Importance of Intestinal Environment and Cellular Plasticity of Islets in the Development of Postpancreatectomy Diabetes

Diabetes Care 2021;44:1002–1011 | https://doi.org/10.2337/dc20-0864

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Received 17 April 2020 and accepted 18 January 2021

This article contains supplementary material online at https://doi.org/10.2337/figshare.13626788.

OBJECTIVE

To elucidate the pathogenesis of postpancreatectomy diabetes mellitus (PPDM).

RESEARCH DESIGN AND METHODS

Forty-eight patients without diabetes undergoing either pancreatoduodenectomy (PD) ($n = 20$) or distal pancreatectomy (DP) ($n = 28$) were included. A 75-g oral glucose tolerance test was performed every 6 months. Microbiome composition and short-chain fatty acids (SCFAs) in feces were examined before and 6 months after surgery. The association of histological characteristics of the resected pancreas with PPDM was examined.

RESULTS

During follow-up (median 3.19 years), 2 of 20 PD patients and 16 of 28 DP patients developed PPDM. Proteobacteria relative abundance, plasma glucagon-like peptide 1 (GLP-1), and fecal butyrate levels increased only after PD. Postsurgical butyrate levels were correlated with postsurgical GLP-1 levels. With no significant difference in the volume of the resected pancreas between the surgical procedures, both β-cell and α-cell areas in the resected pancreas were significantly higher in DP patients than in PD patients. In DP patients, the progressors to diabetes showed preexisting insulin resistance compared with nonprogressors, and both increased α- and β-cell areas were predictors of PPDM. Furthermore, in DP patients, α-cell and β-cell areas were associated with ALDH1A3 expression in islets.

CONCLUSIONS

We postulate that a greater removal of β-cells contributes to the development of PPDM after DP. Islet expansion along with preexisting insulin resistance is associated with high cellular plasticity, which may predict the development of PPDM after DP. In contrast, PD is associated with alterations of gut microbiome and increases in SCFA production and GLP-1 secretion, possibly protecting against PPDM development.

Partial pancreatectomy, including pancreatoduodenectomy (PD) and distal pancreatectomy (DP), can exacerbate glucose tolerance by reducing insulin secretion (1). However, it is uncertain whether the degree of deterioration in postoperative glucose tolerance by PD and DP is different. PD involves bypass of the proximal intestine and

resection of the pancreatic head, whereas DP involves resection of the pancreatic body and tail without gastric bypass. Bypass of the proximal intestine during metabolic surgeries can lead to increased secretion of glucagon-like peptide 1 (GLP-1) from L cells via early delivery of nutrients to the distal ileum and colon (hindgut hypothesis), presumably resulting in improved glycemic control (2). In addition, a study on the transplantation of fecal microbiota obtained from patients who had undergone bypass of the proximal intestine to germ-free mice demonstrated reduced fat deposition in recipient mice (3), suggesting that the gut microbiota may ameliorate postsurgical glucose metabolism after bypass of the proximal intestine. Short-chain fatty acids (SCFAs) and bile acids (BAs) that are produced by fermentation of gut microbiota have been reported to modulate GLP-1 secretion (4,5); therefore, SCFAs and BAs may play a role in the association between altered gut microbiota and improvement of glucose metabolism. Thus, we hypothesize that the altered intestinal environment after gut bypass results in increased GLP-1 production, affecting glucose metabolism after PD. However, few studies have reported the impact of gut bypass on postsurgical glucose tolerance after partial pancreatectomy.

Considering that DP does not involve gastric bypass, postsurgical glucose metabolism after DP may differ from that after PD. Recent studies have demonstrated that an adaptive increase to metabolic stress of β-cell mass and function may occur before the development of overt type 2 diabetes (6). Furthermore, aldehyde dehydrogenase 1 isoform A3 (ALDH1A3), a progenitor cell marker (7), was recently identified as a biomarker for dysfunctional β-cells in animals (8) and humans (9). Nevertheless, due to difficulties in collecting pancreatic tissue, it remains unknown whether histological features of the pancreas in humans, such as β-cell expansion or high cellular plasticity in islets, are predictive markers for type 2 diabetes (10). Because DP is a simple resection of approximately half of the pancreas, it is important to determine the association between the histological features of the pancreas and postpancreatectomy diabetes mellitus (PPDM), as these may be applicable to type 2 diabetes. However, there are no prospective studies that have investigated the association between pancreas histology and diabetes.

Here, we conducted a prospective observational study of patients without diabetes (including patients with impaired glucose tolerance [IGT]) undergoing partial pancreatectomy. The aims of the current study were to *1*) clarify differences in the incidence of PPDM (using repeated measurements of 75-g oral glucose tolerance test [OGTT]) between patients who underwent PD and patients who underwent DP; *2*) determine the effects of gut microbiota, incretins, BAs, and SCFAs on PPDM; and *3*) investigate whether increased cellular plasticity and islet expansion in the resected pancreas are associated with the development of PPDM.

RESEARCH DESIGN AND METHODS

Study Design and Population

This prospective, observational study was approved by the Ethical Committee of the Tokyo Medical and Dental University (approval no. M2000-1890) and complied with the principles established by the Declaration of Helsinki. All study patients provided written informed consent.

Eligibility criteria were being of age ≥20 years, scheduled for partial pancreatectomy (PD or DP) between 22 September 2014 and 31 March 2017, and without previously known diabetes. Supplementary Fig. 1 briefly demonstrates the procedure of both surgeries with similarities and differences. Subtotal stomach-preserving PD (SSPPD) with Child's reconstruction was performed for all patients in the PD group. Patients were excluded if they had malignancies, including pancreatic cancer and functional neuroendocrine tumor, or if they received hemodialysis. A total of 53 patients were enrolled, and measurements of glucose metabolism (75-g OGTT) and hemoglobin A_{1c} (HbA_{1c}) were performed prior to surgery. Five patients were excluded due to diabetes, as determined by 75-g OGTT. Normal glucose tolerance (NGT), impaired fasting glucose, IGT, and diabetes were determined according to the American Diabetes Association criteria (1). A total of 48 patients without diabetes, comprising 27 patients with NGT and 21 patients with IGT (20 patients undergoing PD and 28 patients undergoing DP), were included in the study (Supplementary Fig. 2).

