ORIGINAL ARTICLE

Germline DNA damage repair gene mutations in pancreatic cancer patients with personal/family histories of pancreas/breast/ovarian/prostate cancer in a Japanese population

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Abstract

Aim: Cancer patients with personal/family histories of pancreatic/breast/ovarian/prostate cancer are associated with a higher likelihood of harboring DNA damage repair (DDR)-related germline mutations. Here, we aimed to obtain a better understanding of DDR-related germline mutations in Japanese pancreatic ductal adenocarcinoma (PDAC) patients with personal and/or family histories of *BRCA*-related cancers of the pancreas, breast, ovary, and prostate.

Methods: We performed next-generation sequencing (NGS) and evaluated germline mutations in nine DDR-related genes (*BRCA1*, *BRCA2*, *ATM*, *PALB2*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*) in PDAC patients with personal and/or family histories.

Results: Of 196 patients with PDAC, 39 (19.9%) fulfilled the criteria for at least one family history of pancreatic/breast/ovarian/prostate cancer in first-degree relatives (sibling–sibling or parent-child) or the personal history of these malignancies. Targeted NGS revealed that four (10.2%) of 39 patients with personal/family histories harbored deleterious germline mutations—two in *BRCA2*, one in *ATM*, and one in *MLH1*. Both the *BRCA2* variants showed frameshift mutations due to short insertion/deletions. In the 39 patients undergoing NGS, a similar distribution of the clinicopathological characteristics was observed between those with deleterious mutations/variants of unknown significance (VUSs) and with benign/wild types. Patients with deleterious germline mutations/VUSs in DDR-related genes showed a significantly more favorable prognosis than those with benign mutations/wild-type genes (hazard ratio: 0.160, *P* = .040).

Conclusions: A significant fraction of PDAC patients with personal/family histories of *BRCA*-related cancers harbored deleterious germline mutations in DDR-related genes. DDR-related germline gene mutations might be a favorable prognostic factor in patients with pancreatic cancer.

1 | INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) remains a devastating disease with a 5-year overall survival rate of less than 10%.[1] Early detection and subsequent surgical resection is the only chance of cure; however, only a subset of patients (~20%) with localized disease is amenable to surgery.[2] Moreover, chemotherapy is the only available treatment option for patients with metastatic PDAC. The current standard chemotherapies for metastatic PDAC consist of cytotoxic regimens of FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan, and leucovorin) or gemcitabine plus nab-paclitaxel. These chemotherapy regimens have been shown to prolong the survival period.[3, 4] Nonetheless, the median overall survival still remains less than a year. To improve prognosis, a better understanding of the patient's mutational profile using genetic testing is of vital importance. This may help predict the treatment response to cytotoxic agents and identify potentially actionable somatic or germline mutations for molecular targeted drugs.

Alterations in DNA damage repair (DDR) pathways contribute to cancer initiation across various types of cancer, including pancreatic cancer.[5] Specifically, homologous recombination repair (HRR)-related genes (e.g. *BRCA1/2*, *ATM*, and *PALB2*) are well-known as pancreatic cancer susceptibility genes.[6] Inactivation of these genes and subsequent HRR deficiency may impart sensitivity to DNA-strand damaging cytotoxic agents such as platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors.[7] Prior studies also demonstrated that PDAC patients with HRR-related gene mutations receiving platinum-based chemotherapy showed favorable long-term outcomes compared to those receiving non-platinum chemotherapy.[8, 9] Furthermore, in PDAC patients undergoing platinum chemotherapy, better survival was observed in patients with HRR-related mutations than in those without mutations.[10, 11] Additionally, germline mutations of mismatch repair (MMR)-related genes (e.g. *MLH1*, *MSH2*, *MSH6*, and *PMS2*) also predispose to pancreatic cancer. MMR-related gene mutations and mismatch repair deficiency are associated with microsatellite instability, high mutation burden, and hypersensitivity to immune checkpoint inhibitors.[12, 13]

Germline *BRCA1/2* mutations are found in approximately 5%-10% of familial PDAC and approximately 3% of apparently sporadic PDAC.[6, 14] Additionally, recent reports suggested the relatively high prevalence of such gene mutations in PDAC patients not only with a family history of pancreatic cancer but also a personal or family history of other BRCA-related malignancies such as breast and ovarian cancer.[15] Moreover, in a subgroup of PDAC patients undergoing platinum-based chemotherapy, significantly longer survival was observed in patients with a family history of breast/ovarian/pancreatic cancer than in those without.[16]

Based on these backgrounds, a recent Japanese multicenter trial was conducted to evaluate the efficacy of gemcitabine plus oxaliplatin in metastatic pancreatic cancer patients with a family/personal history of BRCA-related malignancies.[17] This study concluded that family and/or personal history is insufficient information to enrich the subgroup of metastatic PDAC patients expected to respond well to platinum-based chemotherapy. Authors in the study discussed and suggested the necessity of genetic profiling in chemotherapy. However, little is known about the prevalence of DDR-related gene mutations in Japanese PDAC patients, especially in those stratified by the existence of personal/family history of BRCA-related malignancies.

