의학박사 학위논문

Detection of circulating tumor DNA in resectable pancreatic ductal adenocarcinoma

절제가능 췌관선암에서 순환 종양 DNA의 검출 연구

2022년 2월

서울대학교 대학원

의학과 검사의학 전공

이 지 수

절제가능 췌관선암에서 순환 종양 DNA의 검출 연구

2022년

이 지 수

Detection of circulating tumor DNA in resectable pancreatic ductal adenocarcinoma

지도 교수 박 성 섭

이 논문을 의학박사 학위논문으로 제출함
2021년 10월

서울대학교 대학원
의학과 검사의학 전공
이 지 수

이지수의 의학박사 학위논문을 인준함
2022년 1월

위 원 장 장진영 (인)

부위원장 박성섭 (인)

위 원 성문우 (인)

위 원 오도연 (인)

위 원 김소연 (인)

Abstract

Detection of circulating tumor DNA in resectable pancreatic ductal adenocarcinoma

Jee-Soo Lee

Department of Medicine

The Graduate School

Seoul National University

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, where only 20% of the patients are suitable for curative resection at the time of diagnosis, but 80% of them eventually have tumor recurrence. Therefore, reliable biomarkers for the detection of tumors especially in the early stages of PDAC and for the monitoring of residual tumors after curative resection are critical for PDAC treatment. Circulating tumor DNA (ctDNA) is a promising blood-based biomarker because of its easily and serially accessible nature, but accurate quantification of ctDNA in the early stage of disease is challenging. To overcome this challenge, we applied an optimized next-generation sequencing

(NGS) technology for ctDNA analysis in a prospective cohort of patients with resectable PDAC. In this study, we aimed to investigate the feasibility of ctDNA profiling using optimized NGS for resectable PDAC.

Methods: A total of 70 consecutive patients diagnosed with resectable PDAC and undergoing curative resection for pancreatic tumor were enrolled from August 2020 through October 2021. We performed CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) NGS with integrated digital error suppression (iDES) of triple-matched samples [plasma cell-free DNA (cfDNA), tumor tissue, and germline DNA] targeting 77 selected genes. cfDNA isolated from pooled plasma samples of 16 healthy control individuals was also sequenced.

Results: We were able to detect preoperative ctDNA in 37.7% of the evaluable patients, with a median variant allele frequency (VAF) of 0.09% [interquartile range (IQR), 0.04% – 0.16%], which was approximately 40-fold lower than that previously reported for metastatic PDAC. *TP53* gene (29.1%) was most frequently identified, followed by *KRAS* (18.9%), *GNAS* (11.3%), *SMAD4* (1.9%), *PIK3CA* (1.9%), and *CDKN2A* (1.9%). In total, 34.0% (18/53) of patients had clinically

relevant mutations detected in both plasma and tissue, 13.2% (7/53) were detected exclusively in tissue analysis, and 3.8% (2/53) were detected exclusively in ctDNA analysis. In addition, 12 additional oncogenic mutations (*TP53*, n = 6; *GNAS*, n = 5; *SMAD4*, n = 1) that were not detected in tissue samples were detected in ctDNA. In addition, we observed a 15.1% decrease in the proportion of patients with detectable ctDNA after curative resection. VAF in each patient was significantly decreased in postoperative plasma (preoperative ctDNA, median 0.08%, IQR 0.04%–0.15%; postoperative ctDNA, median 0.00%, IQR 0.00%–0.03%; P < 0.001). Among 20 patients with detectable ctDNA before surgery, the risk of recurrence at 1 year tended to be higher in patients with still detectable ctDNA after surgery than in patients with negative conversion of ctDNA after surgery (48.6% vs. 25.0%, P = 0.064). We also found that cfDNA from 24.5% of patients had features compatible with clonal hematopoiesis.

Conclusion: The fraction of ctDNA in resectable PDAC patients was very low. However, an optimized NGS approach may add value beyond tissue analysis through a highly sensitive detection of ctDNA in resectable PDAC. ctDNA analysis

after surgery could be a potential prognostic marker. Moreover, paired sequencing of matched leukocytes may be required to accurately detect clinically relevant ctDNA.

Keywords: Circulating tumor DNA, Pancreatic ductal adenocarcinoma, resectable pancreatic cancer, CAPP-Seq
Student Number: 2016-21962

Table of Contents

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies that generally has a very poor prognosis with a 5-year survival rate of 6-8%, mainly owing to its silent nature in the early stages and late diagnosis. Only 20% of the patients are suitable for curative resection at the time of diagnosis, but even after radical resection, the tumor recurrence rate reaches up to 80%, due to micrometastases present at the time of diagnosis. Therefore, reliable biomarkers are required for the detection of tumors especially in the early stages of the disease and for monitoring the residual tumors after curative resection.

Circulating tumor-derived cell-free DNA [i.e., circulating tumor DNA (ctDNA)] is a promising blood-based biomarker in cancer management because of its easily and serially accessible nature. A majority of previous reports have summarized the benefits of ctDNA as a non-invasive marker for cancer genotyping at diagnosis, minimal residual disease (MRD) assessment after surgery, monitoring

treatment response, and prognosis prediction.

The application of next-generation sequencing (NGS) in ctDNA analysis is appealing because this approach covers a broad range of genes of interest, which provides comprehensive molecular profiling of the tumor. However, this NGS approach has mostly been applied in patients with advanced cancers. In early-stage cancer, the fraction of ctDNA is usually found to be <1% of the total cfDNA, and accurate quantification of ctDNA is technically challenging because it is difficult to reliably distinguish among true variants with low variant allele frequency (VAF) and background errors. Recently, an optimized NGS technology, cancer personalized profiling by deep sequencing (CAPP-Seq) with integrated digital error suppression (iDES) has been developed and achieved very sensitive detection of ctDNA found at as low as 0.002%. However, well-designed prospective studies are still required to provide useful evidence for applicability of this optimized NGS technology for the detection of minute amount of ctDNA in early-stage cancer.

In this study, we aimed to determine the feasibility of ctDNA profiling in a prospective cohort of patients with resectable PDAC utilizing iDES-enhanced CAPP-Seq. We investigated the molecular landscape of identified ctDNA, compared clinically relevant mutations detected in ctDNA with those detected in matched tumor tissues, evaluated potential prognostic value of ctDNA, and described factors that should be considered in ctDNA analysis.

2. Materials and Methods

2.1. Study design and Patients

We prospectively enrolled patients with resectable pancreatic cancer at Seoul National University hospital from August 2020 through October 2021. Eligible patients included in this study were of age > 18 years with previously untreated primary PDAC. Baseline whole blood samples and matched tumor tissue samples were collected before surgery, and postoperative whole blood samples

were collected at 1–2 weeks after surgery. Whole blood samples from 16 healthy adult individuals were additionally collected. All patients provided fully informed written consent on enrolment. The study was approved by the Institutional Review Board of the Seoul National University Hospital (IRB No. H-2007-151-1143).

2.2. Plasma preparation

To obtain plasma, 10-mL whole blood samples were collected in cell-free DNA Blood Collection Tubes (cfDNA BCT tube, Streck, Inc., Omaha, NE) containing cell-stabilizing agents. Each tube was further processed within 6 h. Blood samples were centrifuged at 1600× g for 10 min at room temperature, and plasma fractions were sequentially centrifuged at 13,000 × g for 10 min. Supernatants were transferred to 1.5-mL microtubes (Eppendorf, Hamburg, Germany) for further analysis. The remaining plasma-depleted whole blood was also processed for germline DNA isolation.

2.3. Cell-free DNA isolation

cfDNA was isolated from 3-5mL of plasma using AVENIO cfDNA Isolation kit (Roche Diagnostics, Indianapolis, IN, USA), and was subsequently quantified with Qubit 2.0 fluorometer Qubit dsDNA HS High Assay Kit (Invitrogen, Carlsbad, CA, USA). Size distribution of the isolated cfDNA was analyzed using microfluidic platform-based electrophoresis 4200 TapeStation instrument and high-sensitivity D1000 Tape (Agilent Technologies, Santa Clara, CA, USA). All protocols were performed according to the manufacturers' instructions.

2.4. Matched tissue and germline samples

DNA was extracted from matched tumor tissue and buffy coat samples using Gentra Puregene Blood kit (Gentra Systems, Minneapolis, MN, USA) and QIAamp DNA mini kit (Qiagen, GmbH, Hilden, Germany), respectively.

2.5. NGS Library preparation and sequencing

iDES-enhanced CAPP-Seq NGS libraries using cfDNA, matched tissue DNA, and matched germline DNA were prepared for 77 genes (Table 1), using AVENIO ctDNA Expanded kit (Roche Diagnostics), and AVENIO Tumor Tissue Expanded kit (Roche Diagnostics), according to the manufacturer's instructions. Libraries were sequenced on the Illumina NextSeq 550 or HiSeq X platform (Illumina, San Diego, CA, USA) using paired-end sequencing (2 x 151 cycles). Data was analyzed using the AVENIO ctDNA Analysis Software version 2.0 (Roche Diagnostics).

For variant detection, the following criteria were applied: (1) deduped depth ≥ 1500 × in plasma, depth ≥ 500 × in matched germline DNA, and depth ≥100 × in tumor DNA, (2) variants shared by at least two molecular barcode families, corresponding to two independent mutated alleles, (3) variants not detected in healthy control individuals, (4) variants not detected in germline DNA. All

filtered variants were manually verified using Integrative Genomics Viewer. Variants bioinformatically identified were interpreted based on AMP/ASCO/CAP guidelines, and databases including Exome Aggregation Consortium, Single Nucleotide Polymorphism Database, Catalog of Somatic Mutations in Cancer (COSMIC), and The Cancer Genome Atlas. To define clinically relevant mutations, OncoKB (https://www.oncokb.org/) was used.

