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ORIGINAL RESEARCH ARTICLE

Methylation Analyses Reveal Promoter Hypermethylation as a Rare Cause of "Second Hit" in Germline *BRCA1*-Associated Pancreatic Ductal Adenocarcinoma

Binbin Zheng-Lin[1] · Michael Rainone[2] · Anna M. Varghese[3] · Kenneth H. Yu[3] · Wungki Park[3,7,8] · Michael Berger[4] · Miika Mehine[4] · Joanne Chou[5] · Marinela Capanu[5] · Diana Mandelker[4] · Zsofia K. Stadler[3,7,8] · Ozge Birsoy[4] · Sowmya Jairam[4] · Ciyu Yang[4] · Yirong Li[4] · Donna Wong[4] · Jamal K Benhamida[4] · Marc Ladanyi[4] · Liying Zhang[6] · Eileen M. O'Reilly[3,7,8]

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Abstract

Background and Objective Pancreatic ductal adenocarcinoma (PDAC) is characterized by the occurrence of pathogenic variants in *BRCA1/2* in 5–6% of patients. Biallelic loss of *BRCA1/2* enriches for response to platinum agents and poly (ADP-ribose) polymerase 1 inhibitors. There is a dearth of evidence on the mechanism of inactivation of the wild-type *BRCA1* allele in PDAC tumors with a germline *BRCA1* (*gBRCA1*) pathogenic or likely pathogenic variant (P/LPV). Herein, we examine promotor hypermethylation as a "second hit" mechanism in patients with *gBRCA1*-PDAC.

Methods We evaluated patients with PDAC who underwent Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) somatic and germline testing from an institutional database. DNA isolated from tumor tissue and matched normal peripheral blood were sequenced by MSK-IMPACT. In patients with *gBRCA1*-PDAC, we examined the somatic *BRCA1* mutation status and promotor methylation status of the tumor *BRCA1* allele via a methylation array analysis. In patients with sufficient remaining DNA, a second methylation analysis by pyrosequencing was performed.

Results Of 1012 patients with PDAC, 19 (1.9%) were identified to harbor a *gBRCA1* P/LPV. Fifteen patients underwent a methylation array and the mean percentage of *BRCA1* promotor methylation was 3.62%. In seven patients in whom sufficient DNA was available, subsequent pyrosequencing confirmed an unmethylated *BRCA1* promotor. Loss of heterozygosity was detected in 12 of 19 (63%, 95% confidence interval 38–84) patients, demonstrating loss of heterozygosity is the major molecular mechanism of *BRCA1* inactivation in PDAC. Two (10.5%) cases had a somatic *BRCA1* mutation.

Conclusions In patients with *gBRCA1*-P/LPV-PDAC, loss of heterozygosity is the main inactivating mechanism of the wild-type *BRCA1* allele in the tumor, and methylation of the *BRCA1* promoter is a distinctly uncommon occurrence.

1 Introduction

Pancreatic ductal adenocarcinoma (PDAC) has a rising incidence and is projected to surpass colorectal cancer in 2026 as the second cause of cancer death in the USA [1]. The extraordinarily challenging prognosis of PDAC is partially driven by inherent resistance to cytotoxic therapy and immunotherapy and a lack of early detection with validated screening intervention along with intrinsic tumor resistance mechanisms innate to the tumor microenvironment and immune system and other considerations [2]. Up to 9.7% of patients with PDAC have a pathogenic or likely pathogenic variant (P/LPV) that affects DNA damage repair genes [2]. Among these, altered BReast CAncer gene 1 (*BRCA1*) and BReast CAncer gene 2 (*BRCA2*) expression impairs DNA double-strand break repair resulting in homologous recombination deficiency (HRD). Pancreatic ductal adenocarcinoma tumor cells with HRD experience synthetic lethality when exposed to DNA-damaging agents, which enables treatment opportunities with poly (ADP-ribose) polymerase (PARP) 1 inhibitors and platinum chemotherapy [3, 4]. In individuals with a germline *BRCA1/2* (*gBRCA1/2*) P/LPV, an acquired mutation in the tumor wild-type

Liying Zhang and Eileen M. O'Reilly contributed equally as senior co-authors.

