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ESPGHAN and NASPGHAN Report on the Assessment of Exocrine Pancreatic Function and Pancreatitis in Children

*Christopher J. Taylor, †Kathy Chen, ‡Karoly Horvath, *David Hughes, §Mark E. Lowe, ‡Devendra Mehta, §Abrahim I. Orabi, ||Jeremy Screws, *Mike Thomson, ¶Stephanie Van Biervliet, #Henkjan J. Verkade, §Sohail Z. Husain, and **Michael Wilschanski***

ABSTRACT

The purpose of this clinical report is to discuss several recent advances in assessing exocrine pancreatic insufficiency (EPI) and pancreatitis in children, to review the array of pancreatic function tests, to provide an update on the inherited causes of EPI, with special emphasis on newly available genetic testing, and to review newer methods for evaluating pancreatitis.

Key Words: exocrine pancreatic insufficiency, nonstimulatory PFTs, pancreatic function test, pancreatitis, steatorrhea, stimulatory PFTs

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ANATOMY AND PHYSIOLOGY OF THE PANCREAS

The pancreas forms from the fusion of the dorsal and ventral outgrowths, which develop from the embryologic foregut. Throughout development, the pancreas maintains a close relation with the biliary ductal system and the main pancreatic duct such that the main pancreatic duct and common bile duct empty into the duodenum at the same location via the ampulla of Vater (Fig. 1).

Pancreatic enzymes are synthesized in the pancreatic acinar cells, stored in secretory vesicles as inactive zymogens, and secreted into the duodenum in response to luminal fatty acids, peptides, and amino acids. Secretion is mediated by cholecystokinin (CCK) and secretin, peptide hormones released by I cells and S cells, respectively, in the mucosal epithelium of the small intestine. Proteolytic proenzymes, or zymogens, are activated by enteropeptidase, which is localized in the brush border of the duodenum and proximal jejunum (1). The activation of the principle zymogen trypsinogen to trypsin results in the subsequent activation of the entire cascade of zymogens (2). In adults, 6 to 20 g of digestive enzymes are secreted daily into the duodenum along with about 2.5 L of bicarbonate (HCO_3^-)-rich fluid, which neutralizes gastric acid and provides an optimum pH for pancreatic enzyme function.

Bicarbonate is secreted by the pancreatic ductal epithelium. Secretin is the main stimulant of fluid and bicarbonate release, and thus it mediates the flow of pancreatic juice into the duodenum. Secretion is regulated by the cystic fibrosis transmembrane conductance regulator (CFTR). The generated bicarbonate ion is also actively transported into the ductal lumen together with sodium and passive movement of water into the duct, which facilitates the flow of pancreatic fluid into the small intestine. Bicarbonate secretion in the proximal pancreatic ducts is largely mediated by SLC26A6, which is a Cl^-/HCO_3^- exchanger. In distal ducts, however, where the luminal bicarbonate concentration is already high, most of the bicarbonate secretion is mediated by bicarbonate conductance via the CFTR (3).

PANCREATIC INSUFFICIENCY AND STEATORRHEA

Exocrine pancreatic insufficiency (EPI) is defined as reduced pancreatic enzyme and bicarbonate secretion, or both, which results in the malabsorption of nutrients. Although pancreatic enzymes digest all of the 3 macronutrients—fat, protein, and carbohydrates—the inability to digest fat leads to steatorrhea, the main clinical symptom of EPI. Fats, mainly ingested as long-chain triglycerides, are deesterified by pancreatic lipases, which make up <10% of the total pancreatic enzyme output (4). Pancreatic lipase easily and irreversibly degraded when the luminal pH drops <4. The other major digestive lipase, gastric lipase, cannot fully compensate for the absence of pancreatic lipase. In infants, other enzymes, particularly pancreatic triglyceride lipase (PTL)-related protein 2 and bile salt-stimulated lipase (BSSL), are the key enzymes secreted from the pancreas that act with gastric lipase to achieve efficient fat absorption (5). BSSL is also present in

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From the *Sheffield Children's Hospital, Sheffield, UK, the †St Christopher's Hospital for Children, Philadelphia, PA, the ‡Arnold Palmer Hospital for Children, Orlando, FL, the §Children's Hospital of Pittsburgh of UPMC and the University of Pittsburgh, Pittsburgh, PA, the ||Children's Hospital of Erlanger, Chattanooga, TN, the ¶Ghent University Hospital, Ghent, Belgium, the #Beatrix Children's Hospital, University Medical Center, University of Groningen, Groningen, The Netherlands, and the **Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Address correspondence and reprint requests to Sohail Z. Husain, MD, Rangos Research Center, Suite 7123, 4401 Penn Ave, Pittsburgh, PA 15224 (e-mail: sohail.husain@chp.edu).

***Collaborators: Vandenplas Y, Gottrand F, Lionetti P, Papadopoulos A, Rümmele F, Tempia-Schäppi M, Thapar N, Orel R, Heuschkel R, Falconer J, and Karelis S.

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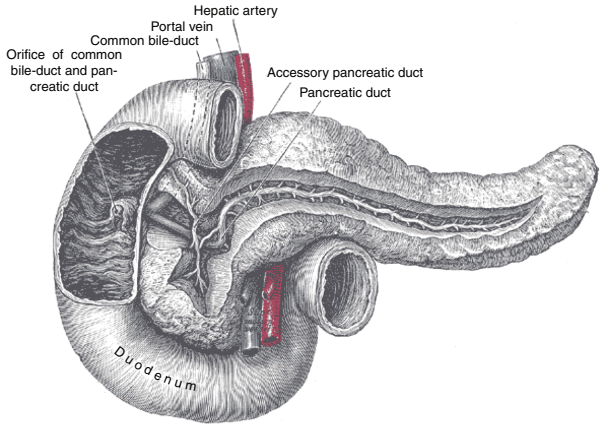

FIGURE 1. Normal pancreatic anatomy. The illustration is from the complete 20th US edition of *Gray's Anatomy of the Human Body* (Gray, 1918), which is in the public domain (downloaded from *www.commons.wikimedia.org*).

human milk, which facilitates fat absorption and growth in breast-fed preterm infants. Proteins and carbohydrates are digested by pancreatic proteases (or zymogens) and pancreatic amylase, respectively. Proteins can also be hydrolyzed to some extent by gastric pepsins, and carbohydrates can be hydrolyzed by salivary amylase.

