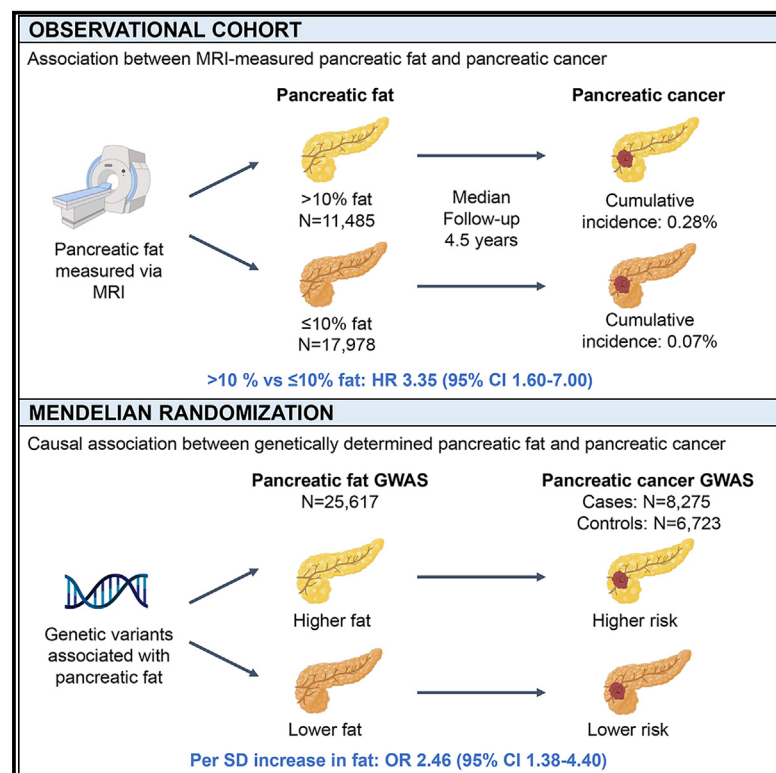


# Evidence for a causal link between intra-pancreatic fat deposition and pancreatic cancer: A prospective cohort and Mendelian randomization study

## Graphical abstract



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## In brief

Yamazaki et al. investigate the causal relationship between intra-pancreatic fat deposition (IPFD) and pancreatic cancer. Their observational cohort study (N = 29,463) shows that individuals with >10% IPFD have a 3-fold increased risk of pancreatic cancer. Furthermore, their Mendelian randomization study provides evidence suggesting a causal link between IPFD and pancreatic cancer.

## Highlights

- Cohort study of 29,463 individuals assessed for intra-pancreatic fat deposition (IPFD)
- Individuals with >10% IPFD face a three times higher risk of pancreatic cancer
- Mendelian randomization (MR) analysis used 8 genetic variants linked to IPFD
- MR analysis provides evidence of causal link between IPFD and pancreatic cancer



## Article

# Evidence for a causal link between intra-pancreatic fat deposition and pancreatic cancer: A prospective cohort and Mendelian randomization study

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## SUMMARY

Prior observational studies suggest an association between intra-pancreatic fat deposition (IPFD) and pancreatic ductal adenocarcinoma (PDAC); however, the causal relationship is unclear. To elucidate causality, we conduct a prospective observational study using magnetic resonance imaging (MRI)-measured IPFD data and also perform a Mendelian randomization study using genetic instruments for IPFD. In the observational study, we use UK Biobank data (N = 29,463, median follow-up: 4.5 years) and find that high IPFD (>10%) is associated with PDAC risk (adjusted hazard ratio [HR]: 3.35, 95% confidence interval [95% CI]: 1.60–7.00). In the Mendelian randomization study, we leverage eight out of nine IPFD-associated genetic variants ( $p < 5 \times 10^{-8}$ ) from a genome-wide association study in the UK Biobank (N = 25,617) and find that genetically determined IPFD is associated with PDAC (odds ratio [OR] per 1-standard deviation [SD] increase in IPFD: 2.46, 95% CI: 1.38–4.40) in the Pancreatic Cancer Cohort Consortium I, II, III (PanScan I-III)/Pancreatic Cancer Case-Control Consortium (PanC4) dataset (8,275 PDAC cases and 6,723 non-cases). This study provides evidence for a potential causal role of IPFD in the pathogenesis of PDAC. Thus, reducing IPFD may lower PDAC risk.

## INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC), responsible for 90% of all pancreatic cancers, is highly lethal, with only a 10% 5-year survival rate.<sup>1</sup> It is expected to become the second leading cause of cancer-related mortality by 2030.<sup>1</sup> Thus, understanding risk factors and etiologic mechanisms is imperative for identifying susceptible populations and reducing the burden of disease. One important modifiable risk factor for many cancers is body fat,<sup>2</sup> which can be stored in the classical subcutaneous and visceral depots, as well as accumulate within various organs.<sup>3</sup>

For instance, excessive fat storage in the liver is a well-established risk factor for liver cancer, presumably through pro-inflammatory and pro-fibrotic mechanisms.<sup>4</sup> While fat deposition in the pancreas, also known as intra-pancreatic fat deposition (IPFD), is a long-known phenomenon,<sup>5</sup> it has received less attention due to past challenges in accurately quantifying IPFD in this small and irregularly shaped organ.<sup>6</sup> Histologically, fat in the pancreas is present in adipocytes that reside between pancreas cells.<sup>6</sup> Adipocytes in the pancreas secrete a variety of proteins, including chemokines and cytokines, thereby promoting tissue inflammation.<sup>7</sup>



**Table 1. Characteristics of the 29,463 participants in the prospective observational study of IPFD and PDAC in the UK Biobank, stratified by high vs. low IPFD**

	High-IPFD group (N = 11,485)	Low-IPFD group (N = 17,978)
Age (years)	67 (61–71)	63 (57–69)
Male, N (%)	7,390 (64.3)	6,882 (38.3)
White ethnicity, N (%)	11,221 (98.0)	17,316 (96.6)
BMI (kg/m <sup>2</sup> )	27.9 (25.5–30.8)	24.7 (22.6–27.2)
Intra-pancreatic fat deposition (%)	15.8 (12.4–22.3)	5.5 (3.8–7.4)
Hepatic fat deposition (%)	4.3 (2.8–7.9)	2.5 (1.9–3.9)
Current smoker, N (%)	420 (3.7)	536 (3.0)
Daily drinker, N (%)	2,027 (17.8)	2,787 (15.6)
Follow-up period (years)	4.5 (3.8–5.4)	4.5 (3.8–5.4)

High and low IPFD were defined based on the mean IPFD among all participants (10%). Continuous data were expressed as median (interquartile range). Missing data: ethnicity (N = 82), BMI (N = 862), hepatic fat deposition (N = 2,255), current smoker (N = 273), and daily drinker (N = 185). IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; BMI, body mass index.