✉ Liying Zhang
LiyingZhang@mednet.ucla.edu

✉ Eileen M. O'Reilly
oreillye@mskcc.org

Extended author information available on the last page of the article

Key Points

Biallelic loss of *BRCA* function is associated with a higher response to DNA-damaging agents. Nevertheless, inactivating mechanisms of the wild-type *BRCA1* allele are poorly understood in patients with pancreatic ductal adenocarcinoma and a germline *BRCA1* variant.

Epigenetic silencing via *BRCA1* promotor methylation has been reported in ovarian and breast tumors, but not in pancreatic ductal adenocarcinoma. By applying a methylation array and pyrosequencing, we observed *BRCA1* promotor hypermethylation was a distinctly uncommon mechanism of wild-type *BRCA1* allele inactivation in patients with germline *BRCA1*-pancreatic ductal adenocarcinoma.

allele may result in complete abrogation of *BRCA* function, which has been associated with a higher response to DNA-damaging agents [5–7].

In addition to acquired somatic mutations and inherited germline variants, epigenetic modifications may silence *BRCA1/2* gene expression. In triple-negative breast cancer and epithelial ovarian cancer, hypermethylated *BRCA1* promotor is frequent and has been reported in up to 57% and 20% of patients, respectively [8, 9]. Furthermore, concomitant somatic *BRCA1* (*sBRCA1*) promoter methylation has been described in 35% of *gBRCA1*-breast tumors and 20% of *gBRCA1*-ovarian tumors [5]. In contrast, hypermethylation of *BRCA1/2* promotors appears to be an uncommon occurrence in PDAC tumors unselected for *gBRCA1/2* status [10]. Prior studies have reported loss of heterozygosity (LOH) of the wild-type *BRCA1* allele as one mechanism of "second hit" in PDAC [11–13], whereas the role of epigenetic silencing remains unexplored. Herein, we aimed to explore promotor hypermethylation as a mechanism of inactivation of the wild-type *BRCA1* allele in patients with PDAC and a *gBRCA1* P/LPV.

2 Methods

This is a single-center retrospective study overseen by the Institutional Review Board at Memorial Sloan Kettering Cancer Center. Inclusion criteria included patients with PDAC who consented and underwent Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) testing for both somatic and germline P/LPVs between July 2015 and January 2019. MSK-IMPACT is a hybridization, capture-based, next-generation sequencing panel that is capable of detecting all protein-coding mutations, copy number alterations, and selected promoter mutations and structural rearrangements in up to 505 oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies [14]. MSK-IMPACT panel included *APC, ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDKN2, CHEK2, EPCAM, ERCC3, FAM175A, FANCA, FANCC, FH, FLCN, HOXB13B, MITF, MLH1, MRE11, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, RAD50, RAD51, RECQL, RET, SDHA, SDHC, STK11, TERT,* and *TSC1* and other cancer predisposition genes [14, 15]. Formalin-fixed paraffin-embedded (FFPE) tumor tissues and normal peripheral blood were used for somatic and germline DNA sequencing, respectively.

2.1 Somatic BRCA1 Mutation Status and LOH Analyses

Genomic DNA from FFPE tumor tissues and matched normal peripheral blood from each patient were extracted and sequenced on Illumina HiSeq2500. Genomic DNA was extracted from FFPE tissue using the Chemagic DNA Tissue kit (PerkinElmer Chemagen Technologie, GmbH, Baesweiler, Germany) after manual macrodissection to ensure at least a 10% tumor content. Please refer to our previous publication for detailed wet-lab used procedures, quality control, and variant calling pipelines [15].

We followed the established workflow for calling somatic mutations in oncology genes [15]. As *BRCA1* is a cancer predisposition gene, we follow the American College of Medical Genetics and Genomics guidelines as well as Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists guidelines for variant interpretations of germline [16] and somatic variants [17]. Germline variants are classified as pathogenic, likely pathogenic, variants of uncertain significance, likely benign, or benign based on the scoring scheme delineated in the American College of Medical Genetics and Genomics guidelines [14]. In individuals who harbored a gBRCA1 P/LPV, we examined the LOH status in the tumor tissue using a FACETS analysis [18].

2.2 Methylation Analysis

In individuals found to have a *gBRCA1* P/LPV, we examined the *BRCA1* promoter methylation status in the tumor using a methylation array analysis. In patients with sufficient leftover DNA samples, a second methylation analysis by pyrosequencing was performed.

