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Progress in individualizing autologous islet isolation techniques for pediatric islet autotransplantation after total pancreatectomy in children for chronic pancreatitis

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Total pancreatectomy with islet autotransplantation is performed to treat chronic pancreatitis in children. Successful islet isolation must address the challenges of severe pancreatic fibrosis and young donor age. We have progressively introduced modifications to optimize enzymatic and mechanical dissociation of the pancreas during islet isolation. We evaluated 2 islet isolation metrics in 138 children—digest islet equivalents per gram pancreas tissue (IEQ/g) and digest IEQ per kilogram body weight (IEQ/kg), using multiple regression to adjust for key disease and patient features. Islet yield at digest had an average 4569 (standard deviation 2949) islet equivalent (IEQ)/g and 4946 (4009) IEQ/kg, with 59.1% embedded in exocrine tissue. Cases with very low yield (<2000 IEQ/g or IEQ/kg) have decreased substantially over time, 6.8% and 9.1%, respectively, in the most recent tertile of time compared to 19.2% and 23.4% in the middle and 34.1% and 36.4% in the oldest tertile. IEQ/g and IEQ/kg adjusted for patient and disease factors improved in consistency and yield in the modern era. Minimal mechanical disruption during digestion, warm enzymatic digestion using enzyme collagenase:NP activity ratio < 10:1, coupled with extended distension and trimming time during islet isolation of younger and fibrotic pediatric pancreases, gave increased islet yield with improved patient outcomes.

KEYWORDS
clinical research/ practice, diabetes, islet isolation, pediatrics

Abbreviations: BMI, body mass index; CP, chronic pancreatitis; CV, coefficient of variation; HbA1c, hemoglobin A1c; IAT, islet autotransplantation; IEQ, islet equivalent; NEM, new enzyme mixture; NP, neutral protease; SD, standard deviation; TDE, tissue dissociation enzyme(s); TP, total pancreatectomy.

1 | INTRODUCTION

Chronic pancreatitis (CP) is rare in children, most often resulting from predisposing genetic mutations affecting trypsin activation or bicarbonate secretion.[1] For children with CP, the disease burden is substantial: recurrent hospitalizations, missed school, severe pain, and opioid use are common.[1-3] When disease is severe and refractory to medical or endoscopic therapy, total pancreatectomy (TP) with islet autotransplantation (IAT) is an option for relieving chronic pain and restoring quality of life.[4] The goal of TP is to relieve pain while the IAT ameliorates postoperative diabetes. Over the past decade, TPIAT has gained favor as a treatment for children with severe and debilitating CP and has been performed in children as young as 3 years.[5]

For children with severe CP, TPIAT gives improved quality of life and favorable metabolic outcomes, with 40% achieving insulin independence.[4,6-9] However, successful islet isolation is necessary for favorable diabetes outcomes, as graft function and insulin independence depend highly on transplanting enough islets. Children transplanted with > 5000 islet equivalents (IEQ)/kg have a 75% chance of being insulin independent at 1 year, compared to 15% in those who receive < 2500 IEQ/kg, and islet graft failure is 25-fold more common with low islet mass (<2000 IEQ/kg).[4,10] Having at least partial islet graft function is paramount for maintaining goal glycemic control, reducing glycemic variability, and avoiding "brittle" forms of diabetes, while insulin independence is associated with better quality of life after TPIAT.[7]

To successfully isolate islets from children, one must adapt islet isolation procedures to consider unique challenges inherent with fibrotic CP pancreata and young donor age. While islet isolation from normal human deceased donor pancreata has become a standardized procedure performed at several institutions,[11-13] little information is available on success of islet isolation techniques for pancreata from adolescent and preadolescent (ie, very young) donors with CP.

This report presents an analysis of techniques used at our institution since the first pediatric TPIAT case in 1989,[14] emphasizing the modern era since 2009 (ie, after introducing the "new enzyme mixture [NEM]"[15]) when most of our pediatric TPIAT cases and process specializations have occurred. We have conducted 138 islet isolations for pediatric patients with CP undergoing IAT, including the first very young cohort of 17 patients aged 3-9 years.[5] We hypothesize that these pediatric cases, particularly preadolescent patients, constitute a distinct subpopulation of CP patients requiring a different paradigm for islet isolation to maximize islet yield and quality. We present our historical experience and process modifications for islet isolation from pediatric pancreata.

2 | METHODS

2.1 | Patients

Among patients undergoing TPIAT at the University of Minnesota between July 7, 1989 and December 31, 2017, 138 were pediatric (≤18 years old). For this article, patients ≤ 12 years old were considered preadolescent and ages 12-18 years old were referred to as adolescent. Patient demographics, islet manufacturing, and IAT infusion data were collected from medical and batch production records under 2 institutional review board–approved protocols, and parental consent and assent (as relevant) were obtained from all participants. Data from 288 adult cases in the most recent era (since October 1, 2009) were collected for additional analysis as described below.