OGTT and Clinical and Laboratory Data

All patients were administered a 75-g OGTT after a 12-h overnight fast within 2 weeks prior to pancreatectomy and every 6 months after surgery. Details are provided in the Supplementary Material.

Postsurgical Follow-up and End Point

After partial pancreatectomy, patients visited our hospital every 6 months for evaluation of fasting plasma glucose and HbA_{1c} levels and to undergo a 75-g OGTT. The end point was defined as the development of diabetes, diagnosed according to the American Diabetes Association criteria (11). The follow-up period was defined as the time between the date of surgery and the date of last visit to our hospital, for patients who did not develop PPDM, or the time between the date of surgery and the date of diagnosis of diabetes for patients who developed PPDM. The total observational period was defined as the time between the date of surgery and the date of last visit to our hospital.

Estimation of Resected Volume of Normal Pancreatic Region

The resected volume of the normal pancreas was determined with ImageJ/Fiji (12) as described in the Supplementary Material.

Microbiota Analysis

Fecal samples were collected from patients without prior use of antibiotics during the day before surgery and 6 months after surgery. Samples were stored immediately at −80°C until further analysis. Fecal DNA extraction and sequencing by Illumina MiSeq (Illumina, Inc., San Diego, CA) were performed as previously described (13,14). Details are provided in the Supplementary Material.

Measurement of Fecal SCFAs and BAs

Fecal SCFAs and BAs were analyzed in samples collected before surgery and 6 months after surgery as previously described (15,16). Details are provided in the Supplementary Material.

Immunohistochemistry and Morphometry of Pancreas

Pancreatic tissue processing is detailed in the Supplementary Material. Stained sections were observed with a BZ9000 microscope (KEYENCE, Osaka, Japan), and α-cell and β-cell areas were determined by two independent investigators

(T.F. and T.T.) as the area stained by glucagon or insulin antibodies, respectively, divided by the total pancreas area with Image J (National Institutes of Health, Bethesda, MD) (https://imagej.nih.gov/ij/). The mean values of the measurements determined by the two independent investigators were used. α-Cell or β-cell areas determined by the two investigators were significantly comparable ($r = 0.82$, $P < 0.01$). The α-to-β ratio was defined as the ratio of the α-cell area to the β-cell area. Immunostaining of ALDH1A3 is described in the Supplementary Material.

Statistical Analysis

Statistical analysis was performed with SPSS (version 21.0; IBM, Armonk, NY) or R software (version 3.3.0) (available from https://www.r-project.org). Details are provided in the Supplementary Material.

RESULTS

Baseline Characteristics

The baseline clinical characteristics of the patients and the medications taken are listed in Table 1. Patients who underwent PD tended to be older and had lower estimated glomerular filtration rate and higher insulin levels at 120 min during the 75-g OGTT compared with those who underwent DP, although these differences were not statistically significant. Both β-cell and α-cell areas were significantly higher in the resected samples from those in the DP group than in the PD group. The patients did not receive any medications that could seriously affect the outcome of diabetes development during the study period.

Postsurgical Changes in Glucose Tolerance

During the median follow-up period of 3.19 years (range 0.02–5.54), 18 patients reached the end point (2 and 16 patients undergoing PD and DP, respectively). Detailed patient data are listed in Supplementary Table 1. The total observational period (means ± SD) was comparable between patients in the PD and DP groups (PD, 4.2 ± 1.0 years; DP, 4.0 ± 1.2 years; $P = 0.534$). Figure 1*A* shows the Kaplan-Meier curve of time to end point in the PD and DP groups. Patients who underwent DP had a significantly higher cumulative incidence rate of diabetes compared with those who underwent PD ($P < 0.001$, log-rank test). The proportion of patients with improved glucose tolerance after surgery (those with impaired fasting glucose or IGT prior to surgery and achievement of NGT 6 months after surgery) was significantly higher for patients in the PD group than in the DP group (44.4% vs. 8.3%, $P = 0.001$). As shown in Supplementary Fig. 3, the BMI was significantly decreased in PD group patients at 1 and 6 months after surgery; however, no difference was observed between presurgical BMI and the BMI after 12 months of surgery. Moreover, there was no significant reduction in BMI after surgery in the DP group. Resected volumes of normal pancreas were similar between patients in both the PD and DP groups (Supplementary Fig. 4*A*). Changes in glucose, insulin, and GLP-1 concentrations in response to 75-g OGTT before and 6 months after pancreatectomy are shown in Fig. 1*B–G* and Supplementary Fig. 4*B–D*. Interestingly, glucose levels measured at each time point and the area under the curve (AUC) of glucose levels during 75-g OGTT remained unchanged after PD. Furthermore, insulin levels at 60 min and GLP-1 levels at 30 and 60 min of 75-g OGTT were significantly increased after PD. In contrast, the AUC of glucose during 75-g OGTT was significantly increased after DP (Supplementary Fig. 4*B*). Insulin levels at 60 min and the AUC of insulin during 75-g OGTT were significantly decreased at 6 months after DP, whereas GLP-1 levels were unchanged after DP (Fig. 1*D* and *G*). Changes in Matsuda index, HOMA of β-cell function (HOMA-β), and insulinogenic index before and 6 months after pancreatectomy are shown in Supplementary Fig. 4*E–G*. The Matsuda index was unchanged after both surgical procedures. However, HOMA-β and insulinogenic index levels were significantly decreased after DP but did not change after PD. We also measured gastric inhibitory polypeptide (GIP) levels in some patients who underwent PD ($n = 5$) and DP ($n = 8$). The AUC of GIP during 75-g OGTT remained unchanged after both surgeries (Supplementary Fig. 4*H–J*).