2.2.1 Methylation Array

For each sample of genomic DNA, 250 nanograms (ng) of input was used for bisulfite conversion (EZ DNA Methylation Kit; Zymo Research; catalog number D5002), followed by an FFPE restoration step using the Infinium HD FFPE DNA Restore Kit (Illumina; catalog number WG-321-1002).

All samples were processed on the Infinium 850k array and scanned using the Illumina iScan, according to the manufacturer's recommended protocol. Each CpG site interrogated by the Infinium array is identified by a unique cg identifier in the format of cg#, where # is a number (e.g., cg17301289 is the cg identifier of a *BRCA1* promoter CpG site) [19]. CpG loci associated with the *BRCA1* promoter covered by the assay included cg17301289, cg04658354, cg04110421, cg21253966, cg16630982, cg16963062, cg15419295, cg20187250, and cg24806953 (Table 1). Methylation level was measured using beta values and a beta value less than 0.20 indicated a qualitatively unmethylated CpG loci.

2.2.2 Pyrosequencing

The assay was designed to detect the level of methylation in a region of transcription start site in exon 1 of the *BRCA1* gene (Ensembl Transcript ID ENST00000357654.9). Tumor DNA was extracted and bisulfite treated using the EZ-DNA Methylation Kit (Cat#D5020; Zymo Research, Irvine, CA, USA). Five hundred nanograms of DNA was used for each analysis [15]. A positive control specimen (CpGenome universal methylated DNA, Cat# S7821; Millipore Corporate, Billerica, MA, USA) and a negative control specimen (peripheral blood DNA) were included in the entire procedure along with patient samples. A single polymerase chain reaction (PCR) fragment spanning a transcription start site of *BRCA1* exon 1 was amplified, and the degree of methylation of 11 CpG sites was analyzed in a single pyrosequencing reaction (Qiagen, Germantown, MD, USA) (Fig. 1). The PCR products (10 µL) were sequenced by pyrosequencing on a PyroMark Q24 Workstation (Qiagen) following the manufacturer's instructions. The *BRCA1* methylation levels were graded as unmethylated or methylated if the average level of methylation across all 11 CpG sites is lower or higher than 10%, respectively.

2.3 Statistics

Baseline sociodemographic characteristics and genomic features were summarized using the frequency and percentages for categorical covariates, and the median and interquartile range for continuous variables. No formal statistical analyses were conducted, and summary descriptive statistics were used to describe what we have observed from this cohort of patients.

3 Results

From our institutional database, we identified 1182 patients with pancreatic tumors, including 1012 patients with PDAC (Fig. 2). All patients underwent somatic and germline testing. Germline P/LPVs were detected in 212 (20.9%) patients with PDAC. The most frequently altered genes were *BRCA2* ($N = 45$ out of 1012, 4.4%), *APC* ($N = 27$, 2.7%), and *ATM* ($N = 21$, 2.1%) (Fig. 3). Nineteen (1.9%) patients with PDAC harbored a *gBRCA1* P/LPV. Of these, the median age at diagnosis was 53 years (interquartile range 47–66), 15 (79%) were male, and four patients (21%) had Ashkenazi Jewish heritage (Table 2).

Of the 19 patients with a *gBRCA1* P/LPV, we analyzed the tumor for the secondary hits that inactivate the normal allele. Notably, 12 (63%, 95% confidence interval [CI] 38–84]) tumors had LOH, indicating LOH is the major molecular mechanism that inactivates the *BRCA1* normal allele in the tumor. Two (10.5%) cases had a s*BRCA1* P/LPV. Interestingly, in addition to a *gBRCA1* P/LPV, a concomitant *sBRCA1* variant and LOH were detected in the tumor of case #16. Altogether, a second hit by LOH and/or somatic mutations was detected in 13 of

Table 1 Description of CpG sites associated with *BRCA1* gene variants

CpG site	Illumina cg identifier	GRCh37 coordinate on chromosome 17	Position relative to transcription start site
1	cg17301289	41277462	−81
2	cg04658354	41277444	−63
3	NA	41277436	−55
4	cg04110421	41277428	−47
5	cg21253966	41277426	−45
6	NA	41277400	−19
7	cg16630982	41277394	−13
8	cg16963062	41277392	−11
9	cg15419295	41277389	−8
10	cg20187250	41277381	−1
11	cg24806953	41277364	18