Selection of TPIAT candidates and the surgical procedure have evolved over time, as described elsewhere.[4] Outcomes for pediatric cases, including a very young subset, have been described recently, emphasizing differences between adult and pediatric cases.[4,5]

2.2 | Islet isolation

Our institution's current standardized approach to islet isolation of CP pancreata has been detailed elsewhere[12]; the procedure and patient population have evolved since the first pediatric surgery was performed in 1989. Upon arrival to the islet laboratory, fibrosis severity is estimated by a consistent laboratory member based on feel and visual assessment. Much of the process (Figure 1) and clinical data collection were standardized after switching to a new enzyme mixture[15] in October 2009. However, because of the wide spectrum of pancreata obtained from patients with CP, we have implemented several procedures to individualize the isolation approach to maximize each patient's islet yield. Where relevant to our pediatric isolation approach, these techniques are described below. Figure 1 depicts implementation of these procedures over time, as well as different approaches for tissue dissociation enzymes (TDEs).

2.3 | Isolation modifications: enzyme combinations and techniques

As Figure 1 shows, our program has used 4 different TDE blends: Sigma XI (Sigma Chemical Company, St. Louis, MO; first 3 pediatric cases, 1989-1998); Liberase HI (Rochelle Molecular Biochemicals, Mannheim, Germany; cases #4-26, 1998-2006); SERVA/Nordmark Collagenase NB1 + Neutral Protease NB (SERVA Electrophoresis GmbH; cases #27-43, 2007-2009); and "NEM": Vitacyte CIzyme Collagenase HA (Vitacyte LLC) + SERVA/Nordmark Neutral Protease NB (cases #44-present, 2009-present). These choices followed the general trend of the islet isolation field, progressing from cruder preparations to highly purified enzymes used as a modular system with components dosed independently based on donor/pancreas characteristics.[12] In November 2009, the NEM was introduced based on research isolations and other data indicating that the intact C1 component of collagenase was critical for human islet isolation.[15] We hypothesized that the increased effectiveness of C1 fibrillar collagen was even more critical for organs with pancreatitis where collagen fiber accumulation characterizes the disease and increases with disease duration and severity.

