type sample. Relative specific activities were determined by dividing the enzyme activities to the relative protein intensities.

2.10. Confocal fluorescence microscopy

COS-1 cells were seeded on coverslips, transfected with SI constructs and fixed 48 h post-transfection with 4% paraformaldehyde. The samples were prepared as described before [44] using a combination of mouse anti-SI primary antibodies to detect SI and rabbit anti-calnexin antibody as an ER marker. ProLong Gold Antifade Reagent with DAPI was used to visualize the cell nucleus and for mounting of the coverslips. The samples were examined by a Leica TCS SP5 confocal microscope with a HCPL APO 63 × 1.3 glycerol immersion objective.

2.11. Tryptic structural analysis

Transfected COS-1 cells were labeled for 6 h with [^{35}S]-methionine. Then SI was immunoprecipitated, washed and treated with 500 BAEE units of trypsin for 1 h at 37 °C. The reaction was stopped by the addition of Laemmli buffer plus DTT and cooking for 5 min at 95 °C. The samples were finally analyzed by SDS-PAGE. For differential identification of the bands corresponding to the sucrase or the isomaltase subunit, lysates of unlabeled cells were similarly treated with trypsin and subjected to immunoblotting with either HBB2/614/88 or HBB3/705/60 antibodies to visualize sucrase or isomaltase subunits, respectively.

2.12. Bioinformatic and statistical analysis

Alignment of the SI sequences has been performed by PRofile ALIgNEment (PRALINE) multiple sequence alignment application [45]. Immunoblot bands were quantified by the Quantity One 1-D Analysis Software (Bio-Rad Laboratories GmbH). Data analysis was performed by Microsoft Excel and the indicated error bars were calculated according to the standard error of the mean (SEM) from at least three independent repeats. Results are expressed as means ± SEM of $n \geq 3$ independent experiments. Statistical analysis was performed using paired Student *t*-test compared to a wild type control. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3. Results

3.1. Description of the SI mutations

The focus of this study was to define the molecular pathogenicity of 13 missense mutations in the *SI* gene. Therefore the functional influence of the genetic defect on the resulting SI protein variant was analyzed *in vitro*. The 13 mutations were among 56 different abnormal alleles, which were identified by screening the entire *SI* gene coding region in 31 patients with confirmed small bowel biopsy diagnosis of CSID [30]. Pathogenic variants identified in individuals with clinical symptoms were selected for this study. All individuals were of Northern European ethnic background, had symptoms of abdominal bloating, pain and diarrhea when ingesting complex carbohydrates. Duodenal biopsies were performed to document the enzymatic deficiency of sucrase and isomaltase in tissue samples (Table 2). The biopsies from the patients revealed no histological changes and reduced or absent enzymatic activities of isomaltase and sucrase [30]. Pathogenic variants were subsequently identified in DNA isolated from peripheral blood. All of the mutations were found to be inherited in a compound heterozygous pattern (Table 2). For the mutations Q307Y, F139Y and S594P no *ex vivo* data are available. All amino acid exchanges targeted motifs of the SI protein which are highly conserved among different species, indicating the structural or functional importance of these regions (Fig. 1).

3.2. Biosynthetic studies reveal three trafficking-relevant classes of SI mutants

Investigation of the maturation of each individual SI variant from the mannose-rich glycosylated SI precursor protein (SI_h, ~210 kDa) to its complex N- and O-glycosylated mature form (SI_c, ~245 kDa) in transiently transfected COS-1 cells led to the identification of three distinct biosynthetic phenotypes of the novel mutants. After 8 h of biosynthetic labeling 70% of the SI wild type protein was complex glycosylated and reached the Golgi apparatus or the cell surface (Fig. 2). SI variants

Table 2
Comparison of the activities of purified SI mutants *versus* their counterparts in the patients' intestinal biopsy specimens. The individual SI mutants were immunoprecipitated and their activities towards sucrose and isomaltose were measured. These activities are shown as a percentage of the SI wild type activities. Activities of SI in the biopsy specimens were measured using sucrose and palatinose (instead of isomaltose) and are shown in units. All studied sucrase-isomaltase mutations were found in a compound heterozygote background of inheritance.
References for sucrase (35–131 U) and isomaltase (32–139 U) activities are according to Dahlqvist [60], palatinose (3.8–41.54 U) activities are according to Gupta [61].