Recent developments in imaging methods such as magnetic resonance imaging (MRI) have not only revealed that IPFD is common<sup>8,9</sup> but have also enabled a better understanding of the clinical significance of IPFD.<sup>10–12</sup> Several imaging studies have shown that individuals with higher levels of IPFD have an increased diabetes risk, presumably due to signals from local adipocytes impairing insulin secretion.<sup>13,14</sup> Most importantly, higher levels of IPFD are also hypothesized to cause PDAC.<sup>6,15</sup>

In the current epidemiologic literature, it has been suggested that IPFD is associated with precancerous lesions and PDAC.<sup>16,17</sup> However, these results are based on several cross-sectional studies and a retrospective case-control study with a short observational period (i.e., 1–36 months). Because of the long latency of tumorigenesis, the results from these studies could be potentially biased due to reverse causation (i.e., IPFD could be a consequence, rather than a risk factor, of PDAC). Thus, a prospective assessment of IPFD and PDAC in a cohort with substantial follow-up time is needed to overcome this limitation and provide stronger evidence of a causal association.

Causality can further be evaluated using Mendelian randomization, a method that uses genetic variants (e.g., single-nucleotide polymorphisms [SNPs]) to assess the causal effect of an exposure (e.g., IPFD) on a disease (e.g., PDAC).<sup>18</sup> Mendelian randomization studies are considered natural randomized trials, in which the random inheritance of genetic variants works as random treatment assignments. Because genetic variants are assigned randomly at conception and are not affected by acquired diseases or environmental factors, Mendelian randomization is less prone to reverse causation and confounding.<sup>19,20</sup>

We conducted a prospective observational study of MRI-measured IPFD and PDAC incidence and performed a Mendelian randomization study to evaluate the causal association of genetically determined IPFD with PDAC. We thereby aimed to clarify if IPFD is indeed a causal contributor to PDAC, which

could ultimately lead to improvement in prevention, early detection, or treatment of this highly fatal cancer.

## RESULTS

### Observational study for the association of IPFD with PDAC

We conducted an observational prospective cohort study to investigate the association between IPFD and PDAC in 29,463 UK Biobank participants who underwent pancreas MRI.<sup>21</sup> Characteristics of the study participants are shown in [Table 1](#). The high-IPFD group (i.e., participants whose IPFD values were more than the mean IPFD value of 10%) tended to be older, predominantly male, and had a higher body mass index (BMI) compared to the low-IPFD group (IPFD ≤ 10%). Correlation between IPFD and BMI was moderate (Pearson correlation coefficient = 0.40). During the median follow-up period of 4.5 years (interquartile range: 3.8–5.4), the cumulative incidence of PDAC was 0.28% (32 cases out of 11,485 individuals) in the high-IPFD group and 0.07% (12 cases out of 17,978 individuals) in the low-IPFD group. High IPFD was associated with a 3-fold increased risk of PDAC (multivariable adjusted hazard ratio: 3.35, 95% confidence interval [95% CI]: 1.60–7.00, p = 0.001). Similar results were observed when IPFD was analyzed as tertiles, while the analysis using continuous IPFD showed a comparable trend ([Table 2](#)).

### Mendelian randomization study

We conducted a two-sample Mendelian randomization study using data from two large-scale genome-wide association studies (GWASs) for MRI-measured IPFD and PDAC in individuals of European ancestry.<sup>21–23</sup> The details of assumptions required in Mendelian randomization are shown in [Figure S1](#). Data sources and selection of genetic instruments are shown in [Figure 1](#). [Table 3](#) shows descriptive information of the GWAS datasets used in this Mendelian randomization study. We leveraged eight out of nine genetic variants associated with IPFD (p < 5 × 10<sup>−8</sup>) from a GWAS in the UK Biobank (N = 25,617 individuals)<sup>21</sup> and assessed their association with PDAC in the Pancreatic Cancer Cohort Consortium I, II, III (PanScan I–III) and Pancreatic Cancer Case-Control Consortium (PanC4) dataset (8,275 PDAC cases and 6,723 non-cases).<sup>22,23</sup>

### Primary Mendelian randomization analysis for the association of IPFD with PDAC

Characteristics of the eight IPFD-associated genetic variants are shown in [Table 4](#). All eight genetic variants were strongly associated with IPFD: mean F-statistics 54 (min 33, max 103). The odds ratios (ORs) of PDAC for each of the eight genetic variants were greater than the reference value of one.

In the primary Mendelian randomization analysis using the inverse-variance weighted (IVW) method, genetically determined IPFD levels were associated with PDAC risk ([Figure 2](#)). The OR of PDAC per 1-standard deviation (SD) increase in genetically determined IPFD level (i.e., per 7.9% increase in fat fraction percentage within the pancreas) was 2.46 (95% CI: 1.38, 4.40; p = 0.002), an average 146% increased risk of PDAC per 1-SD (7.9%) increase in IPFD.