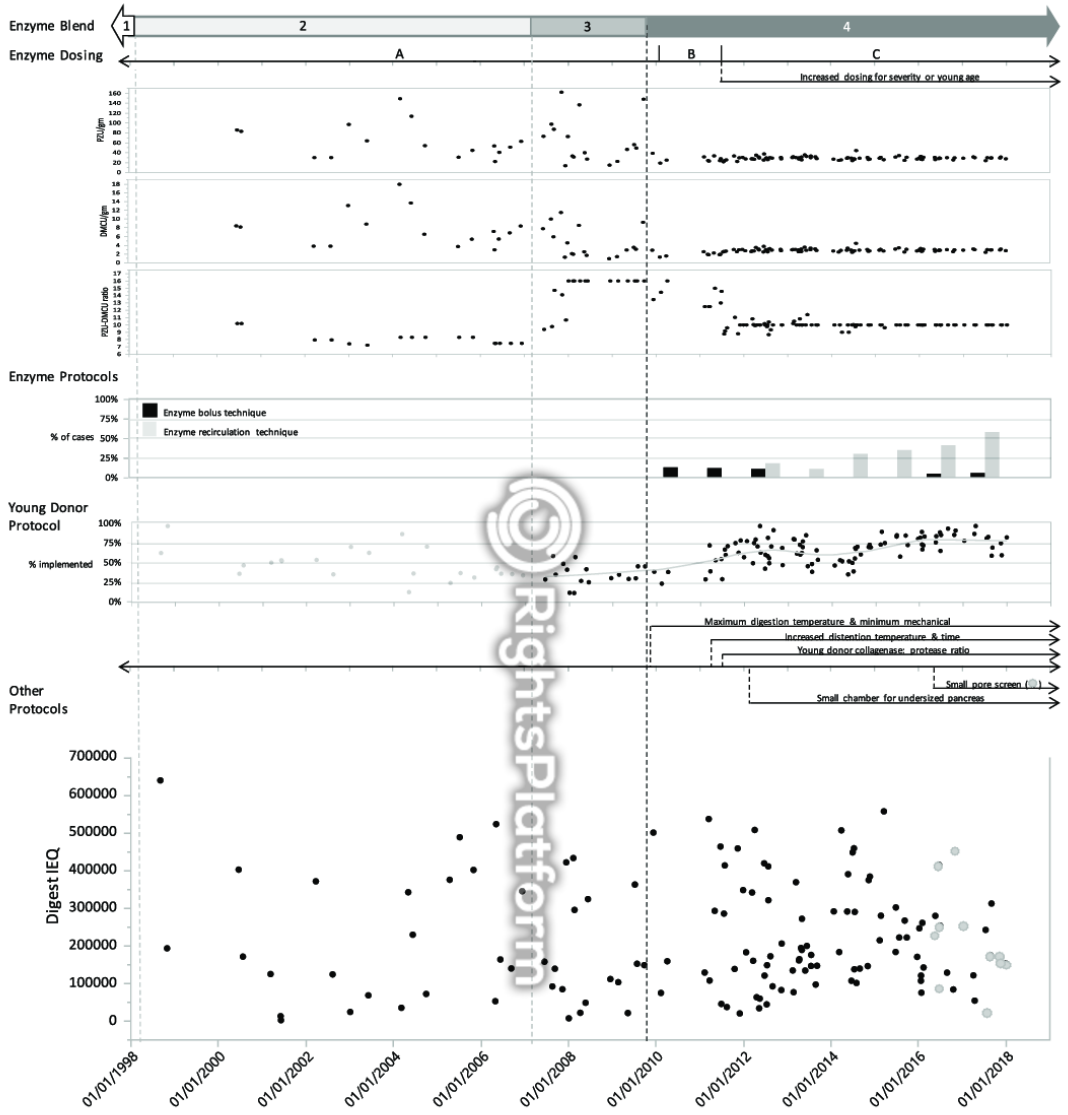

FIGURE 1 History of enzyme use, young donor and other protocols for islet isolation at University of Minnesota. Enzyme Blend (1 = Sigma XI; 2 = LibHI; 3 = SERVA/SERVA [start: March 26, 2007]; 4 = NEM [start: October 1, 2009]); Enzyme Dosing Protocol (A = Fixed dose; B = Dose per g [start: February 5, 2010]); C = Dose formal min/max range [start: June 30, 2011]); Other (gray starred circle = small pore screen used). DMCU, dimethylcasein unit; PZU, Pz-peptide unit

Historically, dosing of TDEs was fixed: for example, 1 full vial of Sigma XI or Liberase HI per pancreas. During the SERVA era, practice evolved toward a fixed amount of enzyme per pancreas, which seemed more suited to this TDE blend. Lot-to-lot variability of the cruder early enzymes, changes in sizes of Ricordi chambers used, and pancreas size or disease fibrosis severity may have contributed to variable results in early eras. NEM was introduced at a fixed dose but was changed to dosing based on pancreas weight around February 2010 because a fixed dose led to frequent over- or underdigestion of small or large pancreata, respectively. Because digesting young or severely fibrotic pancreata was often more difficult, increased doses were instituted for these cases around June 2010, with an altered ratio of activity for collagenase to neutral protease (NP) for younger donors (see below).

Further specialization of enzyme dosing for digestion-resistant pancreata was implemented in 2010, with more frequent use of an enzyme bolus technique, in which extra collagenase and/or NP is added directly to the digestion circuit when a large amount of undigested pancreas is observed after the expected switch time of 20-25 minutes. The timing and amount of additional TDE are determined by experienced manufacturing personnel, but generally a bolus of 25%-50% of the original dose is added 25-40 minutes after digestion begins. These can be more or less aggressive depending on the digested tissue's profile at the time of addition (ie, fragile/fragmented islets or fine acinar may require a more conservative approach). This technique of enzyme bolusing has been used infrequently to avoid overdigesting the tissue, but recent reports suggest that far more enzyme than previously deemed appropriate can be used safely in human islet isolation.[12,16,17] The process of adding supplemental enzyme was further refined in 2012 by adding an enzyme recirculation option (based on ref. 18), where tissue freed early in the digestion process is collected 100-200 mL at a time (≈15%-25% of total circuit volume) in 5-10-minute intervals, with supernatant containing enzyme returned to the digest circuit each interval. This allows continued digestion of the pancreas's resistant portion while protecting liberated tissue from fragmentation or other stressors. As Figure 1 shows, we have used this technique more frequently over time; in 2017 we used recirculation in 58% and enzyme bolus in 6% of pediatric cases. Our current protocol allows both to be used together in the most difficult cases.

2.4 | Isolation modifications: young donor protocol

The Pittsburgh group first reported a substantially modified isolation protocol for digesting young human pancreas,[18] intended to reduce the amount of embedded islets (embedded in acinar tissue), thereby reducing tissue volume, increasing purity of the final islet product, and increasing safety of infusion and the odds of intraportal infusion of the entire islet graft. Starting about 2010, we began a phased implementation of elements of this protocol, emphasizing enzymatic digestion over mechanical digestion of young donor pancreata (Figure 1) without drastically altering our overall isolation protocol.

Modifications we have made for young donors include the following:

1. Minimal mechanical disruption during digestion (mildly shaking Ricordi chamber).
2. Maximizing enzymatic activity during digestion (digest at 37°C instead of 30°C-35°C).
3. Extending enzymatic digestion to include the early distension step: using warm enzyme solutions during distension[17], extending distension, and trimming time duration, cumulatively adding up to 15 minutes to early distension time.
4. Altered TDE ratio (decrease in collagenase:NP ratio to ≤10:1 compared to standard dose of ≈15:1).

These changes were implemented over ≈2 years; for the present article, the degree of implementation was assessed semiquantitatively using isolation data from individual cases compared to a present-day theoretical best-practice value for each modification. Each modification was scored from 0% to 100%, with the overall score being the average of the individual scores (Figure 1).

Further specialized modifications to the isolation protocol for young donors included (Figure 1):

1. Screens in the Ricordi chamber with smaller pore size or altered dimensions (circular vs square mesh) to encourage continued digestion of larger tissue fragments.
2. Smaller Ricordi chambers for smaller pancreata to increase the exogenous concentration of enzyme in the digest circuit. This adjustment compensates for small enzyme doses per g pancreas, used after switching from the fixed-dose approach.