Postsurgical Changes in Gut Microbiota, Fecal BAs, and SCFAs

The gut microbiota reportedly modulates the levels of fecal SCFAs and BAs, which are thought to stimulate GLP-1 secretion (4,5). Therefore, to examine whether the gut microbiota, fecal BAs, and SCFAs affect the association of PD with increased GLP-1, we evaluated changes in the microbiota and fecal SCFAs and BAs before surgery and 6 months after surgery in 16 patients (8 patients per PD and DP group). Bacterial abundance at the phylum level between the groups indicated that Proteobacteria significantly increased after PD ($P < 0.05$) but not after DP (Fig. 2*A* and *B*). Principal coordinates analysis highlighted differences in postsurgical fecal samples between patients who underwent PD and DP ($P = 0.0013$, analysis of similarities [ANOSIM]) (Fig. 2*C*). To investigate postsurgical changes in microbial richness, we determined the Chao1 index and Shannon score (Fig. 3*D*) but found no significant differences between presurgical and postsurgical samples from patients who underwent DP and PD.

Figure 2*E* shows the fecal levels of SCFAs, including acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate. There were significant increases in total SCFA ($P = 0.074$) and butyrate ($P < 0.001$) levels after PD, whereas SCFA levels did not significantly change after DP. In postsurgical fecal samples, valerate and isobutyrate levels were significantly higher after PD than after DP. Correlation analysis between SCFAs and GLP-1 revealed that butyrate levels were positively correlated with serum GLP-1 concentrations in postsurgical fecal samples (Fig. 2*F*). Thus, we investigated the changes in butyrate-producing bacteria after both PD and DP. It is reported that at the family level, *Ruminococcaceae* and *Lachnospiraceae* contain several butyrate-producing species and that *Roseburia* spp. and *Coprococcus* spp. of the family *Ruminococcaceae* as well as *Faecalibacterium* of the family *Lachnospiraceae* can produce butyrate (17); however, the relative abundance of these genera and families did not change after both PD and DP (Supplementary Fig. 5*A* and *B*).

Given that composition of the BA pool is regulated by the gut microbiota (18) and metabolism of BAs may be involved in glucose homeostasis (4), we examined changes in fecal BA levels before and 6 months after PD and DP. However, we observed no changes in fecal BA levels after PD or DP (Supplementary Fig. 6).

Proteobacteria are generally considered harmful due to their proinflammatory properties, including the production of lipopolysaccharide (LPS), which can exacerbate insulin resistance (19,20).

Table 1—Baseline clinical characteristics and medications taken by patients in the PD and DP groups

	PD	DP	*P*
n	20	28	
Clinical characteristics			
Age (years)	61.0 ± 9.1	56.0 ± 11.0	0.088
Sex (% male)	50.0	35.7	0.322
BMI (kg/m^2)	23.0 ± 4.1	23.6 ± 3.1	0.501
Current smoker (%)	5.0	7.1	0.759
SBP (mmHg)	119.2 ± 14.8	119.3 ± 19.7	0.992
DBP (mmHg)	71.0 ± 13.9	73.3 ± 12.5	0.565
HbA_{1c} (%)	5.7 ± 0.4	5.9 ± 0.3	0.251
HbA_{1c} (mmol/mol)	38.4 ± 4.6	39.8 ± 3.3	0.251
GA (%)	14.2 ± 1.8	14.0 ± 1.8	0.674
Triglycerides (mmol/L)	1.1 (0.7–1.6)	1.1 (0.9–1.7)	0.961
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.5 ± 0.5	0.609
LDL cholesterol (mmol/L)	3.2 ± 0.8	3.0 ± 0.7	0.474
Total bilirubin (mg/dL)	0.9 ± 0.4	0.8 ± 0.3	0.694
AST (units/L)	18.0 (14.3–21.5)	18.5 (16.5–22.8)	0.413
ALT (units/L)	15.0 (10.0–23.5)	16.5 (11.0–22.0)	0.645
γ-GTP (units/L)	25.0 (15.3–45.8)	22.0 (16.3–35.0)	0.623
UA (μmol/L)	324.8 (256.7–388.6)	284.2 (250.9–353.8)	0.180
Amylase (units/L)	67.5 (58.5–81.3)	79.5 (66.0–94.0)	0.109
Lipase (units/L)	32.5 (27.3–38.5)	31.0 (27.3–40.5)	0.892
Creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.2	0.068
eGFR (mL/min/1.73 m^2)	69.5 ± 16.5	79.1 ± 18.2	0.064
CRP	0.070 (0.028–0.120)	0.050 (0.028–0.082)	0.245
75-g OGTT			
Glucose (mg/dL)			
Fasting	88.3 ± 7.7	90.8 ± 8.2	0.288
30 min	153.6 ± 23.3	146.9 ± 30.0	0.405
60 min	149.1 ± 42.9	162.3 ± 41.9	0.291
120 min	137.1 ± 29.5	138.8 ± 26.0	0.837
Insulin (μU/mL)			
Fasting	5.3 (3.7–6.1)	5.1 (3.6–6.9)	0.770
30 min	36.7 (27.0–54.2)	30.3 (22.2–51.5)	0.184
60 min	29.0 (18.4–65.1)	41.3 (23.9–64.9)	0.246
120 min	35.0 (21.9–47.9)	42.8 (29.6–73.3)	0.094
Glucagon (pg/mL)			
Fasting	111.5 (101.5–122.0)	106.5 (92.0–113.5)	0.770
30 min	106.5 (96.5–116.0)	102.0 (90.0–112.5)	0.770
60 min	98.5 (88.0–110.5)	98.0 (87.5–102.0)	0.961
120 min	101.0 (88.5–107.3)	96.0 (87.8–104.0)	0.591
GLP-1 (pmol/L)		(*n* = 26)	
Fasting	1.4 (0.8–2.9)	1.2 (0.8–1.7)	0.372
30 min	5.8 (3.8–11.8)	6.2 (3.3–8.0)	0.766
60 min	5.2 (2.8–7.8)	4.4 (2.1–5.2)	0.723
120 min	3.9 (2.5–6.1)	4.5 (3.3–5.4)	0.784
HOMA-IR	1.2 ± 0.4	1.3 ± 0.6	0.472
Matsuda index	7.9 ± 0.9	7.3 ± 0.8	0.597
HOMA-β	77.8 ± 29.0	74.7 ± 35.3	0.742
Insulinogenic index	0.5 (0.4–0.7)	0.6 (0.4–0.8)	0.851
Urinary C-peptide (nmol/day)	16.6 (11.6–22.6)	16.8 (13.4–22.0)	0.826
β-Cell area (%)	0.79 ± 0.31	1.32 ± 0.59	<0.001
α-Cell area (%)	0.29 ± 0.21	0.62 ± 0.39	0. 001
α-to-β ratio	0.37 ± 0.23	0.43 ± 0.19	0.307
Primary disease, *n* (%)			
NET	13 (65)	16 (57)	0.583
IPMN	6 (30)	5 (18)	0.323
MCN	1 (5)	5 (18)	0.378
Splenic varices	0 (0)	2 (7)	0.503
Performance status			
0 (%)	100	100	1.000
≥1 (%)	0	0	1.000