Table 1. List of 77 genes targeted in this study

Gene symbol			
ABL1	*DPYD*	*KDR*	*PMS2*
AKT1	*EGFR*	*KEAP1*	*PTCH1*
AKT2	*ERBB2*	*KIT*	*PTEN*
ALK	*ESR1*	*KRAS*	*RAF1*
APC	*EZH2*	*MAP2K1*	*RB1*
AR	*FBXW7*	*MAP2K2*	*RET*
ARAF	*FGFR1*	*MET*	*RNF43*
BRAF	*FGFR2*	*MLH1*	*ROS1*
BRCA1	*FGFR3*	*MSH2*	*SMAD4*
BRCA2	*FLT1*	*MSH6*	*SMO*
CCND1	*FLT3*	*MTOR*	*STK11*
CCND2	*FLT4*	*NF2*	*TERT*
CCND3	*GATA3*	*NFE2L2*	*TP53*
CD274	*GNA11*	*NRAS*	*TSC1*
CDK4	*GNAQ*	*NTRK1*	*TSC2*
CDK6	*GNAS*	*PDCD1LG2*	*UGT1A1*
CDKN2A	*IDH1*	*PDGFRA*	*VHL*
CSF1R	*IDH2*	*PDGFRB*	
CTNNB1	*JAK2*	*PIK3CA*	
DDR2	*JAK3*	*PIK3R1*	

2.6. Statistical analysis

Significant differences in VAF between ctDNA vs. matched tissue, and preoperative ctDNA vs. postoperative ctDNA were evaluated using Wilcoxon signed-rank tests. Correlation between VAF in cfDNA and VAF in the matched leukocytes was assessed using Spearman correlation. *P*-values of less than 0.05 were considered significant. The primary outcome was recurrence free survival (RFS) defined as the interval from the surgery to recurrence. The Kaplan-Meier method was used to construct survival curves, compared with a log-rank test. GraphPad Prism V.6.0 (GraphPad Software, San Diego, California, USA) was used for statistical analyses

3. Results

3.1. Clinicopathologic characteristics

A total of 70 consecutive patients diagnosed with resectable PDAC, who were undergoing curative resection for pancreatic tumor were enrolled from August 2020 through October 2021. Among these patients, 17 (24.3 %) patients were excluded according to the exclusion criteria (pathologically different diagnosis, n = 10; double primary cancers, n = 1; metastasis, n = 3, received neoadjuvant therapy, n = 2; and failed to follow up, n = 1); and the remaining 53 patients were finally evaluable (**Figure 1**). Clinicopathological characteristics of the patients evaluated in this study are summarized in **Table 2A**. Thirty-three (62.3%) patients were male and the median age was 66 y (IQR, 60-72 y). Patients with tumor stages IA, IB, IIA, IIB, and III (8^{th} edition of the AJCC/UICC staging system) accounted for 8 (15.1%), 10 (18.9%), 3 (5.7%), 25 (47.2%), and 7 (13.2%) patients, respectively. The median radiologic tumor size at

baseline was 2.3 cm (IQR, 1.5–3.8): Patients with radiological tumor diameter < 2 cm, 2–4 cm, and ≥ 4 cm were 14 (26.9%), 36 (69.2%), and 2 (3.8%) in number, respectively. Baseline characteristics of a subset of patients with mutation in tissue samples are presented in Table 2B.

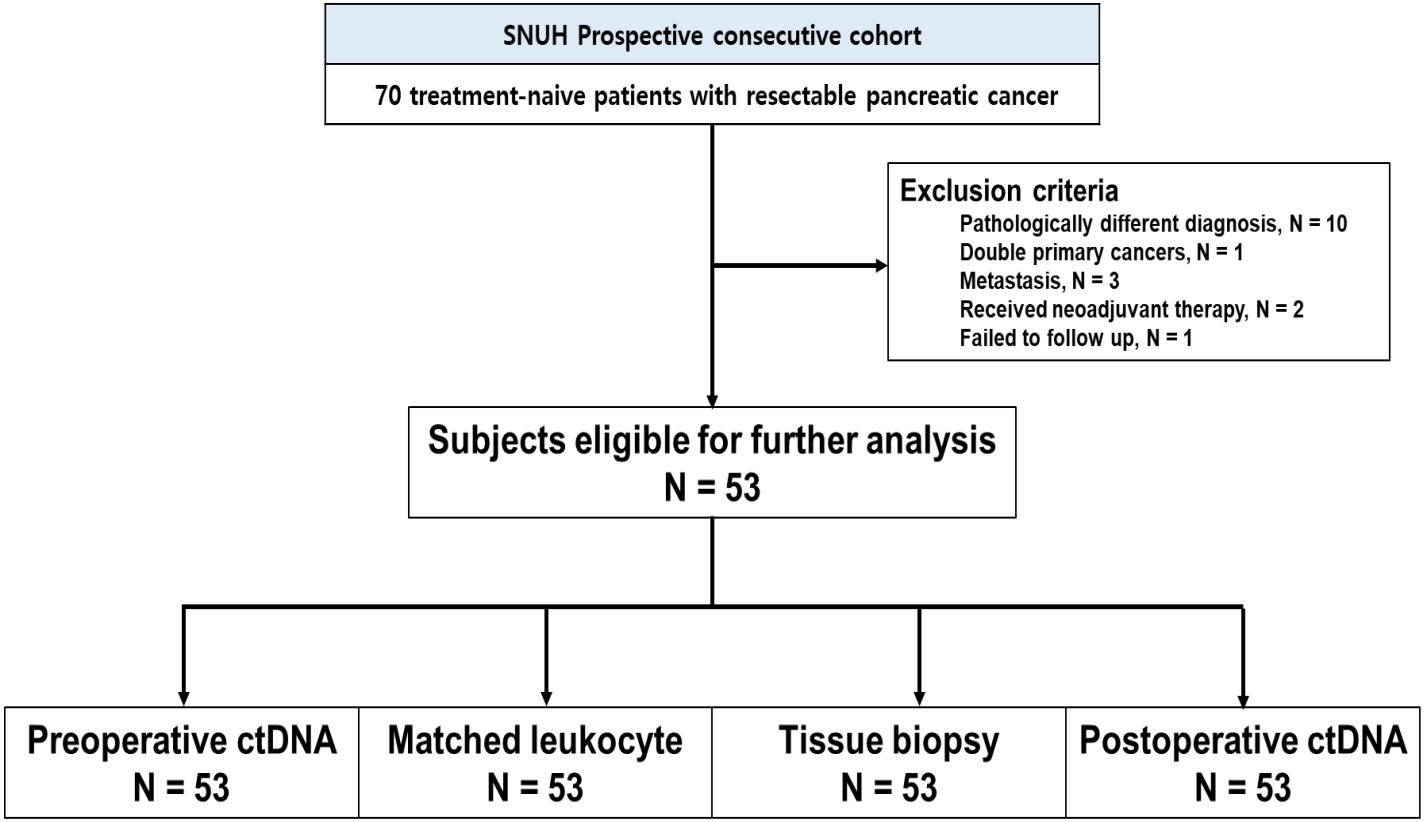


Figure 1. Flow chart for illustrating patient enrollment and analysis.

Table 2A. Baseline characteristics of patients included in this study (N = 53)

	Total (n=53)	Preoperative ctDNA (+) (n=20)	Preoperative ctDNA (−) (n=33)	*P* − value
Age (median, IQR)	66 (60−72)	69 (62–76)	65 (58–70)	
Sex				0.078
Male	33 (62.3)	9 (45.0)	24 (72.7)	
Female	20 (37.7)	11 (55.0)	9 (27.3)	
CA19−9				0.768
< 36 U/mL	17 (32.1)	7 (35.0)	10 (30.3)	
> 36 U/mL	36 (67.9)	13 (65.0)	23 (69.7)	
CEA				0.272
< 5 ng/mL	44 (83.0)	15 (75.0)	29 (87.9)	
> 5 ng/mL	9 (17.0)	5 (25.0)	4 (12.1)	
Recurrence				0.286
Yes	43 (81.1)	18 (90.0)	25 (75.8)	
No	10 (18.9)	2 (10.0)	8 (24.2)	
Location of tumor				0.759
Head	25 (47.2)	12 (60.0)	13 (39.4)	
Head and uncinate process	2 (3.8)	1 (5.0)	1 (3.0)	
Body	9 (17.0)	3 (15.0)	6 (18.2)	
Body and tail	3 (5.7)	0 (0.0)	3 (9.1)	
Tail	10 (18.9)	3 (15.0)	7 (21.2)	
Uncinate process	3 (5.7)	1 (5.0)	2 (6.1)	
Pancreaticoduodenal groove	1 (1.9)	0 (0.0)	1 (3.0)	
Radiologic size of tumor (cm)				1.000
< 2 cm	14 (26.9)	5 (25.0)	9 (28.1)	
≥ 2, < 4 cm	36 (69.2)	14 (70.0)	22 (68.8)	
≥ 4 cm	2 (3.8)	1 (5.0)	1 (3.1)	
T stage				0.837
T1	10 (18.9)	4 (20.0)	6 (18.2)	
T2	35 (66.0)	14 (70.0)	21 (63.6)	
T3	8 (15.1)	2 (10.0)	6 (18.2)	
N status				0.928
N0	21 (39.6)	7 (35.0)	14 (42.4)	
N1	25 (47.2)	10 (50.0)	15 (45.5)	
N2	7 (13.2)	3 (15.0)	4 (12.1)	
Stage (AJCC/UICC) 8th ed				0.646
IA	8 (15.1)	4 (20.0)	4 (12.1)	
IB	10 (18.9)	3 (15.0)	7 (21.2)	
IIA	3 (5.7)	0 (0.0)	3 (9.1)	
IIB	25 (47.2)	10 (50.0)	15 (45.5)	
III	7 (13.2)	3 (15.0)	4 (12.1)	

Operation name				0.528
Whipple's op	6 (11.3)	3 (15.0)	3 (9.1)	
PPPD	26 (49.1)	10 (50.0)	16 (48.5)	
Distal pancreatectomy	16 (30.2)	4 (20.0)	12 (36.4)	
Total pancreatectomy	1 (1.9)	1 (5.0)	0 (0.0)	
Pancreatosplenectomy	4 (7.5)	2 (10.0)	2 (6.1)	
Differentiation degree				0.856
Well	1 (1.9)	1 (5.0)	0 (0.0)	
Moderate	48 (90.6)	17 (85.0)	31 (93.9)	
Moderate to poor	2 (3.8)	1 (5.0)	1 (3.0)	
Poor	2 (3.8)	1 (5.0)	1 (3.0)	
Depth of invasion				0.657
Limited to pancreas	4 (7.5)	1 (5.0)	3 (9.1)	
Beyond pancreas	49 (92.5)	19 (95.0)	30 (90.9)	
Resection margin				0.728
R0	42 (79.2)	15 (75.0)	27 (81.8)	
R1	11 (20.8)	5 (25.0)	6 (18.2)	
Lymphatic invasion				0.270
Absent	24 (45.3)	7 (35.0)	17 (51.5)	
Present	29 (54.7)	13 (65.0)	16 (48.5)	
Vascular invasion				0.779
Absent	30 (56.6)	12 (60.0)	18 (54.5)	
Present	23 (43.4)	8 (40.0)	15 (45.5)	
Perineural invasion				0.695
Absent	8 (15.1)	4 (20.0)	4 (12.1)	
Present	45 (84.9)	16 (80.0)	29 (87.9)	