2.5 | Statistical analysis

Summary statistics are expressed as mean ± standard deviation (SD) or count and proportion (%). Two-sample *t* tests were used to compare numerical means, Pearson's χ^2 for differences in categorical variable frequencies.

Many time trends are described by splitting the TPIAT procedures into 3 equal-sized groups at the tertiles of procedure dates.

Analyses of the association with the 2 yield metrics (digest IEQ/g and digest IEQ/kg) were preceded by a Box-Cox analysis based on a linear regression that included many potential predictors, which indicated that analyzing the square roots of the metrics would give more valid tests. Subsequent analyses used multiple linear regression with 1 of the 2 transformed metrics as the dependent variable. For each metric, a first round of analyses considered simple associations between the metric and 1 patient characteristic (eg, fibrosis severity). A second round of analyses considered, for each characteristic, whether the time trend in the metric depended on the characteristic, using a multiple linear regression including time entered linearly, the characteristic, and their interaction. Time, in all analyses, is in years—specifically, [days from August 9, 2011]/365.25—because August 9, 2011 is the average date of these TPIATs and centering time this way avoids numerical problems arising from collinearity but without changing regression coefficients. A third round of analyses allowed the time trend to change its slope when the NEM was introduced (October 1, 2009) by adding a predictor for the change in slope and for its interaction with the characteristic. Finally, for each metric, predictors that were significant in any of the preceding analyses were included in 1 multiple regression, followed by a backward-elimination procedure in which at each step the characteristic with the largest *P* value was eliminated (except that time trend and slope change at October 1, 2009 were retained, and if an interaction was retained, so were the main effects of the 2 interacting characteristics).

All analyses used JMP Pro v14.1.0 (SAS Institute). All tests were 2-tailed; $P < .05$ was considered statistically significant.

TABLE 1 Characteristics of TPIAT between July 7, 1989 and December 31, 2017

	Mean ± SD	N	%
Number of pediatric TPIAT cases		138	
Patient characteristics			
Age (y)	12.9 ± 4.2		
Sex, female		88	63.8
BMI (kg/m^2)	21.3 ± 6.0		
Cause			
Familial/hereditary		101	73.2
PRSS1		61	(60.4)
SPINK1		18	(17.8)
CFTR		18	(17.8)
Idiopathic		25	18.1
Alcohol and drugs		2	1.4
Other (ie, obstructive, etc)		10	7.2
Pancreatitis duration (y)	5.5 ± 4.2		
Pain duration (y)	7.2 ± 4.1		
Previous pancreas procedure		104	75.4
Previous endoscopic		99	71.7
Previous pancreas surgery		23	16.9
Previous pancreas resection		11	8
Pretransplant metabolic testing			
Diagnosed diabetes		6	4.3
HbA1c (%)	5.3 ± 0.8		
Fasting glucose (mg/dL)	88.5 ± 12.2		
1 h glucose during MMTT (mg/dL)	94.2 ± 28.8		
2 h glucose during MMTT (mg/dL)	92.2 ± 32.2		
Fasting c-peptide (ng/mL)	1.6 ± 1.0		
Stimulated c-peptide (ng/mL)	5.8 ± 3.3		
Pancreas and islet isolation characteristics			
Severity of fibrosis (0-10)	7.2 ± 2.1		
Pancreas weight (g)	50.2 ± 23.1		
Digested pancreas (%)	86.8 ± 11.1		
Digested islet yield (IEQ/g)	4569 ± 2949		
Digested islet yield (IEQ/kg)	4946 ± 4009		
Digested tissue (cm^3)	10.1 ± 10.6		
Embedded islets (%)	59.1 ± 26.5		
COBE purification (%)		19	13.8
Islet recovery after COBE (%)	91.1 ± 19.2		
Isolation time (h)	4.1 ± 0.7		
Final product and transplant characteristics			
Transplanted islet dose (IEQ)	206 627 ± 140 344		
Transplanted islet dose (IEQ/kg)	4604 ± 3163		
Transplanted islet number (IPN/kg)	5649 ± 4068		
Transplanted pellet (cm^3)	8.4 ± 8.0		
Transplanted pellet (cm^3/kg)	0.18 ± 0.15		
Alternate transplant site used (%)		29	21
Dose intraportal (IEQ/kg)	4336 ± 2907		

Abbreviations: BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; HbA1c, hemoglobin A1c; IEQ, islet equivalent; IPN, islet particle number; MMTT, mixed meal tolerance test; *PRSS1*, serine protease 1 gene; SD, standard deviation; SPINK1, serine protease inhibitors of the Kazal type; TPIAT, total pancreatectomy islet auto transplants.