NA Not available

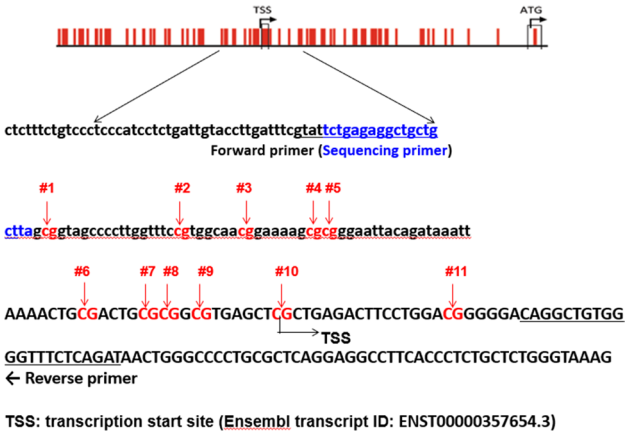


Fig. 1 *BRCA1* promotor sequence. The forward and reverse primers used for polymerase chain reaction (PCR) are underlined. The sequencing primer is in blue. Note that the forward PCR primer and sequencing primer overlap with each other. The 11 CpG sites interrogated by this assay are shown in *red*. *TSS* transcription start site

19 (68.4%) *gBRCA1*-PDAC tumors. Table 3 summarizes the molecular events that inactivate both *BRCA1* alleles in the tumor in 19 patients included in this study.

As five out of 19 (26%) cases had no second hit detected in the tumor DNA, we decided to pursue a methylation analysis to explore the role of DNA methylation in *BRCA1* inactivation in pancreatic cancer. Using the leftover DNA from clinical testing, we first conducted a methylation array that was successfully completed in 15 (79%) cases, while four cases had an insufficient sample for analysis. The mean percentage of methylated DNA of the *BRCA1* CpG islands was 3.62% (range 2.6–5.1%), which suggested that neither *BRCA1* allele was methylated. Seven patients had sufficient leftover DNA for a second analysis by pyrosequencing. All seven cases had methylation levels less than 10% across all 11 CpG sites, suggesting unmethylated *BRCA1* promotors.

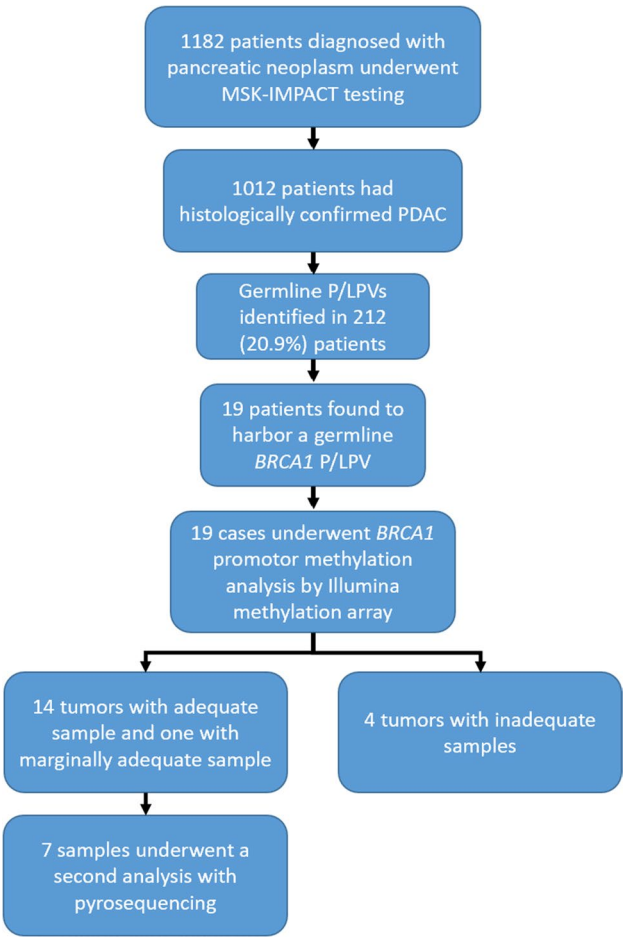
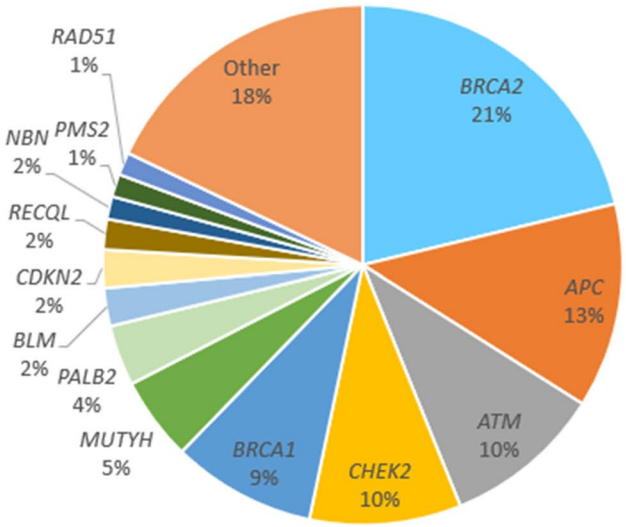