This study aimed to obtain a better understanding of DDR-related germline mutations in Japanese PDAC patients in relation to the presence of personal and/or family histories of BRCA-related cancers of the pancreas, breast, ovary, and prostate. Here, we tested the hypothesis that accounting for extended personal/family histories of BRCA-related malignancies, such as pancreatic, breast, ovarian, and prostate cancers, might contribute to further enrichment of PDAC patients harboring DDR-related germline variants.

2 | METHODS

2.1 | Patients

From November 2017 to August 2020, patients with histologically proven PDAC, who visited the Department of Surgery, Tohoku University Hospital (Japan), were prospectively enrolled in our biobank project. Demographic and clinicopathological data, including personal and family histories, were extracted from a prospectively maintained database and medical records. The study was approved by the Medical Ethics Committee of Tohoku University Graduate School of Medicine (institutional review board approval number: 2019-1–119). Written informed consent was obtained from all patients.

2.2 | Sample collection and DNA extraction

Peripheral blood samples were prospectively collected using cell-free DNA BCT tubes (Streck, Omaha, NE, USA). Harvested blood samples were immediately processed by centrifugation at 1900 rpm for 15 minutes at room temperature, and the buffy coat fraction was selectively collected, aliquoted, and stored at −80°C until further use. All the specimens were subjected to linkable anonymization. All downstream experiments were conducted in a blinded fashion without any prior knowledge of patient information.

Genomic DNA was extracted from 100 μL of buffy coat fraction using the DNeasy Blood and Tissue kit (QIAGEN) according to the manufacturer's instructions. The extracted DNA was quantified using a SYBR Green real-time PCR-based method to evaluate the amount of amplifiable DNA. The standard calibration curve was valid for 5-fold serial dilutions of human genomic DNA (Promega).

2.3 | Targeted next-generation sequencing of germline DNA

Targeted next-generation sequencing (NGS) was performed using the Ion AmpliSeq technology (Thermo Fisher Scientific Inc, Waltham, MA) and custom panel (Thermo Fisher Scientific), which contains 344 amplicons divided into two primer pools covering 100% whole exons for nine DDR-related genes (*BRCA1*, *BRCA2*, *ATM*, *PALB2*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*). Sequencing libraries were prepared using 5 ng of DNA and Ion AmpliSeq Library Kit Plus with Ion Xpress™ Library Barcode Adapters (Thermo Fisher Scientific). Amplified libraries were cleaned and subjected to clonal amplification on microspheres in the emulsion on an Ion OneTouch™ 2 System, followed by purification from empty microspheres on an Ion OneTouch™ ES Instrument (Thermo Fisher Scientific) according to the manufacturer's instructions. Sequencing was performed on an Ion Torrent Personal Genome Machine (PGM; Thermo Fisher Scientific) using 316v2 chips. Post-sequencing data processing, including alignment to the GRCh37/hg19 human reference genome and variant calling, were conducted using the Torrent Suite software (version 5.0) and Torrent Variant Caller (Thermo Fisher Scientific). Genetic variants were annotated using the ANNOVAR software. Alignments and putative mutations were visually verified using the Integrative Genomics Viewer (IGV, v2.4.19; Broad Institute, Cambridge, MA).

2.4 | Classification of detected variants

Non-synonymous, exonic variants in multiple databases, including 1000 Genomes Project, Human Genetic Variation Database (HGVD), Genome Aggregation Database (gnomAD), and Japanese Multi Omics Reference Panel (jMorp, Tohoku Medical Megabank, 8.3KJPN), were filtered by their allele frequency (<1% cutoff). The clinical significance of all variants was classified according to the ClinVar database. Nonsense variants and frameshift insertions and deletions were considered deleterious. For missense variants with uncertain or conflicting significance defined by ClinVar, further in silico analyses, using SIFT, PolyPhen-2, PROVEAN, MutationTaster, and FATHMM, were conducted to assess the clinical significance of pathogenicity. We also referred to the results of a recent large-scale germline test in the Japanese population.[18] Thus, we finally classified each identified variant as deleterious, benign, or variants of unknown significance (VUS).

2.5 | Sanger sequencing

Deleterious mutations identified with NGS were validated by direct sequencing of the PCR product. Paired primers for specific amplification of the regions of interest are listed in Table S1. The amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen), and the sequence was determined with the dideoxy chain-termination method using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. The products were analyzed using a 3130xl Genetic Analyzer (Applied Biosystems).