**Table 2. Results from the prospective observational study of the association between IPFD and incidence of PDAC in the UK Biobank**

	Cumulative incidence, <sup>a</sup> % (N cases/N total)	Crude HR (95% CI) <sup>c</sup>	p value	Age and BMI adjusted HR (95% CI) <sup>c</sup>	p value	Multivariable adjusted <sup>b</sup> HR (95% CI) <sup>c</sup>	p value
Categorization (mean value) <sup>d</sup>							
Low IPFD ( $\leq 10\%$ )	0.07 (12/17,978)	1.00 (reference)	–	1.00 (reference)	–	1.00 (reference)	–
High IPFD ( $> 10\%$ )	0.28 (32/11,485)	4.20 (2.16–8.15)	<0.001	3.53 (1.72–7.26)	0.001	3.35 (1.60–7.00)	0.001
Categorization (tertiles)							
Low IPFD ( $\leq 5.8\%$ )	0.06 (6/9,821)	1.00 (reference)	–	1.00 (reference)	–	1.00 (reference)	–
Moderate IPFD (5.8–11%)	0.09 (9/9,821)	1.49 (0.53–4.19)	0.45	1.34 (0.47–3.87)	0.58	1.28 (0.44–3.73)	0.65
High IPFD ( $> 11\%$ )	0.30 (29/9,821)	4.83 (2.01–11.6)	<0.001	3.85 (1.47–10.1)	0.006	3.57 (1.32–9.62)	0.012
Continuous IPFD <sup>e</sup>							
IPFD (per 1-SD increase)	0.15 (44/29,463)	1.69 (1.25–2.27)	0.001	1.48 (1.05–2.08)	0.025	1.42 (0.99–2.02)	0.056

IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; HR, hazard ratio; CI, confidence interval; BMI, body mass index; SD, standard deviation.

<sup>a</sup>Median follow-up period was 4.5 years (interquartile range: 3.8–5.4).

<sup>b</sup>The multivariable analysis was adjusted for age, gender, BMI, current smoking status, and daily drinking.

<sup>c</sup>HR and 95% CI estimated using Cox regression models.

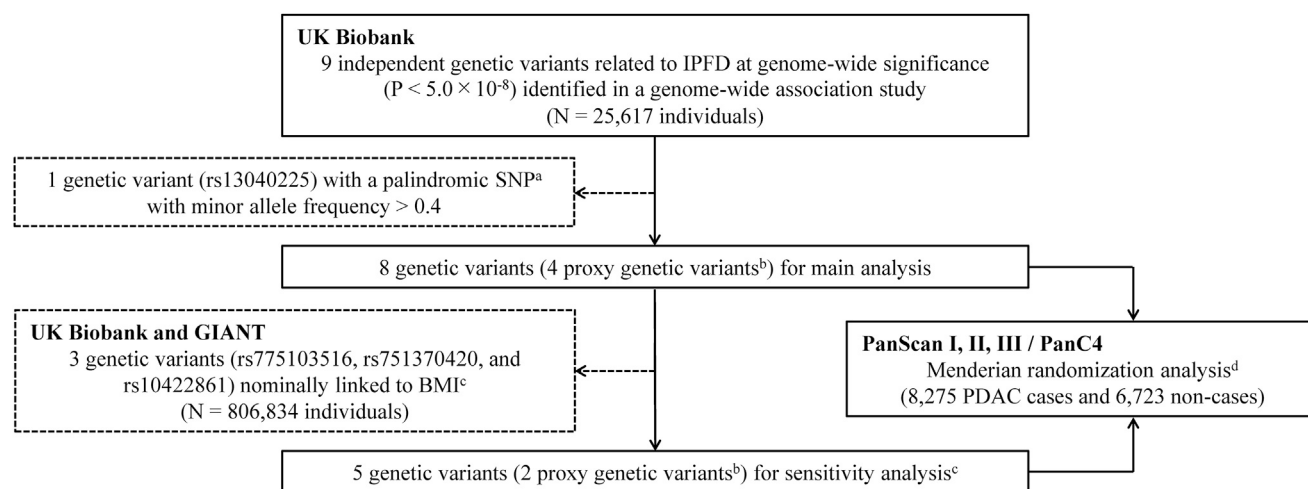
<sup>d</sup>High and low IPFD were defined based on the mean IPFD among all participants (10%).

<sup>e</sup>Log transformation was applied to continuous IPFD to correct for skewness. Models evaluated 1-SD increase in the log-transformed value.

### Sensitivity Mendelian randomization analyses for the association of IPFD with PDAC

To account for potential pleiotropy (i.e., a violation of assumptions for Mendelian randomization), we conducted several sensitivity analyses: the IVW method with leave-one-out analysis and pleiotropy-robust statistical methods. These sensitivity analyses also showed consistent associations between genetically deter-

mined IPFD levels and PDAC (Figure S2; Table 5). All of the leave-one-out ORs and 95% CIs indicated a statistically significant association with PDAC. The weighted median method and the Mendelian randomization-pleiotropy residual sum and outlier (MR-PRESSO) method showed similar associations (ORs [95% CI]: 1.79 [1.13, 2.83] and 2.29 [1.61, 3.26], respectively), while the MR-Egger method showed a wide CI (OR 4.56 [95%



**Figure 1. Data sources and selection of genetic instruments for the Mendelian randomization analysis**

<sup>a</sup>Palindromic SNPs are those where the alleles are complementary (G/C or A/T).

<sup>b</sup>Proxy genetic variants were used when selected genetic variants did not exist in PanScan I–III or PanC4.

<sup>c</sup>Association of each genetic variant with BMI was evaluated using summary statistics obtained from meta-analysis results of the UK Biobank and GIANT consortium. Although none of the eight genetic variants were associated with BMI at the genome-wide significance, we further conducted a sensitivity analysis excluding the three genetic variants with a nominal BMI association.

<sup>d</sup>The estimates for the association of each genetic variant with PDAC were combined using the inverse-variance weighted method, with summary statistics for PDAC obtained from PanScan I–III and PanC4. IPFD, intra-pancreatic fat deposition; SNP, single-nucleotide polymorphism; MAF, minor allele frequency; GIANT, genetic investigation of anthropometric traits; BMI, body mass index; PanScan, Pancreatic Cancer Cohort Consortium; PanC4, Pancreatic Cancer Case-Control Consortium; PDAC, pancreatic ductal adenocarcinoma.