	Biosynthetic phenotype	Sucrase	Isomaltase/palatinase
L741P	III	5	6
F1745C	III	0	0
Patient		0	0
R1124X	III	0	0
G1073D	III	0	4
Patient		0	2
D536V	I	58	5.6
V577G	III	0	0
Patient		0	0
W105C	I	30	61
W931X	III	0	0
Patient		2.61	7.17
G1073D	III	0	4
C1531Y	II	7	109
Patient		5.9	3.5
Q930R	I	80	58
R1544C	III	3	71
Patient		15.3	22.5
W931R	III	0	11
T1606I	III	19	76
Patient		0	0

grouped in the first biosynthetic phenotype (W105C, F139Y, D536V and Q930R) mature in the cell at essentially similar maturation rates to the wild type SI. A proportion of 50% to 70% of total SI is present as the mature complex glycosylated protein after 8 h of labeling (Fig. 2). In two other SI protein variants (Q307Y and C1531Y) the genetic alteration elicited a partial and delayed maturation of SI with low amounts of complex glycosylated protein after 8 h of labeling. These SI variants were grouped in a second biosynthetic phenotype. A third biosynthetic phenotype was caused by SI variants (S594P, L741P, W931R, R1544C and T1606I) that do not traverse the Golgi and persist as mannose-rich glycosylated proteins concomitant with their block in the early secretory pathway, most likely in the ER (Fig. 2). The third phenotype comprised also two stop codon mutations, R1124X and W931X. These mutations resulted in truncated protein variants that revealed no sucrase subunit and respectively only a small part of the isomaltase subunit (Fig. 2). The R1124X variant was even not detectable by all our monoclonal antibodies utilized under native conditions, indicating a severe malfolding. Both SI variants are likely targeted to ER associated degradation.

The cellular localization of the SI mutants was found to be concomitant with the biosynthetic labeling data representing three classes with (i) strong Golgi-like and cell surface appearance of SI, (ii) partial Golgi-like localization, and (iii) complete co-localization with calnexin and ER arrest (Fig. 3).

As shown in Fig. 4, tryptic profiles of the SI mutant varied from a complete or partial degradation to a wild type-like cleavage pattern. The results are summarized in Table 3. Notably, misfolding as represented by the level of sensitivity to trypsin has a direct association with trafficking arrest in the ER and loss of function.

From the *trans*-Golgi network, the sorting of SI to the apical cell surface requires association with membrane microdomains or, lipid rafts, as a trafficking platform [8]. We examined the association of the SI mutants with lipid microdomains to seek for any correlation between the mutation and biosynthetic phenotype in terms of the trafficking and ultimately function. Fig. 5 demonstrates that SI mutants that have