Steatorrhea is defined as the presence of excess fat in the stool (6). It can manifest as diarrhea, large bulky, oily, or greasy stools, increased gas content, or stool floating on the toilet water. Patients with fat malabsorption can have weight loss, failure to thrive, and nutritional deficiencies. Steatorrhea is exacerbated by low luminal pH, because, as mentioned, acid inactivates lipase. Diseases resulting in duct cell dysfunction decrease bicarbonate secretion leading to an acidic intraluminal pH.

PANCREATIC FUNCTION TESTS

The common indications for pancreatic function tests (PFTs) are shown in Table 1. During the last 50 years, several PFTs have evolved to test for EPI. In adults, secretin stimulation tests are more sensitive in detecting early stages of chronic pancreatitis (CP) than even the newer pancreatic imaging tests (7). Imaging studies are usually able to detect CP only when >50% of the gland is fibrotic. Some PFTs, however, can detect damage involving as little as 30% of the pancreas (7,8). The types of PFTs can be divided into indirect nonstimulatory tests and direct stimulatory tests, as shown in Table 2.

NONSTIMULATORY PFTS (INDIRECT)

Nonstimulatory tests measure pancreatic enzymes or their substrate byproducts at baseline from stool, serum, or breath. These include fecal fat, fecal elastase-1 (FE-1), stool chymotrypsin, steatocrit, serum markers, and the ^{13}C-mixed triglyceride breath test.

TABLE 1. Common indications for PFT

To evaluate for EPI in patients with chronic diarrhea, overt steatorrhea, or failure to thrive
To define pancreatic function in patients with CF
To assess efficacy of PERT in patients with previously diagnosed EPI
To rule out CP with inconclusive or normal imaging findings, particularly in the child with unremitting, chronic abdominal pain

CF = cystic fibrosis; CP = chronic pancreatitis; EPI = exocrine pancreatic insufficiency; PERT = pancreatic enzyme replacement therapy; PFT = pancreatic function test.

Fecal Fat

Microscopic evaluation of fecal samples can reveal an increased amount of fat droplets; microscopic interpretation can be enhanced by Sudan red staining (>2.5 droplets/high-power field). This is, however, not specific for pancreatic insufficiency as high fat intake or other causes of malabsorption or increased gut transit time will also result in a positive test.

Fat quantification in stool using the modified van de Kamer method of fat extraction is widely considered the criterion standard test for steatorrhea. Fecal fat measures the coefficient of fat absorption (CFA) using the formula

$$\mathrm{CFA} = \frac{\text{fat intake fat measured in stool, g}}{\text{fat intake, g}} \times 100$$

In infants <6 months of age, reference values are >85%, and above that age, reference values are >93% to 95% (9,10). The standardized collection time is 72 hours, although some reports argue that 24-hour collections are adequate (11).

A key factor in successfully performing fecal fat testing is that the patient should consume a standardized high-fat diet to provoke some degree of fecal fat excretion. A diet consisting of 100 g of fat per day is recommended for adolescents and adults and 2 g/kg in infants and younger children. The standardized fat diet is started 3 days beforehand and then continued for the full 3 days of stool collection (12). Another way to gauge when to begin collecting stool is to ingest a nonabsorbable marker such as charcoal, methylene blue, or carmine red at the start of the high-fat diet and to begin collection with the first discolored stool (13). Markers, however, are generally considered unreliable in children. It is an acceptable practice to calculate the 72-hour fat intake in children by primarily keeping a strict dietary record and not requiring a minimum intake of fat (14).

The disadvantage of fecal fat determination is that the 72-hour collection is laborious and unpleasant for both patient families and technicians to handle. The fecal fat test also requires that the family refrigerate the stool during home collection to avoid hydrolysis by bacterial enzyme metabolism and breakdown.

Near-infrared reflectance analysis could simplify quantification because it is able to differentiate between the nitrogen, fat, and water content of stool samples, and it correlates well with the classical van de Kamer method (15–17). The reliability of the van de Kamer method decreases when dealing with watery stools and is unsuitable for quantifying fecal fat in stool samples with >75% water content. Another technical issue is that the van de Kamer method does not detect medium-chain triglycerides (14). Moreover, the fecal fat test measures fecal fat excretion. Some nonabsorbed dietary fat, however, may be formed by bacterial action.

The exocrine pancreas has a large functional reserve, which limits interpretation of the fecal fat assay. In the classic report by DiMagno et al, (6) stimulated lipase output was plotted against fecal fat excretion in patients with varying degrees of EPI. The fascinating observation was that fecal fat excretion was increased in patients only when pancreatic lipase output fell <10%. In another study of patients with Shwachman-Diamond syndrome (SDS) and cystic fibrosis (CF), lipase levels fell <2%, or levels of another surrogate pancreatic protein colipase were <1%, before steatorrhea developed (18). Thus, abnormal fecal fat testing because of EPI indicates an already advanced state of insufficiency.