**Table 3. Descriptive information of the GWAS used in the Mendelian randomization study**

	UK Biobank <sup>21</sup>	PanScan I-III + PanC4 <sup>23</sup>
Aim of GWAS	IPFD	PDAC
Participants, N	25,617	14,998
		(8,275 cases and 6,723 non-cases)
Age (years)		
Mean (SD)	64.2 (7.5)	N/A
<50; 50–60;	N/A	1,159; 3,088; 5,275;
60–70; 70–80;		4,354; 1,122
>80, N		
Female (%)	51.2	45.8
European ancestry (%)	100	100
BMI (kg/m <sup>2</sup> ), mean (SD)	26.5 (4.3)	N/A
IPFD (%), mean (SD)	10.4 (7.9)	N/A

IPFD (i.e., fat fraction percentage within the pancreas) was measured on MRI. IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; PanScan, Pancreatic Cancer Cohort Consortium; PanC4, Pancreatic Cancer Case-Control Consortium; GWAS, genome-wide association study; SD, standard deviation; BMI, body mass index; N/A, not applicable; MRI, magnetic resonance imaging.

CI: 0.14, 144.9]). Using MR-PRESSO, we found evidence of outliers ( $p_{\text{global test}} < 0.001$ ), but the Mendelian randomization estimates for PDAC did not alter the inference of the results after removal of the outliers ( $p_{\text{distortion}} = 0.57$ ). Although Cochrane's Q value was high (31.6), there was no evidence of pleiotropy in the MR-Egger method (MR-Egger intercept:  $-0.042$ ;  $p = 0.72$ ).

Although none of the eight genetic variants were associated with BMI at genome-wide significance in a past GWAS in the UK Biobank and the Genetic Investigation of Anthropometric Traits (GIANT) consortium (N = 806,834 individuals),<sup>24</sup> we further conducted a sensitivity analysis excluding genetic variants with a nominal BMI association. After removal of the three genetic variants nominally linked to BMI, the association between genet-

cally determined IPFD levels and PDAC remained significant (OR [95% CI]: 3.79 [1.66, 8.65],  $p = 0.002$ ) (Table 5).

### Additional Mendelian randomization analyses for the association of IPFD with PDAC

We conducted an additional Mendelian randomization analysis using only the significant genetic variants for PDAC risk (rs4733612, chromosome 9 [chr9]: 136138765, rs2270911 as listed in Table 4). The results showed that genetically determined higher levels of IPFD were significantly associated with an increased risk of PDAC (OR [95% CI] per 1-SD increase in IPFD: 7.09 [4.41–11.4],  $p < 0.001$ ). Furthermore, we performed another Mendelian randomization analysis using only the non-significant genetic variants for PDAC risk (rs775103516, rs11679492, rs751370420, rs7405380, and rs10422861, also in Table 4). The result still indicated a significant association; higher genetically determined levels of IPFD corresponded with an elevated risk of PDAC (OR [95% CI] per 1-SD increase in IPFD: 1.47 [1.05–2.05],  $p = 0.024$ ).

### DISCUSSION

Triangulating evidence from our prospective observational study and Mendelian randomization study suggests that IPFD plays a causal role in increasing the risk of PDAC. Firstly, our observational study (with median follow-up of 4.5 years) found that participants with IPFD >10% had a 3-fold increased risk of PDAC. Secondly, our Mendelian randomization study provided evidence supporting a causal association between IPFD and PDAC. Consequently, IPFD represents a pathogenic contributor of PDAC. This is likely independent of general adiposity, as suggested by the BMI-adjusted result from our observational study and the sensitivity analysis result from our Mendelian randomization study that excluded genetic variants nominally linked to BMI.

While our study provides genetic evidence for causality using Mendelian randomization, it is well in line with the present

**Table 4. Characteristics of the IPFD-associated genetic variants**

Genetic variant	Nearby gene	Chromosome	Position	Effect allele <sup>a</sup>	Proxy SNP	Association with IPFD		Association with PDAC	
						Beta (%) <sup>b</sup>	p value <sup>b</sup>	OR (95% CI) <sup>c</sup>	p value <sup>c</sup>
rs775103516	FAF1	1	51397564	AAT	rs113170275	0.52	$3.4 \times 10^{-13}$	1.04 (0.99, 1.09)	0.13
rs11679492	PLEKHM3	2	208834477	T	–	0.39	$1.3 \times 10^{-8}$	1.01 (0.96, 1.05)	0.77
rs4733612	–	8	129569999	G	–	0.52	$8.8 \times 10^{-12}$	1.12 (1.06, 1.18)	$6.1 \times 10^{-5}$
chr9: 136138765	ABO	9	136138765	G	rs495828	0.64	$2.7 \times 10^{-13}$	1.21 (1.14, 1.27)	$8.4 \times 10^{-11}$
rs2270911	FAM25C	10	49313245	T	–	0.49	$1.8 \times 10^{-8}$	1.10 (1.03, 1.18)	0.0041
rs751370420	PARP11	12	4122179	AAAG	rs7307879	0.46	$2.4 \times 10^{-11}$	1.04 (0.99, 1.10)	0.11
rs7405380	PABPN1L	16	88975910	C	rs12444726	0.5	$6.1 \times 10^{-13}$	1.03 (0.98, 1.08)	0.26
rs10422861	PEPD	19	33894846	T	–	0.69	$2.1 \times 10^{-22}$	1.02 (0.97, 1.07)	0.5

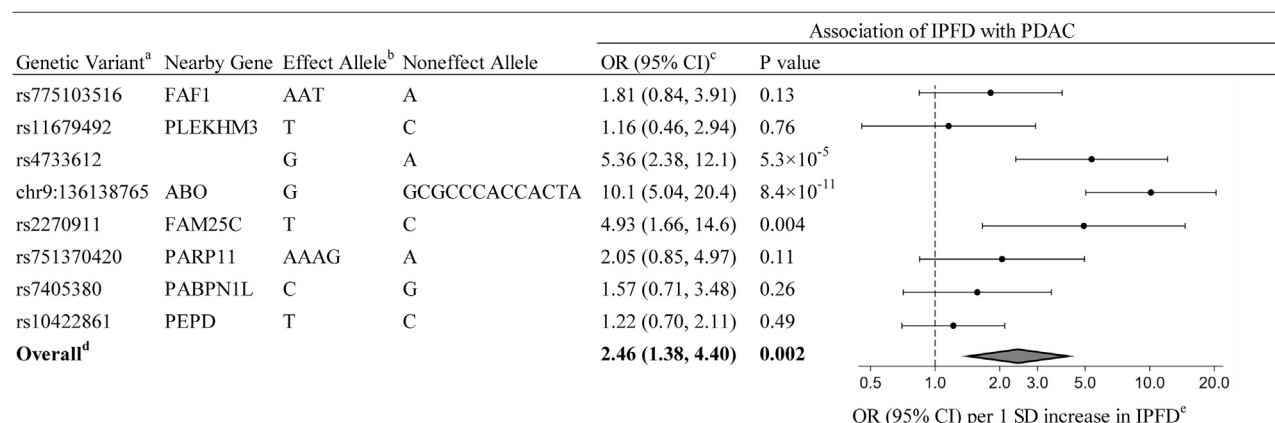
IPFD results were obtained from a genome-wide association study of 25,617 individuals of European ancestry in the UK Biobank.<sup>21</sup> PDAC results were obtained from the PanScan I-III and PanC4, comprising a total of 8,275 PDAC cases and 6,723 non-cases of European ancestry.<sup>23</sup> IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