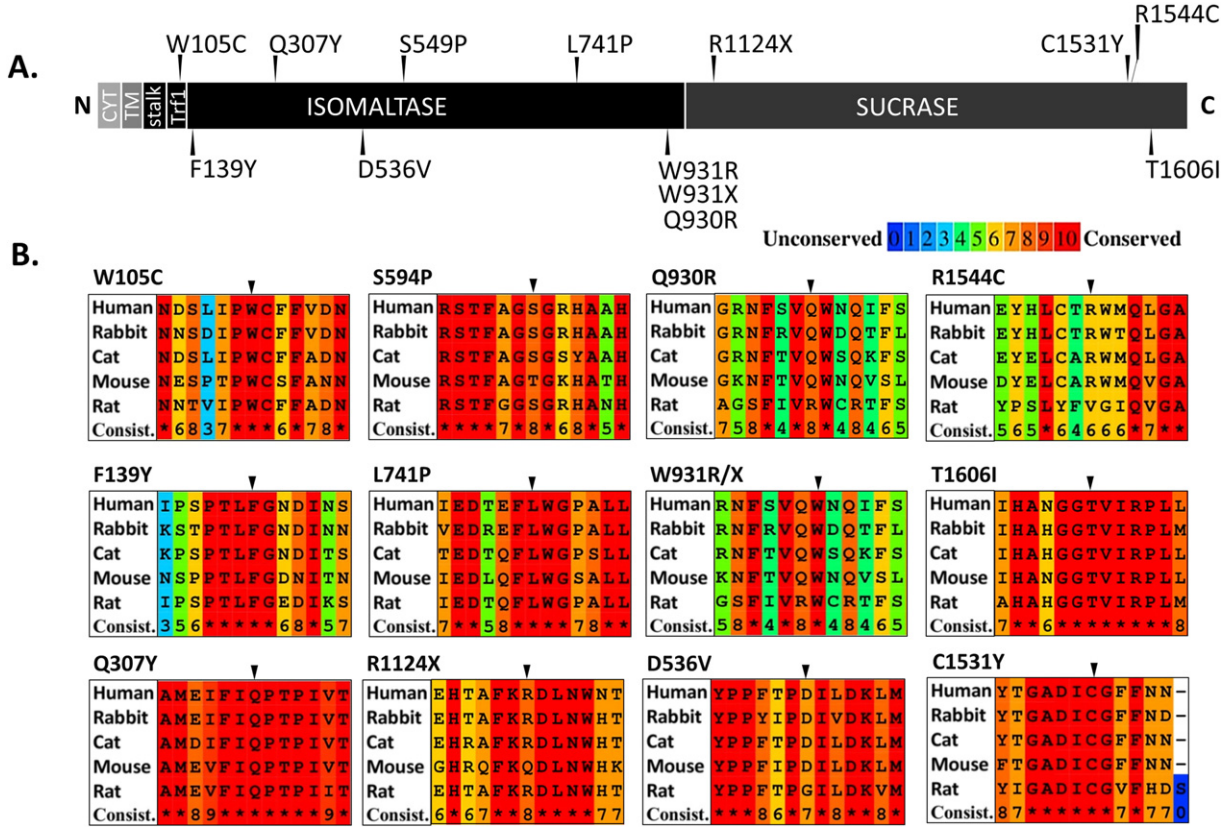

Fig. 1. The mutations studied target mainly highly conserved amino acid residues in consensus motifs of SI. (A) Schematic presentation of the position of the mutations within the SI molecule. CYT: cytosolic tail, TM: transmembrane domain, Trf1: trefoil 1. (B) Homology-extended multiple sequence alignment of the primary structure of SI among different mammalian species for the motifs containing the mutation site (dark arrow). The color spectrum represents the degree of amino acid conservation in these motifs.

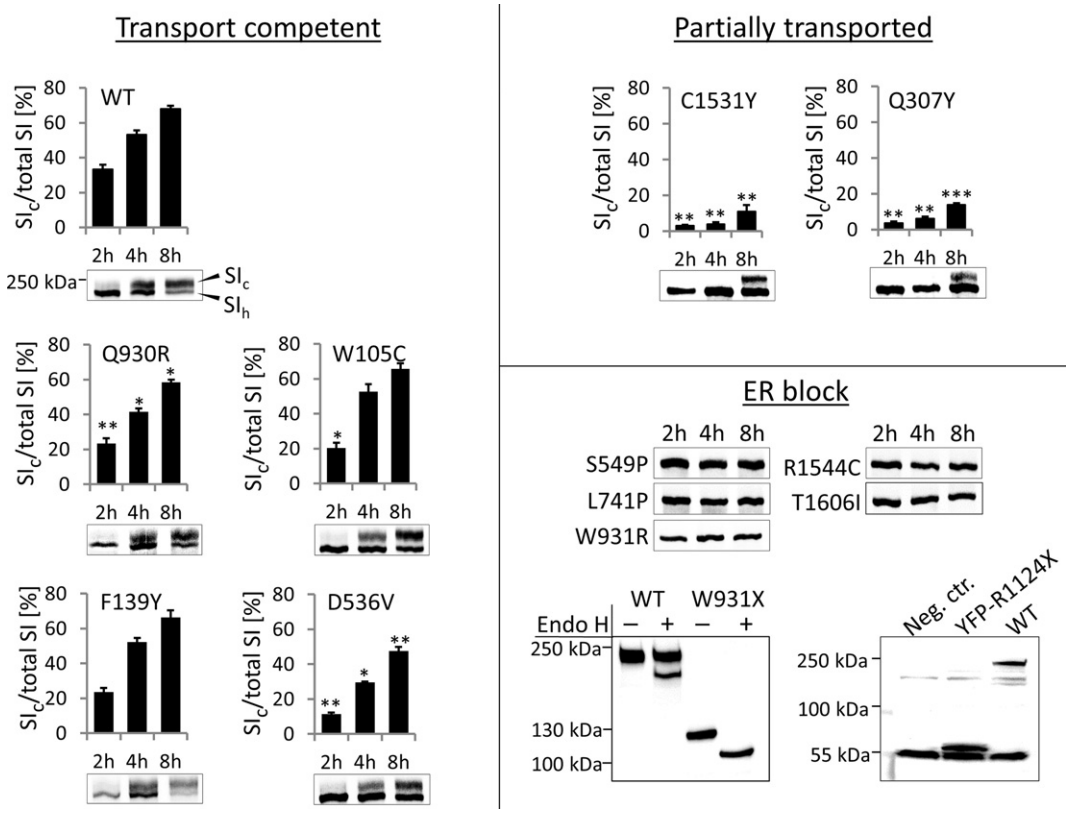

Fig. 2. Maturation rate of the SI mutants compared to the wild type SI. (A) Detection of SI from transiently transfected COS-1 cells after biosynthetic labeling. SI_h: immature mannose-rich form, SI_c: mature complex N- and O-glycosylated form, $n \geq 3$. The stop codon variants SI-W931X and SI-R1124X were transiently expressed in COS-1 cells and detected by immunoblotting. The SI-W931X mutant could be only detected in western blots as an ER-located endo H-sensitive protein and the SI-R1124X mutant was partially degraded in the cell and detectable only when tagged with YFP at its N-terminus.