Abnormal excretion of fecal fat has other etiologies beyond EPI. Therefore, an abnormal fecal fat test should also prompt consideration of nonpancreatic causes of fat malabsorption, including acute, self-limited diarrheal diseases; gut mucosal injury (eg, because of celiac disease); small bowel bacterial overgrowth; short bowel syndrome; Crohn disease; liver disease with cholestasis and

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TABLE 2. Types of PFTs

	Comments
Indirect (nonstimulatory)	
Stool: fat, FE-1, and chymotrypsin	Nonspecific and can only detect severe EPI
Serum: nutritional markers, IRT, lipase, and amylase	Mainly used to support a diagnosis of EPI
Breath: ^{13}C-mixed triglyceride breath test	Not widely available
Urine: pancreolauryl	Not widely available
Direct (stimulatory)	
Pancreatic stimulation test (Dreiling tube test)	Direct collection of output but cumbersome to patients
ePFT	Expanding role in the future

ePFT = endoscopic pancreatic function test; EPI = exocrine pancreatic insufficiency; FE-1 = fecal elastase-1; IRT = immunoreactive trypsinogen; PFT = pancreatic function test.

reduced micelle formation because of compromised bile secretion (19,20). Patients with CF have multiple reasons for abnormal fecal fat excretion. In addition to having a deficiency in pancreatic enzyme secretion, patients with CF also have abnormal gastrointestinal motility, gastric hypersecretion, and diminished bicarbonate secretion, all of which can contribute to fat malabsorption (21).

Fecal Elastase-1

Measuring the zymogen elastase-1 in stool has become the most widely used indirect PFT because it offers several advantages. First, FE-1 is resistant to degradation by endoluminal bacterial proteases and, once collected, is biochemically stable over a broad range of pH and temperatures (22). Second, the FE-1 test uses monoclonal antibodies against human pancreatic elastase, which do not cross-react with elastase in pancreatic enzyme replacement therapy (PERT). Therefore, it is unnecessary for patients to discontinue PERT use when performing the FE-1 test (23). A third advantage of the FE-1 is that a spot sample is adequate. A normal level is >200 μg/g of dry stool; <200 μg/g indicates EPI, and <100 μg/g correlates well with steatorrhea (24). The sensitivity of the FE-1 for proven cases of CF in children is between 86% and 100% (25–27).

A limitation of the FE-1 is that pancreatic sufficient (PS) patients with watery diarrhea can have low FE-1 levels because of stool dilution, despite normalizing to total stool weight. In this situation, the test could be reported after lyophilizing the stool and using dry weight for calculations (28). The FE-1 can only detect EPI in the severe range, but it is still more sensitive than fecal fat. FE-1, however, will not detect isolated enzyme deficiencies, for example, lipase or colipase deficiencies that lead to steatorrhea (29).

Stool Chymotrypsin

Chymotrypsin in stool can be assayed using a simple photometric assay (30). The test, however, is less sensitive than FE-1 and requires discontinuation of pancreatic enzymes. Conversely, this limitation can be exploited as a method of assessing compliance to PERT (31).

Steatocrit

The steatocrit is a ratio of stool fat to total stool from a spot sample. A small amount of stool (as low as 0.5 gm) is collected, homogenized, and then centrifuged at 12,000*g* for 15 minutes. The height of the fat layer is measured in a column as a percentage of the height of the total solid layer. Since the first demonstration in 1981 in infants (32), the method has gained popularity in many parts of the world. The advantage is that the method is simple, cheap, and provides rapid results. The test, however, only gives an estimate of fat content and has poor sensitivity and specificity. The sensitivity can be increased by acidification of the sample before centrifugation (ie, an ''acid steatocrit'') (33).

SERUM TESTS

Serum tests are mostly used to support a diagnosis of EPI.

Nutritional Markers

Abnormal nutritional markers associated with EPI include fat-soluble vitamins, apolipoproteins, total cholesterol, magnesium, retinol-binding protein, calcium, zinc, selenium, and carotene (34). Patients with EPI tend to develop selective vitamin E deficiency (35,36). Patients with EPI can also have abnormal levels of hemoglobin, albumin, prealbumin, and HbA1C, as well as diminished bone density (37).

Immunoreactive Trypsinogen, Lipase, and Amylase

Small amounts of pancreatic enzymes are either physiologically released or leak out of the acinar cell into the systemic circulation. Thus, abnormally low serum levels of a pancreatic enzyme may indicate EPI. There are 3 commonly measured serum enzymes: immunoreactive trypsinogen (IRT), lipase, and amylase. During pancreatic inflammation, their levels are elevated. For instance, the IRT is high at birth in patients with CF, which is the basis for newborn screening (29). Serum lipase and amylase are elevated during acute pancreatitis. The serum IRT, lipase, and amylase, however, are low in older patients with CF who have EPI (38,39). The IRT and amylase are reduced in patients with SDS (40). These markers have low sensitivity and specificity for EPI, and for this reason, their role is, at best, to support a diagnosis (41).

BREATH TESTS

^{13}C-Mixed Triglyceride Breath Test

The most widely published breath test for EPI is the ^{13}C-mixed triglyceride breath test (42–45). The ^{13}C is a natural nonradioactive form of the carbon. The test measures ^{13}C-labelled CO_2, which is one of the breakdown products of digested triglycerides. After an overnight fast, the patient ingests a combination of ^{13}C-labelled mixed triglycerides and butter (or similar fat) on toast. The substrate is hydrolyzed in the intestinal lumen by pancreatic lipases, and ^{13}C-labelled octanoate, an 8 carbon medium-chain fatty acid originating from the sn-2 position of the triglyceride molecule, is absorbed in the gut. ^{13}C-octanoate is metabolized in the liver and peripheral tissues, after which ^{13}C-labelled CO_2 appears in the expired air of the patient. The ^{13}C is measured by mass spectrometry or near-infrared analysis. The amount of ^{13}C-labelled CO_2 is related

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to the activity of lipases present, and thus, the test indirectly measures pancreatic function.