<sup>a</sup>Allele associated with increasing IPFD levels.

<sup>b</sup>Effect size estimates and p values for the association between each effect allele and IPFD levels (i.e., fat fraction percentage within the pancreas).

<sup>c</sup>Effect size estimates and p values for the association between each effect allele and PDAC.





**Figure 2. Primary Mendelian randomization estimates of the association between IPFD and PDAC**

<sup>a</sup>Proxy SNPs were used for rs775103516 (rs113170275), chr9:136138765 (rs495828), rs751370420 (rs7307879), and rs7405380 (rs12444726).

<sup>b</sup>Allele associated with increasing IPFD levels.

<sup>c</sup>OR (95% CI) of PDAC per-1 SD increase in genetically determined IPFD levels (i.e., per 7.9% increase in fat fraction percentage within the pancreas).

<sup>d</sup>Random-effects inverse-variance weighted method was used to obtain the overall estimate for the association of genetically determined IPFD with PDAC.

<sup>e</sup>Data markers indicate the OR for the association of genetically determined IPFD with PDAC, which was estimated using each genetic variant. Error bars indicate 95% CIs. IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; OR, odds ratio; CI, confidence interval; SD, standard deviation.

prospective observational study and earlier epidemiologic work on the topic.<sup>17,25–27</sup> These studies reported that IPFD is more frequently found in patients with precancerous lesions (i.e., intra-epithelial neoplasia) or PDAC and that it can even be a predictor for PDAC.<sup>17,25–27</sup> Similar to our current findings, these prior studies also reported that the relationship between IPFD and PDAC is independent of overall body fat (i.e., BMI).<sup>17,25,27</sup> The correlation between IPFD and BMI was only 0.40 in the present cohort study, which indicates that there are individual differences in fat deposition patterns (e.g., high IPFD without obesity and high IPFD without fatty liver), a finding also highlighted in our earlier research.<sup>3,14</sup> Furthermore, no colocalization was observed between IPFD traits and hepatic fat deposition traits in a previous GWAS, suggest-

ing that the mechanisms of tumorigenesis related to fat deposition vary between these two organs.<sup>21</sup> Thus, our data, along with previous work, support the idea that IPFD may have features that differ from fat deposition in other locations (e.g., the liver).<sup>7,21,28</sup>

One possible mechanism linking IPFD and PDAC is through the enhanced production of cytokines and adipokines from adipocytes residing within the pancreas.<sup>7</sup> By stimulating inflammation, suppressing apoptosis, and promoting cell proliferation and migration, these cytokines and adipokines can contribute to cancer development or progression.<sup>9,29</sup> In fact, findings from an observational study showed that ultrasound-measured IPFD is a risk factor for future subclinical chronic pancreatitis,<sup>30</sup> supporting the hypothesis that IPFD can contribute to chronic

**Table 5. Comprehensive Mendelian randomization estimates of the association between IPFD and PDAC**

No. of genetic variants	Analysis	Association of IPFD with PDAC			
		OR (95% CI) <sup>a</sup>	p value	p for pleiotropy <sup>b</sup>	p for distortion <sup>c</sup>
8	IVW	2.46 (1.38, 4.40)	0.002	–	–
8	weighted median	1.79 (1.13, 2.83)	0.013	–	–
8	MR-Egger	4.56 (0.14, 144.9)	0.39	0.72	–
6 (exclusion of outlier variants <sup>d</sup> )	MR-PRESSO	2.29 (1.61, 3.26)	<0.001	–	0.57
5 (exclusion of variants nominally linked to BMI <sup>e</sup> )	IVW	3.79 (1.66, 8.65)	0.002	–	–

IPFD, intra-pancreatic fat deposition; PDAC, pancreatic ductal adenocarcinoma; OR, odds ratio; CI, confidence interval; BMI, body mass index; IVW, inverse-variance weighted method; SD, standard deviation. See also Figure S2.

<sup>a</sup>OR (95% CI) of PDAC per 1-SD increase in genetically determined IPFD levels (i.e., per 7.9% increase in fat fraction percentage within the pancreas).

<sup>b</sup>p for pleiotropy was obtained from p value of MR-Egger intercept. Less than 0.05 indicates a possible pleiotropic effect.

<sup>c</sup>p for distortion was obtained from MR-PRESSO. Less than 0.05 indicates a difference between estimates before and after exclusion of outlier genetic variants.

<sup>d</sup>Two outlier genetic variants (chr9: 136138765 and rs10422861) were detected in MR-PRESSO.

<sup>e</sup>Although none of the eight genetic variants were associated with BMI at genome-wide significance, we further conducted a sensitivity analysis excluding three genetic variants with a nominal BMI association.

low-grade inflammation of the pancreas, a well-established driver of PDAC.<sup>9,15</sup> Another important consideration is the heterogeneous cellular localization of IPFD.<sup>15,31</sup> Fat in the pancreas is present in adipocytes that reside between pancreas cells; however, fat also accumulates within pancreas cells such as acinar cells, endocrine cells, and stellate cells.<sup>6,15</sup> While imaging modalities such as MRI, computed tomography (CT), and ultrasonography cannot distinguish the cellular location of fat accumulation, it is possible that different types of fat in the pancreas may affect the risk of PDAC differently.<sup>15</sup> Our Mendelian randomization results, which show heterogeneity in the effect estimates of each IPFD-related genetic variant on PDAC risk, may reflect this heterogeneous localization of IPFD. Further mechanistic research is warranted to elucidate the effects of IPFD across different cellular compartments on PDAC.