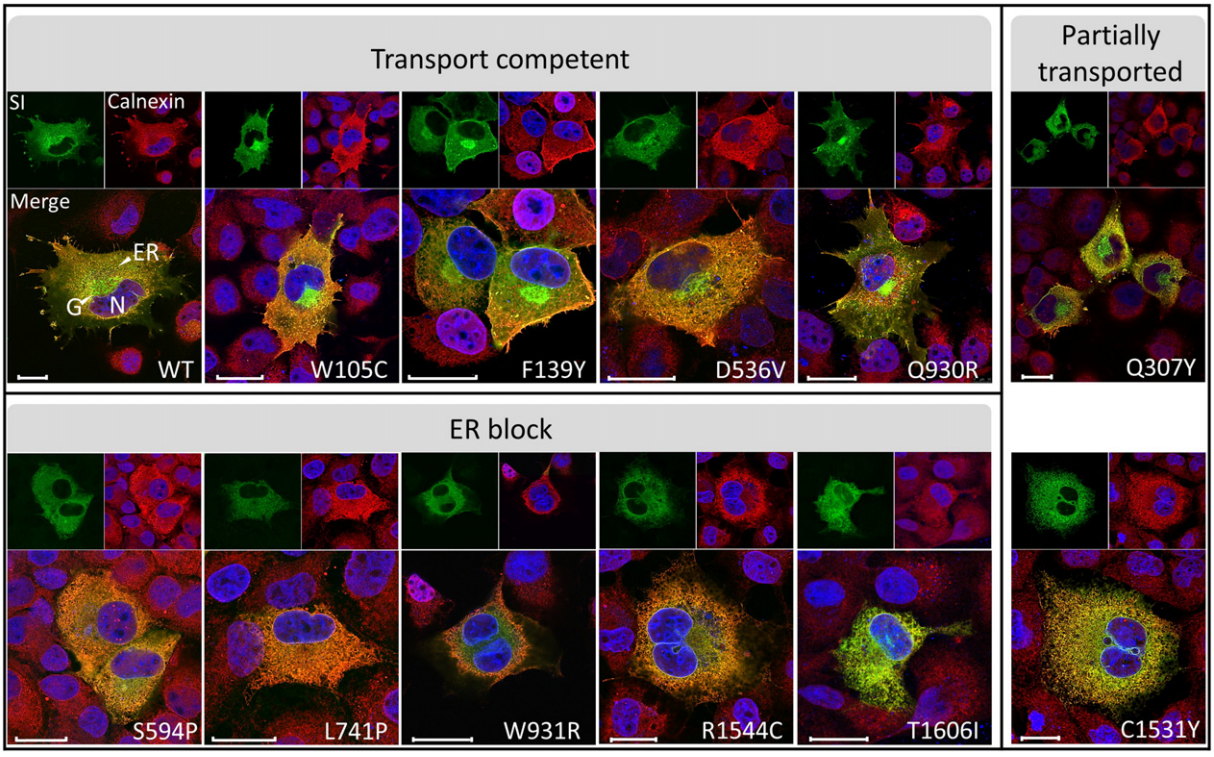

Fig. 3. Confocal microscope analysis of the SI wild type and mutants expressed in COS-1 cells. SI (green) was visualized in combination with the cell nucleus (blue) and endogenous calnexin (red) as an ER marker using indirect fluorescence. Scale bars: 25 μm, G: Golgi apparatus, N: nucleus.

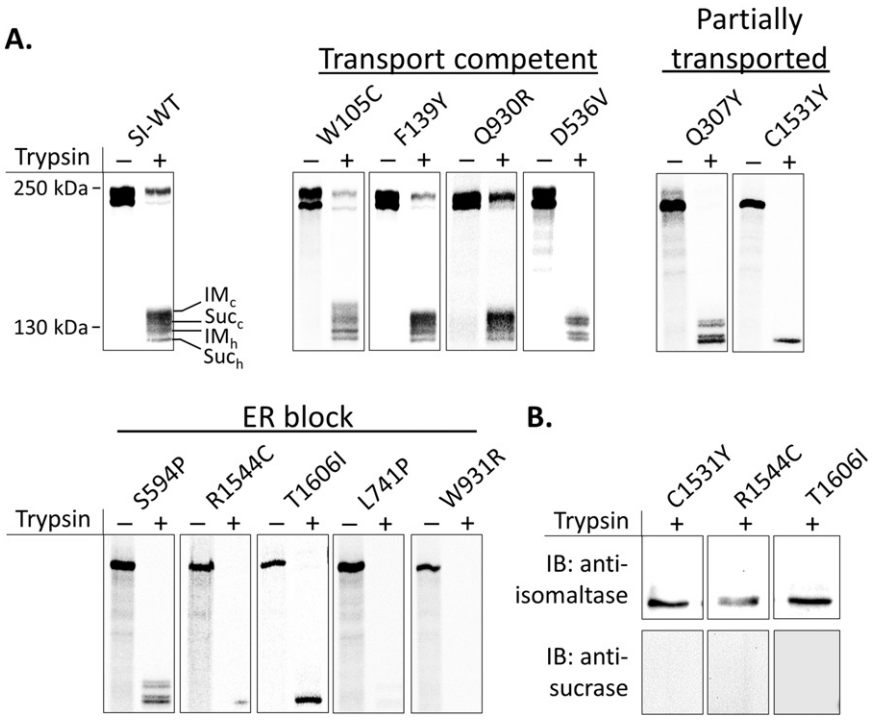

Fig. 4. Examination of the protein folding of the SI mutants by trypsin treatment. (A) After biosynthetic labeling and immunoprecipitation, the wild type and different mutants of SI were treated with 500 BAEE units trypsin for 1 h. IM_c/Suc_c: complex N- and O-glycosylated isomaltase and sucrase subunits, IM_h/Suc_h: mannose-rich forms of the isomaltase and sucrase subunits. (B) The single-band cleavage products of SI-C1531Y, SI-R1544C and SI-T1606I mutants were further characterized using differential immunoblotting of the trypsinized unlabeled material with anti-isomaltase (HBB3/705/60) or anti-sucrase (HBB2/614/88) monoclonal antibodies.