An advantage of the ^{13}C-mixed triglyceride breath test is that it can be used to assess the efficacy of PERT, and it is noninvasive. The disadvantages are that the test has wide variability and the amount of expired ^{13}C-labelled CO_2 fluctuates with activity level (46–48). Furthermore, the breath test results could be influenced by gastric emptying rate, liver disease, intestinal diseases that affect absorption, lung disease, and endogenous CO_2 production.

Another drawback is the lack of availability of ^{13}C-labelled substrate. At present, the test is being performed in only a few countries in Europe and in Australia. The breath test is also difficult to perform in infants and toddlers. Similar to the fecal fat, the ^{13}C-mixed triglyceride breath test is a test of fat maldigestion, not just EPI.

URINE TEST

Pancreolauryl Test

This test is based on the digestion of fluorescein dilaurate by pancreatic aryl esterases. The free fluorescein is systemically absorbed from the intestinal lumen and can be measured in the urine (49) (and also from blood (50)). The original test was modified by adding a second marker mannitol, to correct for changes in intestinal permeability (51). The results are reported as a fluorescein/mannitol ratio. A spot urine test was developed for infants, and the reference value for EPI is a ratio of <30 (52). In comparison with the FE-1, however, the pancreolauryl test is less accurate (53).

DIRECT (STIMULATORY PFTs)

Direct or stimulatory tests of pancreatic function measure the enzyme activity of pancreatic secretions. More than 90% of the pancreatic parenchyma comprise acinar and duct cells (Fig. 2) (54). As mentioned earlier, acinar cells secrete pancreatic enzymes, primarily in response to neurogenic signals that are transduced by CCK. Meanwhile, duct cells secrete fluid and bicarbonate in response to secretin (55). Thus, the direct assessment of EPI includes the administration of 1 or both of the 2 secretagogues.

Pancreatic Stimulation Test (Dreiling Tube Test)

Although little used, the pancreatic stimulation test (Dreiling tube test) is considered the criterion standard for the assessment of exocrine pancreatic function (56). Figure 3 (57) provides a schematic of the procedure. The test involves the placement of a duodenal tube that has an aspiration port to collect intestinal fluid (58). CCK at $40\,ng \cdot kg^{-1} \cdot h^{-1}$ or secretin, 0.2 μg/kg during 1 minute, is infused after a baseline collection. In the combination regimen, CCK is infused 30 minutes after the secretin. In most protocols, intestinal secretions are collected every 15 minutes for 1 hour (7). Secretions are collected on ice and should be expeditiously frozen to prevent loss of enzyme activity. The volume of aspirate, pH, bicarbonate concentration, total protein concentration, and pancreatic enzyme activity are recorded. Amylase, trypsin, chymotrypsin, and lipase are often assayed, although most investigators focus on the peak bicarbonate concentration because it reflects the function of duct cells and is useful in the diagnosis of CP (59).

Multiple confounders may affect interpretation. Mixing of gastric acid with intestinal fluid can alter pancreatic enzyme activity. Therefore, there is a need for constant aspiration of gastric contents via a gastric port. The duodenal tube cannot reliably aspirate all of the secreted fluid, which makes assessing total volume and output a challenge. For this reason, an accurate measurement of the secreted volume requires the use of a double lumen duodenal tube, which, in addition to a distal aspiration port, also has a proximal infusion port. From this port, a known concentration of a nonabsorbable marker, such as polyethylene glycol, gentamicin, or cobalamin, is continuously infused. The concentration of the marker is assessed from the aspirated fluid, and the degree of dilution is directly proportional to the amount of fluid secreted into the duodenum. Some direct PFT protocols avoid the need to use markers by instead maximally capturing fluid secretions through occlusion of the distal duodenum with a balloon.

Although the pancreatic stimulation test can detect mild or at least moderate EPI, the major disadvantages are that it is invasive, impractical, and not available in most centers. The test is burdensome to patients, requires radiation exposure to verify tube and balloon positioning, and can be laborious to perform.

Endoscopic PFT

The endoscopic pancreatic function test (ePFT) has evolved to serve as a more practical option for direct testing (60) (Fig. 4) (61). Patients are infused with either CCK (0.02 μg/kg) or secretin (0.2 μg/kg), or both. Standard upper endoscopy is performed, and the stomach is emptied of gastric contents. Secretions from the

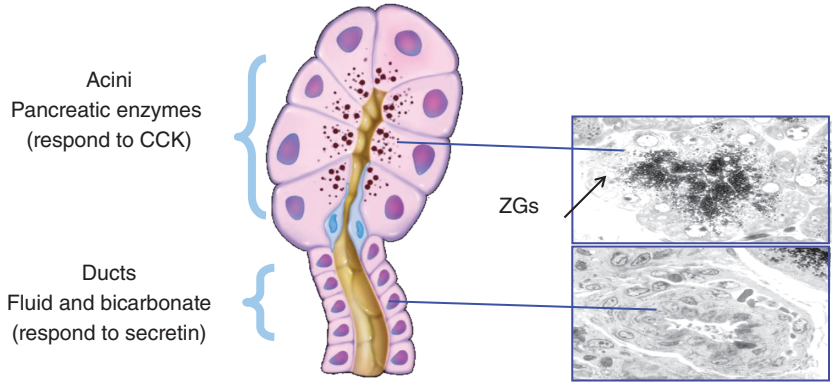

FIGURE 2. Physiologic basis for the stimulatory (or direct) PFTs. The exocrine pancreas is made up of acini and ducts. Pancreatic acinar cells within acini secrete pancreatic enzymes in response to CCK, and ducts cells within the duct network secrete fluid and bicarbonate in response to secretin ZGs. CCK = cholecystokinin; PFT = pancreatic function test; ZG = zymogen granule. Modified with permission from the American Gastroenterology Association's slide set (2005).