2.6 | Statistical analysis

All statistical analyses were conducted using the JMP Pro 15.0.0 statistical software (SAS Institute Inc, Cary, NC) and GraphPad Prism Version 9.0.0 (GraphPad Software). Continuous and categorical variables are presented as median (range) and as whole numbers and percentages. The non-parametric Mann-Whitney U test was used to compare continuous variables. Categorical variables were compared using the Fisher's exact test. Survival analyses were performed using the Kaplan-Meier method, and differences were compared using a univariate log-rank test. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Personal and family histories of included patients

A total of 196 PDAC patients were included in this study. Of these, 39 (19.9%) fulfilled the criteria of personal/family histories of BRCA-related malignancies, i.e. at least one family history of pancreatic/breast/ovarian/prostate cancer in first-degree relatives (FDRs: sibling-sibling or parent-child) or the personal history of these malignancies (Table 1). Five PDAC patients had two FDRs with BRCA-related malignancies. PDAC with a family history of FDRs was observed in 12 patients (6.1%). Likewise, approximately 6% (n = 13) of patients with PDAC had a family history of breast cancer in FDRs. Collectively, 32 (16.3%) patients had a family history of such cancer types in FDRs. On the other hand, in all 196 patients with PDAC, prior breast cancer had the highest prevalence (n = 6, 3.1%) of personal history across the BRCA-related malignancies of breast, pancreatic, ovarian, and prostate cancer.

3.2 | Patient characteristics with or without personal/family histories

Patient characteristics at the initial diagnosis were compared among the 39 PDAC patients with personal/family histories and 157 patients without (Table 2). Females showed a higher proportion of

TABLE 1 Personal and family histories in patients with pancreatic cancer included in this study

			n	(% of total 196)
Family history of FDR	Total		32	(16.3)
	Number of FDRs	One	27	
		Two	5	
	Types of malignancies	Pancreatic cancer	12	(6.1)
		Breast cancer	13	(6.6)
		Ovarian cancer	2	(1.0)
		Prostate cancer	7	(3.6)
Personal history	Total		11	(5.6)
	Types of malignancies	Pancreatic cancer	2	(1.0)
		Breast cancer	6	(3.1)
		Ovarian cancer	1	(0.5)
		Prostate cancer	2	(1.0)

Abbreviations: FDR, first-degree relatives.

patients with personal/family history than those without; however, the difference was not significant ($P =$.071). Patients with personal/family histories were more likely to be young PDAC patients aged <50 years (10.3%) than those without (3.8%). Of all, 112 patients (57%) underwent pancreatic resection with or without preoperative therapy. Neoadjuvant chemotherapy was performed in 56 patients and all of them were treated by either one of gemcitabine with S-1 regimen or gemcitabine with nab-paclitaxel regimen (Table S2). Platinum-based chemotherapy was finally administered to 24 (12.6%) of all 196 patients with or without pancreatic resection (Table 2). Among the 112 patients who underwent resection, the distribution of histological grade in resected pancreas differs between the two groups with (n = 22) or without (n = 90) personal/family histories ($P =$.033, Table S3). Well to moderately differentiated carcinoma was predominant in patients with personal/family histories. On the other hand, higher proportion of poorly differentiated carcinoma in resected pancreas was observed in patients without personal/family histories than those with histories. There were no significant differences of survival outcomes between the patients with and without personal/family histories (Figure S1).

3.3 | Targeted next-generation sequencing

We then performed targeted sequencing for 39 patients who had at least one personal/family history of BRCA-related malignancies. Four (10.2%) of the 39 patients harbored deleterious mutations—two in *BRCA2*, one in *ATM*, and one in *MLH1* (Table 3). These mutations were successfully validated with Sanger sequencing (Figure 1). Both the *BRCA2* variants showed frameshift mutations due to short insertion/deletions. Details of the diagnosis and treatment for the

four patients are summarized in Table 3. Two of the four patients underwent pancreatectomy. Platinum-based chemotherapy of FOLFIRNOX was administered to only one patient with locally advanced PDAC (#224) harboring a *MLH1* deleterious mutation. None of the four patients underwent microsatellite instability testing. We also classified the six mutations into VUS (Table 4), all of which were altered as single nucleotide variants. Of the six patients harboring VUS, two (#29 and #224) also harbored deleterious variants. Among the nine targets in our custom panel, no deleterious mutations/VUSs were found in *BRCA1*, *CHEK2*, and *PMS2*.