IQR, interquartile range; n, number

Table 2B. Baseline characteristics of patients with mutation in tissue samples (n = 44)

	Preoperative ctDNA (+)* (n=13)	Preoperative ctDNA (−) (n=31)	*P* − value
Age (median, Q1 – Q3)	68 (61 – 77)	66 (58 – 72)	0.455
Sex			0.313
Male	7 (53.8)	22 (71.0)	
Female	6 (46.2)	9 (29.0)	
CA19−9			
< 36 U/mL	4 (30.8)	10 (32.3)	
> 36 U/mL	9 (69.2)	21 (67.7)	
CEA			
< 5 ng/mL	10 (76.9)	26 (83.9)	
> 5 ng/mL	3 (23.1)	5 (16.1)	
Recurrence			0.402
No	12 (92.3)	24 (77.4)	
Yes	1 (7.7)	7 (22.6)	
Location of tumor			0.806
Head	8 (61.5)	14 (45.2)	
Head and uncinate process	0 (0.0)	2 (6.5)	
Body	1 (7.7)	6 (19.4)	
Body and tail	0 (0.0)	2 (6.5)	
Tail	3 (23.1)	5 (16.1)	
Uncinate process	1 (7.7)	1 (3.2)	
Pancreaticoduodenal groove	0 (0.0)	1 (3.2)	
Radiologic size of tumor (cm)			1.000
< 2 cm	3 (23.1)	7 (23.3)	
≥ 2, < 4 cm	9 (69.2)	22 (73.3)	
≥ 4 cm	1 (7.7)	1 (3.3)	
T stage			0.066
T1	0 (0.0)	9 (29.0)	
T2	11 (84.6)	18 (58.1)	
T3	2 (15.4)	4 (12.9)	
N status			0.225
N0	2 (15.4)	13 (41.9)	
N1	8 (61.5)	14 (45.2)	
N2	3 (23.1)	4 (12.9)	
Stage (AJCC/UICC) 8th ed			0.369
IA	0 (0.0)	7 (22.6)	
IB	2 (15.4)	5 (16.1)	
IIA	0 (0.0)	1 (3.2)	
IIB	8 (61.5)	14 (45.2)	
III	3 (23.1)	4 (12.9)	

Operation name			0.111
Whipple's op	3 (23.1)	3 (9.7)	
PPPD	5 (38.5)	17 (54.8)	
Distal pancreatectomy	2 (15.4)	10 (32.3)	
Total pancreatectomy	1 (7.7)	0 (0.0)	
Pancreatosplenectomy	2 (15.4)	1 (3.2)	
Differentiation degree			0.209
Well	1 (7.7)	0 (0.0)	
Moderate	10 (76.9)	29 (93.5)	
Moderate to poor	1 (7.7)	1 (3.2)	
Poor	1 (7.7)	1 (3.2)	
Depth of invasion			0.544
Limited to pancreas	0 (0.0)	3 (9.7)	
Beyond pancreas	13 (100.0)	28 (90.3)	
Resection margin			0.256
R0	8 (61.5)	25 (80.6)	
R1	5 (38.5)	6 (19.4)	
Lymphatic invasion			0.335
Absent	4 (30.8)	15 (48.4)	
Present	9 (69.2)	16 (51.6)	
Vascular invasion			1.000
Absent	7 (53.8)	15 (48.4)	
Present	6 (46.2)	16 (51.6)	
Perineural invasion			1.000
Absent	2 (15.4)	5 (16.1)	
Present	11 (84.6)	26 (83.9)	

*Tissue-specific ctDNA (+)

3.2. Mutational landscape of preoperative ctDNA

We detected genomic alterations of preoperative cfDNA in 27 patients (50.9 %) at an average of 1.0 alterations per patient with a median VAF of 0.10%. After excluding variants with uncertain significance, 20 patients were positive for preoperative oncogenic ctDNA mutations with a detection rate of 37.7 % (20/53) (**Figure 2**). *TP53* (n = 12, 22.6%) was most frequently identified, followed by *KRAS* (n=10, 18.9%), *GNAS* (n=6, 11.3%), *SMAD4* (n=1, 1.9%), *PIK3CA* (n=1, 1.9%), and *CDKN2A* (n=1, 1.9%). Notably, median VAF of these ctDNA mutations was 0.09% (IQR, 0.04%–0.16%), approximately 40−fold lower than previously reported in metastatic PDAC. *KRAS* mutations clustered in codon 12: G12D and G12V were detected in 7 (70.0%) and 3 (30.0%) patients, respectively.

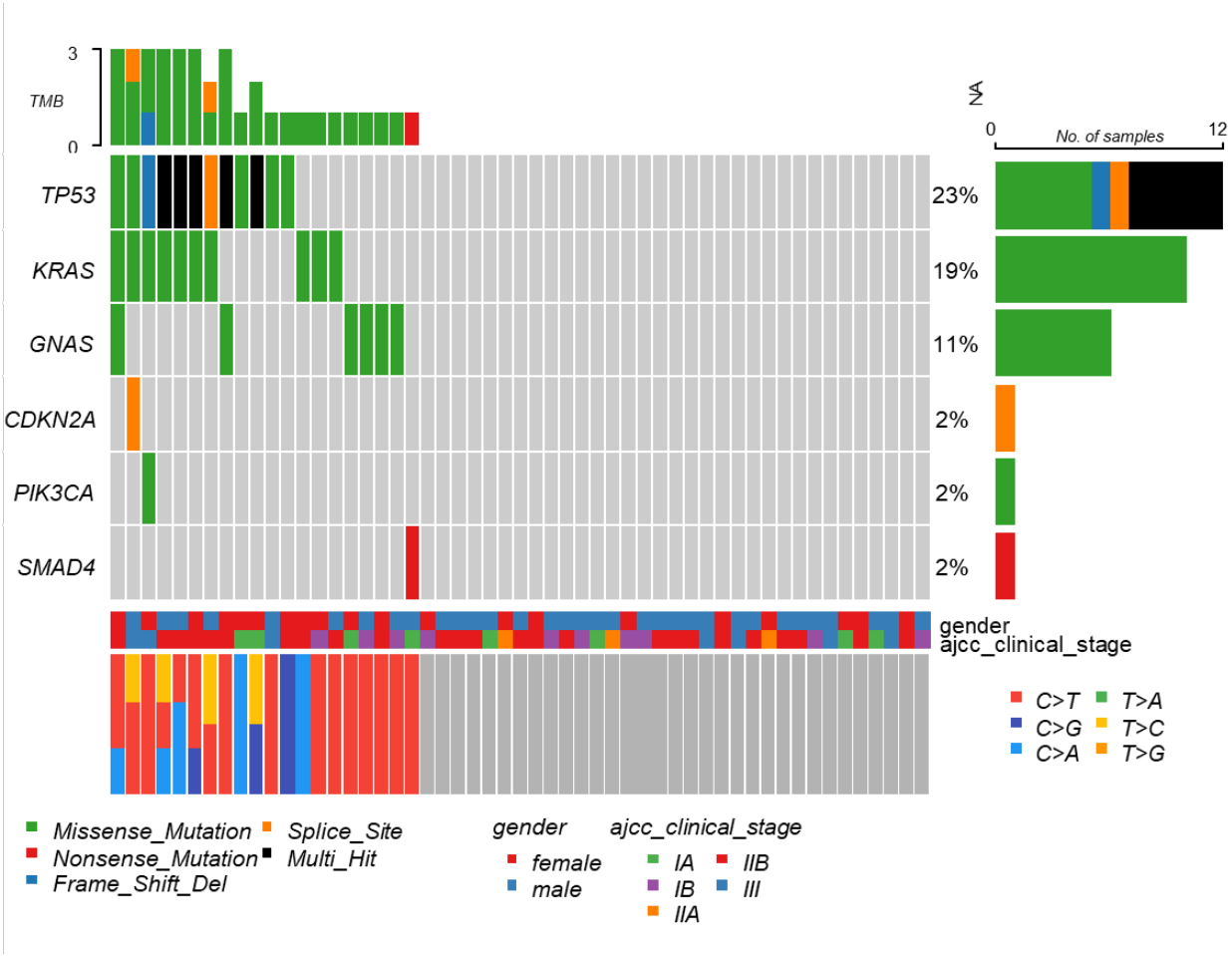


Figure 2. Mutational landscape of preoperative ctDNA.

3.3. Comparison of ctDNA and tissue analysis

All of the 53 patients had paired tumor tissue samples available for ctDNA/tissue-matched analysis. In total, 34.0% (18/53) of patients had clinically relevant mutations detected in both plasma and tissue, 13.2% (7/53) were detected exclusively in tissue analysis, and 3.8% (2/53) were detected exclusively in ctDNA anlaysis. (**Figure 3**).

Next, we attempted to compare the prevalence of mutated genes between ctDNA and tissue samples. *KRAS* and *TP53* were most frequently mutated in both samples, while *GNAS* mutations were more frequently detected in ctDNA than in tissue samples (**Figure 4**). Detailed results in terms of individual mutations are shown in **Table 3**. A total of 24 mutations (*KRAS*, n = 10; *TP53*, n = 11; *CDKN2A*, n = 1; *PIK3CA*, n = 1; *GNAS*, n = 1) were detected in both plasma and tissue samples. Interestingly, plasma iDES-enhanced CAPP-Seq allowed the detection of 12 additional oncogenic mutations in ctDNA that were not detected in tissue samples (*TP53*, n = 6; *GNAS*, n = 5;

SMAD4, n = 1). VAF was significantly lower for ctDNA (median, 0.08%; IQR, 0.04%–0.37%) than for tissue (median, 12.99%, IQR, 7.65%–24.96%) ($P < 0.001$) (**Figure 5**).