Fig. 2 Flow chart for *BRCA1* methylation analysis in patients with pancreatic ductal adenocarcinoma (PDAC). *MSK-IMPACT* Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets, *P/LPV* pathogenic or likely pathogenic variant

4 Discussion

The tumor suppressor genes *BRCA1/2* and their encoded proteins are crucial components of DNA double-strand break repair by homologous recombination. In *gBRCA1/2*-associated tumors, an acquired alteration of the wild-type allele leads to complete loss of *BRCA* function and a higher sensitivity to DNA-damaging agents [5–7]. There is a complete lack of data on epigenetic silencing of the wild-type *BRCA1* in *gBRCA1*-PDAC, albeit it has been described in breast and ovarian tumors. Maxwell et al. reported *BRCA1* locus-specific LOH in 37 of 41 (90%) *gBRCA1* carriers diagnosed with breast cancer, and in 48 of 52 (93%) of patients with *gBRCA1*-ovarian tumors [5]. In their sub-cohort of patients who underwent methylation-specific PCR, somatic promoter methylation was observed in eight of 23 (35%, range not reported) *gBRCA1*-breast tumors and three of 15 (20%, range not reported) *gBRCA1*-ovarian tumors. In our study, we examined the methylation status of the *BRCA1* gene using two distinct assays and observed that promotor hypermethylation is an uncommon phenomenon, and in fact did not occur as a mechanism of wild-type *BRCA1* allele inactivation. Our results are consistent with a recent study by Zhou et al. who measured the promoter methylation of germline *ATM*, *BRCA1*, and *BRCA2* genes using real-time PCR in 655 patients with PDAC. They observed minimal levels of promotor methylation affecting these three genes. Of note, 113 (17.2%) of the cohort met criteria for familial pancreatic cancer, defined as having a family history of at least two first-degree relatives with PDAC, albeit the frequency of g*BRCA1/2* variants in this cohort was not reported [10].

Other molecular inactivating mechanisms of wild-type *BRCA1* have been described in patients with *gBRCA1*-PDAC. In our analysis, LOH was detected in 12 of 19 (63%, 95% CI 38–84) of



Mutated gene	N	Mutated gene	N
BRCA2	45	*SDHA*	2
APC	27	*BARD1*	2
ATM	21	*BRIP1*	2
CHEK2	20	*ERCC3*	2
BRCA1	19	*FANCA*	2
MUTYH	11	*FH*	2
PALB2	8	*FAM175A*	2
BLM	5	*EPCAM*	1
CDKN2	5	*FANCC*	1
RECQL	4	*FLCN*	1
NBN	3	*HOXB13B*	1
PMS2	3	*MLH1*	1
RAD51	3	*MSH3*	1
MITF	2	*NF1*	1
MRE11	2	*RET*	1
MSH2	2	*SDHC*	1
MSH6	2	*STK11*	1
NTHL1	2	*TERT*	1
RAD50	2	*TSC1*	1

Fig. 3 Distribution of germline mutations in a cohort of patients with pancreatic ductal adenocarcinoma. Of 1012 patients with pancreatic ductal adenocarcinoma who underwent Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) testing, 212 were identified to harbor a germline pathogenic or likely pathogenic variant. The pie chart displays the frequency of most detected variants in these 212 patients. The table contains the number of individuals affected by each of the listed germline variants. *N* number of patients