3.4 | Patient characteristics and their prognosis with or without germline mutations

To evaluate the clinical significance of DDR-related mutations in PDAC patients with personal/family histories of BRCA-related malignancies, we compared the characteristics and demographics of the eight patients with deleterious mutations and/or VUS and 31 patients with wild-type and/or benign mutations. Table 5 shows that the two groups did not differ significantly in any of the evaluated variables except for blood type ($P =$.049). Across the 22 patients with surgery, resected primary lesions harboring germline deleterious/VUS variants were more likely to show a high abundance of stroma ($P =$.046) and diffusely infiltrating patterns of growth characterized by an indistinct border with the surrounding tissue ($P =$.023) (Table 6). We then evaluated the prognostic impact of DDR-related germline mutations (Figure 2, Figure S2). Patients with deleterious mutations/VUSs in DDR-related genes showed a significantly more favorable prognosis than those with benign mutations/wild-type gene (HR: 0.160, $P =$.040 by log-rank test). Regarding the response to chemotherapy, Figure S3 showed the heterogeneous backgrounds across the 24 patients undergoing platinum-based chemotherapy, there were no associations between the platinum-based chemosensitivity and the characteristics of patients such as the personal/family histories and genetic features.

4 | DISCUSSION

To improve the chemotherapeutic efficacy and long-term outcomes in patients with PDAC, molecular-based precision/personalized medicine should be introduced in the clinical setting with easier accessibility and applicability and much more affordable cost.[19] Due to the relative lower incidence rate of actionable mutations in PDAC than other types of malignancies, pancreatic cancer patients usually have a rare chance to receive the molecular-based precision/personalized medicine.[20, 21] Currently, the most frequent actionable targets, based on strong evidence, are mutations in DDR-genes such as *BRCA1/2* genes. Recently, the large-scale clinical trial "POLO study" revealed the usefulness of PARP inhibitor as a maintenance therapy after platinum-based cytotoxic chemotherapy in patients with PDAC harboring *BRCA* mutations.[22] To

TABLE 2 Patient characteristics

Characteristics	Total (n = 196)		Patients with personal/ family history (n = 39)		Patients without personal/ family history (n = 157)		P
Sex, n (%)							
Male	108	(55.1)	16	(41.0)	92	(58.6)	.071
Female	88	(44.9)	23	(59.0)	65	(41.4)	
Age							
Median (range), years	69	(39-89)	70	(41-87)	69	(39-89)	.617
<50 years, n (%)			4	(10.3)	6	(3.8)	.113
Blood type, n (%)							
A	84	(42.9)	17	(43.6)	67	(42.7)	.948
B	36	(18.4)	6	(15.4)	30	(19.1)	
O	54	(27.6)	11	(28.2)	43	(27.4)	
AB	22	(11.2)	5	(12.8)	17	(10.8)	
Tumor diameter, n (%)							
≤20 mm	74	(37.8)	11	(28.2)	63	(40.1)	.199
>20 mm	122	(62.2)	28	(71.8)	94	(59.9)	
Tumor location, n (%)							
Head	108	(55.1)	22	(56.4)	86	(54.8)	.999
Body and tail	88	(44.9)	17	(43.6)	71	(45.2)	
Tumor marker, median (range)							
CEA (ng/mL)	3.1	(0.5-74.6)	3.6	(0.7-74.6)	3	(0.5-59.2)	.357
CA 19-9 (U/mL)	102.3	(0.6-49 771)	129.5	(0.6-49 771)	100.8	(0.6-10 125)	.558
Stage, n (%)[a]							
0	7	(3.6)	1	(2.6)	6	(3.8)	.257
IA	39	(19.9)	10	(25.6)	29	(18.5)	
IB	57	(29.1)	13	(33.3)	44	(28.0)	
IIA	14	(7.1)	0	(0.0)	14	(8.9)	
IIB	17	(8.7)	1	(2.6)	16	(10.2)	
III	34	(17.3)	7	(17.9)	27	(17.2)	
IV	28	(14.3)	7	(17.9)	21	(13.4)	
Treatment, n (%)							
Resection (upfront)	35	(17.9)	7	(17.9)	28	(17.8)	.918
CT→resection (neoadjuvant)	56	(28.6)	12	(30.8)	44	(28.0)	
C(R)T→resection (conversion)	21	(10.7)	3	(7.7)	18	(11.5)	
C(R)T only (unresected)	84	(42.9)	17	(43.6)	67	(42.7)	
Platinum-based chemotherapy, n (%)[b]							
Yes	24	(12.6)	9	(23.1)	15	(9.9)	.054
No	166	(87.4)	30	(76.9)	136	(90.1)	

Abbreviations: C(R)T, chemo(radio)therapy; CT, chemotherapy.
[a]According to UICC/TNM staging classification 8th edition.
[b]Exclusion of six patients due to unavailable information.

the best of our knowledge, this is the first study testing for the germline variants in DDR-related genes in a unique subgroup of Japanese PDAC patients with extended personal/family histories of BRCA-related malignancies. Despite the lower prevalence than we expected, this study definitively revealed the significant proportion of DDR-related mutations in such cases.