3 | RESULTS

3.1 | Patient characteristics

Table 1 summarizes demographics, presurgical metabolic testing, isolation, and transplant characteristics for our 138 pediatric patients undergoing TPIAT since 1989. Average patient age was 12.9 (SD 4.2) years with 63.8% female. The principal cause of pancreatitis was familial/hereditary (73.2%), with PRSS1 being the most common mutation (60.4% of hereditary cases), and average disease duration 5.5 (4.2) years. Few patients had preexisting diabetes (4.3%) or evidence of prediabetes based on fasting blood glucose or HbA1c. Average fibrosis severity was 7.2 (2.1) on our 10-point scale, with pancreas weight 50.2 (23.1) g. Islet yield at digest was 4569 (2949) IEQ per g of pancreas and 4946 (4009) IEQ per kg body weight. As typically seen, islets from young pancreata are frequently embedded in exocrine tissue (59.1% [26.5]), and purification is usually avoided (86.2%) unless packed tissue volume exceeds 0.25 mL/kg. Due to smaller patient size (and liver volume), some islets are transplanted at a nonhepatic site more often than in adult cases (21% of cases), although most islets are transplanted intraportally (4336 [2907] IEQ per kg).

3.2 | Pediatric population vs adult population over the same time period

To analyze more closely how pediatric patients differ from adults, we considered data from adults in the modern process era with

more complete data collection (since October 1, 2009) compared to either preadolescent (<12 years) or adolescent patients (Table S1). Both pediatric subsets differ significantly from adults in most demographic and isolation characteristics considered important for specializing the isolation process and interpreting outcomes. Besides age, both groups had lower body mass index (BMI), different disease cause (more often genetic), fewer previous pancreas procedures (especially endoscopic), lower fasting glucose, higher fibrosis severity, and lower pancreas weight. Regarding isolation outcomes, pediatric cases had higher percent pancreas digestion, lower tissue volume recovered, higher percent embedded islets, and greater use of alternate transplant sites.

Only the preadolescent subset had shorter pancreatitis and pain duration, lower pancreas surgery incidence, lower HbA1c, fasting c-peptide, and stimulated glucose during mixed meal tolerance test, higher islet yield (7185 [5417] vs 4549 [2847] IEQ/g), shorter isolation time, higher transplanted islet dose (total IEQ, IEQ/kg, or islet particle number /kg) and pellet volume (cm^3 or cm^3/kg), and higher intraportal dose transplanted (5625 [3132] vs 3991 [2252] IEQ/kg). Thus, although adolescent and preadolescent patients are both distinct from adults in key features including high prevalence of genetic disease, more severe fibrosis, and more embedded islets, the preadolescent subset has additional features distinguishing it from both adolescents and adults.

3.3 | Shifts over time in characteristics of the pediatric population

Key patient demographics including age, fibrosis severity, and cause have shifted substantially over time (Figure 2), resulting in more difficult cases. Average patient age started decreasing about 2011, with more very young patients treated. Fibrosis severity has increased gradually, with average 7.8 in the most recent tertile of cases. Patients with genetic cause, especially PRSS1 mutations, have increased over time, peaking in the middle tertile (91.5% genetic; 67.4% PRSS1) and falling off slightly in the most recent tertile (72.7% genetic; 53.9% PRSS1). In contrast, pancreatitis and pain duration have trended downward in each tertile (6.8 [5.0], 5.2 [3.9], 4.6 [3.5] years pancreatitis; 8.3 [4.4], 7.3 [4.2], 6.2

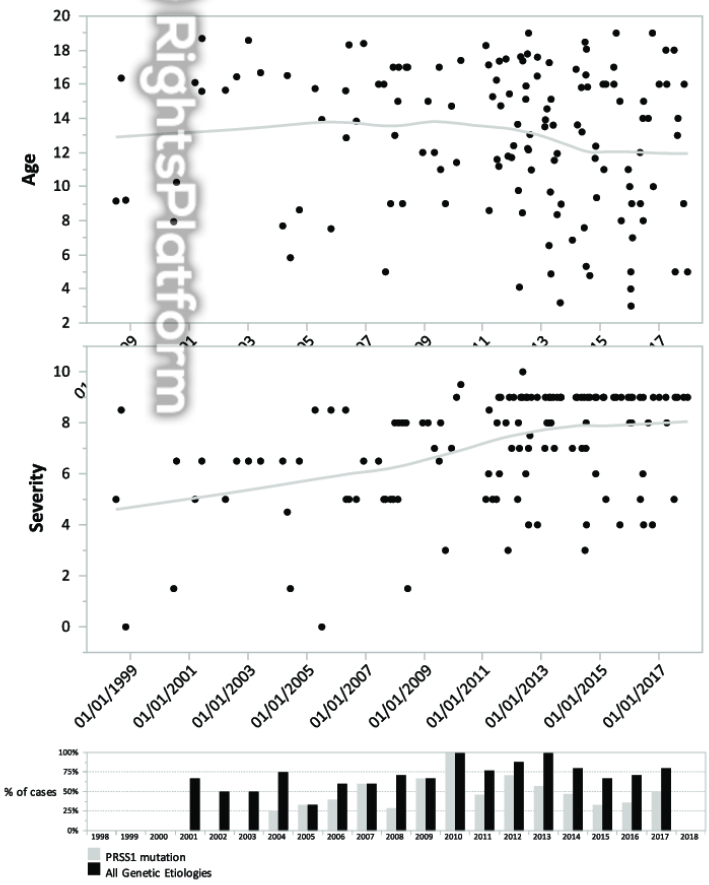

FIGURE 2 Changes in patient demographics associated with islet yield over time in pediatric total pancreatectomy with islet autotransplantation cases