acquired complex glycosylation were associated to a large extent with the lipid rafts revealed in the floating fractions of the sucrose gradients. Other mutants displayed substantially lower levels or no incorporation into the lipid rafts (Fig. 5). Here, flotillin-2 in fractions 1–3 serves as a lipid raft marker [46] and Rho A in fractions 8–10 delineates the non-raft fractions. We conclude that the lipid raft association of SI variants correlate well with their maturation pattern and constitute another criterion that describes the impact of a particular mutation on the SI function.

In conclusion, the analyses of the SI variants at the biochemical and cell biological levels revealed three biosynthetic phenotypes (I–III). Phenotype I comprise SI mutants that showed comparable trafficking to wild type SI as well as virtually normal protein folding. The second phenotype, phenotype II, harbors two mutants with delayed trafficking and normal or partial folding. Phenotype III, the most severe one, includes mutants located in the ER that are either partially folded or completely misfolded.

3.3. Functional variations elicited by the mutations

The digestive capacities of the individual SI mutants were assessed *in vitro* and correlated with the sucrase (SUC) and isomaltase (IM) *ex vivo* activities measured in small bowel biopsies of CSID patients (Table 2). For this purpose, wild type SI or SI variants were purified by immunoprecipitation from the transiently transfected COS-1 cells and the SUC and IM specific activities were determined. Fig. 6 demonstrates that mutants from phenotype I and phenotype II showed reduced but varying enzymatic activities of SUC and IM. Only one of the mutants from phenotype I, Q930R, showed normal activities. Nearly all transport incompetent mutants

Table 3
Overview of the biochemical and functional features of the single SI variants compared to the wild type. A categorization of the SI mutants into 3 biosynthetic phenotypes was performed according to the influence of the respective mutation on the SI biochemical features and overall function.

	SI mutation	Domain of mutation	Maturation [%]	Isomaltase activity [%]	Sucrase activity [%]	Tryptic cleavage pattern	Lipid raft association [%]
WT	–	–	100	100	100	Normal	100
Biosynthetic phenotype I	W105C	Trefoil 1	96	61	30	Normal	92
	F139Y	Isomaltase	97	66	47	Normal	63
	Q930R	Isomaltase	87	98	102	Normal	107
	D536V	Isomaltase	70	1	58	Normal	77
Biosynthetic phenotype II	C1531Y	Sucrase	16	109	7	Part. degraded	35
	Q307Y	Isomaltase	20	2	22	Normal	42
Biosynthetic phenotype III	T1606I	Sucrase	0	76	19	Part. degraded	39
	R1544C	Sucrase	0	71	3	Part. degraded	30
	S594P	Isomaltase	0	4	4	Normal	20
	W931R	Isomaltase	0	11	11	Degraded	0.1
	L741P	Isomaltase	0	6	6	Degraded	0
	W931X	Isomaltase	0	0	0	Degraded	0
	R1124X	Sucrase	0	0	0	Degraded	0