Our current findings can have major clinical implications for reducing PDAC risk, as IPFD is a reversible condition.<sup>32</sup> Several randomized controlled trials have demonstrated that IPFD can be reduced through dietary interventions.<sup>15,33,34</sup> However, it is possible that a targeted approach to reducing IPFD through a healthy diet may require greater specificity than a general weight reduction diet.<sup>15,33,34</sup> For example, a reduction in IPFD was observed following a diet change to a Mediterranean diet with low carbohydrates or an isocaloric multifactorial diet, but not a diet change to a low-fat diet or a diet rich in monounsaturated fatty acids.<sup>33,34</sup> Promisingly, emerging pharmacological therapies for weight loss have been shown to dramatically reduce fat mass<sup>35,36</sup>; however, their potential to reduce IPFD has yet to be proven. Further studies are needed to clarify if and to what extent a reduction of IPFD ultimately translates into decreased PDAC incidence.

One major strength of this study is the use of both a prospective observation investigation and Mendelian randomization. Mendelian randomization, in particular, is a robust approach less susceptible to reverse causation and confounding compared to conventional observational studies. Our observational study and Mendelian randomization study yielded similar findings, supporting the evidence of a causal link between IPFD and PDAC. Secondly, we utilized data from the largest GWAS on IPFD and PDAC to date, providing robust genetic associations for our Mendelian randomization analyses. Third, we also incorporated a third data source (i.e., GIANT) to address potential pleiotropic associations with BMI, a potential confounder, which could have biased our Mendelian randomization findings. Lastly, we used IPFD data measured with MRI, which has been well validated against histologic IPFD measurements and the most sensitive non-invasive modality for detection of IPFD.<sup>12</sup>

In conclusion, IPFD is a risk factor that could causally contribute to PDAC development, likely independent of overall body fat. Mechanisms linking IPFD to PDAC may include inflammatory and cancer-promoting signals from the local adipocytes. IPFD may serve as a non-invasive biomarker of PDAC risk and may be of even greater utility for individuals who already have elevated risk due to other reasons, such as chronic pancreatitis, adult-onset diabetes, inheritance of predisposing mutations, or family history.<sup>1</sup> More importantly, our work further raises the possibility that reduction of IPFD could lower the incidence of PDAC.

### Limitations of the study

There are some limitations to our study. The unknown exact mechanism linking the genetic variants, IPFD, and PDAC could theoretically involve pleiotropic effects that may violate assumptions of Mendelian randomization. To minimize the influence of potential pleiotropic effects, we confirmed the robustness of our results through several sensitivity analyses including leave-one-out analyses, pleiotropy-robust statistical methods, and the analysis excluding genetic variants nominally linked to BMI. Another limitation is the restriction to individuals of European ancestry for our Mendelian randomization study. Future studies should expand this analysis to non-European populations, as well as incorporate genetic variants for IPFD identified in other racial/ethnic groups.<sup>37</sup> Lastly, our Mendelian randomization study was unable to evaluate potential non-linear relationships between IPFD and PDAC or the combined effect of IPFD with other risk factors on PDAC. Further Mendelian randomization studies using large-scale individual-level data are warranted to address these issues.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Statistical analysis

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2023.101391>.

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### AUTHOR CONTRIBUTIONS

H.Y., S.A.S., and B.Z.H. designed the study. H.Y., S.A.S., L.W., and B.Z.H. collected the data. H.Y. wrote the draft. H.Y., S.A.S., L.W., and B.Z.H. analyzed the data for Mendelian randomization analyses. H.Y. analyzed individual data on UK Biobank. H.Y., S.A.S., L.W., S.F., R.W., M.H., S.R.G., H.-J.L., V.W.S., L.L.M., and B.Z.H. reviewed, made critical revisions, and approved the article before submission. H.Y. 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.

### DECLARATION OF INTERESTS

L.W. provided consulting service to Pupil Bio, Inc., and received honorarium.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Deposited data</b>		
Observational data	UK Biobank	<a href="https://www.ukbiobank.ac.uk/">https://www.ukbiobank.ac.uk/</a>
IPFD GWAS summary statics	UK Biobank Liu et al. <sup>21</sup>	<a href="https://cdn.elifesciences.org/articles/65554/elifesciences-suppl-v1.xlsx">https://cdn.elifesciences.org/articles/65554/elifesciences-suppl-v1.xlsx</a>
PDAC GWAS summary statics	Pancreatic Cancer Cohort Consortium I-III and the Pancreatic Cancer Case-Control Consortium Liu et al. <sup>23</sup>	<a href="https://www.ncbi.nlm.nih.gov/gap/dbGaP/accession/phs000206.v5.p3">https://www.ncbi.nlm.nih.gov/gap/dbGaP/accession/phs000206.v5.p3</a> and <a href="https://www.ncbi.nlm.nih.gov/gap/dbGaP/accession/phs000648.v1.p1">phs000648.v1.p1</a>
BMI GWAS summary statics	UK Biobank and Genetic Investigation of Anthropometric Traits consortium Pulit et al. <sup>24</sup>	<a href="https://zenodo.org/record/1251813#.Y9n8YnbP1D8">https://zenodo.org/record/1251813#.Y9n8YnbP1D8</a>
BMI-adjusted waist-to-hip ratio GWAS summary statistics	UK Biobank and Genetic Investigation of Anthropometric Traits consortium Pulit et al. <sup>24</sup>	<a href="https://zenodo.org/record/1251813#.Y9n8YnbP1D8">https://zenodo.org/record/1251813#.Y9n8YnbP1D8</a>
Visceral fat volume GWAS summary statistics	UK Biobank van der Meer et al. <sup>38</sup>	<a href="https://www.ebi.ac.uk/gwas/studies/GCST90267357">https://www.ebi.ac.uk/gwas/studies/GCST90267357</a>
<b>Software and algorithms</b>		
STATA version 18	StataCorp, College Station, TX	<a href="https://www.stata.com/">https://www.stata.com/</a>
R version 4.0.5	R Foundation for Statistical Computing, Vienna, Austria	<a href="https://www.r-project.org/">https://www.r-project.org/</a>
MendelianRandomization package	R project Broadbent et al. <sup>39</sup>	<a href="https://cran.r-project.org/web/packages/MendelianRandomization/index.html">https://cran.r-project.org/web/packages/MendelianRandomization/index.html</a>
MR-PRESSO package	Github Verbanck et al. <sup>40</sup>	<a href="https://github.com/rondolab/MR-PRESSO">https://github.com/rondolab/MR-PRESSO</a>
PhenoScanner V2	Kamat et al. <sup>41</sup>	<a href="http://www.phenoscanter.medschl.cam.ac.uk/">http://www.phenoscanter.medschl.cam.ac.uk/</a>