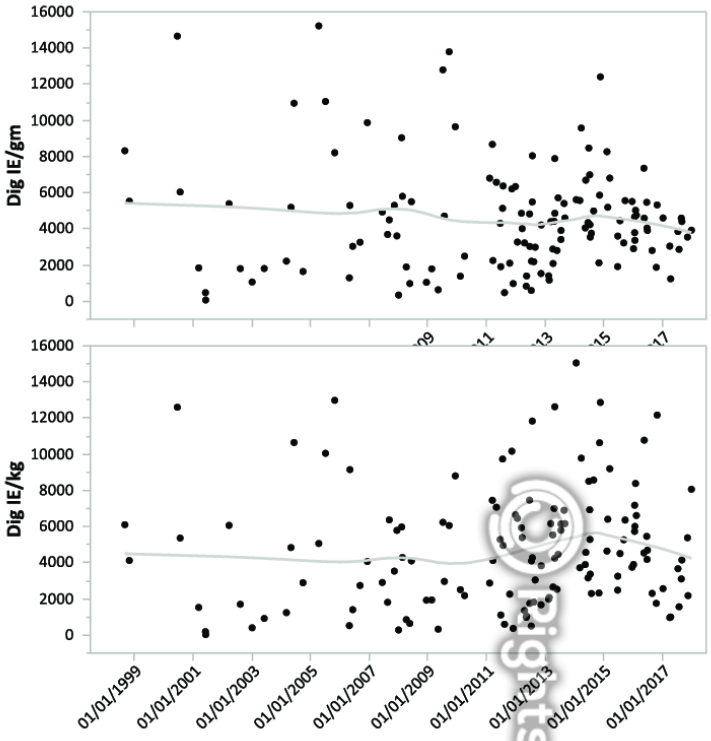

FIGURE 3 Raw (unadjusted) islet yields over time in pediatric total pancreatectomy with islet autotransplantation cases

[3.7] years pain). Also, fewer patients have a history of significant procedures associated with lower islet yield (pseudocyst drainage, Puestow, Frey, or distal pancreatectomy; 40.4%, 10.6%, and 6.8% per tertile).

3.4 | Changes over time in islet isolation outcomes

Due to the patient population's complex evolution, high variability between patients in isolation outcomes, and relatively low overall case volume, it is difficult to evaluate the impact of individual modifications to the islet isolation procedure, so we examined general trends within the context of the increasing difficulty of cases over time. We examined the general trend in islet yield over time using 2 key metrics: digest IEQ per g pancreas and digest IEQ per kg body weight (Figure 3). While digest IEQ/g has decreased slightly over time, it has been fairly flat during the most recent NEM era, and the trend in digest IEQ/kg has been generally flat over time, peaking in 2014 and increasing slightly during the NEM era. Cases with very low yield (<2000 IE/g or IE/kg) have decreased substantially over time: 6.8% and 9.1%, respectively, in the most recent tertile compared to 19.2% and 23.4% in the middle tertile and 34.1% and 36.4% in the oldest tertile. Consistency of the isolation process has increased over time, with variability in islet yield decreasing in each tertile (SD in digest IEQ/g: 4157 (81.3% coefficient of variation [CV]), 2198 [54.8%]), and 2001 [43.3%]) and variability in digest IEQ/kg also lowest in the most recent tertile (2943 [56.1% CV]), though highest in the middle tertile (5193 [93.8%]).

Regarding other isolation outcomes, both the average and variability of pancreas digestion (% original pancreas weight digested) have increased dramatically over the program's history (Figure S1). Similarly, islet size index (ratio of islet number to IEQ) and total isolation time have decreased and trended towards lower variability. The percent of islets embedded in exocrine tissue has varied over time (Figure S1), as have other isolation measures such as tissue volume, frequency of COBE purification, or recovery from purification, which showed no or modest improvements (data not shown). More process innovation may be necessary to further improve some of these isolation outcomes.

3.5 | Impact of patient features and process modifications on isolation outcomes

To assess the impact of process modifications, we analyzed patient factors that might influence islet yield for their association with yields over time. We first considered simple associations of our 2 islet yield metrics with key patient characteristics (Table 2). Longer pancreatitis and pain duration, history of significant pancreas procedure, higher fibrosis severity, pretransplant diabetes

TABLE 2 Significance of bivariate associations between key patient demographic factors and islet yield and interaction with time

	Digest IEQ/g	Digest IEQ/kg
Patient factor	***P* value**	***P* value**
Time, linear	.980	.058
Pre-adolescent vs adolescent	.110	<.0001
Age, linear	.140[b]	.140
Sex	.130	.410
BMI	.440	<.0001
Pancreatitis duration (y)	<.0001[b]	<.0001[b]
Pain duration (y)	<.0001[b]	<.0001[b]
Cause[c]	.062	.580
Total pancreatectomy vs subtotal	.970	.100
Significant procedure[a]	.0056	.0002
Severity of fibrosis (0-10)	.0001[b]	.025
Pre-tx diabetes	.0003	<.0001
Pre-tx HbA1c (%)	.0011[b]	.0012

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; IEQ, islet equivalent; tx, transplant.