Continued on p. 1006

Therefore we further measured serum levels of LPS, which are produced by Proteobacteria, in 23 patients (PD, $n = 8$; DP, $n = 15$) and found that LPS levels were significantly increased after PD ($P < 0.05$) but not after DP (Supplementary Fig. 7). Nevertheless, insulin resistance assessed with use of the Matsuda index was unchanged after PD.

Histological Analysis of Resected Pancreas in Patients Undergoing DP

In contrast to PD, DP robustly increased the risk of PPDM with no changes in the intestinal environment (Fig. 1*A*). Approximately 40% of patients who underwent DP immediately developed PPDM within 1 year after surgery, and the cumulative incidence of PPDM gradually increased thereafter, suggesting that factors other than resection itself are associated with the risk of PPDM. Therefore, we performed histological analysis of resected pancreas from patients who underwent DP to clarify the mechanism involved in PPDM development. Kaplan-Meier analysis, based on the median of β-cell area, α-cell area, or α-to-β ratio, was performed for determination of how long it took patients to develop diabetes after DP, indicated in Fig. 3*A*. Patients with values greater than the median β-cell and α-cell areas were at a significantly higher risk of developing diabetes after DP compared with those with values below the median of each histological index ($P = 0.005$ for β-cell area and $P = 0.032$ for α-cell area, log-rank test). Cox regression analysis revealed that both β-cell area (hazard ratio [HR] 2.54, 95% CI 1.07–5.98, $P = 0.033$) and α-cell area (HR 4.28, 95% CI 1.28–14.21, $P = 0.018$) were significantly associated with the development of diabetes after DP (Supplementary Table 2), and these associations remained significant even after adjustment for covariates including Matsuda index (β-cell area HR 3.68, 95% CI 1.25–10.82, $P = 0.018$; α-cell area HR 6.46, 95% CI 1.61–25.79, $P = 0.008$).

Next, we measured ALDH1A3 immunoreactivity of the resected pancreas in a subset of patients with presurgical NGT who underwent DP ($n = 16$) to investigate islet plasticity. The first group comprised six patients with NGT prior to DP who experienced PPDM (progressors), and the second group comprised 10 patients with NGT prior to DP who did not

Table 1—Continued

	PD	DP	*P*
Medications			
RAS inhibitors (%)	15.0	14.3	0.944
CCBs (%)	25.0	10.7	0.193
Lipid-lowering drugs (%)	15.0	25.0	0.393
UA-lowering agents (%)	10.0	7.2	0.726

Data are shown as mean ± SD or median (interquartile range) unless otherwise indicated. *P* value represents the difference among the groups in percent (χ^2 test), means (*t* test), or median (Mann-Whitney *U* test). CCBs, calcium channel blockers; CRP; C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GA, glycoalbumin; γ-GTP, γ-glutamyl transpeptidase; HOMA-IR, HOMA of insulin resistance; IPMN, intraductal papillary; MCN, mucinous cystic neoplasm; NET, neuroendocrine tumor; RAS, renin-angiotensin-aldosterone system; SBP, systolic blood pressure; UA, uric acid.