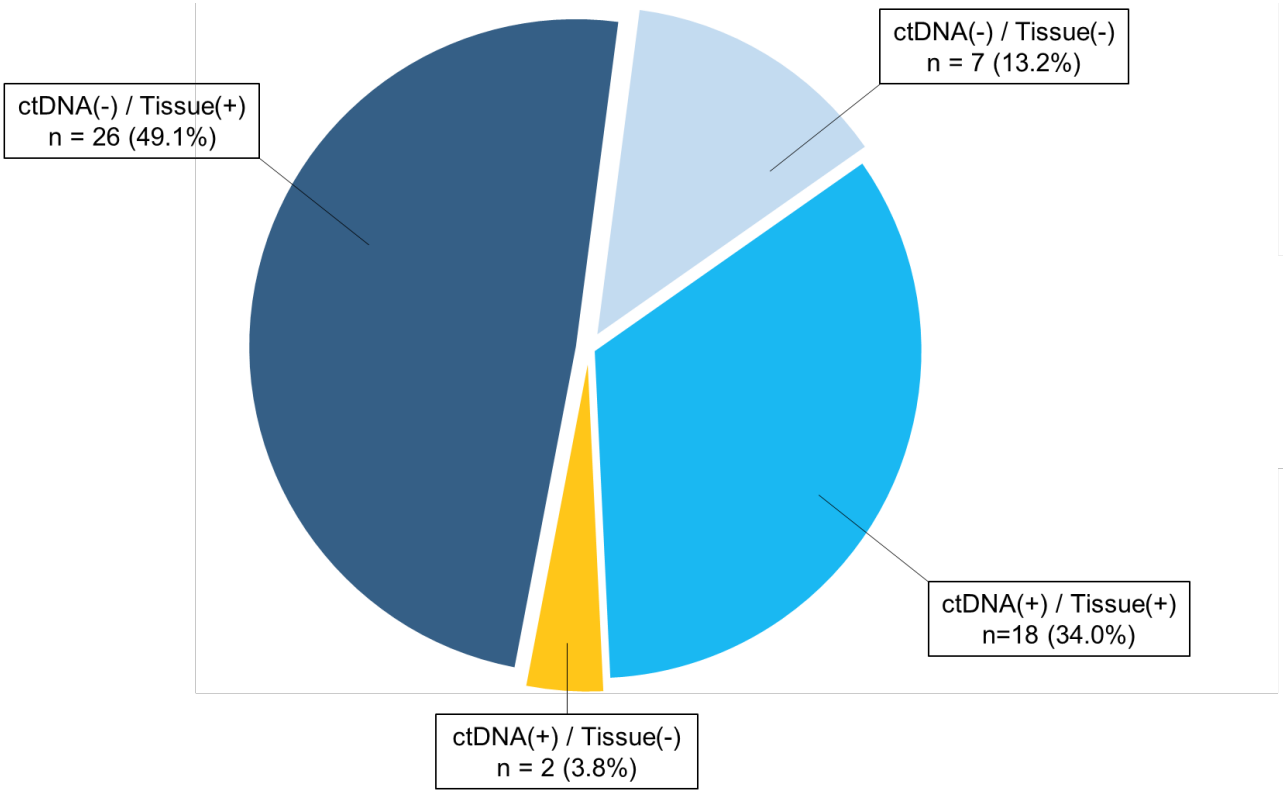


Figure 3. Mutation detection in patients with ctDNA and tissue.

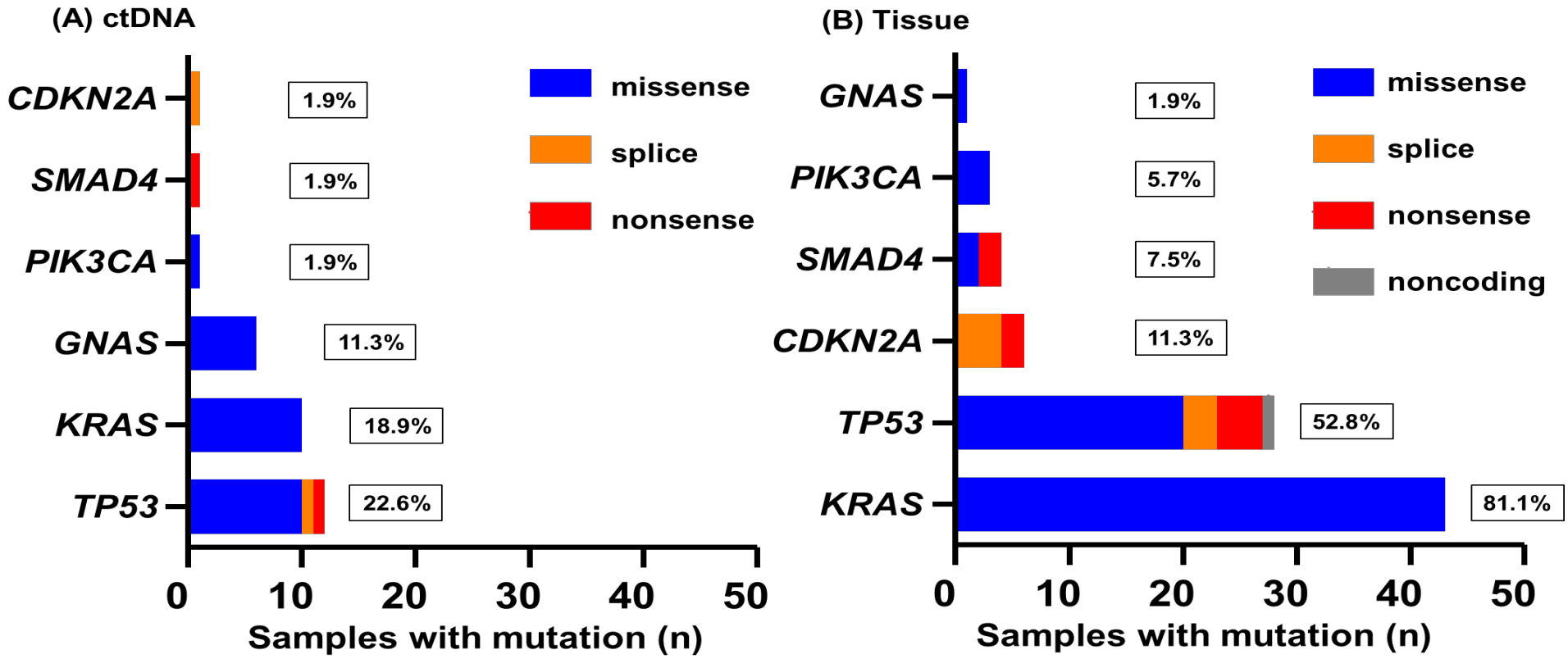


Figure 4. Frequency of mutated genes identified in the (A) ctDNA and (B) tumor tissue of patients with resectable PDAC.

Table 3. Comparison of genetic alterations detected in ctDNA and tissue samples

Case ID	Input DNA (ng)		Genetic alteration			VAF	
	Tissue	cfDNA	Gene	Nucleotide	Amino acid	Tissue	ctDNA
Concordant positive							
2	200.0	26.3	*KRAS*	c.35G>T	p.G12V	4.36%	0.04%
10	200.0	28.6	*TP53*	c.817C>T	p.R273C	9.33%	0.04%
11	200.0	22.4	*KRAS*	c.35G>A	p.G12D	24.87%	0.36%
11	200.0	22.4	*TP53*	c.818G>T	p.R273L	13.53%	0.03%
12	200.0	32.2	*KRAS*	c.35G>T	p.G12V	39.49%	0.05%
12	200.0	32.2	*TP53*	c.743G>A	p.R248Q	32.03%	0.07%
20	200.0	32.1	*KRAS*	c.35G>A	p.G12D	25.21%	0.07%
23	200.0	21.7	*KRAS*	c.35G>A	p.G12D	10.20%	0.02%
33	200.0	50.0	*KRAS*	c.35G>T	p.G12V	33.62%	0.02%
33	200.0	50.0	*TP53*	c.524G>T	p.R175L	0.14%	0.23%
33	200.0	50.0	*TP53*	c.517G>A	p.V173M	37.67%	0.04%
41	200.0	14.3	*TP53*	c.743G>A	p.R248Q	9.04%	0.38%
41	200.0	14.3	*KRAS*	c.35G>A	p.G12D	18.10%	0.11%
41	200.0	14.3	*TP53*	c.380C>G	p.S127C	9.82%	0.09%
51	200.0	26.5	*TP53*	c.711G>A	p.M237I	5.68%	0.03%
59	200.0	50.0	*KRAS*	c.35G>A	p.G12D	24.23%	0.60%
59	200.0	50.0	*PIK3CA*	c.1633G>A	p.E545K	5.84%	0.02%
59	200.0	50.0	*TP53*	c.873_874delinsTT	p.K292X	35.14%	0.71%
60	200.0	30.0	*KRAS*	c.35G>A	p.G12D	7.95%	0.42%
60	200.0	30.0	*TP53*	c.993+2T>C		6.03%	0.20%
64	200.0	26.0	*CDKN2A*	c.151-1G>A		5.84%	0.15%
64	200.0	26.0	*KRAS*	c.35G>A	p.G12D	12.45%	0.44%
64	200.0	26.0	*TP53*	c.659A>G	p.Y220C	18.05%	0.54%
69	200.0	24.2	*GNAS*	c.602G>A	p.R201H	6.73%	0.04%
Concordant negative							
3	200.0	6.3				ND	ND
8	200.0	44.6				ND	ND
37	200.0	22.1				ND	ND