Familial pancreatic cancer is widely defined by the presence of at least one pair of FDRs in the family.[23] A family history of pancreatic cancer is a well-known significant risk factor for pancreatic cancer; however, there is no evidence of a clear relationship between the family history of breast cancer and the risk of pancreatic cancer. Here, 6.1% of patients with PDAC were familial, similar to prior

TABLE 3 Characteristics of four pancreatic cancer patients harboring deleterious germline variants

Case #	Age/sex	Personal history	Family history in FDRs	Genome position	Gene	Nucleic change	AA change	Type of mutation	Clin Var	Max MAF in databases[a]
29	74/F	Breast cancer (bilateral)	Prostate cancer (father)	chr13:032 907 503	BRCA2	c.1888dupA	p. Thr630Asnfs	Frameshift	Pathogenic	N/A
107	42/F	None	Breast cancer (mother)	chr13:032 912 956	BRCA2	c.4462_4463CA[1]	p. His1488fs	Frameshift	Pathogenic	0.0001
224	74/F	Ovarian cancer	None	chr03:037 090 446	MLH1	c.2041G>A	p. Ala681Thr	Missense	Pathogenic	N/A
401	70/F	None	Pancreatic cancer (father)	chr11:108 205 807	ATM	c.8122G>A	p. Asp2708Asn	Missense	Pathogenic/ Likely pathogenic	0.0001

| Case #	Location	Tumor size (mm)	CA 19-9 (U/mL)	Stage	Resectability[b]	Treatment	Hist. grade[c]	Pathological stage[c]	Therapeutic effect[d]	Adjuvant therapy	Long-term outcome
29	Body	30	18.9	III	UR-LA	GnP→CRT →resection (DP-CAR)	G2	T4N1, Stage III	IIb	S-1	25.9 months deceased with disease
107	Head	56	6.9	IV	UR-M (HEP)	GnP	-	-	-	-	18.4 months alive with disease
224	Body	42	4461	III	UR-LA	GnP→FFX	-	-	-	-	20.3 months alive with disease
401	Head	24	9.5	IB	R	GS→resection (PD)	G1	T2N2, Stage III	IIa	S-1	15.0 months alive without disease

Abbreviations: AA, amino acid; CRT, chemoradiotherapy; DP-CAR, distal pancreatectomy with celiac axis resection; FFX, FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan, and leucovorin); FDR, first-degree relatives; GnP, gemcitabine and nab-paclitaxel; GS, gemcitabine and S-1; HEP, hepatic metastasis; MAF, mutant allele frequency; N/A, not applicable; PD, pancreaticoduodenectomy; R, resectable; UR-LA, unresectable-locally advanced; UR-M, unresectable-metastatic.

aBased on multiple database search including 1000 Genomes Project, Human Genetic Variation Database (HGVD), the Genome Aggregation Database (gnomAD), Japanese Multi Omics Reference Panel (jMorp, Tohoku Medical Megabank, 8.3KJPN).

bAccording to the Classification of Pancreatic Carcinoma 4th English edition by the Japan Pancreas Society

cAccording to UICC/TNM staging classification 8th edition.

dAccording to Evans classification (Evans DB, et al Arch Surg 1992).
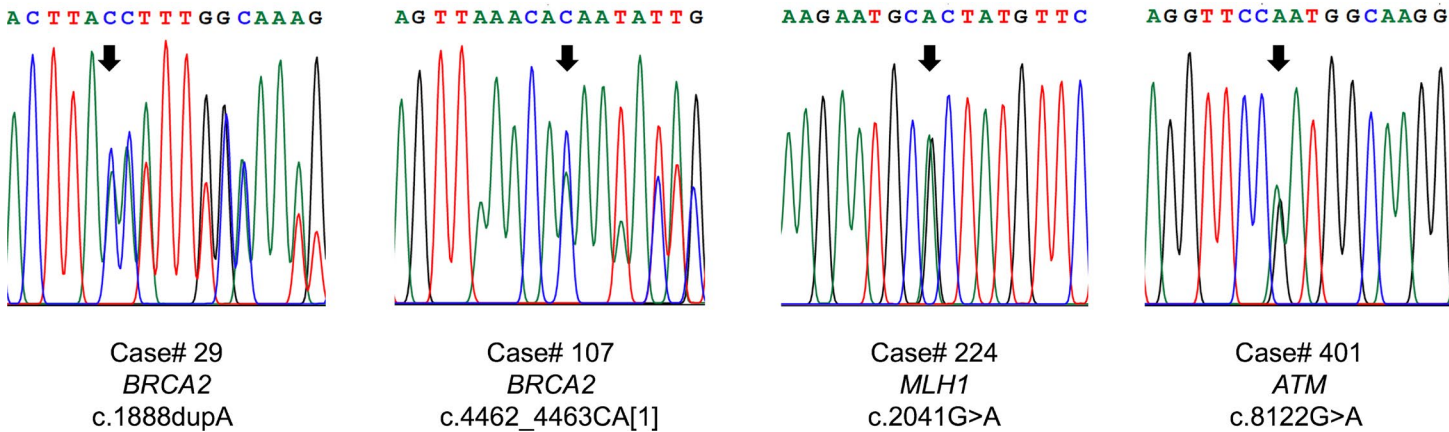
FIGURE 1 Chromatograms by sanger sequencing validation analysis targeting deleterious germline mutations identified by next-generation sequencing