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Hajime Yamazaki ([yamazaki.hajime.7n@kyoto-u.ac.jp](mailto:yamazaki.hajime.7n@kyoto-u.ac.jp)).

#### Materials availability

This study did not generate new unique reagents.

#### Data and code availability

This study uses data from the UK Biobank and dbGaP (accession phs000206.v5.p3 and phs000648.v1.p1). Data can be accessed following approval of a research access application to the UK Biobank and/or dbGaP. This paper also uses publicly available summary statistics from prior GWAS in the UK Biobank and the Genetic Investigation of Anthropometric Traits consortium. The source and identifier of these datasets can be found in the Deposited Data section of the [key resources table](#). This paper does not report the original code. Any additional information required to reanalyze the data reported in this report is available from the [lead contact](#) upon request.

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

#### Source of data

For the prospective observational study using individual-level UK Biobank data, we included 29,463 participants without prevalent PDAC who underwent IPFD measurement using MRI between December 2015 and March 2020. Participants were followed from the date of baseline IPFD measurement to the date of PDAC diagnosis, date of death, or November 2022, when the most recent comprehensive death data was available. Incident cases of PDAC were identified using International Classification of Diseases, 10<sup>th</sup> Revision

(ICD-10) codes C25.0-C25.9 from the national cancer registry, hospital admissions, and cause of death records. We excluded potential neuroendocrine tumors using International Classification of Disease for Oncology, 3rd Edition (ICD-O-3) histology codes (8150; islet cell carcinoma, 8240: carcinoid tumor, 8246: neuroendocrine carcinoma).

For the Mendelian randomization analyses, we obtained IPFD-related genetic variants from a prior GWAS study in the UK Biobank (N = 25,617 individuals).<sup>21,42</sup> In the GWAS study, IPFD levels were measured on MRI and shown as fat fraction percentage within the pancreas.<sup>21</sup> IPFD fraction measured on MRI represents histological IPFD fraction, defined as the percentage of pancreatic intraparenchymal fat in the total pancreatic parenchyma.<sup>12</sup> For PDAC genetic associations, we used information from the PanScan I, II, III and the PanC4 GWAS dataset (N = 14,998 individuals).<sup>23</sup>

As sensitivity Mendelian randomization analyses to address potential pleiotropic associations with obesity-related measures, we used summary statistics from previous GWAS of BMI, BMI-adjusted waist-to-hip ratio, and visceral fat volume. For BMI and BMI-adjusted waist-to-hip ratio, we used data from a meta-analysis of the UK Biobank and the GIANT consortium (N = 806,834 individuals).<sup>24</sup> For visceral fat volume, we used data from a prior GWAS in the UK Biobank (N = 33542).<sup>38</sup>

The UK Biobank has approval from the North West Multi-centre Research Ethics Committee, and our UK Biobank data usage for this study was also approved by the ethics committee of Kyoto University (R3854). We used publicly available data for GIANT consortium, for which no ethical approval was required. PanScan/PanC4 data usage for this study has been approved by the University of Hawaii Institutional Review Board (2019-00402).

## METHOD DETAILS

### IPFD measurement

IPFD was quantified using the proton density fat fraction of the pancreas with multi-echo MRI, as described in a prior publication.<sup>21</sup> A modified convolutional neural network (CNN) was trained using manually measured IPFD and then applied to the data from all participants.<sup>21</sup> In a prior validation study, the CNN was trained on 68 subjects and tested on 30 subjects.<sup>43</sup> There were only minor differences between the IPFD measurements obtained using the CNN and those obtained through manual measurements in the test subjects.<sup>43</sup>

### Selection of genetic instruments

Genetic variants used in the Mendelian randomization analyses were selected as follows (Figure 1). All nine independent genetic variants associated with IPFD levels at genome-wide significance ( $p < 5.0 \times 10^{-8}$ ) were selected from the UK Biobank GWAS, in which age, age squared, gender, imaging center, scan date, scan time, genotyping batch, and genetic relatedness were controlled for in the analysis.<sup>21</sup> We further excluded one genetic variant (rs13040225) with a palindromic SNP (i.e., those where the alleles are complementary, G/C or A/T) and minor allele frequency (MAF) above 0.4 to avoid ambiguity of effect direction. The remaining eight genetic variants were used for the primary Mendelian randomization analysis. Among the eight genetic variants, four were not found in the GWAS summary statistics for PDAC in the PanScan I-III/PanC4 GWAS. We used the same proxy SNPs in linkage disequilibrium ( $r^2 > 0.7$ ) for these genetic variants (Table 4), as done in a previous Mendelian randomization study for IPFD and diabetes mellitus.<sup>42</sup> About 1.6% of the variation in IPFD levels was explained by the eight genetic variants.<sup>42</sup> This modest proportion of genetically explained phenotype variation is within the range typically observed in Mendelian randomization studies.<sup>44,45</sup> The value of Mendelian randomization lies in elucidating causal associations, not in individual risk prediction, and this modest explained phenotype variation is not a limitation in Mendelian randomization studies.<sup>46</sup>