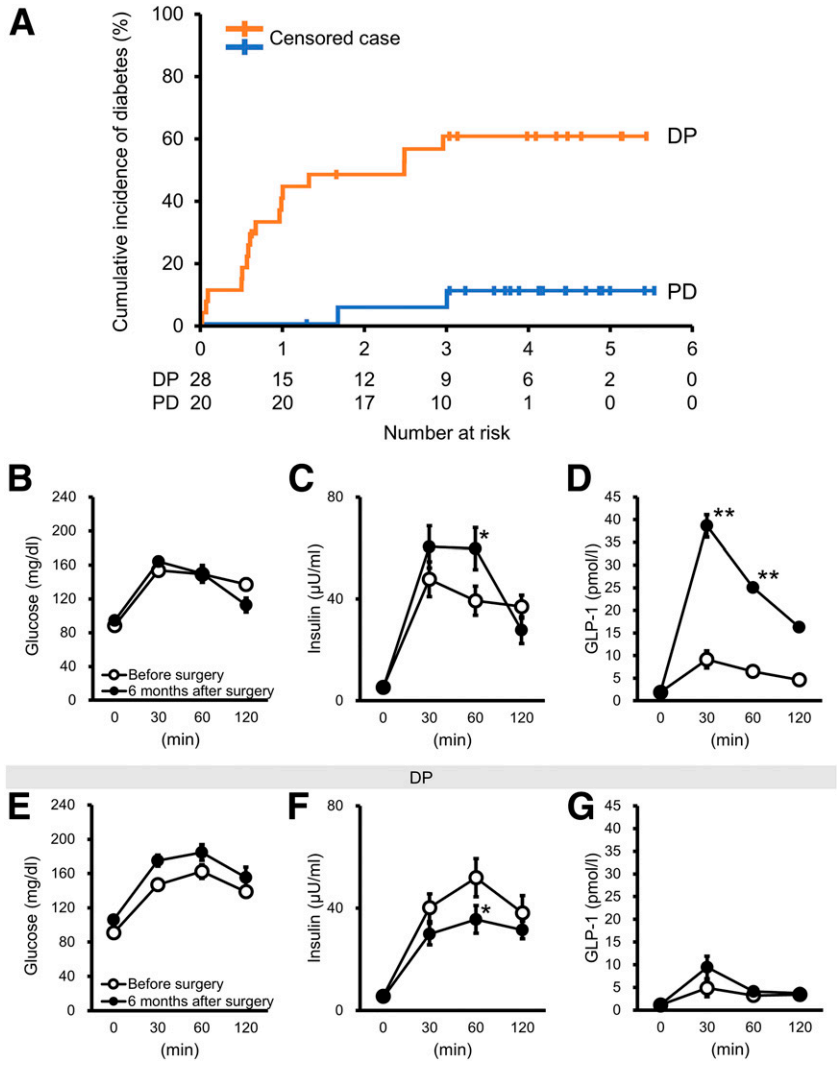

Figure 1—Glucose metabolism before and after partial pancreatectomy. *A*: Kaplan-Meier curves for estimating the cumulative incidence of diabetes during follow-up in the PD and DP groups. *B–D*: Blood glucose ($n = 20$) (*B*), insulin ($n = 20$) (*C*), and GLP-1 ($n = 9$) (*D*) concentrations during 75-g OGTT performed before and 6 months after surgery for patients who underwent PD. *E–G*: Blood glucose (before surgery, $n = 28$; 6 months after surgery, $n = 27$) (*E*), insulin (before surgery, $n = 28$; 6 months after surgery, $n = 27$) (*F*), and GLP-1 ($n = 9$) (*G*) concentrations during 75-g OGTT performed before and 6 months after surgery for patients who underwent DP. Data are presented as means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ at individual time points.

develop PPDM (nonprogressors). The baseline characteristics of the progressors and nonprogressors after DP are listed in Supplementary Table 3. Age, sex, and BMI were similar between the progressors and nonprogressors, while the median counts of ALDH1A3$^+$ cells/islet did not significantly differ between them ($P = 0.237$) (Fig. 3*B* and *C*). As shown in Fig. 3*D*, the β-cell and α-cell areas were both significantly correlated with ALDH1A3$^+$ cells/islet (β-cell area, $r = 0.515$, $P = 0.041$; α-cell area, $r = 0.580$, $P = 0.019$).

We further performed immunostaining of Ki67 (triple staining of Ki67, insulin, and glucagon in randomly selected 25 islets per DP patient; $n = 16$), M30 (double staining of M30 with insulin and glucagon in randomly selected 50 islets per progressor patient in the DP group; $n = 6$), and c-kit (randomly selected 30 islets per DP patient; $n = 16$) to assess cell replication, apoptosis, and transdifferentiation, respectively, in the islets. We found that replication, apoptosis, or transdifferentiation rarely occurred in islet endocrine cells of DP patients without diabetes (data not shown).

Histological Analysis of Resected Pancreas in Patients Undergoing PD

Similar to the analysis described above, we attempted to examine the association between PPDM and islet expansion in patients who underwent PD; however, this was not possible due to the small number of patients who experienced PPDM after PD ($n = 2$). Thus, we instead examined the association between islet expansion and deterioration of glucose tolerance after surgery, which is defined as the progression of presurgical NGT to postsurgical IGT or diabetes or of presurgical IGT to postsurgical diabetes in patients with PD. As shown in Supplementary Fig. 8, β-cell area, α-cell area, and α-to-β ratio were not significantly associated with deterioration of glucose tolerance after PD.

We also measured ALDH1A3 immunoreactivity of the resected pancreas in a subset of patients who underwent PD ($n = 11$). The first group comprised five patients with presurgical NGT who experienced deterioration of glucose tolerance (progressors), and the second group comprised six patients with presurgical NGT who did not experience glucose tolerance deterioration (nonprogressors);