reports showing 4.1%~7.5% familial PDACs.[15, 24-26] Furthermore, 6.6% of PDAC patients studied here had family histories of breast cancer in FDRs. The similar proportions of family histories observed between pancreatic and breast cancers here and in previous studies might be attributed to the incidence rate differences.[24] We found that 3.1% of the PDAC patients included in this study had a personal history of breast cancer, similar to prior study results (3.4%).[15] Future studies are warranted to evaluate the causal relationship between the personal/family histories of breast cancer and the risk of pancreatic cancer development.

In this study, only one patient with deleterious MMR-gene mutation (MLH1) received platinum-based FOLFIRINOX regimen as a second line followed by GEM-nab-paclitaxel therapy and therefore the impact of MMR-gene mutations on treatment efficacy could not be assessed. Indeed, function of VUS identified in this study affecting the platinum-based chemosensitivity needs further elucidation. Although protein-truncating mutations have usually been definitively used in clinical management because of their deleterious impact on protein function, several rare non-synonymous variants in this study were classified as VUS, thereby probably leading to underestimation of their clinical significance. Additional analyses of the POLO study recently reported the newly identified BRCA mutations and their geographic and ethnic heterogeneities across patients screened for entry into that clinical trial.[27] Nonetheless, it is still challenging to fully clarify which mutation status can really affect platinum-based chemosensitivity. Future validation studies stratified by geographic and racial variability are required to assess the relationship between BRCA mutation status and platinum-based chemosensitivity.

Notably, the survival analysis conducted here suggests that the presence of DDR-related gene germline mutations in patients with personal/family histories is a strong prognostic factor. Likewise, a few previous studies also reported that the DDR-related gene mutation carriers had a better survival rate even in a subgroup of PDAC patients not receiving platinum-based chemotherapy.[28-30] Although the underlying mechanism remains largely unknown, our results and previous studies suggest that the better prognosis cannot be explained wholly by good chemotherapeutic response

to platinum-based chemotherapy. Interestingly, recent large-scale sequencing studies have shown that PDAC with germline variants in pancreatic cancer susceptibility genes tend to harbor KRAS wild-type.[12, 31, 32] Furthermore, previous reports demonstrated that KRAS wild-type PDAC has better long-term prognosis than KRAS-mutated PDAC.[33, 34] Although KRAS mutation status could not be investigated here because of tissue sample non-availability, especially in unresectable cases, one possible explanation for better prognosis in PDAC patients with DDR-related genes is that somatic gene alterations characterizing tumor aggressiveness and invasiveness might depend on the presence of germline variants in pancreatic cancer susceptibility genes. Another possibility is that mismatch repair deficiency caused by MMR gene mutations might contribute to longer survival, as suggested by the prior reports.[12]

In this study, we identified the presence of the deleterious/VUS mutations was associated with several variables such as blood type of patients and histological findings in the resected pancreas. Regarding the ABO blood type and pancreatic cancer prognosis, several previous reports demonstrated that O blood type indicated better prognosis compared to non-O type.[35, 36] Furthermore, a recent Japanese cohort study revealed the PDAC patients harboring A alleles showed worse survival than those with non-A alleles.[37] In this study, the significant difference of blood type distribution implies the higher prevalence of A type in patients with wild-type/benign mutations than those with deleterious/VUS mutations. Collectively, there may be a possible inverse association between higher prevalence of A blood type and lower prevalence of DDR-related mutation, and this may be a risk of worse prognosis. However, underlying associated factors remain unelucidated. Moreover, there have been no prior reports suggesting the reason for the association between the DDR-related mutations and histological findings of cancer-stroma relationship and growth patterns. Future study is needed to clarify the clinical and histological characteristics of patients harboring the DDR-related mutations.