39	200.0	16.9				ND	ND
53	200.0	26.9				ND	ND
63	200.0	37.0				ND	ND
68	200.0	39.9				ND	ND
Disconcordant result							
10	200.0	28.6	*GNAS*	c.601C>T	p.R201C	ND	0.15%
10	200.0	28.6	*TP53*	c.713G>A	p.C238Y	ND	0.10%
11	200.0	22.4	*TP53*	c.614A>G	p.Y205C	ND	0.12%
12	200.0	32.2	*GNAS*	c.602G>A	p.R201H	ND	0.07%
17	200.0	40.3	*GNAS*	c.602G>A	p.R201H	ND	0.05%
30	200.0	50.0	*GNAS*	c.602G>A	p.R201H	ND	0.12%
31	200.0	45.0	*TP53*	c.713G>T	p.C238F	ND	0.04%
38	200.0	27.7	*TP53*	c.536A>G	p.H179R	ND	0.08%
38	200.0	27.7	*TP53*	c.388C>G	p.L130V	ND	0.10%
42	200.0	48.5	*GNAS*	c.601C>T	p.R201C	ND	0.15%
45	200.0	34.9	*SMAD4*	c.1333C>T	p.R445X	ND	0.08%
58	200.0	16.7	*TP53*	c.472C>G	p.R158G	ND	0.07%
1	200.0	10.5	*KRAS*	c.35G>A	p.G12D	7.89%	ND
1	200.0	10.5	*TP53*	c.577C>T	p.H193Y	16.21%	ND
4	200.0	23.8	*KRAS*	c.35G>T	p.G12V	3.25%	ND
4	200.0	23.8	*TP53*	c.578A>G	p.H193R	8.95%	ND
5	200.0	10.5	*CDKN2A*	c.238C>T	p.R80X	27.72%	ND
5	200.0	10.5	*KRAS*	c.35G>A	p.G12D	13.95%	ND
7	200.0	26.5	*KRAS*	c.35G>T	p.G12V	15.59%	ND
9	200.0	16.2	*CDKN2A*	c.151-2A>G		25.39%	ND
9	200.0	16.2	*KRAS*	c.35G>A	p.G12D	13.33%	ND
9	200.0	16.2	*PIK3CA*	c.3140A>G	p.H1047R	13.36%	ND
10	200.0	28.6	*KRAS*	c.34G>T	p.G12C	8.84%	ND
14	200.0	9.9	*KRAS*	c.35G>A	p.G12D	5.20%	ND
15	200.0	31.1	*KRAS*	c.34G>C	p.G12R	13.06%	ND
15	200.0	31.1	*TP53*	c.1024C>T	p.R342X	21.51%	ND
16	200.0	31.4	*SMAD4*	c.1157G>A	p.G386D	10.95%	ND
16	200.0	31.4	*TP53*	c.772G>T	p.E258X	13.74%	ND
17	200.0	40.3	*KRAS*	c.35G>T	p.G12V	4.22%	ND

17	200.0	40.3	*PIK3CA*	c.1358A>G	p.E453G	3.71%	ND
20	200.0	32.1	*TP53*	c.994−1G>C		31.65%	ND
21	200.0	5.2	*KRAS*	c.35G>A	p.G12D	14.52%	ND
21	200.0	5.2	*TP53*	c.742C>T	p.R248W	16.76%	ND
23	200.0	21.7	*TP53*	c.375+5G>A		11.73%	ND
26	200.0	19.7	*CDKN2A*	c.132_133delinsAA	p.Y44X	16.10%	ND
26	200.0	19.7	*KRAS*	c.34G>C	p.G12R	15.21%	ND
26	200.0	19.7	*SMAD4*	c.1194G>A	p.E398X	15.27%	ND
26	200.0	19.7	*SMAD4*	c.1571_1572delinsTT	p.E524F	3.53%	ND
26	200.0	19.7	*TP53*	c.797G>A	p.G266E	17.99%	ND
27	200.0	19.2	*KRAS*	c.35G>A	p.G12D	7.72%	ND
28	200.0	19.6	*KRAS*	c.35G>T	p.G12V	4.59%	ND
28	200.0	19.6	*TP53*	c.785G>T	p.G262V	1.77%	ND
30	200.0	50.0	*KRAS*	c.35G>T	p.G12V	7.14%	ND
30	200.0	50.0	*TP53*	c.422G>A	p.C141Y	10.87%	ND
31	200.0	45.0	*KRAS*	c.35G>A	p.G12D	3.45%	ND
31	200.0	45.0	*TP53*	c.712T>G	p.C238G	3.11%	ND
32	200.0	16.3	*KRAS*	c.35G>T	p.G12V	6.83%	ND
32	200.0	16.3	*TP53*	c.394A>G	p.K132E	6.41%	ND
35	200.0	32.9	*KRAS*	c.35G>A	p.G12D	7.86%	ND
36	200.0	26.4	*KRAS*	c.34G>C	p.G12R	17.88%	ND
38	200.0	27.7	*KRAS*	c.35G>A	p.G12D	1.72%	ND
42	200.0	48.5	*KRAS*	c.35G>T	p.G12V	4.39%	ND
43	200.0	50.0	*KRAS*	c.35G>T	p.G12V	26.27%	ND
43	200.0	50.0	*TP53*	c.334−1T>G		33.92%	ND
46	200.0	44.2	*KRAS*	c.34G>C	p.G12R	5.98%	ND
47	200.0	50.0	*KRAS*	c.35G>T	p.G12V	1.50%	ND
48	200.0	35.1	*KRAS*	c.35G>T	p.G12V	14.69%	ND
49	200.0	19.3	*KRAS*	c.35G>A	p.G12D	18.44%	ND
49	200.0	19.3	*TP53*	c.524G>A	p.R175H	20.19%	ND
51	200.0	26.5	*CDKN2A*	c.150+1G>A		4.08%	ND
51	200.0	26.5	*KRAS*	c.35G>A	p.G12D	4.56%	ND
52	200.0	50.0	*KRAS*	c.35G>A	p.G12D	6.74%	ND

52	200.0	50.0	*TP53*	c.949C>T	p.Q317X	5.60%	ND
54	200.0	18.4	*KRAS*	c.35G>A	p.G12D	8.23%	ND
54	200.0	18.4	*TP53*	c.659A>G	p.Y220C	10.32 %	ND
61	200.0	32.1	*CDKN2A*	c.151-1G>T		17.32 %	ND
61	200.0	32.1	*KRAS*	c.35G>T	p.G12V	10.14 %	ND
61	200.0	32.1	*SMAD4*	c.247C>T	p.Q83X	12.63 %	ND
65	200.0	21.2	*KRAS*	c.35G>A	p.G12D	1.78%	ND
67	200.0	45.3	*KRAS*	c.34G>C	p.G12R	27.03 %	ND
67	200.0	45.3	*TP53*	c.712T>C	p.C238R	19.76 %	ND
69	200.0	24.2	*KRAS*	c.35G>A	p.G12D	6.21%	ND
69	200.0	24.2	*TP53*	c.797G>A	p.G266E	9.51%	ND
70	200.0	50.0	*KRAS*	c.35G>A	p.G12D	21.63 %	ND
70	200.0	50.0	*TP53*	c.814G>A	p.V272M	14.26 %	ND

cfDNA, cell-free DNA; ctDNA, circulating-tumor DNA; ND, not detected; VAF, variant allele frequency

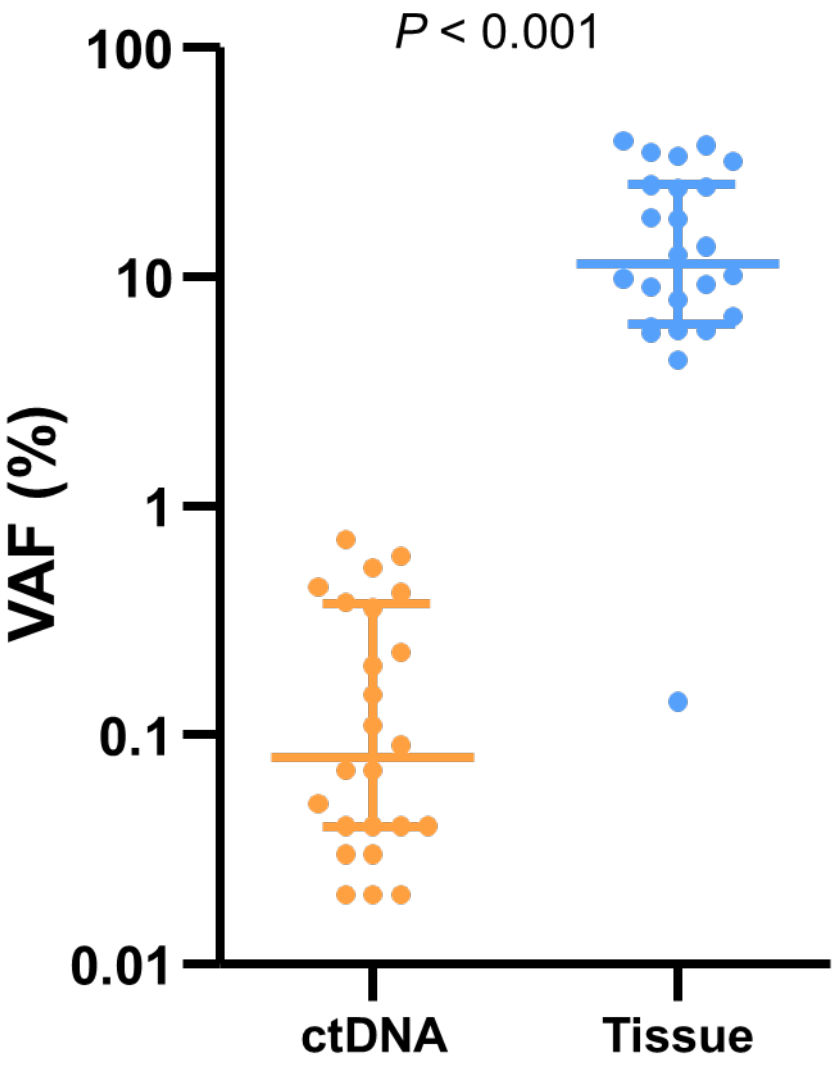


Figure 5. Variant allele frequency (VAF) of detected mutations in paired ctDNA and tissue samples.

3.4. Pre-operative vs. post-operative ctDNA

We then analyzed matched pre- and post-operative plasma samples. Among 20 (37.7% of total patients) patients with detectable ctDNA before surgery, 12 (22.6% of total patients) patients were still detectable after curative resection (median VAF 0.04%, IQR 0.03%-0.06%), which implies 15.1% decrease in the proportion of patients with detectable ctDNA after curative resection. We observed that the VAF in each patient was significantly decreased in postoperative plasma (preoperative ctDNA, median 0.08%, IQR 0.04%-0.15%; postoperative ctDNA, median 0.00%, IQR 0.00%-0.03%; $P < 0.001$) (**Figure 6**). Next, we evaluated the association between change of ctDNA after surgery and prognosis of patients. Among 20 patients with detectable ctDNA before surgery, the risk of recurrence at 1 year tended to be higher in patients with still detectable ctDNA after surgery than in patients with negative conversion of ctDNA after surgery (48.6% vs. 25.0%, $P = 0.064$) (**Figure 7**).