[a]Any of: Puestow, Frey, Pseudocyst, Distal.

[b]Significant interaction with time.

[c]Hereditary, PRSS1 (61); hereditary, other (39); idiopathic (23); other (12).

diagnosis, and higher HbA1c levels were strongly associated with both islet yield metrics. Age subset (preadolescent vs adolescent) and BMI were also strongly associated with digest IEQ/kg. Longer pancreatitis, pain duration, and pretransplant HbA1c were significantly associated with time for both islet yield metrics, with higher fibrosis severity associated with digest IEQ/g only.

We next examined the change over time in the *association* of yield with patient factors, to see whether process modifications have improved isolation outcomes, particularly for more difficult cases. For example, considering the association between pancreatitis duration and digest IEQ/g (Figure 4), longer duration was strongly associated with lower yield in early cases but that association has gradually disappeared. Pain duration, age, fibrosis severity, cause, and pretransplant diabetes showed similar patterns, though not as strikingly as pancreatitis duration (data not shown). This suggests that process modifications have improved isolation outcomes for more difficult cases.

To analyze the trend in islet yields over time more globally, accounting for patient factors that influence islet yield, we created multivariate models for both islet yield metrics. Candidate factors were chosen based on results of the simple analyses, with time dependency assessed in 2 ways as described in the Methods section. Details of the full multivariate models are included in Figure S2.

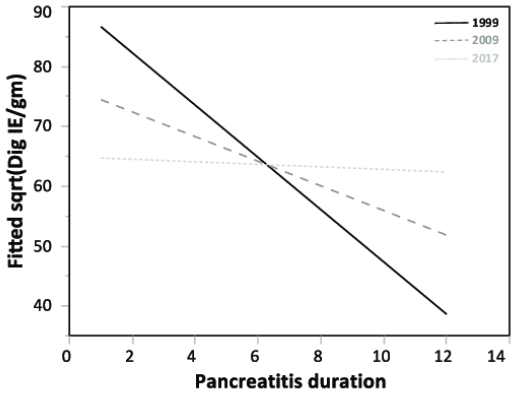

FIGURE 4 Association of pancreatitis duration and islet yield (digest islet equivalent/g) over time in pediatric total pancreatectomy with islet autotransplantation cases

For digest IEQ/g, the full model had $R^2 = 0.522$, and factors reaching statistical significance were fibrosis severity ($P = .008$) and pretransplant HbA1c ($P = .038$), as well as the interaction of fibrosis severity and the 2 time-dependent factors ($P = .0009$ for the interaction of fibrosis severity and time [severity*time] and $P < .0001$ for the interaction of fibrosis severity with the change in time trend when NEM was introduced [severity*slope-change]). For digest IEQ/kg, the full model had $R^2 = 0.444$, and factors reaching significance were severity ($P = .018$), BMI ($P = .028$), and pain duration ($P = .037$). Backwards elimination was performed for both models; many predictors included on the basis of the simpler analyses were removed but doing so did not result in noteworthy changes in the model fit or adjusted islet yields. Figure 5 shows adjusted islet yields over time for both models. After adjusting for patient characteristics that significantly affected islet yields, the trend over time (Figure 5) supports the hypothesis that changes in islet processing have resulted in better and/or more consistent results, particularly in the most recent historical era and for digest IEQ/g, which is the best metric for assessing impact of process changes.

4 | DISCUSSION

This study presents our approach to modifying the isolation process over a long period to optimize the yield of islets from increasingly younger and more fibrotic pancreata from pediatric patients. In our experience with 138 isolations from pediatric CP pancreata, adjustments to enzymatic dissociation and minimizing mechanical disruption improved the consistency of islet isolation and resulted in fewer low-yield islet products in the most recent era.

When accounting for patient and disease factors that affect islet yield, we have shown a general trend toward increased islet yield over time, expressed as digest IEQ per g pancreas, as well as reduced