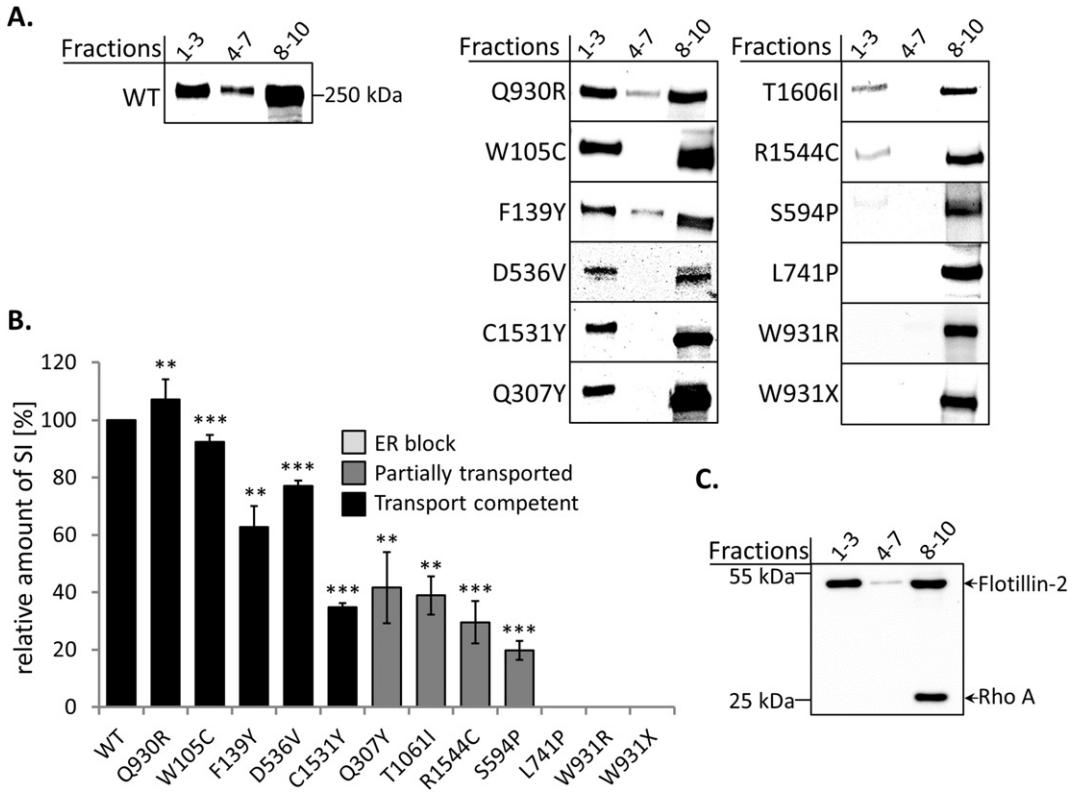

Fig. 5. Association of SI mutants with cholesterol-enriched membrane microdomains or lipid rafts. (A) Transiently transfected COS-1 cells were solubilized with 1% Lubrol WX and lipid raft and non-raft fractions were separated using sucrose density gradients. Lipid raft fractions (1–3) and non-raft fractions (4–7, 8–10) were pooled and analyzed by immunoblotting with anti-SI antibodies. (B) Graphical presentation of the lipid raft enrichment factor of SI mutants *versus* the wild type. The amount of wild type SI associated with lipid rafts was set to 100% and lipid raft association of the SI mutants was normalized to the wild type. (C) Representative control immunoblot for the lipid raft preparations illustrating the distribution of flotillin-2 which is mainly associated with lipid rafts and Rho A as a non-raft marker.

from phenotype III, S594P, L741P, W931R, W931X and R1124X, were also enzymatically inactive. The two remaining mutants from phenotype III, R1544C and T1606I, displayed isomaltase activity but cannot reach the cell surface due to their trafficking defect to fulfil their functions (Fig. 6). The loss of *in vitro* function of phenotype III mutants was reflected by the enzymatic activities measured in small bowel biopsies of the CSID patients (Table 2). Patients with inheritance of one mutation from phenotype III on each allele also showed a complete loss of intestinal SI activities *in vivo*. The combination of mutations from phenotype III with that of phenotype I or II in patients led to reduced but varying activities matching to a certain extent with our *in vitro* data (Table 2).

Notably, our *in vitro* modelling revealed that mutations in one subunit can also influence the activity of the other subunit most likely by

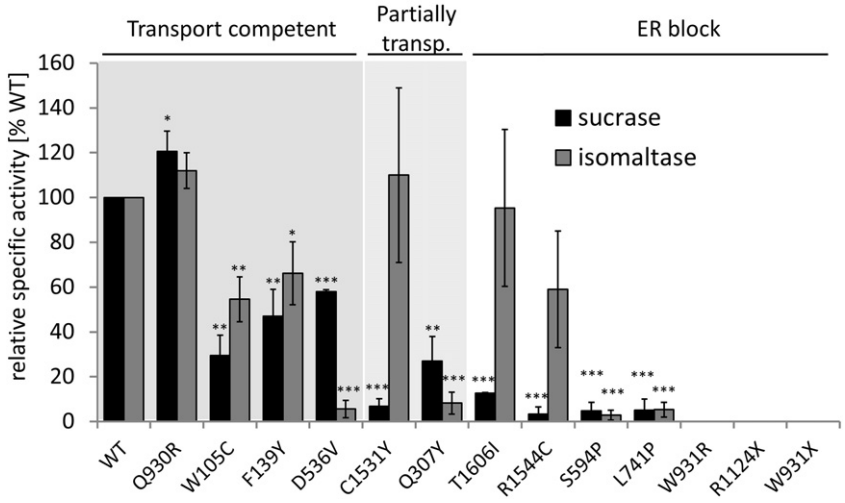

Fig. 6. Specific enzyme activities of the SI mutants *versus* wild type SI. Wild type or mutants of SI were immunoprecipitated from transfected COS-1 cells and assessed for sucrase and isomaltase activities. The relative SI content of each sample was determined by immunoblotting. Relative specific activities are recorded in comparison to wild type SI. SI mutants are grouped based on their similar trafficking characteristics.

imposing structural changes. Interestingly, mutations in the IM affected activities in both subunits and that in SUC mostly led to loss of the SUC activity, with preserved the IM activity.

The IM-located mutations, Q307Y and D536V, completely abolished the IM activity and substantially reduced the SUC activity. The SUC mutations on the other hand C1531Y, R1544C and T1606I were associated with complete reduction in SUC, while IM was either not affected or its activity was only slightly reduced (Fig. 6).