Table 2 Baseline demographic characteristics of patients with pancreatic ductal adenocarcinoma and a germline *BRCA1* pathogenic or likely pathogenic variant

Parameter	*N* = 19
Sex, *N* (%)	
Female	4 (21)
Male	15 (79)
Ethnicity, *N* (%)	
African American	1 (5.3)
Ashkenazi Jewish/Caucasian	4 (21)
Caucasian	13 (68)
Declined to answer	1 (5.3)
Age at diagnosis, years, median (IQR)	53 (47–66)
Initial stage at diagnosis, *N* (%)	
Stage 2	2 (11)
Stage 3	3 (16)
Stage 4	14 (74)
Past history of other malignancy, *N* (%)	
Ampullary cancer	1 (5.3)
Breast cancer	1 (5.3)
No	17 (89)
First-degree relative with pancreas cancer, *N* (%)	2 (11)

IQR interquartile range

patients, demonstrating LOH is the major molecular mechanism of "second hit" for *BRCA1* inactivation in PDAC. Additionally, we observed a low prevalence of a second somatic mutation of *BRCA1*, which occurred in two (10.5%) patients in our cohort. In a prior study, Sokol et al. analyzed 12,248 patients with pancreatic neoplasms, 5.2% of whom had a *BRCA1/2* variant. Among patients with computationally predicted *gBRCA1* and *gBRCA2* variants, respectively, 79.2% and 79.7% had a biallelic alteration in the tumor. The group did not report the frequency of different types of biallelic alteration, which included LOH of the wild-type allele, homozygous deletion, and two or more *BRCA1/2* alterations in the same tumor sample [11]. In a cohort of seven patients with *gBRCA1*-associated PDAC, Al-Sukhni et al. observed that LOH of the *BRCA1* locus occurred in five cases. Of these, three had tumor DNA sequenced, which all had loss of the wild-type *BRCA1* allele [13]. Nguyen et al. examined the HRD landscape of a large pan-cancer cohort. Among 370 patients with PDAC, three were carriers of a *gBRCA1* P/LPV. They observed LOH in all three patients, albeit the exact mechanism was not reported [20]. Similarly, Lowery et al. interrogated the somatic and germline profiles of 615 patients with exocrine pancreatic tumors, which included primarily PDAC, adenosquamous carcinoma, acinar cell carcinoma, and undifferentiated tumors. Loss of heterozygosity analysis was performed in ten patients with a *gBRCA1* variant. Of these, three had LOH and three had copy neutral LOH [12].

Table 3 Molecular characteristics of germline and somatic *BRCA1* pathogenic or likely pathogenic variants in patients with pancreatic ductal adenocarcinoma

Case number	Germline *BRCA1* change	Germline *BRCA1* amino acid change	Somatic *BRCA1* variants	Loss of heterozygosity
1	c.427G>T	p.Glu143*	No	No
2	c.4986+5G>A	n/a	No	Yes
3	c.1953dupG	p.Lys652Glufs*21	No	Yes
4	c.5266dupC	p.Gln1756Profs*74	No	Yes
5	c.68_69delAG	p.Glu23Valfs*17	No	No
6	c.68_69delAG	p.Glu23Valfs*17	No	Yes
7	c.68_69delAG	p.Glu23Valfs*17	*BRCA1* in-frame deletion V1688del (c.5062_5064del)	No
8	c.68_69delAG	p.Glu23Valfs*17	No	Yes
9	c.5266dupC	p.Gln1756Profs*74	No	Yes
10	c.65T>C	p.Leu22Ser	No	No
11	c.5266dupC	p.Gln1756Profs*74	No	Yes
12	c.68_69delAG	p.Glu23Valfs*17	No	Yes
13	c.68_69delAG	p.Glu23Valfs*17	No	No
14	Deletion exons 13-19	n/a	No	Indeterminate
15	c.68_69delAG	p.Glu23Valfs*17	No	Yes
16	c.181T>G	p.Cys61Gly	*BRCA1* (NM_007294) exon10 p.E1258* (c.3772G>T)	Yes
17	c.2389G>T	p.Glu797*	No	Yes
18	c.68_69delAG	p.Glu23Valfs*17	No	No
19	c.68_69delAG	p.Glu23Valfs*17	No	Yes

In unselected populations, somatic alterations of *BRCA1/2* are rare and have been reported in 1.5–3.9% of PDAC tumors [21, 22]. In patients with *gBRCA1*-PDAC, prior studies have found a high prevalence of somatic *BRCA1* mutations, albeit their findings were limited in the sample size. Yurgelun et al. described concomitant *sBRCA1* mutations in one (33%) out of three patients [23], whereas Borazanci et al. observed the same finding in one of two PDAC tumors [24]. Our results indicate that second *sBRCA1* mutations are infrequently associated with *gBRCA1* P/LPVs and occurred in only two (10.5%) of 19 patients in our cohort.