To enrich PDAC patients harboring DDR-related germline gene mutations, we focused on the personal/family histories of breast, ovarian, and prostate cancers in addition to pancreatic cancer. However,

TABLE 4 Germline variants classified as VUS

Case #	Age/sex	Personal history	Family history in FDRs	Gene	Genome position	Nucleic change	AA change	ClinVar	SIFT	PolyPhen-2	PROVEAN	FATHMM	Mutation Taster	Max MAF in Databases[a]
2	66/F	None	Breast cancer (mother)	BRCA2	chr13:032 910 800	c.2308A>G	p.Ile770Val	Uncertain significance	–	B	N	T	P	N/A
29	74/F	Breast cancer (bilateral)	Prostate cancer (father)	PALB2	chr16:023 646 488	c.1379A>G	p.Gln460Arg	Conflicting	T	B	N	T	P	0.007
43	66/F	None	Pancreatic cancer (father)	MSH2	chr02:047 630 448	c.118G>A	p.Gly40Ser	Conflicting	T	B	N	DAMAGING	Disease causing	0.0012
224	74/F	Ovarian cancer	None	MSH2	chr02:047 703 697	c.2197G>A	p.Ala733Thr	Uncertain significance	–	PROBABLY DAMAGING	Deleterious	DAMAGING	Disease causing	0.0006
256	55/M	None	Pancreatic cancer (sister)	BRCA2	chr13:032 893 328	c.182T>C	p.Leu61Pro	Conflicting	–	B	N	T	P	0.0004
389	76/M	Prostate cancer (father)	Prostate cancer (brother)	MSH6	chr02:048 027 527	c.2405C>G	p.Pro802Arg	–	T	B	N	DAMAGING	Disease causing	0.0001

Abbreviations: B, benign; FDR, first-degree relatives; MAF, mutant allele frequency; N, neutral; N/A, not applicable; P, polymorphism; T, tolerated; VUS, variants of unknown significance.

[a]Based on multiple database search including 1000 Genomes Project, Human Genetic Variation Database (HGVD), the Genome Aggregation Database (gnomAD), Japanese Multi Omics Reference Panel (jMorp, Tohoku Medical Megabank, 8.3KJPN).

TABLE 5 Characteristics of pancreatic cancer patients with personal/family histories stratified by the presence of potentially pathogenic germline variants

Characteristics	Deleterious/VUS mutations (n = 8)		Wild type and benign mutations (n = 31)		P
Sex, n (%)					
Male	2	(25.0)	14	(45.2)	.432
Female	6	(75.0)	17	(54.8)	
Age, median (range), years	68	(42-76)	71	(41-87)	.443
Blood type, n (%)					
A	1	(12.5)	16	(51.6)	**.049**
B	1	(12.5)	5	(16.1)	
O	3	(37.5)	8	(25.8)	
AB	3	(37.5)	2	(6.5)	
Tumor diameter, n (%)					
≤20 mm	2	(25.0)	9	(29.0)	.999
>20 mm	6	(75.0)	22	(71.0)	
Tumor location, n (%)					
Head	4	(50.0)	18	(58.1)	.709
Body and tail	4	(50.0)	13	(41.9)	
Stage, n (%)[a]					
0	1	(12.5)	0	(0.0)	.495
IA	1	(12.5)	9	(29.0)	
IB	3	(37.5)	10	(32.3)	
IIA	0	(0.0)	0	(0.0)	
IIB	0	(0.0)	1	(3.2)	
III	2	(25.0)	5	(16.1)	
IV	1	(12.5)	6	(19.4)	
Treatment, n (%)					
Resected	6	(75.0)	16	(51.6)	.426
Unresected	2	(25.0)	15	(48.4)	
Platinum-based chemotherapy, n (%)					
Yes	1	(12.5)	8	(25.8)	.653
No	7	(87.5)	23	(74.2)	

Note: Bold values indicate statistical significance.

Abbreviation: VUS, variants of unknown significance.

[a]According to UICC/TNM staging classification 8th edition.

deleterious DDR-related germline gene mutations were identified in only four (10.2%) of the 39 patients with PDAC. This prevalence appeared to be similar to that reported by a previous study on Japanese familial PDAC.[15] One possible reason is that only nine genes known to be associated with hereditary predispositions for pancreatic, breast, and ovarian cancers, were included. A recent whole-exome study on Japanese familial PDAC identified various DDR-related genes such as *ERCC4*.[31] Future studies are needed to test whether the combination of extended criteria of personal/family histories with genome-wide comprehensive investigation of DDR-related genes contributes to achieving further enrichment of the select patients who can benefit from platinum-based chemotherapy and/or PARP inhibitors.

In conclusion, we found possibly deleterious germline variants in PDAC patients with personal/family histories of pancreatic/breast/ovarian/prostate cancers. A better understanding of clinical and genomic features in such cases might provide new opportunities for early germline testing in order to select the best therapy early on in the treatment course of a patient.