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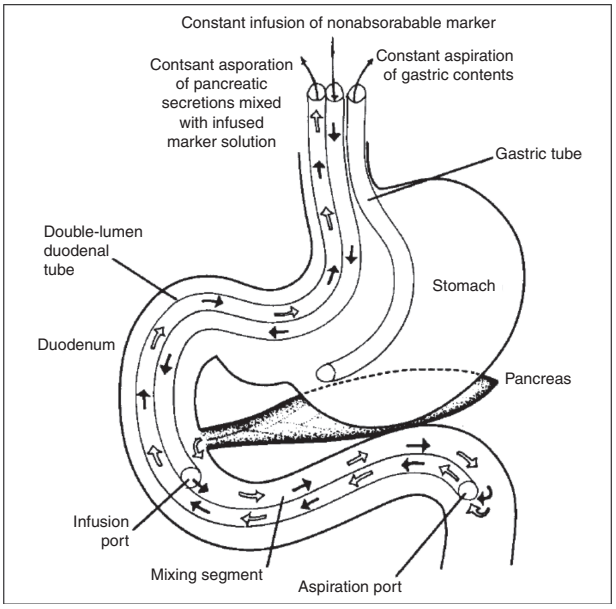

FIGURE 3. Schematic of the Dreiling tube test. As described in the text, the Dreiling tube has several key components that include a distal aspiration port, a proximal infusion port for a nonabsorbable marker, and a gastric aspiration port. Modified with permission from (57).

second portion of the duodenum close to the ampulla of Vater are collected, in many instances, every 15 minutes up to an hour. Chloride and bicarbonate concentrations are measured, and an increase in bicarbonate >80 mmol/L indicates normal function (62). The patient should fast for the endoscopy and should stop taking PERT for ≥48 hours before the procedure.

The ePFT has several advantages and some limitations, particularly in children. A major advantage is that it is a direct PFT, which can potentially detect mild to moderate EPI. Furthermore, the ePFT can be performed as a combined procedure with endoscopic ultrasound (EUS), which would allow direct visualization of the pancreas (see Endoscopic Ultrasound in Pancreatitis and Pancreatic Pseudocysts). This combination vastly improves the ability to provide both functional and structural information about the pancreas. A major limitation of the ePFT is that standard protocols and reference values are lacking. It is also unclear whether brief aspiration periods without marker perfusion underestimate the pancreatic secretion capacity and thereby misclassify normal patients as having EPI (63). The ePFT also requires general anesthesia in children. Most of the anesthesia drugs used do not influence the test; however, atropine should be avoided (64). Furthermore, enzyme activity rapidly degrades in stored samples, and most centers are unable to measure activities within their institutional labs. Frequent shortages of secretin or CCK limit the availability of the test. Part of the reason for the shortage is that few companies manufacture the secretagogues, and production can, therefore, be hindered by regulatory and manufacturing issues.

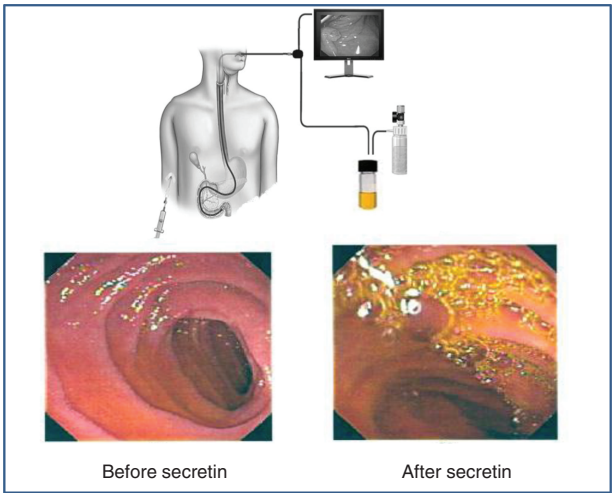

FIGURE 4. Schematic of the ePFT. Pancreatic secretions can be easily visualized in the second portion of duodenum through the endoscope just minutes after injection of secretin. ePFT = endoscopic pancreatic function test; PFT = pancreatic function test. Modified from reference 61 with permission.

GENETIC ASSOCIATIONS WITH SYNDROMES OF EPI

Within the last decade, several syndromic forms of EPI in children have been linked to specific genetic mutations (Table 3). Knowing these genetic associations is useful in evaluating a child with EPI. Because the landscape of genomic technologies is dramatically changing, especially with the advent of whole-exome sequencing (65–67), we recommend that clinicians work with their geneticists to formulate the most feasible plan for evaluating the genetic basis of EPI in a child. An update of the known genetic syndromes of EPI is provided below.

Cystic Fibrosis

CF is the most common cause of EPI in children (68). Most patients (85%) with classic CF (class I, II, or III mutations) have severe EPI. More than half (65%) of the infants with classic CF have EPI at birth, and another 15% to 20% develop progressive loss of pancreatic function by school age (69). Patients with CF have mutations in the *CFTR* gene, and there are currently nearly 2000 reported mutations (70). Nonclassic patients with CF (class IV or V) can have varying degrees of exocrine pancreatic function. These patients are usually exocrine PS and are at a higher risk of developing pancreatitis (71).