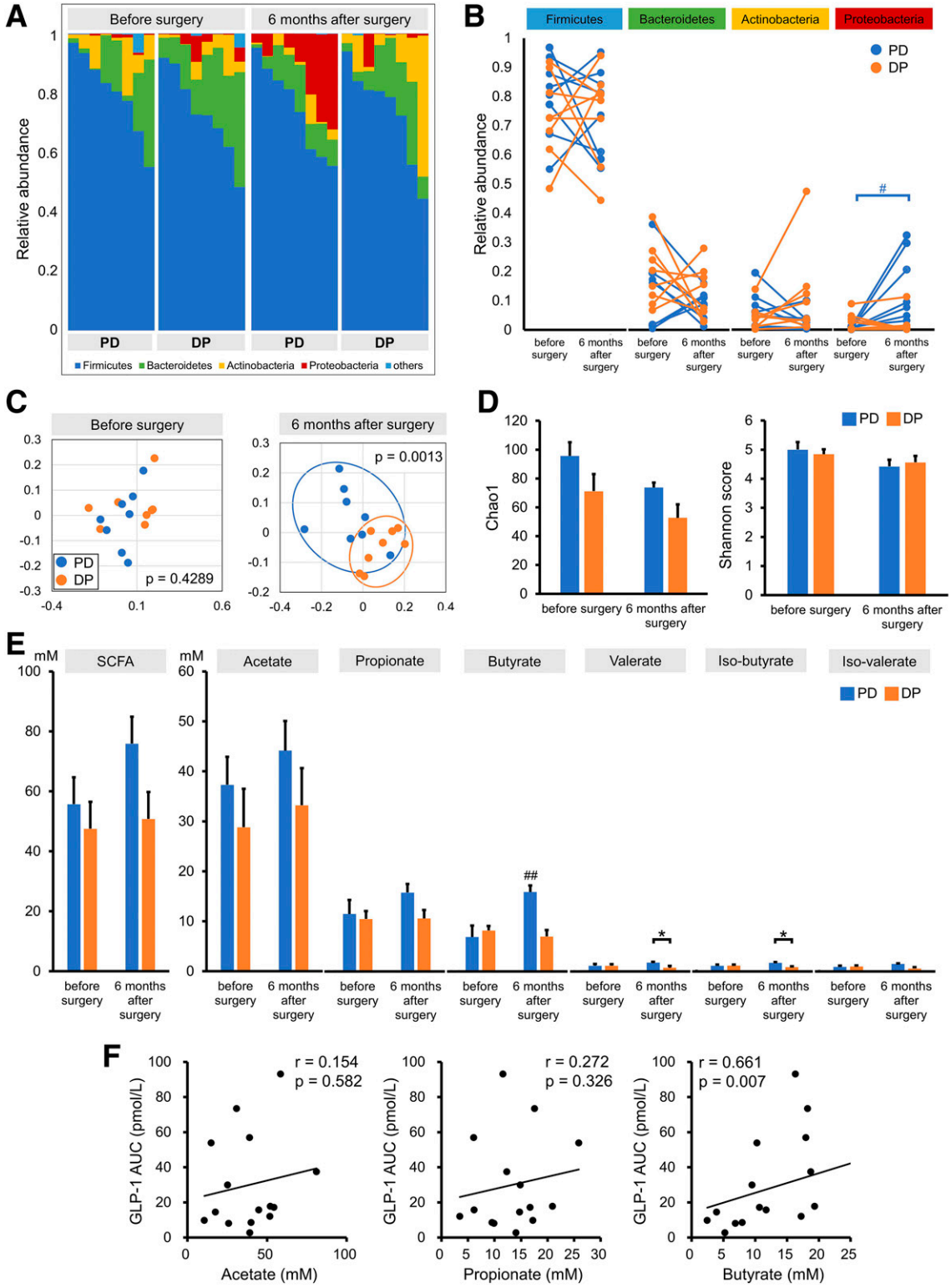

Figure 2—Gut microbiome and fecal concentrations of SCFAs in patients who underwent PD and DP. *A* and *B*: Relative abundance of bacterial phyla in fecal microbiota collected before and 6 months after surgery in patients who underwent PD ($n = 8$) or DP ($n = 8$). *B*: Black and gray lines represent the change in relative abundance of bacterial phyla in each patient. *C* and *D*: Principal coordinates analysis of the unweighted UniFrac distances (*C*) and α diversity indices of Shannon score and Chao1 (*D*) of fecal microbiota collected before and 6 months after surgery in patients who underwent PD ($n = 8$) or DP ($n = 8$). *E*: SCFA levels in fecal samples before and 6 months after surgery in patients who underwent PD ($n = 8$) or DP ($n = 8$). *F*: Spearman correlations between AUC of plasma GLP-1 levels during 75-g OGTT and acetate, propionate, and butyrate levels in fecal samples at 6 months after surgery in 16 patients (PD, $n = 8$; DP, $n = 8$). Data are presented as means ± SEM. $^*P < 0.05$, differences between surgical procedures; $\#P < 0.05$, $\#\#P < 0.01$ vs. before surgery.