Considering possible pleiotropic effects of the genetic variants on potential confounders, we also evaluated the association of the eight genetic variants with obesity-related measures (e.g., BMI, BMI-adjusted waist-to-hip ratio, and visceral fat volume) using GWAS summary statistics from the UK Biobank and the GIANT consortium.<sup>24,38</sup> None of the eight genetic variants were associated with BMI, BMI-adjusted waist-to-hip ratio, or visceral fat volume at a genome-wide significance threshold ( $p < 5.0 \times 10^{-8}$ ). However, using the Bonferroni corrected threshold of  $p < 0.00625$  ( $[p < 0.05]/8$  genetic variants) as done in a previous Mendelian randomization study by Larsson, Burgess, and Michaelsson (2017),<sup>47</sup> three of the genetic variants (rs775103516, rs751370420, and rs10422861) had a nominal association with BMI. Rs10422861 was also nominally associated with BMI-adjusted waist-to-hip ratio. We conducted a sensitivity analysis excluding these three obesity-related genetic variants to minimize potential residual pleiotropy. In addition to obesity, we also checked pleiotropic association of the eight genetic variants with other potential confounders (current smoking, alcohol intake, and high risk diet<sup>1</sup>) using PhenoScanner,<sup>41,48</sup> but none of the eight genetic variants had a nominal association with these potential confounders.

### GWAS data for PDAC

GWAS data for PDAC in PanScan I, PanScan II, PanScan III, and PanC4 were downloaded from dbGaP (study accession nos.: phs000206.v5.p3 and phs000648.v1.p1). The detailed information for these data has been described in previous publications.<sup>22,49–52</sup> In brief, genotyping was performed on the Illumina HumanHap550, 610-Quad, OmniExpress, and OmniExpressExome arrays, respectively. Standard QC was conducted according to the guidelines recommended by the consortia.<sup>22,23</sup> Study subjects who were related to each other, had missing information on age or gender, had gender discordance, had non-European ancestry based on genetic estimation, or had a low call rate (less than 94% and 98% in PanScan and PanC4, respectively) were excluded. Duplicated

SNPs and those with a high missing call rate (of at least 6% and 2% in PanScan and PanC4, respectively), or violations of Hardy-Weinberg equilibrium (of  $p < 1 \times 10^{-7}$  and  $p < 1 \times 10^{-4}$  in PanScan and PanC4, respectively) were also excluded. In the PanC4 dataset, we also excluded SNPs that had a MAF  $< 0.005$ , more than one Mendelian error in HapMap control trios, or more than two discordant calls in study duplicates. SNPs with gender differences in allele frequency  $> 0.2$  or in heterozygosity  $> 0.3$  for autosomes/XY were further excluded. We conducted the genotype imputation with the Haplotype Reference Consortium reference panel (r1.1 2016), using Minimac4 after phasing with Eagle v2.4.<sup>53,54</sup> Imputed SNPs with an imputation quality of  $> 0.3$  were retained. All of the genetic variants used in the Mendelian randomization analysis had an imputation quality of  $> 0.8$  except for rs2270911, which had an imputation quality of 0.6. The associations between individual SNPs and PDAC risk were further assessed with logistic regression adjusting for age, gender, and the top 10 principal components. In the final analyses, we included 8,275 PDAC cases and 6,723 non-cases of European ancestry.<sup>23</sup>

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Statistical analysis

For the prospective observational study evaluating the association between IPFD and PDAC incidence, we used Cox regression models adjusting for age, gender, BMI, current smoking status, and daily drinking. To account for missing covariates data, we also conducted multiple imputation using multivariate imputation by chained equations. IPFD was dichotomized as high ( $> 10\%$ ) and low ( $\leq 10\%$ ) based on the mean value among all participants. IPFD was also assessed as tertiles and as a continuous measure (per 1 SD increase in log-transformed IPFD).

To evaluate the strength of the association between each genetic variant and IPFD (assumption 1 in Figure S1), we calculated F-statistics. F-statistics should be more than 10 to be valid genetic variants for Mendelian randomization.<sup>18</sup> Cochran's Q value was calculated to evaluate the heterogeneity among estimates obtained using different genetic variants.

For the primary Mendelian randomization analysis, the association of each genetic variant with PDAC was weighted by its association with IPFD, and estimates were combined using the random-effects IVW method. This method is the most efficient and provides valid causal estimates when the average pleiotropic effect is zero. If the genetic variants used in this study are additionally associated with another risk factor for PDAC (i.e., presence of pleiotropic effect), then either assumption 2 or 3 for Mendelian randomization in Figure S1 is violated.

To account for potential pleiotropy, we conducted five sensitivity Mendelian randomization analyses: IVW method with leave-one-out analysis, MR-Egger regression method, weighted median method, MR-PRESSO, and IVW method after exclusion of the three genetic variants nominally linked to BMI. For leave-one-out analysis, each genetic variant was excluded one at a time, and the IVW method was used on the remaining genetic variants to evaluate the causal association of genetically determined IPFD with PDAC. Under the Instrument Strength Independent of Direct Effect (InSIDE) assumption,<sup>55</sup> MR-Egger can provide valid causal estimates even if the average pleiotropic effect is not zero, and the intercept in MR-Egger can be tested to judge whether pleiotropic effects exist. However, the drawback of MR-Egger is the wide confidence intervals. Weighted median method can provide valid causal estimates even if up to 50% of genetic variants have pleiotropic effects. MR-PRESSO can detect outlier genetic variants and calculate IVW estimates after exclusion of the outliers.<sup>19</sup> Lastly, to address potential pleiotropy with BMI, a potential confounder, we excluded the three genetic variants nominally linked to BMI and conducted IVW analysis.

As additional analyses, we conducted Mendelian randomization analyses using only the significant genetic variants associated with PDAC risk (rs4733612, chr9:136138765, rs2270911 as listed in Table 4), and separately using only the non-significant genetic variants for PDAC risk (rs775103516, rs11679492, rs751370420, rs7405380, rs10422861 also in Table 4).

We considered  $p < 0.05$  as statistically significant for our Mendelian randomization analyses. Statistical analyses were performed using Stata 18 (StataCorp, College Station, TX) and R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). We used the MendelianRandomization package<sup>39,56</sup> and MR-PRESSO package<sup>40</sup> in R to conduct the Mendelian randomization analysis.