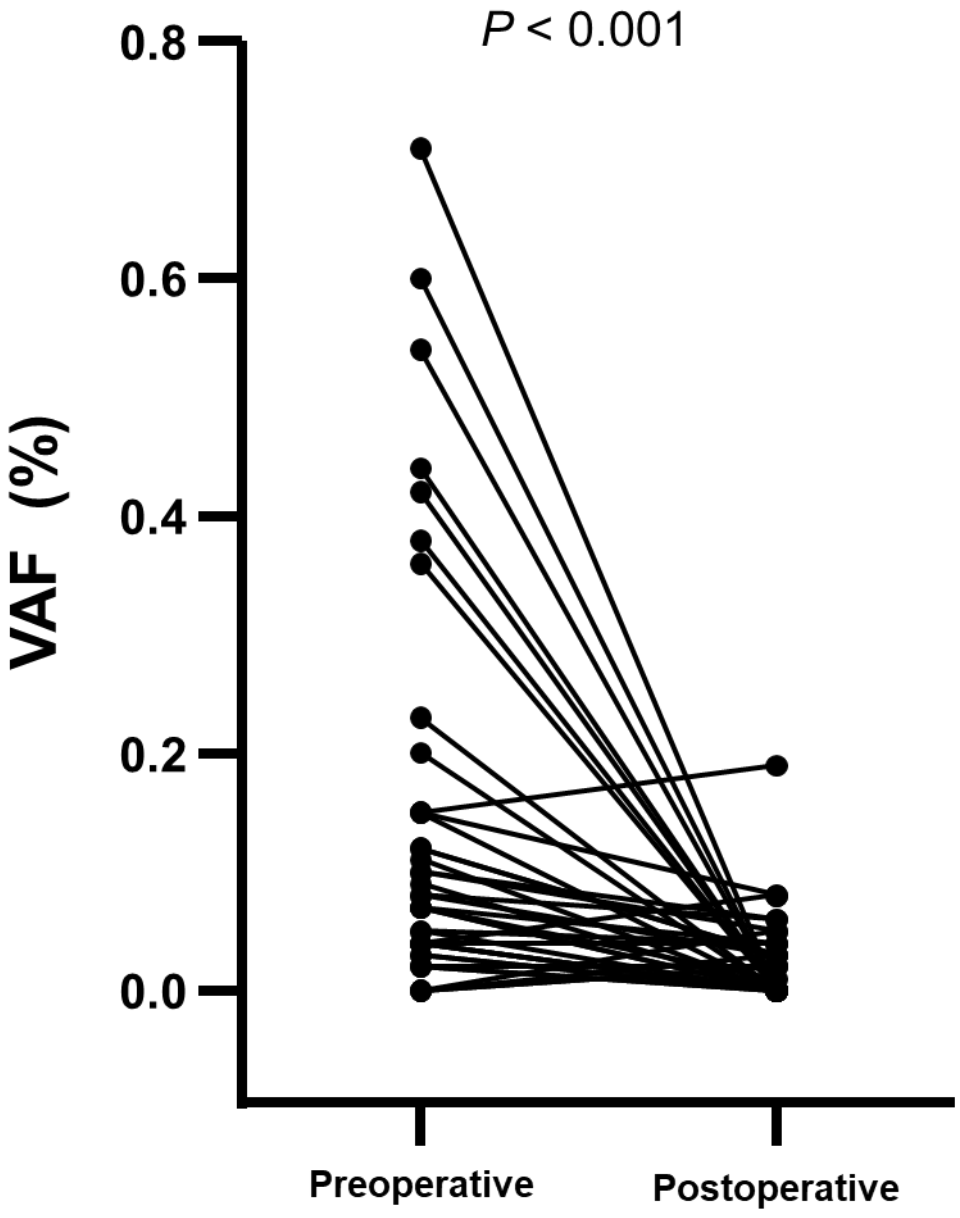


Figure 6. Variant allele frequency (VAF) of detected mutations in paired preoperative and postoperative ctDNA.

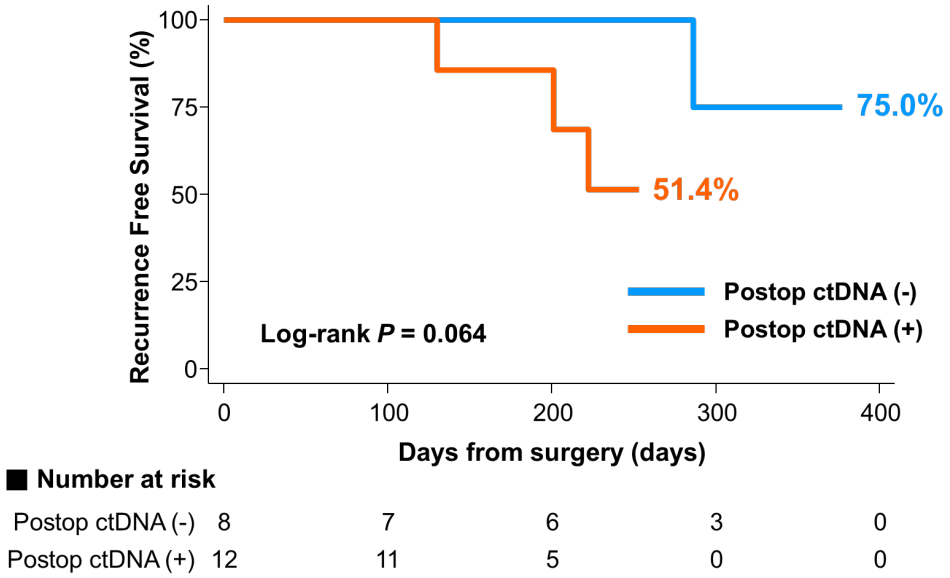


Figure 7. Kaplan–Meier estimates of recurrence free survival (RFS) for patient with detectable ctDNA before surgery, stratified by post-operative ctDNA changes: postoperative ctDNA negative vs. postoperative ctDNA positive.

3.5. False positive results due to clonal hematopoiesis

We further investigated the presence of somatic mutations in the matched leukocyte sequencing results, which revealed that 13 cfDNA variants in 13 (24.5 %) patients were detected in matched leukocytes. Six variants were detected in canonical clonal hematopoiesis genes (*JAK2, GNAS, KRAS,* and *TP53*) including *JAK2* V617F mutation (**Figure 8A**). The VAFs detected in leukocytes were significantly correlated with VAFs of the matched cfDNA variants (**Figure 8B**). As clonal hematopoiesis is related to age, we examined the prevalence of clonal hematopoiesis of indeterminate potential (CHIP) mutations in canonical clonal hematopoiesis genes detected in cfDNA in each age group. All CHIP mutations were identified in the age group of over 60 years (**Figure 8C**).

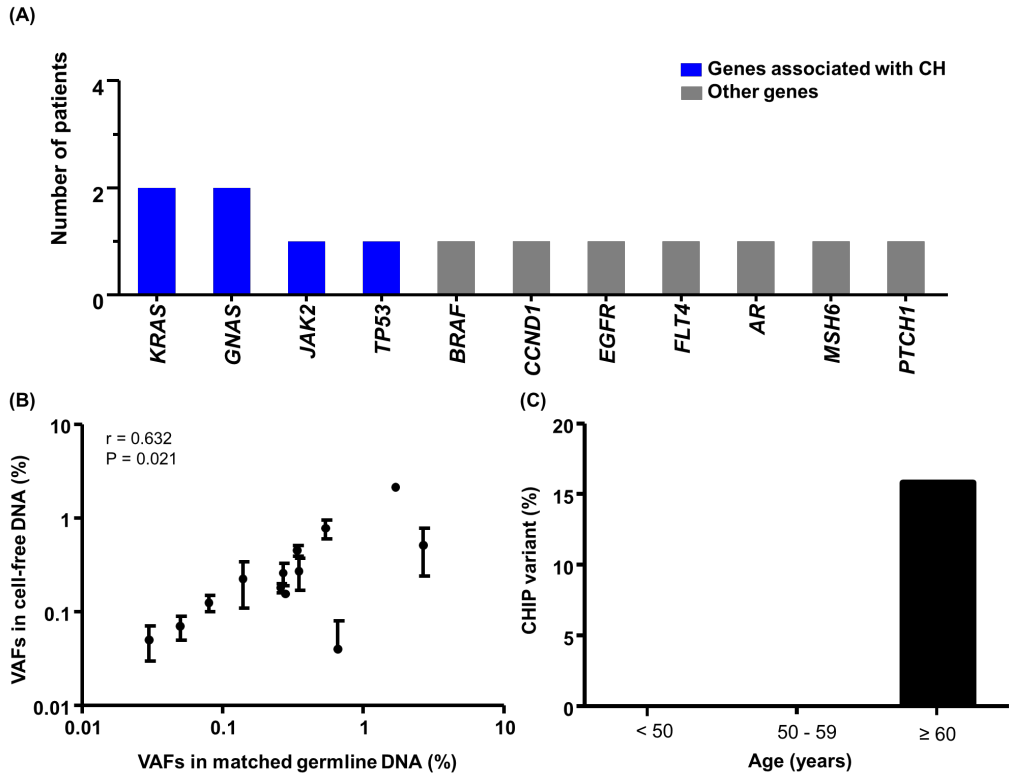


Figure 8. Variants detected in matched leukocyte DNA. (A) Genes mutated in matched leukocyte DNA. (B) Correlation between variant allele frequency (VAF) in cell-free DNA (cfDNA) and VAF in matched leukocyte DNA. The mean value of each VAF of pre-operative cfDNA and post-operative cfDNA was plotted as a dot, and error bars representing each value of pre-operative cfDNA and post-operative cfDNA were represented. (C) The prevalence of clonal hematopoiesis of indeterminate potential (CHIP) mutations in *JAK2, TP53, KRAS,* and *GNAS* detected in cfDNA in each age group. The prevalence of variants in canonical clonal hematopoiesis genes was 0.0 %, 0.0 %, and 15.8 % in patients of < 50 years, 50–59 years, and ≥60 years, respectively.

4. Discussion

This prospective consecutive cohort study applied iDES-enhanced CAPP-Seq, an optimized NGS technology, for preoperative and postoperative ctDNA analysis using triple-matched samples from 53 patients in the early stage of resectable PDAC. This iDES-enhanced CAPP-Seq approach allowed us to detect preoperative ctDNA in 37.7% of treatment-naïve patients with resectable PDAC and detect low level of ctDNA with oncogenic mutations in patients with resectable PDAC (preoperative VAF, 0.02%–0.71%; postoperative VAF, 0.01%–0.19%). The detection rate of ctDNA observed in our study was comparable to that of previous studies in which the majority of advanced stage patients with PDAC were included, but the detectable VAF of ctDNA in our study was much lower than that in previous studies (**Supplementary Table 1**, **Supplementary Figure 1**). As baseline ctDNA correlates to the tumor burden of patients, our findings imply that highly selective resectable PDAC sheds a minute amount of ctDNA; thus, highly sensitive

optimized NGS technology is necessary to sensitively detect ctDNA in these patients.