Moreover the detection of IM activity in some mannose-rich glycosylated transport incompetent mutants suggests that complex N- and O-glycosylation of IM is required for proper transport but not essentially for its enzyme activity. Nevertheless, the active enzyme will not be available at the cell surface, which is required for disaccharide digestion in the intestinal lumen.

4. Discussion

In the present study we have analyzed the pathobiochemistry of 13 naturally occurring missense mutations in SI, which have been identified in patients with CSID. This study has unraveled a remarkable heterogeneity in the molecular pathogenesis of CSID revealing the unique molecular etiologies of this multifaceted intestinal malabsorption disorder. CSID belongs to a group of protein folding diseases that can be triggered by improper folding like CFTR in cystic fibrosis and β-glucosidase in Gaucher disease [47–50], improper targeting and localization like AAT deficiency [51], dominant negative mutations like Keratin in epidermolysis bullosa simplex [52] or accumulation of aggregated proteins like amyloid accumulation [53]. Unlike many of the aforementioned diseases, however, multiple mechanisms can elicit CSID rendering this intestinal disorder unique among other protein folding diseases. In fact, mutations identified in patients of CSID are associated with severe biosynthetic phenotypes of altered folding, aberrant trafficking, missorting and even functional deficits in a correctly folded protein [26,31–33,54].

The molecular and cellular analyses of these 13 novel mutants have led to the categorization of CSID into three major biosynthetic phenotypes based on criteria that comprised protein trafficking, enzyme function and lipid raft association of SI mutants (Fig. 8).

The first group of mutants includes W105C, F139Y, D536V and Q930R with comparable maturation rates to wild type SI. These mutants do not reveal gross structural alterations and are properly trafficked along the secretory pathway, but not necessarily to the cell surface. The normal maturation is not always associated with normal activity levels. In fact, while the Q930R mutant is almost as active as wild type SI, the remaining mutants of this category reveal reduced activities of down to 30% *versus* the wild type SI (Table 3).

The second group includes Q307Y and C1531Y and is characterized by delayed maturation and reduced digestive capacities. The third group consists of L741P, S594P, R1544C, T1606I, W931R, W931X, and R1124X and constitutes the most severe effect on the SI function *in vitro*. The major characteristics of the mutants in this group are their intracellular block in the ER as immature mannose-rich glycosylated forms as well as the functional deficits with complete loss of sucrase and isomaltase activities. The residual enzymatic activities of a few mutants cannot be made accessible to the substrate *in vivo* due to the failure of these mutants to reach the cell surface. In particular the complete loss of function of mutations grouped into the phenotype III indicates a high molecular pathogenicity of these mutants.

The classification of the mutants into three groups may provide an explanation for the variations in the enzymatic activities and subsequently the underlying pathomechanisms that elicit severe, intermediate or mild symptoms in several cases of CSID. While the level of severity in CSID cases elicited by compound heterozygous mutations belonging to phenotype III would be expected to be the highest, a combination of a mutation of phenotype III, for example R1544C with a mutation from phenotype I Q930R may generate a milder form of CSID. In fact, measurements of the enzyme activity levels support this view.

The current data propose that the pathogenesis of CSID is a multifactorial network of events that altogether elicit the wide heterogeneity in the overall pattern of CSID. The biosynthetic, trafficking, functional features as well as the lipid raft association of the individual mutants comprise most essential elements in the regulation of the carbohydrate digestive capacity of the SI isoforms. Nevertheless, the degree of a putative regulatory influence of these mutants on each other in compound heterozygosity should be addressed. Particularly in cases when mutants with wild type-like trafficking behaviour are co-expressed with highly pathogenic ER-located mutants a potential interaction of the mutants with ultimate influence on their subsequent targeting along the secretory pathway and functional characteristics can be anticipated. In fact, SI is a type II membrane glycoprotein and an SI monomer may interact with another SI monomer in an oligomer-like fashion similar to that described for type II membrane proteins of the *medial*-Golgi and referred to as kin recognition [55]. This mode of interaction is supported by the fact that SI acquires dimeric topology along the secretory pathway [56] and retains this topology at the brush border membrane [57] (Fig. 7).

Another factor that offers an explanation for the wide variations in the SI activities in compound heterozygote subjects is the mosaic expression pattern of the disaccharidases including SI. Regardless of any abnormalities in the structure or function of SI, the gene expression of this enzyme can be downregulated in different regions of the intestinal epithelium that is ultimately associated with reduced carbohydrate digestion capacity of the intestine [58,59]. Hitherto individuals with an *a priori* reduced expression of wild type SI will be more susceptible to develop gastrointestinal symptoms in a heterozygote background. This possibility is similar to a haploinsufficiency model, in which one gene allele produces low levels of a functionally active protein, while the other allele is pathogenic. The difference, however, to the mosaic expression pattern of SI is the heterogeneous expression levels of wild type SI in the enterocytes that vary from low to normal [58].