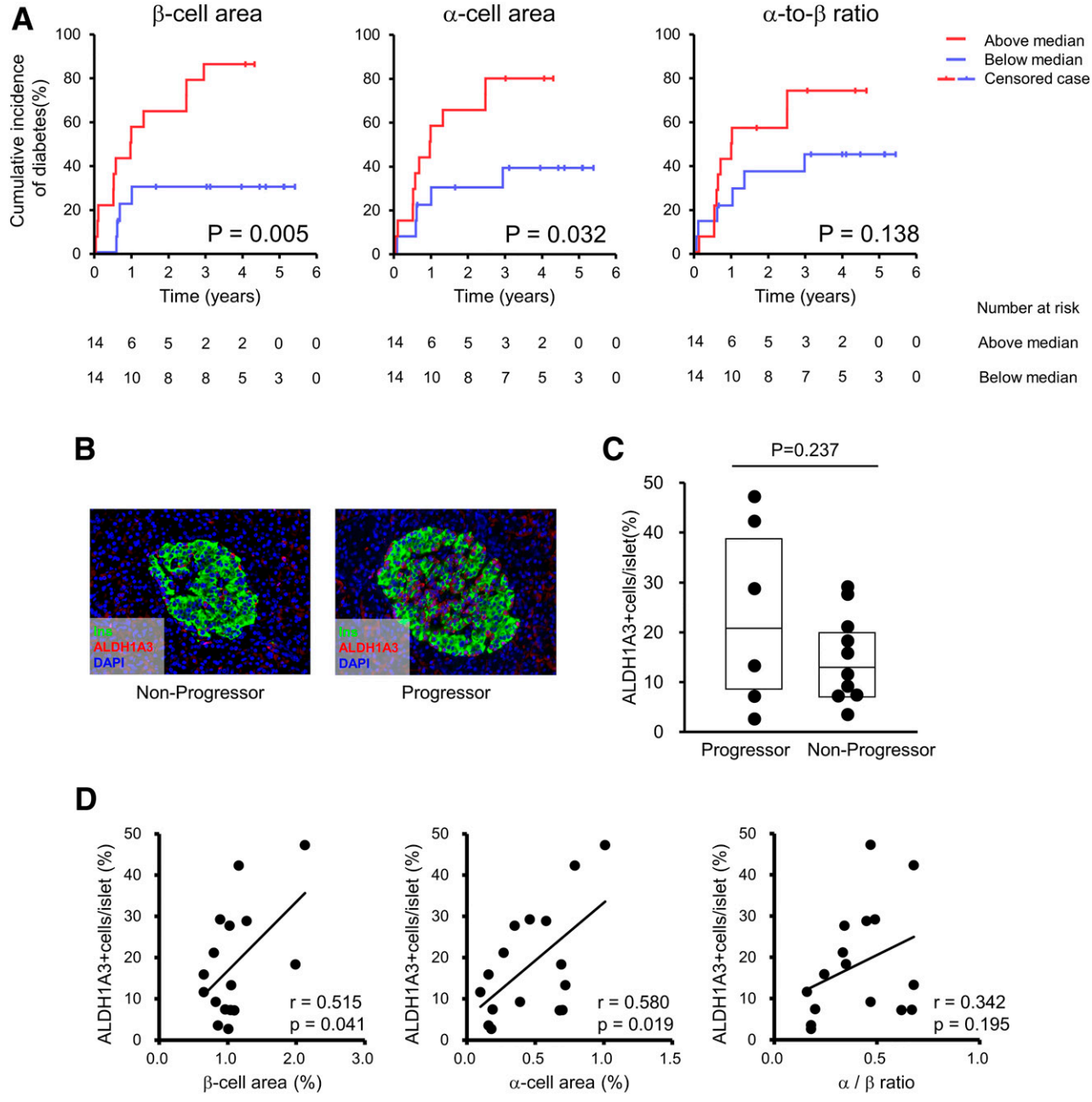

Figure 3—Histological analysis of the resected pancreas. *A*: Kaplan-Meier curves for estimating the cumulative incidence of diabetes after DP, based on the median of the α-cell area, β-cell area, or α-to-β ratio. *B*: Representative images of immunofluorescence of ALDH1A3 (red) with insulin (green) and DAPI (blue) in resected pancreas of the progressors and nonprogressors who underwent DP. *C*: Quantitative analysis of ALDH1A3$^+$ cells per islet in resected pancreas from progressors ($n = 6$) and nonprogressors ($n = 10$) who underwent DP. Box plot indicates the median and interquartile range. *D*: Correlations between α-cell area, β-cell area, or α-to-β ratio and ALDH1A3$^+$ cells per islet in patients who underwent DP ($n = 12$).

their baseline characteristics are listed in Supplementary Table 4. Age, sex, BMI, and indices of insulin resistance were similar between the progressors and nonprogressors. We found a significant difference in the number of ALDH1A3$^+$ cells/islet (means ± SD) between the DP and PD groups (18.3 ± 13.4% and 8.9 ± 5.9%, respectively; $P = 0.042$). Moreover, the median counts of ALDH1A3$^+$ cells/islet in the progressor group were significantly higher than those in the nonprogressor group ($P = 0.017$) (Supplementary Fig. 9*A*). As shown in Supplementary Fig. 9*B*, the α-cell area and α-to-β ratio were both significantly correlated with ALDH1A3$^+$ cells/islet (α-cell area, $r = 0.691$ and $P = 0.023$; α-to-β ratio, $r = 0.818$ and $P = 0.004$). We further performed c-kit immunostaining (randomly selected 30 islets per 11 patients in the PD group) to assess cell transdifferentiation in the islets and found that transdifferentiation rarely occurred in islet endocrine cells of patients with PD without diabetes (data not shown).

CONCLUSIONS

In this study, we revealed that the incidence of PPDM was much lower after PD than after DP. We excluded malignant

pancreatic disease and chronic pancreatitis from our analysis, as they can lead to changes in presurgical to postsurgical glucose metabolism, and performed consecutive evaluations of 75-g OGTT (at baseline and every 6 months after surgery), which is the gold standard for diabetes diagnosis. Close collaboration between diabetologists and surgeons allowed for strict follow-up of glucose tolerance throughout the study period. Considering that previous studies have frequently included patients with cancer or chronic pancreatitis or had short follow-up periods (21,22), the incidence of PPDM measured in the current study may be the most reliable to date.

We considered several reasons for the difference in PPDM incidence between the surgeries. First, as the β-cell area was significantly higher in the resected pancreas after DP but the resected volume was comparable between patients after PD and DP, the higher incidence of PPDM after DP may result, in part, from the greater removal of β-cells. Second, there is significant correlation between increased β- and α-cell areas and ALDH1A3 expression in DP patients, and DP patients with NGT who developed PPDM (progressors) showed preexisting insulin resistance compared with those who did not (nonprogressors). These findings may suggest that islet expansion and cellular plasticity have already been induced in response to insulin sensitivity deterioration before surgery, consequently reducing insulin secretion and finally leading to an increased risk of PPDM in patients after DP. Third, intestinal bypass surgeries, including PD, markedly increase GLP-1 secretion, SCFA production, and alterations in the gut microbiota, eventually reducing PPDM risk in patients who underwent PD.

In an effort to elucidate the mechanism underlying the low incidence of diabetes after PD, we found a significant increase in GLP-1 secretion with preserved insulin secretion after PD. Bypass of the proximal intestine was reported to mainly contribute to enhanced GLP-1 secretion after meal intake (23,24); therefore, it is likely that GLP-1 levels are increased in response to the bypass performed during PD. This finding is in agreement with a previous study that examined patients undergoing pylorus-preserving PD (25); however, the underlying mechanism has not been fully elucidated. Therefore, we focused on the changes in gut microbiota and fecal SCFA and BA levels, both of which are thought to modulate GLP-1 secretion (4,5), and found that SCFA levels (butyrate) tend to be significantly increased after PD. Furthermore, a study on baboons demonstrated that a sufficient increase in GLP-1 induces an increase in β-cells (26). Although it is difficult to confirm such changes in the human pancreas, a robust increase in GLP-1 secretion after PD can lead to an increase in β-cells, resulting in preserved insulin secretion after surgery. Unexpectedly, we did not find any changes in fecal BA levels after PD or DP. Although gut BAs were previously reported to play a critical role in regulating intestinal GLP-1 secretion via transmembrane G-protein–coupled receptor 5 (5), administration of colesevelam, a BA sequestrant that increases fecal BA excretion by disrupting its enterohepatic circulation, to patients with type 2 diabetes did not affect GLP-1 secretion (27). Other clinical studies have also reported no significant effect of BA sequestration on the GLP-1 response (28,29). Thus, further studies are required to determine the effects of fecal BAs on the GLP-1 response.