BRCA1/2-associated PDAC delineates a subpopulation of patients with key clinical features. Healthy individuals with a *gBRCA1/2* P/LPV carry a two-fold to six-fold increase in a lifetime risk of PDAC and are typically diagnosed with this disease at a median age of 60 years, younger than sporadic cases [25–27]. Moreover, tumors with deficient DNA repair experience synthetic lethality when exposed to DNA-damaging agents including PARP inhibitors and platinum chemotherapy. *gBRCA1/2* status is a validated surrogate biomarker for HRD in patients with PDAC, albeit its predictive value differs based on zygosity status [5, 28]. In a study by Momtaz et al. with 136 patients with metastatic PDAC, 116 had a *gBRCA1/2* variant and 20 had a *sBRCA1*/2 mutation. Zygosity analysis identified biallelic *BRCA1/2* alterations in 65 (56%) patients within the germline cohort and in 12 (60%) patients in the somatic cohort. Survival analyses showed numerically higher median overall survival in the biallelic group versus the group of patients with a heterozygous g*BRCA1/2* or *sBRCA1/2* when treated with frontline platinum therapy (respective median overall survival were 26 months, 95% CI 20–52 months vs 8.7 months, 95% CI 6.2 months to not reached) and when treated with a PARP inhibitor (26 months, 95% CI 24–53 months versus 8.7 months, 95% CI 7.2 months to not reached, respectively) [28]. Despite not reaching statistical significance because of the small sample size, these results suggest that monoallelic loss of *BRCA1* is likely a bystander passenger alteration instead of a driver of PDAC tumor phenotype in some patients. Similarly, Park et al. reported higher median overall survival in patients with advanced PDAC with HRD, defined as having a somatic or germline pathogenic variant of *BRCA1, BRCA2, PALB2, ATM, BAP1, BARD1, BLM, BRIP1, CHEK2, FAM175A, FANCA, FANCC, NBN, RAD50, RAD51, RAD51C,* or *RTEL1* [29]. Of note, the survival benefit seen in the HRD group remained statistically significant after adjusting for first-line platinum treatment, which suggests an independent prognostic impact of the underlying tumor biology.

The inherent resistance of PDAC to the one-size-fits-all chemotherapy points to the necessity of new therapy development centered on precision medicine strategies. Presently, *gBRCA1/2* variant status is used to select patients with PDAC who may benefit from platinum-based chemotherapy and PARP inhibitors [3, 4]. However, only a small portion of individuals benefit from such therapies because of the rarity of *gBRCA1/2* variants. Therefore, it is an imperative to examine and potentially expand surrogate

biomarkers for HRD in this patient population. Beyond *BRCA1/2*, the alteration of less frequently mutated genes with lower pathogenicity generates a "BRCAness" phenotype akin to the molecular features of *BRCA*-mutant tumors [30]. In a dataset of 391 patients, Golan et al. examined their whole-genome sequencing data and applied HRD classifiers with the genomic instability scores. The authors observed the genomic HRD signature was frequently associated with biallelic inactivation of *BRCA1/2* and *PALB2*, as well as *XRCC2, RAD51C*, and *RAD51D* [31]. This study reported two clinically relevant highlights. First, approximately one in eight (12%) of patients with *gBRCA1/2*-PDAC did not have hallmarks of HRD and had retention of the wild-type allele. Second, in patients with advanced PDAC without a germline *BRCA1/2* or *PALB2* variant, genomic HRD classifiers identified somatic HRD defects in an additional 7–10% of patients who may benefit from DNA-damaging agents [31]. Beyond PDAC, Sokol et al. demonstrated that biallelic alteration of *BRCA1/2* was associated with a functional HRD status and elevated genomic instability regardless of the primary tumor type [11]. This may represent a renewed therapeutic opportunity with DNA-damaging agents in all *BRCA1/2*-mutated tumors, akin to prior tumor-agnostic trials with tropomyosin receptor kinase inhibitors and pembrolizumab [32–34].