TABLE 6 Histological characteristics of resected pancreas in patients with personal/family histories stratified by the presence of potentially pathogenic germline variants

Characteristics	Deleterious/VUS mutations (n = 6)		Wild type and benign mutations (n = 16)		P
Procedures, n (%)					
PD	2	(33.3)	10	(62.5)	.514
DP	4	(66.7)	5	(31.3)	
TP	0	(0.0)	1	(6.3)	
PV/SMV resection, n (%)					
Yes	0	(0.0)	2	(12.5)	.999
No	6	(100.0)	14	(87.5)	
Histological grade, n (%)[a]					
G1	3	(50.0)	7	(43.8)	.471
G2	2	(33.3)	7	(43.8)	
G3	1	(16.7)	0	(0.0)	
G4	0	(0.0)	2	(12.5)	
Pathological T factor, n (%)[a]					
T1	3	(50.0)	10	(62.5)	.129
T2	1	(16.7)	3	(18.8)	
T3	0	(0.0)	3	(18.8)	
T4	2	(33.3)	0	(0.0)	
Pathological N factor, n (%)[a]					
N0	3	(50.0)	7	(43.8)	.999
N1	2	(33.3)	7	(43.8)	
N2	1	(16.7)	2	(12.5)	
Margins at resection, n (%)					
Negative	5	(83.3)	14	(87.5)	.999
Positive	1	(16.7)	2	(12.5)	
Cancer-stroma relationship, n (%)[b]					
Intermediate type	0	(0.0)	9	(56.3)	**.046**
Scirrhous type	6	(100.0)	7	(43.8)	
Growth patterns, n (%)[b]					
INFa	0	(0.0)	0	(0.0)	**.023**
INFb	1	(16.7)	12	(75.0)	
INFc	5	(83.3)	4	(25.0)	
Lymphatic invasion, n (%)[b]					
ly0	2	(33.3)	2	(12.5)	.693
ly1	4	(66.7)	11	(68.8)	
ly2	0	(0.0)	1	(6.3)	
ly3	0	(0.0)	2	(12.5)	
Venous invasion, n (%)[b]					
v0	1	(16.7)	0	(0.0)	.264
v1	3	(50.0)	6	(37.5)	
v2	1	(16.7)	8	(50.0)	
v3	1	(16.7)	2	(12.5)	
Nerve invasion, n (%)[b]					
ne0	2	(33.3)	3	(18.8)	.296
ne1	1	(16.7)	7	(43.8)	

TABLE 6 (Continued)

| Characteristics	Deleterious/VUS mutations (n = 6)		Wild type and benign mutations (n = 16)		P
ne2	1	(16.7)	5	(31.3)	
ne3	2	(33.3)	1	(6.3)	
Therapeutic effect, n (%)[c]					
N/A (upfront surgery)	1	(16.7)	6	(37.5)	.370
I	0	(0.0)	3	(18.8)	
IIa	4	(66.7)	4	(25.0)	
IIb	1	(16.7)	3	(18.8)	
Adjuvant therapy, n (%)					
Yes	5	(83.3)	14	(87.5)	.999
No	1	(16.7)	2	(12.5)	

Note: Bold values indicate statistical significance.

Abbreviations: DP, distal pancreatectomy; N/A, not applicable; PD, pancreaticoduodenectomy; PV/SMV, portal vein/superior mesenteric vein; TP, total pancreatectomy; VUS, variants of unknown significance.

[a]According to UICC/TNM staging classification 8th edition.

[b]According to the Classification of Pancreatic Carcinoma fourth English edition by the Japan Pancreas Society.

[c]According to Evans classification (Evans DB, et al Arch Surg 1992).
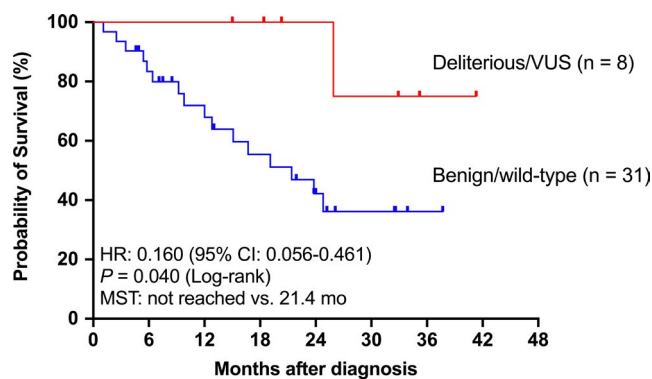
FIGURE 2 Kaplan-Meier curves for overall survival in patients with personal/family histories stratified by the germline mutation status of DNA damage repair genes between deleterious/VUS mutations group (n = 8) with benign mutation/wild-type group (n = 31). VUS; variants of unknown significance; HR, hazard ratio; CI, confidence interval; MST, median survival time

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.