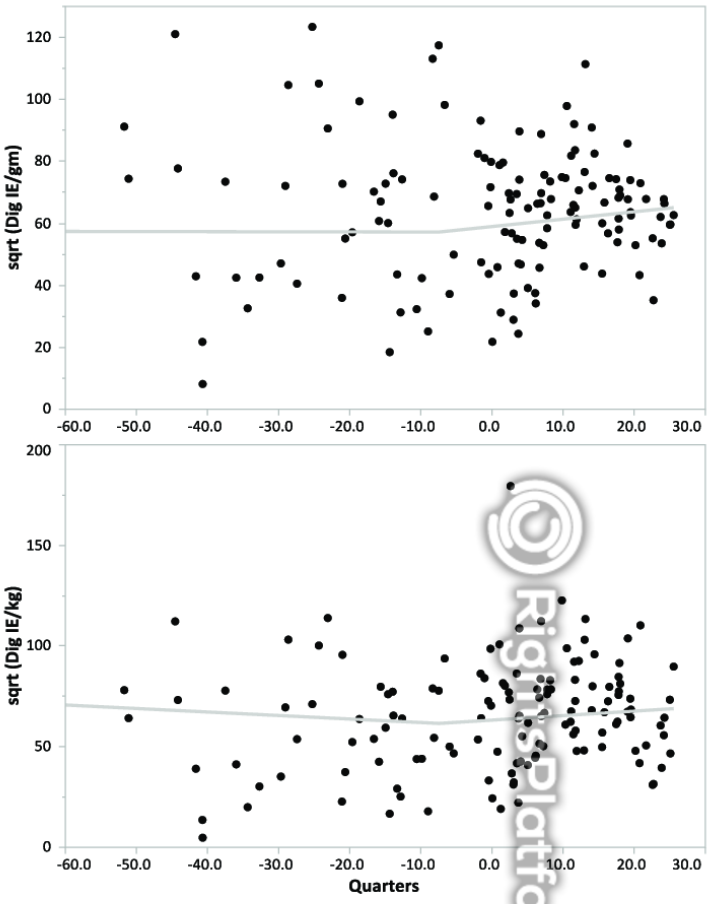

FIGURE 5 Adjusted islet yields over time in pediatric total pancreatectomy with islet autotransplantation cases

variability in yield, and notable improvements in complete digestion of the pancreas and reduction in percentage of cases with very low islet yield. Over time, we observed a trend toward a younger population with increased genetic disease and severity of pancreatic fibrosis. Although the average duration of pancreatitis before TPIAT decreased over time, we particularly observed more consistency in successful isolations, even with a pancreatitis of long duration, in the modern isolation era. Due to the challenges of patient variability and low case volumes, it is difficult to determine exactly which modifications of the isolation procedure led to improved outcomes; therefore, we have taken a more macro-scale approach to analysis. Randomized validations of these modification procedures would be difficult.

Optimizing islet isolation outcomes is a critical step towards attaining optimal diabetes outcomes. Most children after TPIAT have partial or full islet graft function (ie, partial insulin dependence or insulin independence). However, those with <2000 IEQ/kg are at greatly elevated risk for islet graft failure.[10] In the absence of a functioning islet graft, the diabetes outcome becomes similar to that of TP alone, with more difficulty achieving target glucose levels, high glycemic variability, and risk of severe hypo- and hyperglycemia. Thus, the substantial reduction in low islet yields over time is a clinically meaningful improvement. Furthermore, achieving better islet yields from more difficult cases may also increase the likelihood of insulin independence, which we have previously observed to correspond with higher quality of life scores.[7] Thus, tailoring islet isolation techniques to this unique pediatric CP population is an important component of our TPIAT processes.

More aggressive changes in the isolation process could also be attempted such as highly modified or additional digestion procedures for embedded islets, or adjustment in the mesh size in the Ricordi chamber to produce smaller tissue fragments.[12,13,15] However, most islet manufacturing centers are likely reluctant to change a process that works quite well for a benefit of unknown significance. In addition, procedures that may fragment or damage islets could result in less functional islet tissue despite a preserved islet mass. Use of pancreatitis animal models or access to fibrotic pancreas from patients undergoing partial or total resections

without IAT (ie, pancreas cancer) are needed to test more aggressive modalities. Whether our approaches apply to pediatric deceased donor islet isolations is uncertain. An increased protease dose also reduces the percentage of embedded islet tissue from young deceased donors,[16] as for our approach, but some modifications introduced for young donors in TPIAT are specific to pancreatic fibrosis and cannot be summarily applied to deceased donor pancreata.

This study is limited in that it relies on data from 1 center over a long period and is therefore subject to era effects of unknown significance. As mentioned, the high variability in the patient population also makes signal-to-noise ratio a major limitation of these analyses. While we have developed a parameter to describe gross pancreatic fibrosis severity based on visual inspection and feel, this is a subjective assessment. Also, enzyme preparations may have batch-to-batch variability, though concern is reduced in the modern era with better stability of collagenase and tighter quality control ranges for enzyme activity and purity. Because these are islet autografts, we do not have sufficient in vitro functional islet assessments to characterize islet function after isolation, though diabetes outcomes have been previously published.[4]

Even given these challenges, we continue to modify our islet isolation procedures to try to individualize the process for each patient and to maximize their clinical outcome and benefit. Our current process is to implement the young donor protocol in pediatric pancreata, while adapting to the unique condition of each pancreas.

In summary, our islet isolation process has evolved over the years. We have introduced changes to optimize enzymatic dissociation by using warm enzymatic digestion to increase enzyme activity, adding supplemental enzyme for digestion-resistant pancreata, and reducing the collagenase:NP ratio to < 10:1, coupled with extended distension and trimming time during islet isolation of younger and fibrotic pediatric pancreata, while at the same time minimizing mechanical disruption. The changes have resulted in improved islet yield, more consistent islet isolation outcomes, and fewer low islet yield preparations, all of which are critical to optimizing the likelihood for successful diabetes outcomes after TPIAT.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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