Shwachman-Diamond Syndrome

SDS is an autosomal recessive disorder consisting of EPI, bone marrow dysfunction, and skeletal anomalies (40). The typical symptoms are malabsorption, malnutrition, growth failure, hematologic abnormalities with single-lineage or multilineage cytopenia, susceptibility to myelodysplasia syndrome, and metaphyseal dysostosis. In almost all of the affected children, persistent or intermittent neutropenia is a common presenting finding. Short stature and recurrent infections are also common. Pancreatic activity improves with age in some patients with SDS, such that about a half become relatively PS by 4 years of age. In 2003, mutations in a gene called Shwachman-Bodian-Diamond syndrome (*SBDS*) were found in about 90% of SDS patients (72). Gene testing for SDS is commercially performed by many laboratories

Johanson-Blizzard Syndrome

The key findings in Johanson-Blizzard syndrome (JBS) are EPI, severe developmental delay, hypoplasia or aplasia of the nasal

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TABLE 3. Genetic associations with syndromes of EPI

Disease	OMIM	Gene/locus	Comments
CF	219700	*CFTR*	Most common
SDS	260400	*SBDS*	Hematologic abnormalities, short stature, skeletal anomalies, and malignancies
JBS	243800	*UBR1*	Nasal alar hypoplasia and congenital deafness
PMPS	557000	*mtDNA*	Refractory anemia in infancy
Pancreatic agenesis	260370	*IPF1*	Both endocrine and EPI
Congenital lipase deficiency	614338	*PNLIP*	Steatorrhea but usually without FTT
Congenital enterokinase deficiency	226200	*PRSS7*	Protein malabsorption and no steatorrhea
Syndrome of EPI, dyserythropoietic anemia, calvarial hyperostosis	612714	*COX4I2*	Steatorrhea, FTT, and anemia
PACA	609069	*PTF1A*	Diabetes mellitus and cerebellar agenesis

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; EPI = exocrine pancreatic insufficiency; FTT = ??; JBS = Johanson-Blizzard syndrome; mtDNA = mitochondrial DNA; OMIM = ??; PACA = pancreatic and cerebellar agenesis; PMPS = Pearson marrow pancreas syndrome; SBDS = Shwachman-Bodian-Diamond syndrome; SDS = Shwachman-Diamond syndrome.

wings, hypothyroidism, and congenital deafness (73). EPI is the most consistent feature, associated with replacement of the pancreas by fat and connective tissue. There is selective acinar cell loss, and endocrine insufficiency develops in adulthood. JBS is an autosomal recessive disorder, and, in 2005, mutations in the *UBR1* (the ubiquitin protein ligase E3 component n-recognin 1) gene were reported (74). UBR1 is highly expressed in acinar cells, and its absence is thought to lead to a destructive pancreatitis of intrauterine onset. To date, 59 different mutations have been identified (73).

Pearson Marrow Pancreas Syndrome

Pearson marrow pancreas syndrome (PMPS) is a rare, often fatal, disorder characterized by refractory, transfusion-dependent sideroblastic anemia and exocrine pancreatic fibrosis with EPI (75). PMPS has a non-Mendelian inheritance pattern that is because of mutations or deletions of mitochondrial DNA (mtDNA). PMPS is diagnosed by its clinical picture, along with a characteristic lactic acidosis and high serum lactate to pyruvate ratio. Molecular analysis of mtDNA can demonstrate the common point mutations.

PANCREATITIS IN CHILDREN

Pancreatitis can be categorized as the following (76):

1. Acute pancreatitis, with histological resolution after full clinical recovery
2. Acute recurrent pancreatitis (ARP)
3. CP, leading to irreversible inflammatory fibrosis. It can be classified as mild, moderate, and severe CP.

There is mounting evidence that many patients with ARP will progress to CP. More than 80% of cases of acute pancreatitis in adult are biliary tract disease or alcohol abuse (77). In children, the etiology of acute pancreatitis is more diverse compared with adults. The number of patients presenting with pancreatitis during childhood increases with age. Major recognized etiologies include biliary causes in 33% (gallstones, microlithiasis, structural, pancreas divisum, and sphincter of Oddi dysfunction), medications in 26% (valproic acid, prednisone, mesalamine, trimethoprim/sulfamethoxazole, 6-mercaptopurine/azathioprine, L-asparaginase, furosemide, tacrolimus, and antiretrovirals), idiopathic in 20%, systemic in 10% (sepsis and systemic diseases), trauma in 9%, viral infection in 8%, metabolic conditions in 5% (diabetic ketoacidosis, hypertriglyceridemia, inborn error of metabolism, and hypercalcemia), endoscopic retrograde cholangiopancreatography (ERCP) in 4%, CF in 2%, and finally alcohol in 1%. In 21% of patients, >1 etiology is identified (78,79). Failure of the ventral and dorsal pancreatic ducts to merge, termed pancreas divisum (Fig. 5), affects 5% to 10% of the population (80). There is evidence that pancreas divisum, particularly in combination with genetic factors, can predispose to pancreatitis (81). The preferred diagnostic test for pancreas divisum is a secretin-enhanced magnetic resonance cholangiopancreatography (MRCP) (82). Pancreas divisum is a combinatorial risk factor for pancreatitis in children.

Diagnosis of Pancreatitis

The definition of acute pancreatitis in children is by ≥2 of the following: abdominal pain, elevated amylase or lipase ≥3 times the upper limit of normal, or imaging findings of acute pancreatitis (76). These enzyme levels are elevated because of leakage from pancreatic acinar cells into the interstitial space and subsequent absorption into the circulation. The amylase level becomes elevated within hours of the development of pain and can remain elevated for 3 to 5 days. The differential diagnosis for hyperamylasemia includes intestinal obstruction, visceral perforation, tubo-ovarian abscess, renal failure, and salivary gland disease (79).

Gene Mutations in Children With ARP and CP

In 2001, the Third International Symposium of Inherited Diseases of the Pancreas recommended that in children with an unexplained, repeat episode of pancreatitis, genetic testing for cationic trypsinogen (PRSS1) mutations was warranted (83). Since 2001, other mutations have been implied in pancreatitis, including serine protease inhibitor Kazal type 1 (SPINK1), and more recently, chymotrypsin C (CTRC).