Current concepts have established CSID to be inherited as homozygous or compound heterozygous traits [22]. Interestingly, a study with a cohort of Hungarian patients suffering from symptoms similar to those in CSID patients revealed that 5 out of 11 subjects are heterozygotes for the severe mutations G1073D, F1745C, C1229Y and T694P [9]. The high frequency of heterozygotes in the Hungarian patients suggests that heterozygous insufficiency is a potentially novel phenotype of CSID. The fact that the mutants with this pattern of inheritance are highly pathogenic suggests that an interaction between a wild type SI and one of these mutants may downregulate the activity of the wild type

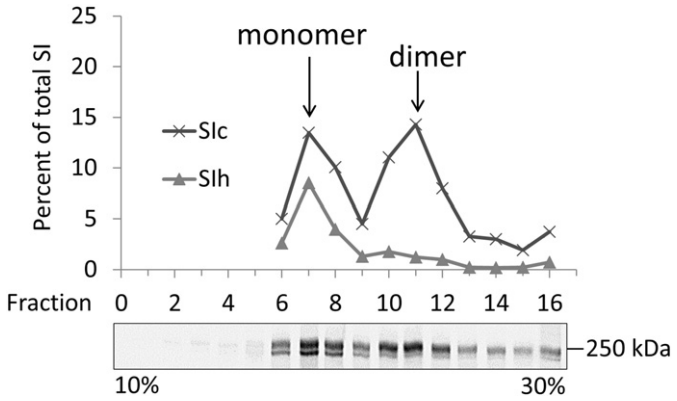

Fig. 7. Quaternary structure of sucrase-isomaltase. The complex glycosylated biosynthetic form of SI is revealed in two peaks of the gradient compatible with two different quaternary structures. SI_h: immature mannose-rich form, SI_c: mature complex N- and O-glycosylated form.

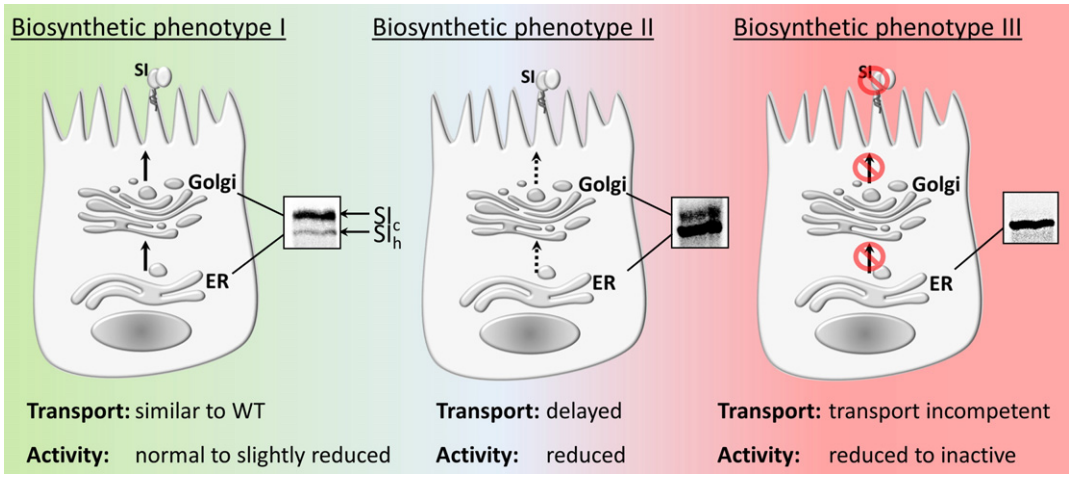

Fig. 8. Classification of SI variants into three major biosynthetic phenotypes. In this model SI mutants are classified based on their molecular and functional characteristics *in vitro*. For expression of functional SI at the brush border membrane of the intestinal epithelium, the polypeptide should be properly folded, complex N- and O-glycosylated along the secretory pathway and sorted to the apical cell surface *via* association with cholesterol- and sphingolipid-enriched membrane microdomains. The intracellular trafficking behaviour of the mutants within the enterocyte is depicted. Representative radioactive micrographs show the maturation of SI. SI_c: complex N- and O-glycosylated SI; SI_h: mannose-rich immature form of SI.

substantially in the formed oligomer. As a consequence, SI-related malabsorption could be considered to be a more common disorder than initially thought. Nevertheless, this pattern of inheritance requires more detailed analyses at the molecular and cellular levels to establish itself as an independent novel class of CSID.

In conclusion, the current study supports the existence of heterogeneous forms of CSID that vary in their degree of enzymatic activity and probably clinical severity in light of a homozygous and compound heterozygote inheritance pattern. The mild to severe gradient in the SI function due to the mutants supports the view that a proper diagnosis of CSID could presumably be missed in several cases and introduces a new paradigm into CSID, which seems to occur in more individuals than initially thought.

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Author contributions

BG and MA performed the experiments, interpreted results and drafted the manuscript. CRS provided patients' data and revised the manuscript. HYN designed the study, interpreted results and drafted the manuscript.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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