The present study has a number of significant limitations. Our analysis stems from a single-center experience in a tertiary cancer care center. Our sample size was small. Our cohort size was limited as was ethnic and racial diversity. We reported an unmethylated *BRCA1* promotor after evaluation using two different assays. However, we did not evaluate for other epigenetic modifications that may affect *BRCA1* expression, such as histone acetylation. Last, we did not assess epigenetic changes of other genes (e.g., *BRCA2* and *ATM*) involved in the homologous recombination repair pathway.

5 Conclusions

In summary, we have demonstrated that LOH is the major molecular mechanism of "second hit" in patients with PDAC and a *gBRCA1* P/LPV. Concomitant *sBRCA1* mutations are rare, whereas methylation of promoter CpG islands of *BRCA1* is a distinctly uncommon mechanism of inactivation of the wild-type *BRCA1* in these patients. Further investigation will clarify whether epigenetic modifications (e.g., histone acetylation and microRNAs) occur as potential mechanisms for biallelic loss of DNA damage repair genes in PDAC.

Declarations

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Conflicts of interest/competing interests EOR: research funding to MSK: Genentech/Roche, Celgene/BMS, BioNTech, AstraZeneca, Arcus, Elicio, Parker Institute. Consulting/DSMB: Cytomx Therapeutics (DSMB), Rafael Therapeutics (DSMB), Seagen, Boehringer Ingelheim, BioNTech, Ipsen, Merck, IDEAYA, AstraZeneca, Noxxon, BioSapien, Cend Therapeutics, Novartis, Thetis, Agios (spouse), Genentech-Roche (spouse), Eisai (spouse). LZ: honoraria (Future Technology Research LLC, BGI, Illumina); honoraria and travel and accommodation expenses (Roche Diagnostics Asia Pacific). Family members hold leadership positions and ownership interests in Decipher Medicine. ML: honoraria for advisory board participation from Merck, Astra-Zeneca, Bristol Myers Squibb, Blueprint Medicines, Janssen Pharmaceuticals, Takeda Pharmaceuticals, Lilly Oncology, LOXO Oncology, Bayer, ADC Therapeutics, Riken Genesis/Sysmex, and Paige. AI: research support from LOXO Oncology, Merus, Elevation Oncology, and Helsinn Therapeutics.

Ethics approval The study was overseen by the Institutional Review Board at Memorial Sloan Kettering. The analyses took place under IRB #18-514 and #12-245.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because they contain information that could compromise research participant privacy/consent.

Code availability Not applicable.

Author contributions EOR, LZ, and MR contributed to the study conception and design. Material preparation, data collection, and analysis were performed by EOR, LZ, JC, and MC. BZ, LZ, and EOR drafted the manuscript. All authors read and approved the final manuscript.

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Authors and Affiliations

Binbin Zheng-Lin[1] · Michael Rainone[2] · Anna M. Varghese[3] · Kenneth H. Yu[3] · Wungki Park[3,7,8] · Michael Berger[4] · Miika Mehine[4] · Joanne Chou[5] · Marinela Capanu[5] · Diana Mandelker[4] · Zsofia K. Stadler[3,7,8] · Ozge Birsoy[4] · Sowmya Jairam[4] · Ciyu Yang[4] · Yirong Li[4] · Donna Wong[4] · Jamal K Benhamida[4] · Marc Ladanyi[4] · Liying Zhang[6] · Eileen M. O'Reilly[3,7,8]

1 Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

2 Department of Medical Oncology, City of Hope National Medical Center, Duarte, CA, USA

3 Gastrointestinal Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, Office 1021, New York, NY 10065, USA

4 Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

5 Department of Epidemiology and Biostatistics, Weill Cornell Medical College, New York, NY, USA

6 Department of Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), 10833 Le Conte Ave, Los Angeles, CA 90095, USA

7 Weill Cornell Department of Medicine, Weill Cornell Medicine, New York, NY, USA

8 David M. Rubenstein Center for Pancreatic Research, Memorial Sloan Kettering Cancer Center, New York, NY, USA