Technically, standard NGS detects a wide range of mutation, but sensitivity is limited to a VAF of 2–5% by background errors that is generated during library preparation and sequencing and masks the detection of true positive variants. The iDES–enhanced CAPP–Seq incorporates in silico removal of artifacts detected in sequencing data, the majority of which are G>T followed by C>T and G>A. Moreover, two kinds of combined molecular barcodes, insert barcodes and index barcodes, are integrated with this platform, which enable us to reduce background error rates and maximize sensitivity.

One of our important findings in this study is the prognostic value of ctDNA change after surgery. Patients with detectable ctDNA after surgery tended to show higher risk of recurrence. In resectable PDAC, the presence of ctDNA after surgery may suggest micrometastasis of disease.

We observed several disconcordant results between tissue and

ctDNA analysis. A total of 13.2 % of patients had one or more mutations detected in tissue biopsy, but no tumor-derived mutations were found in ctDNA analysis. These disconcordant cases with negative ctDNA might be probably due to low tumor burden in early-stage cancer and intrinsic tumor biology of PDAC (i.e., stromagenic microenvironment). In contrast, we noticed that in another 3.8% of individuals, mutations were identified exclusively in cfDNA. Twelve additional oncogenic mutations in *GNAS, TP53*, and *SMAD4* were exclusively detected in cfDNA, probably due to intratumoral heterogeneity resulting in uneven distribution of subclones. These findings suggest that ctDNA may provide additional information on the molecular profile of tumor in an individual patient in addition to tumor biopsies. Of course, failure to detect mutation in ctDNA assay requires testing of tumor tissue.

We found that cfDNA from 24.5% of patients had features compatible with clonal hematopoiesis, and variants in canonical clonal hematopoiesis genes were notably observed in patients over 60 years

of age. These CHIP mutations frequently overlap with genes recurrently mutated in solid cancers, which may result in false positives in ctDNA analysis and complicate the interpretation results. Many previous studies reported that CHIP mutations remain constant during treatment, which has no prognostic consequence in patients with CHIP mutations. Our findings suggest that clinically relevant ctDNA mutation should be relevantly interpreted by filtering out CHIP mutations using sequencing of matched leukocytes. In addition, several variants of unknown significance in other driver genes of solid tumors (*BRAF, CCND1, EGFR, FLT4, AR, MSH6*, and *PTCH1*), were detected in matched leukocyte. These variants could be passenger variants randomly acquired during clonal hematopoiesis or somatic mosaicism. Additionally, previous history of solid malignancy was observed more frequently in patients with a CHIP mutation (23.0%, 3/13) than in patients without a CHIP mutation (7.5%, 3/40). Further studies would be required to clarify the relationship between clonal hematopoiesis and solid malignancy. Moreover, VAF of CHIP

mutations in cfDNA was significantly correlated with VAF detected in leukocytes, which underscores the importance of sequencing matched leukocytes to a depth comparable to cfDNA to avoid misunderstandings.

In our cohort, the *KRAS* and *TP53* mutation was detected in 81.1% and 52.8% of tissue samples, which is comparable to the results from a previous reports. The detection rate of *CDKN2A* and *SMAD4* was 11.3%, and 7.5%, respectively, which is lower than the results from a previous reports. It could be due to a technological limitation of our iDES-enhanced CAPP-Seq approach. Large deletion in *CDKN2A*, frequently observed in PDAC is technologically limited to be detected by our sequencing platform. Whole-exome sequencing and whole genome sequencing may be alternative approaches to increase detection rate of *CDKN2A* deletion. The lower detection rate of *CDKN2A* and *SMAD4* also could be due to intratumoral heterogeneity.

The major limitation of our study was relatively small number

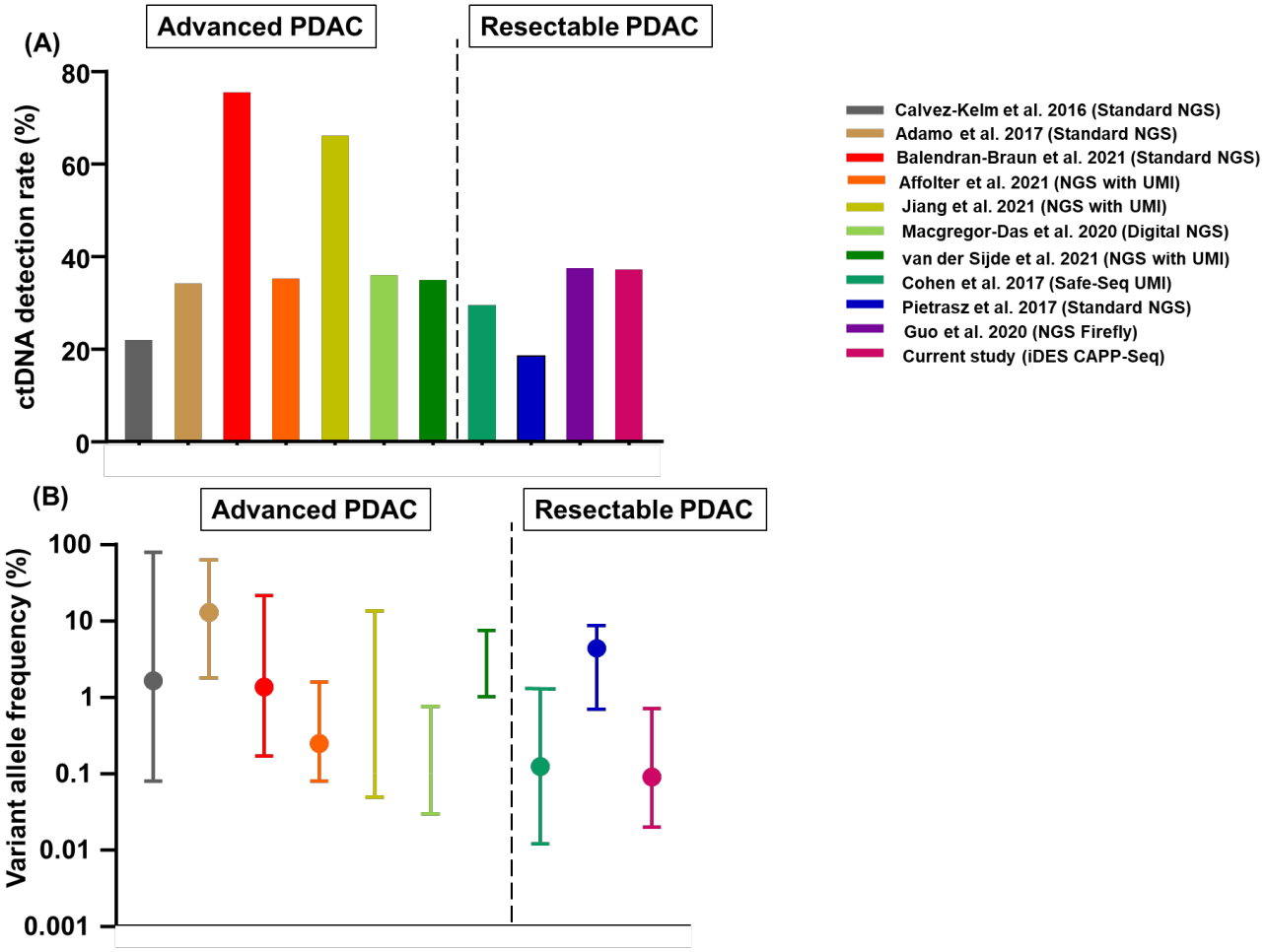
of patients, prospectively enrolled for a limited period of time, which that may limit statistical significance. Despite this limitation, the strengths of this study are analysis of triple-matched samples from each consecutive patients with PDAC, prospectively enrolled.

In summary, a fraction of preoperative ctDNA in resectable PDAC patients was very low at 0.09%, which could be detected and molecularly profiled using highly sensitive NGS technology. Moreover, paired sequencing of matched leukocytes may be required to accurately detect clinically relevant ctDNA. An optimized NGS approach may add value beyond tissue analysis by highly sensitive detection of ctDNA in resectable PDAC, which could have a strong impact on the diagnosis, monitoring, and treatment of pancreatic cancer.

Supplementary Table 1. Summary of studies of ctDNA analysis using NGS in PDAC

Year	Author	N	Stage	Technology	Detection rate (%)	VAF (median, range)	Tissue	Leukocyte
2016	Calvez-Kelm et al.	40	Local (n=6, 15%) Regional (n=17, 42.5%) Systemic (n=16, 40%) NA (n=1, 2.5%)	standard NGS	22.5	1.64% (0.08%-79.0%)	Matched	Not matched
2017	Adamo et al.	26	Resectable (n=6, 23.0%) Non-resectable (n=5, 19.2%) Metastatic (n=15, 57.7%)	standard NGS	34.7	13.0% (1.8%-62.9%)	Matched	Not matched
2017	Cohen et al.	221	IA (n=11, 5%) IB (n=18, 8%) IIA (n=22, 10%) IIB (n=170, 77%)	Safe-SeqS	30.0	0.13% (0.01%-1.31%)	Matched	Not matched
2017	Pietrasz et al.	135	Resectable (n= 31; 23%) Locally advanced (n=36; 27%) Metastatic (n=68; 50%)	standard NGS	19.4	4.4% (0.7%-8.7%)	Not matched	Not matched
2021	F van der Sijde et al.	48	Resectable (n=18, 37.5%) Locally advanced (n=16, 33.3%)	NGS with UMI	35.4	1.02-7.56%	Not matched	Not matched

			Metastatic (n=14, 29.2%)					
2021	Balendran-Braun et al.	21	Locally advanced (n=3, 14.3%) Metastatic (n=18, 85.7%)	standard NGS	76.0	1.37% (0.17%-21.73%)	Matched	Not matched
2021	K.E. Affolter et al.	14	IIB (n=4, 28.6%) III (n=8, 57.1%) IV (n=2, 14.3%)	NGS with UMI	35.7	0.25% (0.08%-1.59%)	Matched	Not matched
2020	Jiang et al.	27	I (n=13, 48.15%) II (n=9, 33.34%) IV (n=5, 18.52%))	NGS with UMI	66.7	0.05-13.64%	Matched	Not matched
2020	Guo et al.	113	IA (n=15, 13.3%) IB (n=34, 30.0%) IIA (n=14, 12.4%) IIB (n=43, 38.1%) III (n=7, 6.2%)	Firefly NGS	38.1	NA	Matched	Not matched
2020	Macgregor-Das et al.	67	IA (n=5, 7.5%) IB (n=9, 13.4%) IIA (n=1, 1.5%) IIB (n=22, 32.8%) III (n=11, 16.4%) IV (n=19, 28.4%)	Digital NGS	36.5	0.03%-0.75%	Not matched	Not matched



Supplementary Figure 1. Detection rate and variant allele frequency of ctDNA in recent PDAC studies.