Gene associations include the following:

1. Recent work has identified a mutation in carboxypeptidase A1, primarily in childhood pancreatitis (84).
2. Gain-of-function mutations
   a. *PRSS1*. The *PRSS1* gene encodes for cationic trypsinogen—autosomal dominant, with 80% penetrance. p.R122H and p.N291 are the most common mutations (~80%).
3. Loss-of-function mutations
   a. *SPINK1*. The *SPINK1* gene encodes for serine protease inhibitor Kazal type 1, which is strongly associated with

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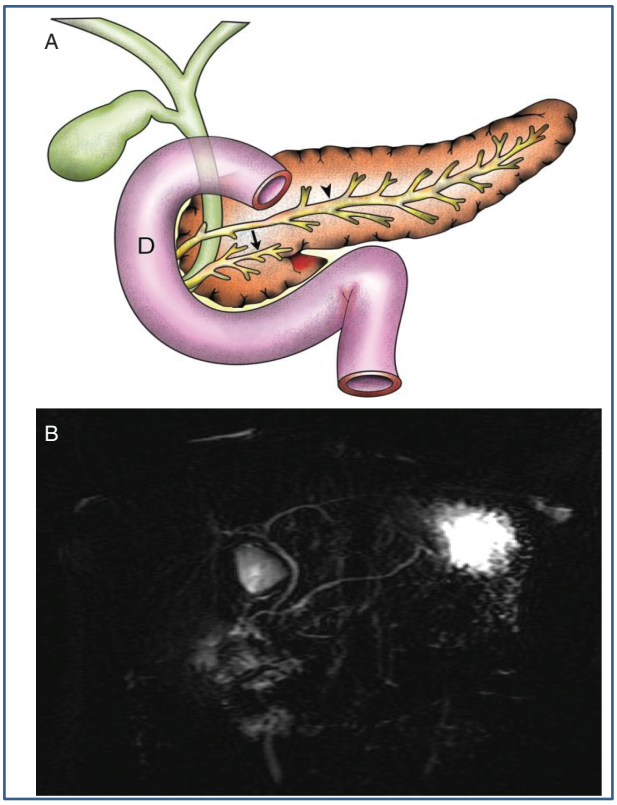

FIGURE 5. Pancreas divisum. A, Ventral and dorsal ducts fail to fuse together resulting in pancreas divisum, the most common congenital anomaly of the pancreas. B, Thick-slab maximum intensity projection MRCP image showing pancreas divisum with the typical crossing appearance of the common bile duct and main pancreatic duct. MRCP = magnetic resonance cholangiopancreatography. (Courtesy of Dr D Hughes).

idiopathic CP. The mutation is disease modifying but not causative (85,86).

b. *CTRC*. The *CTRC* gene encodes for CTRC, which acts as a defense mechanism against intrapancreatic trypsin autodigestion by catalyzing rapid trypsin degradation. A loss of function mutation has been identified in patients with CP (86,87).

c. *CFTR*. A higher frequency of mutations in the *CFTR* gene in patients with idiopathic CP (upward of 30% in the group and representing a 2- to 6-fold increase above the control population) was first demonstrated in 1998 (88,89). There is an up to 40-fold risk for pancreatitis in individuals with compound heterozygote *CFTR* mutations (90). In patients with CF, pancreatitis occurs in 20% of those who are PS. To evaluate genotype-phenotype correlations, the Pancreatic Insufficiency Prevalence score was developed and validated to determine severity in a large number of *CFTR* mutations. Specific *CFTR* genotypes are associated with pancreatitis. Patients who carry genotypes with more mild phenotypic effects have a greater risk of developing pancreatitis than patients carrying genotypes with moderate-severe phenotypic consequences at any given time (71).

In a single-center study in children ($\leq$18 years) diagnosed as having ARP or CP between 2000 and 2009, 23 of the 29 patients (79%) were positive for mutations in CFTR, PRSS1, or SPINK1. The family history was positive in 5 of the 29 (17%) of the gene-tested patients (91).

Another study by Lucidi et al (92) included 78 young patients (39 female; mean age at diagnosis, 8.8 ± 5.1 years) with ARP. The patients had a high prevalence of positive family history for CP (21%). The sweat test was abnormal in 1 patient (1%) and borderline in 7 patients (9%). Overall, genetic analysis showed mutations in *CFTR*, *SPINK1*, and *PRSS1* genes in 40%, 7%, and 5% of patients, respectively.

Recently recognized pancreas-associated genes include *CLDN2*, *CASR*, *CEL-MODY*, and *CPA1*, but few data are available from studies in children (93). Although chronic alcoholic pancreatitis usually develops in the fourth or fifth decade of life after years of alcohol abuse, patients with hereditary pancreatitis often develop pancreatitis in the first or second decades of life (94,95).

Chronic Pancreatitis

CP is characterized by progressive fibrotic destruction of the pancreatic secretory parenchyma, leading to loss of the lobular morphology and structure of the pancreas, deformation of the large ducts, and severe changes in the arrangement and composition of the islets (96,97). There is resultant impairment of both exocrine and endocrine functions. As the disease progresses from a mild to severe form, the pathological changes in the pancreatic ducts and parenchyma become easier to visualize with imaging modalities such as transabdominal ultrasound, computed tomography, MRCP, endoscopic ultrasonography (EUS), and ERCP. Structural abnormalities include a dilated and irregular main pancreatic duct, diffuse pancreatic calcifications, an irregular pancreatic contour, and pancreatic atrophy or fatty infiltration of the pancreas.