Previous animal and human studies that examined changes in the gut microbiota after Roux-en-Y gastric bypass have consistently reported an increase in Proteobacteria (3,30) and that intestinal exposure to an aerobic environment due to surgery can contribute to the increase in aerobic bacteria mainly belonging to Proteobacteria. Consistent with these studies, we found a significant increase in Proteobacteria after PD. Proteobacteria are generally considered harmful due to their proinflammatory characteristics, such as the production of LPS, which can exacerbate insulin resistance (19,20). Indeed, we observed an increase in serum LPS levels, but insulin resistance did not worsen after PD. It is possible that decreased body weight after PD attenuates the association between increased Proteobacteria and increased LPS. Moreover, given that fecal butyrate levels increased after PD, we performed relative abundance analysis for butyrate-producing bacteria; however, their abundance was not significantly altered after PD (Supplementary Fig. 5). Because with this relative abundance analysis we were not able to accurately assess the absolute amount of gut microbiota species, future studies should elucidate which bacterial species contribute to the increase in fecal butyrate levels after PD. Finally, alterations to the gut microbiota after PD may stimulate GLP-1 secretion by increasing fecal levels of SCFAs, especially butyrate, resulting in maintenance of glucose metabolism, despite increases in Proteobacteria and LPS in feces and blood, respectively.

Considering that GIP-secreting K cells are abundant in the duodenum (31), it is assumed that a reduction in GIP in patients after PD mainly occurs as a result of duodenectomy. Indeed, in a previous study by Muscogiuri et al. (25), GIP levels decreased by one-third after pylorus-preserving PD; however, postsurgical GIP levels in patients after PD in our study did not decrease to such an extent. The GIP secretion response to meal intake is reported to be exaggerated in patients who underwent Billroth I gastrectomy (32); here, the SSPPD performed includes resection of the pylorus, which causes early exposure of the distal intestine to undigested nutrients, possibly stimulating GIP secretion in the residual small intestine (jejunum and ileum) where K cells are present. Therefore, resection of the pylorus may have attenuated the decrease in GIP after SSPPD compared with after pylorus-preserving PD.

Next, we revealed that both α- and β-cell areas in the resected pancreas from patients after DP were independently associated with PPDM, even after adjustment for covariates including the Matsuda index. It was previously reported that people with insulin resistance have a higher number of β-cells than those without (33,34) and that the increase in β-cells may be a compensatory response to the elevated metabolic demand (6). Thus, our data suggest that the increased β-cell area represents an early adaptation that cannot be detected by clinical parameters such as the Matsuda index. In the PD group, we found no significant association between α- and β-cell areas with the deterioration of glucose tolerance after surgery. It is possible that drastic changes in the gut environment, including increased GLP-1 secretion after surgery, may attenuate this association. Further studies are needed to elucidate whether the β-cell area is associated with PPDM after PD.

Regarding immunostaining of ALDH1A3, we demonstrated that increased expression of ALDH1A3 was accompanied by increased β- and α-cell area in patients with NGT and without cancer who underwent DP. In NGT patients who underwent PD, ALDH1A3 expression was significantly correlated with the α-cell area, supporting the notion that ALDH1A3 expression is increased along with islet expansion. Increased levels of ALDH1A3 in islets are thought to be a marker of β-cell failure (8), and ALDH1A3 is reported to be abundantly expressed in the islets of patients with chronic pancreatitis and without diabetes (35). Furthermore, a recent study in patients without diabetes revealed that islet ALDH1A3 expression is increased in patients with pancreatic ductal adenocarcinomas compared with those with benign tumors (36). Taken together, the findings indicate that a substantial number of islet endocrine cells may gain cellular plasticity and thus express ALDH1A3 and that inflammation may trigger ALDH1A3 expression even during the very early stages of diabetes development. As inflammation of the pancreas has been suggested to be associated with the pathogenesis of diabetes (37), ALDH1A3 may be a predictive marker for diabetes development. Moreover, cellular plasticity increased with an increase in β-cells, even in patients without diabetes. The current study excluded patients with cancer or pancreatitis to eliminate the possible effect of neoplastic inflammation or pancreatitis. However, clarifying whether high cellular plasticity in islets of subjects without diabetes is associated with the development of future type 2 diabetes is not possible due to difficulties in collecting human pancreatic tissue. Nevertheless, we believe that our findings provide insights into the mechanism of type 2 diabetes development in humans and support ALDH1A3 as a marker for dysfunctional islet cells in addition to β-cell expansion.

This study has several limitations. First, the sample size was small, and we only included Japanese subjects; therefore, it is unknown whether our findings are applicable to other ethnicities. Second, the 90-min value of the 75-g OGTT is missing, limiting the accurate assessment of Matsuda index or the AUC of glucose, insulin, GLP-1, and GIP. Third, we were unable to obtain information about diet, which can modulate the gut microbiota (38) and fecal SCFAs (39) throughout the study period. Fourth, we were unable to examine associations between histological characteristics and PPDM in patients who underwent PD, since few exhibited PPDM. Finally, we were unable to repeatedly collect pancreatic tissue during the follow-up period; thus, it remains unclear whether increased β-cell mass and increased ALDH1A3$^+$ cells represent early adaptation to metabolic stress in humans.

In conclusion, our results provide evidence that PPDM incidence is much lower after PD than after DP and that changes in the intestinal environment involving increased fecal SCFA production and altered gut microbiota composition can prevent the deterioration of glucose metabolism via increased GLP-1 secretion after PD. Moreover, islet expansion is associated with increased cellular plasticity in islets and is a valuable predictor of PPDM after DP.

Acknowledgments. The authors thank all of the staff of Department of Molecular Endocrinology and Metabolism, Department of Hepatobiliary and Pancreatic Surgery, and Department of Human Pathology of Tokyo Medical and Dental University for their contributions.

Funding. This work was funded by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (15K-19507), the Japan Foundation for Applied Enzymology, Takeda Science Foundation, and AstraZeneca.

The study funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation.

Duality of Interest. This work was partially supported by the grant from AstraZeneca. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. R.B. and Y.O. designed this study. T.F., R.B., and T.T. researched data, and T.F. wrote and edited the manuscript. A.K., S.T., and M.T. performed partial pancreatectomy and participated in data collection. T.A., K.A.-S., K.T., and Y.T. performed pathological analyses of pancreas. T.O. analyzed the gut microbiota sequence data. M.I. and I.K. quantified the levels of SCFAs and BAs in feces. K.H., Y.O., and T.Y. contributed to interpretation of data. All authors were involved in the discussion of the results and commented on the manuscript. R.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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