References

1. Detection of Circulating Tumor Cells: A Potential Prognostic Factor. The breast journal 1999; 5(6): 351-3.

2. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. Nature reviews Clinical oncology 2010; 7(3): 163-72.

3. Moletta L, Serafini S, Valmasoni M, Pierobon ES, Ponzoni A, Sperti C. Surgery for Recurrent Pancreatic Cancer: Is It Effective? Cancers 2019; 11(7).

4. Evans DB. What Makes a Pancreatic Cancer Resectable? American Society of Clinical Oncology educational book American Society of Clinical Oncology Meeting 2018; 38: 300-5.

5. Merker JD, Oxnard GR, Compton C, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2018; 36(16): 1631-41.

6. Siravegna G, Mussolin B, Venesio T, et al. How liquid biopsies can change clinical practice in oncology. Annals of oncology : official journal of the European Society for Medical Oncology 2019; 30(10):

1580-90.

7. Lee JS, Park SS, Lee YK, Norton JA, Jeffrey SS. Liquid biopsy in pancreatic ductal adenocarcinoma: current status of circulating tumor cells and circulating tumor DNA. Molecular oncology 2019; 13(8): 1623-50.

8. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Science translational medicine 2017; 9(403).

9. Newman AM, Lovejoy AF, Klass DM, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. Nature biotechnology 2016; 34(5): 547-55.

10. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. The Journal of molecular diagnostics : JMD 2017; 19(1): 4-23.

11. Strijker M, Soer EC, de Pastena M, et al. Circulating tumor DNA quantity is related to tumor volume and both predict survival in metastatic pancreatic ductal adenocarcinoma. International journal of

cancer 2020; 146(5): 1445-56.

12. Calvez-Kelm FL, Foll M, Wozniak MB, et al. KRAS mutations in blood circulating cell-free DNA: a pancreatic cancer case-control. Oncotarget 2016.

13. Adamo P, Cowley CM, Neal CP, et al. Profiling tumour heterogeneity through circulating tumour DNA in patients with pancreatic cancer. Oncotarget 2017; 8(50): 87221-33.

14. Xiong A, Ma N, Wei G, Li C, Li K, Wang B. Genomic alterations in tumor tissue and ctDNA from Chinese pancreatic cancer patients. American journal of cancer research 2021; 11(9): 4551-67.

15. van der Sijde F, Azmani Z, Besselink MG, et al. Circulating TP53 mutations are associated with early tumor progression and poor survival in pancreatic cancer patients treated with FOLFIRINOX. Ther Adv Med Oncol 2021; 13: 17588359211033704.

16. Balendran-Braun S, Kieler M, Liebmann-Reindl S, et al. Bead-Based Isolation of Circulating Tumor DNA from Pancreatic Cancer Patients Enables High Fidelity Next Generation Sequencing. Cancer Manag Res 2021; 13: 6249-61.

17. Affolter KE, Hellwig S, Nix DA, et al. Detection of circulating tumor DNA without a tumor-informed search using next-generation

sequencing is a prognostic biomarker in pancreatic ductal adenocarcinoma. Neoplasia (New York, NY) 2021; 23(9): 859-69.

18. Jiang J, Ye S, Xu Y, et al. Circulating Tumor DNA as a Potential Marker to Detect Minimal Residual Disease and Predict Recurrence in Pancreatic Cancer. Frontiers in oncology 2020; 10: 1220.

19. Macgregor-Das A, Yu J, Tamura K, et al. Detection of Circulating Tumor DNA in Patients with Pancreatic Cancer Using Digital Next-Generation Sequencing. The Journal of molecular diagnostics : JMD 2020; 22(6): 748-56.

20. Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC - challenges to implementing ctDNA-based screening and MRD detection. Nature reviews Clinical oncology 2018; 15(9): 577-86.

21. Bai Y, Wang Z, Liu Z, Liang G, Gu W, Ge Q. Technical progress in circulating tumor DNA analysis using next generation sequencing. Molecular and cellular probes 2020; 49: 101480.

22. Jeffrey SS, Toner M. Liquid biopsy: a perspective for probing blood for cancer. Lab on a chip 2019; 19(4): 548-9.

23. Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic - implementation issues and future challenges. Nature reviews

Clinical oncology 2021; 18(5): 297-312.

24. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nature reviews Clinical oncology 2018; 15(2): 81-94.

25. Abbosh C, Swanton C, Birkbak NJ. Clonal haematopoiesis: a source of biological noise in cell-free DNA analyses. Annals of oncology : official journal of the European Society for Medical Oncology 2019; 30(3): 358-9.

26. Liu J, Chen X, Wang J, et al. Biological background of the genomic variations of cf-DNA in healthy individuals. Annals of oncology : official journal of the European Society for Medical Oncology 2019; 30(3): 464-70.

국문 초록

서론: 췌관선암은 대표적인 난치암으로, 진단 당시 환자의 20%만이 근치적 절제술에 적합하지만 80%는 결국 종양이 재발한다. 따라서, 조기에 종양을 감지하고 근치적 절제후 미세잔존질환을 모니터링하기 위한 민감하고 특이적인 종양 표지자의 확립이 필요하다. 순환 종양 DNA는 최소 침습적인 방법으로 쉽게 연속적으로 모니터링할 수 있는 유망한 바이오마커이지만, 질병의 초기단계에서 순환 종양 DNA의 민감한 검출 및 정량화는 어려운 실정이다. 본 연구에서는 전향적으로 모집한 절제가능한 췌관선암 환자군에서 순환 종양 DNA 분석을 위한 최적화된 차세대 염기서열기법 기술을 적용하여, 실제 순환 종양 DNA 검출 양상과 조직 DNA 분석과의 일치도를 평가하여 순환 종양 DNA 검출의 절제가능 췌관선암에의 적용 가능성을 살펴보고자 하였다.

방법: 본 연구는 2020년 8월부터 2021년 10월까지 췌장 종양에 대한 근치적 절제술을 받는 절제 가능한 췌관선암으로 진단된 총 70명의 환자를 전향적 및 연속적으로 등록하였고, 이들 환자에서 짝지어진 말초혈액 (진단 당시, 그리고 수술 후) 및 조직 검체를 수집하였다. 이 중 선정 기준에 적합하여 분석이 가능했던 53명의 환자에서, 각 시점의 세포 유리 DNA와 조직 및 배선 DNA를 추출하여 77개의 선택된 유전자를 표적으로 하는 integrated digital error suppression (iDES)– CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) 방법으로 최적화된 차세대 염기서열 분석을 시행하였다. 또한 16명의 건강한 대조군으로부터 수집된 혈장 검체에서 추출한 세포 유리 DNA도 같은 방법으로 분석하였다.

결과: 평가 가능한 환자의 37.7%에서 수술 전 순환 종양 DNA를 검출할 수 있었는데, 변이 대립유전자 빈도(variant allele frequency)의 중간

값은 0.09% (사분위 범위, 0.04% – 0.16%)로 이는 전이성 췌관선암에서 기보고된 것 보다 약 40배 낮다. 빈도 순으로는 *TP53, KRAS, GNAS, SMAD4, PIK3CA*, 그리고 *CDKN2A* 순이었다. 전체적으로, 환자의 34.0%(18/53)가 혈장 검체와 조직 모두에서 임상적으로 관련된 돌연변이가 검출되었고, 13.2%(7/53)는 조직 DNA에서만 검출되었으며, 3.8%(2/53)는 혈장 검체에서만 검출되었다. 변이별로 비교를 해보았을 때, 조직 검체에서 검출되지 않은 12개 (TP53, n = 6; GNAS, n = 5; SMAD4, n = 1)의 추가 돌연변이가 혈장 검체에서 검출되었다. 변이 대립유전자 빈도는 조직(중앙값, 12.99%, 사분위 범위, 7.65% – 24.96%)보다 순환 종양 DNA에서(중앙값, 0.08%; 사분위 범위, 0.04% – 0.37%)에서 유의하게 낮았다 ($P < 0.001$). 근치적 절제술 후 순환 종양 DNA 검출을 분석하였을 때, 근치적 절제술 후에는 검출 가능한 순환 종양 DNA를 가진 환자의 비율이 15.1% 감소하였고, 순환 종양 DNA의 변이 대립유전자 빈도는 수술 후 혈장에서 유의하게 감소했다 ($P < 0.001$). 수술 전 ctDNA가 검출된 20명의 환자 중 수술 후 ctDNA가 음성으로 전환된 환자보다 수술 후에도 여전히 ctDNA가 검출된 환자에서 1년 후 재발 위험이 더 높은 경향을 확인하였다(48.6% vs. 25.0%, P=0.064). 추가적으로 환자의 백혈구 DNA와 비교 분석을 한 결과, 환자의 24.5 %에서 혈장 검체에 클론성 조혈증 유래 돌연변이가 검출되었다.

결론: 절제 가능한 췌관선암 환자에서 검출되는 순환 종양 DNA의 분율은 매우 낮았으며, 최적화된 차세대 염기서열 분석법을 적용하여 절제 가능한 췌관선암에서 순환 종양 DNA의 극대화된 민감한 검출이 가능함을 확인하였고, 이는 조직 DNA 분석과 병렬적으로 사용되었을 때 추가적인 이점을 제공할 수 있을 것으로 기대된다. 또한, 임상적으로 의미있는 순환

종양 DNA를 정확하게 검출하기 위해 짝지어진 백혈구 DNA의 염기서열분석이 필요할 것으로 생각된다.