Diagnostic Modalities

Ultrasound is often the first imaging test employed and can assess pancreatic size and contour, peripancreatic fluid, main duct diameter, and irregularity and the presence of calcifications. Its sensitivity is 50% to 80% in adults (98). MRCP is the test of choice because it is noninvasive and can image ducts as small as 1 mm (99). It is useful to exclude biliary stones and anatomical variants, such as pancreas divisum (Fig. 5). Duct visualization can be improved by the administration of secretin, which induces fluid secretion (100). The role of secretin-enhanced MRCP, however, has not been fully clarified in children (101,102). ERCP is thought to be the criterion standard imaging test but is invasive, technically challenging in small children, and carries a complication rate of 0% to 11%. It is mainly reserved for therapeutic intervention (103). Because of the high radiation dose and relatively poor visualization of the pancreatic duct, CT is not the preferred modality in children.

As CP progresses, abnormalities in exocrine and endocrine pancreatic functions will be evident. These abnormalities include steatorrhea and diabetes mellitus. PFTs can help identifying exocrine gland (ductal and acinar) insufficiencies and can be useful to assess the progression of the decline in secretory capacity (8).

Endoscopic Ultrasound in Pancreatitis and Pancreatic Pseudocysts

Endoscopic ultrasound (EUS) is feasible in children as young as 5 years of age, but it is invasive, highly operator dependent, and also not fully evaluated in children (104). Reports on the utility of EUS in children with CP have recently emerged (105,106). One of the first papers was published in 2005 (107). In children, the main role of EUS is diagnostic. The EUS-guided fine needle aspiration or

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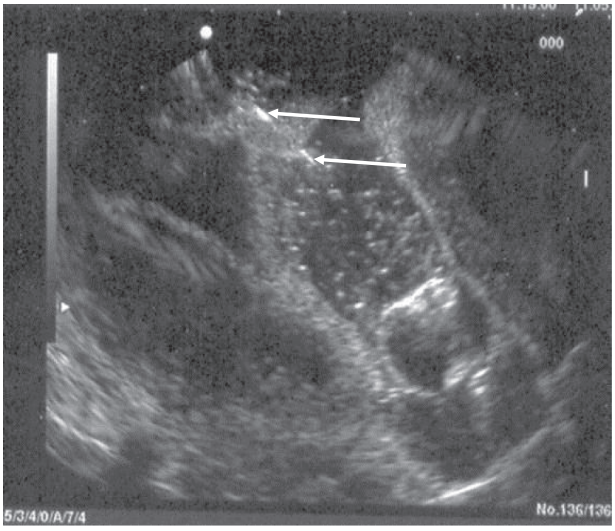

FIGURE 6. Transgastric linear endoultrasound needle puncture of a pancreatic pseudocyst. The linear needle (arrows) can be seen as a straight white line in the upper part of the picture. (Courtesy of Dr M Thomson.)

TABLE 4. Suggested plan for investigating pancreatitis in children

Single episode of acute pancreatitis without positive family history
Confirm diagnosis with measurement of amylase/lipase (note lipase assay is more specific; amylase levels fall rapidly and nonpancreatic amylase may confound result)
Look for potentially causal agents
Drugs
Alcohol
Hypertriglyceridemia
Hypercalcemia
Viral agents
Abdominal ultrasound scan to review anatomy and assess for gallstones
Single episode of pancreatitis with positive family history of chronic or recurrent pancreatitis
Screen for genetic mutations (CFTR, SPINK1, CTRC, and PRSS1)
Consider connective tissue disease and autoimmune disease
Imaging for analysis of anatomical variants and complications (MRCP and US)

CFTR = cystic fibrosis transmembrane conductance regulator; CTRC = chymotrypsin C; MRCP = magnetic resonance cholangiopancreatography; SPINK1 = serine protease inhibitor Kazal type 1; US = ??.

biopsy, however, can be useful in the diagnosis of idiopathic fibrosing pancreatitis or autoimmune pancreatitis. Microlithiasis can be identified by EUS as a possible contributor to chronic intermittent pancreatitis in children (106).

An increasingly popular indication of EUS in children is the internal drainage of pancreatic pseudocysts as a complication of acute pancreatitis because of trauma, medications, and so on. The clinical presentation of a pseudocyst includes persistent amylase/lipase elevation, chronic pain, presence of an abdominal mass, and nausea/vomiting. Treatment for small, noninfected cysts is conservative unless infected. In larger cysts, treatment is surgical but a trial with antisecretory agents such as octreotide or its longer-acting analogues (eg, lanreotide), or ERCP if it is accessible through the main duct may be attempted. Endoscopic transgastric cystostomies have been performed in children (105). These are guided by either fluoroscopy or EUS, with the latter being a safer option to help avoid the gastric vessels (Fig. 6). The linear endoultrasound is ideal, but radial endoultrasound may be at times sufficient and has been described in children. EUS has become the accepted procedure for drainage of pancreatic fluid collections in the past decade. EUS has been shown to be safe and effective, and it has been the first-line therapy for pseudocysts. Where walled-off pancreatic necrosis was originally thought to be a contraindication for endoscopic treatment, multiple case series have now shown that these fluid collections also can be treated endoscopically with low morbidity.

SUMMARY AND FUTURE DIRECTIONS

Assessment of pancreatic disease including exocrine pancreatic function and of pancreatitis is complex and dependent on the clinical presentation and manifestations of pancreatic disease. The available indirect PFTs can detect severe EPI, but they miss mild or moderate cases. Among the indirect nonstimulatory tests, FE-1 is the most convenient PFT, and it is the recommended screening tool. The ePFT may be helpful in children, and standardized protocols and reference values in children are necessary. The gene mutations associated with syndromes of EPI in children will further aid diagnosis. Ultimately, PFTs in combination with biochemical parameters, imaging, and genetic data will provide the best method to diagnose and assess pancreatic exocrine function. A suggested plan for investigating pancreatitis in children is given in Table 4. To streamline work in the field of pediatric pancreatology, pediatric gastroenterologists and multidisciplinary teams must work together to construct multicenter databases of children with pancreatic disorders (76,108).

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